Derivati nesteroidnih protuupalnih lijekova i aminokiselina - sinteza, karakterizacija i biološko djelovanje

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Doctoral thesis / Disertacija

2008

Degree Grantor / Ustanova koja je dodijelila akademski / stručni stupanj: **University of Zagreb, Faculty of Pharmacy and Biochemistry / Sveučilište u Zagrebu, Farmaceutsko-biokemijski fakultet**

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:163:773431

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SVEUČILIŠTE U ZAGREBU
FARMACEUTSKO-BIOKEMIJSKI FAKULTET

Zrinka Rajić

DERIVATI NESTEROIDNIH PROTUUPALNIH LIJEKOVA I AMINOKISELINA – SINTEZA, KARAKTERIZACIJA I BIOLOŠKO DJELOVANJE

DOKTORSKI RAD

Zagreb, 2008.

Ovaj doktorski rad izrađen je na Zavodu za farmaceutsku kemiju Farmaceutsko-biokemijskog fakulteta Sveučilišta u Zagrebu pod vodstvom prof. dr. sc. Branke Zorc i predan je na ocjenu Fakultetskom vijeću Farmaceutsko-biokemijskog fakulteta Sveučilista u Zagrebu radi stjecanja znanstvenog stupnja doktora znanosti iz područja biomedicina i zdravstvo, polje farmacija, grana farmacija

Zahvaljujem

Prof. dr. sc. B. Zorc i prof. dr. sc. I. Butuli na pruženoj pomoći, savjetima i znanstvenim diskusijama tijekom izrade doktorskog rada.

I. Perković, mr. pharm., na znanstvenim raspravama, zajedničkom vremenu provedenom u laboratoriju i pomoći kod antimikrobnih ispitivanja.

Prof. dr. sc. M. Jadrijević-Mladar Takač i dr. sc. M. Vinković na pomoći kod interpretacije dvodimenzijskih NMR spektara.

Prof. dr. sc. K. Pavelića i dr. sc. M. Kralj na antitumorskim ispitivanjima.

Prof. dr. sc. E. De Clercqu, prof. dr. sc. J. Balzariniju i prof. dr. sc. M. Mintasu na antivirusnim i antitumorskim ispitivanjima.

Prof. dr. sc. S. Pepeljnjaku i dr. sc. I. Kosalecu na pomoći kod antimikrobnih ispitivanja.

Prof. dr. sc. T. Žanić Grubišić na pomoći i savjetima kod ispitivanja inhibicije ureaze.

Prof. dr. sc. D. Hadjipavlou-Litini na antioksidativnim ispitivanjima, ispitivanjima inhibicije lipooksigenaze iz soje i lipidne peroksidacije linolne kiseline.

Prof. dr. sc. T. Ossowskom na određivanju pK_a vrijednosti.

Prof. dr. sc. M. Medić-Šarić i svim članovima Zavoda za farmaceutsku kemiju Farmaceutsko-biokemijskog fakulteta na pruženoj pomoći i susretljivosti.

Svojoj obitelji na nesebičnoj podršci.

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BASIC DOCUMENTATION CARD

KRATICE

AAPH	2,2'-azobis(2-amidinopropan) dihidroklorid
ATCC	American Type Culture Collection
Boc	*t*-butiloksikarbonil
BOP-Cl	bis(2-okso-3-oksazolidinil)fosfonil klorid
BtcCl	klorid 1-benzotriazolkarboksilne kiseline
BtH	benzotriazol
CDI	1,1'-karbonilimidazol
CEM	stanična linija zloćudno preobraženih T-limfocita
CLSI	Clinical and Laboratory Standards Institute
COSY	Correlation Spectroscopy
DCC	*N*,*N*'-dicikloheksilkarbodiimid
Dic	diklofenak
DMAP	4-dimetilaminopiridin
DPPH	1,1-difenil-2-pikrilhidrazil radikal
EDC	1-etil-3-(3-dimetilaminopropil)karbodiimid
Fen	fenoprofen
FM3A/0	stanična linija karcinoma prsne žlijezde
Fmoc	9-fluorenilmetoksikarbonil
H 460	stanična linija karcinoma pluća
HCT 116	stanična linija karcinoma debelog crijeva
HeLa	stanična linija karcinoma grlića maternice
HMBC	Heteronuclear Multiple Bond Correlation
HMQC	Heteronuclear Multiple Quantum Correlation
HSV-1 (KOS)	herpes simpleks virus tip 1
HSV-1 TK KOS ACV^r	herpes simpleks virus tip 1 bez aktivnosti timidin kinaze
HSV-2 (G)	herpes simpleks virus tip 2
HyKet	2-(3-(hidroksi(fenil)metil)fenil)propanska kiselina
Ibu	ibuprofen
Ind	indometacin
Ket	ketoprofen
KetNHOH	ketoprofen hidroksamska kiselina

L1210	stanična linija leukemije porijeklom iz miša
LDA	litijev diizopropilamid
LO	lipooksigenaza iz soje
LP	lipidna peroksidacija
MCC	minimalna citotoksična koncentracija
MCF-7	stanična linija karcinoma dojke
MeKet	2-(3-benzilfenil)propanska kiselina
MiaPaCa-2	stanična linija karcinoma gušterače
MIC	minimalna inhibitorna koncentracija
MMcC	minimalna mikrobicidna koncentracija
MOLT-4	stanična linija akutne limfoblastične leukemije
Molt4/C8	stanična linija zloćudno preobraženih T-limfocita
MW	mikrovalna radijacija
NCCLS	National Committee for Clinical Laboratory Standards
NCI-H727	stanična linija karcinoma pluća velikih stanica
NSAID	nesteroidni protuupalni lijekovi
Pd/C	paladij na ugljenu
Pd/C(en)	Pd-etilendiamin kompleks na ugljenu
Ra-Ni	Raney-nikal
SW620	stanična linija karcinoma debelog crijeva
SK-BR-3	stanična linija metastatskog adenokarcinoma dojke
TEA	trietilamin
TFA	trifluoroctena kiselina
TMS	tetrametilsilan
WI 38	stanična linija normalnih fibroblasta porijeklom iz čovjeka

1. UVOD

Hidantoini, derivati 2,4-imidazolidindiona, su heterociklički spojevi među kojima se nalaze važne ljekovite tvari iz različitih terapijskih skupina.[1,2] Tijekom prijašnjih istraživanja na Zavodu za farmaceutsku kemiju sintetizirana je serija hidantoina te je ispitano njihovo citostatsko i virustatsko djelovanje.[3,4] Najbolje farmakološko djelovanje imali su hidantoini s lipofilnim supstituentima (cikloalkil, fenil, benzhidril). Zbog toga je u okviru ovog doktorskog rada sintetizirana serija novih strukturnih analoga tih vodećih spojeva intramolekulskom ciklizacijom amida *N*-(1-benzotriazolkarbonil)aminokiselina prema ranije opisanom postupku,[3–5] te je ispitano njihovo citostatsko i virustatsko djelovanje.

Ibuproksam i oksametacin su hidroksamske kiseline, derivati nesteroidnih protuupalnih lijekova (NSAID) ibuprofena i indometacina, čija se analgetska, antipiretska i protuupalna svojstva temelje na inhibiciji enzima ciklooksigenaze i 5-lipooksigenaze.[6,7] Brojni radovi i patenti opisuju druge NSAID hidroksamske kiseline kao snažne inhibitore 5-lipooksigenaze i ciklooksigenaze.[6,8,9] Međutim, u literaturi nema podataka o djelovanju NSAID hidroksamskih kiselina na ostale enzime koje hidroksamske kiseline mogu inhibirati (matriksna metaloproteinaza, angiotenzin konvertirajući enzim, leukotrien A_4 hidrolaza, peroksidaze, ureaze, ribonukleotid reduktaze, peptidna deformilaza i deacetilaza histona).[8,10–14]

Pretjerana ekspresija i aktivacija metaloproteinaza uključena je u brojne bolesti, uključujući invaziju tumora i metastaze.[8] Potencijal samih NSAID kao lijekova protiv malignih bolesti sugeriraju brojne epidemiološke, eksperimentalne i kliničke studije.[15]

U okviru ovog doktorskog rada pripravljena je serija NSAID hidroksamskih kiselina i njihovih derivata novim sintetskim postupkom, iz NSAID benzotriazolida i odgovarajućeg hidroksilamina, te je ispitano njihovo antitumorsko, antimikrobno, antivirusno i antioksidacijsko djelovanje, učinak na ureazu, lipooksigenazu i lipidnu peroksidaciju linolne kiseline.

Ketoprofen je lijek iz skupine NSAID s analgetskim, antipiretskim, antireumatskim i protuupalnim djelovanjem. Radi smanjivanja gastrointestinalnih nuspojava tipičnih za NSAID, produljivanja poluvijeka života u plazmi i modificiranja lipofilnosti pripravljeni su njegovi brojni prolijekovi.[16–20] Također je pokazano da amidi ketoprofena imaju snažnije antitumorsko djelovanje od samog ketoprofena.[21]

U ovom radu opisana je priprava serije novih amidnih derivata ketoprofena, kao i ispitivanje njihovog antitumorskog i antioksidacijskog djelovanja, učinka na lipooksigenazu i lipidnu peroksidaciju linolne kiseline.

Isto tako, pripravljeni su 3-hidroksipropilamidi NSAID ibuprofena, fenoprofena, ketoprofena, diklofenaka i indometacina te je ispitano njihovo antitumorsko i antivirusno djelovanje.

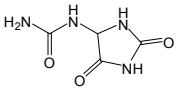
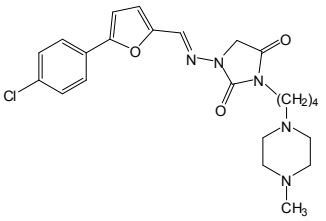
2. OBRAZLOŽENJE TEME

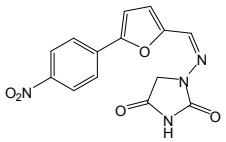
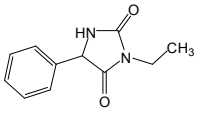
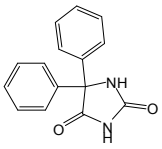
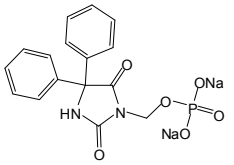
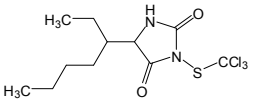
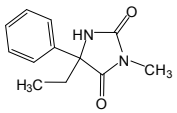
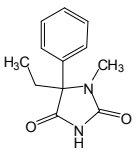
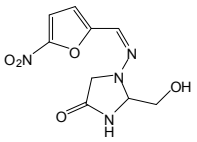
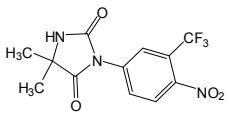
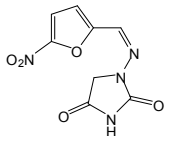
2.1. HIDANTOINI

Hidantoini, derivati 2,4-imidazolidindiona, su heterociklički spojevi među kojima se nalaze važne ljekovite tvari iz različitih terapijskih skupina (antiepileptici, antiaritmici, nesteroidni antiandrogeni, miorelaksansi, fungicidi, uroantiseptici, antipsorijatici, keratolitici, adstringensi i antacidi, Tablica 1).[1,2] Najvažniji među njima su antiepileptici (antikonvulzivi). Hidantoini s antikonvulzivnim djelovanjem imaju po dva supstituenta na položaju C-5 i, ponekad, dodatni supstituent na N-3 (numeracija atoma prikazana je na Slici 1). Važni antikonvulzivi su fenitoin (5,5-difenil-2,4-imidazolidindion), mefenitoin (5-etil-5-fenil-3-metil-2,4-imidazolidindion), metetoin (5-etil-5-fenil-1--metil-2,4-imidazolidindion), etotoin (3-etil-5-fenil-2,4-imidazolidindion) i prolijek fenitoina fosfenitoin natrij (dinatrijev (2,5-diokso-4,4-difenilimidazolidin-1-il)metil fosfat).[1,22] Antikonvulzivno djelovanje imaju i 5,5-ditienilhidantoin, 5,5-dipiridil-hidantoin, spirotiohidantoini, tiohidantoini i ditiohidantoini.

Neki hidantoini također imaju antidepresivno, antivirusno i antitrombotsko djelovanje, kao i inhibitorno djelovanje prema nekim enzimima (humana aldoza reduktaza i humana leukocitna elastaza).[23]

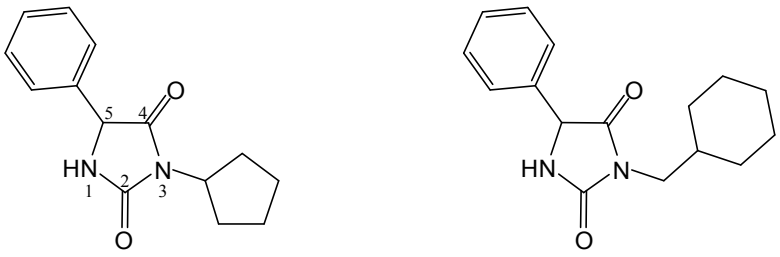
Tablica 1. Pregled derivata hidantoina u suvremenoj terapiji.

Generički naziv [kemijski naziv]	Struktura	Farmakološko djelovanje	Trgovački naziv
Alantoin [(2,5-diokso-4-imidazolidinil)-urea]		antipsorijatik adstringens keratolitik antacid	Actinac,[a] Bahnex, Contractubex, Hepathrombin, Lipo Codres, Psoralon,
Azimilid [1-(((-5-(4-klorfenil)-2-furanil)-metilen)amino)-3-(4-(4-metil-1--piperazinil)butil)-2,4--imidazolidindion]		antiaritmik	Stedicor

Dantrolen 1-(((-5-(4-nitrofenil)-2-furanil)-metilen)amino)-2,4--imidazolidindion]		miotonolitik	Dantramacrin, Dantrium, Dantrolen
Etotoin [3-etil-5-fenil-2,4--imidazolidindion]		antiepileptik	Accenon, Peganone
Fenitoin [5,5-difenil-2,4--imidazolidindion]		antiepileptik	Difetoin,[a] Dilantin, Dintospina, Phenytoin AWD
Fosfenitoin natrij [dinatrijev (2,5-diokso-4,4--difenilimidazolidin-1-il)metil fosfat]		antiepileptik	Cerebyx
Klodantoin [5-(1-etilpentil)-3-((triklormetil)-tio)-2,4-imidazolidindion]		fungicid	Gynelan, Sporostacin
Mefenitoin [5-etil-5-fenil-3-metil-2,4--imidazolidindion]		antiepileptik	Mesantoin, Mesantoina, Sédantoinal
Metetoin [5-etil-5-fenil-1-metil-2,4--imidazolidindion]		antiepileptik	Deltoin
Nifurtoinol [3-(hidroksimetil)-1-(((5-nitro-2--furanil)metilen)amino)-2,4--imidazolidindion]		uroantiseptik	Fultrexin, Urfadyn, Urfadyne
Nilutamid [5,5-dimetil-3-(4-nitro-3-(trifluormetil)pentil)-2,4--imidazolidindion]		nesteroidni antiandrogen	Anandron, Nilandron
Nitrofurantoin [1-(((5-nitro-2-furanil)metilen)-amino)-2,4-imidazolidindion]		uroantiseptik	Cistofuran,[a] Furadantine, Macrobid, Ninur, Nitrofurin

[a] Navedeni su samo neki zaštićeni nazivi lijekova.

Tijekom prijašnjih istraživanja na Zavodu za farmaceutsku kemiju sintetizirana je serija 3,5-disupstituiranih hidantoina te je ispitano njihovo citostatsko i virustatsko djelovanje.[3,4] Najbolju farmakološku aktivnost imali su hidantoini s lipofilnim supstituentima (Slika 1).



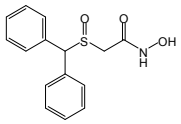
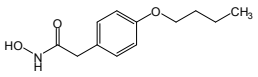
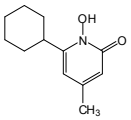
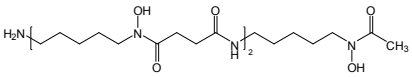
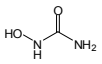
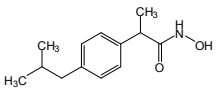
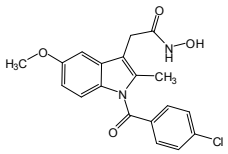
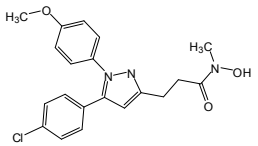
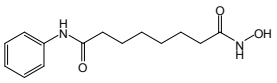
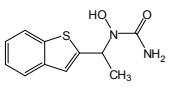
Slika 1. 3-Ciklopentil-5-fenil hidantoin i 3-cikloheksanmetil-5-fenil hidantoin.

Zbog toga je u okviru ovog doktorskog rada pripravljena serija novih strukturnih analoga tih vodećih spojeva s aromatskim supstituentom na C-5, N-3 ili na oba atoma intramolekulskom ciklizacijom amida *N*-(1-benzotriazolkarbonil)aminokiselina, prema ranije opisanom postupku,[3–5] te je ispitano njihovo citostatsko i virustatsko djelovanje.

2.2. NSAID HIDROKSAMSKE KISELINE I DERIVATI

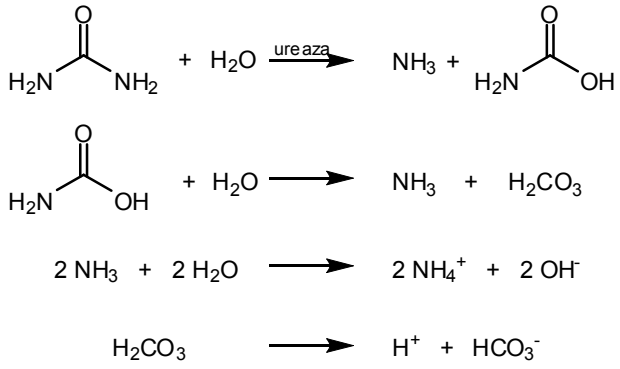
Hidroksamske kiseline skupina su spojeva s vrlo širokim biološkim djelovanjem. U biomedicinskim znanostima imaju ulogu u dizajniranju lijekova protiv malignih i kardiovaskularnih bolesti, AIDS-a, Alzheimerove bolesti, malarije, tuberkuloze, alergija, trovanja metalima i stanja s viškom željeza u krvi.[14,24–32] U Tablici 2 prikazani su registrirani lijekovi s funkcionalnom skupinom hidroksamskih kiselina i njima srodnih hidroksiurea.

Tablica 2. Pregled derivata hidroksamskih kiselina i hidroksiurea u suvremenoj terapiji.

Generički naziv [kemijski naziv]	**Struktura**	**Farmakološko djelovanje**	**Trgovački naziv**
Adrafinil [benzhidrilsulfinilaceto-hidroksamska kiselina]		α-adrenergik antidepresiv	Olmifon
Bufeksamak [2-(4-butoksifenil)--*N*-hidroksiacetamid]		antiinflamatorik analgetik	Bufex, Bufexan, Duradermal, Jomax, Parfenac
Ciklopiroks [6-cikloheksil-1-hidroksi--4-metillpiridin-2(1*H*)--on]		fungicid	Batrafen, Loprox, Penlac, Stieprox
Desferioksamin [N^1-(5-aminopentil)-N^1--hidroksi-N^4-(5-(*N*--hidroksiacetamido)-pentil)sukcinamid]		kelator željeza	Desferal
Hidroksiurea [1-hidroksiurea]		citostatik	Droxia, Hydrea
Ibuproksam [*N*-hidroksi-2-(4--izobutilfenil)propanamid]		antiinflamatorik analgetik	Ibudros, Deflogon
Oksametacin [*N*-hidroksi-1-(4--klorbenzoil)-2-metil-5--metoksi-1*H*-indol-3--acetamid]		antiinflamatorik analgetik	Dinulcid, Flogan, Restid
Tepoksalin [3-(5-(4-klorofenil)-1-(4--metoksifenil)-pirazol-3--il)-*N*-hidroksi-*N*-metil-propanamid]		antiinflamatorik analgetik	Zubrin
Vorinostat [*N*-hidroksi-*N*'-feniloktan-diamid]		citostatik	Zolinza
Zileuton [1-(1-(benzo[b]tiofen-2--il)etil)-1-hidroksiurea]		antiastmatik	Zyflo

Hidroksamske kiseline imaju sposobnost inhibicije različitih enzima, uključujući ureaze, lipooksigenaze, deacetilazu histona, enzime iz skupine metaloproteinaza s cinkom (matriksna metaloproteinaza, angiotenzin konvertirajući enzim, leukotrien A_4 hidrolaza), peroksidaze, ribonukleotid reduktaze i peptidnu deformilazu.[8,10–14] Njihovo djelovanje temelji se na sposobnosti stvaranja kompleksa vrlo velikih konstanti stabilnosti s metalnim ionima koji se nalaze u aktivnom mjestu enzima.

Ureaze kataliziraju hidrolizu uree do amonijaka i karbaminske kiseline, koja spontano hidrolizira u drugu molekulu amonijaka i ugljičnu kiselinu. Molekule amonijaka su protonirane kod fiziološkog pH, dok ugljična kiselina disocira (Shema 1).[33]



Shema 1. Hidroliza uree djelovanjem ureaze.

Stehiometrijske i spektroskopske analize izoliranih ureaza iz biljnih i bakterijskih izvora (*Canavalia ensiformis* (*Jack bean*), *Klebsiella aerogenes*, *Bacillus pasteurri*) pokazale su da sve sadrže dva Ni^{2+} iona po aktivnom mjestu enzima.[33]

Bakterijske ureaze važne su u patogenezi brojnih bolesti kod čovjeka i životinja. Ureaze su uključene u tvorbu infektivnih kamenaca i sudjeluju u patogenezi urolitijaze, pijelonefritisa, jetrene encefalopatije i hepatičke kome.[33] Infekcija bakterijom *Helicobacter pylori* uzrokuje gastritis te je uključena u nastanak čira na želucu i karcinoma želuca.[10,34] *H. pylori* preživljava u jako kiselom okruženju želuca zahvaljujući svojoj ureazi, koja razgrađuje ureu iz želučanog soka te dolazi do povišenja pH želuca.[10,33,34] Hidroksamske kiseline su visokospecifični i potentni inhibitori ureaza biljnog i bakterijskog porijekla[35] i zbog toga nalaze primjenu kao potencijalni antimikrobni i antiulkusni lijekovi. Drugi razlog ispitivanja antimikrobnog djelovanja hidroksamskih kiselina njihova je sposobnost keliranja iona različitih metala, bez kojih mnogi enzimi ne funkcioniraju.

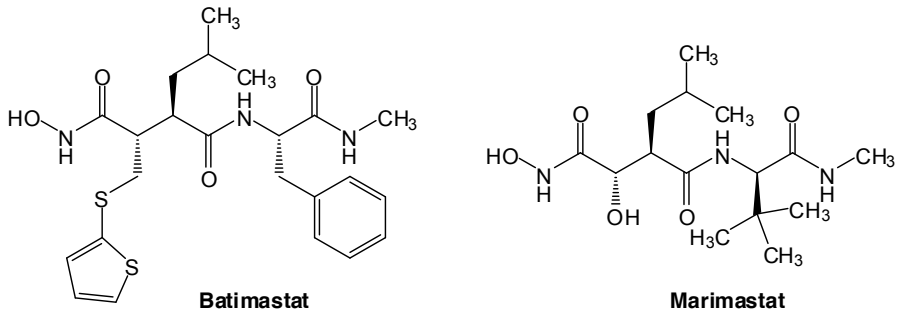
Arahidonska kiselina najčešća je polinezasićena masna kiselina u fosfolipidnom dvosloju stanične membrane. Aktivacijom fosfolipaze A_2 dolazi do otpuštanja arahidonske kiseline, koja se dalje metabolizira preko dva enzimska puta: ciklooksigenaze i 5-lipooksigenaze, te nastaju prostaglandini i leukotrieni, važni medijatori različitih bolesti, uključujući astmu, artritis, psorijazu i alergije.[36]

Ciklooksigenaza (prostaglandin endoperoksid H sintaza) je integralni membranski protein s hemom[37] te je odgovorna za pretvorbu arahidonske kiseline u prostaglandine, koji imaju vrlo široki spektar fizioloških i patofizioloških učinaka.[36]

5-Lipooksigenaza je enzim obitelji lipidnih peroksidaza s jednim nehemskim atomom željeza po molekuli, koji oscilira između Fe^{2+} (inaktivni enzim) i Fe^{3+} (aktivni enzim) tijekom katalitičkog ciklusa.[38] Ona katalizira prvi korak u biokemijskom putu u kojem se arahidonska kiselina transformira u leukotriene.[8,36] 5-Lipooksigenaza može se direktno inhibirati: (*i*) redoks inhibitorima (antioksidansima), koji utječu na redoks-sustav enzima; (*ii*) kelatorima željeza (hidroksamske kiseline i hidroksiuree) i (*iii*) neredoksnim kompetitivnim inhibitorima. Enzim se indirektno može inhibirati FLAP linhibitorima (protein koji aktivira 5-lipooksigenazu, "5-lipoxygenase activating protein"), koji je ključan za interakciju 5-lipooksigenaze i arahidonske kiseline.[36]

Inhibicija ciklooksigenaze nesteroidnim protuupalnim lijekovima, osim što uzrokuje smanjenu sintezu vazodilatatornih i gastroprotektivnih prostaglandina, dovodi do preusmjeravanja metabolizma arahidonske kiseline preko 5-lipooksigenaze, povećanja sinteze leukotriena, doprinosi upali i neželjenim učincima NSAID. Dualni inhibitori ciklooksigenaze i 5-lipooksigenaze bili bi lijekovi s povećanim protuupalnim potencijalom i manje nuspojava (ozljede gastrointestinalnog trakta, alergijske reakcije).[8,36] Inkorporacijom funkcionalne skupine hidroksamskih kiselina u poznate NSAID nastaju takvi analozi s dualnim inhibitornim djelovanjem.[6]

Matriksne metaloproteinaze su skupina enzima s ionom cinka u aktivnom mjestu enzima te razgrađuju brojne proteinske komponente ekstracelularnog matriksa. Pretjerana ekspresija i aktivacija matriksnih metaloproteinaza uključena je u brojne bolesti, uključujući artritis, periodontalnu bolest, multiplu sklerozu, invaziju tumora i metastaze.[39] Spojevi s funkcionalnom skupinom hidroksamskih kiselina najistraživanija su klasa inhibitora matriksnih metaloproteinaza zbog njihove sposobnosti kompleksacije iona cinka iz aktivnog mjesta enzima i mogućnosti tvorbe vodikovih veza s karakterističnim aminokiselinskim ostatcima.[40] Neki od tih lijekova su u fazi III kliničkih studija (batimastat, marimastat, neovastat, Slika 2).[8]



Slika 2. Inhibitori matriksnih metaloproteinaza.

Histoni su proteini čiji oktamer čini okosnicu nukleosoma stanične jezgre. Oko njega je prosječno 150 parova baza DNA 1,75 puta zamotano u obliku lijeve superuzvojnice DNA.[41] Mogu se acetilirati i deacetilirati pod utjecajem acetil transferaze, odnosno deacetilaze histona, čime se regulira količina pozitivnih naboja u molekuli (deacetilirani histon visoko je pozitivno nabijen) i ekspresija gena.[42] Nukleosomi s hipoacetiliranim histonima čvrsto se vežu za fosfatnu okosnicu DNA te dolazi do inhibicije transkripcije. Acetilacija neutralizira naboj histona i generira otvoreniju konformaciju DNA.[42,43] Inhibicijom deacetilaze histona dolazi do akumuliranja acetiliranih histona u nukleosomima i ekspresije specifičnih gena, što vodi do zastoja u rastu stanice, diferencijacije i/ili apoptoze te inhibicije rasta tumora.[14] U aktivnom mjestu deacetilaze histona nalazi se cink, kojeg mogu vezati hidroksamske kiseline, zbog čega dolazi do inhibicije enzima.[44] Vorinostat je registriran kao antitumorski lijek (Tablica 2), a nekoliko derivata hidroksamskih kiselina nalazi se u različitim fazama kliničkih ispitivanja.[14]

Ibuproksam (*N*-hidroksi-2-(4-izobutilfenil)propanamid) i oksametacin (1-(4-klorobenzoil)-*N*-hidroksi-5-metoksi-1*H*-indol-3-acetamid) su protuupalni lijekovi, hidroksamske kiseline ibuprofena i indometacina (Tablica 2). Njihova analgetska, antipiretska i protuupalna svojstva temelje se na inhibiciji enzima ciklooksigenaze i 5-lipooksigenaze te posljedičnoj inhibiciji nastanka prostaglandina i leukotriena.[6–8]

Brojni radovi i patenti opisuju druge NSAID hidroksamske kiseline, derivate diklofenaka, fenoprofena, meklofenaminske kiseline, sulindaka, izoksepaka, kao i antidepresiva dibenzoksepina kao snažne inhibitore 5-lipooksigenaze i ciklooksigenaze.[6–9] Važno je napomenuti da ti spojevi imaju nižu akutnu toksičnost, povoljniji terapijski indeks, manje su štetni za gastrointestinalni trakt i manje ga iritiraju te bolje prodiru kroz površinsku membranu od ishodnih NSAID.[45–47]

U literaturi nema podataka o djelovanju NSAID hidroksamskih kiselina na druge enzime koje hidroksamske kiseline mogu inhibirati.

Brojne epidemiološke, eksperimentalne i kliničke studije sugeriraju da su NSAID potencijalni lijekovi u terapiji malignih bolesti.[15] Ibuprofen, indometacin i neki drugi NSAID su učinkoviti kemopreventivni agensi protiv induciranih karcinoma i genetski manipuliranih animalnih modela karcinogeneze debelog crijeva.[15,48–50] Također su povezani sa smanjenim rizikom od raka dojke, jednjaka, pluća, jajnika, prostate, želuca, jetre, gušterače, jezika i multiformnog glioblastoma.[51] Točan mehanizam koji je odgovoran za antitumorsku aktivnost NSAID još uvijek je nepoznat. Smatra se da je povezan s inhibicijom inducibilne ciklooksigenaze-2, koja je prekomjerno eksprimirana u mnogim epitelnim tumorima, te s aktivacijom apoptoze, inhibicijom angiogeneze ili direktnom inhibicijom rasta tumorskih stanica blokiranjem putova prijenosa signala koji su odgovorni za rast stanica.[50,52,53]

U okviru ovog doktorskog rada pripravljena je serija NSAID hidroksamskih kiselina i njihovih derivata te je ispitano njihovo antitumorsko, antimikrobno, antivirusno i antioksidacijsko djelovanje, učinak na ureazu, lipooksigenazu i lipidnu peroksidaciju linolne kiseline.

S obzirom da u literaturi manjkaju i podaci o njihovim pK_a vrijednostima, ta konstanta je također određena. pK_a je važna fizikalno-kemijska konstanta koja određuje većinu farmakokinetičkih parametara.[54] O pH medija i pK_a supstancije ovisi stupanj disocijacije lijekova koji posjeduju kisele i bazične funkcionalne skupine, što utječe na apsorpciju lijekova iz gastrointestinalnog trakta, distribuciju po tjelesnim tekućinama i tkivima, tubularnu reapsorpciju i renalnu ekskreciju.

Istraživanja strukturnih značajki i ponašanja hidroksamskih kiselina u čvrstom stanju i u otopini predmet su brojnih istraživanja, osobito onih koja se odnose na mogućnost postojanja funkcionalne skupine hidroksamskih kiselina (-C(=O)-NH-OH) u obliku *cis*(Z)- i *trans*(E)-izomera, relativno prema C-N vezi. Ovisno o strukturnim značajkama ostatka molekule i mogućnosti tvorbe vodikove veze, izomeri se stabiliziraju u jednom ili drugom geometrijskom obliku. Isto tako, mogućnost tvorbe vodikove veze ima utjecaja na inhibiciju ciklooksigenaze djelovanjem NSAID. Zbog toga su istražene i opisane strukturne značajke i geometrija molekule u seriji derivata NSAID hidroksamskih kiselina, mogućnost tvorbe vodikove veze te ponašanje u čvrstom stanju i u otopini.

2.3. NSAID AMIDI

Brojni amidi NSAID pokazali su se kao korisni prolijekovi,[55] a imaju i utjecaj na smanjivanje gastrointestinalne toksičnosti NSAID, produljivanje poluvijeka života u plazmi i modificiranje lipofilnosti ishodnih molekula.[16–20,56]

Kao što je već rečeno, sami NSAID potencijalni su lijekovi u terapiji malignih bolesti. Različitim modifikacijama molekule to djelovanje moguće je pojačati. Serija fenolnih estera i amida naproksena pokazuje i antioksidacijsko i antiproliferativno djelovanje. Iako su i esteri i amidi bili učinkovitiji inhibitori stanične proliferacije od samog naproksena, amidni derivati pokazali su snažnije antiproliferativno djelovanje.[57]

Tijekom prijašnjih istraživanja na Zavodu za farmaceutsku kemiju sintetizirana je serija amida fenoprofena i ketoprofena kao potencijalnih prolijekova.[58,59] Ispitivanje njihovog antitumorskog djelovanja pokazalo je da imaju jače antiproliferativno djelovanje od ishodišnih NSAID.[21]

U ovom radu opisana je priprava serije novih amidnih derivata ketoprofena, kao i ispitivanje njihovog antitumorskog i antioksidacijskog djelovanja, učinka na lipooksigenazu i lipidnu peroksidaciju linolne kiseline.

Isto tako, pripravljeni su 3-hidroksipropilamidi NSAID ibuprofena, fenoprofena, ketoprofena, diklofenaka i indometacina te je ispitano njihovo antitumorsko i antivirusno djelovanje.

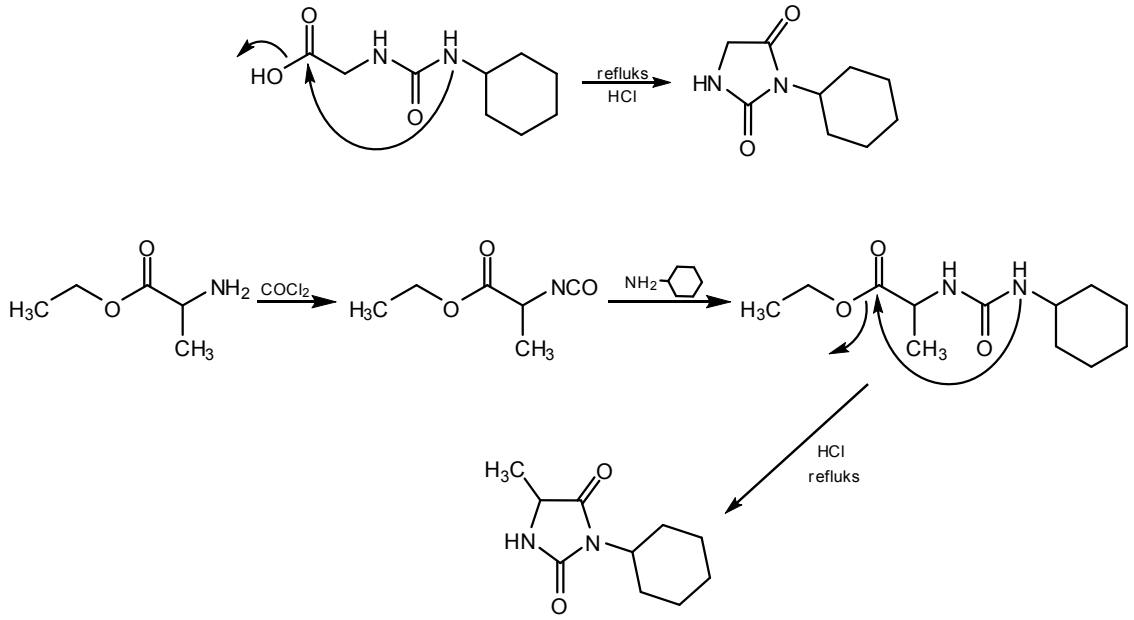
3. PREGLED LITERATURNIH PODATAKA

3.1. SINTEZA HIDANTOINA

Hidantoini se mogu pripraviti klasičnim metodama sinteze u otopini[60–67] te sintezom na čvrstoj fazi.[68–72] Isto tako, novije metode sinteze hidantoina opisuju njihovu pripravu uz pomoć mikrovalne radijacije (MW) u otopini i na čvrstoj fazi.[73,74]

Sintezom u otopini hidantoini se mogu pripraviti iz različitih polaznih spojeva: aminokiselina i njihovih derivata, hidantoinskih kiselina, ketona i aldehida, aminonitrila i amida malonske kiseline.

Ciklizacijom hidantoinskih kiselina[60] ili njihovih estera[61] nastaju 3-cikloheksil hidantoini (Shema 2).

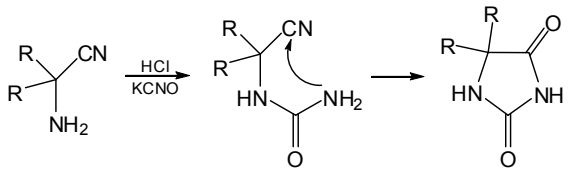


Shema 2. Sinteza hidantoina ciklizacijom hidantoinskih kiselina ili njihovih estera.

3-Supstituirani hidantoini pokazuju herbicidna svojstva, pa su slični spojevi opisani i u patentu GB 1092962.[62] Druge sinteze hidantoina, među kojima i poznatih antiepileptika fenitoina, mefenitoina i drugih, mogu se naći u revijalnom članku E. Ware.[63]

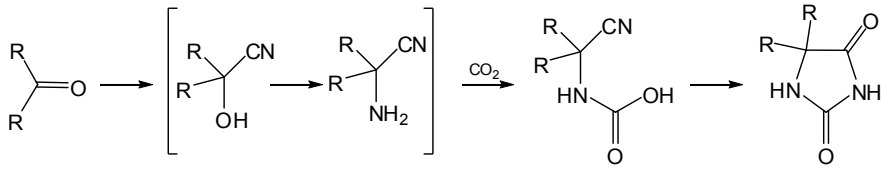
Dobivanje 5-supstituiranih, 5,5-disupstituiranih i 3,5-disupstituiranih hidantoina prikazano je u Heterocyclic Compounds po Elderfieldu (Sheme 3–7).[64]

(*i*) Dobivanje iz aminonitrila: reakcijom aminonitrila i cijanatne kiseline nastaje cijanourea, koja u kiselom mediju hidrolizira i ciklizira.



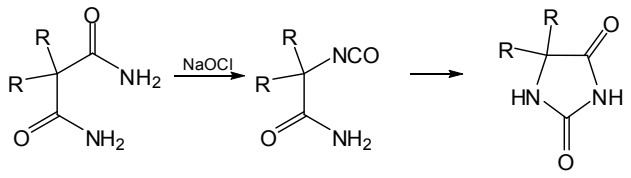
Shema 3. Sinteza hidantoina iz aminonitrila.

(*ii*) Buchererova reakcija: dobivanje iz aldehida ili ketona s kalijevim cijanidom i amonijevim karbonatom u vodeno-alkoholnoj otopini na 60–70 °C.



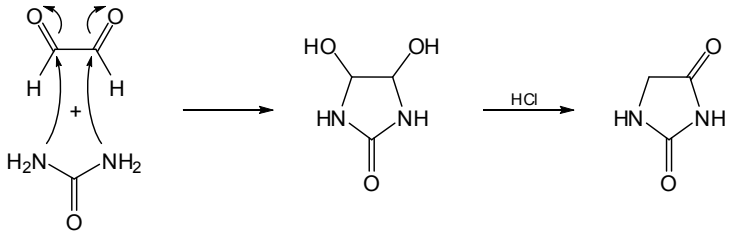
Shema 4. Sinteza hidantoina Buchererovom reakcijom.

(*iii*) Dobivanje iz amida malonske kiseline (primjena Hoffmanove reakcije na malonamide) u prisustvu hipohalita.



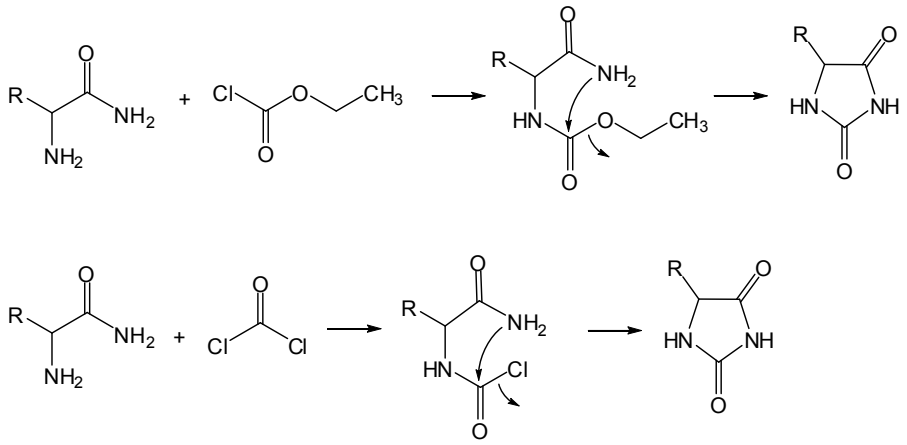
Shema 5. Sinteza hidantoina iz amida malonske kiseline.

(*iv*) Dobivanje iz glioksala i uree: produkt njihove kondenzacije u kiselom mediju prelazi u hidantoin.



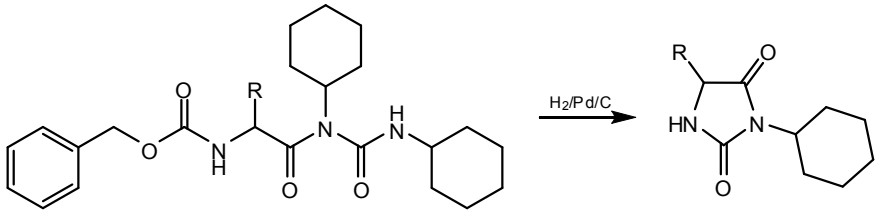
Shema 6. Sinteza hidantoina iz glioksala i uree.

(*v*) Dobivanje iz amida aminokiselina



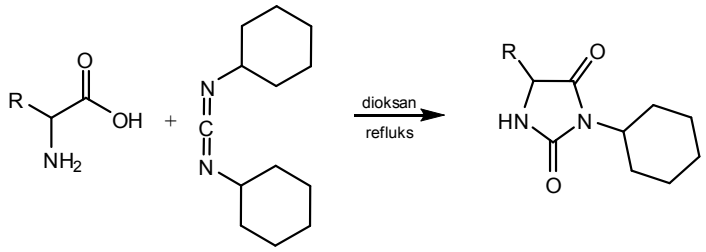
Shema 7. Sinteza hidantoina iz amida aminokiselina.

Ovim reakcijama slične su reakcije derivata aminoacil urea. Tako su npr. Schon i suradnici opisali nastajanje hidantoina kao nusprodukata prilikom uklanjanja benziloksikarbonilne skupine katalitičkim hidrogeniranjem iz zaštićenih aminoacil urea uz paladij na ugljenu (Pd/C) (Shema 8).[65]



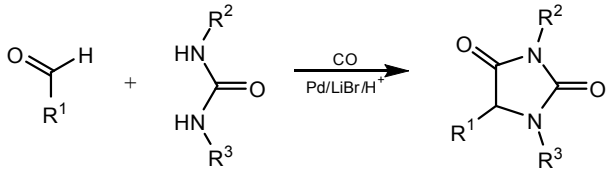
Shema 8. Nastajanje hidantoina kao nusprodukta katalitičkog hidrogeniranja.

Opisana je i sinteza hidantoina izravno iz aminokiselina, u reakciji s *N*,*N*'-diciklohekSilkarbodiimidom (DCC-om) (Shema 9).[66]



Shema 9. Sinteza hidantoina uz DCC.

Beller i suradnici opisali su sintezu 5-, 3,5- i 1,3,5-supstituiranih hidantoina koja se temelji na karbonilaciji aldehida u prisutnosti derivata uree te uz Pd kao katalizator (Shema 10). Prednosti opisane metode su jednostavnost izvođenja, dobra iskorištenja, kemo- i regioselektivnost te mogućnost primjene na multigramsku proizvodnju.[67]



Shema 10. Sinteza hidantoina metodom Bellera i suradnika.

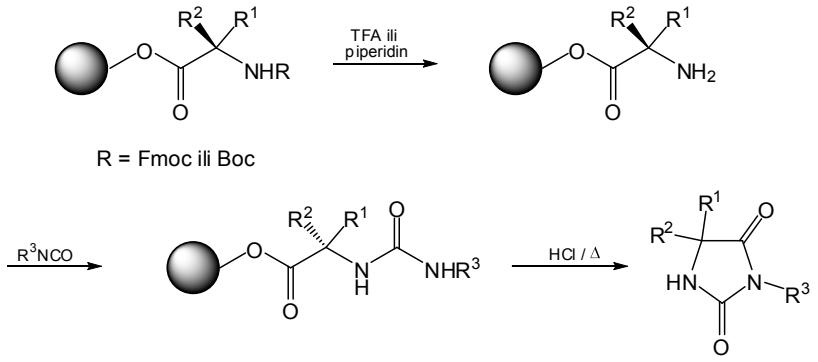
Novije metode sinteze hidantoina su sinteza hidantoina na čvrstoj fazi, sinteza na topljivom polimernom nosaču uz mikrovalnu radijaciju i sinteza na čvrstoj fazi uz mikrovalnu radijaciju, te su ovdje dani primjeri za svaki od tih sintetskih postupaka.

Dvije su temeljne metode sinteze hidantoina na čvrstoj fazi:

(*i*) Aminokiselina se veže na polimerni nosač esterskom vezom, nakon čega u reakciji s izocijanatom nastaje urea, koja se u jednom koraku skida s polimernog nosača (hidroliza esterske veze) i ciklizira u hidantoin.[68]

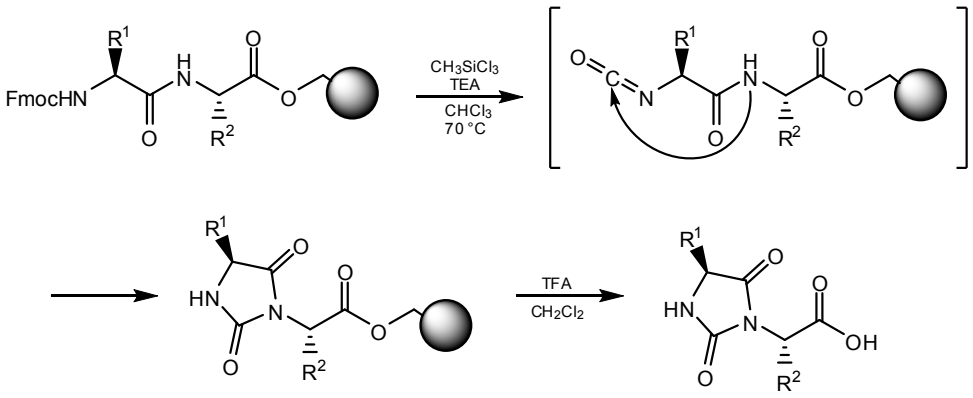
(*ii*) Na polimerni nosač vezan je dipeptid sa slobodnom terminalnom amino skupinom koja reagira s derivatom ugljične kiseline (fozgen, trifozgen, tiofozgen, 1,1'-karbonildiimidazol (CDI), *N*,*N*'-disukcinimidil karbonat) te nastaje aktivni karbamat. Nukleofilnim napadom amida peptidne veze na nastali karbamat dolazi do zatvaranja hidantoinskog prstena.[68]

Hobbs DeWitt i suradnici razvili su uređaj i metodu za višestruku simultanu sintezu na čvrstoj fazi i na taj način pripravili cijeli niz 3,5-disupstituiranih hidantoina.[69] Prvi korak je 9-fluorenilmetoksikarbonilna (Fmoc) ili *t*-butiloksikarbonilna (Boc) deprotekcija aminokiselinskog ostatka na polimernoj smoli, nakon čega slijedi reakcija s izocijanatom te nastaju uree vezane na polimerni nosač, koje reakcijom s kloridnom kiselinom (c = 6 mol L^{-1}) u jednom koraku daju hidantoine (Shema 11).



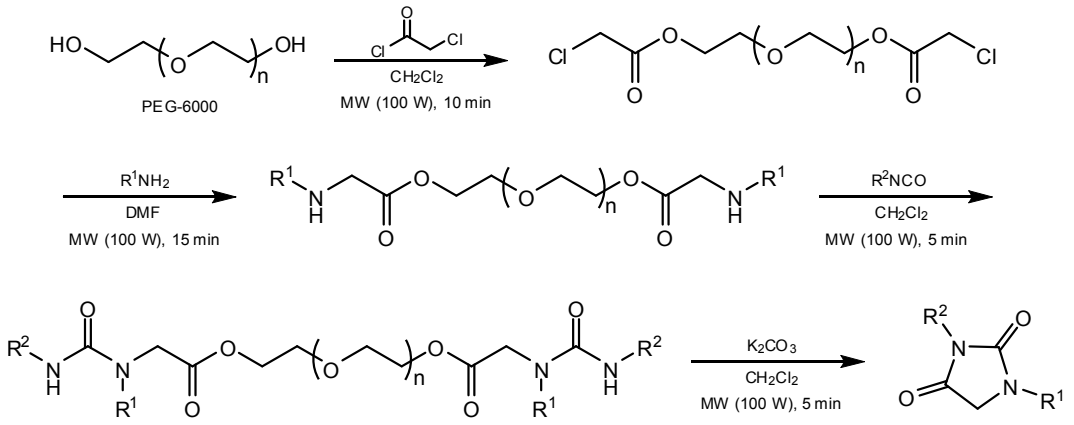
Shema 11. Sinteza hidantoina na čvrstoj fazi iz Fmoc- (Boc-) zaštićenih aminokiselina.

Chong i suradnici opisali su sintezu hidantoina na čvrstoj fazi u jednom koraku direktno iz Fmoc zaštićenih dipeptida (Shema 12).[70] Dipeptidi vezani na polimerni nosač reakcijom s triklormetilsilanom i trietilaminom (TEA) u kloroformu daju odgovarajuće izocijanate. Oni zagrijavanjem na 70 °C kroz 24 h cikliziraju i nastaju hidantoini koji se skidaju s polimernog nosača pomoću trifluoroctene kiseline (TFA).



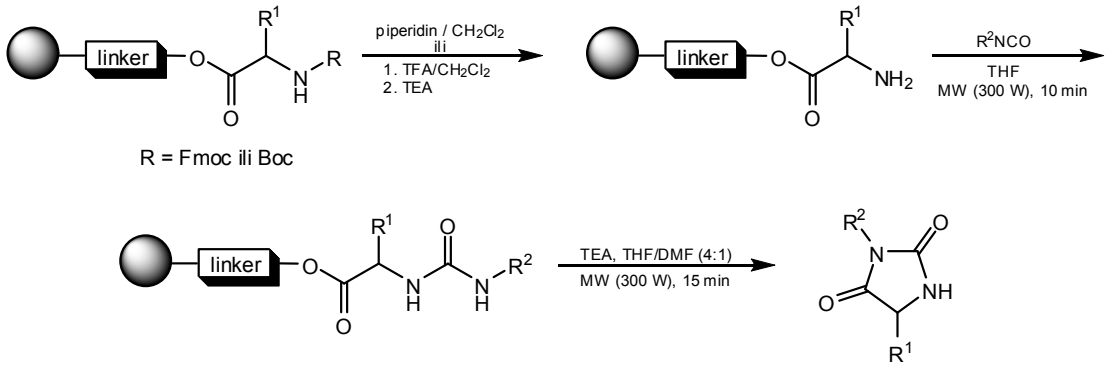
Shema 12. Sinteza hidantoina na čvrstoj fazi iz Fmoc zaštićenih dipeptida.

Korištenje mikrovalne radijacije u organskoj sintezi skraćuje trajanje reakcije, te poboljšava njezinu čistoću i iskorištenje. Lee i suradnici prvi su opisali sintezu 1,3-disupstituiranih hidantoina uz primjenu mikrovalne tehnologije (Shema 13).[73] Polimerni nosač polietilenglikol (PEG-6000) otopljen u diklormetanu u reakciji s kloridom kloroctene kiseline daje kloracetilni ester. Slijedi reakcija nukleofilne supstitucije s primarnim aminima, pri čemu nastaju odgovarajući amidi, koji s različitim izocijanatima daju uree. Konačno, u blago lužnatim uvjetima u jednom koraku dolazi do ciklizacije i skidanja s polimernog nosača. Sve reakcije izvođene su uz mikrovalnu radijaciju snage 100 W.



Shema 13. Sinteza hidantoina na topljivom polimernom nosaču korištenjem mikrovalne radijacije.

Colacino i suradnici opisali su sintezu hidantoina na čvrstoj fazi upotrebom mikrovalne radijacije.[74] Same reakcije vrlo su slične već opisanima,[69] međutim nakon N-deprotekcije na sobnoj temperaturi, iduća dva koraka izvedena su uz mikrovalnu radijaciju (Shema 14). Iste reakcije izvedene su i pod konvencionalnim uvjetima te je utvrđeno da su hidantoini uz mikrovalnu radijaciju dobiveni za puno kraće vrijeme.[74] Reakcija uz mikrovalnu radijaciju trajala je samo 25 min, a pod konvencionalnim uvjetima 22 h.

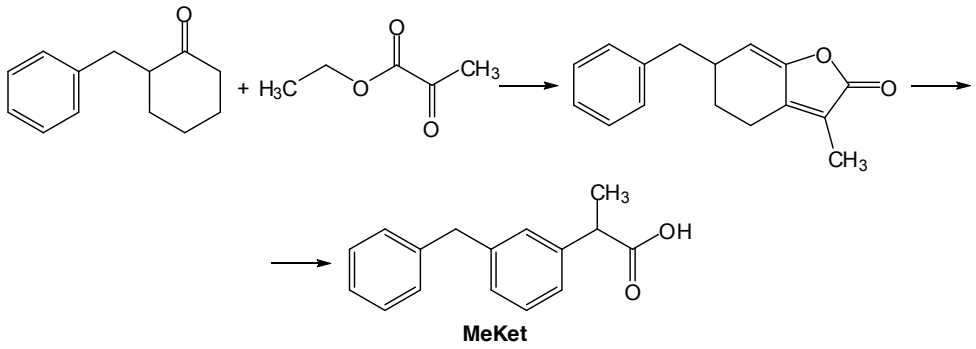


Shema 14. Sinteza hidantoina na čvrstoj fazi upotrebom mikrovalne radijacije.

3.2. SINTEZA REDUCIRANIH DERIVATA KETOPROFENA

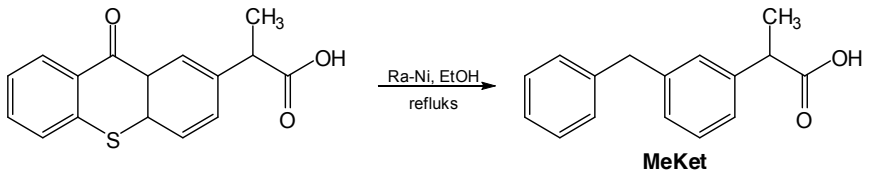
Ovdje će biti prikazane sintetske metode priprave reduciranih derivata ketoprofena: 2-(3-benzilfenil)propanske kiseline (MeKet) i 2-(3-(hidroksi(fenil)metil)-fenil)propanske kiseline (HyKet).

MeKet može se pripraviti aromatizacijom iz dihidrobenzofuranona uz piridin hidroklorid. Polazni dihidrobenzofuranon dobiven je kondenzacijom 2-benzilcikloheksanona i etil-2-oksopropanoata (Shema 15).[75]



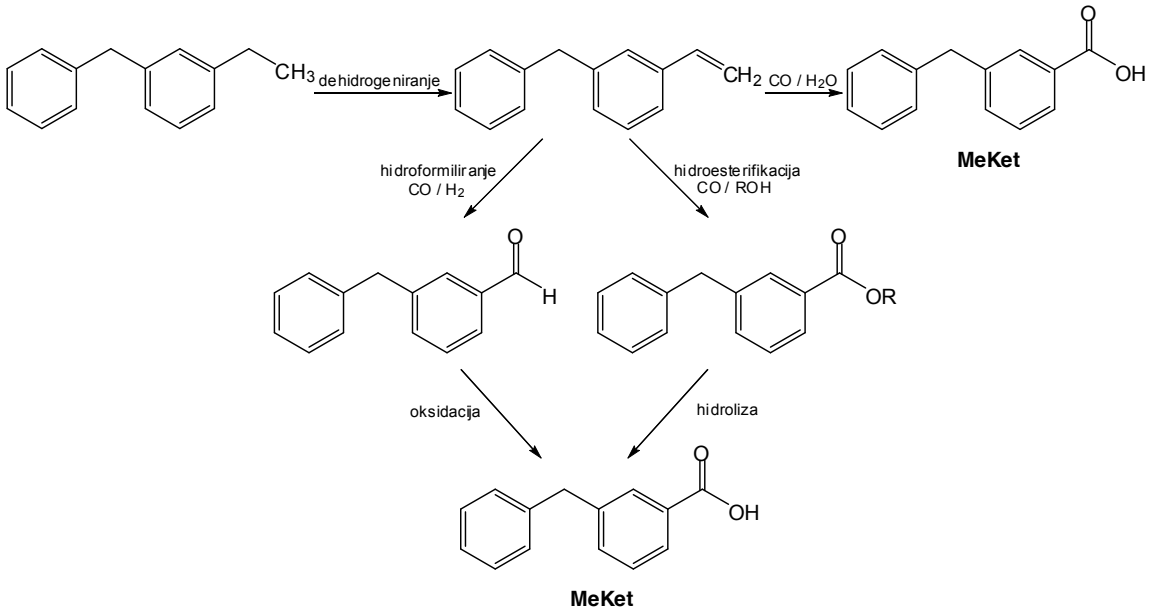
Shema 15. Sinteza MeKet iz 2-benzilcikloheksanona i etil-2-oksopropanoata.

Patent US 3641127 opisuje pripravu MeKet iz 2-(2-tiaksantonil)propanske kiseline uz Raney-nikal (Ra-Ni) kao katalizator, u etanolu i na temperaturi refluksa (Shema 16).[76]



Shema 16. Sinteza MeKet iz 2-(2-tiaksantonil)propanske kiseline uz Ra-Ni.

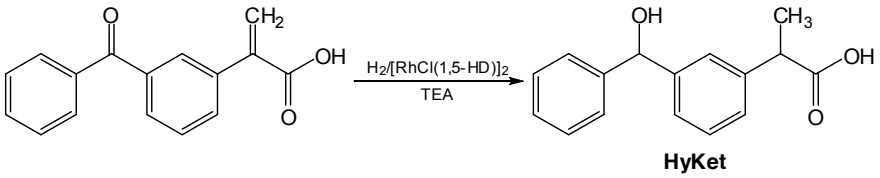
Patent WO 8807032 prikazuje sintezu MeKet u 4 koraka (Shema 17).[77] Prvi korak je sinteza etilbenzena alkilacijom benzena etenom u prisutnosti zeolita kao katalizatora. Kao nusprodukt u reakciji nastaje uljasta smjesa produkata. Jedan od njih je (3-etilfenil)fenilmetan, koji se odvaja frakcijskom destilacijom. Slijedi dehidrogeniranje dobivene frakcije uz katalizator Fe-oksid, Cr-oksid ili njihovu smjesu, na 550–650 °C, pri tlaku do 3 bara. Produkt reakcije, (3-vinilfenil)fenilmetan, odvaja se destilacijom, te se potom karboniliranjem uz komplekse platinskih metala (Pd, Ir, Rh, Ru, Re) prevodi sa smjesom CO/H_2 u odgovarajući aldehid ili sa smjesom CO/H_2O ili CO/alkohol u odgovarajuću kiselinu, odnosno ester. Oksidacijom aldehida ili hidrolizom estera nastaje arilkarboksilna kiselina MeKet.



Shema 17. Sinteza MeKet iz (3-etilfenil)fenilmetana.

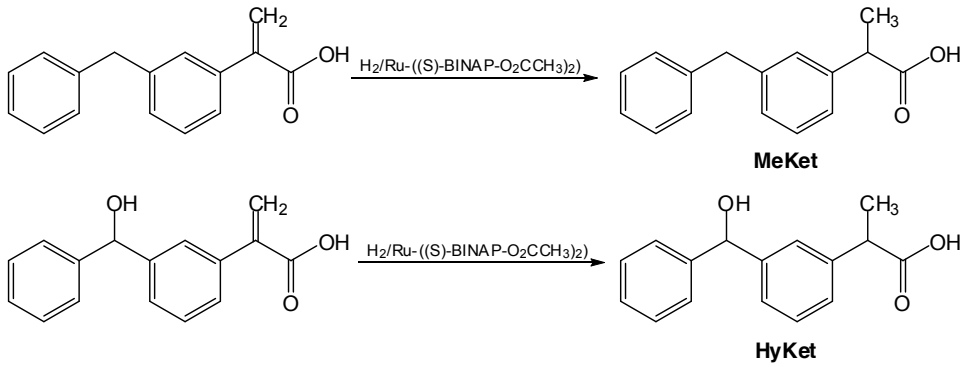
Hamon i suradnici prikazali su novu metodu sinteze racemata i (*S*)-enantiomera ketoprofena i ibuprofena, te su tu istu metodu primijenili i na sintezu MeKet.[78,79] Reakcijski put uključuje kombinaciju Sharplessove epoksidacije i stereoselektivne katalitičke hidrogenolize veze ugljik-kisik uvedenog benzilnog epoksida, čime nastaje stereogeni centar. Slijedi oksidacija dobivenog diola pri čemu se uvodi željena funkcionalna skupina. Dioli također podliježu elektrofilnoj supstituciji s jodovim monokloridom te nastaju odgovarajući jododioli. Oksidacija (rutenijev triklorid, natrijev perjodat) tih diola rezultira optički aktivnim jodokiselinama koje su prikladni supstrati za paladijem katalizirane reakcije kondenzacije s različitim organocinkovim reagensima.[78,79]

Comisso i suradnici opisali su dobivanje MeKet iz ketoprofena Wolff-Kishnerovom redukcijom (hidrazin hidrat i kalijev hidroksid u dietilenglikolu), te su dobivene enantiomere razdvojili kristalizacijom dijestereomernih soli s (–)-α-feniletilaminom.[80] Isti autori prikazali su sintezu racemičnog HyKet katalitičkim hidrogeniranjem 2-(3-benzoilfenil)propenske kiseline uz katalizator $[RhCl(1,5\text{-}HD)]_2$ (HD = heksadien, 0,025 ekvivalenata katalizatora).[81] Reakcija se zbivala u prisustvu TEA (1,26 ekvivalenata), u smjesi otapala benzen/etanol 1:1 (Shema 18). Tijekom reakcije dolazi do odlaganja elementarnog rodija. Iskorištenje reakcije nije dano.



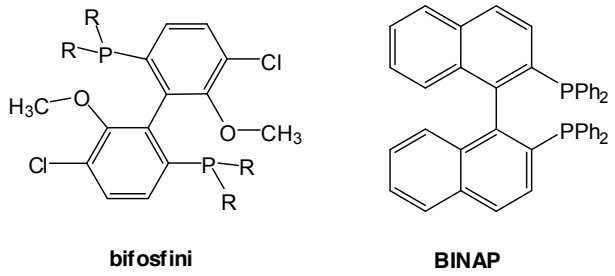
Shema 18. Sinteza HyKet katalitičkim hidrogeniranjem uz $[RhCl(1,5\text{-}HD)]_2$.

U patentu US 5362907 prikazana je sinteza MeKet i HyKet iz analognih propenskih kiselina (Shema 19). Katalitičko hidrogeniranje provedeno je u autoklavu pod tlakom vodika od 100 atm, uz Ru-((*S*)-BINAP-$O_2CCH_3)_2$) kao katalizator (Slika 3), u metanolu i na sobnoj temperaturi tijekom 48 h. Na ovaj način dobiveni su *S*-enantiomeri željenih spojeva, u visokim iskorištenjima (preko 90%) i velike enantiomerne čistoće (preko 80%).[82]



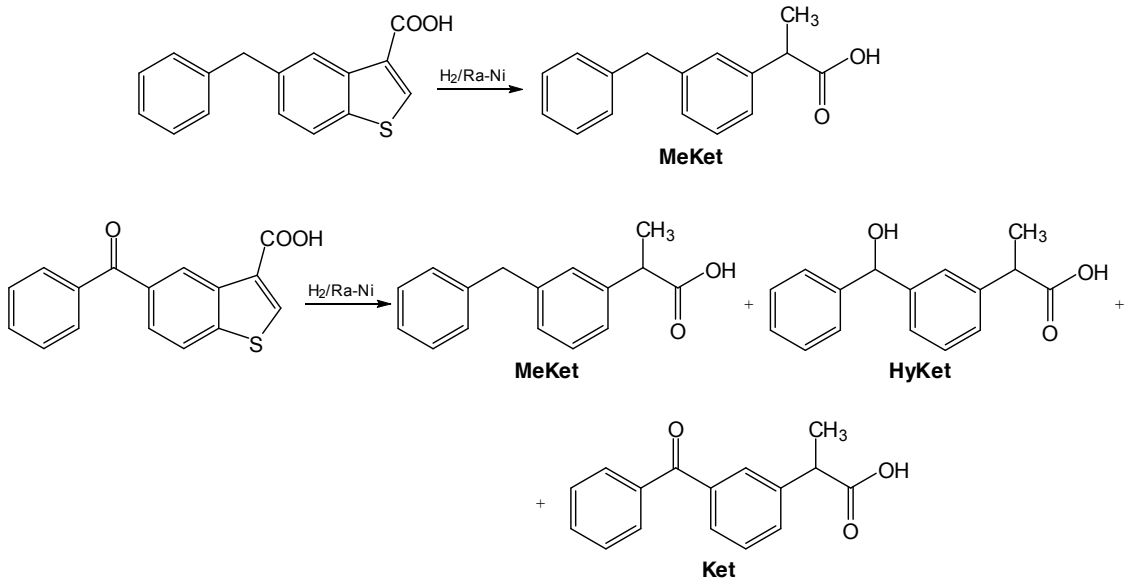
Shema 19. Sinteza MeKet i HyKet katalitičkim hidrogeniranjem uz Ru-((*S*)-BINAP-$O_2CCH_3)_2$).

Patent US 5710339 opisuje bifosfine opće formule prikazane na Slici 3. Enantiomerno su čisti, te se njihovi metalni kompleksi koriste za asimetrično hidrogeniranje. Jedan od primjera je i redukcija 2-(3-benzilfenil)propenske kiseline u MeKet, koja se provodi pod tlakom vodika od 90 atm, u metanolu i na sobnoj temperaturi. Iskorištenje reakcije je kvantitativno, a enantiomerna čistoća 88% (prevladava *S*-enantiomer).[83]



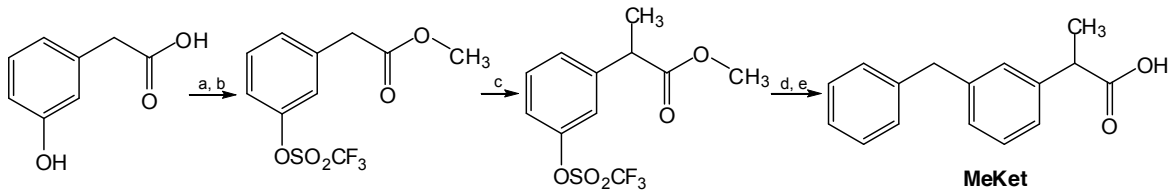
Slika 3. Opća formula bifosfina i struktura BINAP-a.

Hannoun i suradnici prikazali su dobivanje MeKet i HyKet postupkom desulfuriranja (Shema 20).[84] Sinteza MeKet iz 5-benzilbenzo[*b*]tiofen-3-karboksilne kiseline uz Ra-Ni u smjesi otopine natrijevog hidroksida (w = 5%) i etanola (1,5:1) napreduje bez poteškoća, te je željeni produkt dobiven u visokom iskorištenju (preko 90%). Izvođenjem iste reakcije s 5-benzoilbenzo[*b*]tiofen-3-karboksilnom kiselinom dobivena je smjesa ketoprofena, MeKet i HyKet. Hidrogeniranje uz deaktivirani Ra-Ni (refluksiranje tijekom nekoliko sati u acetonu), rezultiralo je isključivo ketoprofenom i spojem HyKet, koji su dobiveni u omjeru 2:3. Također je prikazana i sinteza obje ishodne benzo[*b*]tiofen-3-karboksilne kiseline.



Shema 20. Sinteza MeKet i HyKet reakcijom desulfuriranja uz Ra-Ni.

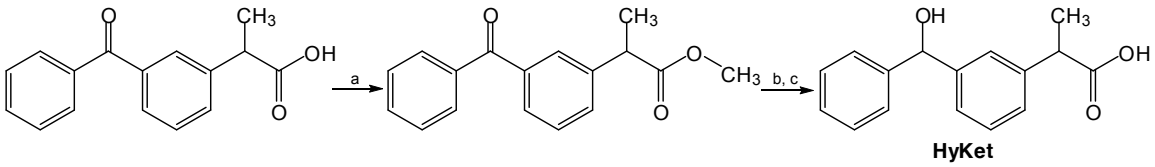
Allegretti i suradnici opisali su dobivanje MeKet i HyKet iz (3-hidroksi-fenil)octene kiseline. Početna kiselina prvo se prevodi u metilni ester, koji stupa u reakciju s trifluormetansulfonanhidridom te nastaje metil 2-(4-(trifluormetansulfonil-oksi)fenil)propanoat. Iduća reakcija je alkiliranje dobivenog estera s metil-jodidom, nakon čege slijedi reakcija s benzil-cink-bromidom i lužnata hidroliza dobivenog estera (Shema 21).[85]



Reakcijski uvjeti: (a) konc. H_2SO_4, MeOH, sobna temperatura; (b) *i*-Pr_2EtN, $(CF_3SO_2)_2O$, CH_2Cl_2, –15 °C; (c) 60% NaH, CH_3I, THF, –25 °C; (d) $PhCH_2ZnBr$, LiCl, $Pd(PPh_3)_4$, THF, refluks; (e) KOH, MeOH, sobna temperatura.

Shema 21. Sinteza MeKet iz (3-hidroksifenil)octene kiseline.

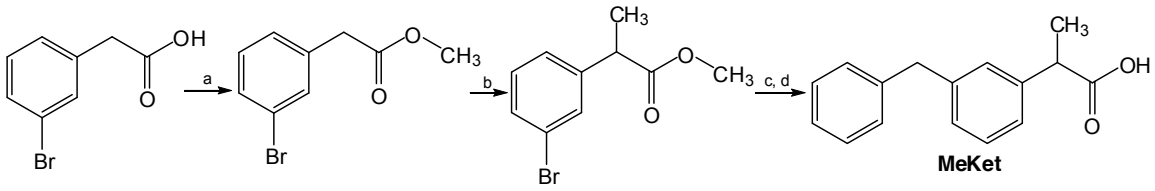
HyKet sintetiziran je iz metilnog estera ketoprofena katalitičkim hidrogeniranjem uz 10%-tni Pd/C:TEA 1:1 (u odnosu na Pd, *m*/*m*). Nakon izolacije, reducirani ester podvrgnut je lužnatoj hidrolizi te je dobiven HyKet. Ukupno iskorištenje nakon dva koraka bilo je 75% (Shema 22).[85]



Reakcijski uvjeti: (a) konc. H_2SO_4, MeOH, sobna temperatura; (b) H_2, Pd/C:TEA 1:1, EtOH; (c) KOH, MeOH, sobna temperatura.

Shema 22. Sinteza HyKet iz ketoprofena uz Pd/C:TEA 1:1.

Patent WO 2003043625 istih autora opisuje slične sinteze MeKet i HyKet. Polazni spoj za sintezu MeKet bila je (3-bromfenil)octena kiselina. Nakon prevođenja u odgovarajući metilni ester, alkilirana je na položaju 2 uz litijev diizopropilamid (LDA) koji se generira *in situ* iz *i*-Pr_2EtN i *n*-BuLi. Slijedi reakcija s benzil-cink-bromidom te lužnata hidroliza dobivenog estera (Shema 23).[86]



Reakcijski uvjeti: (a) konc. H_2SO_4, MeOH, sobna temperatura; (b) *i*-Pr_2EtN, *n*-BuLi, CH_3I, THF; –4 °C do sobne temperature; (c) $PhCH_2ZnBr$, $Pd(PPh_3)_4$, THF, refluks; (c) NaOH, MeOH, sobna temperatura.

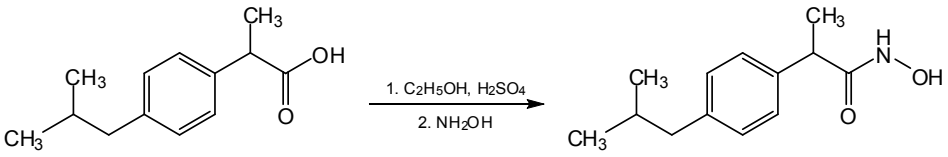
Shema 23. Sinteza MeKet iz (3-bromfenil)octene kiseline.

Katalitičkim hidrogeniranjem ketoprofena uz 5%-tni Pd/C i TEA (TEA je bio prisutan u velikom suvišku) u etanolu, dobiven je HyKet u 85% iskorištenju.[86]

3.3. SINTEZA NSAID HIDROKSAMSKIH KISELINA I NJIHOVIH DERIVATA

3.3.1. SINTEZA IBUPROFEN HIDROKSAMSKE KISELINE I SRODNIH SPOJEVA

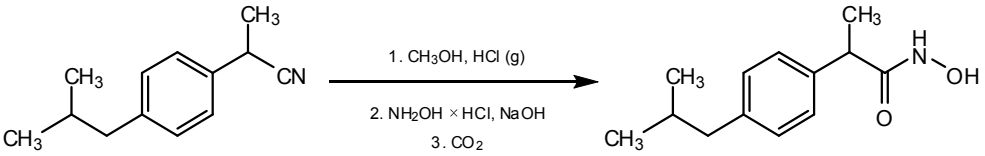
U prvoj patentnoj prijavi opisana je sinteza ibuprofen hidroksamske kiseline (ibuproksama) iz estera ibuprofena i hidroksilamina (Shema 24).[1,87] U prvom reakcijskom koraku karboksilna skupina ibuprofena esterificirana je etanolom (refluks 4 h, u prisutnosti koncentrirane sumporne kiseline). Dobiveni ester preveden je pomoću hidroksilamina u ibuproksam (refluks 15 min).



Shema 24. Sinteza ibuproksama iz estera ibuprofena i hidroksilamina.

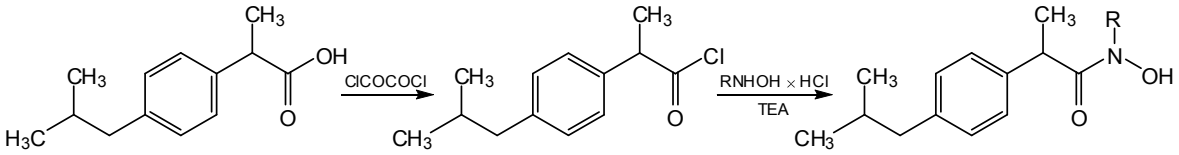
Hidroksilamin je oslobođen iz hidroklorida na dva načina: (*i*) otopini hidroklorida hidroksilamina u apsolutnom metanolu dodana je otopina natrijevog metoksida dobivenog iz elementarnog natrija i metanola. Istaloženi natrijev klorid je odsisan, a matičnica s otopljenim hidroksilaminom odmah upotrebljena u daljnjoj reakciji; (*ii*) otopini hidroklorida hidroksilamina u mezanolu dodana je otopina kalijevog hidroksida u metanolu na 0 °C. Istaloženi kalijev klorid odsisan je od otopine hidroksilamina.

U drugom patentu sinteza ibuproksama polazi iz odgovarajućeg nitrila.[9] U prvom reakcijskom koraku otopina nitrila u apsolutnom metanolu zasićena je suhim klorovodikom (3 dana, sobna temperatura), a u drugom je dodana smjesa hidroklorida hidroksilamina, metanola i otopine natrijevog hidroksida (w = 50%) na temperaturi < 10 °C tijekom 48 h. Produkt je iz reakcijske smjese istaložen uvođenjem ugljikovog(IV) oksida (Shema 25).



Shema 25. Sinteza ibuproksama iz odgovarajućeg nitrila.

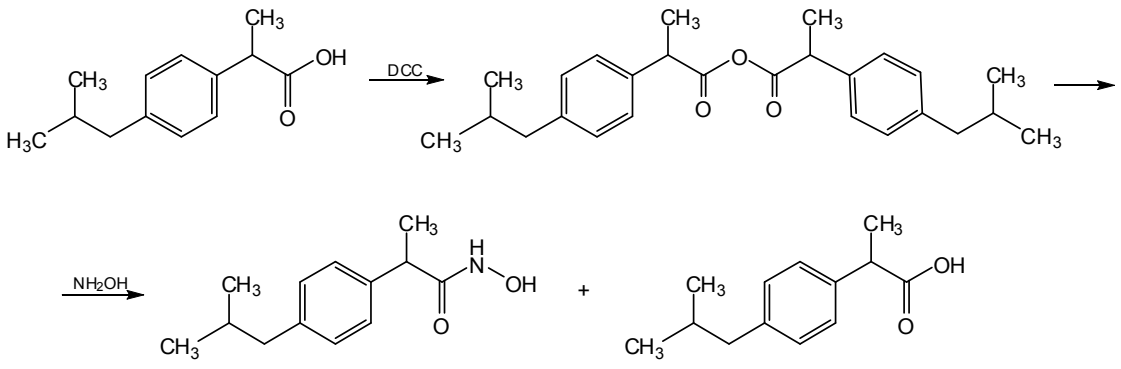
U publikaciji Summersa i suradnika opisana je sinteza serije hidroksamskih kiselina, derivata ibuprofena.[88] Početna kiselina je pomoću oksalil-klorida u dimetilformamidu prevedena u kiselinski klorid koji je bez daljnjeg čišćenja upotrebljen u reakciji s odgovarajućim hidroksilaminom (Shema 26, R = H, CH_3, $CH(CH_3)_2$, C_6H_5).



Shema 26. Sinteza ibuproksama i derivata preko oksalil-klorida.

Sintezu ibuproksama preko kiselinskog klorida opisali su i Allegretti i suradnici, međutim oni su kao reagens umjesto oksalil-klorida koristili tionil-klorid.[85]

Ibuprofen hidroksamsku kiselinu moguće je sintetizirati i iz anhidrida ibuprofena (nastaje u reakciji s DCC-om) i hidroksilamina (Shema 27).[89]



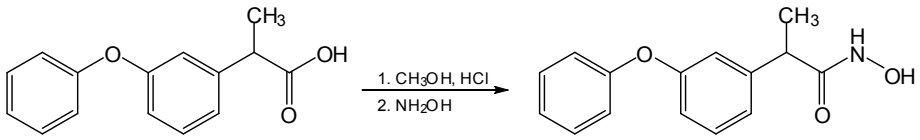
Shema 27. Sinteza ibuprofen hidroksamske kiseline iz anhidrida ibuprofena.

Ibuprofen hidroksamska kiselina također je sintetizirana izravno iz ibuprofena, u reakciji kataliziranoj lipazom iz gljivice *Candida rugosa* stabiliziranom u obliku poprečno povezanih kristala, pri čemu iz racemičnog ibuprofena nastaje (*S*)-ibuprofen hidroksamska kiselina.[90]

O-metilni derivat ibuprofen hidroksamske kiseline sintetiziran je iz ibuprofena, prevođenjem u kiselinski klorid pomoću tionil-klorida[85] ili oksalil-klorida,[88] te reakcijom s *O*-metilhidroksilaminom.

3.3.2. SINTEZA FENOPROFEN HIDROKSAMSKE KISELINE

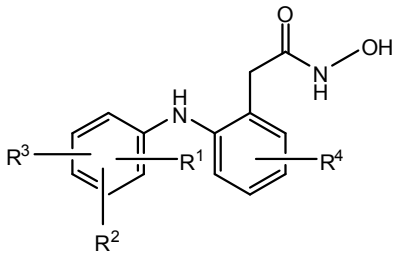
U patentu FR 2015728 opisana je sinteza fenoprofen hidroksamske kiseline iz estera fenoprofena i hidroksilamina (Shema 28).[91] U prvom reakcijskom koraku metanolna otopina fenoprofena zasićena je suhim klorovodikom, a potom refluksirana 12 h. Nakon izolacije, dobiveni metilni ester preveden je u odgovarajuću hidroksamsku kiselinu reakcijom s hidroksilaminom (0,5 h na sobnoj temperaturi, potom 1,5 h refluks). Hidroksilamin je oslobođen iz hidroklorida pomoću natrijevog metoksida u metanolu.



Shema 28. Sinteza fenoprofen hidroksamske kiseline.

3.3.3. SINTEZA DIKLOFENAK HIDROKSAMSKE KISELINE I SRODNIH SPOJEVA

Patent DE 2144641 opisuje sintezu hidroksamskih kiselina opće formule prikazane na Slici 4.

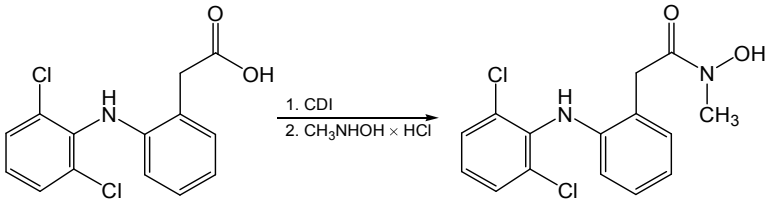


Slika 4. Opća formula hidroksamskih kiselina iz patenta DE 2144641.

U njoj R^1, R^3 i R^4 mogu biti atom vodika, niži alkili, niži alkoksidi i atomi halogena (Cl, F ili Br), a R^2 atom vodika, niži alkili, niži alkoksidi, atomi halogena (Cl, F ili Br) ili trifluormetilna skupina.[92] Sinteza polazi iz odgovarajućeg estera ili amida i hidroksilamina (oslobođen je iz hidroklorida pomoću natrijevog metoksida u metanolu). Kao esteri su pogodni niži esteri (metil, etil) ili aktivirani esteri kao što su *p*-nitrobenzil, etoksikarbonilmetil, metoksimetil, *p*-nitrotiofenil, a naročito cijanometil i *p*-nitrofenil. Najpovoljniji amid je amid bez supstituenata na dušikovom atomu.

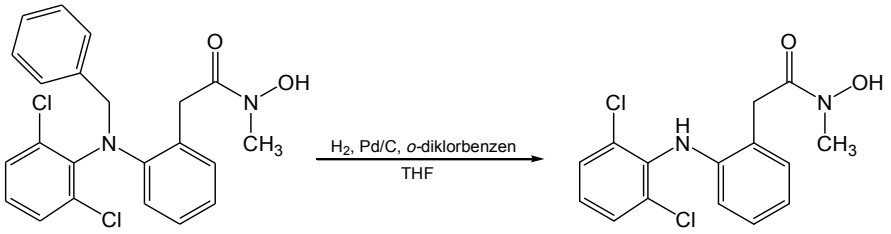
Dani su eksperimentalni podaci za sintezu sljedećih hidroksamskih kiselina: ((*o*-2,6-dikloranilino)fenil)acethidroksamsku kiselinu (diklofenak hidroksamska kiselina), ((*o*-2,6-diklor-*m*-toluidino)fenil)acethidroksamsku kiselinu, ((*o*-6-klor-*o*--toluidino)fenil)-acethidroksamsku kiselinu, ((*o*-2,6-ksilidino)fenil)acethidroksamsku kiselinu i ((*o*-3-klor-*o*-toluidino)fenil)acethidroksamsku kiselinu.

Sinteza i farmakološko djelovanje diklofenak *N*-metilhidroksamske kiseline opisani su u dva patenta.[7,93] U oba patenta karboksilna skupina diklofenaka aktivirana je pomoću CDI-a (Shema 29).



Shema 29. Sinteza diklofenak *N*-metilhidroksamske kiseline aktivacijom karboksilne skupine pomoću CDI-a.

Diklofenak *N*-metilhidroksamska kiselina može se dobiti katalitičkim hidrogeniranjem *N*-hidroksi-2-(2-(2,6-diklorfenil)-*N*-benzilamino)fenil)-*N*-metilacetamida uz Pd/C i dodatak *o*-diklorbenzena u tetrahidrofuranu (Shema 30).[93]



Shema 30. Sinteza diklofenak *N*-metilhidroksamske kiseline katalitičkim hidrogeniranjem.

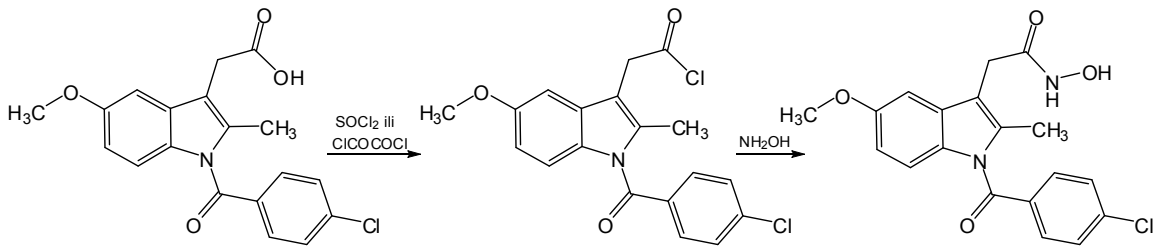
U istom patentu opisana je i treća sintetska metoda. Diklofenak *N*-metilhidroksamska kiselina sintetizirana je iz odgovarajućeg estera (metilnog, *p*-nitrofenilnog, cijanometilnog i sukcinimidnog estera) reakcijom s hidrokloridom *N*-metilhidroksilamina u prisustvu TEA.[93]

O-metilni derivat diklofenak hidroksamske kiseline dobiven je na analogan način kao i diklofenak *N*-metilhidroksamska kiselina.[7]

3.3.4. SINTEZA INDOMETACIN HIDROKSAMSKE KISELINE I SRODNIH SPOJEVA

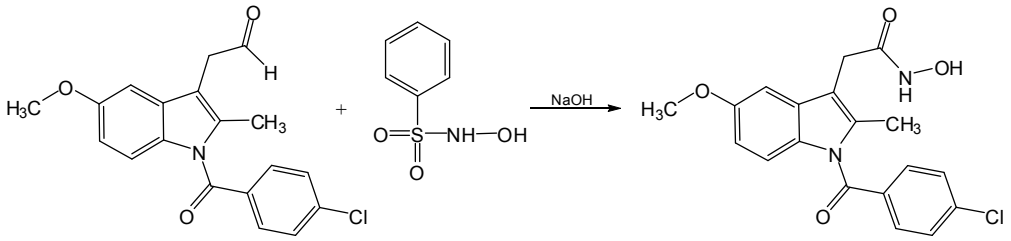
U prvom patentu indometacin hidroksamska kiselina (oksametacin) sintetizirana je iz odgovarajućeg metilnog estera i hidroksilamina oslobođenog iz hidroklorida pomoću natrijevog metoksida.[94]

Najčešća metoda sinteze oksametacina je iz kiselinskog klorida indometacina reakcijom s hidroksilaminom (Shema 31). Kiselinski klorid može se dobiti reakcijom s tionil-kloridom[45,47,95] ili oksalil-kloridom.[89,96] Hidroksilamin se oslobađa iz hidroklorida pomoću natrijevog hidroksida,[45,95] kalijevog karbonata,[47] 4-dimetilaminopiridina (DMAP)[96] ili TEA[88].



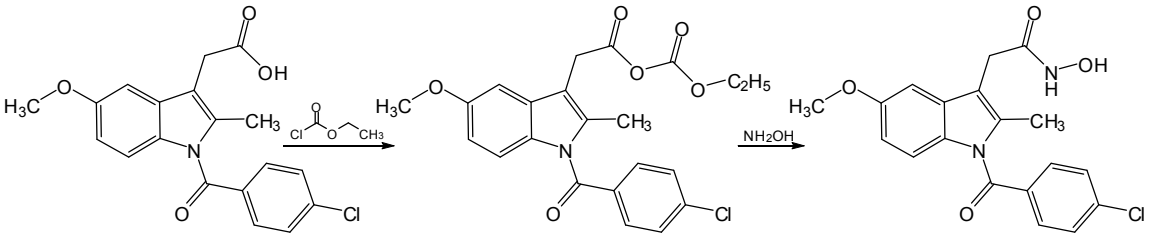
Shema 31. Sinteza oksametacina iz kiselinskog klorida indometacina.

U patentu DE 2708646 opisan je postupak sinteze oksametacina iz 1-*p*-klorobenzoil-2-metil-5-metoksi-3-indolacetaldehida i *N*-hidroksibenzensulfonamida u molarnom omjeru 1:2 (Shema 32).[97]



Shema 32. Sinteza oksametacina iz odgovarajućeg aldehida.

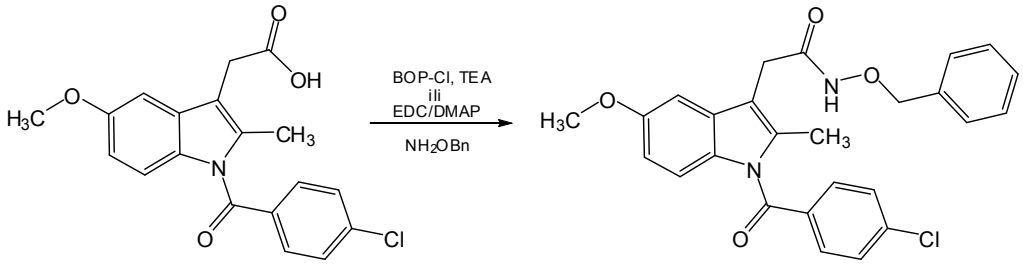
Poboljšana metoda sinteze oksametacina opisuje reakciju indometacina s alkil halokarbonatom, pri čemu nastaje odgovarajući mješoviti anhidrid koji reagira s hidroksilaminom (Shema 33).[98]



Shema 33. Sinteza indometacin hidroksamske kiseline iz mješovitog anhidrida.

O-metilni derivat oksametacina sintetiziran je preko odgovarajućeg kiselinskog klorida (nastaje u reakciji s oksalil-kloridom), koji je bez daljnjeg čišćenja upotrebljen za reakciju s *O*-metilhidroksilaminom, analogno sintezi oksametacina.[7,88]

O-benzilni derivat oksametacina sintetiziran je iz indometacina pomoću bis(2-okso-3-oksazolidinil)fosfonil klorida (BOP-Cl-a) ili hidroklorida 1-etil-3-(3--dimetilaminopropil)karbodiimida (EDC-a) kao aktivatora karboksilne kiseline (Shema 34).[96,99] U oba slučaja dobiveno je slabo iskorištenje (37–38%).



Shema 34. Sinteza *O*-benzilnog derivata oksametacina.

3.4. SINTEZA AMIDA KETOPROFENA

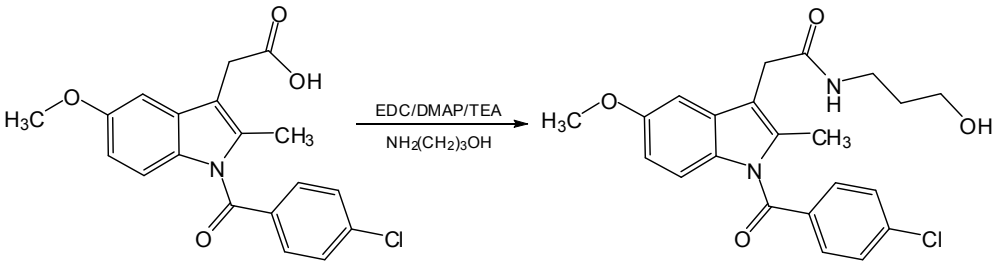
Na Zavodu za farmaceutsku kemiju opisana je sinteza amida ketoprofena benzotriazolskom metodom, koja je korištena i u ovom radu. Karboksilna skupina ketoprofena aktivira se pomoću klorida 1-benzotriazolkarboksilne kiseline, a nastali benzotriazolid u idućem koraku reagira s različitim aminima.[58] Reakcija s lipofilnim aminima provodi se uz 5 ekvivalenata amina, u toluenu i na sobnoj temperaturi.

Reakcija s hidrofilnim bifunkcionalnim aminima (2-hidroksietilamin, 3-hidroksipropilamin, dietanolamin) provedena je u acetonitrilu. Reakcija je izvedena na nižoj temperaturi (10 °C) dodatkom otopine benzotriazolida suvišku hidroksilamina. Amino skupina je snažniji nukleofil od hidroksilne skupine, reagira prva te ne dolazi do nastanka estera.[58]

3.5. SINTEZA 3-HIDROKSIPROPILAMIDA NSAID

3-Hidroksipropilamidi fenoprofena i ketoprofena sintetizirani su benzotriazolskom metodom na Zavodu za farmaceutsku kemiju.[58,59]

3-Hidroksipropilamid indometacina sintetiziran je iz indometacina, aktiviranjem njegove karboksilne skupine pomoću EDC-a (Shema 35).[96,100]



Shema 35. Sinteza 3-hidroksipropilamida indometacina.

4. MATERIJALI I METODE

4.1. SINTEZE

4.1.1. MATERIJALI I INSTRUMENTI

Tališta (t_t) su određena na Stuart SMP3 instrumentu za određivanje tališta (Barloworld Scientific, UK) i nisu korigirana. Iskorištenja nisu optimirana. CHN analiza je izvršena na CHN-LECO-932 instrumentu za elementarnu analizu (LECO Corporation, USA) te su sve elementarne analize bile unutar 0,4%.

Za tankoslojnu kromatografiju upotrebljene su silikagel ploče 60 F_{254} (Kemika, Hrvatska), te cikloheksan/etil-acetat/metanol (3:1:0,5), kloroform/metanol (95:5 i 9:1), cikloheksan/etil-acetat (1:1) i etil-acetat kao pokretna faza. Za kromatografiju na koloni kao nepokretna faza korišten je silikagel veličine čestica 0,063–0,200 nm (Merck, Njemačka), a cikloheksan/etil-acetat/metanol (3:1:0,5 i 3:1:0,6), kloroform/metanol (93:7 i 95:5), diklormetan/metanol (93:7), petroleter/etil-acetat (3:1 i 1:1), cikloheksan/etil-acetat (4:1, 3:1, 2:1 i 1:1), etil-acetat→etil-acetat/metanol (8:1) i etil-acetat kao pokretne faze. Analizirani spojevi detektirani su UV zračenjem (λ = 254 nm), parama joda i otopinom željezovog(III) klorida (w = 1%).

IR spektri snimljeni su na Paragon FT-IR spektrofotometru (Perkin Elmer, UK). Jedno- i dvodimenzijski homo- i heteronuklearni NMR spektri snimljeni su Bruker AV-600 spektrometrom (Bruker, USA) kod 600,13 ili 300,13 MHz za ^{1}H, odnosno kod 150,917 ili 75,47 MHz za ^{13}C jezgru. Uzorci su mjereni u DMSO-d_6 otopinama kod 27 °C u NMR cjevčicama od 5 mm. Kemijski pomaci dani su u ppm u odnosu na tetrametilsilan (TMS) kao unutarnji standard. FID rezolucija u ^{1}H NMR i ^{13}C NMR spektrima bila je 0,29 Hz i 0,54 Hz po točki. Korištene su sljedeće mjerne tehnike i metode: standardni ^{1}H, ^{13}C, COSY (Correlation Spectroscopy), NOESY (Nuclear Overhauser Effect Spectroscopy), HMQC (Heteronuclear Multiple Quantum Correlation) i HMBC (Heteronuclear Multiple Bond Correlation).

Standardni gradijentni ^{1}H-^{1}H COSY spektar snimljen je s 2K točaka u F2 dimenziji i 512 prirasta u F1 dimenziji uz 1024 točke nultog punjenja u F1. Po prirastu su napravljena 4 pulsa na 8012 Hz spektralne širine i vrijeme kašnjenja od 1 s. FID rezolucija je bila 3,91 Hz/točki i 15,65 Hz/točki u F2, odnosno F1 dimenziji.

Standardni ^{1}H-^{1}H fazno-osjetljivi NOESY spektri snimljeni su s 2K točaka u F2 dimenziji i 512 prirasta u F1 dimenziji uz 1024 točke nultog punjenja u F1. Po prirastu je napravljeno 16 pulseva na 6127 Hz spektralne širine i vrijeme kašnjenja od 1 s. Vrijeme

miješanja je bilo 0,5 s. Dobivena FID rezolucija bila je 2,99 Hz/točki i 11,96 Hz/točki u F2, odnosno F1 dimenziji.

^{1}H-^{13}C HMQC ($^{1}J_{C,H}$ postavljena je na 145 Hz) spektri su snimljeni s 1K točaka u F2 dimenziji i 256 prirasta u F1 dimenziji uz 1024 točke nultog punjenja u F1. Za svaki prirast primijenjeno je 32 pulsa, a vrijeme relaksacije bilo je 1 s. Spektralne širine bile su 6070 Hz u F2 i 25000 Hz u F1, a rezolucija 2,96 i 97,65 Hz/točki u F2, odnosno u F1 dimenziji.

^{1}H-^{13}C HMBC spektar snimljen je s 1K točaka u F2 dimenziji i 256 prirasta u F1 dimenziji uz 1024 točke nultog punjenja u F1. Za svaki prirast primijenjeno je 32 pulsa. Spektralne širine bile su 8012 Hz u F2 i 33560 Hz u F1. Vrijeme relaksacije bilo je 1 s, a dodatno vrijeme od 0,065 s korišteno je za detekciju C,H sprega dugog dosega. FID rezolucija bila je 3,91 i 131,08 Hz/točki u F2, odnosno F1 dimenziji. 2D NMR spektri, osim NOESY spektara, izmjereni su u pulsnom gradijentu (z-gradijent).

Benzotriazol (BtH), trifozgen, *N*-metilhidroksilamin hidroklorid, *O*-metilhidroksilamin hidroklorid, *O*-benzilhidroksilamin hidroklorid, ciklopentilamin, cikloheksilamin, cikloheksanmetilamin, benzilamin, feniletilamin, benzhidrilamin, 2-hidroksietilamin, 3-hidroksipropilamin, dietanolamin, etilendiamin i 10%-tni Pd/C nabavljeni su od tvrtke Aldrich (Njemačka), hidroksilamin hidroklorid od Carlo Erba (Italija), *O*-etilhidroksilamin hidroklorid od Fluke (Njemačka), TEA od Sigme (USA), L-valin, L-leucin, L-fenilalanin i natrijev ditionit od Kemike (Hrvatska). D-fenilglicin i NSAID (diklofenak, ketoprofen, fenoprofen, ibuprofen i indometacin) dobiveni su susretljivošću tvornica lijekova Pliva i Belupo (Hrvatska) i Sveučilišta u Potchefstroomu (Južnoafrička republika).

U eksperimentalnom dijelu korištena su bezvodna otapala. Bezvodni toluen dobiven je sljedećim postupkom: toluen je ekstrahiran vodom, zatim je osušen nad bezvodnim kalcijevim kloridom, destiliran i čuvan nad elementarnim natrijem. Bezvodni eter priređen je na sljedeći način: eter je ekstrahiran 3 puta otopinom kalcijevog klorida (w = 10%), 1 put otopinom sumporne kiseline (w = 5%), 1 put vodom, 1 put otopinom natrijevog karbonata (w = 5%) i 1 put otopinom željezovog(II) sulfata (w = 5%). Nakon toga eter je opran do neutralnog pH, sušen nad bezvodnim kalcijevim kloridom 24 h, predestiliran i čuvan nad elementarnim natrijem. Bezvodni dioksan: dioksan je refluksiran 24 h nad elementarnim natrijem, predestiliran i čuvan nad elementarnim natrijem.

Sve ostale kemikalije bile su *p*. *a*. čistoće.

4.1.2. SINTEZA KLORIDA 1-BENZOTRIAZOLKARBOKSILNE KISELINE (**1**)

Otopina BtH (1,191 g, 10 mmol) i trifozgena (2,523 g, 8,5 mmol) u bezvodnom toluenu (20 mL) refluksirana je 3 h. Reakcijska smjesa uparena je pod sniženim tlakom i nekoliko puta naparena toluenom. Dobiveni klorid 1-benzotriazolkarboksilne kiseline (BtcCl, **1**) upotrebljen je u daljnjim reakcijama bez čišćenja.

4.1.3. SINTEZA *N*-(1-BENZOTRIAZOLKARBONIL)AMINOKISELINA **2a-d**

Opća metoda. Suspenziji odgovarajuće aminokiseline (20 mmol) u bezvodnom dioksanu (40 mL) uz miješanje je dokapana otopina BtcCl (**1**) (1,810 g, 10 mmol) u bezvodnom dioksanu (10 mL) tijekom 0,25 h. Nakon dokapavanja reakcijska smjesa miješana je 24 h na sobnoj temperaturi. Talog hidroklorida aminokiseline je odsisan, a otapalo upareno pod sniženim tlakom. Dobivena sirova *N*-(1-benzotriazolkarbonil)aminokiselina **2a-d** kristalizira obradom sa smjesom etera i petroletera. Produkt je zatim ispran vrućim toluenom (**2a**) ili prekristaliziran iz toluena (**2c**). IR spektri i tališta spojeva **2a-d** u potpunosti odgovaraju ranije sintetiziranim spojevima.[101]

4.1.4. SINTEZA KLORIDA *N*-(1-BENZOTRIAZOLKARBONIL)AMINOKISELINA **3a-d**

Opća metoda. Otopina odgovarajuće *N*-(1-benzotriazolkarbonil)aminokiseline **2a-d** (4 mmol) u tionil-kloridu (20 mL) miješana je na sobnoj temperaturi 24 h. Tionil-klorid uparen je pod sniženim tlakom, a dobiveni ostatak nekoliko puta naparen bezvodnim toluenom. Dobiveni kloridi **3a-d** upotrebljeni su u daljnjim reakcijama bez čišćenja.

4.1.5. SINTEZA AMIDA *N*-(1-BENZOTRIAZOLKARBONIL)AMINOKISELINA **4a-h**

Opća metoda. U otopinu odgovarajućeg klorida *N*-(1-benzotriazolkarbonil)aminokiseline **3a-d** (4 mmol) u bezvodnom toluenu (40 mL) dokapana je otopina odgovarajućeg amina (4 mmol) i TEA (0,558 mL, 4 mmol) u toluenu (30 mL) na 0 °C. Reakcijska smjesa miješana je 24 h na sobnoj temperaturi. Istaloženi TEA hidroklorid je odsisan, a matičnica ekstrahirana vodom (3×70 mL), kloridnom kiselinom (w = 1%, 3×70 mL) i vodom do neutralnog pH, a zatim osušena nad bezvodnim natrijevim sulfatom. Nakon uparavanja otapala pod sniženim tlakom dobiven je sirovi produkt koji kristalizira. Produkti **4a-h** prekristalizirani su iz odgovarajućeg otapala.[3] IR spektri i tališta spojeva **4b-h** u potpunosti odgovaraju ranije sintetiziranim spojevima,[3] a za spoj **4a** navedeni su na kraju opisa za pripravu **4a**.

4.1.5.1. *N*-Btc-L-valin benzhidrilamid (4a)

Količina reaktanata: 1,122 g spoja **3a**, 0,690 mL benzhidrilamina. Nakon završetka reakcije, talog, koji sadrži produkt **4a** i TEA hidroklorid, odsisan je i otopljen u smjesi diklormetana i vode (1:1, 40 mL). Organski sloj ekstrahiran je kloridnom kiselinom (w = 1%, 3×40 mL) i vodom (3×40 mL), osušen nad bezvodnim natrijevim sulfatom i uparen pod sniženim tlakom.

Sirovi produkt **4a** pročišćen je rastrljavanjem eterom.

Iskorištenje: 0,205 g (48%).

t_t 155–160 °C.

IR (KBr): ν_{max} 3288, 3111, 3063, 2966, 1713, 1650, 1556, 1524, 1449, 1232, 1077, 760, 698 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 9,19 (d, 1H, 3, J = 8,42), 8,68 (d, 1H, 1", J = 8,53), 8,25–7,14 (m, 14H, arom.), 6,20 (d, 1H, 2", J = 8,54), 4,49 (d, 1H, 2, J = 7,89), 2,34–2,23 (m, 1H, 5), 0,96–0,70 (m, 6H, 6, 7).

^{13}C NMR (DMSO-d_6, δ ppm): 169,29 (1), 148,59 (4), 145,45, 142,00, 131,17 (1', 6', 3", 9"), 130,08, 128,30, 128,15, 127,24, 127,15, 126,99, 125,61, 119,82, 113,37 (2'–5', 4"–8", 10"–14"), 63,90 (2), 56,04 (2") 30,77 (5), 19,17 (6), 18,20 (7).

4.1.6. SINTEZA 3,5-DISUPSTITUIRANIH HIDANTOINA **5a-h**

Opća metoda. Otopini odgovarajućeg amida *N*-(1-benzotriazolkarbonil)aminokiseline **4a-h** (1 mmol) u acetonu (60 mL) dodana je vodena otopina natrijevog karbonata (w = 5%, 5 mL). Reakcijska smjesa miješana je 2 h na sobnoj temperaturi. Aceton je uparen pod sniženim tlakom, a produkti **5a-h** odsisani, prani vodom i prekristalizirani iz smjese acetona i vode.

4.1.6.1. 3-Benzhidril-5-izopropil hidantoin (5a)

Količina reaktanta: 0,428 g spoja **4a**.

Iskorištenje: 0,265 g (86%).

Ulje.

IR (film): ν_{max} 3249, 3063, 3030, 2963, 2930, 1772, 1711, 1496, 1450, 1417, 1347, 1176, 1116, 748, 697 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 8,40 (s, 1H, 1), 7,38–7,23 (m, 10H, arom.), 6,35 (s,1H, 1'), 4,07 (d, 1H, 5, J = 3,33), 2,08–1,97 (m, 1H, 6), 0,92 (d, 3H, 7, J = 6,92), 0,71 (d, 3H, 8, J = 6,78).

^{13}C NMR (DMSO-d_6, δ ppm): 173,63 (4), 156,87 (2), 138,63, 138,37 (2', 8'), 128,56, 128,37, 128,32, 128,22, 127,64, 127,53 (3'–7', 9'–13'), 61,06 (1'), 57,14 (5), 29,92 (6), 18,64 (7), 15,84 (8).

4.1.6.2. 3-Benzhidril-5-izobutil hidantoin (5b)

Količina reaktanta: 0,442 g spoja **4b**.

Iskorištenje: 0,284 g (88%).

t_t 139–141 °C.

IR (KBr): ν_{max} 3265, 3110, 3069, 3032, 2956, 2930, 1773, 1711, 1496, 1450, 1422, 1373, 1320, 1175, 1113, 714, 699, 668 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 8,51 (s, 1H, 1), 7,38–7,22 (m, 10H, arom.), 6,34 (s, 1H, 1'), 4,18 (q, 1H, 5, J = 4,11), 1,79–1,74 (m, 1H, 7), 1,56–1,36 (m, 2H, 6), 0,87 (2d, 6H, 8, 9, J = 4,09).

^{13}C NMR (DMSO-d_6, δ ppm): 174,34 (4), 156,22 (2), 138,30, 138,21 (2', 8'), 128,15, 128,12, 127,33 (3'–7', 9'–13'), 56,85 (1'), 54,44 (5), 40,75 (6), 23,96 (7), 22,98 (8), 21,27 (9).

4.1.6.3. 3-Cikloheksil-5-fenil hidantoin (5c)

Količina reaktanta: 0,377 g spoja **4c**.

Iskorištenje: 0,096 g (37%).

t_t 180–184 °C; lit. t_t 178–179 °C.[5]

IR (KBr): ν_{max} 3228, 3100, 3037, 2927, 2856, 1765, 1706, 1458, 1429, 1383, 1349, 1194, 1115, 763, 726, 706 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 8,64 (s, 1H, 1), 7,44–7,29 (m, 5H, arom.), 5,13 (s, 1H, 5), 3,81–3,70 (m, 1H, 1'), 2,12–1,02 (m, 10H, 2'–6').

^{13}C NMR (DMSO-d_6, δ ppm): 172,36 (4), 156,61 (2), 135,91 (6), 128,62, 128,23, 126,68 (7–11), 59,08 (5), 50,22 (1'), 28,92 (2'), 28,83 (6'), 25,27 (3'), 25,24 (5'), 24,72 (4').

4.1.6.4. 3-Benzhidril-5-fenil hidantoin (5d)

Količina reaktanta: 0,462 g spoja **4c**.

Iskorištenje: 0,082 g (24%).

t_t 172–177 °C.

IR (KBr): ν_{max} 3230, 3093, 3032, 2825, 1774, 1712, 1495, 1455, 1423, 1373, 1283, 1176, 826, 763, 722, 701 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 8,91 (s, 1H, 1), 7,43–7,20 (m, 15H, arom.), 6,39 (s, 1H, 1'), 5,34 (s, 1H, 5).

^{13}C NMR (DMSO-d_6, δ ppm): 172,35 (4), 156,54 (2), 138,96, 138,30, 135,85 (6, 2', 8'), 128,94–126,87 (7–11, 3'–7', 9'–13'), 59,66 (1'), 57,35 (5).

4.1.6.5. 3-Ciklopentil-5-benzil hidantoin (5e)

Količina reaktanta: 0,377 g spoja **4e**.

Iskorištenje: 0,189 g (73%).

t_t 121–124 °C.

IR (KBr): ν_{max} 3258, 3091, 3034, 2959, 2869, 1760, 1718, 1431, 1387, 1213, 760, 700 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 8,18 (s, 1H, 1), 7,28–7,13 (m, 5H, arom.), 4,29 (t, 1H, 5, J = 4,38), 4,09–4,02 (m, 1H, 1'), 2,94 (d, 2H, 6, J = 4,22), 1,67–1,42 (m, 8H, 2'–5').

^{13}C NMR (DMSO-d_6, δ ppm): 175,20 (4), 158,25 (2), 136,80 (7), 131,64, 129,81, 128,55 (8–12), 58,11 (5), 51,90 (1'), 38,30 (6), 30,30 (5'), 30,02 (2'), 26,39 (3', 4').

4.1.6.6. 3-Cikloheksil-5-benzil hidantoin (5f)

Količina reaktanta: 0,391 g spoja **4f**.

Iskorištenje: 0,231 g (85%).

t_t 119–120 °C; lit. t_t 133–135 °C.[65]

IR (KBr): ν_{max} 3271, 3093, 3033, 2926, 2856, 1768, 1707, 1629, 1560, 1498, 1436, 1384, 1111, 725, 696 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 8,15 (s, 1H, 1), 7,28–7,13 (m, 5H, arom.), 4,29 (t, 1H, 5, J = 4,39), 3,52–3,44 (m, 1H, 1'), 2,93 (t, 2H, 6, J = 3,53), 1,85–0,94 (m, 10H, 2'–6').

^{13}C NMR (DMSO-d_6, δ ppm): 173,20 (4), 156,22 (2), 134,78 (7), 129,76, 127,82, 126,60 (8–12), 56,04 (5), 49,64 (1'), 36,28 (6), 28,58 (2'), 28,39 (6'), 25,18 (3', 5'), 24,68 (4').

4.1.6.7. 3-Cikloheksanmetil-5-benzil hidantoin (5g)

Količina reaktanta: 0,405 g spoja **4g**.

Iskorištenje: 0,229 g (80%).

t_t 150–153 °C.

IR (KBr): ν_{max} 3314, 3085, 3030, 2925, 2852, 1768, 1698, 1496, 1454, 1428, 1360, 1124, 1099, 755, 726, 700 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 8,21 (s, 1H, 1), 7,27–7,14 (m, 5H, arom.), 4,38 (t, 1H, 5, J = 4,22), 3,05–2,87 (m, 4H, 6, 1'), 1,61–0,50 (m, 11H, 2'–7').

^{13}C NMR (DMSO-d_6, δ ppm): 173,27 (4), 156,60 (2), 134,78 (7), 129,80, 127,82, 126,58 (8–12), 56,71 (5), 43,37 (1'), 35,95 (6), 35,66 (2'), 29,79 (3'), 29,52 (7'), 25,65 (4'), 25,17 (6'), 25,12 (5').

4.1.6.8. 3-Benzhidril-5-benzil hidantoin (5h)

Količina reaktanta: 0,476 g spoja **4h**.

Iskorištenje: 0,103 g (29%).

t_t 76 °C (raspad).

IR (KBr): ν_{max} 3269, 3030, 2925, 1774, 1711, 1602, 1496, 1452, 1419, 1374, 1178, 752, 699 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 8,44 (s, 1H, 1), 7,30–6,73 (m, 15H, arom.), 6,13 (s, 1H, 1'), 4,52 (t, 1H, 5, J = 4,28), 3,07–2,94 (m, 2H, 6).

^{13}C NMR (DMSO-d_6, δ ppm): 172,87 (4), 155,72 (2), 138,01, 137,95, 134,65 (7, 2', 8'), 126,78–130,01 (8–12, 3'–7', 9'–13'), 56,62 (5, 1'), 35,78 (6).

4.1.7. SINTEZA 10%-TNOG Pd/C(en)

Suspenzija komercijalnog 10%-tnog Pd/C (0,300 g, 0,28 mmol Pd) i etilendiamina (1,2 mL, 19,74 mmol) u metanolu (10 mL) pod atmosferom argona miješana je na sobnoj temperaturi 48 h. Krutina je odsisana, višestruko isprana metanolom (5×20 mL) i eterom (5×20 mL) te sušena na vakuum-pumpi na sobnoj temperaturi tijekom 48 h. Dobiven je 10%-tni Pd/C(en). Iskorištenje: 100%.

4.1.8. SINTEZA REDUCIRANIH DERIVATA KETOPROFENA **6a**,**b**

Opća metoda. Otopini ketoprofena (2,543 g, 10 mmol) u odgovarajućem otapalu dodan je katalizator. Nastala suspenzija miješana je na sobnoj temperaturi pod atmosferskim tlakom vodika. Nakon završetka reakcije katalizator je odfiltriran, a otapalo upareno pod sniženim tlakom te je dobiven čisti produkt.

4.1.8.1. 2-(3-(Hidroksi(fenil)metil)fenil)propanska kiselina (6a)

Katalizator: 10%-tni Pd/C(en) (0,254 g); otapalo: tetrahidrofuran; trajanje reakcije 23 h. Iskorištenje: 2,563 g (100%).

t_t 116–118 °C; lit. t_t 103–104 °C;[81] 117–118 °C;[84] 104–107 °C.[85]

IR (KBr): ν_{max} 3417, 3028, 2988, 2918, 2713, 2610, 1709, 1601, 1490, 1454, 1327, 1237, 1020, 914, 839, 799, 708, 698 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 12,23 (s, 1H, 1), 7,38–7,11 (m, 9H, arom.), 5,89 (s, H, 12), 5,68 (s, 1H, 11), 3,64 (q, 1H, 3, J = 7,05), 1,33 (d, 3H, 4, J = 7,08).

^{13}C NMR (DMSO-d_6, δ ppm): 175,78 (2), 146,35, 146,07, 141,54 (5, 9, 13), 128,61, 128,53, 127,17, 126,68, 126,19, 125,69, 125,26 (6–8, 10, 14–18), 74,69 (11), 45,20 (3), 19,08 (4).

4.1.8.2. 2-(3-Benzilfenil)propanska kiselina (6b)

Katalizator: 10%-tni Pd/C (0,254 mg); otapalo: metanol; trajanje reakcije 4 h.

Iskorištenje: 2,409 g (100%).

t_t 53–54 °C; lit. ulje;[79,85] t_t 58–59 °C.[84]

IR (KBr): ν_{max} 3060, 3027, 2987, 2931, 2733, 2642, 1700, 1598, 1494, 1451, 1417, 1238, 1213, 1085, 939, 756, 717, 699 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 12,23 (s, 1H, 1), 7,29–7,06 (m, 9H, arom.), 3,90 (s, 3H, 11), 3,60 (q, 1H, 3, J = 7,06), 1,31 (d, 3H, 4, J = 7,12).

^{13}C NMR (DMSO-d_6, δ ppm): 175,76 (2), 141,86, 141,84, 141,61 (5, 9, 12), 129,13, 128,98, 128,88, 128,26, 127,63, 126,43, 125,45 (6–8, 10, 13–17), 45,09 (3), 41,53 (11), 19,01 (4).

4.1.9. SINTEZA NSAID BENZOTRIAZOLIDA **7a-h**

Opća metoda sinteze spojeva 7a-g. U otopinu odgovarajućeg NSAID (ibuprofena, fenoprofena, diklofenaka, indometacina, ketoprofena i reduciranih derivata ketoprofena **6a**,**b**) (10 mmol) i TEA (1,393 mL, 10 mmol) u bezvodnom toluenu (30 mL) dokapana je otopina BtcCl (**1**) (1,696 g, 10 mmol) u bezvodnom toluenu (30 mL) tijekom 0,25 h. Reakcijska smjesa miješana je na sobnoj temperaturi 2 h, a zatim ekstrahirana vodom (3×60 mL). Organski sloj sušen je nad bezvodnim natrijevim sulfatom, profiltriran i uparen pod sniženim tlakom.

4.1.9.1. Benzotriazolid ibuprofena (7a)

Količina reaktanta: 2,063 g ibuprofena. Sirovi produkt **7a** prekristaliziran je iz smjese acetona i vode.

Iskorištenje: 2,516 g (82%).

t_t 57–59 °C; lit. t_t 56–59 °C.[102]

IR (KBr): ν_{max} 3027, 2954, 1739, 1379, 949, 752 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 8,27–8,21 (m, 2H, arom.), 7,80–7,75 (m, 1H, arom.), 7,62–7,57 (m, 1H, arom.), 7,35 (d, 2H, arom., J = 8,02), 7,12 (d, 2H, arom., J = 8,05), 5,31 (q, 1H, 2, J = 6,96), 2,37 (d, 2H, 10, J = 7,14), 1,79–1,75 (m, 1H, 11), 1,64 (d, 3H, 3, J = 6,99), 0,81 (d, 6H, 12, 13, J = 6,60).

^{13}C NMR (DMSO-d_6, δ ppm): 173,07 (1), 145,33, 140,19, 136,87, 130,65 (4, 7, 1', 6'), 130,82, 129,31, 127,37, 126,46, 119, 97, 113,92 (5, 6, 8, 9, 2'–5'), 44,11 (2), 44,04 (10), 29,41 (3), 22,04 (12, 13), 18,52 (11).

4.1.9.2. Benzotriazolid fenoprofena (7b)

5,226 g kalcijevog fenoprofena otopljeno je u vodi (80 mL), zakiseljeno kloridnom kiselinom (w = 1%) do pH 1, te ekstrahirano diklormetanom (3×80 mL). Organski sloj ekstrahiran je vodom (3×80 mL), sušen nad bezvodnim natrijevim sulfatom, profiltriran i

uparen. Iskorištenje: 2,423 g (100%) fenoprofena, koji je upotrebljen u daljnjoj reakciji prema Općoj metodi.

Sirovi produkt **7b** prekristaliziran je iz smjese acetona i vode.

Iskorištenje: 2,575 g (75%).

t_t 63–66 °C; lit. t_t 64–66 °C.[103]

IR (KBr): ν_{max} 3064, 2985, 1729, 1580, 1487, 1450, 1374, 1243, 954, 770, 692 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 8,26–8,22 (m, 2H, arom.), 7,81–7,76 (m, 1H, arom.), 7,64–7,58 (m, 1H, arom.), 7,39–7,33 (m, 3H, arom.), 7,22–7,12 (m, 3H, arom.), 6,99–6,96 (m, 2H, arom.), 6,88-6,85 (m, 1H, arom.), 5,34 (q, 1H, 2, J = 6,97), 1,66 (d, 3H, 3, J = 6,99).

^{13}C NMR (DMSO-d_6, δ ppm): 172,80 (1), 157,02, 156,25, 145,50, 141,89, 130,78 (4, 8, 10, 1', 6'), 130,99, 130,52, 130,10, 126,63, 123,72, 122,64, 120,14, 118,81, 118,09, 117,20, 114,07 (5–7, 9, 11–15, 2'–5'), 44,59 (2), 18,61 (3).

4.1.9.3. Benzotriazolid diklofenaka (7c)

Količina reaktanta: 2,962 g diklofenaka. Sirovi produkt **7c** pročišćen je rastrljavanjem eterom.

Iskorištenje: 3,059 g (77%).

t_t 163–165 °C; lit. t_t 162–164 °C.[102]

IR (KBr): ν_{max} 3321, 3076, 2925, 1728, 1710, 1590, 1450, 1382, 1006, 750 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 8,27 (dd, 2H, arom., J = 8,29, J = 3,10), 7,82–7,77 (m, 1H, arom.), 7,65–7,60 (m, 1H, arom.), 7,53 (d, 2H, arom., J = 8,07), 7,36–7,08 (m, 4H, arom., 9), 6,89–6,84 (m, 1H, arom.), 6,24 (d, 1H, arom., J = 8,01), 4,94 (s, 2H, 2).

^{13}C NMR (DMSO-d_6, δ ppm): 170,08 (1), 145,35, 143,62, 136,92, 132,01, 130,78, 120,91 (3, 8, 10, 11, 15, 1', 6'), 131,96, 130,64, 129,04, 128,03, 126,48, 126,26, 119,92, 119,78, 114,91, 113, 97 (4–7, 12–14, 2'–5'), 38,11 (2).

4.1.9.4. Benzotriazolid indometacina (7d)

Količina reaktanta: 3,578 g indometacina. Sirovi produkt **7d** pročišćen je rastrljavanjem eterom i prekristalizacijom iz toluena.

Iskorištenje: 3,947 g (86%).

t_t 105–107 °C; lit. t_t 105–107 °C.[102]

IR (KBr): ν_{max} 3097, 2926, 1739, 1679, 1609, 1598, 1483, 1368, 1315, 1227, 1066, 965, 745 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 8,19 (d, 1H, arom., J = 4,14), 8,12 (d, 1H, arom., J = 4,11), 7,68 (t, 1H, arom., J = 3,70). 7,62 (d, 2H, arom., J = 4,20), 7,56 (d, 2H, arom., J = 4,20), 7,53 (t, 1H, arom., J = 3,69), 7,18 (d, 1H, arom., J = 1,08), 6,87 (d, 1H, arom., J = 4,50), 6,64 (dd, 1H, arom., J = 1,16, J = 3,33), 4,83 (s, 2H, 2), 3,65 (s, 3H, 2''), 2,25 (s, 3H, 1'').

^{13}C NMR (DMSO-d_6, δ ppm): 169,43 (1), 167,87 (11), 155,61, 145,49, 137,71, 136,38, 134,02, 130,79, 130,72, 130,26, 111,40 (3–5, 8, 10, 12, 15, 1', 6'), 131,17, 130,69, 129,04, 126,36, 119,99, 114,51, 113,95, 111,51, 102,00 (6, 7, 9, 13, 14, 16, 17, 2'–5'), 55,39 (2''), 30,89 (2), 13,29 (1'').

4.1.9.5. Benzotriazolid ketoprofena (7e)

Količina reaktanta: 2,543 g ketoprofena. Sirovi produkt **7e** pročišćen je rastrljavanjem eterom.

Iskorištenje: 3,092 g (87%).

t_t 95–98 °C; lit. t_t 96–98 °C.[102]

IR (KBr): ν_{max} 17245, 1653, 1449, 1380, 1319, 1282, 961, 942, 750, 713, 610 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 8,26–8,22 (m, 2H, arom.), 7,84–7,49 (m, 11H, arom.), 5,45 (q, 1H, 2, J = 7,05), 1,70 (d, 3H, 3, J = 7,05).

^{13}C NMR (DMSO-d_6, δ ppm): 195,33 (10), 172,74 (1), 140,80, 137,38, 136,80, 130,96, 114,05 (4, 8, 11, 1', 6'), 145,48, 132,78, 132,13, 129,66, 129,22, 129,21, 129,12, 128,87, 128,57, 126,60, 120,11 (5–7, 9, 12–16, 2'–5'), 44,35 (2), 18,60 (3).

4.1.9.6. Benzotriazolid 2-(3-(hidroksi(fenil)metil)fenil)propanske kiseline (7f)

Količina reaktanta: 2,561 g spoja **6a**. Sirovi produkt **7f** pročišćen je rastrljavanjem eterom.

Iskorištenje: 2,681 g (75%).

t_t 96–99 °C.

IR (KBr): ν_{max} 3342, 3072, 3027, 3003, 2942, 2874, 1738, 1060, 1597, 1486, 1452, 1376, 959, 771, 751, 746, 710 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 8,26–8,21 (m, 2H, arom.), 7,80–7,75 (m, 1H, arom.), 7,62–7,57 (m, 1H, arom.), 7,52–7,50 (m, 1H, arom.), 7,33–7,14 (m, 8H, arom.), 5,88 (d, 1H, 11, J = 4,00), 5,65 (d, 1H, 10, J = 3,83), 5,32 (q, 1H, 2, J = 6,89), 1,63 (d, 3H, 3, J = 6,94).

^{13}C NMR (DMSO-d_6, δ ppm): 173,50 (1), 146,82, 145,88, 145,83, 140,01, 131,18 (4, 8, 12, 1', 6'), 131,39, 129,08, 128,45, 127,14, 127,02, 126,66, 126,63, 126,58, 125,84, 120,53, 114,44 (5–7, 9, 13–17, 2'–5'), 74,49 (10), 45,12 (2), 19,07 (3).

4.1.9.7. Benzotriazolid 2-(3-benzilfenil)propanske kiseline (7g)

Količina reaktanta: 2,403 g spoja **6b**. Sirovi produkt **7g** pročišćen je rastrljavanjem eterom.

Iskorištenje: 2,663 g (78%).

t_t 59–60 °C.

IR (KBr): ν_{max} 3027, 2937. 2918, 2852, 1730, 1597, 1485, 1452, 1390, 1372, 1168, 955, 780, 756, 738, 708, 692 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 8,25–8,20 (m, 2H, arom.), 7,79–7,74 (m, 1H, arom.), 7,61–7,56 (m, 1H, arom.), 7,33–7,07 (m, 9H, arom.), 5,30 (q, 1H, 2, J = 6,94), 3,90 (s, 2H, 10), 1,63 (d, 3H, 3, J = 6,99).

^{13}C NMR (DMSO-d_6, δ ppm): 173,47 (1), 145,88, 142,33, 141,39, 140,32, 131,18 (4, 8, 11, 1', 6'), 131,38, 129,41, 129,06, 128,82, 128,57, 128,21, 127,01, 126,41, 125,77, 120,52, 114,45 (5–7, 9, 12–16, 2'–5'), 45,05 (2), 41,37 (10), 19,06 (3).

4.1.9.8. Benzotriazolid 2-(3-(*N*-benzotriazolkarboniloksi)(fenil)metil)fenil)-propanske kiseline (7h)

U otopinu **6a** (0,256 g, 1 mmol) i TEA (0,390 mL, 2,8 mmol) u bezvodnom toluenu (20 mL) dokapana je otopina BtcCl (**1**) (0,508 g, 2,8 mmol) u bezvodnom toluenu (15 mL) tijekom 0,25 h. Reakcijska smjesa miješana je na sobnoj temperaturi 1 h, a zatim ekstrahirana vodom (5×40 mL). Organski sloj sušen je nad bezvodnim natrijevim sulfatom, profiltriran i uparen pod sniženim tlakom. Sirovi produkt **7h** pročišćen je rastrljavanjem eterom.

Iskorištenje: 0,352 g (70%).

t_t 124–127 °C.

IR (KBr): ν_{max} 3091, 3032, 2980, 2937, 1764, 1732, 597, 1486, 1451, 1398, 1250, 1036, 951, 781, 760, 748, 708, 583 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 8,28–7,98 (m, 3H, arom.), 7,78–7,28 (m, 15H, arom.), 7,24 (s, 1H, 10), 5,38 (q, 1H, 2, J = 6,84), 1,66 (d, 3H, 3, J = 6,94).

^{13}C NMR (DMSO-d_6, δ ppm): 173,28 (1), 147,91 (1"), 145,80, 145,77, 140,72, 140,02, 139,17, 131,73 (4, 8, 11, 1', 6', 2", 7"), 131,36, 131,11, 129,83, 129,20, 128,29, 127,36, 127,23, 126,97, 126,79, 126,55, 126,38, 120,70, 120,45, 114,39, 113,67 (5–7, 9, 12–16, 2'–5', 3"–6"), 81,48 (10), 45,04 (2), 18,98 (3).

4.1.10. SINTEZA NSAID HIDROKSAMSKIH KISELINA I NJIHOVIH DERIVATA **8a-y**

Metoda A. Otopina odgovarajućeg hidroksilamina,* natrijevog ditionita (10 mg) i NSAID benzotriazolida **7a-e** (1 mmol) u kloroformu (10 mL) miješana je na sobnoj temperaturi 24 h. Reakcijska smjesa ekstrahirana je vodom kod pH 7–8 (3×10 mL), uz zaluživanje otopinom natrijevog hidroksida (w = 5%). Organski sloj opran je vodom, sušen nad bezvodnim natrijevim sulfatom, profiltriran i uparen pod sniženim tlakom.
*Hidroksilamin, *N*-metilhidroksilamin i *O*-benzilhidroksilamin prethodno su oslobođeni iz hidroklorida na sljedeći način: u otopinu natrijevog metoksida pripravljenu iz natrija (0,115 g, 5 mmol) i bezvodnog metanola (5 mL) dodan je odgovarajući hidroksilamin hidroklorid (5 mmol). Nakon hlađenja na −15 °C (10 min) istaloženi natrijev klorid je odfiltriran, a matičnica odmah upotrebljena u daljnjoj reakciji.

Metoda B. Otopina benzotriazolida **7a-e** i **7g** (1 mmol), hidroklorida odgovarajućeg hidroksilamina (1,2 mmol), TEA (1,3 mmol ili 5 mmol) i natrijevog ditionita (10 mg) u kloroformu, toluenu ili dioksanu (10 mL) miješana je na sobnoj temperaturi 0,5–48 h ili refluksirana 1–5,5 h (iznimka je spoj spoj **8t**). Spojevi **8b**,**f**,**g**,**l**,**r**,**s**,**u**,**x**,**y** pripravljeni su uz 1,3 mmol TEA, a spojevi **8i-k**,**m-q**,**t**,**v** uz 5 mmol TEA. U pripravi spojeva **8f** i **h** dobivena su viša iskorištenja korištenjem peterostrukog suviška hidroksilamina i šesterostrukog suviška TEA. Spojevi **8b**,**f-h**,**l**,**r**,**y** dobiveni su na sljedeći način: otapalo je uklonjeno pod sniženim tlakom. Ostatak je otopljen u smjesi acetona i vode, a dobivena otopina zakiseljena do pH 1. Aceton je uparen pod sniženim tlakom bez zagrijavanja. Istaloženi produkt je odsisan i ispran vodom do neutralnog pH. Postupak obrade za ostale spojeve bio je isti kao u Metodi A.

Metoda C. Suspenzija *O*-benzilnog derivata **8s-y** (0,5 mmol ili 1 mmol) i 10%-tnog Pd/C u metanolu, etil-acetatu ili acetonu (10 mL) hidrogenirana je pod atmosferskim tlakom vodika i na sobnoj temperaturi tijekom 0,15–17 h. Katalizator je odsisan, a otapalo upareno pod sniženim tlakom. U pripravi spoja **8f** reakcijska smjesa zakiseljena je s nekoliko kapi sulfatne kiseline (c = 1 mol L^{-1}).

4.1.10.1. 2-(3-Benzilfenil)propanamid (8a)

Metoda C: količina reaktanta: 0,359 g (1 mmol) spoja **8v**; količina katalizatora: 50 mg; otapalo: metanol; trajanje reakcije 17 h.

Iskorištenje: 0,239 g (100%).

t_t 76–78 °C.

IR (KBr): ν_{max} 3393, 3177, 3084, 3026, 2980, 1653, 1599, 1451, 1406, 1368, 1288, 1123, 786, 710, 694, 646, 628 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 7,41–7,04 (m, 9H, arom.), 6,78 (s, 2H, 1), 3,91 (s, 2H, 11), 3,53 (q, 1H, 3, J = 7,01), 1,28 (d, 3H, 4, J = 7,06).

^{13}C NMR (DMSO-d_6, δ ppm): 175,69 (2), 143,02, 141,68, 141,46 (5, 9, 12), 129,12, 128,87, 128,15, 127,30, 126,41, 125,41 (6–8, 10, 13–17), 45,33 (3), 41,65 (11), 18,97 (4).

4.1.10.2. *N*-hidroksi-2-(4-izobutilfenil)propanamid (8b)

Metoda A: količina reaktanata: 0,307 g spoja **7a**, 0,347 g hidroklorida hidroksilamina. Iskorištenje: 0,100 g (45%).

Metoda B: količina reaktanata: 0,307 g spoja **7a**, 0,083 g hidroklorida hidroksilamina; otapalo: kloroform; trajanje reakcije 0,6 h; sobna temperatura. Sirovi produkt **8b** prekristaliziran je iz toluena. Iskorištenje: 0,091 g (41%).

Metoda C: količina reaktanta: 0,156 g (0,5 mmol) spoja **8s**; količina katalizatora: 50 mg; otapalo: metanol; trajanje reakcije 0,25 h. Sirovi produkt **8b** prekristaliziran je iz toluena. Iskorištenje: 0,106 g (96%).

t_t 117–119 °C; lit. t_t 119–121 °C.[9,89]

pK_a 10,89.

IR (KBr): ν_{max} 3191, 3025, 2955, 1634, 1513, 1462, 1025, 939 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 10,59 (s, 1H, 1), 8,75 (s, 1H, 2), 7,21 (d, 2H, arom., J = 8,07), 7,07 (d, 2H, arom., J = 8,07), 3,49 (q, 1H, 4, J = 7,03), 2,40 (d, 2H, 12, J = 7,11), 1,84–1,75 (m, 1H, 13), 1,31 (d, 3H, 5, J = 7,06), 0,84 (d, 6H, 14, 15, J = 6,61).

^{13}C NMR (DMSO-d_6, δ ppm): 170,52 (3), 139,46, 139,23 (6, 9), 128,83, 127,10 (7, 8, 10, 11), 44,36 (12), 41,86 (4), 29,76 (5), 22,29 (14, 15), 18,30 (13).

4.1.10.3. *N*-hidroksi-2-(3-benzilfenil)propanamid (8c)

Metoda C: količina reaktanta 0,359 g (1 mmol) spoja **8v**; količina katalizatora 50 mg; otapalo: metanol; trajanje reakcije 2,50 h. Sirovi produkt **8c** prekristaliziran je iz toluena. Iskorištenje: 0,204 g (80%).

t_t 135–137 °C.

IR (KBr): ν_{max} 3189, 3023, 2904, 1632, 1533, 1486, 1072, 1029, 723, 702 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 10,61 (s, 1H, 1), 8,77 (s, 1H, 2), 7,31–7,05 (m, 9H, arom.), 3,91 (s, 2H, 12), 3,39 (q, 1H, 4, J = 7,06), 1,30 (d, 3H, 5, J = 7,02).

^{13}C NMR (DMSO-d_6, δ ppm): 170,67 (3), 142,46, 141,64, 141,49 (6, 10, 13), 129,13, 128,88, 128,71, 128,11, 127,47, 126,43, 125,43 (7–9, 11, 14–18), 42,54 (5), 41,62 (12), 18,71 (4).

4.1.10.4. *N*-hidroksi-2-(3-fenoksifenil)propanamid (8d)

Metoda A: količina reaktanata 0,343 g spoja **7b,** 0,347 g hidroklorida hidroksilamina. Sirovi produkt **8d** prekristaliziran je iz smjese toluena i cikloheksana. Iskorištenje: 0,103 g (40%).

Metoda C: količina reaktanta: 0,347 g (1 mmol) spoja **8u**; količina katalizatora 50 mg; otapalo: metanol; trajanje reakcije 0,62 h. Iskorištenje: 0,242 g (94%).

t_t 118–119 °C; lit. t_t 121–122 °C.[91]

pK_a 10,66.

IR (KBr): ν_{max} 3214, 3035, 2921, 1629, 1583, 1536, 1488, 1248, 694 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 10,63 (s, 1H, 1), 8,81 (s, 1H, 2), 7,42–7,37 (m, 2H, arom.), 7,31 (t, 1H, arom., J = 7,04), 7,17–7,08 (m, 2H, arom.), 7,01–6,99 (m, 3H, arom.), 6,85–6,82 (m, 1H, arom.), 3,43 (q, 1H, 4, J = 7,05), 1,31 (d, 3H, 5, J = 6,99).

^{13}C NMR (DMSO-d_6, δ ppm): 169,77 (3), 156,46, 156,37, 143,98 (6, 10, 11), 129,96, 129,62, 123,34, 122,32, 118,48, 117,47, 116,58 (7–9, 11, 13–17), 41,88 (4), 18,06 (5).

4.1.10.5. *N*-hidroksi-2-(3-(hidroksi(fenil)metil)fenil)propanamid (8e)

Metoda C: količina reaktanta: 0,180 g (0,5 mmol) spoja **8v**; količina katalizatora: 20 mg; otapalo: metanol; trajanje reakcije 3 h. Sirovi produkt **8e** prekristaliziran je iz smjese etil-acetata i cikloheksana.

Iskorištenje: 0,099 g (74%).

t_t 148–152 °C.

IR (KBr): ν_{max} 3378, 3214, 2979, 2915, 1645, 1520, 1492, 1447, 1022, 945, 724, 701 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 10,62 (s, 1H, 1), 8,77 (s, 1H, 2), 7,38–7,16 (m, 9H, arom.), 5,87 (d, 1H, 13, J = 3,90), 5,66 (d, 1H, 12, J = 3,60), 3,41 (q, 1H, 4, J = 7,00), 1,31 (d, 3H, 5, J = 7,00).

^{13}C NMR (DMSO-d_6, δ ppm): 170,65 (3), 146,12, 146,02, 142,09 (6, 10, 14), 128,52, 128,33, 127,16, 126,73, 126,13, 125,64, 125,11 (7–9, 11, 15–19), 74,80 (12), 42,62 (4), 18,77 (5).

4.1.10.6. *N*-hidroksi-2-(2-(2,6-diklorfenilamino)fenil)acetamid (8f)

Metoda B: količina reaktanata 0,397 g spoja **7c**, 0,347 g hidroklorida hidroksilamina; otapalo: kloroform; trajanje reakcije 0,5 h; sobna temperatura. Sirovi produkt **8f** prekristaliziran je iz smjese acetona i petroletera. Iskorištenje: 0,202 g (65%).

Metoda C: količina reaktanta: 0,201 g (0,5 mmol) spoja **8x**; količina katalizatora 50 mg; otapalo: etil-acetat; trajanje reakcije 0,15 h. Nakon uparavanja sirovi produkt **8f** rastrljan je vodom, odsisan i sušen. Iskorištenje: 0,138 g (89%).

t_t 158–162 °C; lit. t_t 164–165 °C.[92]

pK_a 10,64.

IR (KBr): ν_{max} 3289, 3235, 3031, 2963, 2891, 1660, 1626, 1589, 1507, 1453, 774, 749 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 10,87 (s, 1H, 1), 8,97 (s, 1H, 2), 8,44 (s, 1H, 11), 7,43 (d, 2H, arom., J = 8,05), 7,09–7,06 (m, 2H, arom.), 6,97 (t, 1H, arom., J = 7,15), 6,78 (t, 1H, arom., J = 7,22), 6,23 (d, 1H, arom., J = 7,92), 3,39 (s, 2H, 4).

^{13}C NMR (DMSO-d_6, δ ppm): 168,11 (3), 142,99 , 137,16, 129,27, 125,13 (5, 10, 12, 13, 17), 130,29, 129,15, 127,28, 124,93, 120,71, 116,04 (6–9, 14–16), 36,57 (4).

4.1.10.7. *N*-hidroksi-1-(4-klorbenzoil)-2-metil-5-metoksi-1*H*-indol-3-acetamid (8g)

Metoda B: količina reaktanata: 0,459 g spoja **7d**, 0,083 g hidroklorida hidroksilamina; otapalo: dioksan; trajanje reakcije 24 h, sobna temperatura. Sirovi produkt **8g** prekristaliziran je iz dioksana. Iskorištenje: 0,198 g (53%).

Metoda C: količina reaktanta: 0,463 g (1 mmol) spoja **8y**; količina katalizatora 50 mg; otapalo: aceton; trajanje reakcije 0,5 h. Sirovi produkt **8g** rastrljan je eterom. Iskorištenje: 0,291 g (78%).

t_t 175–178 °C; lit. t_t 181–182 °C;[45] 147–150 °C;[47] 181–184 °C;[95] 181–183 °C.[98]

pK_a 10,97.

IR (KBr): ν_{max} 3243, 3015, 2965, 2840, 1652, 1635, 1630, 1604, 1483, 1468, 1402, 1383, 1225, 1090, 870, 786 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 10,59 (s, 1H, 1), 8,76 (s, 1H, 2), 7,61 (d, 2H, arom., J = 8,28), 7,56 (d, 2H, arom., J = 8,45), 7,07 (s, 1H, arom.), 6,84 (d, 1H, arom., J = 8,96), 6,63 (dd, 1H, arom., J = 1,90, J = 6,84), 3,70 (s, 3H, 2''), 3,32 (s, 2H, 4), 2,18 (s, 3H, 1'').

^{13}C NMR (DMSO-d_6, δ ppm): 167,81 (3), 166,31 (13), 155,50, 137,57, 135,23, 134,19, 130,72, 130,25, 113,74 (5–7, 10, 12, 14, 17), 131,12, 129,00, 114,45, 111,12, 102,09 (8, 9, 11, 15, 16, 18, 19), 55,43 (2''), 28,36 (4), 13,29 (1'').

4.1.10.8. *N*-hidroksi-2-(2-(2,6-diklorfenilamino)fenil)-*N*-metilacetamid (8h)

Metoda A: količina reaktanata: 0,397 g spoja **7c,** 0,418 g hidroklorida *N*-metilhidroksilamina. Iskorištenje: 0,085 g (26%).

Metoda B: količina reaktanata: 0,397 g spoja **7c**, 0,418 g hidroklorida *N*-metilhidroksilamina; otapalo: kloroform; trajanje reakcije 4 h; sobna temperatura. Sirovi produkt **8h** prekristaliziran je iz smjese acetona i petroletera. Iskorištenje: 0,280 g (86%).

t_t 134–138 °C; lit. t_t 135–138 °C;[7] lit. 135–137 °C.[93]

pK_a 9,91.

IR (KBr): ν_{max} 3272, 3066, 2925, 1614, 1581, 1509, 1452, 770, 752 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 10,30 (s, 1H, 1), 7,82 (s, 1H, 11), 7,51 (d, 2H, arom., J = 8,07), 7,20–7,14 (m, 2H, arom.), 7,08–7,02 (m, 1H, arom.), 6,88–6,83 (m, 1H, arom.), 6,30 (d, 1H, arom., J = 7,78), 3,86 (s, 2H, 4), 3,16 (s, 3H, 1').

^{13}C NMR (DMSO-d_6, δ ppm): 171,22 (3), 143,30, 137,33, 129,60, 125,08 (5, 10, 12, 13, 17), 131,19, 129,32, 127,36, 125,29, 120,90, 116,18 (6–9, 14–16), 35,98 (4), 35,26 (1').

4.1.10.9. 2-(3-Benzilfenil)-*N*-metoksipropanamid (8i)

Metoda B: 0,341 g spoja **7g**, 0,100 g hidroklorida *O*-metilhidroksilamina; otapalo: toluen; trajanje reakcije 48 h; sobna temperatura. Sirovi produkt **8i** pročišćen je kromatografijom na koloni, uz pokretnu fazu diklormetan/metanol 97:3. Dobiveno ulje **8i** kristalizira.

Iskorištenje: 0,159 g (59%).

t_t 61–64 °C.

IR (KBr): ν_{max} 3153, 3083, 2971, 938, 2854, 1661, 1640, 493, 1069, 1054, 720, 706 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 11,19 (s, 1H, 2), 7,30–7,07 (m, 9H, arom.), 3,91 (s, 2H, 12), 3,38–3,32 (m, 4H, 4, 1'), 1,30 (d, 3H, 5, J = 7,09).

^{13}C NMR (DMSO-d_6, δ ppm): 170,48 (3), 141,99, 141,67, 141,62 (6, 10, 13), 129,13, 128,87, 128,00, 127,57, 126,42, 125,30 (7–9, 11, 14–18), 63,48 (1'), 42,52 (4), 41,59 (12), 18,65 (5).

4.1.10.10. 2-(3-Benzoilfenil)-*N*-metoksipropanamid (8j)

Metoda B: 0,359 g spoja **7e**, 0,100 g hidroklorida *O*-metilhidroksilamina; otapalo: toluen; trajanje reakcije 26 h; sobna temperatura. Sirovi produkt **8j** pročišćen je kromatografijom na koloni, uz pokretnu fazu petroleter/etil-acetat 1:1. Dobiveno ulje **8j** kristalizira. Iskorištenje: 0,201 g (71%).

t_t 105–107 °C.

IR (KBr): ν_{max} 3164, 2984, 2945, 882, 1664, 1651, 1642, 1603, 1445, 1318, 1289, 722, 703, 692 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, *J*/Hz): 11,30 (s, 1H, 2), 7,75–7,49 (m, 9H, arom.), 3,55–3,47 (m, 4H, 4, 1'), 1,37 (d, 3H, 5, *J* = 7,03).

^{13}C NMR (DMSO-d_6, δ ppm): 196,14 (11), 170,18 (2), 142,25, 137,46, 137,43 (5, 9, 12), 133,19, 132,03, 130,07, 129,13, 129,05, 128,84, 128,78 (6–8, 10, 13–17), 63,59 (1'), 42,44 (3), 18,61 (4).

4.1.10.11. 2-(2-(2,6-Diklorfenilamino)fenil)-*N*-metoksiacetamid (8k)

Metoda B: količina reaktanata: 0,397 g spoja **7c**, 0,100 g hidroklorida *O*-metilhidroksilamina; otapalo: toluen; trajanje reakcije 24 h; sobna temperatura. Sirovi produkt **8k** prekristaliziran je iz toluena.

Iskorištenje: 0,128 g (39%).

t_t 178–179 °C; lit. t_t 177–178 °C.[7]

IR (KBr): ν_{max} 3277, 3152, 2979, 2866, 1644, 1592, 1510, 1452, 1280, 1064, 977, 949, 772, 750 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, *J*/Hz): 11,44 (s, 1H, 2), 8,03 (s, 1H, 11), 7,44 (d, 2H, arom., *J* = 8,08), 7,10–7,07 (m, 2H, arom.), 6,99–6,96 (m, 1H, arom.), 6,80–6,77 (m, 1H, arom.), 6,22 (d, 1H, arom., *J* = 7,95), 3,54 (s, 3H, 1'), 3,37 (s, 2H, 4).

^{13}C NMR (DMSO-d_6, δ ppm): 168,02 (3), 142,93 , 137,03, 129,50, 124,48 (5, 10, 12, 13, 17), 130,38, 129,17, 127,44, 124,19, 120,77, 116,01 (6–9, 14–16), 63,33 (1'), 36,57 (4).

4.1.10.12. 1-(4-Klorbenzoil)-2-metil-*N*,5-dimetoksi-1*H*-indol-3-acetamid (8l)

Metoda B: količina reaktanata: 0,459 g spoja **7d**; otapalo: toluen; trajanje reakcije 48 h; sobna temperatura. Sirovi produkt **8l** prekristaliziran je iz toluena.

Iskorištenje: 0,251 g (65%).

t_t 182–184 °C; lit. t_t 184–185 °C.[7]

IR (KBr): ν_{max} 3222, 3093, 2991, 2929, 1677, 1660, 1615, 1596, 1479, 1318, 1224, 1072, 849, 755 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, *J*/Hz): 11,28 (s, 1H, 2), 7,70 (d, 2H, arom., *J* = 8,70), 7,65 (d, 2H, arom., *J* = 8,71), 7,12 (d, 1H, arom., *J* = 2,17), 6,93 (d, 1H, arom., *J* = 8,98), 6,71 (dd, 1H, arom., *J* = 2,50, *J* = 6,50), 3,77 (s, 3H, 2''), 3,59 (s, 3H, 1'), 3,40 (s, 2H, 4), 2,24 (s, 3H, 1'').

^{13}C NMR (DMSO-d_6, δ ppm): 168,31 (3), 166,72 (13), 156,02, 138,08, 135,86, 134,65, 131,13, 130,71, 113,75 (5–7, 10, 12, 14, 17), 131,64, 129,51, 115,04, 111,77, 102,22 (8, 9, 11, 15, 16, 18, 19), 63,70 (1'), 55,91 (2''), 28,86 (4), 13,77 (1'').

4.1.10.13. *N*-etoksi-2-(4-izobutilfenil)propanamid (8m)

Metoda B: količina reaktanata: 0,307 g spoja **7a**, 0,117 g hidroklorida *O*-etilhidroksilamina; otapalo: toluen; trajanje reakcije 40 h; sobna temperatura. Sirovi produkt **8m** pročišćen je kromatografijom na koloni, uz pokretnu fazu cikloheksan/etil-acetat 2:1.

Iskorištenje: 0,162 g (64%).

t_t 54–56 °C.

IR (KBr): ν_{max} 3178, 2933, 2870, 1659, 1512, 1464, 1042, 934, 850 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, *J*/Hz): 11,07 (s, 1H, 2), 7,20 (d, 2H, arom., *J* = 8,03), 7,08 (d, 2H, arom., *J* = 8,03), 3,75 (q, 2H, 1', *J* = 7,03), 3,38 (q, 1H, 4, *J* = 7,05), 2,40 (d, 2H, 12, *J* = 7,13), 1,87–1,73 (m, 1H, 13), 1,31 (d, 3H, 5, *J* = 7,04), 1,09 (t, 3H, 2', *J* = 7,03), 0,85 (d, 6H, 14, 15, *J* = 6,60).

^{13}C NMR (DMSO-d_6, δ ppm): 170,78 (3), 139,87, 139,20 (6, 9), 129,27, 127,34 (7, 8, 10, 11), 70,87 (1'), 44,68 (12), 42,20 (4), 30,08 (5), 22,62 (14, 15), 18,63 (13), 13,84 (2').

4.1.10.14. 2-(3-Benzilfenil)-*N*-etoksipropanamid (8n)

Metoda B: 0,341 g spoja **7g**, 0,117 g hidroklorida *O*-etilhidroksilamina; otapalo: toluen; trajanje reakcije 46 h; sobna temperatura. Sirovi produkt **8n** pročišćen je kromatografijom na koloni, uz pokretnu fazu cikloheksan/etil-acetat 1:1. Dobiveno ulje **8n** kristalizira.

Iskorištenje: 0,130 g (46%).

t_t 114–116 °C.

IR (KBr): ν_{max} 3170, 3023, 2987, 1671, 1650, 1600, 1492, 1047, 721, 707 cm^{-1}.

^1H NMR (DMSO-d_6, δ ppm, *J*/Hz): 11,07 (s, 1H, 2), 7,31–7,07 (m, 9H, arom.), 3,91 (s, 2H, 12), 3,71 (q, 1H, 4, *J* = 7,03), 3,37 (q, 2H, 1', *J* = 7,07), 1,30 (d, 3H, 5, *J* = 7,04), 1,07 (t, 3H, 2', *J* = 7,03).

^{13}C NMR (DMSO-d_6, δ ppm): 170,55 (2), 142,11, 141,62 (5, 9, 12), 129,11, 128,86, 128,82, 127,99, 127,55, 126,41, 125,28 (6–8, 10, 13–17), 70,85 (1'), 42,51 (3), 41,60 (11), 18,65 (4), 13,83 (2').

4.1.10.15. *N*-etoksi-2-(3-fenoksifenil)propanamid (8o)

Metoda B: 0,343 g spoja **7b**, 0,117 g hidroklorida *O*-etilhidroksilamina; otapalo: toluen; trajanje reakcije 24 h; sobna temperatura. Sirovi produkt **8o** prekristaliziran je iz smjese etera i petroletera.

Iskorištenje: 0,168 g (59%).

t_t 60–61 °C.

IR (KBr): ν_{max} 3145, 2946, 2880, 1673, 1654, 1582, 1488, 1251, 1226, 945, 694 cm^{-1}.

^1H NMR (DMSO-d_6, δ ppm, *J*/Hz): 11,10 (s, 1H, 2), 7,42–7,30 (m, 3H, arom.), 7,14 (t, 1H, arom., *J* = 7,36), 7,07 (d, 1H, arom., *J* = 7,65), 7,01–6,96 (m, 3H, arom.), 6,86 (dd, 1H, arom., *J* = 1,89, *J* = 6,14), 3,72 (q, 2H, 1', *J* = 7,02), 3,41 (q, 1H, 4, *J* = 7,03), 1,31 (d, 3H, 5, *J* = 7,01), 1,08 (t, 3H, 2', *J* = 7,02).

^{13}C NMR (DMSO-d_6, δ ppm): 170,26 (3), 157,03, 156,99, 144,14 (6, 10, 12), 130,51, 130,33, 123,93, 122,72, 119,08, 117,82, 117,27 (7–9, 11, 13–17), 70,91 (1'), 42,41 (4), 18,50 (5), 13,83 (2').

4.1.10.16. 2-(3-Benzoilfenil)-*N*-etoksipropanamid (8p)

Metoda B: 0,355 g spoja **7e**, 0,117 g hidroklorida *O*-etilhidroksilamina; otapalo: toluen; trajanje reakcije 24 h; sobna temperatura. Nakon ekstrakcije spoj **8p** pročišćen je kromatografijom na koloni, uz pokretnu fazu petroleter/etil-acetat 3:1. Dobiveni sirovi produkt **8p** nakon rastrljavanja eterom kristalizira.

Iskorištenje: 0,199 g (67%).

t_t 75–77 °C.

IR (KBr): ν_{max} 3192, 2980, 2936, 1660, 1597, 1448, 1319, 1284, 1042, 718, 643 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, *J*/Hz): 11,17 (s, 1H, 2), 7,74–7,67 (m, 4H, arom.), 7,64–7,49 (m, 5H, arom.), 3,75 (q, 2H, 1', *J* = 7,02), 3,54 (q, 1H, 4, *J* = 7,02), 1,37 (d, 3H, 5, *J* = 7,04), 1,11 (t, 3H, 2', *J* = 7,03).

^{13}C NMR (DMSO-d_6, δ ppm): 196,16 (12), 170,25 (3), 142,38, 137,48, 137,43 (6, 10, 13), 133,19, 132,00, 130,06, 129,10, 129,05, 128,82, 128,73 (7–9, 11, 14–18), 70,97 (1'), 42,43 (4), 18,59 (5), 13,83 (2').

4.1.10.17. *N*-etoksi-2-(2-(2,6-diklorfenilamino)fenil)acetamid (8q)

Metoda B: količina reaktanata: 0,397 g spoja **7c**, 0,117 g hidroklorida *O*-etilhidroksilamina; otapalo: kloroform; trajanje reakcije 6,5 h; sobna temperatura. Dio produkta taloži se iz reakcijske smjese te je odsisan i ispran vodom. Matičnica je obrađena prema općem propisu. Sirovi produkt **8q** prekristaliziran je iz toluena.

Iskorištenje: 0,261 g (77%).

t_t 181–182 °C.

IR (KBr): ν_{max} 3298, 3152, 2980, 2882, 1642, 1578, 1507, 1454, 1304, 1060, 975, 782, 750 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, *J*/Hz): 11,40 (s, 1H, 2), 8,12 (s, 1H, 11), 7,52 (d, 2H, arom., *J* = 8,08), 7,20–7,14 (m, 2H, arom.), 7,08–7,03 (m, 1H, arom.), 6,89–6,84 (m, 1H, arom.), 6,30 (d, 1H, arom., *J* = 7,88), 3,83 (q, 2H, 1', *J* = 7,03), 3,46 (s, 2H, 4), 1,14 (t, 3H, 2', *J* = 7,03).

^{13}C NMR (DMSO-d_6, δ ppm): 168,59 (3), 143,42 , 137,53, 129,99, 125,12 (5, 10, 12, 13, 17), 130,83, 129,67, 127,91, 125,68, 121,27, 116,50 (6–9, 14–16), 71,21 (1'), 36,93 (4), 13,88 (2').

4.1.10.18. *N*-etoksi-1-(4-klorbenzoil)-2-metil-5-metoksi-1*H*-indol-3-acetamid (8r)

Metoda B: količina reaktanata: 0,459 g spoja **7d**, 0,117 g hidroklorida *O*-etilhidroksilamina; otapalo: kloroform; trajanje reakcije 43 h; sobna temperatura. Sirovi produkt **8r** prekristaliziran je iz toluena.

Iskorištenje: 0,317 g (79%).

t_t 184–186 °C.

IR (KBr): ν_{max} 3220, 3090, 2988, 2933, 2889, 1678, 1659, 1614, 1595, 1479, 1316, 1225, 1071, 849, 755 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 11,17 (s, 1H, 2), 7,69 (d, 2H, arom., J = 8,71), 7,64 (d, 2H, arom., J = 8,39) 7,13 (d, 1H, arom., J = 2,17), 6,93 (d, 1H, arom., J = 8,98), 6,71 (dd, 1H, arom., J = 2,48, J = 6,53), 3,80 (q, 2H, 1', J = 7,05), 3,77 (s, 3H, 2''), 3,41 (s, 2H, 4), 2,24 (s, 3H, 1''), 1,13 (t, 3H, 2', J = 7,02).

^{13}C NMR (DMSO-d_6, δ ppm): 168,31 (3), 166,78 (13), 156,02, 138,07, 135,83, 134,66, 131,13, 130,72, 113,90 (5–7, 10, 12, 14, 17), 131,64, 129,51, 115,04, 111,78, 102,20 (8, 9, 11, 15, 16, 18, 19), 71,02 (1'), 55,91 (2''), 28,86 (4), 13,89 (2'), 13,78 (1'').

4.1.10.19. *N*-benziloksi-2-(4-izobutilfenil)propanamid (8s)

Metoda B: količina reaktanata: 0,307 g spoja **7a**, 0,191 g hidroklorida *O*-benzilhidroksilamina.

Otapalo kloroform: trajanje reakcije 48 h; sobna temperatura. Sirovi produkt **8s** pročišćen je rastrljavanjem petroleterom. Iskorištenje: 0,218 g (70%).

Otapalo toluen: trajanje reakcije 48 h; sobna temperatura. Sirovi produkt **8s** pročišćen je rastrljavanjem petroleterom. Iskorištenje: 0,193 g (62%).

t_t 66–68 °C.

IR (KBr): ν_{max} 3156, 3068, 2975, 2948, 2866, 1674, 1648, 1530, 1454, 1376, 1042, 733, 696 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 11,19 (s, 1H, 2), 7,37–7,29 (m, 5H, arom.), 7,21 (d, 2H, arom., J = 8,01), 7,10 (d, 2H, arom., J = 8,04), 4,75 (s, 2H, 1'), 3,40 (q, 1H, 4, J = 7,04), 2,43 (d, 2H, 12, J = 7,10), 1,87–1,78 (m, 1H, 13), 1,33 (d, 3H, 5, J = 7,03), 0,87 (d, 6H, 14, 15, J = 6,60).

^{13}C NMR (DMSO-d_6, δ ppm): 170,57 (3), 139,57, 138,77, 136,03 (6, 9, 2'), 129,08, 128,93, 128,36, 127,09 (7, 8, 10, 11, 3'–7'), 76,69 (1'), 44,36 (12), 41,80 (4), 29,77 (13), 22,30 (14, 15), 18,22 (5).

4.1.10.20. *N*-benziloksi-2-(3-benzilfenil)propanamid (8t)

Metoda B: 0,341 g spoja **7g**, 0,191 g hidroklorida *O*-benzilhidroksilamina; otapalo: toluen; trajanje reakcije 11 h; temperatura 75 °C. Nakon ekstrakcije spoj **8t** pročišćen je kromatografijom na koloni, uz pokretnu fazu cikloheksan/etil-acetat/metanol 3:1:0,5. Iskorištenje: 0,231 g (67%).

Ulje.

IR (film): ν_{max} 3181, 3062, 3028, 2975, 2934, 2876, 1658, 1601, 1495, 1454, 1027, 749, 725, 698 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 11,18 (s, 1H, 2), 7,34–7,07 (m, 14H, arom.), 4,70 (s, 2H, 1'), 3,92 (s, 2H, 12), 3,38 (q, 1H, 4, J = 7,01), 1,30 (d, 3H, 5, J = 7,03).

^{13}C NMR (DMSO-d_6, δ ppm): 170,71 (2), 141,99, 141,62 (5, 9, 12), 136,34 (2'), 129,36, 128,71 (3'–7'), 129,12, 128,86, 128,82, 128,04, 127,56, 126,42, 125,36 (6–8, 10, 13–17), 77,06 (1'), 42,44 (3), 41,60 (11), 18,59 (4).

4.1.10.21. *N*-benziloksi-2-(3-fenoksifenil)propanamid (8u)

Metoda A: 0,343 g spoja **7b,** 0,798 g hidroklorida *O*-benzilhidroksilamina. Sirovi produkt **8u** pročišćen je rastrljavanjem smjesom etera i petroletera. Iskorištenje: 0,181 g (52%).

Metoda B: 0,343 g spoja **7b**; 0,191 g hidroklorida *O*-benzilhidroksilamina.

Otapalo kloroform: trajanje reakcije 48 h, sobna temperatura. Sirovi produkt **8u** pročišćen je rastrljavanjem petroleterom. Iskorištenje: 0,240 g (69%).

Otapalo toluen: trajanje reakcije 5,5 h; temperatura refluksa. Sirovi produkt **8u** pročišćen je kromatografijom na koloni, uz pokretnu fazu cikloheksan/etil-acetat/metanol 3:1:0,5. Iskorištenje: 0,316 g (91%).

Ulje.

IR (film): ν_{max} 3181, 3065, 3033, 2973, 1658, 1583, 1487, 1453, 751, 695 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, , J/Hz): 11,13 (s, 1H, 2),7,32–7,22 (m, 8H, arom.), 7,07–7,05 (m, 1H, arom.), 6,99 (d, 1H, arom., J = 7,56), 6,94–6,90 (m, 3H, arom.), 6,79 (dd, 1H, arom., J = 1,74, J = 6,22), 4,65 (s, 2H, 1'), 3,34 (q, 1H, 4, J = 6,90), 1,25 (d, 3H, 5, J = 7,01).

^{13}C NMR (DMSO-d_6, δ ppm): 169,93 (3), 156,52, 143,55, 135,82 (6, 10, 12, 2'), 130,00, 129,80, 128,89, 128,22, 123,41, 122,30, 118,56, 117,48, 116,78 (7–9, 11, 13–17, 3'–7'), 76,59 (1'), 41,88 (4), 17,97 (5).

4.1.10.22. *N*-benziloksi-2-(3-benzoilfenil)propanamid (8v)

Metoda B: količina reaktanata: 0,355 g spoja **7e,** 0,191 g hidroklorida *O*-benzilhidroksilamina; otapalo: toluen; trajanje reakcije 48 h; sobna temperatura. Sirovi produkt **8v** pročišćen je rastrljavanjem smjesom etera i petroletera.

Iskorištenje: 0,270 g (75%).

t_t 82–85 °C.

IR (KBr): ν_{max} 3188, 3063, 3031, 2974, 2875, 1660, 1597, 1578, 1497, 1448, 1319, 1284, 1026, 719, 698 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 11,27 (s, 1H, 2), 7,75–7,49 (m, 10H, arom.), 7,31 (s, 5H, arom.), 4,73 (s, 2H, 1'), 3,54 (q, 1H, 4, J = 7,08), 1,37 (d, 3H, 5, J = 6,99).

^{13}C NMR (DMSO-d_6, δ ppm): 196,16 (12), 179,37 (3), 142,28, 137,48, 137,42, 136,26 (6, 10, 13, 2'), 133,19, 132,03, 130,08, 129,41, 129,05, 128,92, 128,76, 128,71 (7–9, 11, 14–18, 3'–7'), 77,08 (1'), 42,36 (4), 18,50 (5).

4.1.10.23. *N*-benziloksi-2-(2-(2,6-diklorfenilamino)fenil)acetamid (8x)

Metoda A: količina reaktanata: 0,397 g spoja **7c**, 0,798 g hidroklorida *O*-benzilhidroksilamina. Sirovi produkt **8x** pročišćen je rastrljavanjem petroleterom. Iskorištenje: 0,357 g (89%).

Metoda B: količina reaktanata: 0,397 g spoja **7c**, 0,191 g hidroklorida *O*-benzilhidroksilamina.

Otapalo kloroform: trajanje reakcije 27 h; sobna temperatura. Sirovi produkt **8x** pročišćen je prekristalizacijom iz toluena. Iskorištenje: 0,229 g (57%).

Otapalo toluen: trajanje reakcije 1 h, temperatura refluksa. Dio produkta taloži se iz reakcijske smjese te je odsisan i ispran vodom. Matičnica je obrađena prema općem propisu. Sirovi produkt **8x** prekristaliziran je iz toluena. Iskorištenje: 0,353 g (88%).

t_t 161–164 °C.

IR (KBr): ν_{max} 3265, 3153, 3065, 2974, 1655, 1636, 1588, 1507, 1454, 1304, 751, 733, 696 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 11,47 (s, 1H, 2), 8,09 (s, 1H, 11), 7,53 (d, 2H, arom., J = 8,06), 7,37 (s, 5H, arom.), 7,20–7,14 (m, 3H, arom.), 7,09–7,04 (m, 1H, arom.), 6,89–6,84 (m, 1H, arom.), 6,31 (d, 1H, arom., J = 7,80), 4,82 (s, 2H, 1'), 3,47 (s, 2H, 4).
^{13}C NMR (DMSO-d_6, δ ppm): 168,16 (3), 142,87, 136,98, 135,75, 129,54, 124,45 (5, 10, 12, 13, 17, 2'), 130,35, 129,11, 128,78, 128,23, 127,35, 125,18, 120,66, 115,89 (6–9, 14–16, 3'–7'), 76,77 (1'), 36,29 (4).

4.1.10.24. *N*-benziloksi-1-(4-klorbenzoil)-2-metil-5-metoksi-1*H*-indol-3-acetamid (8y)

Metoda A: količina reaktanata: 0,459 g spoja **7d**, 0,798 g hidroklorida *O*-benzilhidroksilamina. Sirovi produkt **8y** pročišćen je rastrljavanjem smjesom etera i petroletera i prekristalizacijom iz toluena. Iskorištenje: 0,264 g (57%).
Metoda B: količina reaktanata: 0,459 g spoja **7d**, 0,191 g hidroklorida *O*-benzilhidroksilamina; otapalo: kloroform; trajanje reakcije 48 h; sobna temperatura. Iskorištenje: 0,421 g (91%).
t_t 156–158 °C; lit. t_t 152–153 °C;[96] 158–160 °C.[99]
IR (KBr): ν_{max} 3240, 3065, 3033, 2997, 2925, 2864, 1685, 1656, 1609, 1518, 1479, 1452, 1370, 1321, 1227, 1089, 836, 753, 699 cm^{-1}.
^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 11,20 (s, 1H, 2), 7,61 (d, 2H, arom., J = 8,44), 7,57 (d, 2H, arom., J = 8,30), 7,27 (s, 5H, arom.), 7,05 (d, 1H, arom., J = 1,77), 6,86 (d, 1H, arom., J = 8,98), 6,64 (dd, 1H, arom., J = 2,11, J = 6,81), 4,75 (s, 2H, 1'), 3,72 (s, 3H, 2''), 3,39 (s, 2H, 4), 2,20 (s, 3H, 1'').
^{13}C NMR (DMSO-d_6, δ ppm): 168,32 (3), 167,03 (13), 156,03, 138,07, 136,38, 135,86, 134,66, 131,17, 130,71, 113,83 (5–7, 10, 12, 14, 17, 2'), 131,63, 129,52, 129,35, 128,74, 115,01, 111,74, 102,34 (8, 9, 11, 15, 16, 18, 19, 3'–7'), 77,25 (1'), 55,92 (2''), 28,80 (4), 13,79 (1'').

4.1.11. SINTEZA DERIVATA KETOPROFENA **9a-i**, **10a-f**, **11a-f**

Opća metoda sinteze spojeva 9a-f i 10a-f. Otopina odgovarajućeg benzotriazolida **7e**,**g** (1 mmol), odgovarajućeg amina (1,1 mmol) i TEA (0,696 mL, 5 mmol) u toluenu (10 mL) miješana je na sobnoj temperaturi 1–8 h ili na 78 °C 11–16 h. Reakcijska smjesa ekstrahirana je zasićenom otopinom natrijevog klorida (5×10 mL), kloridnom kiselinom (w = 1%, 2×5 mL) i vodom (3×10 mL). Nakon sušenja nad bezvodnim natrijevim sulfatom i uparavanja pod sniženim tlakom, dobiven je sirovi produkt.

Opća metoda sinteze spojeva 9g i 9h. Otopina **7g** (0,341 g, 1 mmol), odgovarajućeg amina (1,1 mmol) i TEA (0,139 mL, 1 mmol) u smjesi toluen/etil-acetat (2:1, 15 mL) miješana je na sobnoj temperaturi 0,25 h. Nakon uparavanja reakcijske smjese i kromatografije na koloni, uz pokretnu fazu etil-acetat, dobiven je odgovarajući produkt.

Opća metoda sinteze spojeva 11a-f. Suspenzija amida ketoprofena **10a-f** (0,5 mmol) i 10%-tnog Pd/C(en) (20 mg) u metanolu (10 mL) miješana je pod atmosferskim tlakom vodika na sobnoj temperaturi 5–9 h. Katalizator je odsisan, a otapalo upareno te je dobiven sirovi produkt.

4.1.11.1. 2-(3-Benzilfenil)-*N*-ciklopentilpropanamid (9a)

Količina reaktanata: 0,341 g spoja **7g**, 0,109 mL ciklopentilamina; trajanje reakcije 5 h; sobna temperatura. Sirovi produkt **9a** rastrljan je eterom.

Iskorištenje: 0,252 g (82%).

t_t 92–93 °C.

IR (KBr): ν_{max} 3274, 3061, 3027, 2962, 2936, 2864, 1635, 1599, 1548, 1448, 785, 731, 711, 699 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 7,85 (d, 1H, 1, J = 7,15), 7,30–7,04 (m, 9H, arom.), 3,99–3,87 (m, 3H, 11, 1'), 3,52 (q, 1H, 3, J = 7,01), 1,83–1,16 (m, 8H, 2'–5'), 1,27 (d, 3H, 4, J = 7,03).

^{13}C NMR (DMSO-d_6, δ ppm): 173,02 (2), 143,11, 141,69, 141,42 (5, 9, 12), 129,09, 128,83, 128,68, 128,03, 127,21, 126,39, 125,26 (6–8, 10, 13–17), 50,64 (1'), 45,22 (3), 41,67 (11), 32,68, 32,63 (2', 5'), 23,96, 23,92 (3', 4'), 19,01 (4).

4.1.11.2. 2-(3-Benzilfenil)-*N*-cikloheksilpropanamid (9b)

Količina reaktanata: 0,341 g spoja **7g**, 0,126 mL cikloheksilamina; trajanje reakcije 7 h; sobna temperatura. Sirovi produkt **9b** rastrljan je eterom.

Iskorištenje: 0,273 g (85%).

t_t 89–93 °C.

IR (KBr): ν_{max} 3297, 3062, 3026, 2980, 2936, 2854, 1639, 1585, 1546, 1448, 779, 750, 698 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 7,74 (d, 1H, 1, J = 7,79), 7,29–7,04 (m, 9H, arom.), 3,90 (s, 2H, 11), 3,52 (q, 1H, 3, J = 7,04), 3,49–3,40 (m, 1H, 1'), 1,73–1,54, 1,21–0,93 (2m, 8H, 2'–6'), 1,27 (d, 3H, 4, J = 7,03).

^{13}C NMR (DMSO-d_6, δ ppm): 172,59 (2), 143,15, 141,69, 141,41 (5, 9, 12), 129,10, 128,82, 128,67, 128,00, 127,19, 126,37, 125,26 (6–8, 10, 13–17), 47,84 (1'), 45,27 (3), 41,66 (11), 32,81, 32,78 (2', 6'), 25,69, 25,04, 24,96 (3'–5'), 19,00 (4).

4.1.11.3. 2-(3-Benzilfenil)-*N*-(cikloheksanmetil)propanamid (9c)

Količina reaktanata: 0,341 g spoja **7g**, 0,143 mL cikloheksanmetilamina; trajanje reakcije 8 h; sobna temperatura. Sirovi produkt **9c** pročišćen je kromatografijom na koloni, uz pokretnu fazu cikloheksan/etil-acetat 3:1.

Iskorištenje: 0,275 g (82%).

t_t 50–54 °C.

IR (KBr): ν_{max} 3295, 3061, 3027, 2968, 2918, 2851, 1647, 1602, 1585, 1558, 1442, 1221, 1155, 711, 698 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 7,84 (t, 1H, 1, J = 5,31), 7,30–7,03 (m, 9H, arom.), 3,90 (s, 2H, 11), 3,56 (q, 1H, 3, J = 6,97), 2,85 (dd, 2H, 1', J = 6,11 , J = 6,50), 1,66–1,51, 1,40–1,24, 1,17–1,04, 0,84–0,72 (4m, 4H, 3'–7'), 1,28 (d, 3H, 4, J = 7,02).

^{13}C NMR (DMSO-d_6, δ ppm): 173,55 (2), 143,10, 141,67, 141,36 (5, 9, 12), 129,07, 128,83, 128,66, 128,11, 127,27, 126,39, 125,31 (6–8, 10, 13–17), 45,46 (3), 45,24 (1'), 41,65 (11), 37,88 (2'), 30,81, 30,77 (2', 7'), 26,49, 25,85 (4'–6'), 19,02 (4).

4.1.11.4. *N*-benzil-2-(3-benzilfenil)propanamid (9d)

Količina reaktanata: 0,341 g spoja **7g**, 0,120 mL benzilamina; trajanje reakcije 3,5 h; sobna temperatura. Sirovi produkt **9d** pročišćen je kromatografijom na koloni, uz pokretnu fazu cikloheksan/etil-acetat 2:1. Sirovi produkt **9d** kristalizira iz etera.

Iskorištenje: 0,290 g (88%).

t_t 70–74 °C.

IR (KBr): ν_{max} 3292, 3062, 3028, 2971, 2930, 1648, 1602, 1546, 1495, 1453, 722, 697 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 8,44 (t, 1H, 1, J = 5,65), 7,31–7,06 (m, 14H, arom.), 4,24 (d, 2H, 1', J = 5,52), 3,91 (s, 2H, 11), 3,64 (q, 1H, 3, J = 6,96), 1,34 (d, 3H, 4, J = 7,01).

^{13}C NMR (DMSO-d_6, δ ppm): 173,69 (2), 142,88, 141,66, 141,48 (5, 9, 12), 140,00 (2'), 129,11, 128,86, 128,67, 128,16, 127,23, 126,41, 125,41 (6–8, 10, 13–17), 128,76, 127,51, 127,41, 127,14 (3'–7'), 45,52 (3), 42,48 (1'), 41,64 (11), 19,04 (4).

4.1.11.5. 2-(3-Benzilfenil)-*N*-fenetilpropanamid (9e)

Količina reaktanata: 0,341 g spoja **7g**, 0,139 mL feniletilamina; trajanje reakcije 2 h; sobna temperatura. Sirovi produkt **9e** prekristaliziran je iz smjese etera i petroletera.

Iskorištenje: 0,295 g (86%).

t_t 60–64 °C.

IR (KBr): ν_{max} 3306, 3064, 3026, 2925, 1642, 1548, 1496, 1454, 753, 712, 698 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 7,97 (t, 1H, 1, J = 5,43), 7,29–7,05 (m, 14H, arom.), 3,91 (s, 2H, 11), 3,52 (q, 1H, 3, J = 7,00), 3,29–3,16 (m, 2H, 1'), 2,64 (t, 2H, 2', J = 7,23), 1,27 (d, 3H, 4, J = 7,04).

^{13}C NMR (DMSO-d_6, δ ppm): 173,53 (2), 142,90, 141,67, 141,47 (5, 9, 12), 139,91 (3'), 129,13, 128,86, 128,71, 128,12, 127,30, 126,40, 125,40 (6–8, 10, 13–17), 129,10, 128,67, 126,44 (4'–8'), 45,50 (3), 41,66 (11), 40,83 (1'), 35,37 (2'), 19,02 (4).

4.1.11.6. *N*-Benzhidril-2-(3-benzilfenil)propanamid (9f)

Količina reaktanata: 0,341 g spoja **7g**, 0,190 mL benzhidrilamina; trajanje reakcije 16 h; temperatura 78 °C. Sirovi produkt **9f** pročišćen je kromatografijom na koloni, uz pokretnu fazu cikloheksan/etil-acetat 4:1, te rastrljavanjem eterom.

Iskorištenje: 0,284 g (70%).

t_t 122–123 °C.

IR (KBr): ν_{max} 3297, 3087, 3064, 3025, 2979, 1667, 1641, 1601, 1586, 1529, 1494, 1452, 757, 741, 718, 703 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 8,86 (d, 1H, 1, J = 8,45), 7,36–7,04 (m, 19H, arom.), 6,07 (d, 1H, 1', J = 8,45), 3,90 (s, 2H, 11), 3,80 (q, 1H, 3, J = 6,94), 1,32 (d, 3H, 4, J = 7,00).

^{13}C NMR (DMSO-d_6, δ ppm): 172,97 (2), 142,95, 141,67, 141,40 (5, 9, 12), 142,86, 142,74 (2', 8'), 129,09, 128,85, 128,72, 128,23, 127,28, 126,40, 125,38 (6–8, 10, 13–17), 128,68, 127,85, 127,55, 127,41, 127,40 (3'–7', 9'–13'), 56,28 (1'), 45,06 (3), 41,65 (11), 18,86 (4).

4.1.11.7. 2-(3-Benzilfenil)-*N*-(2-hidroksietil)propanamid (9g)

Količina amina: 0,066 mL 2-hidroksietilamina.

Iskorištenje: 0,238 g (84%).

Ulje.

IR (film): ν_{max} 3414, 3312, 3084, 3027, 2972, 2931, 1648, 1601, 1560, 1540, 1494, 1453, 1363, 1072, 723, 700 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 7,92 (t, 1H, 1, J = 5,24), 7,31–7,04 (m, 9H, arom.), 4,64 (t, 1H, 3', J = 5,36), 3,91 (s, 2H, 11), 3,57 (q, 1H, 3, J = 7,03), 3,39–3,33 (m, 2H, 2'), 3,17–3,01 (m, 2H, 1'), 1,28 (d, 3H, 4, J = 7,03).

^{13}C NMR (DMSO-d_6, δ ppm): 173,81 (2), 143,11, 141,67, 141,47 (5, 9, 12), 129,13, 128,86, 128,71, 128,14, 127,27, 126,41, 125,37 (6–8, 10, 13–17), 60,30 (1'), 45,40 (3), 41,98 (2'), 41,65 (11), 19,21 (4).

4.1.11.8. 2-(3-Benzilfenil)-*N*-(3-hidroksipropil)propanamid (9h)

Količina amina: 0,084 mL 3-hidroksipropilamina.

Iskorištenje: 0,229 g (77%).

Ulje.

IR (film): ν_{max} 3301, 3062, 3027, 2970, 2935, 2877, 1652, 1601, 1558, 1548, 1494, 1453, 721, 699 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 7,89 (t, 1H, 1, J = 5,29), 7,31–7,04 (m, 9H, arom.), 4,38 (t, 1H, 4'), 3,91 (s, 2H, 11), 3,53 (q, 1H, 3, J = 7,00), 3,34 (q, 2H, 1', J = 6,19), 3,06 (q, 2H, 3', J = 6,51), 1,54–1,45 (m, 2H, 2'), 1,28 (d, 3H, 4, J = 7,04).

^{13}C NMR (DMSO-d_6, δ ppm): 173,64 (2), 143,02, 141,66, 141,47 (5, 9, 12), 129,12, 128,86, 128,72, 128,08, 127,28, 126,40, 125,30 (6–8, 10, 13–17), 58,80 (1'), 45,50 (3), 41,65 (11), 36,20 (3'), 32,83 (2'), 19,07 (4).

4.1.11.9. 2-(3-Benzilfenil)-*N*,*N*-bis(2-hidroksietil)propanamid (9i)

Otopina **7g** (0,341 g, 1 mmol), dietanolamina (0,116 g, 1,1 mmol) i TEA (0,139 mL, 1 mmol) u smjesi toluen/acetonitril (2:1, 15 mL) miješana je na sobnoj temperaturi 5 h. Nakon uparavanja reakcijske smjese i kromatografije na koloni uz pokretnu fazu etil-acetat→etil-acetat/metanol 8:1, dobiven je **9i**.

Iskorištenje: 0,249 g (76%).

Ulje.

IR (film): ν_{max} 3370, 3060, 3026, 2931, 1622, 1494, 1473, 1454, 1442, 1061, 745, 699 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 7,31–7,04 (m, 9H, arom.), 4,82 (s, 1H, 3'), 4,46 (s, 1H, 3''), 4,10 (q, 1H, 3, J = 6,74), 3,91 (s, 2H, 11), 3,56–3,34, 3,19–3,10 (m, 8H, 1', 2', 1'', 2''), 1,24 (d, 3H, 4, J = 6,77).

^{13}C NMR (DMSO-d_6, δ ppm): 173,73 (2), 143,10, 141,89, 141,63 (5, 9, 12), 129,09, 128,86, 128,00, 127,32, 126,40, 125,40 (6–8, 10, 13–17), 59,73, 59,07 (1', 1''), 50,64, 48,93 (2', 2''), 41,64 (11), 41,60 (3), 21,26 (4).

4.1.11.10. 2-(3-Benzoilfenil)-*N*-ciklopentilpropanamid (10a)

Količina reaktanata: 0,355 g spoja **7e**, 0,109 mL ciklopentilamina; trajanje reakcije 1,5 h; sobna temperatura. Sirovi produkt **10a** rastrljan je eterom.

Iskorištenje: 0,257 g (80%).

t_t 94–95 °C.

IR (KBr): ν_{max} 3355, 3066, 2964, 2870, 1663, 1648, 1596, 1528, 1449, 1312, 1284, 723, 702, 642 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, *J*/Hz): 7,99 (d, 1H, 1, *J* = 7,01), 7,72–7,45 (m, 9H, arom.), 3,99–3,87 (m, 1H, 1'), 3,66 (q, 1H, 3, *J* = 6,73), 1,83–1,17 (m, 8H, 2'–5'), 1,32 (d, 3H, 4, *J* = 7,00).

^{13}C NMR (DMSO-d_6, δ ppm): 196,23 (11), 172,71 (2), 143,34, 137,53, 137,28 (5, 9, 12), 133,14, 131,99, 130,04, 129,03, 128,97, 128,88, 128,41 (6–8, 10, 13–17), 50,73 (1'), 45,08 (3), 32,68 (2', 5'), 23,94, 23,90 (3', 4'), 18,95 (4).

4.1.11.11. 2-(3-Benzoilfenil)-*N*-cikloheksilpropanamid (10b)

Količina reaktanata: 0,355 g spoja **7e**, 0,126 mL cikloheksilamina; trajanje reakcije 6 h; sobna temperatura. Sirovi produkt **10b** rastrljan je eterom.

Iskorištenje: 0,258 g (77%).

t_t 120–121 °C; lit. t_t 120–121 °C.[58]

IR (KBr): ν_{max} 3355, 3067, 2976, 2936, 2851, 1660, 1648, 1596, 1526, 1448, 1311, 1285, 1220, 768, 722, 701, 644 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, *J*/Hz): 7,91 (d, 1H, 1, *J* = 7,97), 7,74–7,47 (m, 9H, arom.), 3,69 (q, 1H, 3, *J* = 7,02), 3,54–3,42 (m, 1H, 1'), 1,76–1,51, 1,27–0,98 (2m, 10H, 2'–6'), 1,34 (d, 3H, 4, *J* = 7,07).

^{13}C NMR (DMSO-d_6, δ ppm): 196,23 (11), 172,29 (2), 143,40, 137,55, 137,29 (5, 9, 12), 133,13, 131,98, 130,05, 129,02, 128,96, 128,88, 128,37 (6–8, 10, 13–17), 47,93 (1'), 45,14 (3), 32,98 (2', 6'), 25,67, 25,00, 24,92 (3'–5'), 18,96 (4).

4.1.11.12. 2-(3-Benzoilfenil)-*N*-(cikloheksanmetil)propanamid (10c)

Količina reaktanata: 0,355 g spoja **7e**, 0,143 mL cikloheksanmetilamina; trajanje reakcije 2 h; sobna temperatura. Sirovi produkt **10c** rastrljan je eterom.

Iskorištenje: 0,315 g (90%).

t_t 96–98 °C.

IR (KBr): ν_{max} 3298, 3094, 2919, 2853, 1676, 1660, 1642, 1597, 1563, 1447, 1289, 1271, 1234, 729, 707, 640 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, *J*/Hz): 7,98 (t, 1H, 1, *J* = 7,98), 7,74–7,47 (m, 9H, arom.), 3,72 (q, 1H, 3, *J* = 6,99), 2,87 (dd, 2H, 1', *J* = 6,18, *J* = 4,38), 1,67–1,51, 1,41–1,30, 1,18–1,03, 0,84–0,73 (4m, 4H, 3'–7'), 1,35 (d, 3H, 4, *J* = 7,00).

^{13}C NMR (DMSO-d_6, δ ppm): 196,22 (11), 173,24 (2), 143,41, 137,53, 137,30 (5, 9, 12), 133,17, 132,00, 130,04, 129,03, 128,92, 128,44 (6–8, 10, 13–17), 45,30 (3), 45,26 (1'), 37,89 (2'), 30,78, 30,75 (3', 7'), 26,46, 25,84 (4'–6'), 18,92 (4).

4.1.11.13. *N*-benzil-2-(3-benzoilfenil)propanamid (10d)

Količina reaktanata: 0,355 g spoja **7e**, 0,120 mL benzilamina; trajanje reakcije 1 h; sobna temperatura. Sirovi produkt **10d** rastrljan je eterom.

Iskorištenje: 0,285 g (83%).

t_t 97–99 °C.

IR (KBr): ν_{max} 3314, 3287, 3066, 3033, 2926, 2870, 1644, 1551, 1449, 1349, 1015, 712, 701 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, *J*/Hz): 8,56 (t, 1H, 1, *J* = 5,75), 7,76–7,49 (m, 9H, arom.), 7,29–7,14 (m, 5H, arom.), 4,25 (d, 2H, 1', *J* = 5,84), 3,80 (q, 1H, 3, *J* = 7,03), 1,40 (d, 3H, 4, *J* = 7,03).

^{13}C NMR (DMSO-d_6, δ ppm): 196,22 (11), 173,37 (2), 143,15, 137,51, 137,38 (5, 9, 12), 139,86 (2'), 133,17, 132,08, 130,07, 129,04, 129,02, 128,58 (6–8, 10, 13–17), 128,70, 127,50, 127,21 (3'–7'), 45,36 (3), 42,54 (1'), 18,91 (4).

4.1.11.14. 2-(3-Benzoilfenil)-*N*-fenetilpropanamid (10e)

Količina reaktanata: 0,355 g spoja **7e**, 0,139 mL feniletilamina; trajanje reakcije 1 h; sobna temperatura. Sirovi produkt **10e** kristalizira iz smjese etera i petroletera.

Iskorištenje: 0,329 g (92%).

t_t 66–69 °C.

IR (KBr): ν_{max} 3255, 3062, 2936, 2876, 1661, 1634, 1599, 1559, 1543, 1448, 1288, 748, 702 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 8,11 (t, 1H, 1, J = 5,45), 7,75–7,48 (m, 9H, arom.), 7,24–7,07 (m, 5H, arom.), 3,69 (q, 1H, 3, J = 6,94), 3,32–3,18 (m, 2H, 1'), 2,66 (t, 2H, 2', J = 7,13), 1,34 (d, 3H, 4, J = 7,02).

^{13}C NMR (DMSO-d_6, δ ppm): 196,22 (11), 173,22 (2), 143,19, 137,54, 137,32 (5, 9, 12), 139,85 (2'), 133,16, 132,09, 130,08, 129,04, 128,99, 128,97, 128,52 (6–8, 10, 13–17), 129,10, 128,64, 126,44 (4'–8'), 45,33 (3), 40,71 (1'), 35,40 (2'), 18,91 (4).

4.1.11.15. *N*-benzhidril-2-(3-benzoilfenil)propanamid (10f)

Količina reaktanata: 0,355 g spoja **7e**, 0,190 mL benzhidrilamina; trajanje reakcije 11 h; temperatura 78 °C. Sirovi produkt **10f** rastrljan je eterom.

Iskorištenje: 0,306 g (73%).

t_t 115–119 °C.

IR (KBr): ν_{max} 3316, 3251, 3057, 3030, 2932, 1656, 1649, 1598, 1528, 1494, 1320, 1285, 723, 704, 696 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 8,98 (d, 1H, 1, J = 8,46), 7,78–7,48 (m, 9H, arom.), 7,37–7,08 (m, 10H, arom.), 6,08 (d, 1H, 1', J = 8,40), 3,96 (q, 1H, 3, J = 6,99), 1,39 (d, 3H, 4, J = 7,01).

^{13}C NMR (DMSO-d_6, δ ppm): 196,23 (11), 172,64 (2), 143,01, 137,51, 137,35 (5, 9, 12), 142,80, 142,77 (2', 8'), 133,16, 132,07, 130,05, 129,03, 128,98, 128,53 (6–8, 10, 13–17), 128,88, 128,68, 127,86, 127,45, 127,33 (3'–7', 9'–13'), 56,36 (1'), 44,98 (3), 18,81 (4).

4.1.11.16. *N*-ciklopentil-2-(3-(hidroksi(fenil)metil)fenil)propanamid (11a)

Količina reaktanta: 0,161 g spoja **10a**; trajanje reakcije 5 h. Sirovi produkt **11a** rastrljan je eterom.

Iskorištenje: 0,126 (78%).

t_t 120–125 °C.

IR (KBr): ν_{max} 3274, 3062, 3031, 2964, 2933, 2866, 1645, 1603, 1549, 1453, 1231, 1195, 1021, 730, 701 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, *J*/Hz): 7,86 (d, 1H, 1, *J* = 6,92), 7,36–7,13 (m, 9H, arom.), 5,86 (d, 1H, 12, *J* = 3,83), 5,65 (d, 1H, 11, *J* = 3,04), 3,98–3,87 (m, 1H, 1'), 3,54 (q, 1H, 3, *J* = 6,94), 1,81–1,18 (m, 8H, 2'–5'), 1,27 (d, 3H, 4, *J* = 6,99).

^{13}C NMR (DMSO-d_6, δ ppm): 173,00 (2), 146,16, 145,99, 142,76 (5, 9, 13), 128,47, 128,29, 127,11, 126,69, 126,01, 125,56, 124,81 (6–8, 10, 14–18), 74,84 (11), 50,63 (1'), 45,29 (3), 32,69, 32,65 (2', 5'), 23,97, 23,93 (3', 4'), 19,10 (4).

4.1.11.17. *N*-cikloheksil-2-(3-(hidroksi(fenil)metil)fenil)propanamid (11b)

Količina reaktanta: 0,166 g spoja **10b**; trajanje reakcije 6 h. Sirovi produkt **11b** pročišćen je kromatografijom na koloni, uz pokretnu fazu diklormetan/metanol 93:7.

Iskorištenje: 0,127 g (75%).

t_t 94–98 °C.

IR (KBr): ν_{max} 3366, 3308, 3058, 2973, 2924, 2855, 1641, 1603, 1539, 1449, 1015, 712, 701 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, *J*/Hz): 7,74 (dd, 1H, 1, *J* = 5,24, *J* = 2,39), 7,35–7,11 (m, 9H, arom.), 5,84 (d, 1H, 12, *J* = 3,84), 5,64 (d, 1H, 11, *J* = 3,57), 3,53 (q, 1H, 3, *J* = 6,98), 3,48–3,38 (m, 1H, 1'), 1,72–1,46, 1,27–0,92 (2m, 10H, 2'–6'), 1,25 (d, 3H, 4, *J* = 6,56).

^{13}C NMR (DMSO-d_6, δ ppm): 172,59 (2), 146,16, 145,98, 142,82 (5, 9, 13), 128,45, 128,28, 127,11, 126,71, 126,02, 125,55, 124,81 (6–8, 10, 14–18), 74,84 (11), 47,83 (1'), 45,34 (3), 32,83, 32,80 (2', 6'), 25,70 (3'), 25,05 (5'), 24,98 (4'), 19,10 (4).

4.1.11.18. *N*-cikloheksanmetil-2-(3-(hidroksi(fenil)metil)fenil)propanamid (11c)

Količina reaktanta: 0,175 g spoja **10c**; trajanje reakcije 7 h. Sirovi produkt **11c** pročišćen je kromatografijom na koloni, uz pokretnu fazu kloroform/metanol 95:5.

Iskorištenje: 0,141 g (80%).

Ulje.

IR (KBr): ν_{max} 3312, 3063, 2925, 2852, 1649, 1604, 1550, 1493, 1450, 1025, 731, 701 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 7,87 (t, 1H, 1, J = 5,56), 7,37–7,14 (m, 9H, arom.), 5,86 (dd, 1H, 12, J = 2,84, J = 1,05), 5,65 (d, 1H, 11, J = 3,50), 3,58 (q, 1H, 3, J = 7,01), 2,85 (t, 2H, 1', J = 6,26), 1,68–1,51, 1,39–1,24, 1,19–1,05, 0,89–0,73 (4m, 4H, 3'–7'), 1,28 (d, 3H, 4, J = 6,85).

^{13}C NMR (DMSO-d_6, δ ppm): 173,58 (2), 146,15, 145,93, 142,71 (5, 9, 13), 128,47, 128,28, 127,13, 126,69, 126,03, 125,62, 124,90 (6–8, 10, 14–18), 74,82 (11), 45,53 (3), 45,28 (1'), 37,87 (2'), 30,83 (3'), 30,81 (7'), 26,49 (4'), 25,85 (5', 6'), 19,13 (4).

4.1.11.19. *N*-benzil-2-(3-(hidroksi(fenil)metil)fenil)propanamid (11d)

Količina reaktanta: 0,172 g spoja **10d**; trajanje reakcije 6 h. Sirovi produkt **11d** pročišćen je kromatografijom na koloni, uz pokretnu fazu kloroform/metanol 93:7.

Iskorištenje: 0,142 g (82%).

t_t 100–103 °C.

IR (KBr): ν_{max} 3331, 3285, 1648, 1603, 1539, 1494, 1454, 1238, 1017, 730, 701 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 8,46 (t, 1H, 1, J = 5,57), 7,41–7,15 (m, 15H, arom.), 5,88 (d, 1H, 12, J = 3,21), 5,66 (s, 1H, 11), 4,24 (d, 2H, 1', J = 5,74), 3,66 (q, 1H, 3, J = 6,97), 1,34 (d, 3H, 4, J = 6,97).

^{13}C NMR (DMSO-d_6, δ ppm): 173,71 (2), 146,11, 146,02, 142,50 (5, 9, 13), 139,96 (2'), 128,69, 127,53 (3'–7'), 128,50, 128,38, 127,14, 126,73, 126,09, 125,67, 124,04 (6–8, 10, 14–18), 74,83 (11), 45,59 (3), 42,51 (1'), 19,11 (4).

4.1.11.20. 2-(3-(Hidroksi(fenil)metil)fenil)-*N*-fenetilpropanamid (11e)

Količina reaktanta: 0,179 g spoja **10e**; trajanje reakcije 6 h. Sirovi produkt **11e** pročišćen je kromatografijom na koloni, uz pokretnu fazu kloroform/metanol 93:7.

Iskorištenje: 0,131 g (73%).

Ulje.

IR (KBr): ν_{max} 3412, 3312, 3062, 3028, 2973, 2932, 1652, 1603, 1558, 1540, 1522, 1454, 1368, 1239, 1025, 750, 700 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 8,04 (t, 1H, 1, J = 5,47), 7,42–7,14 (m, 14H, arom.), 5,91 (dd, 1H, 12, J = 3,25, J = 0,63), 5,70 (d, 1H, 11, J = 3,75), 3,58 (q, 1H, 3, J = 6,93), 3,26 (q, 2H, 1', J = 7,22), 2,68 (t, 2H, 2', J = 7,28), 1,32 (d, 3H, 4, J = 6,89).

^{13}C NMR (DMSO-d_6, δ ppm): 173,55 (2), 146,16, 146,02, 142,55 (5, 9, 13), 139,90 (3'), 129,10, 128,71, 126,45 (4'–8'), 128,49, 128,34, 127,14, 126,73, 126,17, 125,63, 124,95 (6–8, 10, 14–18), 74,83 (11), 45,58 (3), 40,74 (1'), 35,53 (2'), 19,12 (4).

4.1.11.21. *N*-benzhidril-2-(3-(hidroksi(fenil)metil)fenil)propanamid (11f)

Količina reaktanta: 0,210 g spoja **10f**; trajanje reakcije 9 h. Sirovi produkt **11f** prekristaliziran je iz smjese acetona i petroletera.

Iskorištenje: 0,190 g (90%).

t_t 51–55 °C.

IR (KBr): ν_{max} 3410, 3304, 3062, 3029, 2975, 2930, 1649, 1602, 1531, 1494, 1451, 1028, 743, 699 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 8,88 (d, 1H, 1, J = 8,39), 7,44–7,10 (m, 19H, arom.), 6,06 (d, 1H, 1', J = 8,38), 5,87 (d, 1H, 12, J = 3,86), 5,65 (d, 1H, 11, J = 3,19), 3,82 (q, 1H, 3, J = 6,78), 1,32 (d, 3H, 4, J = 6,20).

^{13}C NMR (DMSO-d_6, δ ppm): 173,00 (2), 146,13, 145,97, 142,82 (5, 9, 13), 143,01, 142,36 (2', 8'), 128,85, 128,70, 127,81, 127,58, 127,55, 127,41, 127,26 (3'–7', 9'–13'), 128,49, 128,33, 127,13, 126,73, 126,05, 125,76, 125,04 (6–8, 10, 14–18), 74,83 (11), 56,30 (1'), 45,14 (3), 18,95 (4).

4.1.12. SINTEZA 3-HIDROKSIPROPILAMIDA NSAID **12a-e**

Opća metoda. Otopina odgovarajućeg benzotriazolida **7a-e** (2 mmol), 3-hidroksipropilamina (0,165 g, 2,2 mmol) i TEA (0,279 mL, 2 mmol) u smjesi toluen/etil-acetat (2:1, 30 mL) miješana je 1 h na sobnoj temperaturi. Spojevi **12a**,**b**,**e**: otapalo je upareno, a ostatak nakon uparavanja kromatografiran je na koloni, uz pokretnu fazu etil-acetat. Spojevi **12c**,**d**: Otapalo je uklonjeno pod sniženim tlakom. Ostatak je otopljen u smjesi acetona i vode, a dobivena otopina zakiseljena kloridnom kiselinom (w = 5%) do pH 1. Aceton je uparen pod sniženim tlakom bez zagrijavanja. Istaloženi produkt je odsisan i ispran vodom do neutralnog pH.

4.1.12.1. *N*-(3-hidroksipropil)-2-(4-izobutilfenil)propanamid (12a)

Količina reaktanta: 0,615 g spoja **7a**.
Iskorištenje: 0,450 g (85%).
Ulje.
IR (film): ν_{max} 3299, 3088, 2954, 2932, 2869, 1652, 1548, 1512, 1065, 849 cm^{-1}.
^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 7,88 (t, 1H, 1', J = 5,40), 7,21 (d, 2H, arom., J = 8,03), 7,07 (d, 2H, arom., J = 8,10), 4,39 (t, 1H, 5', J = 5,25), 3,54 (q, 1H, 2, J = 7,04), 3,35 (q, 2H, 4', J = 6,27), 3,13–3,01 (m, 2H, 2'), 2,40 (d, 2H, 10, J = 7,12), 1,87–1,74 (m, 1H, 11), 1,56–1,47 (m, 2H, 3'), 1,30 (d, 3H, 3, J = 7,05), 0,86 (d, 6H, 12, 13, J = 6,61).
^{13}C NMR (DMSO-d_6, δ ppm): 173,52 (1), 139,79, 139,25 (4, 7), 128,85, 127,03 (5, 6, 8, 9), 58,46 (4'), 44,86 (2), 44,37 (10), 35,87 (2'), 32,50 (3'), 29,75 (11), 22,32 (12, 13), 18,74 (3).

4.1.12.2. 2-(3-Fenoksifenil)-*N*-(3-hidroksipropil)propanamid (12b)

Količina reaktanta: 0,687 g spoja **7b**.
Iskorištenje: 0,491 g (82%).
Ulje; lit. ulje.[59]

IR (film): ν_{max} 3400, 3298, 2937, 1648, 1582, 1486, 1443, 1246, 1209, 1071, 926, 757, 693 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 8,01 (bs, 1H, 1'), 7,38 (t, 2H, arom., J = 7,62), 7,29 (t, 1H, arom., J = 7,83), 7,13 (t, 1H, arom., J = 7,11), 7,07 (d, 1H, arom., J = 7,80), 6,98 (s, 3H, arom.), 6,83 (d, 1H, arom. J = 7,80), 4,48 (bs, 1H, 5'), 3,57 (q, 1H, 2, J = 7,14), 3,34 (t, 2H, 4', J = 6,00), 3,06 (t, 2H, 2', J = 5,83), 1,49 (t, 2H, 3', J = 6,49), 1,28 (d, 3H, 3, J = 6,81).

^{13}C NMR (DMSO-d_6, δ ppm): 173,06 (1), 156,72, 156,56, 144,77 (4, 8, 10), 130,16, 129,87, 123,51, 122,48, 118,67, 117,63, 116,70 (5–7, 9, 11–15), 58,41 (4'), 45,02 (2), 35,86 (2'), 32,46 (3'), 18,57 (3).

4.1.12.3. *N*-(3-hidroksipropil)-2-(2-(2,6-diklorfenilamino)fenil)acetamid (12c)

Količina reaktanta: 0,795 g spoja **7c**. Sirovi produkt **12c** pročišćen je rastrljavanjem petroleterom.

Iskorištenje: 0,676 g (96%).

t_t 130–133 °C.

IR (KBr): ν_{max} 3495, 34323, 3297, 3253, 3082, 2947, 2882, 1618, 1565, 1508, 1453,1286, 1070, 775, 747 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 8,45 (s, 1H, 9), 8,35 (t, 1H, 1', J = 5,27), 7,51 (d, 2H, arom., J = 8,06), 7,20–7,12 (m, 2H, arom.), 7,06–7,01 (m, 1H, arom.), 6,87–6,82 (m, 1H, arom.), 6,29 (d, 1H, arom., J = 7,81), 4,45 (t, 1H, 5', J = 5,16), 3,57 (s, 1H, 2), 3,42 (q, 2H, 4', J = 5,90), 3,13 (q, 2H, 2', J = 6,49), 1,62–1,53 (m, 2H, 3').

^{13}C NMR (DMSO-d_6, δ ppm): 172,03 (1), 143,43, 137,65, 129,83, 126,03 (3, 8, 10, 11, 15), 130,82, 129,65, 127,63, 125,44, 121,11, 116,40 (4–7, 12–14), 58,77 (4'), 40,04 (2'), 36,45 (3'), 32,67 (2).

4.1.12.4. *N*-(3-hidroksipropil)-1-(4-klorbenzoil)-2-metil-5-metoksi-1*H*-indol-3--acetamid (12d)

Količina reaktanta: 0,918 g spoja **7d**. Sirovi produkt **12d** prekristaliziran je iz smjese cikloheksana i toluena.

Iskorištenje: 0,498 g (60%).

t_t 130–131 °C; lit. t_t 106–107 °C.[96]

IR (KBr): ν_{max} 3428, 3314, 3093, 2928, 2884, 1673, 1620, 1561, 1478, 1317, 1071, 835, 755 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 8,02 (t, 1H, 1', J = 5,24), 7,69 (d, 3H, arom., J = 8,63), 7,64 (d, 2H, J = 8,38), 7,12 (d, 1H, arom., J = 1,96), 6,95 (d, 1H, arom., J = 9,03), 6,71 (dd, 1H, arom., J = 2,09, J = 6,80), 4,43 (t, 1H, 5', J = 5,10), 3,77 (s, 3H, 2''), 3,49 (s, 2H, 2), 3,40 (q, 2H, 4', J = 5,79), 3,12 (q, 2H, 2', J = 6,39), 2,23 (s, 3H, 1''), 1,60–1,51 (m, 2H, 3').

^{13}C NMR (DMSO-d_6, δ ppm): 169,82 (1), 168,31 (11), 156,01, 138,01, 135,56, 134,74, 131,35, 130,74, 114,90 (3–5, 8, 10, 12, 15), 131,61, 129,49, 115,02, 111,75, 102,25 (6, 7, 9, 13, 14, 16, 17), 58,85 (4'), 55,87 (2''), 36,41 (2'), 32,89 (3'), 31,66 (2), 13,84 (1'').

4.1.12.5. 2-(3-Benzoilfenil)-*N*-(3-hidroksipropil)propanamid (12e)

Količina reaktanta: 0,683 g spoja **7e**.

Iskorištenje: 0,498 g (80%).

Ulje; lit. ulje.[58]

IR (film): ν_{max} 3301, 3064, 2936, 2876, 1651, 1597, 1546, 1448, 1319, 1285, 1237, 722, 705 cm^{-1}.

^{1}H NMR (DMSO-d_6, δ ppm, J/Hz): 8,06 (t, 1H, 1', J = 5,30), 7,47–7,40 (m, 9H, arom.), 4,43 (t, 1H, 5', J = 4,90), 3,70 (q, 1H, 2, J = 6,70), 3,36 (q, 2H, 4', J = 4,90), 3,08 (q, 2H, 2', J = 6,60), 1,56–1,47 (m, 2H, 3'), 1,35 (d, 3H, 3, J = 6,89).

^{13}C NMR (DMSO-d_6, δ ppm): 195,66 (10), 172,76 (1), 142,75 136,97, 136,77 (4, 8, 11), 132,59, 131,48, 129,51, 128,48, 128,43, 128,35, 127,94 (5–7, 9, 12–16), 58,22 (4'), 44,78 (2), 35,71 (2'), 32,25 (3'), 18,44 (3).

4.2. BIOLOŠKA ISPITIVANJA

4.2.1. INHIBICIJA UREAZE

4.2.1.1. Materijali

Fenolno crvenilo i DMSO nabavljeni su od Kemike (Hrvatska), a ureaza iz soje (5 U mg^{-1}) od Mercka (Njemačka). Fenolno crvenilo korišteno je kao otopina (c = 1 g L^{-1}, otapalo 96% (V/V) etanol). Kontrolni pufer pH 7,70 pripravljen je razrjeđivanjem otopine pripravljene iz otopine kalijevog dihidrogenfosfata (c = 1 mol L^{-1}, 0,1 mL) i otopine natrijevog hidrogenfosfata (c = 1 mol L^{-1}, 0,7 mL) vodom do 80 mL. Pufer pH 6,70 pripravljen je razrjeđivanjem otopine pripravljene iz otopine kalijevog dihidrogenfosfata (c = 1 mol L^{-1}, 1 mL) i otopine natrijevog hidrogenfosfata (c = 1 mol L^{-1}, 1 mL) vodom do 100 mL. Puferirana otopina uree (pH 6,70) pripravljena je otapanjem uree (3 g) u puferu pH 6,70 (100 mL).

4.2.1.2. Metoda

U određivanju postotka inhibicije ureaze korištena je kolorimetrijska metoda.[104,105] Otopina fenolnog crvenila (25 μL) dodana je u otopinu C (DMSO (50 μL) razrijeđen kontrolnim puferom pH 7,70 (175 μL)) i u otopinu S (otopina uzorka u DMSO (50 μL) razrijeđena puferom pH 6,70 (175 μL)). Nakon dodatka otopine ureaze (460 jedinica u 25 mL vode, 250 μL) obje otopine su preinkubirane na 30 ºC tijekom 30 min. Nakon razrjeđivanja otopine C kontrolnim puferom pH 7,70 (2,5 mL) mjerena je apsorbancija na 560 nm. Otopina S razrijeđena je puferiranom otopinom uree pH 6,70 (2,5 mL) te je na 560 nm mjeren vremenski interval potreban da apsorbancija otopine S stigne do izmjerene apsorbancije otopine C. Postotak inhibicije (%) svakog uzorka izračunat je prema sljedećoj jednadžbi:

$$\text{postotak inhibicije (\%)} = [(t - t_0)/t] \times 100$$

t – vremenski interval (s) mjeren za svaki uzorak određene molarne koncentracije ispitivanog spoja; t_0 – vremenski interval (s) mjeren za uzorak bez ispitivanog spoja (slijepa proba). Svaki uzorak mjeren je u triplikatu.

4.2.2. ANTIMIKROBNO ISPITIVANJE

4.2.2.1. Mikrobiološke vrste i materijali

Mikrobiološke vrste (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Kocuria rhizophila* ATCC 9341, *Escherichia coli* ATCC 10535, *Salmonella enterica* subsp. *enterica* ATCC 13076, *Pseudomonas aeruginosa* ATCC 27853 i *Candida albicans* ATCC 10231) korištene u ispitivanju nabavljene su od American Type Culture Collection (ATCC, LGC Promochem, UK). Prije ispitivanja, mikrobiološke vrste presađene su iz hranjivog agara (zbirka Zavoda za mikrobiologiju Farmaceutsko-biokemijskog fakulteta), te su dva puta subkultivirane na triptikaza-sojin agar (Merck, Njemačka) za bakterijske vrste i na Sabouraud 2% (*m*/*V*)-glukoza agar (BBL, Njemačka) za gljivice tijekom 24 h na 37 °C. Svi inokulumi iz mikrobioloških kultura upotrebljeni za ispitivanje antimikrobnog djelovanja uzeti su iz svježe pripravljene kulture (24 h) u fiziološkoj otopini. Oksitetraciklin hidroklorid i nistatin nabavljeni su od Plive (Hrvatska), a norfloksacin od Krke (Slovenija), te su korišteni kao kontrola osjetljivosti mikrobiološke kulture u metodi difuzije. Radne otopine antibiotika pripravljene su u puferskim otopinama prema smjernicama Europske farmakopeje.[106]

4.2.2.2. Metode antimikrobnog učinka

U uvjetima *in vitro* antimikrobna aktivnost ispitana je korištenjem difuzijske metode prema smjernicama Europske farmakopeje[106] kako bi se dobile zone inhibicije. Müller-Hinton agar (Merck, Njemačka) korišten je kao hranjivi medij za utvrđivanje zona inhibicije rasta. 50 µL spoja otopljenog u DMSO-u unešeno je u rupice, te je inkubirano prvo na 4 ºC, a onda tijekom 24 h na 37 ºC. DMSO je korišten kao kontrola.

Za određivanje minimalne inhibitorne (*MIC*) i minimalne mikrobicidne koncentracije (*MMcC*) korištena je metoda serijalnog mikrorazrijeđenja u mikrotitar pločama s 96 jažica (TTG, Švicarska) u Müller-Hinton bujonu (Merck, Njemačka), prema smjernicama Clinical and Laboratory Standards Institute (CLSI, prije National Committee for Clinical Laboratory Standards, NCCLS) M7-A4.[107] Inokulum je pripravljen s fiziološkom otopinom kako bi dobili približnu gustoću mikrobnih stanica od

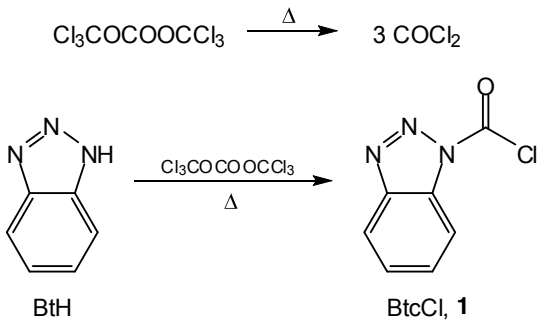
0,5 McFarlandovih jedinica. *MIC* i *MMcC* određene su nakon perioda inkubacije (24 h) i nakon subkultivacije svih razrjeđenja na površini Müller-Hinton agara. Nakon 24 h inkubacije, *MIC* je određena kao najniža koncentracija koja uzrokuje rast ≤ 20% od kontrolne razine, a *MMcC* kao najniža koncentracija kod koje nije došlo do vizualnog rasta.

Kod najviše koncentracije (min. 99%) DMSO nije pokazao antimikrobnu aktivnost.

5. REZULTATI I RASPRAVA

5.1. SINTEZE

Početni spoj za pripravu derivata aminokiselina: *N*-(1-benzotriazolkarbonil)-aminokiselina (*N*-Btc-aminokiselina) **2**, njihovih klorida **3**, amida **4**, 3,5-disupstituiranih hidantoina **5** i derivata NSAID: NSAID benzotriazolida **7**, NSAID hidroksamskih kiselina i njihovih derivata **8**, amida ketoprofena **9**, **10**, **11** i 3-hidroksipropilamida NSAID **12**, bio je klorid 1-benzotriazolkarboksilne kiseline (BtcCl, **1**). Taj spoj dobiven je refluksiranjem benzotriazola i trifozgena u toluenskoj otopini (Shema 36).[108] Klorid **1** može se prirediti i iz benzotriazola i 20%-tne otopine fozgena u toluenu ili uvođenjem plinovitog fozgena iz metalnog spremnika u toluensku otopinu benzotriazola.[109] Zbog otežanog rukovanja i sigurnosti na radu u laboratoriju, danas se sve više upotrebljava trimer fozgena, odnosno čvrsti trifozgen, koji zagrijavanjem prelazi u fozgen prema jednadžbi prikazanoj na Shemi 36.



Shema 36. Sinteza klorida 1-benzotriazolkarboksilne kiseline (BtcCl, **1**).

5.1.1. SINTEZA 3,5-DISUPSTITUIRANIH HIDANTOINA **5a-h**

Prvi korak u sintezi hidantoina je sinteza *N*-Btc-aminokiselina **2a-d**. Klorid 1-benzotriazolkarboksilne kiseline reagira s amino skupinom aminokiselina (L-valin, L-leucin, D-fenilglicin, L-fenilalanin) pri čemu nastaju *N*-Btc-aminokiseline.[101] Reakcija se provodi u bezvodnom dioksanu, na sobnoj temperaturi, uz molarni omjer reaktanata 1:2, pri čemu 1 mol aminokiseline služi kao akceptor u reakciji oslobođenog klorovodika. Nastali hidroklorid aminokiseline praktički je netopljiv u dioksanu i odsiše se, a uparavanjem matičnice dobije se sirovi produkt **2**.

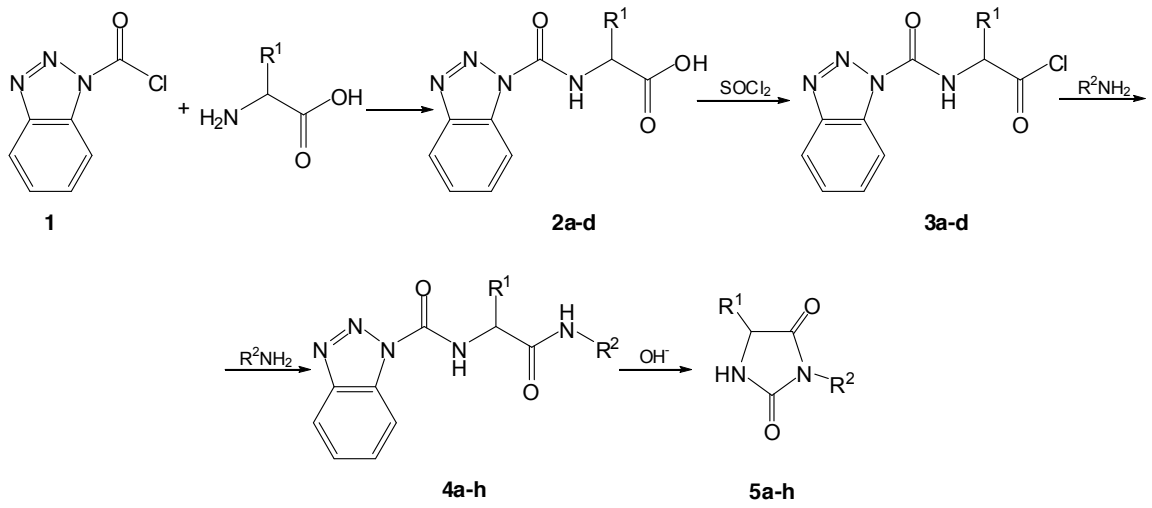
N-(1-benzotriazolkarbonil)aminokiseline **2a-d** prevedene su pomoću tionil-klorida u odgovarajuće kiselinske kloride **3a-d**, koji su upotrebljeni u daljnjim reakcijama bez čišćenja. Reakcijom s odgovarajućim aminima priređeni su amidi *N*-Btc-aminokiselina **4** (Shema 37).[3,5]

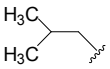
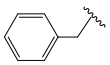
Pripravljeni su sljedeći amidi:
N-(1-benzotriazolkarbonil)-L-valin benzhidrilamid (**4a**),
N-(1-benzotriazolkarbonil)-L-leucin benzhidrilamid (**4b**),
N-(1-benzotriazolkarbonil)-D-fenilglicin cikloheksilamid (**4c**),
N-(1-benzotriazolkarbonil)-D-fenilglicin benzhidrilamid (**4d**),
N-(1-benzotriazolkarbonil)-L-fenilalanin ciklopentilamid (**4e**),
N-(1-benzotriazolkarbonil)-L-fenilalanin cikloheksilamid (**4f**),
N-(1-benzotriazolkarbonil)-L-fenilalanin cikloheksanmetilamid (**4g**),
N-(1-benzotriazolkarbonil)-L-fenilalanin benzhidrilamid (**4h**).

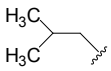
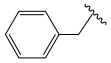
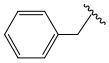
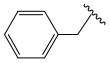
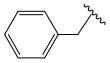
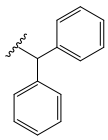
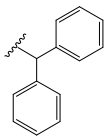
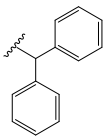
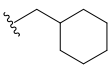
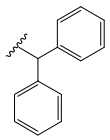
Svi amidi sintetizirani su prema ranije razvijenom postupku,[5] a sinteza i potpuna karakterizacija (tališta, elementarne analize, IR, ^{1}H i ^{13}C NMR) tih i srodnih spojeva također je opisana ranije.[3] Novi Btc-amid je **4a**, pa su u Tablicama 3–5 dani i njegovi podaci.

Amidi *N*-Btc-aminokiselina **4** u lužnatom mediju vrlo lako cikliziraju u 3,5-disupstituirane hidantoine **5** uz odvajanje benzotriazola (Shema 37).[3,5] Supstituent u položaju 5 ovisi o upotrebljenoj aminokiselini, a supstituent u položaju 3 o upotrebljenom aminu u sintezi amida **4**. Reakcija je vrlo brza i jednostavna. Otopini amida **4** u acetonu dodaje se vodena otopina natrijevog karbonata (w = 5%). Reakcija završava nakon kratkotrajnog miješanja na sobnoj temperaturi. Hidantoin **7** taloži se

nakon uparavanja acetona te se odsiše, a natrijeva sol benzotriazola zaostaje u vodenom sloju.



	2a, 3a	2b, 3b	2c, 3c	2d, 3d
R^1				

	4a, 5a	4b, 5b	4c, 5c	4d, 5d	4e, 5e	4f, 5f	4g, 5g	4h, 5h
R^1								
R^2								

Shema 37. Sinteza 3,5-disupstituiranih hidantoina **5a-h**.

Priređeni su sljedeći hidantoini:

3-benzhidril-5-izopropil hidantoin (**5a**),

3-benzhidril-5-izobutil hidantoin (**5b**),

3-cikloheksil-5-fenil hidantoin (**5c**),

3-benzhidril-5-fenil hidantoin (**5d**),

3-ciklopentil-5-benzil hidantoin (**5e**),

3-cikloheksil-5-benzil hidantoin (**5f**),

3-cikloheksanmetil-5-benzil hidantoin (**5g**),

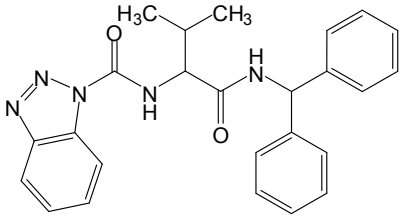
3-benzhidril-5-benzil hidantoin (**5h**).

Od sintetiziranih spojeva novi spojevi su **4a** i svi hidantoini, osim **5c** i **5f**.[5,65,66] Sinteza hidantoina i njihova čistoća praćeni su tankoslojnom kromatografijom, a strukture spojeva **4a** te **5a-h** potvrđene su IR, ^{1}H i ^{13}C NMR spektroskopijom i elementarnom analizom (sve elementarne analize bile su unutar 0,4%).

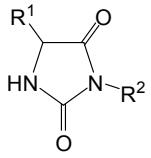
IR spektri hidantoina pokazuju karakteristične vrpce kod 3227–3314 (NH), 1760–1774 i 1698–1718 (C=O) cm^{-1}. ^{1}H NMR spektri ispitivanih spojeva otkrivaju da se NH nalazi u području 8,15 do 8,91 ppm. Analizom ^{13}C NMR spektara utvrđeno je da se karbonil u položaju 4 nalazi u području 172,35–175,20 ppm, a karbonil u položaju 2 u području 156,22–158,25 ppm. Kemijski pomaci drugih protona u ^{1}H, te atoma ugljika u ^{13}C NMR spektrima u skladu su s predloženim strukturama novih spojeva.

Analitički i spektroskopski podaci dani su u Tablicama 3–5.

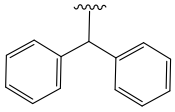
Tablica 3. Analitički i IR podaci za *N*-(1-benzotriazolkarbonil)-L-valin benzhidrilamid (**4a**) i 3,5-disupstituirane hidantoine **5a-h**.

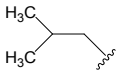
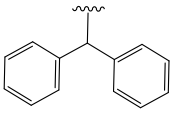
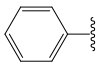
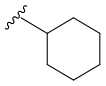
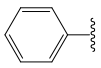
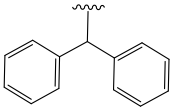
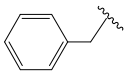
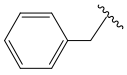
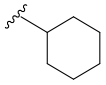
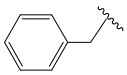
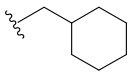
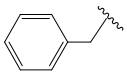
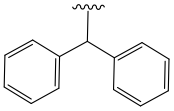


4a

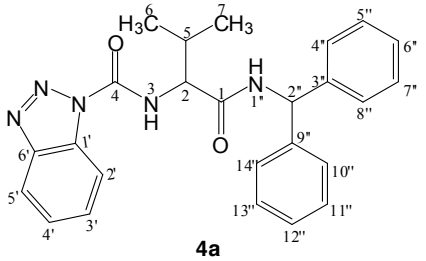


5a-h

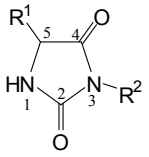
Spoj	R^1	R^2	Iskor. (%)	t_t (°C)	Molekulska formula (M_r)	IR (KBr/film) ν_{max} (cm^{-1})
4a	–	–	48	155–160	$C_{25}H_{25}N_5O_2$ (427,25)	3288, 3111, 3063, 2966, 1713, 1650, 1556, 1524, 1449, 1232, 1077, 760, 698
5a			86	ulje	$C_{19}H_{20}N_2O_2$ (308,37)	3249, 3063, 3030, 2963, 2930, 1772, 1711, 1496, 1450, 1417, 1347, 1176, 1116, 748, 697

5b			88	139–141	$C_{20}H_{22}N_2O_2$ (322,40)	3265, 3110, 3069, 3032, 2956, 2930, 1773, 1711, 1496, 1450, 1422, 1373, 1320, 1175, 1113, 714, 699, 668
5c			37	180–184	$C_{15}H_{18}N_2O_2$ (258,32)	3228, 3100, 3037, 2927, 2856, 1765, 1706, 1458, 1429, 1383, 1349, 1194, 1115, 763, 726, 706
5d			24	172–177	$C_{22}H_{18}N_2O_2$ (342,39)	3230, 3093, 3032, 2825, 1774, 1712, 1495, 1455, 1423, 1373, 1283, 1176, 826, 763, 722, 701
5e			73	121–124	$C_{15}H_{18}N_2O_2$ (258,32)	3258, 3091, 3034, 2959, 2869, 1760, 1718, 1431, 1387, 1213, 760, 700
5f			85	119–120	$C_{16}H_{20}N_2O_2$ (272,34)	3271, 3093, 3033, 2926, 2856, 1768, 1707, 1629, 1560, 1498, 1436, 1384, 1111, 725, 696
5g			80	150–153	$C_{17}H_{22}N_2O_2$ (286,37)	3314, 3085, 3030, 2925, 2852, 1768, 1698, 1496, 1454, 1428, 1360, 1124, 1099, 755, 726, 700
5h			29	76 (raspad)	$C_{23}H_{22}N_2O_2$ (356,42)	3269, 3030, 2925, 1774, 1711, 1602, 1496, 1452, 1419, 1374, 1178, 752, 699

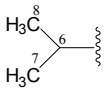
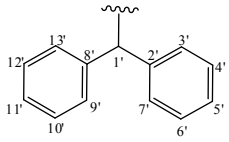
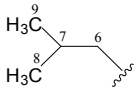
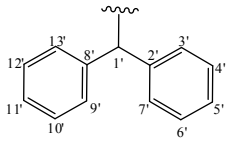
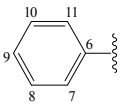
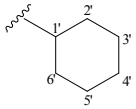
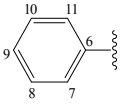
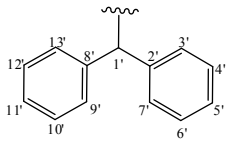
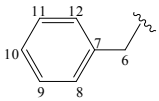
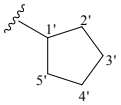
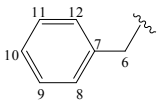
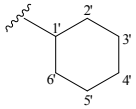
Tablica 4. ^{1}H NMR spektroskopski podaci za *N*-(1-benzotriazolkarbonil)-L-valin benzhidrilamid **4a** i 3,5-disupstituirane hidantoine **5a-h**.

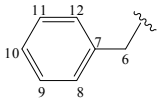
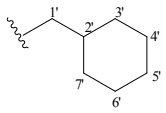
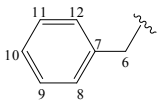
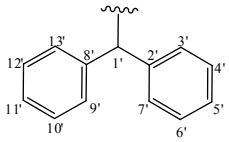


4a

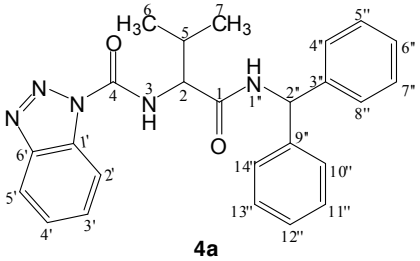


5a-h

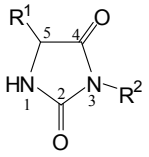
Spoj	R^1	R^2	^{1}H NMR (DMSO-d_6, δ ppm, J/Hz)
4a	–	–	9,19 (d, 1H, 3, J = 8,42), 8,68 (d, 1H, 1", J = 8,53), 8,25–7,14 (m, 14H, arom.), 6,20 (d, 1H, 2", J = 8,54), 4,49 (d, 1H, 2, J = 7,89), 2,34–2,23 (m, 1H, 5), 0,96–0,70 (m, 6H, 6, 7)
5a			8,40 (s, 1H, 1), 7,38–7,23 (m, 10H, arom.), 6,35 (s, 1H, 1'), 4,07 (d, 1H, 5, J = 3,33), 2,08–1,97 (m, 1H, 6), 0,92 (d, 3H, 7, J = 6,92), 0,71 (d, 3H, 8, J = 6,78)
5b			8,51 (s, 1H, 1), 7,38–7,22 (m, 10H, arom.), 6,34 (s, 1H, 1'), 4,18 (q, 1H, 5, J = 4,11), 1,79–1,74 (m, 1H, 7), 1,56–1,36 (m, 2H, 6), 0,87 (2d, 6H, 8, 9, J = 4,09)
5c			8,64 (s, 1H, 1), 7,44–7,29 (m, 5H, arom.), 5,13 (s, 1H, 5), 3,81–3,70 (m, 1H, 1'), 2,12–1,02 (m, 10H, 2'–6')
5d			8,91 (s, 1H, 1), 7,43–7,20 (m, 15H, arom.), 6,39 (s, 1H, 1'), 5,34 (s, 1H, 5)
5e			8,18 (s, 1H, 1), 7,28–7,13 (m, 5H, arom.), 4,29 (t, 1H, 5, J = 4,38), 4,09–4,02 (m, 1H, 1'), 2,94 (d, 2H, 6, J = 4,22), 1,67–1,42 (m, 8H, 2'–5')
5f			8,15 (s, 1H, 1), 7,28–7,13 (m, 5H, arom.), 4,29 (t, 1H, 5, J = 4,39), 3,52–3,44 (m, 1H, 1'), 2,93 (t, 2H, 6, J = 3,53), 1,85–0,94 (m, 10H, 2'–6')

5g			8,21 (s, 1H, 1), 7,27–7,14 (m, 5H, arom.), 4,38 (t, 1H, 5, *J* = 4,22), 3,05–2,87 (m, 4H, 6, 1'), 1,61–0,50 (m, 11H, 2'–7')
5h			8,44 (s, 1H, 1), 7,30–6,73 (m, 15H, arom.), 6,13 (s, 1H, 1'), 4,52 (t, 1H, 5, *J* = 4,28), 3,07–2,94 (m, 2H, 6)

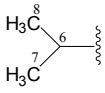
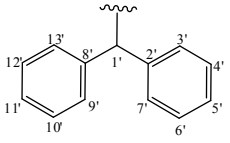
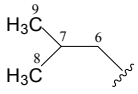
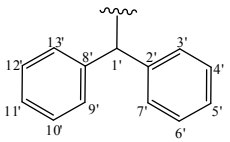
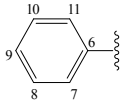
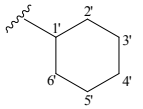
Tablica 5. ^{13}C NMR spektroskopski podaci za *N*-(1-benzotriazolkarbonil)-L-valin benzhidrilamid (**4**) i 3,5-disupstituirane hidantoine **5a-h**.

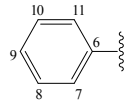
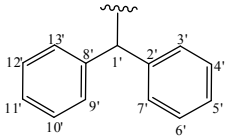
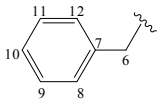
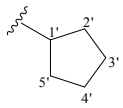
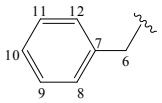
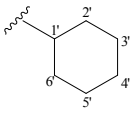
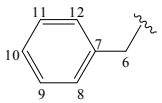
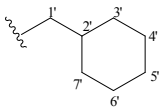
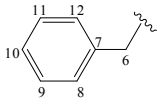
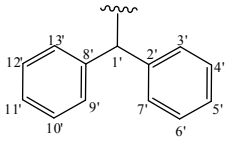


4a



5a-h

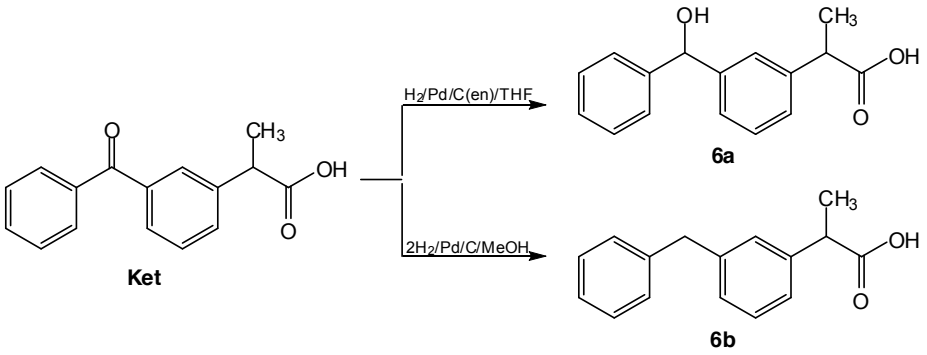
Spoj	R^1	R^2	^{13}C NMR (DMSO-d_6, δ ppm)
4a	–	–	169,29 (1), 148,59 (4), 145,45, 142,00, 131,17 (1', 6', 3", 9"), 130,08, 128,30, 128,15, 127,24, 127,15, 126,99, 125,61, 119,82, 113,37 (2'–5', 4"–8", 10"–14"), 63,90 (2), 56,04 (2") 30,77 (5), 19,17 (6), 18,20 (7)
5a			173,63 (4), 156,87 (2), 138,63, 138,37 (2', 8'), 128,56, 128,37, 128,32, 128,22, 127,64, 127,53 (3'–7', 9'–13'), 61,06 (1'), 57,14 (5), 29,92 (6), 18,64 (7), 15,84 (8)
5b			174,34 (4), 156,22 (2), 138,30, 138,21 (2', 8'), 128,15, 128,12, 127,33 (3'–7', 9'–13'), 56,85 (1'), 54,44 (5), 40,75 (6), 23,96 (7), 22,98 (8), 21,27 (9)
5c			172,36 (4), 156,61 (2), 135,91 (6), 128,62, 128,23, 126,68 (7–11), 59,08 (5), 50,22 (1'), 28,92 (2'), 28,83 (6'), 25,27 (3'), 25,24 (5'), 24,72 (4')

5d			172,35 (4), 156,54 (2), 138,96, 138,30, 135,85 (6, 2', 8'), 128,94–126,87 (7–11, 3'–7', 9'–13'), 59,66 (1'), 57,35 (5)
5e			175,20 (4), 158,25 (2), 136,80 (7), 131,64, 129,81, 128,55 (8–12), 58,11 (5), 51,90 (1'), 38,30 (6), 30,30 (5'), 30,02 (2'), 26,39 (3', 4')
5f			173,20 (4), 156,22 (2), 134,78 (7), 129,76, 127,82, 126,60 (8–12), 56,04 (5), 49,64 (1'), 36,28 (6), 28,58 (2'), 28,39 (6'), 25,18 (3', 5'), 24,68 (4')
5g			173,27 (4), 156,60 (2), 134,78 (7), 129,80, 127,82, 126,58 (8–12), 56,71 (5), 43,37 (1'), 35,95 (6), 35,66 (2'), 29,79 (3'), 29,52 (7'), 25,65 (4'), 25,17 (6'), 25,12 (5')
5h			172,87 (4), 155,72 (2), 138,01, 137,95, 134,65 (7, 2', 8'), 126,78–130,01 (8–12, 3'–7', 9'–13'), 56,62 (5, 1'), 35,78 (6)

5.1.2. SINTEZA DERIVATA NSAID **6–12**

5.1.2.1. Sinteza reduciranih derivata ketoprofena 6a,b

Dva derivata ketoprofena, **6a** i **b**, pripravljena su katalitičkim hidrogeniranjem ketoprofena. Variranjem otapala i upotrebljenog katalizatora optimirane su metode za potpunu, odnosno djelomičnu redukciju keto skupine (Shema 38).



Shema 38. Redukcija ketoprofena pri različitim reakcijskim uvjetima.

Katalitičkim hidrogeniranjem ketoprofena pri atmosferskom tlaku vodika uz 10%-tni Pd/C i metanol kao otapalo, karbonilna skupina ketoprofena reducira se u metilensku skupinu. Pod tim uvjetima nastali međuprodukt **6a** odmah se dalje reducira u **6b**, pa je krajnji produkt hidrogeniranja difenilmetanski derivat **6b**.

Hattori i suradnici razvili su blagu i kemoselektivnu metodu hidrogeniranja α-aromatskih ketona i aldehida u odgovarajuće benzilne alkohole uz heterogeni paladijev katalizator, Pd-etilendiamin kompleks na ugljenu (Pd/C(en)).[110] On posjeduje slabiju katalitičku aktivnost od komercijalnog Pd/C jer koordinirani etilendiamin djeluje kao slabi otrov za katalizator. Osim selektivnog hidrogeniranja aromatskih aldehida i ketona u benzilne alkohole, on također selektivno katalizira hidrogeniranje različitih spojeva, kao što su olefini, benzilni esteri, azidi i nitro spojevi, pri čemu ne dolazi do hidrogenolize *O*-benzilnih, *N*-benziloksikarbonilnih i *O*-*t*-butildimetilsililnih zaštitnih skupina i epoksida. Isto tako, uz njega se acetati benzilnih alkohola lako deacetoksiliraju te nastaju odgovarajući metilenski derivati, iako on ne katalizira izravnu hidrogenolizu benzilnih alkohola u metilenske derivate.

Pomoću 10%-tnog Pd/C(en) karbonilna skupina ketoprofena selektivno je reducirana u hidroksilnu skupinu. Kao otapalo korišten je tetrahidrofuran, u kojem reakcija napreduje sporije nego u metanolu, ali je selektivnija te ju je moguće zaustaviti na hidroksilnom derivatu **6a**. Nakon završetka reakcije katalizator je odfiltriran, a matičnica uparena, te su dobiveni čisti produkti **6a** i **b** u kvantitativnom iskorištenju.

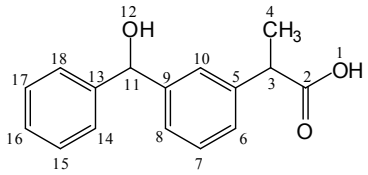
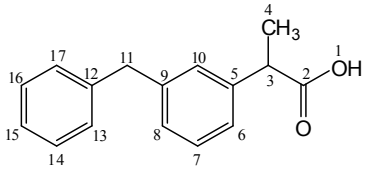
U literaturi je opisana analogna sinteza **6a** uz Pd/C kao katalizator te dodatak TEA.[85,86] Međutim, katalitičkim hidrogeniranjem ketoprofena uz 10%-tni Pd/C(en), **6a** je dobiven u značajno višem iskorištenju.

Sinteza spojeva **6a** i **b** i njihova čistoća praćeni su tankoslojnom kromatografijom, a njihove strukture potvrđene su IR, ^{1}H i ^{13}C NMR spektroskopijom i elementarnom analizom (sve elementarne analize bile su unutar 0,4%).

IR spektri oba spoja pokazuju dvije karakteristične vrpce: široku vrpcu u području 3400–2500 cm^{-1} (OH skupina karboksilne kiseline povezana vodikovom vezom), te usku vrpcu kod 1700, odnosno 1709 cm^{-1} (C=O). U ^{1}H NMR spektrima OH skupina karboksilne kiseline nalazi se na 12,23 ppm, dok se karbonilna skupina u ^{13}C NMR spektrima nalazi se na 175,76, odnosno 175,78 ppm.

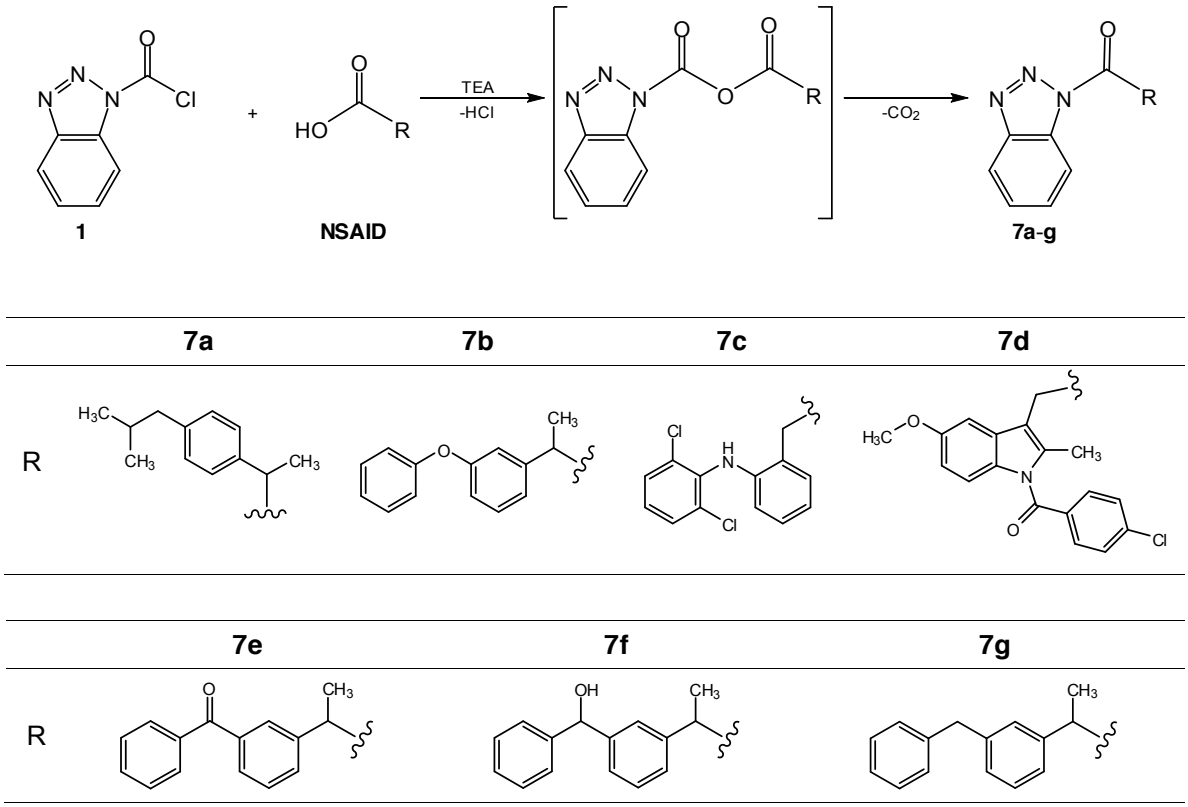
Tališta spojeva **6a** i **b** nisu identična s literaturnim podacima, ali njihovi spektroskopski podaci u skladu su s literaturnim podacima i u skladu s predloženim strukturama,[81,84,85] te su prikazani u Tablici 6.

Tablica 6. Analitički i spektroskopski podaci za derivate ketoprofena **6a** i **b**.

Spoj	 **6a**	 **6b**
Iskor. (%)	100	100
t_t (°C)	116–118	53–54
Molekulska formula	$C_{16}H_{16}O_3$	$C_{16}H_{16}O_2$
M_r	256,30	240,30
IR (KBr) ν_{max} (cm^{-1})	3417, 3028, 2988, 2918, 2713, 2610, 1709, 1601, 1490, 1454, 1327, 1237, 1020, 914, 839, 799, 708, 698	3084, 3060, 3027, 2987, 2931, 2733, 2642, 1700, 1598, 1494, 1451, 1417, 1238, 1213, 1085, 939, 756, 717, 699
^{1}H NMR (DMSO-d_6, δ ppm, J/Hz)	12,23 (s, 1H, 1), 7,38–7,11 (m, 9H, arom.), 5,89 (s, H, 12), 5,68 (s, 1H, 11), 3,64 (q, 1H, 3, J = 7,05), 1,33 (d, 3H, 4, J = 7,08)	12,23 (s, 1H, 1), 7,29–7,06 (m, 9H, arom.), 3,90 (s, 3H, 11), 3,60 (q, 1H, 3, J = 7,06), 1,31 (d, 3H, 4, J = 7,12)
^{13}C NMR (DMSO-d_6, δ ppm)	175,78 (2), 146,35, 146,07, 141,54 (5, 9, 13), 128,61, 128,53, 127,17, 126,68, 126,19, 125,69, 125,26 (6–8, 10, 14–18), 74,69 (11), 45,20 (3), 19,08 (4)	175,76 (2), 141,86, 141,84, 141,61 (5, 9, 12), 129,13, 128,98, 128,88, 128,26, 127,63, 126,43, 125,45 (6–8, 10, 13–17), 45,09 (3), 41,53 (11), 19,01 (4)

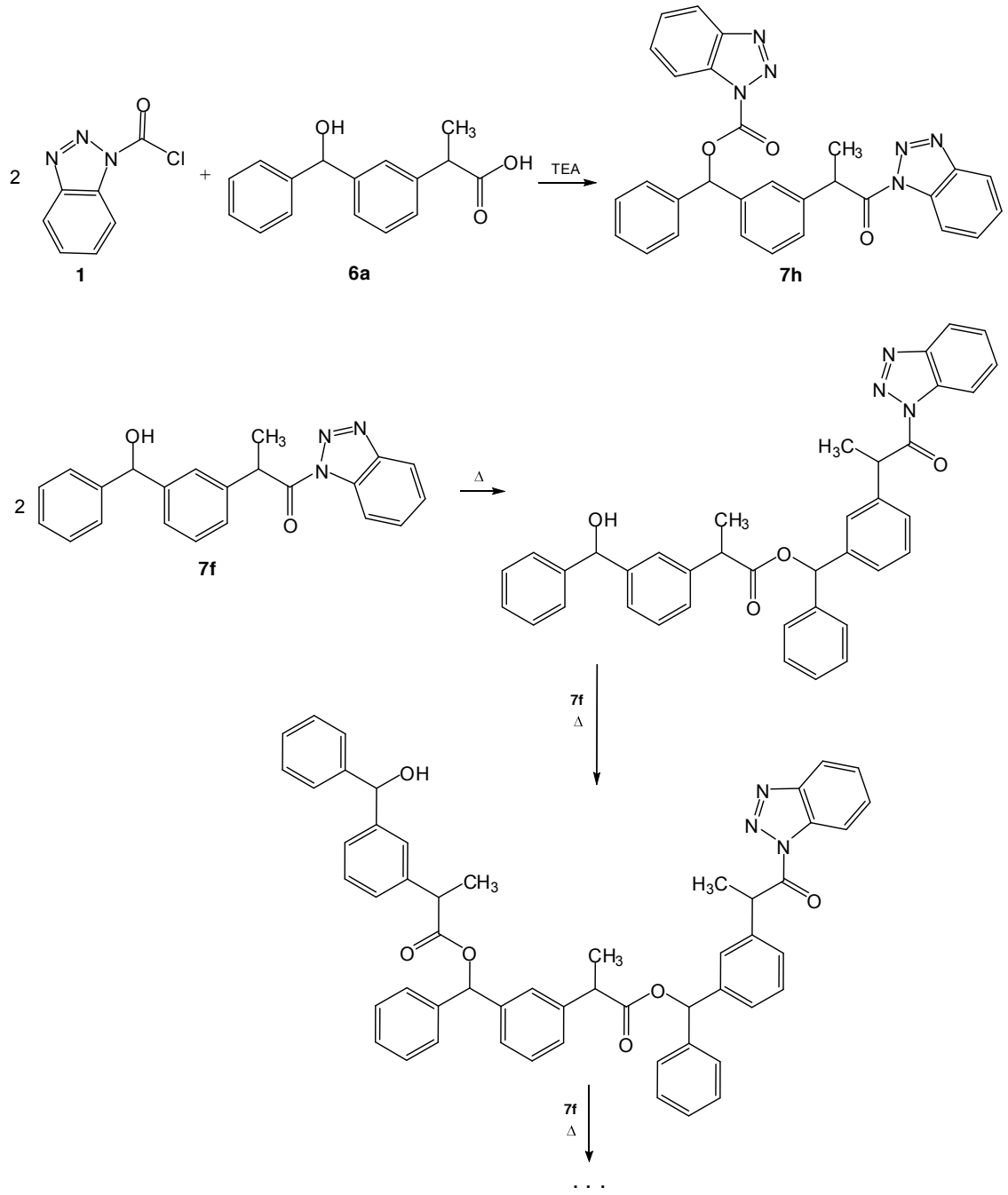
5.1.2.2. Sinteza NSAID benzotriazolida 7a-h

NSAID benzotriazolidi **7a-g** pripravljeni su reakcijom u kojoj klorid **1** reagira s odgovarajućim NSAID (ibuprofen, fenoprofen, diklofenak, indometacin, ketoprofen i njegovi derivati **6a**,**b**) u omjeru 1:1, u prisutnosti TEA.[102,103] Reakcijom prvo nastaje mješoviti anhidrid koji dekarboksilacijom daje benzotriazolid **7** (Shema 39).



Shema 39. Sinteza NSAID benzotriazolida **7a-g**.

Ako se reakcija spoja **6a** provodi s dva ekvivalenta klorida **1** nastaje benzotriazolid **7h,** u kojem je acilirana i hidroksilna skupina, u 70%-tnom iskorištenju (Shema 40). Reakcija kod sobne temperature niti uz suveliki višak klorida **1** ne ide do kraja, pa je spoj **7h** popraćen s više ili manje međuprodukta **7g**. Međutim, na povišenoj temperaturi postoji mogućnost sporedne reakcije u kojoj benzotriazolid **7f** (koji nastaje prvi) reagira s hidroksilnom skupinom iz druge molekule **7f**, te može doći do polikondenzacije (Shema 40).



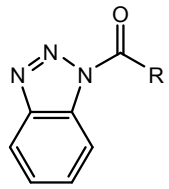
Shema 40. Sinteza benzotriazolida **7h** i moguća sporedna reakcija polikondenzacije.

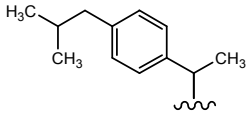
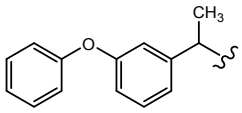
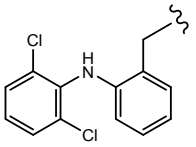
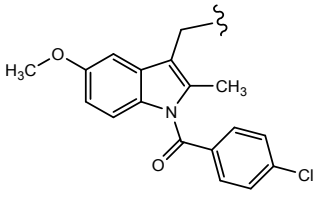
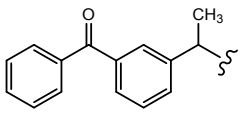
Sinteza i čistoća spojeva **7a-h** i praćeni su tankoslojnom kromatografijom, a njihove strukture potvrđene su IR, ^{1}H i ^{13}C NMR spektroskopijom i elementarnom analizom (elementarne analize bile su unutar 0,4%). Tališta ranije pripravljenih spojeva **7a-e** i njihovi spektroskopski podaci u skladu su s literaturnim podacima.[102,103]

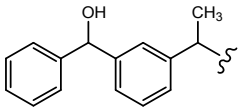
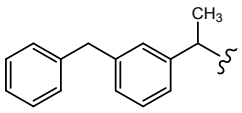
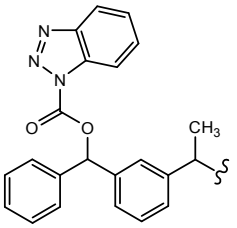
IR spektri NSAID benzotriazolida **7a-h** pokazuju za acilazole karakteristične ν(CO) apsorpcije kod 1724–1739 cm^{-1}, pomaknute prema višim valnim brojevima u odnosu na apsorpcije karbonilne skupine u običnim amidima. Karbonilna skupina u položaju 1 u ^{13}C NMR spektrima nalazi se u području 169,43–173,50 ppm, te je pomaknuta u više polje (manji broj kemijskog pomaka) u odnosu na ishodnu karboksilnu kiselinu zbog njenog zasjenjenja benzotriazolskom skupinom. Kemijski pomaci u ^{1}H i ^{13}C NMR spektrima u skladu su s predloženim strukturama novih spojeva **7f-g**.

Analitički i spektroskopski podaci spojeva **7a-h** prikazani su u Tablicama 7–9.

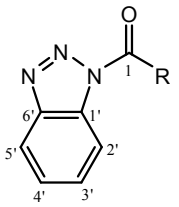
Tablica 7. Analitički i IR podaci za NSAID benzotriazolide **7a-h**.

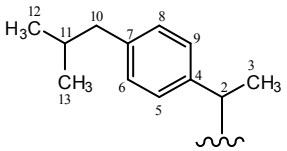
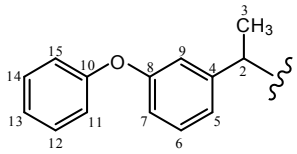


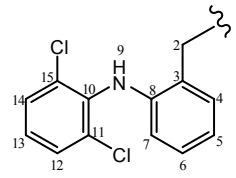
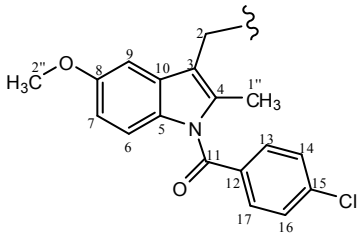
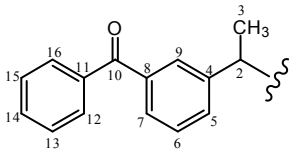
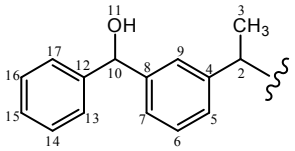
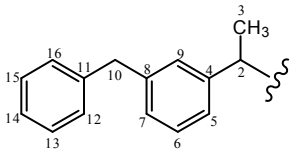
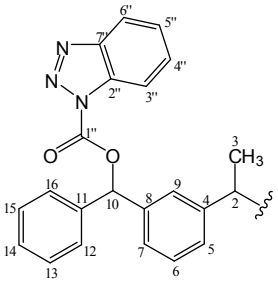
Spoj	R	Iskor. (%)	t_t (°C)	Molekulska formula (M_r)	IR (KBr) ν_{max} (cm^{-1})
7a		82	57–59	$C_{19}H_{21}N_3O$ (307,39)	3027, 2954, 1739, 1379, 949, 752
7b		75	63–67	$C_{21}H_{17}N_3O_2$ (343,38)	3064, 2985, 1729, 1580, 1487, 1450, 1374, 1243, 954, 770, 692
7c		77	163–165	$C_{20}H_{14}Cl_2N_4O$ (397,26)	3321, 3076, 2925, 1728, 1710, 1590, 1450, 1382, 1006, 750
7d		86	105–107	$C_{25}H_{19}ClN_4O_3$ (458,90)	3097, 2926, 1739, 1679, 1609, 1598, 1483, 1368, 1315, 1227, 1066, 965, 745
7e		78	59–60	$C_{22}H_{19}N_3O$ (341,41)	3027, 2937. 2918, 2852, 1730, 1597, 1485, 1452, 1390, 1372, 1168, 955, 780, 756, 738, 708, 692

7f		75	96–99	$C_{22}H_{19}N_3O_2$ (357,41)	3342, 3072, 3027, 3003, 2942, 2874, 1738, 1060, 1597, 1486, 1452, 1376, 959, 771, 751, 746, 710
7g		87	96–98	$C_{22}H_{17}N_3O_2$ (355,39)	3027, 2917, 1724, 1652, 1450, 1382, 1321, 1282, 962, 942, 750, 713, 609
7h		70	124–127	$C_{29}H_{22}N_6O_3$ (502,52)	3091, 3032, 2980, 2937, 1764, 1732, 1597, 1486, 1451, 1398, 1250, 1036, 951, 781, 760, 748, 708, 583

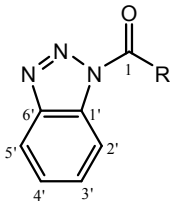
Tablica 8. ^{1}H NMR podaci za NSAID benzotriazolide **7a-h**.

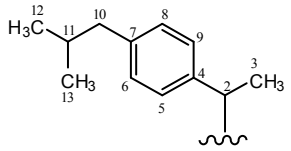
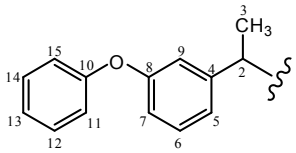
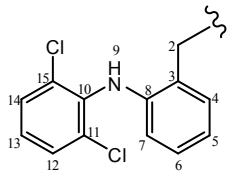
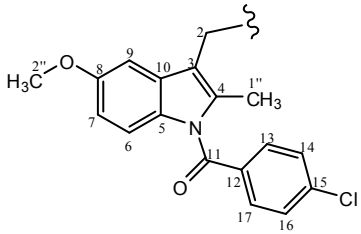
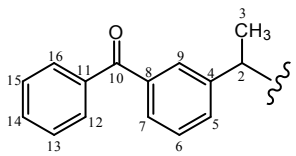
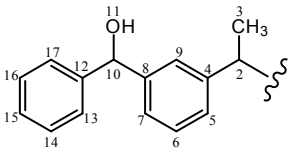


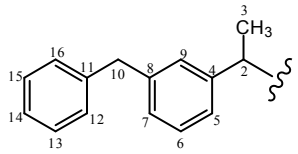
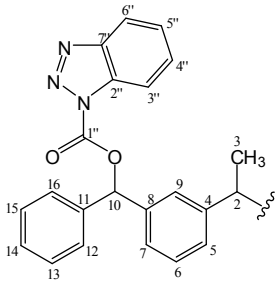
Spoj	R	^{1}H NMR (DMSO-d_6, δ ppm, J/Hz)
7a		8,27–8,21 (m, 2H, arom.), 7,80–7,75 (m, 1H, arom.), 7,62–7,57 (m, 1H, arom.), 7,35 (d, 2H, arom., J = 8,02), 7,12 (d, 2H, arom., J = 8,05), 5,31 (q, 1H, 2, J = 6,96), 2,37 (d, 2H, 10, J = 7,14), 1,79–1,75 (m, 1H, 11), 1,64 (d, 3H, 3, J = 6,99), 0,81 (d, 6H, 12, 13, J = 6,60)
7b		8,26–8,22 (m, 2H, arom.), 7,81–7,76 (m, 1H, arom.), 7,64–7,58 (m, 1H, arom.), 7,39–7,33 (m, 3H, arom.), 7,22–7,12 (m, 3H, arom.), 6,99–6,96 (m, 2H, arom.), 6,88-6,85 (m, 1H, arom.), 5,34 (q, 1H, 2, J = 6,97), 1,66 (d, 3H, 3, J = 6,99)

7c		8,27 (dd, 2H, arom., *J* = 8,29, *J* = 3,10), 7,82–7,77 (m, 1H, arom.), 7,65–7,60 (m, 1H, arom.), 7,53 (d, 2H, arom., *J* = 8,07), 7,36–7,08 (m, 4H, arom., 9), 6,89–6,84 (m, 1H, arom.), 6,24 (d, 1H, arom., *J* = 8,01), 4,94 (s, 2H, 2)
7d		8,19 (d, 1H, arom., *J* = 4,14), 8,12 (d, 1H, arom., *J* = 4,11), 7,68 (t, 1H, arom., *J* = 3,70). 7,62 (d, 2H, arom., *J* = 4,20), 7,56 (d, 2H, arom., *J* = 4,20), 7,53 (t, 1H, arom., *J* = 3,69), 7,18 (d, 1H, arom., *J* = 1,08), 6,87 (d, 1H, arom., *J* = 4,50), 6,64 (dd, 1H, arom., *J* = 1,16, *J* = 3,33), 4,83 (s, 2H, 2), 3,65 (s, 3H, 2''), 2,25 (s, 3H, 1'')
7e		8,26–8,22 (m, 2H, arom.), 7,84–7,49 (m, 11H, arom.), 5,45 (q, 1H, 2, *J* = 7,05), 1,70 (d, 3H, 3, *J* = 7,05)
7f		8,26–8,21 (m, 2H, arom.), 7,80–7,75 (m, 1H, arom.), 7,62–7,57 (m, 1H, arom.), 7,52–7,50 (m, 1H, arom.), 7,33–7,14 (m, 8H, arom.), 5,88 (d, 1H, 11, *J* = 4,00), 5,65 (d, 1H, 10, *J* = 3,83), 5,32 (q, 1H, 2, *J* = 6,89), 1,63 (d, 3H, 3, *J* = 6,94)
7g		8,25–8,20 (m, 2H, arom.), 7,79–7,74 (m, 1H, arom.), 7,61–7,56 (m, 1H, arom.), 7,33–7,07 (m, 9H, arom.), 5,30 (q, 1H, 2, *J* = 6,94), 3,90 (s, 2H, 10), 1,63 (d, 3H, 3, *J* = 6,99)
7h		8,28–7,98 (m, 3H, arom.), 7,78–7,28 (m, 15H, arom.), 7,24 (s, 1H, 10), 5,38 (q, 1H, 2, *J* = 6,84), 1,66 (d, 3H, 3, *J* = 6,94)

Tablica 9. ^{13}C NMR podaci za NSAID benzotriazolide **7a-h**.

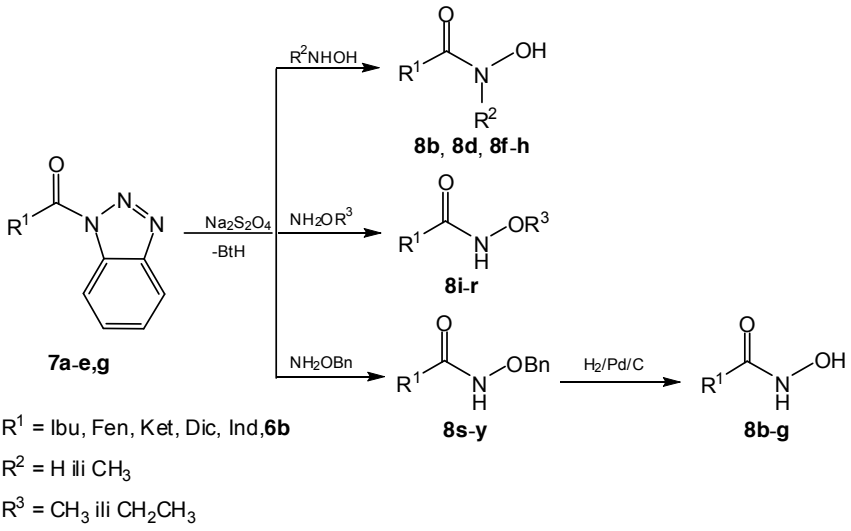


Spoj	R	^{13}C NMR (DMSO-d_6, δ ppm)
7a		173,07 (1), 145,33, 140,19, 136,87, 130,65 (4, 7, 1', 6'), 130,82, 129,31, 127,37, 126,46, 119, 97, 113,92 (5, 6, 8, 9, 2'–5'), 44,11 (2), 44,04 (10), 29,41 (3), 22,04 (12, 13), 18,52 (11)
7b		172,80 (1), 157,02, 156,25, 145,50, 141,89, 130,78 (4, 8, 10, 1', 6'), 130,99, 130,52, 130,10, 126,63, 123,72, 122,64, 120,14, 118,81, 118,09, 117,20, 114,07 (5–7, 9, 11–15, 2'–5'), 44,59 (2), 18,61 (3)
7c		170,08 (1), 145,35, 143,62, 136,92, 132,01, 130,78, 120,91 (3, 8, 10, 11, 15, 1', 6'), 131,96, 130,64, 129,04, 128,03, 126,48, 126,26, 119,92, 119,78, 114,91, 113, 97 (4–7, 12–14, 2'–5'), 38,11 (2)
7d		169,43 (1), 167,87 (11), 155,61, 145,49, 137,71, 136,38, 134,02, 130,79, 130,72, 130,26, 111,40 (3–5, 8, 10, 12, 15, 1', 6'), 131,17, 130,69, 129,04, 126,36, 119,99, 114,51, 113,95, 111,51, 102,00 (6, 7, 9, 13, 14, 16, 17, 2'–5'), 55,39 (2''), 30,89 (2), 13,29 (1'')
7e		195,33 (10), 172,74 (1), 140,80, 137,38, 136,80, 130,96, 114,05 (4, 8, 11, 1', 6'), 145,48, 132,78, 132,13, 129,66, 129,22, 129,21, 129,12, 128,87, 128,57, 126,60, 120,11 (5–7, 9, 12–16, 2'–5'), 44,35 (2), 18,60 (3)
7f		173,50 (1), 146,82, 145,88, 145,83, 140,01, 131,18 (4, 8, 12, 1', 6'), 131,39, 129,08, 128,45, 127,14, 127,02, 126,66, 126,63, 126,58, 125,84, 120,53, 114,44 (5–7, 9, 13–17, 2'–5'), 74,49 (10), 45,12 (2), 19,07 (3)

7g		173,47 (1), 145,88, 142,33, 141,39, 140,32, 131,18 (4, 8, 11, 1', 6'), 131,38, 129,41, 129,06, 128,82, 128,57, 128,21, 127,01, 126,41, 125,77, 120,52, 114,45 (5–7, 9, 12–16, 2'–5'), 45,05 (2), 41,37 (10), 19,06 (3)
7h		173,28 (1), 147,91 (1"), 145,80, 145,77, 140,72, 140,02, 139,17, 131,73 (4, 8, 11, 1', 6', 2", 7"), 131,36, 131,11, 129,83, 129,20, 128,29, 127,36, 127,23, 126,97, 126,79, 126,55, 126,38, 120,70, 120,45, 114,39, 113,67 (5–7, 9, 12–16, 2'–5', 3"–6"), 81,48 (10), 45,04 (2), 18,98 (3)

5.1.2.3. Sinteza NSAID hidroksamskih kiselina i njihovih derivata 8a-y i IR i NMR spektroskopska studija

Reaktivni NSAID benzotriazolidi **7a-e** i **7g** bili su ishodni spojevi u sintezi NSAID hidroksamskih kiselina i njihovih derivata **8a-y**. Benzotriazol je u spojevima **7a-e** i **7g** u reakciji nukleofilne supstitucije zamijenjen odgovarajućim hidroksilaminom (hidroksilamin, *N*-metilhidroksilamin, *O*-metilhidroksilamin, *O*-etilhidroksilamin i *O*-benzilhidroksilamin, Shema 41). Reakcije su izvođene u odsutnosti TEA (Metoda A) ili u prisutnosti TEA (Metoda B), u različitim otapalima i uz natrijev ditionit kao antioksidans kako bi se poboljšala iskorištenja. Općenito, Metodom B dobivena su viša iskorištenja uz kraća reakcijska vremena (Tablica 10). *O*-benzilirani derivati **8s-y** reakcijom katalitičkog hidrogeniranja (Metoda C) dali su slobodne NSAID hidroksamske kiseline **8b-g**.

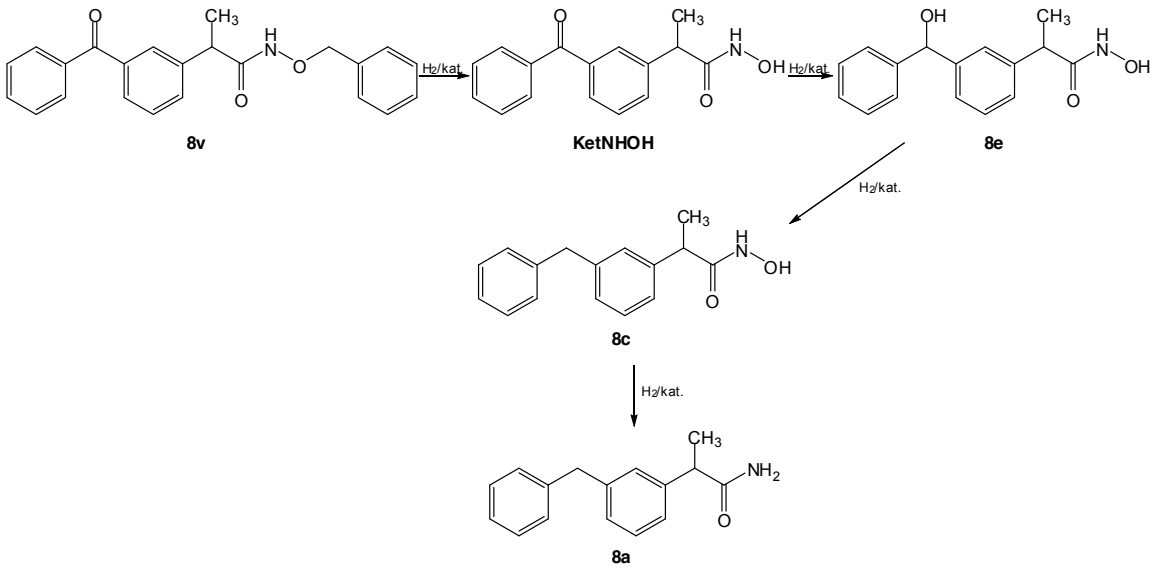


Shema 41. Sinteza derivata hidroksamskih kiselina **8b-y**.

Zanimljiva je reakcija redukcije *N*-benziloksi-2-(3-benzoilfenil)propanamida (**8v**) (Shema 42). Uklanjanjem benzilne skupine nastaje ketoprofen hidroksamska kiselina (KetNHOH). U daljnjoj reakciji njezina keto skupina reducira se u hidroksilnu i potom u metilensku skupinu, te nastaju spojevi **8e**, odnosno **8c**. Ako reakciju hidrogeniranja provodimo dovoljno dugo, funkcionalna skupina hidroksamskih kiselina (–CONHOH) prijeći će u amidnu skupinu ($–CONH_2$). Variranjem katalizatora (10%-tni Pd/C ili Pd/C(en)), njegove količine, otapala (metanol ili tetrahidrofuran) i trajanja

reakcije hidrogeniranja pokušali smo naći optimalne uvjete za selektivnu hidrogenolizu spoja **8v**. Najveći problem predstavljala je sinteza KetNHOH. Naime, kod svih kombinacija otapala, katalizatora i trajanja reakcije, reakcija skidanja benzilne zaštitne skupine i redukcija keto skupine odvijale su se usporedno, reakciju nije bilo moguće zaustaviti na željenom produktu. Sinteza spojeva **8e**, **8c** i **8a** najbolje je napredovala uz 10%-tni Pd/C u metanolu. Utvrdili smo da prilikom redukcije **8v** količina upotrebljenog katalizatora određuje selektivnost reakcije, odnosno manja količina katalizatora omogućuje prekid reakcije te nastaje produkt **8e** uz malu količinu spoja **8c**. Produljenjem reakcije hidrogeniranja, iz spoja **8c** nastaje **8a**.

Reakcije redukcije spoja **8v** uz 10%-tni Pd/C(en) u tetrahidrofuranu bile su prespore i manje selektivne.



Shema 42. Redukcija spoja **8v**.

Priređene su sljedeće NSAID hidroksamske kiseline i njihovi derivati:

2-(3-benzilfenil)propanamid (**8a**),

N-hidroksi-2-(4-izobutilfenil)propanamid (**8b**),

N-hidroksi-2-(3-benzilfenil)propanamid (**8c**),

N-hidroksi-2-(3-fenoksifenil)propanamid (**8d**),

N-hidroksi-2-(3-(hidroksi(fenil)metil)fenil)propanamid (**8e**),

N-hidroksi-2-(2-(2,6-diklorfenilamino)fenil)acetamid (**8f**),

N-hidroksi-1-(4-klorbenzoil)-2-metil-5-metoksi-1*H*-indol-3-acetamid (**8g**),

N-hidroksi-2-(2-(2,6-diklorfenilamino)fenil)-*N*-metilacetamid (**8h**),
2-(3-benzilfenil)-*N*-metoksipropanamid (**8i**),
2-(3-benzoilfenil)-*N*-metoksipropanamid (**8j**),
2-(2-(2,6-diklorfenilamino)fenil)-*N*-metoksiacetamid (**8k**),
1-(4-klorbenzoil)-2-metil-*N*,5-dimetoksi-1*H*-indol-3-acetamid (**8l**),
N-etoksi-2-(4-izobutilfenil)propanamid (**8m**),
2-(3-benzilfenil)-*N*-etoksipropanamid (**8n**),
N-etoksi-2-(3-fenoksifenil)propanamid (**8o**),
2-(3-benzoilfenil)- *N*-etoksipropanamid (**8p**),
N-etoksi-2-(2-(2,6-diklorfenilamino)fenil)acetamid (**8q**),
N-etoksi-1-(4-klorbenzoil)-2-metil-5-metoksi-1*H*-indol-3-acetamid (**8r**),
N-benziloksi-2-(4-izobutilfenil)propanamid (**8s**),
N-benziloksi-2-(3-benzilfenil)propanamid (**8t**),
N-benziloksi-2-(3-fenoksifenil)propanamid (**8u**),
N-benziloksi-2-(3-benzoilfenil)propanamid (**8v**),
N-benziloksi-2-(2-(2,6-diklorfenilamino)fenil)acetamid (**8x**),
N-benziloksi-1-(4-klorbenzoil)-2-metil-5-metoksi-1*H*-indol-3-acetamid (**8y**).

Od sintetiziranih spojeva novi spojevi su **8a**,**c**,**e**,**i**,**j**,**m**-**x**, te su svi pripravljeni novom, do sada neobjavljenom metodom. Sinteza spojeva **8a**-**y** i njihova čistoća praćeni su tankoslojnom kromatografijom, a strukture potvrđene IR, ^{1}H i ^{13}C NMR spektroskopijom i elementarnom analizom. Tališta i spektroskopski podaci ranije pripravljenih spojeva podudaraju se s literaturnim podacima.[1,7,9,45,47,85,87–99] Sve elementarne analize bile su unutar 0,4%. Reakcijski uvjeti priprave prikazani su u Tablici 10, a analitički i spektroskopski podaci u Tablicama 11–13.

pK_a vrijednosti spojeva **8b**,**d**,**f**-**h** određene su potenciometrijskom titracijom s otopinom Bu_4NOH (Tablica 10). Prema očekivanjima, pK_a vrijednosti NSAID hidroksamskih kiselina značajno su više (između 9,91 i 10,97) od pK_a vrijednosti NSAID od kojih potječu (ibuprofen 5,2, diklofenak 4,5, indometacin 4,5).[111]

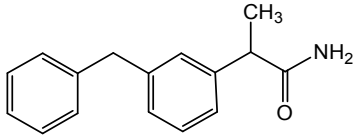
Tablica 10. Reakcijski uvjeti sinteze NSAID hidroksamskih kiselina i njihovih derivata **8a-y**.

8a-y	Metoda A				Metoda B				Metoda C				pK_a
	Otapalo	*t* (°C)	Trajanje (h)	Iskor. (%)	Otapalo	*t* (°C)	Trajanje (h)	Iskor. (%)	Otapalo	*t* (°C)	Trajanje (h)	Iskor. (%)	
a		[a]				[a]			metanol	20	17	100	n.o.[b]
b	kloroform	20	24	45	kloroform	20	0,6	41	metanol	20	0,25	96	10,89
c		[a]				[a]			metanol	20	2,50	80	n.o.
d	kloroform	20	24	40		[c]			metanol	20	0,62	94	10,66
e		[a]				[a]			metanol	20	3	74	n.o.
f		[d]			kloroform	20	0,5	65	etil-acetat	20	0,15	89	10,64
g		[e]			dioksan	20	24	53	metanol	20	0,5	78	10,97
h	kloroform	20	24	26	kloroform	20	4	86		[a]			9,91
i		[a]			toluen	20	48	59		[a]			n.o.
j		[a]			toluen	20	26	71		[a]			n.o.
k		[a]			toluen	20	24	39		[a]			n.o.
l		[a]			toluen	20	48	65		[a]			n.o.
m		[a]			toluen	20	40	64		[a]			n.o.
n		[a]			toluen	20	46	46		[a]			n.o.
o		[a]			toluen	20	24	59		[a]			n.o.

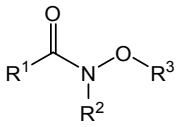
p			[a]			toluen	20	24	47	[a]	n.o.
q			[a]			toluen	20	6,5	77	[a]	n.o.
r			[a]			toluen	20	43	79	[a]	n.o.
s			[c]			kloroform	20	48	70	[a]	n.o.
						toluen	20	48	62		
t			[a]			toluen	75	11	67	[a]	n.o.
u	kloroform	20		24	52	kloroform	20	48	69	[a]	n.o.
						toluen	refluks	5,5	91		
v			[c]			toluen	20	48	75	[a]	n.o.
x	kloroform	20		24	89	kloroform	20	27	57	[a]	n.o.
						toluen	refluks	1	88		
y	kloroform	20		24	57	kloroform	20	48	91	[a]	n.o.

[a] Spoj nije pripravljen opisanom metodom. [b] n.o. – Nije određeno; [c] smjesa produkata; [d] teškoće kod čišćenja; [e] dolazi do pucanja amidne veze.

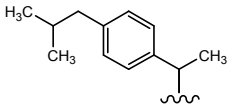
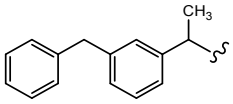
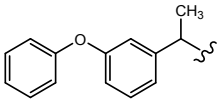
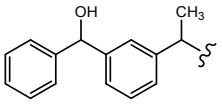
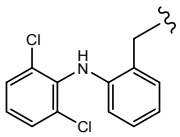
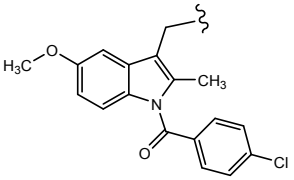
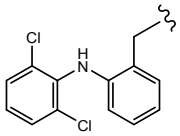
Tablica 11. Analitički i IR podaci za NSAID hidroksamske kiseline i derivate **8a-y**.

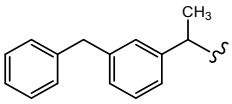
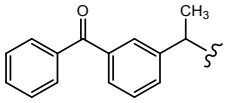
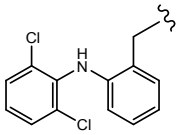
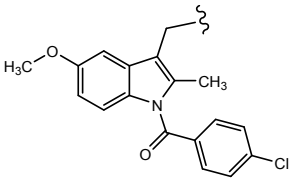
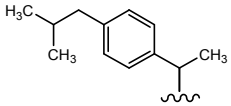
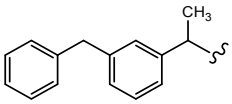
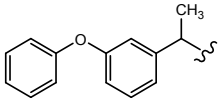
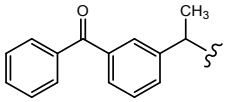
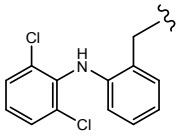


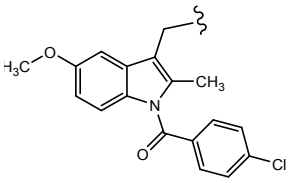
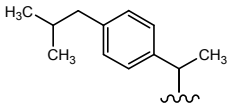
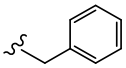
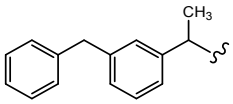
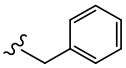
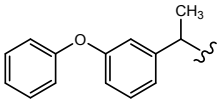
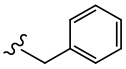
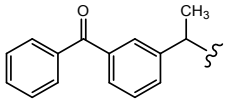
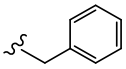
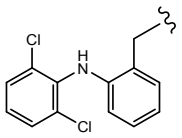
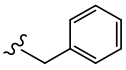
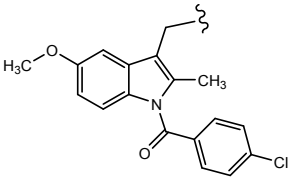
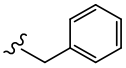
8a



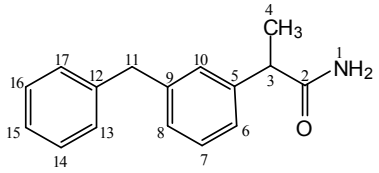
8b-y

Spoj	R^1	R^2	R^3	t_t (°C)	Molekulska formula (M_r)	IR (KBr/film) ν_{max} (cm^{-1})
8a	–	–	–	76–78	$C_{16}H_{17}NO$ (239,31)	3393, 3177, 3084, 3026, 2980, 1653, 1599, 1451, 1406, 1368, 1288, 1123, 786, 710, 694, 646, 628
8b		H	H	117–119 raspad	$C_{13}H_{19}NO_2$ (221,29)	3191, 3025, 2955, 1634, 1513, 1462, 1025, 939
8c		H	H	135–137	$C_{16}H_{17}NO_2$ (255,31)	3189, 3023, 2904, 1632, 1533, 1486, 1072, 1029, 723, 702
8d		H	H	118–119 raspad	$C_{15}H_{15}NO_3$ (257,28)	3426, 3215, 3038, 2920, 1628, 1582, 1536, 1489, 1248, 698
8e		H	H	148–152	$C_{16}H_{17}NO_3$ (271,31)	3378, 3214, 2979, 2915, 1645, 1520, 1492, 1447, 1022, 945, 724, 701
8f		H	H	158–162 raspad	$C_{14}H_{12}Cl_2N_2O_2$ (311,16)	3289, 3235, 3031, 2963, 2891, 1660, 1626, 1589, 1507, 1453, 774, 749
8g		H	H	175–178	$C_{19}H_{17}ClN_2O_4$ (372,80)	3243, 3015, 2965, 2840, 1652, 1635, 1630, 1604, 1483, 1468, 1402, 1383, 1225, 1090, 870, 786
8h		CH_3	H	134–138	$C_{15}H_{14}Cl_2N_2O_2$ (325,19)	3272, 3066, 3036, 2925, 1614, 1581, 1509, 1452, 770, 752

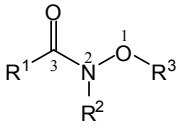
8i		H	CH_3	61–64	$C_{17}H_{19}NO_2$ (269,34)	3153, 3083, 2971, 938, 2854, 1661, 1640, 493, 1069, 1054, 720, 706
8j		H	CH_3	105–107	$C_{17}H_{17}NO_3$ (283,32)	3164, 2984, 2945, 882, 1664, 1651, 1642, 1603, 1445, 1318, 1289, 722, 703, 692,
8k		H	CH_3	178–179	$C_{15}H_{14}Cl_2N_2O_2$ (325,19)	3277, 3152, 2979, 2866, 1644, 1592, 1510, 1452, 1280, 1046, 977, 949, 772, 750
8l		H	CH_3	182–184	$C_{20}H_{19}ClN_2O_4$ (386,83)	3222, 3093, 2991, 2929, 1677, 1660, 1615, 1596, 1479, 1318, 1224, 1072, 849, 755
8m		H	CH_2CH_3	54–56	$C_{15}H_{23}NO_2$ (249,35)	3178, 2933, 2870, 1659, 1512, 1464, 1042, 934, 850
8n		H	CH_2CH_3	114–116	$C_{18}H_{21}NO_2$ (283,36)	3170, 3023, 2987, 1671, 1650, 1600, 1492, 1047, 721, 707
8o		H	CH_2CH_3	60–61	$C_{17}H_{19}NO_3$ (285,34)	3145, 2946, 2880, 1673, 1654, 1582, 1488, 1251, 1226, 945, 694
8p		H	CH_2CH_3	75–77	$C_{18}H_{19}NO_3$ (297,35)	3192, 2980, 2936, 1660, 1597, 1448, 1319, 1284, 1042, 718, 643
8q		H	CH_2CH_3	181–182	$C_{16}H_{16}Cl_2N_2O_2$ (339,22)	3298, 3152, 2980, 2882, 1642, 1578, 1507, 1454, 1304, 1060, 975, 782, 750

8r		H	CH_2CH_3	184–186	$C_{21}H_{21}ClN_2O_4$ (400,86)	3220, 3090, 2988, 2933, 2889, 1678, 1659, 1614, 1595, 1479, 1316, 1225, 1071, 849, 755
8s		H		66–68	$C_{20}H_{25}NO_2$ (311,42)	3156, 3068, 2975, 2948, 2866, 1674, 1648, 1530, 1454, 1376, 1042, 733, 696
8t		H		ulje	$C_{23}H_{23}NO_2$ (345,43)	3181, 3062, 3028, 2975, 2934, 1658, 1601, 1495, 1454, 1027, 749, 725, 698
8u		H		ulje	$C_{22}H_{21}NO_3$ (347,41)	3181, 3065, 3033, 2973, 1658, 1583, 1487, 1453, 751, 695
8v		H		82–85	$C_{23}H_{21}NO_3$ (359,42)	3188, 3088, 3063, 3031, 2974, 2875, 1660, 1597, 1578, 1497, 1448, 1319, 1284, 1026, 719, 698
8x		H		161–164	$C_{21}H_{18}Cl_2N_2O_2$ (401,29)	3265, 3153, 3065, 2974, 1655, 1636, 1588, 1507, 1454, 1304, 751, 733, 696
8y		H		156–158	$C_{26}H_{23}ClN_2O_4$ (462,92)	3240, 3065, 3033, 2997, 2925, 2864, 1685, 1656, 1609, 1518, 1479, 1452, 1370, 1321, 1227, 1089, 836, 753, 699

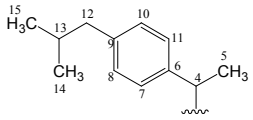
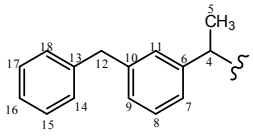
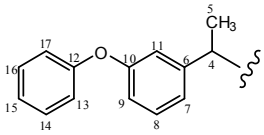
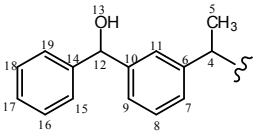
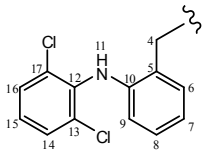
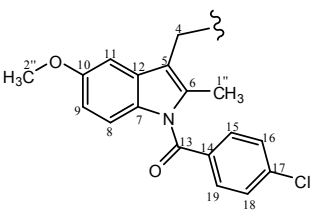
Tablica 12. ^{1}H NMR podaci za NSAID hidroksamske kiseline i njihove derivate **8a-y**.

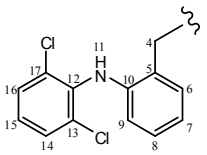
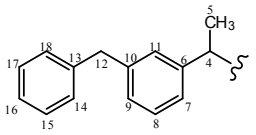
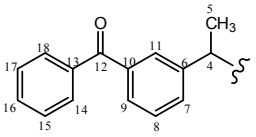
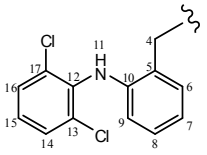
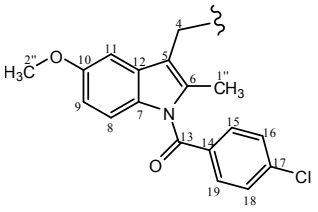
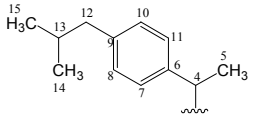
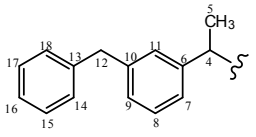
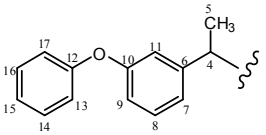


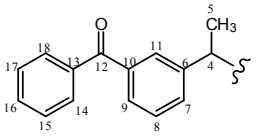
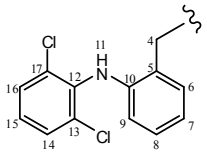
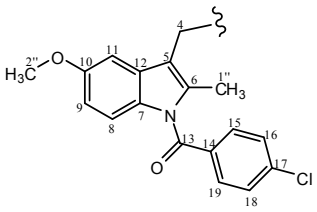
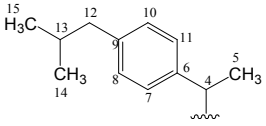
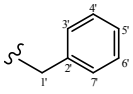
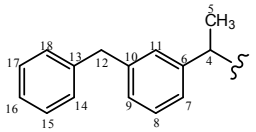
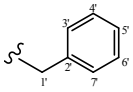
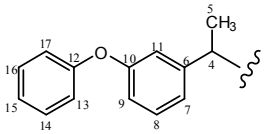
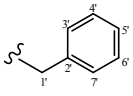
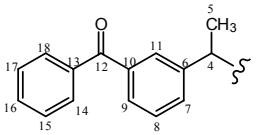
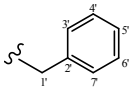
8a

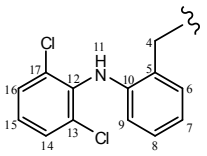
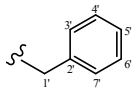
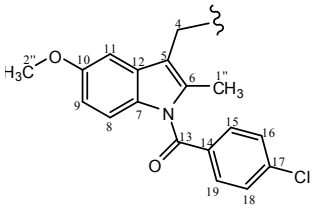
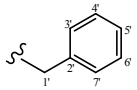


8b-y

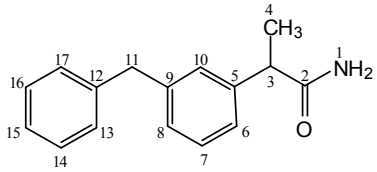
Spoj	R^1	R^2	R^3	^{1}H NMR (DMSO-d_6, δ ppm, J/Hz)
8a	–	–	–	7,41–7,04 (m, 9H, arom.), 6,78 (s, 2H, 1), 3,91 (s, 2H, 11), 3,53 (q, 1H, 3, J = 7,01), 1,28 (d, 3H, 4, J = 7,06)
8b		H	H	10,59 (s, 1H, 1), 8,75 (s, 1H, 2), 7,21 (d, 2H, arom., J = 8,07), 7,07 (d, 2H, arom., J = 8,07), 3,49 (q, 1H, 4, J = 7,03), 2,40 (d, 2H, 12, J = 7,11), 1,84–1,75 (m, 1H, 13), 1,31 (d, 3H, 5, J = 7,06), 0,84 (d, 6H, 14, 15, J = 6,61)
8c		H	H	10,61 (s, 1H, 1), 8,77 (s, 1H, 2), 7,31–7,05 (m, 9H, arom.), 3,91 (s, 2H, 12), 3,39 (q, 1H, 4, J = 7,06), 1,30 (d, 3H, 5, J = 7,02)
8d		H	H	10,63 (s, 1H, 1), 8,81 (s, 1H, 2), 7,42–7,37 (m, 2H, arom.), 7,31 (t, 1H, arom., J = 7,04), 7,17–7,08 (m, 2H, arom.), 7,01–6,99 (m, 3H, arom.), 6,85–6,82 (m, 1H, arom.), 3,43 (q, 1H, 4, J = 7,05), 1,31 (d, 3H, 5, J = 6,99)
8e		H	H	10,62 (s, 1H, 1), 8,77 (s, 1H, 2), 7,38–7,16 (m, 9H, arom.), 5,87 (d, 1H, 13, J = 3,90), 5,66 (d, 1H, 12, J = 3,60), 3,41 (q, 1H, 4, J = 7,00), 1,31 (d, 3H, 5, J = 7,00)
8f		H	H	10,87 (s, 1H, 1), 8,97 (s, 1H, 2), 8,44 (s, 1H, 11), 7,43 (d, 2H, arom., J = 8,05), 7,09–7,06 (m, 2H, arom.), 6,97 (t, 1H, arom., J = 7,15), 6,78 (t, 1H, arom., J = 7,22), 6,23 (d, 1H, arom., J = 7,92), 3,39 (s, 2H, 4)
8g		H	H	10,59 (s, 1H, 1), 8,76 (s, 1H, 2), 7,61 (d, 2H, arom., J = 8,28), 7,56 (d, 2H, arom., J = 8,45), 7,07 (s, 1H, arom.), 6,84 (d, 1H, arom., J = 8,96), 6,63 (dd, 1H, arom., J = 1,90, J = 6,84), 3,70 (s, 3H, 2''), 3,32 (s, 2H, 4), 2,18 (s, 3H, 1'')

8h		$^{1'}CH_3$	H	10,30 (s, 1H, 1), 7,82 (s, 1H, 11), 7,51 (d, 2H, arom., *J* = 8,07), 7,20–7,14 (m, 2H, arom.), 7,08–7,02 (m, 1H, arom.), 6,88–6,83 (m, 1H, arom.), 6,30 (d, 1H, arom., *J* = 7,78), 3,86 (s, 2H, 4), 3,16 (s, 3H, 1')
8i		H	$^{1'}CH_3$	11,19 (s, 1H, 2), 7,30–7,07 (m, 9H, arom.), 3,91 (s, 2H, 12), 3,38–3,32 (m, 4H, 4, 1'), 1,30 (d, 3H, 5, *J* = 7,09)
8j		H	$^{1'}CH_3$	11,30 (s, 1H, 2), 7,75–7,49 (m, 9H, arom.), 3,55–3,47 (m, 4H, 4, 1'), 1,37 (d, 3H, 5, *J* = 7,03)
8k		H	$^{1'}CH_3$	11,44 (s, 1H, 2), 8,03 (s, 1H, 11), 7,44 (d, 2H, arom., *J* = 8,08), 7,10–7,07 (m, 2H, arom.), 6,99–6,96 (m, 1H, arom.), 6,80–6,77 (m, 1H, arom.), 6,22 (d, 1H, arom., *J* = 7,95), 3,54 (s, 3H, 1'), 3,37 (s, 2H, 4)
8l		H	$^{1'}CH_3$	11,28 (s, 1H, 2), 7,70 (d, 2H, arom., *J* = 8,70), 7,65 (d, 2H, arom., *J* = 8,71), 7,12 (d, 1H, arom., *J* = 2,17), 6,93 (d, 1H, arom., *J* = 8,98), 6,71 (dd, 1H, arom., *J* = 2,50, *J* = 6,50), 3,77 (s, 3H, 2''), 3,59 (s, 3H, 1'), 3,40 (s, 2H, 4), 2,24 (s, 3H, 1'')
8m		H	$^{1'}CH_2{}^{2'}CH_3$	11,07 (s, 1H, 2), 7,20 (d, 2H, arom., *J* = 8,03), 7,08 (d, 2H, arom., *J* = 8,03), 3,75 (q, 2H, 1', *J* = 7,03), 3,38 (q, 1H, 4, *J* = 7,05), 2,40 (d, 2H, 12, *J* = 7,13), 1,87–1,73 (m, 1H, 13), 1,31 (d, 3H, 5, *J* = 7,04), 1,09 (t, 3H, 2', *J* = 7,03), 0,85 (d, 6H, 14, 15, *J* = 6,60)
8n		H	$^{1'}CH_2{}^{2'}CH_3$	11,07 (s, 1H, 2), 7,31–7,07 (m, 9H, arom.), 3,91 (s, 2H, 12), 3,71 (q, 1H, 4, *J* = 7,03), 3,37 (q, 2H, 1', *J* = 7,07), 1,30 (d, 3H, 5, *J* = 7,04), 1,07 (t, 3H, 2', *J* = 7,03)
8o		H	$^{1'}CH_2{}^{2'}CH_3$	11,10 (s, 1H, 2), 7,42–7,30 (m, 3H, arom.), 7,14 (t, 1H, arom., *J* = 7,36), 7,07 (d, 1H, arom., *J* = 7,65), 7,01–6,96 (m, 3H, arom.), 6,86 (dd, 1H, arom., *J* = 1,89, *J* = 6,14), 3,72 (q, 2H, 1', *J* = 7,02), 3,41 (q, 1H, 4, *J* = 7,03), 1,31 (d, 3H, 5, *J* = 7,01), 1,08 (t, 3H, 2', *J* = 7,02)

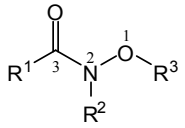
8p		H		11,17 (s, 1H, 2), 7,74–7,67 (m, 4H, arom.), 7,64–7,49 (m, 5H, arom.), 3,75 (q, 2H, 1', *J* = 7,02), 3,54 (q, 1H, 4, *J* = 7,02), 1,37 (d, 3H, 5, *J* = 7,04), 1,11 (t, 3H, 2', *J* = 7,03)
8q		H		11,40 (s, 1H, 2), 8,12 (s, 1H, 11), 7,52 (d, 2H, arom., *J* = 8,08), 7,20–7,14 (m, 2H, arom.), 7,08–7,03 (m, 1H, arom.), 6,89–6,84 (m, 1H, arom.), 6,30 (d, 1H, arom., *J* = 7,88), 3,83 (q, 2H, 1', *J* = 7,03), 3,46 (s, 2H, 4), 1,14 (t, 3H, 2', *J* = 7,03)
8r		H		11,17 (s, 1H, 2), 7,69 (d, 2H, arom., *J* = 8,71), 7,64 (d, 2H, arom., *J* = 8,39) 7,13 (d, 1H, arom., *J* = 2,17), 6,93 (d, 1H, arom., *J* = 8,98), 6,71 (dd, 1H, arom., *J* = 2,48, *J* = 6,53), 3,80 (q, 2H, 1', *J* = 7,05), 3,77 (s, 3H, 2''), 3,41 (s, 2H, 4), 2,24 (s, 3H, 1''), 1,13 (t, 3H, 2', *J* = 7,02)
8s		H		11,19 (s, 1H, 2), 7,37–7,29 (m, 5H, arom.), 7,21 (d, 2H, arom., *J* = 8,01), 7,10 (d, 2H, arom., *J* = 8,04), 4,75 (s, 2H, 1'), 3,40 (q, 1H, 4, *J* = 7,04), 2,43 (d, 2H, 12, *J* = 7,10), 1,87–1,78 (m, 1H, 13), 1,33 (d, 3H, 5, *J* = 7,03), 0,87 (d, 6H, 14, 15, *J* = 6,60)
8t		H		11,18 (s, 1H, 2), 7,34–7,07 (m, 14H, arom.), 4,70 (s, 2H, 1'), 3,92 (s, 2H, 12), 3,38 (q, 1H, 4, *J* = 7,01), 1,30 (d, 3H, 5, *J* = 7,03)
8u		H		11,13 (s, 1H, 2), 7,32–7,22 (m, 8H, arom.), 7,07–7,05 (m, 1H, arom.), 6,99 (d, 1H, arom., *J* = 7,56), 6,94–6,90 (m, 3H, arom.), 6,79 (dd, 1H, arom., *J* = 1,74, *J* = 6,22), 4,65 (s, 2H, 1'), 3,34 (q, 1H, 4, *J* = 6,90), 1,25 (d, 3H, 5, *J* = 7,01)
8v		H		11,27 (s, 1H, 2), 7,75–7,49 (m, 10H, arom.), 7,31 (s, 5H, arom.), 4,73 (s, 2H, 1'), 3,54 (q, 1H, 4, *J* = 7,08), 1,37 (d, 3H, 5, *J* = 6,99)

8x		H		11,47 (s, 1H, 2), 8,09 (s, 1H, 11), 7,53 (d, 2H, arom., *J* = 8,06), 7,37 (s, 5H, arom.), 7,20–7,14 (m, 3H, arom.), 7,09–7,04 (m, 1H, arom.), 6,89–6,84 (m, 1H, arom.), 6,31 (d, 1H, arom., *J* = 7,80), 4,82 (s, 2H, 1'), 3,47 (s, 2H, 4)
8y		H		11,20 (s, 1H, 2), 7,61 (d, 2H, arom., *J* = 8,44), 7,57 (d, 2H, arom., *J* = 8,30), 7,27 (s, 5H, arom.), 7,05 (d, 1H, arom., *J* = 1,77), 6,86 (d, 1H, arom., *J* = 8,98), 6,64 (dd, 1H, arom., *J* = 2,11, *J* = 6,81), 4,75 (s, 2H, 1'), 3,72 (s, 3H, 2''), 3,39 (s, 2H, 4), 2,20 (s, 3H, 1'')

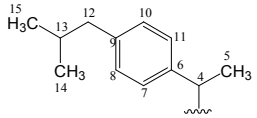
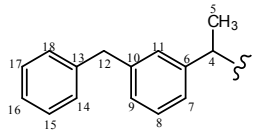
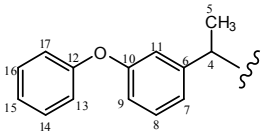
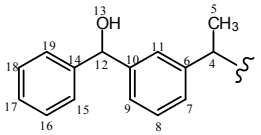
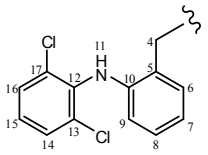
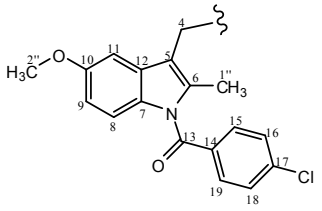
Tablica 13. ^{13}C NMR podaci za NSAID hidroksamske kiseline i njihove derivate **8a-y**.

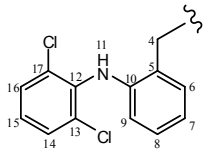
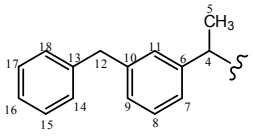
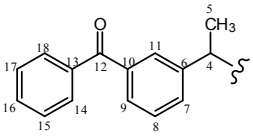
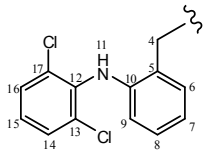
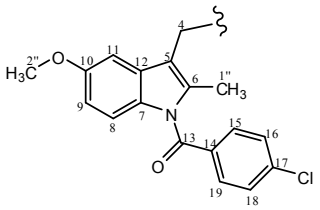
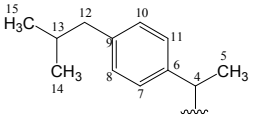
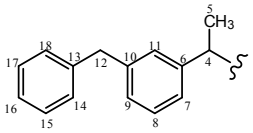
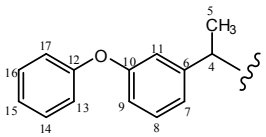
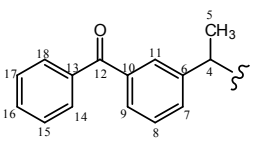


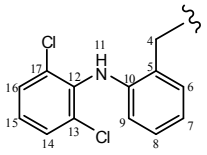
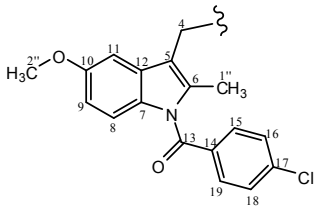
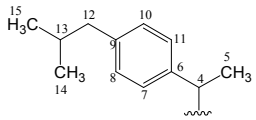
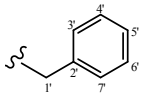
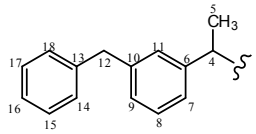
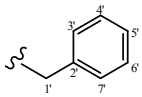
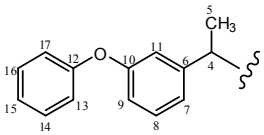
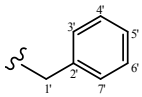
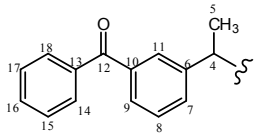
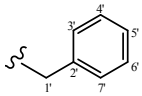
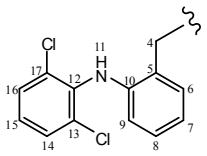
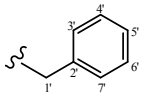
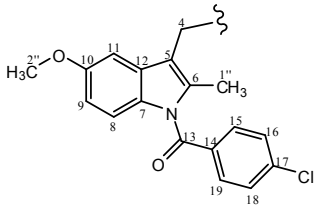
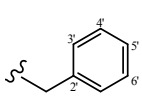
8a



8b-y

Spoj	R^1	R^2	R^3	^{13}C NMR (DMSO-d_6, δ ppm)
8a	–	–	–	175,69 (2), 143,02, 141,68, 141,46 (5, 9, 12), 129,12, 128,87, 128,15, 127,30, 126,41, 125,41 (6–8, 10, 13–17), 45,33 (3), 41,65 (11), 18,97 (4)
8b		H	H	179,39 (3), 139,29, 139,09 (6, 9), 128,68, 126,95 (7, 8, 10, 11), 44,22 (12), 41,73 (4), 29,61 (5), 22,13 (14, 15), 18,16 (13)
8c		H	H	170,67 (3), 142,46, 141,64, 141,49 (6, 10, 13), 129,13, 128,88, 128,71, 128,11, 127,47, 126,43, 125,43 (7–9, 11, 14–18), 42,54 (5), 41,62 (12), 18,71 (4)
8d		H	H	169,77 (3), 156,46, 156,37, 143,98 (6, 10, 11), 129,96, 129,62, 123,34, 122,32, 118,48, 117,47, 116,58 (7–9, 11, 13–17), 41,88 (4), 18,06 (5)
8e		H	H	170,65 (3), 146,12, 146,02, 142,09 (6, 10, 14), 128,52, 128,33, 127,16, 126,73, 126,13, 125,64, 125,11 (7–9, 11, 15–19), 74,80 (12), 42,62 (4), 18,77 (5)
8f		H	H	168,11 (3), 142,99, 137,16, 129,27, 125,13 (5, 10, 12, 13, 17), 130,29, 129,15, 127,28, 124,93, 120,71, 116,04 (6–9, 14–16), 36,57 (4)
8g		H	H	167,81 (3), 166,31 (13), 155,50, 137,57, 135,23, 134,19, 130,72, 130,25, 113,74 (5–7, 10, 12, 14, 17), 131,12, 129,00, 114,45, 111,12, 102,09 (8, 9, 11, 15, 16, 18, 19), 55,43 (2''), 28,36 (4), 13,29 (1'')

8h		$^{1'}CH_3$	H	171,22 (3), 143,30, 137,33, 129,60, 125,08 (5, 10, 12, 13, 17), 131,19, 129,32, 127,36, 125,29, 120,90, 116,18 (6–9, 14–16), 35,98 (4), 35,26 (1')
8i		H	$^{1'}CH_3$	170,48 (3), 141,99, 141,67, 141,62 (6, 10, 13), 129,13, 128,87, 128,00, 127,57, 126,42, 125,30 (7–9, 11, 14–18), 63,48 (1'), 42,52 (4), 41,59 (12), 18,65 (5)
8j		H	$^{1'}CH_3$	196,14 (11), 170,18 (2), 142,25, 137,46, 137,43 (5, 9, 12), 133,19, 132,03, 130,07, 129,13, 129,05, 128,84, 128,78 (6–8, 10, 13–17), 63,59 (1'), 42,44 (3), 18,61 (4)
8k		H	$^{1'}CH_3$	168,02 (3), 142,93, 137,03, 129,50, 124,48 (5, 10, 12, 13, 17), 130,38, 129,17, 127,44, 124,19, 120,77, 116,01 (6–9, 14–16), 63,33 (1'), 36,57 (4)
8l		H	$^{1'}CH_3$	168,31 (3), 166,72 (13), 156,02, 138,08, 135,86, 134,65, 131,13, 130,71, 113,75 (5–7, 10, 12, 14, 17), 131,64, 129,51, 115,04, 111,77, 102,22 (8, 9, 11, 15, 16, 18, 19), 63,70 (1'), 55,91 (2''), 28,86 (4), 13,77 (1'')
8m		H	$^{1'}CH_2\,^{2'}CH_3$	170,78 (3), 139,87, 139,20 (6, 9), 129,27, 127,34 (7, 8, 10, 11), 70,87 (1'), 44,68 (12), 42,20 (4), 30,08 (5), 22,62 (14, 15), 18,63 (13), 13,84 (2')
8n		H	$^{1'}CH_2\,^{2'}CH_3$	170,55 (2), 142,11, 141,62 (5, 9, 12), 129,11, 128,86, 128,82, 127,99, 127,55, 126,41, 125,28 (6–8, 10, 13–17), 70,85 (1'), 42,51 (3), 41,60 (11), 18,65 (4), 13,83 (2')
8o		H	$^{1'}CH_2\,^{2'}CH_3$	170,26 (3), 157,03, 156,99, 144,14 (6, 10, 12), 130,51, 130,33, 123,93, 122,72, 119,08, 117,82, 117,27 (7–9, 11, 13–17), 70,91 (1'), 42,41 (4), 18,50 (5), 13,83 (2')
8p		H	$^{1'}CH_2\,^{2'}CH_3$	196,16 (12), 170,25 (3), 142,38, 137,48, 137,43 (6, 10, 13), 133,19, 132,00, 130,06, 129,10, 129,05, 128,82, 128,73 (7–9, 11, 14–18), 70,97 (1'), 42,43 (4), 18,59 (5), 13,83 (2')

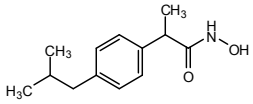
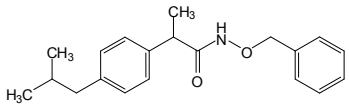
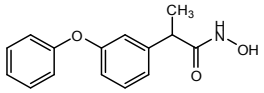
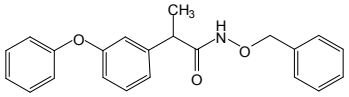
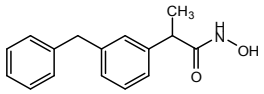
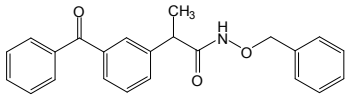
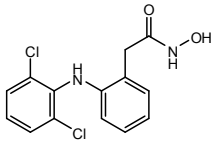
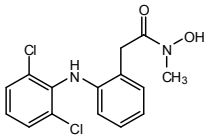
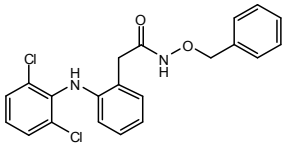
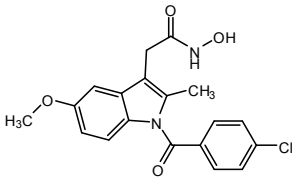
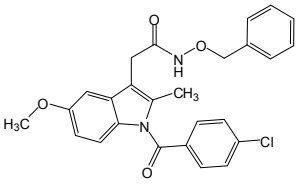
8q		H		168,59 (3), 143,42, 137,53, 129,99, 125,12 (5, 10, 12, 13, 17), 130,83, 129,67, 127,91, 125,68, 121,27, 116,50 (6–9, 14–16), 71,21 (1'), 36,93 (4), 13,88 (2')
8r		H		168,31 (3), 166,78 (13), 156,02, 138,07, 135,83, 134,66, 131,13, 130,72, 113,90 (5–7, 10, 12, 14, 17), 131,64, 129,51, 115,04, 111,78, 102,20 (8, 9, 11, 15, 16, 18, 19), 71,02 (1'), 55,91 (2''), 28,86 (4), 13,89 (2'), 13,78 (1'')
8s		H		170,57 (3), 139,57, 138,77, 136,03 (6, 9, 2'), 129,08, 128,93, 128,36, 127,09 (7, 8, 10, 11, 3'–7'), 76,69 (1'), 44,36 (12), 41,80 (4), 29,77 (13), 22,30 (14, 15), 18,22 (5)
8t		H		170,71 (2), 141,99, 141,62 (5, 9, 12), 136,34 (2'), 129,36, 128,71 (3'–7'), 129,12, 128,86, 128,82, 128,04, 127,56, 126,42, 125,36 (6–8, 10, 13–17), 77,06 (1'), 42,44 (3), 41,60 (11), 18,59 (4)
8u		H		169,93 (3), 156,52, 143,55, 135,82 (6, 10, 12, 2'), 130,00, 129,80, 128,89, 128,22, 123,41, 122,30, 118,56, 117,48, 116,78 (7–9, 11, 13–17, 3'–7'), 76,59 (1'), 41,88 (4), 17,97 (5)
8v		H		196,16 (12), 170,37 (3), 142,28, 137,48, 137,42, 136,26 (6, 10, 13, 2'), 133,19, 132,03, 130,08, 129,41, 129,05, 128,92, 128,76, 128,71 (7–9, 11, 14–18, 3'–7'), 77,08 (1'), 42,36 (4), 18,50 (5)
8x		H		168,16 (3), 142,87, 136,98, 135,75, 129,54, 124,45 (5, 10, 12, 13, 17, 2'), 130,35, 129,11, 128,78, 128,23, 127,35, 125,18, 120,66, 115,89 (6–9, 14–16, 3'–7'), 76,77 (1'), 36,29 (4)
8y		H		168,32 (3), 167,03 (13), 156,03, 138,07, 136,38, 135,86, 134,66, 131,17, 130,71, 113,83 (5–7, 10, 12, 14, 17, 2'), 131,63, 129,52, 129,35, 128,74, 115,01, 111,74, 102,34 (8, 9, 11, 15, 16, 18, 19, 3'–7'), 77,25 (1'), 55,92 (2''), 28,80 (4), 13,79 (1'')

Napravljena je IR i NMR spektroskopska studija hidroksamskih kiselina, derivata ibuprofena (**8b**), fenoprofena (**8d**), ketoprofena (**8c**), diklofenaka (**8f**) i indometacina (**8g**), te njihovih derivata sa supstituiranim dušikom ili kisikom u funkcionalnoj skupini hidroksamskih kiselina: *N*-metilni derivat diklofenaka (**8h**) i *O*-benzilni derivati ibuprofena (**8s**), fenoprofena (**8u**), ketoprofena (**8v**), diklofenaka (**8x**) i indometacina (**8y**). Na temelju nje opisane su strukturne značajke i geometrija molekula u seriji derivata hidroksamskih kiselina **8**, mogućnost tvorbe vodikove veze, te njihovo ponašanje u čvrstom stanju i u otopini.

Ispitivanja su provedena u čvrstom stanju (KBr ili film) pomoću IR metode i u otopini u DMSO-d_6 pomoću jedno- i dvodimenzijskih homo- i heteronuklearnih NMR spektroskopskih metoda: ^{1}H, ^{13}C, COSY, NOESY, HMQC i HMBC.

U IR spektrima vodikova veza opažena je u području –OH i –NH rasteznih vibracija, te u području rasteznih vibracija karbonilne skupine hidroksamskih kiselina (**8b-d**,**f**,**g**), pokazujući pomak tih vibracija prema manjim valnim brojevima, u usporedbi s karbonilnom skupinom u ishodnoj karboksilnoj kiselini i u njihovim *O*-benzilnim derivatima (**8s**,**u-y**) (Tablica 14).

Tablica 14. Rastezne vibracije NSAID hidroksamskih kiselina i njihovih derivata **8**.

Spoj	Struktura	IR (KBr) ν_{max} (cm^{-1})			
		O–H	N–H	C=O	C=C–H
8b		3191 (široka vrpca)		1634	3025
8s		–	3156	1674 1648	3068 3028
8d		3426	3215	1628	3038
8u		–	3181	1658	3065 3033
8c		3189 (široka vrpca)		1632	3023
8v		–	3188	1660	3088 3063 3031
8f		3289	3235	1660	3031
8h		3272 (široka vrpca)		1614	3066 3036
8x		–	3265 3153	1655 1636	3065
8g		3243 (široka vrpca)		1652 1635	3015
8y		–	3240	1685 1657	3090 3070 3033

^{1}H NMR spektri otkrivaju da se NH protoni funkcionalne skupine hidroksamske kiseline u svim *O*-benzilnim derivatima nalaze u području 11,13–11,47 ppm, dok se u hidroksamskim kiselinama pojavljuju od 8,75 do 8,97 ppm. Hidroksilni protoni u hidroksamskoj skupini javljaju se u području 10,30–10,87 ppm, te su u usporedbi s hidroksilnim protonima odgovarajućih NSAID pomaknuti su u više polje za oko 1,5 ppm.

^{13}C NMR spektri ispitivanih spojeva otkrivaju da se karbonilna skupina u svim NSAID nalazi u području 171,97–175,80 ppm, u hidroksamskim kiselinama u području 167,81–179,38 ppm, a u *O*-benzilnim derivatima u području 168,16–170,57 ppm. Najznačajnija razlika u kemijskim pomacima za karbonilnu skupinu nađena je kod ibuprofena, ibuprofen hidroksamske kiseline **8b** i njezinog *O*-benzilnog derivata **8s** (175,37, 179,39 i 170,57).

Nasuprot svim drugim NSAID, karbonilna skupina ibuprofen hidroksamske kiselina **8b** pokazuje značajni pomak u niže polje u usporedbi s ibuprofenom (Δ = – 4,02 ppm) i u usporedbi sa svojim *O*-benzilnim derivatom (Δ = – 9,01 ppm). Kod svih drugih NSAID hidroksamskih kiselina i njihovih derivata, kemijski pomak za karbonilnu skupinu pomaknut je u više polje. U ovim spojevima –NHOBn i –NHOH skupine zasjenjuju njihove susjedne karbonilne skupine, što rezultira manjim brojem kemijskog pomaka karbonilne skupine, odnosno pomakom u više polje.

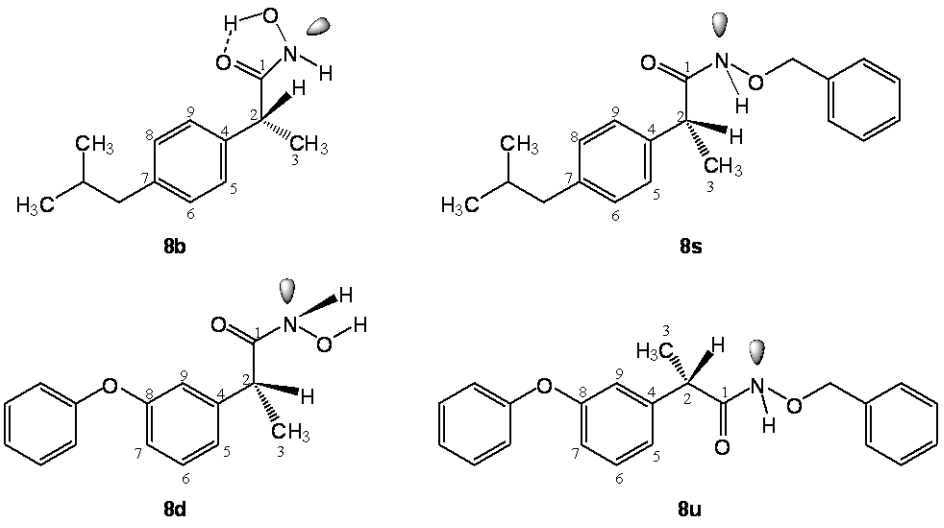
NOESY i HMBC NMR interakcije pokazale su da ibuprofen hidroksamska kiselina u DMSO-d_6 otopini stvara intramolekularne vodikove veze (Tablica 15). Zbog vodikove veze između H-atoma hidroksilne skupine i karbonilnog kisika nastaje 5-člani prsten. Zbog činjenice da vodikova veza slabi elektronsku gustoću karbonilne skupine, ona je pomaknuta u niže polje u ^{13}C NMR spektru spoja **8b**, u odnosu na sami lijek i njegov *O*-benzilni derivat **8s**. Ovaj fenomen, opažen kod ibuprofen hidroksamske kiseline u odnosu na sve druge ispitivane hidroksamske kiseline, može biti objašnjen i pripisan induktivnom učinku *i*-butilne skupine u *p*-položaju prema supstituentu 2-propilkarboksilne kiseline i njezinih derivata.

Tablica 15. Interakcije u NOESY i HMBC NMR spektrima **8b**,**s**,**d** i **u**, u dijelu koji se odnosi na profenski dio molekule, R-CH(CH_3)CONH-R'.

Spoj[a]	NOESY interakcija			HMBC interakcija	
	H-atom	DA	NE	DA	NE
8b	OH CH_3	H-2 H-5 i H-6	CH_3 H-8 i H-9	C=O i OH (s)[b]	C=O i NH
8s	CH_3	H-5 i H-6	H-8, H-9 i NH	n.i.[c]	C=O i NH
8d	OH NH	H-2 (s), CH_3 (w)[d] H-2 (s), CH_3 (w), H-5,6,7,9 (vw)[e]	n.i.	C=O i OH (vs)[f]	C=O i OH (vs)
8d	NH CH_3	H-2 (s), CH_3 (w), H-5,6,7,9 (vw), H-9 (s)	n.i.	C=O i NH (s)	n.i.

[a] Za strukture spojeva i numeraciju vidi Sliku 5. [b] s – Jaka interakcija; [c] n.i. – nije uočena interakcija bitna za geometriju molekule. [d] w – Slaba interakcija; [e] vw – vrlo slaba interakcija; [f] vs – vrlo jaka interakcija.

Snimljeni NMR spektri pokazali su da su sve ispitivane hidroksamske kiseline u keto-obliku. Rezultati NMR analiza u suglasju su s analizama IR spektara hidroksamskih kiselina. Predložene geometrije NSAID hidroksamskih kiselina i njihovih derivata (Slika 5) dobivene su na temelju rezultata analiza NOESY i HMBC spektara.



Slika 5. Geometrije molekula **8b**,**d**,**s** i **u** dobivene na temelju interakcija u NOESY i HMBC NMR spektrima.

5.1.2.4. Sinteza derivata ketoprofena 9a-i, 10a-f i 11a-f

U sklopu ranijih istraživanja na Zavodu za farmaceutsku kemiju sintetizirani su različiti amidi ketoprofena kao citostatici i potencijalni selektivni inhibitori ciklooksigenaze-2.[21,58] U ovom radu pripravljeni su amidi reduciranog ketoprofena **9** i **11**, te novi amidi ketoprofena **10**.

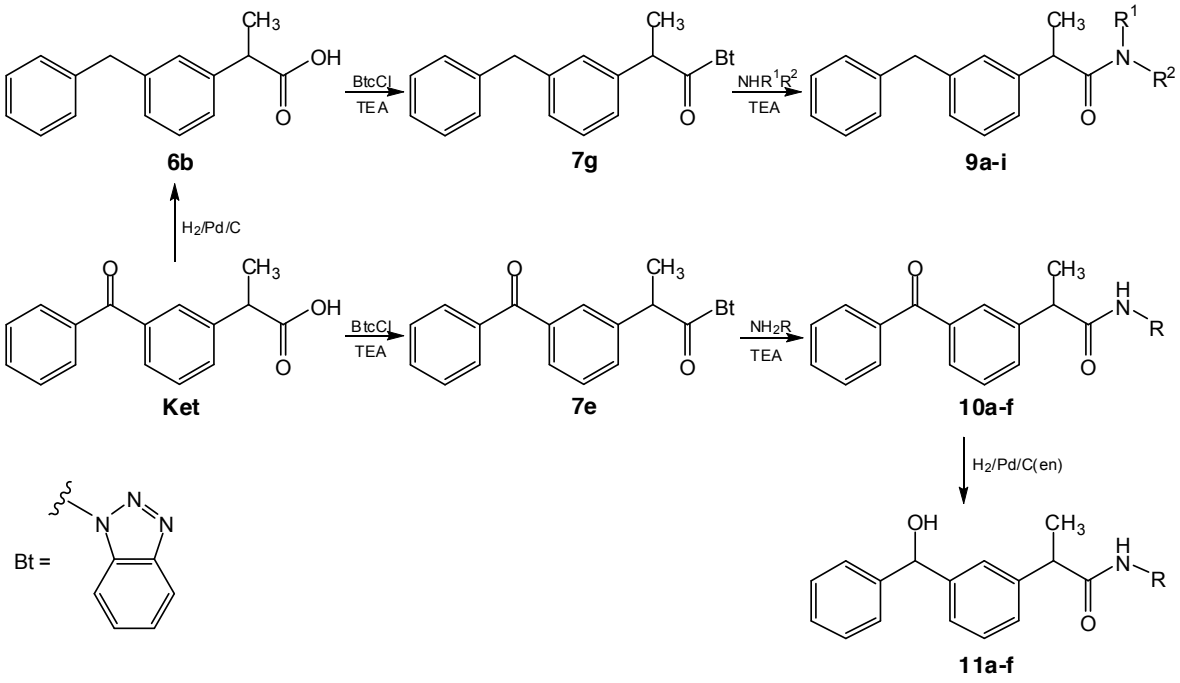
Amidi **9a-i** i **10a-f** pripravljeni su reakcijama amidiranja benzotriazolida **7e** i **7g** odgovarajućim aminima. One su izvedene u prisutnosti 5 ekvivalenata TEA u toluenu, na sobnoj temperaturi u vremenu od 1–8 h ili na 78 °C u vremenu od 11–16 h (Shema 43). Postupak se u odnosu na analogni literaturni[58] razlikuje time što je umjesto viška amina upotrebljen višak TEA. TEA s oslobođenim benzotriazolom stvara sol, te u isto vrijeme uzrokuje bazični pH tijekom ekstrakcije vodom, zbog čega benzotriazol lakše i kvantitativnije prelazi u vodeni sloj.

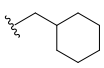
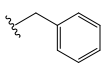
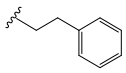
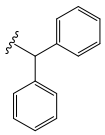
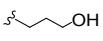
Sinteza spojeva **9a-e** i **10a-e** napredovala je vrlo dobro na sobnoj temperaturi u toluenu te su dobivena visoka iskorištenja. Općenito se priprava amida **10a-e** zbivala brže nego priprava amida **9a-e**. Nasuprot tome, sinteza amida **9f** i **10f** napredovala je vrlo sporo na sobnoj temperaturi, vjerojatno zbog steričkih smetnji u benzhidrilaminu. Na 78 °C (vrelište TEA) reakcije su se zbivale znatno brže.

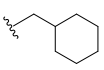
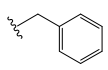
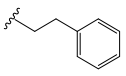
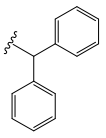
Reakcije s hidrofilnim bifunkcionalnim aminima (2-hidroksietilamin, 3-hidroksipropilamin, dietanolamin) provedene su u smjesi otapala toluen/etil-acetat, odnosno toluen/acetonitril zbog netopljivosti amina u toluenu. Za razliku od ranije opisanog postupka,[58] reakcija je provedena na sobnoj temperaturi, jer je utvrđeno da pri danim reakcijskim uvjetima hidroksilna skupina amina ne reagira. Sinteza **9i** (reakcija s dietanolaminom) napredovala je puno sporije od reakcija priprave **9f** i **9g** (reakcije s 2-hidroksietilaminom, odnosno 3-hidroksipropilaminom). Reakcije su provedene uz 1,1 ekvivalent amina i 1 ekvivalent TEA. Uzrok tome je drugačija obrada reakcijske smjese nakon završetka reakcije. Benzotriazol nije uklonjen ekstrakcijom već kromatografijom na koloni, jer je razlika u R_f vrijednosti benzotriazola i produkta dovoljno velika za njihovo uspješno razdvajanje.

Derivati ketoprofena **11a-f** sintetizirani su katalitičkim hidrogeniranjem spojeva **10a-f** uz 10%-tni Pd/C(en) kao katalizator (Shema 43). Reakcije su izvedene na sobnoj temperaturi kroz 5–9 h. Kao otapalo upotrebljen je metanol, jer u njemu reakcije napreduju brže nego u tetrahidrofuranu. Za razliku od reakcije katalitičkog hidrogeniranja spoja **8v** (vidi Shemu 42), daljnja hidrogenoliza nastale

hidroksilmetilenske skupine do metilenske skupine vrlo je spora te je reakciju moguće zaustaviti kada je nastala tek mala količina metilenskog nusprodukta (spojevi **9a-f**), koji je odvojen kromatografijom na koloni.



	9a	9b	9c	9d	9e	9f	9g	9h	9i
R^1									
R^2	H	H	H	H	H	H	H	H	

	10a, 11a	10b, 11b	10c, 11c	10d, 11d	10e, 11e	10f, 11f
R						

Shema 43. Sinteza derivata ketoprofena **9a-i**, **10a-f** i **11a-f**.

Priređeni su sljedeći amidi:

2-(3-benzilfenil)-*N*-ciklopentilpropanamid (**9a**),

2-(3-benzilfenil)-*N*-cikloheksilpropanamid (**9b**),

2-(3-benzilfenil)-*N*-(cikloheksanmetil)propanamid (**9c**),

N-benzil-2-(3-benzilfenil)propanamid (**9d**),

2-(3-benzilfenil)-*N*-fenetilpropanamid (**9e**),

N-benzhidril-2-(3-benzilfenil)propanamid (**9f**),

2-(3-benzilfenil)-*N*-(2-hidroksietil)propanamid (**9g**),

2-(3-benzilfenil)-*N*-(2-hidroksipropil)propanamid (**9h**),

2-(3-benzilfenil)-*N*,*N*-bis(2-hidroksietil)propanamid (**9i**),

2-(3-benzoilfenil)-*N*-ciklopentilpropanamid (**10a**),

2-(3-benzoilfenil)-*N*-cikloheksilpropanamid (**10b**),

2-(3-benzoilfenil)-*N*-(cikloheksanmetil)propanamid (**10c**),

N-benzil-2-(3-benzoilfenil)propanamid (**10d**),

2-(3-benzoilfenil)-*N*-fenetilpropanamid (**10e**),

N-benzhidril-2-(3-benzoilfenil)propanamid (**10f**),

N-ciklopentil-2-(3-(hidroksi(fenil)metil)fenil)propanamid (**11a**),

N-cikloheksil-2-(3-(hidroksi(fenil)metil)fenil)propanamid (**11b**),

N-cikloheksanmetil-2-(3-(hidroksi(fenil)metil)fenil)propanamid (**11c**),

N-benzil-2-(3-(hidroksi(fenil)metil)fenil)propanamid (**11d**),

2-(3-(hidroksi(fenil)metil)fenil)-*N*-fenetilpropanamid (**11e**),

N-benzhidril-2-(3-(hidroksi(fenil)metil)fenil)propanamid (**11f**).

Svi sintetizirani spojevi su novi, osim spojeva **10b**[58] i **10d**.[112] Priprava **10d** drugačijim sintetskim postupkom ranije je opisana, međutim reakcijski produkt nije izoliran niti karakteriziran.

Tijek sinteze i čistoća spojeva praćeni su tankoslojnom kromatografijom, a njihove strukture potvrđene su IR, ^{1}H i ^{13}C NMR spektroskopijom i elementarnom analizom (sve elementarne analize bile su unutar 0,4%).

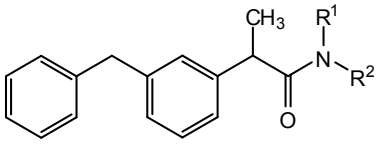
U IR spektrima prisutne su karakteristične vrpce za NH i za amidnu skupinu. U seriji spojeva **9a-i** NH se nalazi u području 3274–3312 cm^{-1}, a amidna karbonilna skupina u području 1635–1652 cm^{-1} (amid I) i 1529–1558 cm^{-1} (amid II). Kod spojeva **10a-f** NH se nalazi u području 3251–3355 cm^{-1}, a amidni karbonil u području 1634–1649 cm^{-1} (amid I) i 1526–1563 cm^{-1} (amid II). NH i OH skupine u spojevima **11a-f** prisutne su u području 3274–3414 cm^{-1}, te se kod nekih spojeva preklapaju. Isto tako, iz IR spektara spojeva **11a-f** vidljivo je da je OH skupina slobodna i ne dolazi do povezivanja vodikovim vezama. Amidna skupina javlja se u području 1641–1652 cm^{-1} (amid I) i 1531–1550 cm^{-1} (amid II).

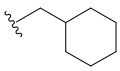
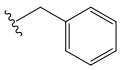
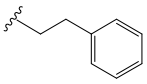
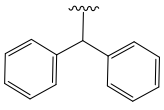
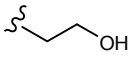
U ^{1}H NMR spektrima spojeva **9a-i** NH se javlja u području 7,74–7,97 ppm. Iznimka su spojevi **9d** (8,44 ppm) i **9f** (8,86 ppm), kod kojih supstituenti s fenilnim prstenovima otkrivaju NH skupinu te dolazi do kemijskog pomaka u niže polje. Taj učinak kod spoja **9e** nije tako izražen zbog udaljenosti fenilnog prstena. U seriji **10a-f** NH se javlja u području 7,91–8,11 ppm, s iznimkom spojeva **10d** (8,56 ppm) i **10f** (8,98 ppm). NH skupina u spojevima **11a-f** nalazi se u području 7,74–8,04 ppm, s nešto višim vrijednostima kod spojeva **11d** (8,46 ppm) i **11f** (8,88 ppm). OH skupina javlja se u području 5,84–5,91 ppm.

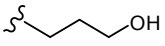
Karbonilna skupina u položaju 2 u ^{13}C NMR spektrima svih sintetiziranih amida nalazi se od 172,29 do 173,82 ppm. Za pojedinu seriju spojeva karakteristični su kemijski pomaci parnih aromatskih ugljikovih atoma. Vrijednosti tih kemijskih pomaka za spojeve **9a-i** su ≈ 143,0, 141,7 i 141,4 ppm, za spojeve **10a-f** ≈ 143,3, 137,5 i 137,3 ppm, te za spojeve **11a-f** ≈ 146,1, 146,0 i 142,7 ppm.

Analitički i spektroskopski podaci dani su u Tablicama 16–24.

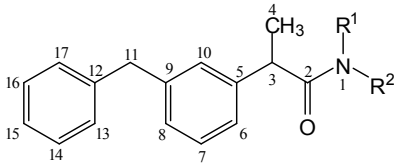
Tablica 16. Analitički i IR podaci za derivate ketoprofena **9a-i**.

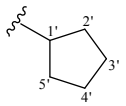
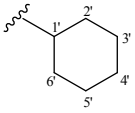
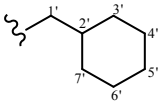
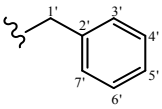
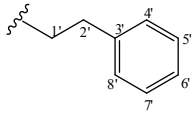


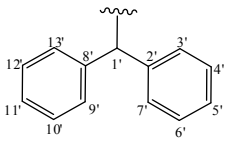
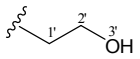
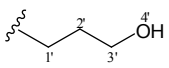
Spoj	R^1	R^2	Iskor. (%)	t_t (°C)	Molekulska formula (M_r)	IR (KBr/film) ν_{max} (cm^{-1})
9a		H	82	92–93	$C_{21}H_{25}NO$ (307,43)	3274, 3061, 3027, 2962, 2936, 2864, 1635, 1599, 1548, 1448, 785, 731, 711, 699
9b		H	85	89–93	$C_{22}H_{27}NO$ (321,46)	3297, 3062, 3026, 2980, 2936, 2854, 1639, 1585, 1546, 1448, 779, 750, 698
9c		H	82	50–54	$C_{23}H_{29}NO$ (335,48)	3295, 3061, 3027, 2968, 2918, 2851, 1647, 1602, 1585, 1558, 1442, 1221, 1155, 711, 698
9d		H	88	70–74	$C_{23}H_{23}NO$ (329,43)	3292, 3062, 3028, 2971, 2930, 1648, 1602, 1546, 1495, 1453, 722, 697
9e		H	86	60–64	$C_{24}H_{25}NO$ (343,46)	3306, 3064, 3026, 2925, 1642, 1548, 1496, 1454, 753, 712, 698
9f		H	70	122–123	$C_{29}H_{27}NO$ (405,53)	3297, 3087, 3064, 3025, 2979, 1667, 1641, 1601, 1586, 1529, 1494, 1452, 757, 741, 718, 703
9g		H	84	ulje	$C_{18}H_{21}NO_2$ (283,36)	3414, 3312, 3084, 3027, 2972, 2931, 1648, 1601, 1560, 1540, 1494, 1453, 1363, 1072, 723, 700

9h		H	77	ulje	$C_{19}H_{23}NO_2$ (297,39)	3301, 3062, 3027, 2970, 2935, 2877, 1652, 1601, 1558, 1548, 1494, 1453, 721, 699
9i			76	ulje	$C_{20}H_{25}NO_3$ (327,42)	3370, 3060, 3026, 2931, 1622, 1494, 1473, 1454, 1442, 1061, 745, 699

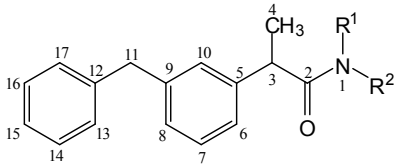
Tablica 17. ^{1}H NMR podaci za derivate ketoprofena **9a-i**.

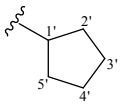
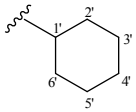


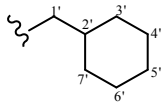
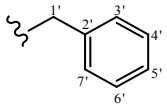
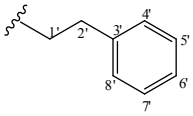
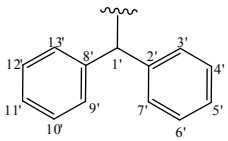
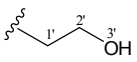
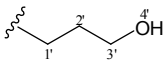
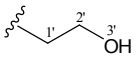
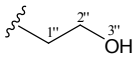
Spoj	R^1	R^2	^{1}H NMR (DMSO-d_6, δ ppm, J/Hz)
9a		H	7,85 (d, 1H, 1, J = 7,15), 7,30–7,04 (m, 9H, arom.), 3,99–3,87 (m, 3H, 11, 1'), 3,52 (q, 1H, 3, J = 7,01), 1,83–1,16 (m, 8H, 2'–5'), 1,27 (d, 3H, 4, J = 7,03)
9b		H	7,74 (d, 1H, 1, J = 7,79), 7,29–7,04 (m, 9H, arom.), 3,90 (s, 2H, 11), 3,52 (q, 1H, 3, J = 7,04), 3,49–3,40 (m, 1H, 1'), 1,73–1,54, 1,21-0,93 (2m, 8H, 2'–6'), 1,27 (d, 3H, 4, J = 7,03)
9c		H	7,84 (t, 1H, 1, J = 5,31), 7,30–7,03 (m, 9H, arom.), 3,90 (s, 2H, 11), 3,56 (q, 1H, 3, J = 6,97), 2,85 (dd, 2H, 1', J = 6,11, J = 6,50), 1,66–1,51, 1,40–1,24, 1,17–1,04, 0,84–0,72 (4m, 4H, 3'–7'), 1,28 (d, 3H, 4, J = 7,02)
9d		H	8,44 (t, 1H, 1, J = 5,65), 7,31–7,06 (m, 14H, arom.), 4,24 (d, 2H, 1', J = 5,52), 3,91 (s, 2H, 11), 3,64 (q, 1H, 3, J = 6,96), 1,34 (d, 3H, 4, J = 7,01)
9e		H	7,97 (t, 1H, 1, J = 5,43), 7,29–7,05 (m, 14H, arom.), 3,91 (s, 2H, 11), 3,52 (q, 1H, 3, J = 7,00), 3,29–3,16 (m, 2H, 1'), 2,64 (t, 2H, 2', J = 7,23), 1,27 (d, 3H, 4, J = 7,04)

9f		H	8,86 (d, 1H, 1, *J* = 8,45), 7,36–7,04 (m, 19H, arom.), 6,07 (d, 1H, 1', *J* = 8,45), 3,90 (s, 2H, 11), 3,80 (q, 1H, 3, *J* = 6,94), 1,32 (d, 3H, 4, *J* = 7,00)
9g		H	7,92 (t, 1H, 1, *J* = 5,24), 7,31–7,04 (m, 9H, arom.), 4,64 (t, 1H, 3', *J* = 5,36), 3,91 (s, 2H, 11), 3,57 (q, 1H, 3, *J* =7,03), 3,39–3,33 (m, 2H, 2'), 3,17–3,01 (m, 2H, 1'), 1,28 (d, 3H, 4, *J* = 7,03)
9h		H	7,89 (t, 1H, 1, *J* = 5,29), 7,31–7,04 (m, 9H, arom.), 4,38 (t, 1H, 4'), 3,91 (s, 2H, 11), 3,53 (q, 1H, 3, *J* = 7,00), 3,34 (q, 2H, 1', *J* = 6,19), 3,06 (q, 2H, 3', *J* = 6,51), 1,54–1,45 (m, 2H, 2'), 1,28 (d, 3H, 4, *J* = 7,04)
9i			7,31–7,04 (m, 9H, arom.), 4,82 (s, 1H, 3'), 4,46 (s, 1H, 3''), 4,10 (q, 1H, 3, *J* = 6,74), 3,91 (s, 2H, 11), 3,56–3,34, 3,19–3,10 (m, 8H, 1', 2', 1'', 2''), 1,24 (d, 3H, 4, *J* = 6,77)

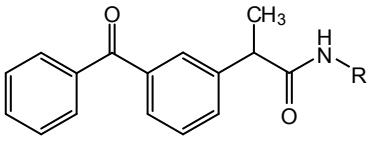
Tablica 18. ^{13}C NMR podaci za derivate ketoprofena **9a-i**.

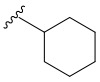
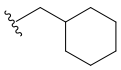
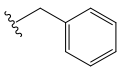
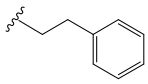
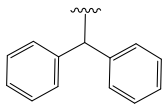


Spoj	R^1	R^2	^{13}C NMR (DMSO-d_6, δ ppm)
9a		H	173,02 (2), 143,11, 141,69, 141,42 (5, 9, 12), 129,09, 128,83, 128,68, 128,03, 127,21, 126,39, 125,26 (6–8, 10, 13–17), 50,64 (1'), 45,22 (3), 41,67 (11), 32,68, 32,63 (2', 5'), 23,96, 23,92 (3', 4'), 19,01 (4)
9b		H	172,59 (2), 143,15, 141,69, 141,41 (5, 9, 12), 129,10, 128,82, 128,67, 128,00, 127,19, 126,37, 125,26 (6–8, 10, 13–17), 47,84 (1'), 45,27 (3), 41,66 (11), 32,81, 32,78 (2', 6'), 25,69, 25,04, 24,96 (3'–5'), 19,00 (4)

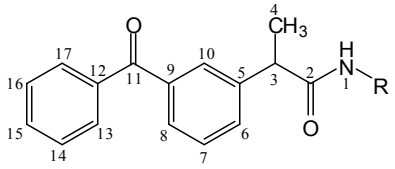
9c		H	173,55 (2), 143,10, 141,67, 141,36 (5, 9, 12), 129,07, 128,83, 128,66, 128,11, 127,27, 126,39, 125,31 (6–8, 10, 13–17), 45,46 (3), 45,24 (1'), 41,65 (11), 37,88 (2'), 30,81, 30,77 (2', 7'), 26,49, 25,85 (4'–6'), 19,02 (4)
9d		H	173,69 (2), 142,88, 141,66, 141,48 (5, 9, 12), 140,00 (2'), 129,11, 128,86, 128,67, 128,16, 127,23, 126,41, 125,41 (6–8, 10, 13–17), 128,76, 127,51, 127,41, 127,14 (3'–7'), 45,52 (3), 42,48 (1'), 41,64 (11), 19,04 (4)
9e		H	173,53 (2), 142,90, 141,67, 141,47 (5, 9, 12), 139,91 (3'), 129,13, 128,86, 128,71, 128,12, 127,30, 126,40, 125,40 (6–8, 10, 13–17), 129,10, 128,67, 126,44 (4'–8'), 45,50 (3), 41,66 (11), 40,83 (1'), 35,37 (2'), 19,02 (4)
9f		H	172,97 (2), 142,95, 141,67, 141,40 (5, 9, 12), 142,86, 142,74 (2', 8'), 129,09, 128,85, 128,72, 128,23, 127,28, 126,40, 125,38 (6–8, 10, 13–17), 128,68, 127,85, 127,55, 127,41, 127,40 (3'–7', 9'–13'), 56,28 (1'), 45,06 (3), 41,65 (11), 18,86 (4)
9g		H	173,81 (2), 143,11, 141,67, 141,47 (5, 9, 12), 129,13, 128,86, 128,71, 128,14, 127,27, 126,41, 125,37 (6–8, 10, 13–17), 60,30 (1'), 45,40 (3), 41,98 (2'), 41,65 (11), 19,21 (4)
9h		H	173,64 (2), 143,02, 141,66, 141,47 (5, 9, 12), 129,12, 128,86, 128,72, 128,08, 127,28, 126,40, 125,30 (6–8, 10, 13–17), 58,80 (1'), 45,50 (3), 41,65 (11), 36,20 (3'), 32,83 (2'), 19,07 (4)
9i			173,73 (2), 143,10, 141,89, 141,63 (5, 9, 12), 129,09, 128,86, 128,00, 127,32, 126,40, 125,40 (6–8, 10, 13–17), 59,73, 59,07 (1', 1''), 50,64, 48,93 (2', 2''), 41,64 (11), 41,60 (3), 21,26 (4)

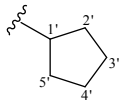
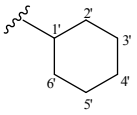
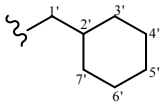
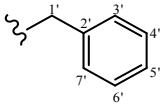
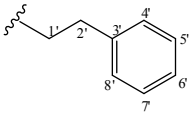
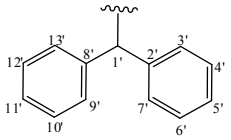
Tablica 19. Analitički i IR podaci za derivate ketoprofena **10a-f**.



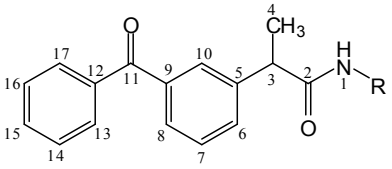
Spoj	R	Iskor. (%)	t_t (°C)	Molekulska formula (M_r)	IR (KBr) ν_{max} (cm^{-1})
10a		80	94–95	$C_{21}H_{23}NO_2$ (321,41)	3355, 3066, 2964, 2870, 1663, 1648, 1596, 1528, 1449, 1312, 1284, 723, 702, 642
10b		77	120–121	$C_{22}H_{25}NO_2$ (335,44)	3355, 3067, 2976, 2936, 2851, 1660, 1648, 1596, 1526, 1448, 1311, 1285, 1220, 768, 722, 701, 644
10c		90	96–98	$C_{23}H_{27}NO_2$ (349,47)	3298, 3094, 2919, 2853, 1676, 1660, 1642, 1597, 1563, 1447, 1289, 1271, 1234, 729, 707, 640
10d		83	97–99	$C_{23}H_{21}NO_2$ (343,42)	3314, 3287, 3066, 3033, 2926, 2870, 1644, 1551, 1449, 1349, 1015, 712, 701
10e		92	66–69	$C_{24}H_{23}NO_2$ (357,44)	3255, 3062, 2936, 2876, 1661, 1634, 1599, 1559, 1543, 1448, 1288, 748, 702
10f		73	115–119	$C_{29}H_{25}NO_2$ (419,51)	3316, 3251, 3057, 3030, 2932, 1656, 1649, 1598, 1528, 1494, 1320,1285, 723, 704, 696

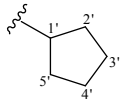
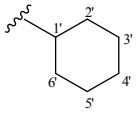
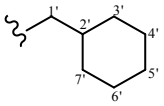
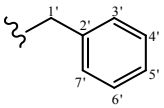
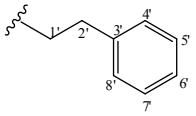
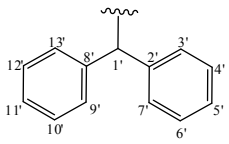
Tablica 20. ^{1}H NMR podaci za derivate ketoprofena **10a-f**.



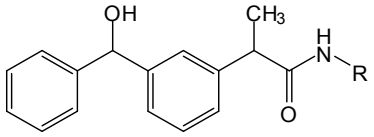
Spoj	R	^{1}H NMR (DMSO-d_6, δ ppm, J/Hz)
10a		7,99 (d, 1H, 1, J = 7,01), 7,72–7,45 (m, 9H, arom.), 3,99–3,87 (m, 1H, 1'), 3,66 (q, 1H, 3, J = 6,73), 1,83–1,17 (m, 8H, 2'–5'), 1,32 (d, 3H, 4, J = 7,00)
10b		7,91 (d, 1H, 1, J = 7,97), 7,74–7,47 (m, 9H, arom.), 3,69 (q, 1H, 3, J = 7,02), 3,54–3,42 (m, 1H, 1'), 1,76–1,51, 1,27–0,98 (2m, 10H, 2'–6'), 1,34 (d, 3H, 4, J = 7,07)
10c		7,98 (t, 1H, 1, J = 7,98), 7,74–7,47 (m, 9H, arom.), 3,72 (q, 1H, 3, J = 6,99), 2,87 (dd, 2H, 1', J = 6,18, J = 4,38), 1,67–1,51, 1,41–1,30, 1,18–1,03, 0,84–0,73 (4m, 4H, 3'–7'), 1,35 (d, 3H, 4, J = 7,00)
10d		8,56 (t, 1H, 1, J = 5,75), 7,76–7,49 (m, 9H, arom.), 7,29–7,14 (m, 5H, arom.), 4,25 (d, 2H, 1', J = 5,84), 3,80 (q, 1H, 3, J = 7,03), 1,40 (d, 3H, 4, J = 7,03)
10e		8,11 (t, 1H, 1, J = 5,45), 7,75–7,48 (m, 9H, arom.), 7,24–7,07 (m, 5H, arom.), 3,69 (q, 1H, 3, J = 6,94), 3,32–3,18 (m, 2H, 1'), 2,66 (t, 2H, 2', J = 7,13), 1,34 (d, 3H, 4, J = 7,02)
10f		8,98 (d, 1H, 1, J = 8,46), 7,78–7,48 (m, 9H, arom.), 7,37–7,08 (m, 10H, arom.), 6,08 (d, 1H, 1', J = 8,40), 3,96 (q, 1H, 3, J = 6,99), 1,39 (d, 3H, 4, J = 7,01)

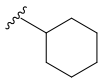
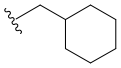
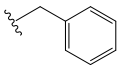
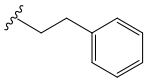
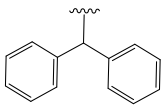
Tablica 21. ^{13}C NMR podaci za derivate ketoprofena **10a-f**.



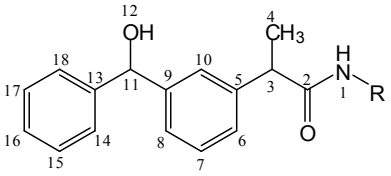
Spoj	R	^{13}C NMR (DMSO-d_6, δ ppm)
10a		196,23 (11), 172,71 (2), 143,34, 137,53, 137,28 (5, 9, 12), 133,14, 131,99, 130,04, 129,03, 128,97, 128,88, 128,41 (6–8, 10, 13–17), 50,73 (1'), 45,08 (3), 32,68 (2', 5'), 23,94, 23,90 (3', 4'), 18,95 (4)
10b		196,23 (11), 172,29 (2), 143,40, 137,55, 137,29 (5, 9, 12), 133,13, 131,98, 130,05, 129,02, 128,96, 128,88, 128,37 (6–8, 10, 13–17), 47,93 (1'), 45,14 (3), 32,98 (2', 6'), 25,67, 25,00, 24,92 (3'–5'), 18,96 (4)
10c		196,22 (11), 173,24 (2), 143,41, 137,53, 137,30 (5, 9, 12), 133,17, 132,00, 130,04, 129,03, 128,92, 128,44 (6–8, 10, 13–17), 45,30 (3), 45,26 (1'), 37,89 (2'), 30,78, 30,75 (3', 7'), 26,46, 25,84 (4'–6'), 18,92 (4)
10d		196,22 (11), 173,37 (2), 143,15, 137,51, 137,38 (5, 9, 12), 139,86 (2'), 133,17, 132,08, 130,07, 129,04, 129,02, 128,58 (6–8, 10, 13–17), 128,70, 127,50, 127,21 (3'–7'), 45,36 (3), 42,54 (1'), 18,91 (4)
10e		196,22 (11), 173,22 (2), 143,19, 137,54, 137,32 (5, 9, 12), 139,85 (2'), 133,16, 132,09, 130,08, 129,04, 128,99, 128,97, 128,52 (6–8, 10, 13–17), 129,10, 128,64, 126,44 (4'–8'), 45,33 (3), 40,71 (1'), 35,40 (2'), 18,91 (4)
10f		196,23 (11), 172,64 (2), 143,01, 137,51, 137,35 (5, 9, 12), 142,80, 142,77 (2', 8'), 133,16, 132,07, 130,05, 129,03, 128,98, 128,53 (6–8, 10, 13–17), 128,88, 128,68, 127,86, 127,45, 127,33 (3'–7', 9'–13'), 56,36 (1'), 44,98 (3), 18,81 (4)

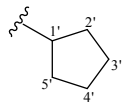
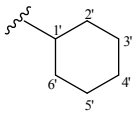
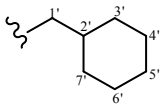
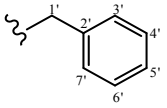
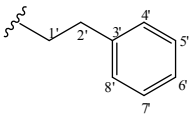
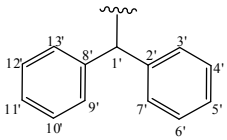
Tablica 22. Analitički i IR podaci za derivate ketoprofena **11a-f**.



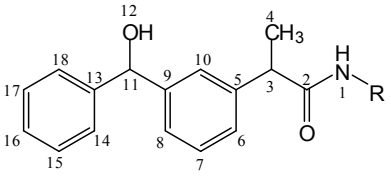
Spoj	R	Iskor. (%)	t_t (°C)	Molekulska formula (M_r)	IR (KBr/film) ν_{max} (cm^{-1})
11a		78	120–125	$C_{21}H_{25}NO_2$ (323,19)	3274, 3062, 3031, 2964, 2933, 2866, 1645, 1603, 1549, 1453, 1231, 1195, 1021, 730, 701
11b		75	94–98	$C_{22}H_{27}NO_2$ (337,46)	3366, 3308, 3058, 2973, 2924, 2855, 1641, 1603, 1539, 1449, 1015, 712, 701
11c		80	ulje	$C_{23}H_{29}NO_2$ (351,48)	3312, 3063, 2925, 2852, 1649, 1604, 1550, 1493, 1450, 1025, 731, 701
11d		82	100–103	$C_{23}H_{21}NO_2$ (343,42)	3331, 3285, 1648, 1603, 1539, 1494, 1454, 1238, 1017, 730, 701
11e		73	ulje	$C_{24}H_{25}NO_2$ (359,46)	3414, 3312, 3062, 3028, 2973, 2932, 1652, 1603, 1558, 1540, 1522, 1454, 1368, 1239, 1025, 750, 700
11f		90	51–55	$C_{29}H_{27}NO_2$ (421,53)	3410, 3304, 3062, 3029, 2975, 2930, 1649, 1602, 1531, 1494, 1451, 1028, 743, 699

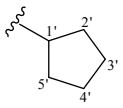
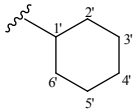
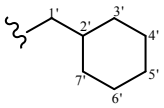
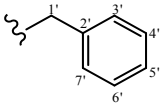
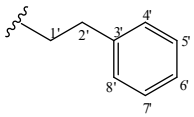
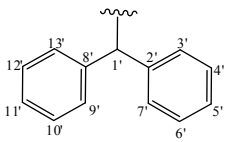
Tablica 23. ^{1}H NMR podaci za derivate ketoprofena **11a-f**.



Spoj	R	^{1}H NMR (DMSO-d_6, δ ppm, J/Hz)
11a		7,86 (d, 1H, 1, J = 6,92), 7,36–7,13 (m, 9H, arom.), 5,86 (d, 1H, 12, J = 3,83), 5,65 (d, 1H, 11, J = 3,04), 3,98–3,87 (m, 1H, 1'), 3,54 (q, 1H, 3, J = 6,94), 1,81–1,18 (m, 8H, 2'–5'), 1,27 (d, 3H, 4, J = 6,99)
11b		7,74 (dd, 1H, 1, J = 5,24, J = 2,39), 7,35–7,11 (m, 9H, arom.), 5,84 (d, 1H, 12, J = 3,84), 5,64 (d, 1H, 11, J = 3,57), 3,53 (q, 1H, 3, J = 6,98), 3,48–3,38 (m, 1H, 1'), 1,72–1,46, 1,27–0,92 (2m, 10H, 2'–6'), 1,25 (d, 3H, 4, J = 6,56)
11c		7,87 (t, 1H, 1, J = 5,56), 7,37–7,14 (m, 9H, arom.), 5,86 (dd, 1H, 12, J = 2,84, J = 1,05), 5,65 (d, 1H, 11, J = 3,50), 3,58 (q, 1H, 3, J = 7,01), 2,85 (t, 2H, 1', J = 6,26), 1,68–1,51, 1,39–1,24, 1,19–1,05, 0,89–0,73 (4m, 4H, 3'–7'), 1,28 (d, 3H, 4, J = 6,85)
11d		8,46 (t, 1H, 1, J = 5,57), 7,41–7,15 (m, 15H, arom.), 5,88 (d, 1H, 12, J = 3,21), 5,66 (s, 1H, 11), 4,24 (d, 2H, 1', J = 5,74), 3,66 (q, 1H, 3, J = 6,97), 1,34 (d, 3H, 4, J = 6,97)
11e		8,04 (t, 1H, 1, J = 5,47), 7,42–7,14 (m, 14H, arom.), 5,91 (dd, 1H, 12, J = 3,25, J = 0,63), 5,70 (d, 1H, 11, J = 3,75), 3,58 (q, 1H, 3, J = 6,93), 3,26 (q, 2H, 1', J = 7,22), 2,68 (t, 2H, 2', J = 7,28), 1,32 (d, 3H, 4, J = 6,89)
11f		8,88 (d, 1H, 1, J = 8,39), 7,44–7,10 (m, 19H, arom.), 6,06 (d, 1H, 1', J = 8,38), 5,87 (d, 1H, 12, J = 3,86), 5,65 (d, 1H, 11, J = 3,19), 3,82 (q, 1H, 3, J = 6,78), 1,32 (d, 3H, 4, J = 6,20)

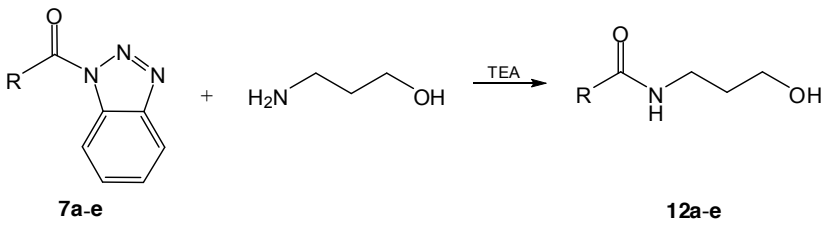
Tablica 24. ^{13}C NMR podaci za derivate ketoprofena **11a-f**.



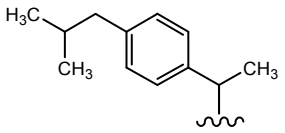
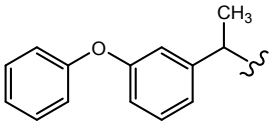
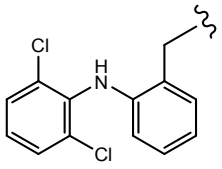
Spoj	R	^{13}C NMR (DMSO-d_6, δ ppm)
11a		173,00 (2), 146,16, 145,99, 142,76 (5, 9, 13), 128,47, 128,29, 127,11, 126,69, 126,01, 125,56, 124,81 (6–8, 10, 14–18), 74,84 (11), 50,63 (1'), 45,29 (3), 32,69, 32,65 (2', 5'), 23,97, 23,93 (3', 4'), 19,10 (4)
11b		172,59 (2), 146,16, 145,98, 142,82 (5, 9, 13), 128,45, 128,28, 127,11, 126,71, 126,02, 125,55, 124,81 (6–8, 10, 14–18), 74,84 (11), 47,83 (1'), 45,34 (3), 32,83, 32,80 (2', 6'), 25,70 (3'), 25,05 (5'), 24,98 (4'), 19,10 (4)
11c		173,58 (2), 146,15, 145,93, 142,71 (5, 9, 13), 128,47, 128,28, 127,13, 126,69, 126,03, 125,62, 124,90 (6–8, 10, 14–18), 74,82 (11), 45,53 (3), 45,28 (1'), 37,87 (2'), 30,83 (3'), 30,81 (7'), 26,49 (4'), 25,85 (5', 6'), 19,13 (4)
11d		173,71 (2), 146,11, 146,02, 142,50 (5, 9, 13), 139,96 (2'), 128,69, 127,53 (3'–7'), 128,50, 128,38, 127,14, 126,73, 126,09, 125,67, 124,04 (6–8, 10, 14–18), 74,83 (11), 45,59 (3), 42,51 (1'), 19,11 (4)
11e		173,55 (2), 146,16, 146,02, 142,55 (5, 9, 13), 139,90 (3'), 129,10, 128,71, 126,45 (4'–8'), 128,49, 128,34, 127,14, 126,73, 126,17, 125,63, 124,95 (6–8, 10, 14–18), 74,83 (11), 45,58 (3), 40,74 (1'), 35,53 (2'), 19,12 (4)
11f		173,00 (2), 146,13, 145,97, 142,82 (5, 9, 13), 143,01, 142,36 (2', 8'), 128,85, 128,70, 127,81, 127,58, 127,55, 127,41, 127,26 (3'–7', 9'–13'), 128,49, 128,33, 127,13, 126,73, 126,05, 125,76, 125,04 (6–8, 10, 14–18), 74,83 (11), 56,30 (1'), 45,14 (3), 18,95 (4)

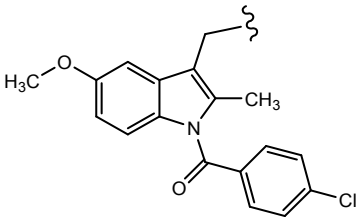
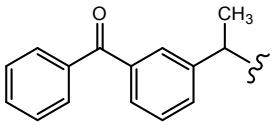
5.1.2.5. Sinteza 3-hidroksipropilamida NSAID 12a-e

3-Hidroksipropilamidi **12a-e** sintetizirani su iz NSAID benzotriazolida **7a-e** reakcijom nukleofilne supstitucije s 3-hidroksipropilaminom (Shema 44). Reakcija se izvodi uz 1,1 ekvivalent amina i 1 ekvivalent TEA u smjesi otapala toluen/etil-acetat, na sobnoj temperaturi tijekom 1 h.



7a-e **12a-e**

	7a, 12a	**7b, 12b**	**7c, 12c**
R			

	7d, 12d	**7e, 12e**
R		

Shema 44. Sinteza 3-hidroksipropilamida NSAID **12a-e**.

Priređeni su sljedeći spojevi:

N-(3-hidroksipropil)-2-(4-izobutilfenil)propanamid (**12a**)**,**

2-(3-fenoksifenil)-*N*-(3-hidroksipropil)propanamid (**12b**)**,**

N-(3-hidroksipropil)-2-(2-(2,6-diklorfenilamino)fenil)acetamid (**12c**)**,**

N-(3-hidroksipropil)-1-(4-klorbenzoil)-2-metil-5-metoksi-1*H*-indol-3-acetamid (**12d**)**,**

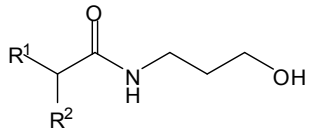
2-(3-benzoilfenil)-*N*-(3-hidroksipropil)propanamid (**12e**).

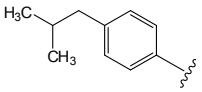
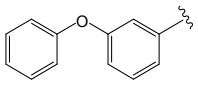
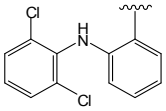
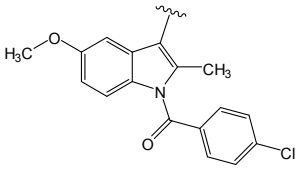
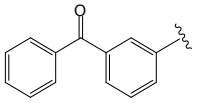
Tijek sinteze i čistoća spojeva praćeni su tankoslojnom kromatografijom, a njihove strukture potvrđene su IR, ^{1}H i ^{13}C NMR spektroskopijom i elementarnom analizom (sve elementarne analize bile su unutar 0,4%).

IR spektri 3-hidroksipropilamida NSAID **12a-e** pokazuju karakteristične vrpce kod 3299–3495 (NH i OH), 1618–1652 (amid I) i 1546–1582 (amid II) cm^{-1}. U ^{1}H NMR spektrima NH se javlja u području 7,88–8,35 ppm, a OH u području 4,39–4,48 ppm. Karbonilna skupina u položaju 1 u ^{13}C NMR spektrima nalazi se u području 169,82–173,52 ppm.

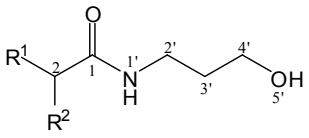
Od sintetiziranih spojeva novi su **12a** i **12c**. Analitički i spektroskopski podaci spojeva **12b,d**,**e** (osim tališta spoja **12d**) podudaraju se s ranije sintetiziranim spojevima,[58,59,96,100] te su zajedno s podacima za nove spojeve dani u Tablicama 25–27.

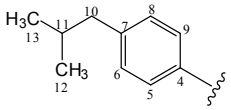
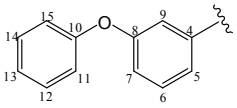
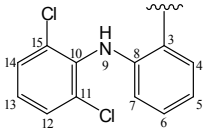
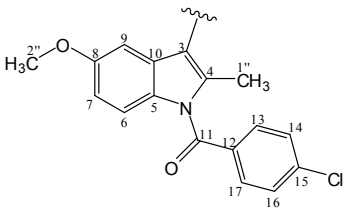
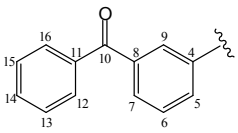
Tablica 25. Analitički i IR podaci za 3-hidroksipropilamide NSAID **12a-e**.



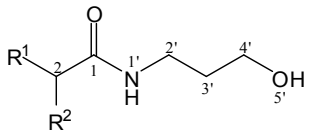
Spoj	R^1	R^2	Iskor. (%)	t_t (°C)	Molekulska formula (M_r)	IR (KBr/film) ν_{max} (cm^{-1})
12a		CH_3	75	ulje	$C_{16}H_{25}NO_2$ (263,38)	3299, 3088, 2954, 2932, 2869, 1652, 1548, 1512, 1065, 849
12b		CH_3	82	ulje	$C_{18}H_{21}NO_3$ (299,36)	3400, 3298, 2937, 1648, 1582, 1486, 1443, 1246, 1209, 1071, 926, 757, 693
12c		H	96	130–133	$C_{17}H_{18}Cl_2N_2O_2$ (353,24)	3495, 3432, 3297, 3253, 3082, 2947, 2882, 1618, 1565, 1508, 1453, 1286, 1070, 775, 747
12d		H	60	130–131	$C_{22}H_{23}ClN_2O_4$ (414,88)	3428, 3314, 3093, 2928, 2884, 1673, 1620, 1561, 1478, 1317, 1071, 835, 755
12e		CH_3	80	ulje	$C_{19}H_{21}NO_3$ (311,38)	3301, 3064, 2936, 2876, 1651, 1597, 1546, 1448, 1319, 1285, 1237, 722, 705

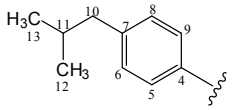
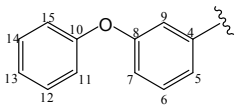
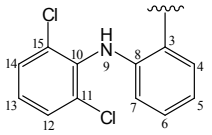
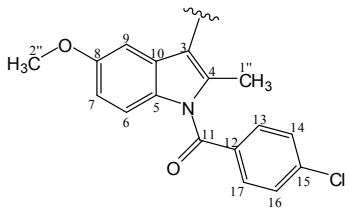
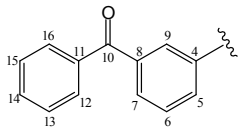
Tablica 26. ^{1}H NMR podaci za 3-hidroksipropilamide NSAID **12a-e**.



Spoj	R^1	R^2	^{1}H NMR (DMSO-d_6, δ ppm, J/Hz)
12a		$^3CH_3$	7,88 (t, 1H, 1', J = 5,40), 7,21 (d, 2H, arom., J = 8,03), 7,07 (d, 2H, arom., J = 8,10), 4,39 (t, 1H, 5', J = 5,25), 3,54 (q, 1H, 2, J = 7,04), 3,35 (q, 2H, 4', J = 6,27), 3,13–3,01 (m, 2H, 2'), 2,40 (d, 2H, 10, J = 7,12), 1,87–1,74 (m, 1H, 11), 1,56–1,47 (m, 2H, 3'), 1,30 (d, 3H, 3, J = 7,05), 0,86 (d, 6H, 12, 13, J = 6,61)
12b		$^3CH_3$	8,01 (bs, 1H, 1'), 7,38 (t, 2H, arom., J = 7,62), 7,29 (t, 1H, arom., J = 7,83), 7,13 (t, 1H, arom., J = 7,11), 7,07 (d, 1H, arom., J = 7,80), 6,98 (s, 3H, arom.), 6,83 (d, 1H, arom. J = 7,80), 4,48 (bs, 1H, 5'), 3,57 (q, 1H, 2, J = 7,14), 3,34 (t, 2H, 4', J = 6,00), 3,06 (t, 2H, 2', J = 5,83), 1,49 (t, 2H, 3', J = 6,49), 1,28 (d, 3H, 3, J = 6,81)
12c		H	8,45 (s, 1H, 9), 8,35 (t, 1H, 1', J = 5,27), 7,51 (d, 2H, arom., J = 8,06), 7,20–7,12 (m, 2H, arom.), 7,06–7,01 (m, 1H, arom.), 6,87–6,82 (m, 1H, arom.), 6,29 (d, 1H, arom., J = 7,81), 4,45 (t, 1H, 5', J = 5,16), 3,57 (s, 1H, 2), 3,42 (q, 2H, 4', J = 5,90), 3,13 (q, 2H, 2', J = 6,49), 1,62–1,53 (m, 2H, 3')
12d		H	8,02 (t, 1H, 1', J = 5,24), 7,69 (d, 3H, arom., J = 8,63), 7,64 (d, 2H, J = 8,38), 7,12 (d, 1H, arom., J = 1,96), 6,95 (d, 1H, arom., J = 9,03), 6,71 (dd, 1H, arom., J = 2,09, J = 6,80), 4,43 (t, 1H, 5', J = 5,10), 3,77 (s, 3H, 2''), 3,49 (s, 2H, 2), 3,40 (q, 2H, 4', J = 5,79), 3,12 (q, 2H, 2', J = 6,39), 2,23 (s, 3H, 1''), 1,60–1,51 (m, 2H, 3')
12e		$^3CH_3$	8,06 (t, 1H, 1', J = 5,30), 7,47–7,40 (m, 9H, arom.), 4,43 (t, 1H, 5', J = 4,90), 3,70 (q, 1H, 2, J = 6,70), 3,36 (q, 2H, 4', J = 4,90), 3,08 (q, 2H, 2', J = 6,60), 1,56–1,47 (m, 2H, 3'), 1,35 (d, 3H, 3, J = 6,89)

Tablica 27. ^{13}C NMR podaci za 3-hidroksipropilamide NSAID **12a-e**.



Spoj	R^1	R^2	^{13}C NMR (DMSO-d_6, δ ppm)
12a		$^3CH_3$	173,52 (1), 139,79, 139,25 (4, 7), 128,85, 127,03 (5, 6, 8, 9), 58,46 (4'), 44,86 (2), 44,37 (10), 35,87 (2'), 32,50 (3'), 29,75 (11), 22,32 (12, 13), 18,74 (3)
12b		$^3CH_3$	173,06 (1), 156,72, 156,56, 144,77 (4, 8, 10), 130,16, 129,87, 123,51, 122,48, 118,67, 117,63, 116,70 (5–7, 9, 11–15), 58,41 (4'), 45,02 (2), 35,86 (2'), 32,46 (3'), 18,57 (3)
12c		H	172,03 (1), 143,43, 137,65, 129,83, 126,03 (3, 8, 10, 11, 15), 130,82, 129,65, 127,63, 125,44, 121,11, 116,40 (4–7, 12–14), 58,77 (4'), 40,04 (2'), 36,45 (3'), 32,67 (2)
12d		H	169,82 (1), 168,31 (11), 156,01, 138,01, 135,56, 134,74, 131,35, 130,74, 114,90 (3–5, 8, 10, 12, 15), 131,61, 129,49, 115,02, 111,75, 102,25 (6, 7, 9, 13, 14, 16, 17), 58,85 (4'), 55,87 (2''), 36,41 (2'), 32,89 (3'), 31,66 (2), 13,84 (1'')
12e		$^3CH_3$	195,66 (10), 172,76 (1), 142,75 136,97, 136,77 (4, 8, 11), 132,59, 131,48, 129,51, 128,48, 128,43, 128,35, 127,94 (5–7, 9, 12–16), 58,22 (4'), 44,78 (2), 35,71 (2'), 32,25 (3'), 18,44 (3)

5.2. BIOLOŠKA ISPITIVANJA

5.2.1. ANTITUMORSKA ISPITIVANJA

Antitumorska ispitivanja provedena su na Institutu Rudjer Bošković i na Rega Institute for Medical Research, Katholieke Universiteit Leuven, Belgija, te je eksperimentalna metoda ispitivanja opisana ranije.[113]

5.2.1.1. 3,5-Disupstituirani hidantoini 5

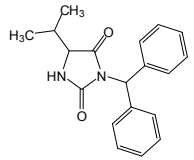
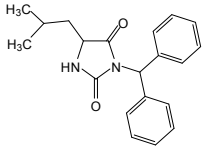
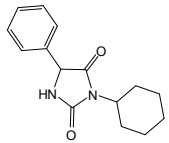
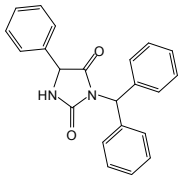
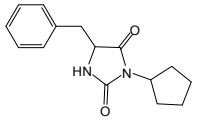
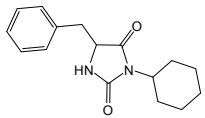
Citostatsko djelovanje hidantoina **5a-h** ispitano je *in vitro* na sljedeće maligne stanične linije: leukemija porijeklom iz miša (L1210), zloćudno preobraženi T-limfociti (Molt4/C8 i CEM), karcinom grlića maternice (HeLa), karcinom dojke (MCF-7), karcinom gušterače (MiaPaCa-2), karcinom pluća (H 460), karcinom debelog crijeva (SW620) i na staničnu liniju normalnih fibroblasta porijeklom iz čovjeka (WI 38) (Tablica 28).[113] Ispitivanja su pokazala je MCF-7 najosjetljivija na djelovanje hidantoina **5a-h**.

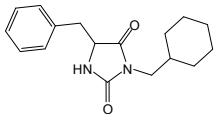
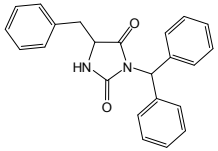
Prema našim očekivanjima, rezultati su pokazali da je citostatsko djelovanje spojeva **5a-h** značajno jače od djelovanja ranije sintetiziranih hidantoina,[3] zbog njihove veće lipofilnosti. Usporedbom hidantoina, derivata istih aminokiselina (L-valina, L-leucina, D-fenilglicina i L-fenilalanina), a uzimajući u obzir i ranije ispitane spojeve,[3] može se zaključiti da derivati L-fenilalanina, najlipofilnije aminokiseline, imaju najsnažnije citostatsko djelovanje. Isto tako, među derivatima L-valina i L leucina, spojevi s benzhidrilnim supstituentom na N-3 (**5a** i **5b**) imaju najbolji učinak.

Međutim, hidantoinski derivati D-fenilglicina i L-fenilalanina ne slijede u potpunosti to pravilo, te je usporedba supstituenata na N-3 pokazala opadanje jačine citostatskog djelovanja sljedećim redosljedom: cikloheksanmetil > benzhidril > cikloheksil > ciklopentil.

Dva hidantoina s najjačim citostatskim djelovanjem na staničnu liniju MCF-7, derivat L-fenilalanina **5g** i derivat D-fenilglicina, 3-cikloheksanmetil-5-fenil hidantoin, (IC_{50} = 2, odnosno 3,3 μmol L^{-1}) ispoljili su značajno različito djelovanje na staničnu liniju normalnih fibroblasta. Dok spoj **5g** pokazuje gotovo jednak učinak, djelovanje 3-cikloheksanmetil-5-fenil hidantoina vrlo je selektivno (IC_{50} = 78,9 μmol L^{-1}).

Tablica 28. Inhibitorno djelovanje 3,5-disupstituiranih hidantoina **5a-h** na rast malignih staničnih linija i normalnih fibroblasta (WI 38).

Spoj	Struktura	Rast tumorskih stanica [IC_{50}[a] (μmol L^{-1})]								
		L1210	**MoltC4/C8**	**CEM**	**HeLa**	**MCF-7**	**MiaPaCa-2**	**H 460**	**SW620**	**WI 38**
5a		102 ± 3	183 ± 38	83 ± 4	> 100	36 ± 13	47 ± 5	65 ± 35	62 ± 20	77 ± 23
5b		57 ± 21	232±194	148 ± 95	47 ± 44	16 ± 0,6	46 ± 29	43 ± 1,5	31 ± 27	42 ± 2
5c		> 500	> 500	406 ± 16	> 100	63 ± 14	> 100	> 100	> 100	> 100
5d		303 ± 9	317 ± 12	266 ± 15	37	18 ± 1	81 ± 10	> 100	78 ± 21	55 ± 14
5e		312 ± 128	> 500	246 ± 85	38 ± 8	22 ± 2	70 ± 19	> 100	74 ± 25	42 ± 2
5f		42 ± 2	53 ± 2	48 ± 4	13 ± 1,4	11 ± 1	14 ± 0,9	19 ± 4	15 ± 2	13 ± 5

5g		172 ± 52	277 ± 45	146 ± 14	5,4 ± 13	2 ± 1,6	58 ± 42	58 ± 25	60 ± 35	1,5 ± 0,3
5h		46 ± 4	52 ± 5	50 ± 4	21 ± 3	20 ± 10	22 ± 0,7	23 ± 0,7	21 ± 0,9	> 100
	Hidroksiurea	n.o.[b]	n.o.	n.o.	42 ± 38	> 100	> 100	> 100	> 100	> 100
	5-Fluorouracil	n.o.	n.o.	n.o.	4 ± 1,5	15 ± 2	10 ± 3	2 ± 0,7	9 ± 2	14 ± 12
	Cisplatina	n.o.	n.o.	n.o.	4 ± 3	12 ± 6	5 ± 1	0,3 ± 0,1	8 ± 6	20 ± 19

[a] IC_{50} – koncentracija spoja kod koje je rast stanica inhibiran za 50%; [b] n.o. – nije određeno.

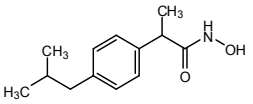
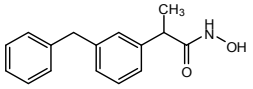
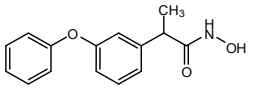
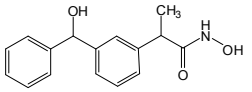
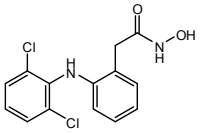
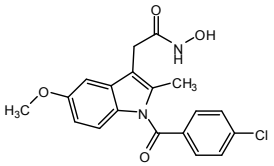
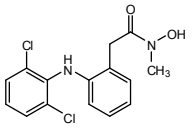
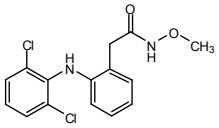
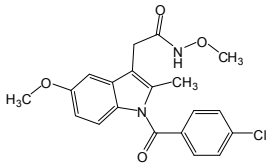
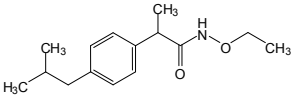
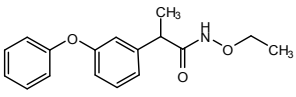
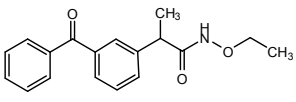
5.2.1.2. NSAID hidroksamske kiseline i njihovi derivati 8

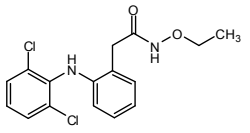
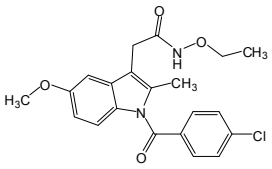
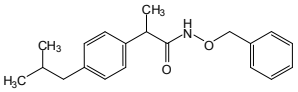
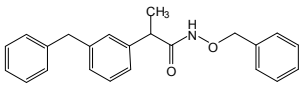
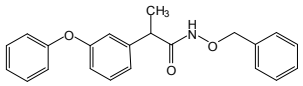
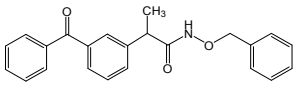
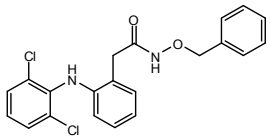
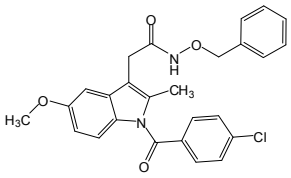
Citostatsko djelovanje spojeva **8** ispitano je *in vitro* na sljedeće maligne stanične linije: L1210, Molt4/C8, CEM, akutna limfoblastična leukemija (MOLT-4), HeLa, MiaPaCa-2, karcinom debelog crijeva (SW620 i HCT 116), karcinom prsne žlijezde (FM3A/0), MCF-7, metastatski adenokarcinom dojke (SK-BR-3), karcinom pluća velikih stanica (NCI-H727) i H 460, te je uspoređeno s njihovim djelovanjem na rast WI 38 (Tablica 29 i 30).

Između NSAID hidroksamskih kiselina **8b-h**, indometacin hidroksamska kiselina (**8g**) pokazala je najsnažnije antiproliferativno djelovanje na ispitane maligne stanične linije, koje nije bilo selektivno u usporedbi s djelovanjem na WI 38. Nasuprot njoj, MeKet hidroksamska kiselina (**8c**) i fenoprofen hidroksamska kiselina (**8d**) pokazale su selektivno i diferencijalno djelovanje na stanične linije MiaPaCa-2, odnosno HeLa, u koncentracijama 1×10^{-6} i 1×10^{-5} mol L^{-1} (IC_{50} = 2,2, odnosno 6,5 μmol L^{-1}). Usporedba citostatskog djelovanja *O*-benzilnih derivata NSAID hidroksamskih kiselina **8s-y** pokazala je da spoj **8t**, derivat MeKet, ima najsnažnije djelovanje (na staničnu liniju MOLT-4, IC_{50} = 5 μmol L^{-1}). *O*-alkilni derivati NSAID hidroksamskih kiselina **8k-m**,**o-r** pokazali su najslabije citostatsko djelovanje prema svim ispitanim staničnim linijama. Međutim, spojevi **8m** i **8r**, *O*-etilni derivati ibuprofen i indometacin hidroksamske kiseline, na staničnu liniju MiaPaCa-2 djeluju diferencijalno i selektivno (IC_{50} = 8, odnosno 9,7 μmol L^{-1}).

Jačina citostatskog djelovanja kod derivata ibuprofena, fenoprofena i ketoprofena opada sljedećim redosljedom: *O*-benzilni derivati hidroksamskih kiselina > hidroksamske kiseline > *O*-alkilni derivati hidroksamskih kiselina. Iznimke su spojevi **8c** i **8m** (na staničnu liniju MiaPaCa-2), te spoj **8d** (na staničnu liniju HeLa). Usporedba citostatskog djelovanja derivata diklofenaka i indometacina pokazala je da njihove hidroksamske kiseline imaju najsnažnije djelovanje, slijede ih *O*-benzilni i *O*-alkilni derivati. Međutim, njihovo djelovanje nije selektivno u usporedbi s djelovanjem na staničnu liniju normalnih fibroblasta (iznimka je spoj **8r** i njegovo djelovanje na staničnu liniju MiaPaCa-2).

Tablica 29. Inhibitorno djelovanje NSAID hidroksamskih kiselina i njihovih derivata **8** na rast malignih staničnih linija.

Spoj	Struktura	Rast tumorskih stanica [IC_{50}[a] (μmol L^{-1})]					
		L1210	Molt4/ C8	MOLT-4	CEM	HeLa	MiaPaCa--2
8b		42 ± 0	37 ± 1	n.o.[b]	50 ± 5	56 ± 12	73 ± 25
8c		27 ± 17	42 ± 11	n.o.	34 ± 7	56,3	2,2
8d[c]		39 ± 0	44 ± 3	n.o.	44 ± 4	6,5 ± 4,6	40 ± 13
8e		n.o.	n.o.	49 ± 31	n.o.	n.o.	n.o.
8f		10 ± 0	11 ± 4	n.o.	12 ± 1	30 ± 10	50 ± 25
8g		8,4 ± 0,1	8,8 ± 1,5	n.o.	7,4 ± 0,3	3,0 ± 1,9	26 ± 6
8h		43 ± 1	28 ± 19	n.o.	25 ± 17	42 ± 19	34 ± 16
8k		≥ 500	> 500	n.o.	> 500	> 100	> 100
8l		34 ± 18	41 ± 2	n.o.	25 ± 8	67,9	53,4
8m		76 ± 49	135 ± 5	n.o.	72 ± 7	> 100	8
8o		66 ± 21	94 ± 51	n.o.	54 ± 10	> 100	71,8
8p		38 ± 20	71 ± 14	n.o.	45 ± 2	> 100	55,7

8q		≥ 500	> 500	n.o.	> 500	> 100	84,8
8r		318 ± 166	> 500	n.o.	> 500	57,1	9,7
8s		41 ± 1	38 ± 2	n.o.	39 ± 0	32 ± 7	34 ± 7
8t[d]		n.o.	n.o.	5 ± 3	n.o.	n.o.	n.o.
8u		39 ± 1	36 ± 3	n.o.	36 ± 1	35 ± 6	38 ± 9
8v		20 ± 4	35 ± 0	n.o.	24 ± 12	96,6	17,7
8x[c]		139 ± 85	138 ± 74	n.o.	221 ± 63	34,4 ± 11	76 ± 8
8y[e]		30 ± 2	63 ± 13	n.o.	73 ± 54	11,9 ± 3,6	≥ 100

[a] IC_{50} – koncentracija spoja kod koje je rast stanica inhibiran za 50%; [b] n.o. – nije određeno; [c] taloži se iz medija (HeLa i MiaPaCa-2) kod koncentracije 1×10^{-4} mol L^{-1}. [d] Taloži se iz medija (MOLT-4) kod koncentracije 1×10^{-4} mol L^{-1}. [e] Taloži se iz medija (HeLa i MiaPaCa-2) kod koncentracija 1×10^{-5} mol L^{-1} i 1×10^{-4} mol L^{-1}.

Tablica 30. Inhibitorno djelovanje NSAID hidroksamskih kiselina i njihovih derivata **8** na rast malignih staničnih linija i normalnih fibroblasta (WI 38).

Spoj[a]	Rast tumorskih stanica [IC_{50}[b] (μmol L^{-1})]							
	FM3A/0	MCF-7	SK-BR-3	SW620	HCT 116	NCI-H727	H 460	WI 38
8b	n.o.[c]	57 ± 20	n.o.	42 ± 16	n.o.	n.o.	n.o.	22 ± 22
8c	27 ± 9	49,8	21	50,7	n.o.	67,1	n.o.	62,7
8d[d]	n.o.	41 ± 15	n.o.	48 ± 13	n.o.	n.o.	n.o.	34 ± 34
8e	n.o.	>100	n.o.	>100	>100	n.o.	>100	n.o.
8f	n.o.	51 ± 10	n.o.	39 ± 14	n.o.	n.o.	n.o.	13 ± 6
8g	n.o.	28 ± 9	n.o.	19 ± 14	n.o.	n.o.	n.o.	1,0 ± 1,0
8h	n.o.	39 ± 11	n.o.	34 ± 12	n.o.	n.o.	n.o.	46 ± 4
8k	> 500	74,2	> 100	> 100	n.o.	> 100	n.o.	69,4
8l	40 ± 4	27,6	35,5	22,7	n.o.	> 100	n.o.	36
8m	218 ± 4	66	> 100	> 100	n.o.	> 100	n.o.	>100
8o	231 ± 58	77	79,1	> 100	n.o.	> 100	n.o.	> 100
8p	292 ± 105	66,8	86	39,4	n.o.	> 100	n.o.	> 100
8q	> 500	96,3	88,2	97,3	n.o.	> 100	n.o.	78,1
8r	> 500	38,7	34,5	29,7	n.o.	86,9	n.o.	36,2
8s	n.o.	31 ± 6	n.o.	30 ± 12	n.o.	n.o.	n.o.	23 ± 14
8t[e]	n.o.	15 ± 1	n.o.	16 ± 0,2	7 ± 5	n.o.	7 ± 1	n.o.
8u	n.o.	30 ± 8	n.o.	43 ± 8	n.o.	n.o.	n.o.	38 ± 26
8v	26 ± 22	25,9	n.o.	23,8	n.o.	64,6	n.o.	61,1
8x[f]	n.o.	75 ± 10	n.o.	40 ± 14	n.o.	n.o.	n.o.	21 ± 6
8y[f]	n.o.	≥ 100	n.o.	46,7 ± 10	n.o.	n.o.	n.o.	30 ± 14

[a] Za strukture vidi Tablicu 29. [b] IC_{50} – koncentracija spoja kod koje je rast stanica inhibiran za 50%; [c] n.o. – nije određeno; [d] taloži se iz medija (MCF-7, SW620, NCI-H727 i WI 38) kod koncentracije 1×10^{-4} mol L^{-1}. [e] Taloži se iz medija (MCF-7, SW620, HCT 116 i H 460) kod koncentracije 1×10^{-4} mol L^{-1}. [f] Taloži se iz medija (MCF-7, SW620, NCI-H727 i WI 38) kod koncentracija 1×10^{-5} mol L^{-1} i 1×10^{-4} mol L^{-1}.

5.2.1.3. Derivati ketoprofena 9–11

U prijašnjim antitumorskim istraživanjima amida ketoprofena i fenoprofena, pokazalo se da lipofilniji amidi ketoprofena i fenoprofena imaju snažnije inhibitorno djelovanje na rast tumorskih staničnih linija.[21] Zbog toga su samo neki novosintetizirani amidi izabrani za to ispitivanje.

Citostatsko djelovanje derivata ketoprofena ispitano je *in vitro* na sljedeće maligne stanične linije: SW620, HCT 116, MCF-7, MOLT-4 i H 460 (Tablica 31). Rezultati su pokazali da svi ispitani spojevi pokazuju slično antiproliferativno djelovanje na ispitane stanične linije.

Najsnažnije djelovanje od svih ispitanih spojeva pokazao je derivat MeKet **9f** (na staničnu liniju HCT 116, IC_{50} = 4 μmol L^{-1}), koji je i najlipofilniji spoj unutar serije. Međutim, ne može se reći da je lipofilnost spojeva jedino i najvažnije svojstvo koje određuje jačinu citostatskog djelovanja. Ako se usporedi antiproliferativno djelovanje spojeva s istim NSAID dijelom molekule, a različitim amidnim supstituentom, ne može se naći pravilnost u opadanju jačine citostatskog djelovanja.

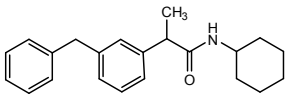
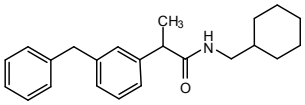
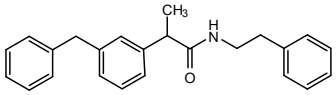
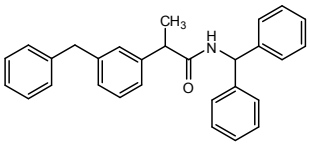
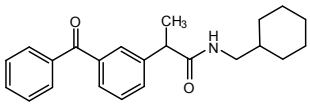
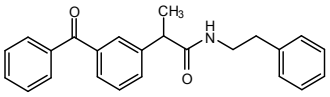
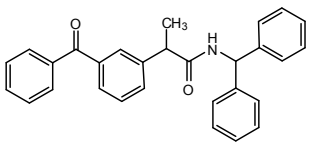
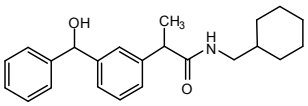
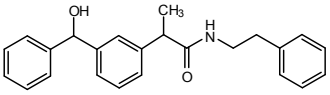
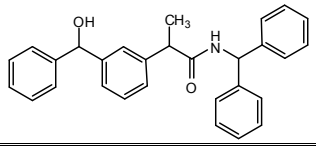
Slični zaključci izvedeni su i usporedbom djelovanja spojeva s jednakim amidnim supstituentom, a različitim NSAID dijelom. Između derivata s cikloheksanmetilnim i feniletilnim amidnim supstituentom na većinu ispitanih staničnih linija najjače djelovanje imaju najlipofilniji spojevi, derivati MeKet **9c**, odnosno **9e**. Njihova djelovanja na ostale stanične linije vrlo su slična. Međutim, usporedba spojeva s benzhidrilamidnim dijelom pokazala je da najmanje lipofilan derivat HyKet **11f** ima snažnije djelovanje od **9f** na sve maligne stanične linije, osim na HCT 116.

Ako se usporedi citostatsko djelovanje amida s feniletilnim supstituentom (**9e**, **10e** i **11e**) te strukturno srodnih *O*-benzilnih derivata hidroksamskih kiselina **8t** i **8v** (derivati MeKet, odnosno ketoprofena), dolazi se do sljedećih rezultata: unutar te skupine spojeva najjače djelovanje na sve maligne stanične linije pokazuje *O*-benzilni derivat hidroksamske kiseline izvedene iz MeKet (**8t**). Amidi **9e**, **10e** i **11e** pokazuju snažnije djelovanje na staničnu liniju MCF-7, a slabije na SW620 od *O*-benzilnog derivata KetNHOH (**8v**).

Od dvije ispitane stanične linije karcinoma debelog crijeva, ispitivani spojevi pokazali su snažnije djelovanje na HCT 116 nego na SW620. Najjače djelovanje na staničnu liniju HCT 116 pokazao je **9f**, dok drugi spojevi na nju imaju slično djelovanje (IC_{50} = 11–18 μmol L^{-1}). Djelovanje na staničnu liniju SW620 značajno je slabije, najsnažnije djeluje **11f** (IC_{50} = 18 μmol L^{-1}). Na staničnu liniju MCF-7 svi spojevi (osim **10f**) pokazuju slično djelovanje (IC_{50} = 13–23 μmol L^{-1}), dok na MOLT-4 najsnažnije djelovanje imaju **9e** i **11f** (IC_{50} = 11 μmol L^{-1}). Spojevi **9c**,**9e**,**10c**,**10e** pokazuju najjače djelovanje na staničnu liniju H 460 (IC_{50} = 19–22 μmol L^{-1}).

Najslabije djelovanje na sve ispitane stanične linije pokazao je spoj **10f**, vjerojatno zbog svog jakog taloženja iz medija, što je kompromitiralo njegovo ispitivanje.

Tablica 31. Inhibitorno djelovanje derivata ketoprofena **9**–**11** na rast malignih staničnih linija.

Spoj	Struktura	Rast tumorskih stanica [IC_{50}[a] (μmol L^{-1})]				
		HCT 116	SW620	MCF-7	MOLT-4	H 460
9b[b]		12 ± 3	56 ± 43	14 ± 1	19 ± 3	53 ± 46
9c		11 ± 0,3	24 ± 2	14 ± 0,1	17 ± 0,2	19 ± 1
9e		15 ± 0,6	25 ± 5	20 ± 2	11 ± 8	21 ± 0,7
9f[b]		4 ± 3	87 ± 10	14 ± 4	40 ± 15	> 100
10c		13 ± 5	53 ± 42	13 ± 0,4	15 ± 0,8	20 ± 1
10e		15 ± 2	30 ± 3	17 ± 5	15 ± 3	22 ± 4
10f[b]		> 100	> 100	> 100	> 100	> 100
11c		13 ± 0,8	37 ± 8	13 ± 0,1	15 ± 0,4	32 ± 8
11e[b]		18 ± 15	50 ± 40	23 ± 16	27 ± 15	57 ± 37
11f[b]		13 ± 2	18 ± 2	13 ± 2	11 ± 4	15 ± 0,01

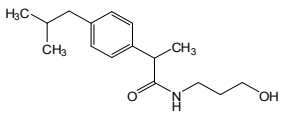
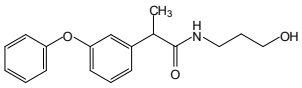
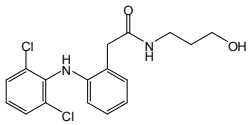
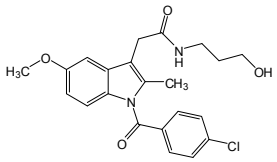
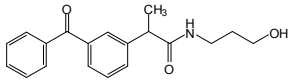
[a] IC_{50} – koncentracija spoja kod koje je rast stanica inhibiran za 50%; [b] taloži se iz medija kod koncentracije 1×10^{-4} μmol L^{-1}.

5.2.1.4. 3-Hidroksipropilamidi NSAID 12

Citostatsko djelovanje 3-hidroksipropilamida NSAID **12a-e** ispitano je *in vitro* na sljedeće maligne stanične linije: L1210, Molt4/C8, CEM, HeLa, MiaPaCa-2, SW620, MCF-7 i H 460 (Tablica 32).[114]

Svi ispitani spojevi pokazali su dosta slabo citostatsko djelovanje (IC_{50} > 100 μmol L^{-1}). Najjače djelovanje imao je spoj **12d**, i to na stanične linije HeLa i MCF-7 (IC_{50} = 26, odnosno 22 μmol L^{-1}).

Tablica 32. Inhibitorno djelovanje 3-hidroksipropilamida NSAID **12a-e** na rast malignih staničnih linija.

Spoj	Struktura	Rast tumorskih stanica [IC_{50}[a] (μmol L^{-1})]							
		L1210	Molt4/C8	CEM	HeLa	MCF-7	MiaPaCa-2	H 460	SW620
12a		213 ± 16	93 ± 7	55 ± 3	> 100	> 100	≥ 100	> 100	> 100
12b		230 ± 6	99 ± 18	71 ± 1	> 100	> 100	> 100	> 100	> 100
12c		200 ± 35	215 ± 12	212 ± 23	46 ± 10	39 ± 29	54 ± 25	68 ± 34	36 ± 3
12d		179 ± 93	113 ± 68	78 ± 26	26 ± 16	22 ± 14	44 ± 20	> 100	47 ± 2
12e		273 ± 14	97± 18	71 ± 9	> 100	> 100	> 100	> 100	> 100

[a] IC_{50} – koncentracija spoja kod koje je rast stanica inhibiran za 50%.

5.2.2. ANTIMIKROBNA ISPITIVANJA NSAID HIDROKSAMSKIH KISELINA I NJIHOVIH DERIVATA **8**

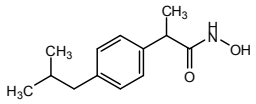
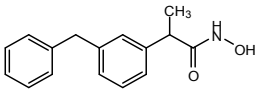
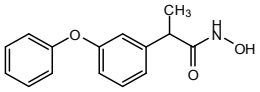
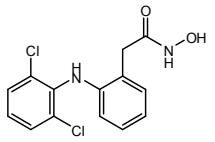
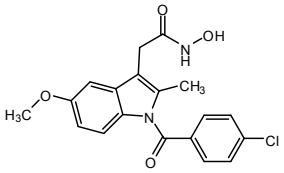
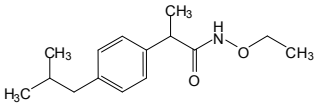
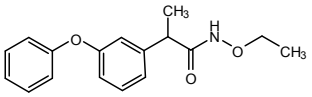
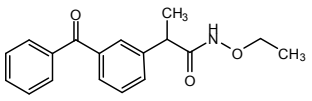
Antimikrobno djelovanje spojeva **8** ispitano je korištenjem difuzijske metode (bušenjem cilindrima: visine 10, vanjskog promjera 8, a unutrašnjeg 6 mm).[104] Utvrđeno je da samo hidroksamske kiseline sa slobodnom i *O*-etil supstituiranom hidroksilnom skupinom pokazuju značajno antimikrobno djelovanje pri koncentraciji 29,6 mg mL^{-1} (Tablica 33). Ostali spojevi nisu pokazali zone inhibicije rasta te su smatrani antimikrobno inaktivnima pri danoj koncentraciji i provedenoj metodi, vjerojatno zbog njihove slabe topljivosti u Müller-Hinton agaru (taloženje supstancije, rezultati nisu prikazani). Ibuprofen i fenoprofen hidroksamske kiseline **8b**,**d** pokazale su široki spektar antimikrobnog djelovanja protiv ispitanih Gram-pozitivnih i Gram-negativnih bakterijskih vrsta. Spojevi **8f**,**g** (diklofenak i indometacin hidroksamske kiseline) pokazali su značajnu baktericidnu aktivnost samo prema *E. faecalis*, dok je MeKet hidroksamska kiselina **8c** pokazala aktivnost prema *S. aureus*, *E. coli*, *S. enterica* subsp. *enterica* i *P. aeruginosa*. *O*-etilni derivati ibuprofen, fenoprofen i ketoprofen hidroksamskih kiselina **8m**,**o**,**p** pokazale su antimikrobno djelovanje protiv *B. subtilis*, dok je spoj **8m** pokazao značajnu aktivnost i prema *K. rhizophila* i *P. aeruginosa*. Antifungalno djelovanje protiv *C. albicans* pokazao je samo *O*-metilni derivat diklofenak hidroksamske kiseline **8k**.

Minimalna inhibitorna (*MIC*) i minimalna mikrobicidna koncentracija (*MMcC*) određene su metodom serijalnog mikrorazrijeđenja (CLSI)[105] (Tablica 34). Spoj **8b** pokazao je jednake vrijednosti *MIC*/*MMcC* kao i **8d** prema *B. subtilis* (0,116/0,232 mg mL^{-1}) i *S. enterica* subsp. *enterica*. Niže vrijednosti *MIC*/*MMcC* spoj **8b** pokazao je prema *E. coli*, dok je **8d** bio aktivniji prema *S. aureus*, *E. faecalis*, *K. rhizophila* i *P. aeruginosa*. Spoj **8c** pokazao je samo slabo djelovanje protiv *S. aureus*, dok su **8m**,**o** pokazali djelovanje protiv *K. rhizophila*. Hidroksamske kiseline sa slobodnom hidroksilnom skupinom i prema ovoj metodi pokazale su bolje antimikrobno djelovanje, međutim njihovo djelovanje općenito je slabo (visoke vrijednosti *MIC*/*MMcC*).

Promjer zona inhibicije rasta u metodi difuzije ovisi, između ostalog i o topljivosti supstancija u agaru te o koncentraciji ispitivanih tvari. Direktno se mogu uspoređivati samo zone inhibicija rasta onih supstancija koje imaju jednake koncentracije. Oksitetraciklin, norfloksacin i nistatin korišteni su kao kontrola

osjetljivosti mikrobnih kultura u metodi difuzije, te se njihove zone inhibicije ne mogu uspoređivati sa zonama inhibicije NSAID hidroksamskih kiselina i njihovih derivata **8**.

Tablica 33. Antimikrobno djelovanje NSAID hidroksamskih kiselina i njihovih derivata **8**.

Spoj	Struktura	Bakterijske vrste[a]							Gljivice
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
8b[b]		24[f]	15	n.a.[g]	10	15	17	15	n.a.
8c[b]		n.a.	12	n.a.	n.a.	9	10	10	n.a.
8d[b]		23	16	11	18	23	21	18	n.a.
8f[b]		n.a.	n.a.	13	n.a.	n.a.	n.a.	n.a.	n.a.
8g[b]		n.a.	n.a.	12	n.a.	n.a.	n.a.	n.a.	n.a.
8m[b]		13	n.a.	n.a.	14	n.a.	n.a.	9	n.a.
8o[b]		11	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
8p[b]		18	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	10
Oksitetraciklin hidroklorid[c]		35	12	13	27	21	n.a.	17	n.o.[h]
Norfloksacin[d]		25	n.a.	19	10	30	21	n.a.	n.o.
Nistatin[e]		n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	20

[a] Mikrobiološke vrste: 1 – *B. subtilis* ATCC 6633; 2 – *S. aureus* ATCC 25923; 3 – *E. faecalis* ATCC 29212; 4 – *K. rhizophila* ATCC 9341; 5 – *E. coli* ATCC 10536; 6 – *S. enterica* subsp. *enterica* ATCC 13076; 7 – *P. aeruginosa* ATCC 27853; 8 – *C. albicans* ATCC 10231. Koncentracije: [b] 29,6; [c] 0,5; [d] 0,4; [e] 5 mg mL^{-1}; [f] zona inhibicije rasta (mm); [g] n.a. – zona inhibicije rasta nije detektirana; [h] n.o. – nije određeno.

Tablica 34. Minimalna inhibitorna (*MIC*) i minimalna mikrobicidna koncentracija (*MMcC*) NSAID hidroksamskih kiselina i njihovih derivata **8** utvrđena metodom serijalnog mikrorazrijeđenja.

Spoj[a]	**Bakterijske vrste**[b]							**Gljivice**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
8b	0,116 (0,232)[c]	1,850 (3,700)	n.o.[d]	n.a.[e]	0,116 (0,232)	3,700 (7,400)	1,850 (3,700)	n.o.
8c	n.o.	1,850 (3,700)	n.o.	n.o.	n.a.	n.a.	n.a.	n.o.
8d	0,116 (0,232)	0,232 (0,463)	0,925 (1,850)	0,925 (1,850)	0,232 (0,463)	3,700 (7,400)	0,463 (0,925)	n.o.
8f	n.o.	n.o.	n.a.	n.o.	n.o.	n.o.	n.o.	n.o.
8g	n.o.	n.o.	n.a.	n.o.	n.o.	n.o.	n.o.	n.o.
8m	n.a.	n.o.	n.o.	0,925 (1,850)	n.o.	n.o.	n.a.	n.a.
8o	n.a.	n.o.	n.o.	3,700 (7,400)	n.o.	n.o.	n.a.	n.a.
8p	n.a.	n.o.	n.o.	n.o.	n.o.	n.o.	n.a.	n.a.

[a] Za strukture spojeva vidi Tablicu 33. [b] Mikrobiološke vrste: 1 – *B. subtilis* ATCC 6633; 2 – *S. aureus* ATCC 25923; 3 – *E. faecalis* ATCC 29212; 4 – *K. rhizophila* ATCC 9341; 5 – *E. coli* ATCC 10536; 6 – *S. enterica* subsp. *enterica* ATCC 13076; 7 – *P. aeruginosa* ATCC 27853; 8 – *C. albicans* ATCC 10231.[c] *MIC* (*MMcC*)/mg mL^{-1}; [d] n.o. – nije određeno; [e] n.a. – nema djelovanja kod koncentracija do 14,8 mg mL^{-1}.

5.2.3. ANTIVIRUSNA ISPITIVANJA

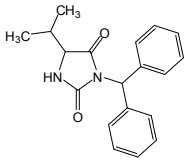
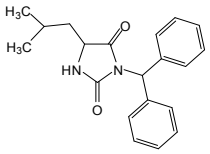
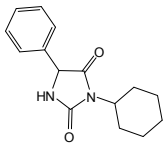
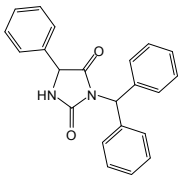
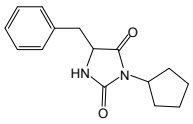
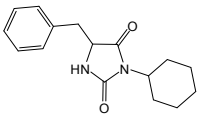
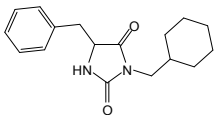
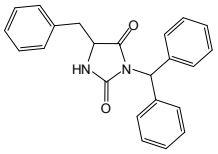
Antivirusna ispitivanja provedena su na Rega Institute for Medical Research, Katholieke Universiteit Leuven, Belgija, te je eksperimentalna metoda ispitivanja opisana ranije.[113]

5.2.3.1. 3,5-Disupstituirani hidantoini 5

Antivirusno djelovanje 3,5-disupstituiranih hidantoina **5a-h** ispitano je na sljedeće viruse: herpes simpleks tip 1 (HSV-1 (KOS)), herpes simpleks virus tip 1 bez aktivnosti timidin kinaze (HSV-1 TK KOS ACV^r), herpes simpleks tip 2 (HSV-2 (G)), vaccinia virus, vesicular stomatitis virus, Coxsackie virus B4, respiracijski sincicijski virus, parainfluenza-3 virus, reovirus-1, Sindbis virus i Punta Toro virus, te je uspoređeno s djelovanjem aciklovira, ganciklovira i ribavirina (Tablica 35 i 36).[113]

Strukturno slični hidantoini **5f** i **5h** pokazali su slično inhibitorno djelovanje na ispitane viruse. Ovi spojevi pokazali su antivirusno djelovanje na Coxsackie virus B4, parainfluenza-3 virus, reovirus-1 i Sindbis virus (EC_{50} = 16 μg mL^{-1}), što je 5 puta niža koncentracija od minimalne citotoksične koncentracije (*MCC*). Spoj **5a** pokazao je inhibitorno djelovanje na vaccinia virus u koncentraciji koja je 25 puta niža od *MCC* (EC_{50} = 16 μg mL^{-1}).

Tablica 35. Antivirusno djelovanje 3,5-disupstituiranih hidantoina **5a-h**.

Spoj	Struktura	*MCC*[a] (μg mL^{-1})	EC_{50}[b] (μg mL^{-1})				
			1[c]	2	3	4	5
5a		400	> 80	80	80	16	80
5b		80	> 16	> 16	> 16	> 16	> 16
5c		> 400	> 400	> 80	240	240	> 400
5d		80	> 16	> 16	> 16	> 16	> 80
5e		400	> 80	> 80	> 80	> 80	> 16
5f		80	> 16	> 16	> 16	16	> 16
5g		80	> 16	> 16	> 16	> 16	> 80
5h		80	> 16	> 16	> 16	> 16	> 16
	Aciklovir	> 100	0,11	13,51	0,18	67,5	> 100
	Ganciklovir	> 25	0,02	0,2	0,02	15,3	> 25
	Ribavirin	> 100	> 100	> 100	73,3	24,4	> 100

[a] *MCC* – minimalna citotoksična koncentracija kod koje je mikroskopski vidljiva promjena morfologije normalne stanice; [b] EC_{50} – efektivna koncentracija kod koje je stvaranje plakova virusa smanjeno za 50%. [c] Virusi: 1 – HSV-1 (KOS); 2 – HSV-1 TK KOS ACV^r; 3 – HSV-2 (G); 4 – vaccinia virus; 5 – vesicular stomatitis virus.

Tablica 36. Antivirusno djelovanje 3,5-disupstituiranih hidantoina **5a-h**.

Spoj[a]	EC_{50}[b] (μg mL^{-1})					
	Coxsackie virus B4	Respiracijski sincicijski virus	Parainfluenza-3 virus	Reovirus-1	Sindbis virus	Punta Toro virus
5a	> 80	> 80	> 80	> 80	48	> 80
5b	> 16	> 16	> 16	> 16	16	> 16
5c	240	> 400	> 400	> 400	240	> 400
5d	80	> 80	> 80	> 80	> 80	> 80
5e	> 16	> 16	> 16	> 16	> 16	> 16
5f	16	> 16	16	16	16	> 16
5g	> 80	> 80	> 80	> 80	> 80	> 80
5h	16	> 16	16	16	16	> 16
Ribavirin	> 100	> 100	73,3	73,3	24,4	73,3

[a] Za strukture spojeva vidi Tablicu 35; [b] EC_{50} – efektivna koncentracija kod koje je stvaranje plakova virusa smanjeno za 50%.

5.2.3.2. NSAID hidroksamske kiseline i njihovi derivati 8

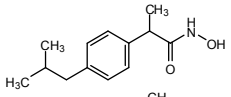
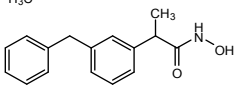
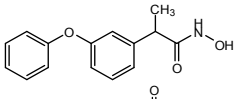
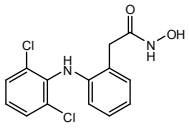
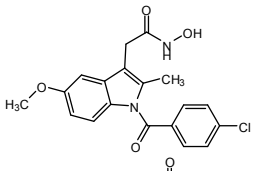
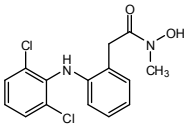
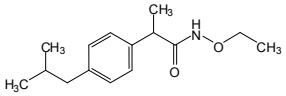
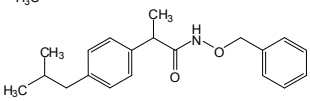
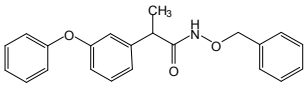
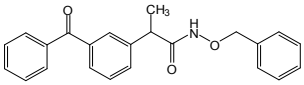
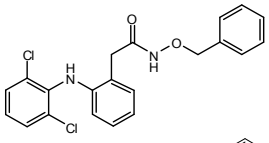
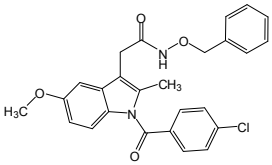
Ispitano je antivirusno djelovanje većine derivata NSAID hidroksamskih kiselina **8** na virus influenze te je uspoređeno s djelovanjem oseltamivir karboksilata, ribavirina, amantadina i rimantadina. Rezultati su pokazali da niti jedan od ispitanih spojeva **8** nije inhibirao citopatološko djelovanje influenze A ili B u subtoksičnim koncentracijama (rezultati nisu pokazani). Jedina iznimka bio je spoj **8s**, koji je pokazao antivirusno djelovanje niske selektivnosti (odnos MCC/EC_{50} = 7–10) i ograničeno na podtip influenza A/H1N1.

Također je ispitano antivirusno djelovanje spojeva **8** na sljedeće viruse: HSV-1 (KOS), HSV-1 TK KOS ACV^r, HSV-2 (G), vaccinia virus i vesicular stomatitis virus. Niti jedan ispitani spoj **8** nije pokazao specifično antivirusno djelovanje (*MIC* najmanje 5 puta niža od *MCC*) kada je ispitivanje provedeno na staničnim linijama ljudskih embrionalnih plućnih stanica (HEL) (rezultati nisu pokazani).

Ispitivanje antivirusnog djelovanja spojeva **8c**,**k-m**,**o-r** i **v** na viruse Feline Corona i Feline Herpes i citotoksičnosti na staničnim kulturama bubrega Crandell-Rees Feline pokazalo je da niti jedan spoj **8** nije značajno inhibirao dane viruse u subtoksičnim koncentracijama (rezultati nisu pokazani).

Međutim, spojevi **8b-d**,**f-h**,**m**,**s** i **u-y** pokazali su antivirusno djelovanje na parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxackie virus B4 i Punta Toro virus u Vero staničnoj kulturi (Tablica 37). Najbolje djelovanje pokazao je spoj **8y** (*MIC* = 12 μmol L^{-1} (5,6 μg mL^{-1}), indeks selektivnosti *MCC*/*MIC* = 8,3).

Tablica 37. Citotoksičnost i antivirusno djelovanje NSAID hidroksamskih kiselina i njihovih derivata **8**.

Spoj	Struktura	*MCC*[a] (μmol L^{-1})	*MIC*[b] (μmol L^{-1})				
			1[c]	2	3	4	5
8b		≥ 100	12	12	> 100	> 100	> 100
8c		100	20	> 20	> 20	> 20	20
8d		≥ 100	20	12	> 100	> 100	> 100
8f		≥ 100	> 100	> 100	> 100	60	> 100
8g		> 100	20	12	60	100	> 100
8h		100	> 20	> 20	> 20	> 20	> 20
8m		> 100	> 100	> 100	> 100	> 100	59
8s		100	> 20	> 20	> 20	> 20	> 20
8u		100	> 20	> 20	> 20	> 20	> 20
8v		≥100	> 100	> 100	> 100	59	> 100
8x		20	> 4	> 4	> 4	> 4	> 4
8y		100	20	> 20	> 20	> 20	12
	Brivudin	> 250	> 250	> 250	> 250	> 250	> 250
	(S)-DHPA	> 250	30	150	> 250	> 250	> 250
	Ribavirin	> 250	150	150	> 250	> 250	50

[a] *MCC* – minimalna koncentracija kod koje je mikroskopski vidljiva promjena morfologije normalne stanice; [b] *MIC* – minimalna inhibitorna koncentracija kod koje je patogenost virusa inhibirana za 50%; [c] Virusi: 1 – parainfluenza-3 virus; 2 – reovirus-1; 3 – Sindbis virus; 4 – Coxackie virus B4; 5 – Punta Toro virus.

5.2.3.3. 3-Hidroksipropilamidi NSAID 12

Antivirusno djelovanje spojeva **12a-e** ispitano je na sljedeće viruse: virus HIV-a tip 1 i tip 2, herpes simpleks tip 1 i tip 2, vaccinia virus, varicella-zoster virus, citomegalovirus, vesicular stomatitis virus, Coxsackie virus B4, respiracijski sincicijski virus, parainfluenza-3 virus, reovirus-1, Sindbis virus i Punta Toro virus.[114]

Niti jedan ispitani spoj **12** nije pokazao specifično antivirusno djelovanje na bilo koji virus (rezultati nisu pokazani), osim spoja **12d** koji je pokazao slabo inhibitorno djelovanje na citomegalovirus i vesicular stomatitis virus u koncentracijama 30–77 μmol L^{-1}.

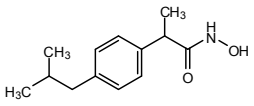
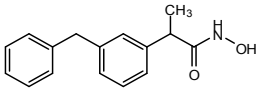
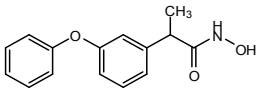
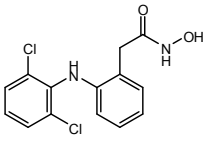
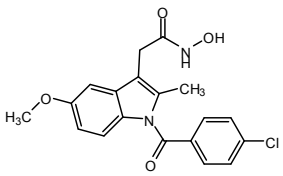
5.2.4. INHIBICIJA UREAZE

Inhibicija ureaze NSAID hidroksamskim kiselinama i njihovim derivatima **8b-d**,**f-h**,**s** i **u-y** određena je korištenjem modificirane kolorimetrijske vremenske metode.[104,105] Iako postoji značajna razlika u inhibiciji *H*. *pylori* ureaze i ureaze iz soje (*Canavalia ensiformis* (*Jack bean*) ureaza),[10] koristili smo ureazu iz soje, jer ureaza iz *H*. *pylori* nije komercijalno dostupna i prilično ju je teško izolirati. Postotak inhibicije svakog uzorka izračunat je prema sljedećoj jednadžbi:

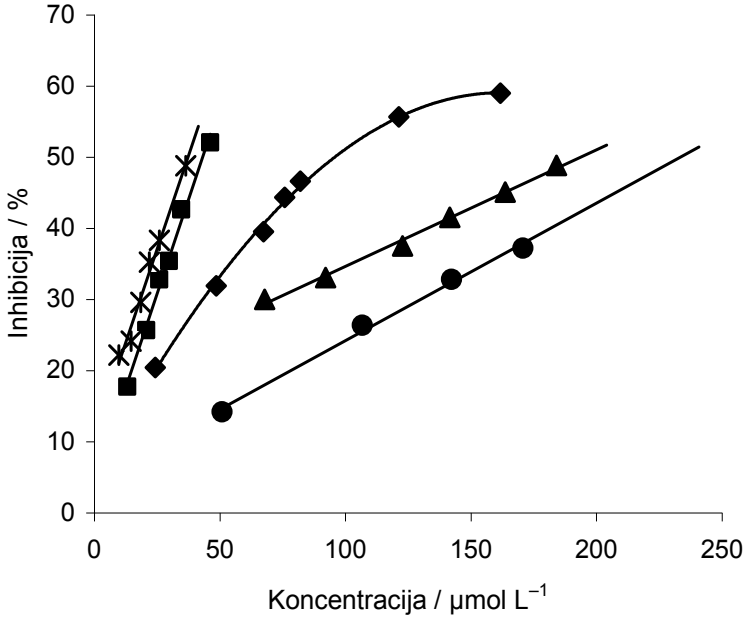
$$\text{postotak inhibicije (\%)} = [(t - t_0)/t] \times 100$$

gdje je t vremenski interval (s) mjeren za svaki uzorak određene molarne koncentracije ispitivanog spoja, a t_0 vremenski interval (s) mjeren za uzorak bez ispitivanog spoja (slijepa proba). Rezultati su pokazali da samo hidroksamske kiseline sa slobodnom hidroksilnom skupinom inhibiraju aktivnost ureaze (Tablica 38, Slika 6). Najjači inhibitor je derivat diklofenaka **8f** (IC_{50} = 37,4 μmol L^{-1}), dok su njegovi *N*-metilni i *O*-alkilni derivati inaktivni.

Tablica 38. Inhibicija ureaze hidroksamskim kiselinama **8**.

Spoj	Struktura	IC_{50} (μmol L^{-1})[a]
8b		97,4
8c		193,8
8d		43,2
8f		37,4
8g		233,3[b]

[a] IC_{50} – koncentracija kod koje je aktivnost ureaze inhibirana za 50%; [b] taloži se iz medija pri koncentracijama ispod IC_{50} (IC_{50} je ekstrapolirana vrijednost).



Slika 6. Ovisnost inhibicije ureaze iz soje o koncentraciji ispitivanog spoja: **8b** (♦), **8c** (▲), **8d** (■), **8f** (∗), **8g** (●).

5.2.5. ANTIOKSIDACIJSKO DJELOVANJE, INHIBICIJA LIPIDNE PEROKSIDACIJE LINOLNE KISELINE I INHIBICIJA LIPOOKSIGENAZE

Ispitivanje antioksidacijskog djelovanja, inhibicije lipidne peroksidacije linolne kiseline i inhibicija lipooksigenaze provedena su na Department of Pharmaceutical Chemistry, School of Pharmacy, Aristotles University of Thessaloniki, Thessaloniki, Grčka, a eksperimentalne metode opisane su ranije.[115–117]

Spojevi s antioksidacijskim djelovanjem mogu služiti za razvoj novih protuupalnih lijekova i lijekova protiv reumatoidnog artritisa.[118] Isto tako, inhibitori lipooksigenaze, ključnog enzima u biokemijskom putu pretvorbe arahidonske kiseline u leukotriene, potencijalni su protuupalni lijekovi.

Lipidna peroksidacija je oksidativna degeneracija lipida. Ako se ne zaustavi, dolazi do oštećenja stanične membrane i membrane staničnih organela, koje se sastoje uglavnom od lipida. Krajnji produkti lipidne peroksidacije mogu biti mutageni i kancerogeni (malondialdehid reagira s deoksiadenozinom i deoksigvanozinom iz DNA te nastaju DNA adukti).[119] Lipidna peroksidacija također se može povezati s patogenezom brojnih bolesti, uključujući aterosklerozu, astmu, Alzheimerovu i Parkinsonovu bolest, šećernu bolest, ozljede uzrokovane radijacijom i kemikalijama.[120,121]

5.2.5.1. NSAID hidroksamske kiseline i njihovi derivati 8

Interakcija supstancija sa stabilnim slobodnim radikalom 1,1-difenil-2--pikrilhidrazil radikalom (DPPH) opisuje njihovu sposobnost hvatanja slobodnih radikala u sustavu bez željeza i pokazuje kolika je njihova reducirajuća aktivnost. Jačina reakcije spojeva s DPPH ovisi o njihovoj koncentraciji.

Na temelju te činjenice ispitana je interakcija derivata NSAID hidroksamskih kiselina **8a**,**c**,**e**,**i**,**j**,**m-y** s DPPH, prema ranije opisanoj metodi,[115] nakon 20 i 60 min kod dvije koncentracije ispitivanih spojeva (0,05 i 0,1 mmol L^{-1}), a rezultati su prikazani u Tablici 39. Nije bilo značajnih promjena ovisnih o vremenu, osim za spojeve **8c**,**e**,**m**,**q**,**s**,**u**,**v**. Promatranje dobivenih vrijednosti kod 0,05 mmol L^{-1} pokazuje da spojevi **8o** i **8c** imaju najsnažnije djelovanje (77 i 58%). Pri koncentraciji 0,1 mmol L^{-1} najaktivniji su spojevi **8o** i **8c**, te ih slijede **8v** > **m** = **x** > **r** > **s**. Nije uočena uloga lipofilnosti cijele molekule na interakciju s DPPH. Nasuprot tome, lipofilni doprinos

supstituenta π je važan za spomenutu interakciju. Niže π vrijednosti povezane su s jačim djelovanjem pri 0,1 mmol L^{-1}: **8m** (76%) > **8s** (63%) (π $-CH_2CH_3$ = 1,02, π $-CH_2C_6H_5$ = 2,01), **8o** (86%) > **8u** (63%) (π $-CH_2CH_3$ = 1,02, π $-CH_2C_6H_5$ = 2,01).

Azo spojevi koji generiraju slobodne radikale spontanom termičkom razgradnjom korisni su za *in vitro* studije nastajanja slobodnih radikala. Jedan od njih je i vodotopljivi 2,2'-azobis(2-amidinopropan) dihidroklorid (AAPH), izvor alkilperoksidnih slobodnih radikala koji se uobičajeno upotrebljava u znanstvenim istraživanjima. U našoj studiji AAPH je korišten kao inicijator slobodnih radikala, što slijede oksidativne promjene linolne kiseline do konjugiranog peroksidnog diena.

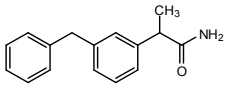
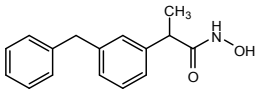
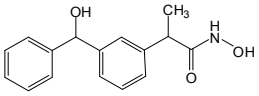
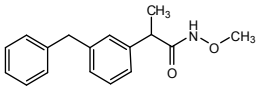
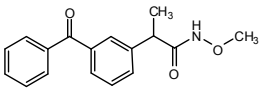
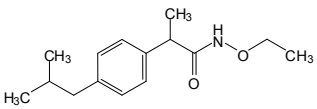
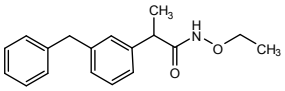
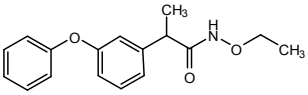
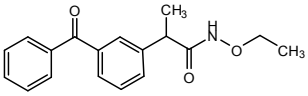
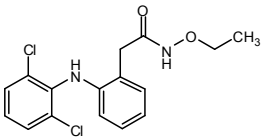
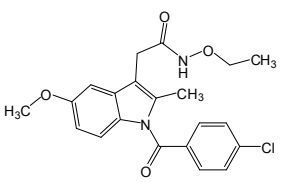
Ispitana je sposobnost inhibicije lipidne peroksidacije linolne kiseline derivatima NSAID hidroksamskih kiselina **8a,c**,**e**,**i**,**j**,**m-y**, prema ranije opisanoj metodi.[116] Rezultati pokazuju da su spojevi **8t**,**y**,**x**,**r**,**o** vrlo dobri inhibitori lipidne peroksidacije linolne kiseline (98,7–93%, Tablica 39).

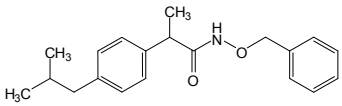
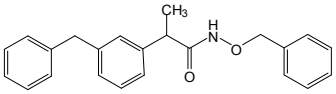
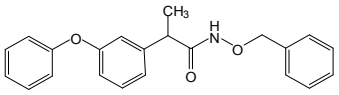
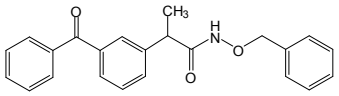
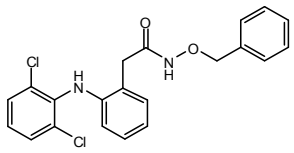
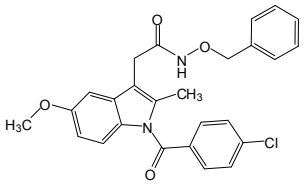
Lipooksigenaze oksidiraju određene masne kiseline, koje u svojoj strukturi sadrže konjugiranu triensku strukturu na specifičnim položajima, do hidroperoksida, prekursora leukotriena, važnih medijatora upale. Poznato je da NSAID i njihovi ranije opisani derivati **8k** i **l** inhibiraju lipooksigenazu iz soje (LO),[7] koja prevodi linolnu kiselinu u 13-hidroperoksilinolnu kiselinu, na sličan način kao i lipooksigenaza iz masnih stanica štakora. Zbog toga se inhibicija LO može uzeti kao pouzdani pokazatelj inhibicije tog enzima. Većina inhibitora lipooksigenaze su antioksidansi ili hvatači slobodnih radikala, zbog toga što se lipooksigenacija događa preko ugljikovih radikala.

Sposobnost inhibicije LO derivatima NSAID hidroksamskih kiselina **8a,c**,**e**,**i**,**j**,**m-y** ispitana je enzimskim testom temeljenim na mjerenju UV apsorpcije (Tablica 39).[115,117] Između ispitanih spojeva, najsnažniji inhibitor je spoj **8j** (IC_{50} = 75 μmol L^{-1}), slijede ga spojevi **8r**,**s**,**u**. Iako je lipofilnost važna fizikalno-kemijska veličina inhibitora lipooksigenaze,[122–124] samo se spoj **8s** pokorava tom pravilu (clog P = 7,03).

Kavena kiselina, nordihidroguaiaretična kiselina (NDGA) i troloks poznati su antioksidansi te je djelovanje spojeva **8** uspoređeno s njihovim djelovanjem. Niti jedan spoj nije stupio u jaču interakciju s DPPH kod obje ispitane koncentracije (0,05 i 0,1 mmol L^{-1}) od NDGA. Nasuprot tome, svi derivati NSAID hidroksamskih kiselina bili su snažniji inhibitori LO od kavene kiseline, dok su spojevi **8t**,**y**,**x**,**r**,**o**,**s**,**j** bili bolji inhibitori lipidne peroksidacije od troloksa.

Tablica 39. Teorijski izračunate clog *P* vrijednosti, interakcija s DPPH, inhibicija lipidne peroksidacije linolne kiseline i *in vitro* inhibicija lipooksigenaze NSAID hidroksamskih kiselina i njihovih derivata **8**.

Spoj	Struktura	clog *P*[a]	DPPH 20 min[b] (%)	DPPH 60 min[b] (%)	DPPH 20 min[c] (%)	DPPH 60 min[c] (%)	Inhibicija LP[c] (%)	LO[d]
8a		2,83	4,5	4,8	30,0	24,1	23,6	325
8c		3,22	58	46	82	68	n.a.[e]	380
8e		1,56	7,4	18,3	26,6	43,0	44,3	200
8i		3,04	4,7	5,3	22,8	26,1	n.a.	n.i.[f]
8j		n.o.[g]	2,1	n.a.	34,7	29,5	68,3	75
8m		3,79	8	30	76	63	4	400
8n		3,57	6,5	5,9	27,6	28,6	n.a.	385
8o		4,73	77	n.a	86	65	93	n.a.
8p		2,88	32	25	26	31	54	190
8q		4,83	32	35	34	55	32	310
8r		4,29	n.a.	n.a.	68	45	96	82

8s		7,03	21	44	63	63	71	96
8t		4,98	6,2	5,4	29,4	29,3	98,7	100
8u		5,76	15	18	20	63	17	125
8v		6,12	18	28	80	93	28	n.a.
8x		8,07	11	34	76	75	96	250
8y		7,53	30	11	38	34	97	340
	Kavena kiselina	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	600
	NDGA	81	83	93	97	n.o.	n.o.	n.o.
	Troloks	n.o.	n.o.	n.o.	n.o.	n.o.	63	n.o.

[a] Lipofilnost molekule teorijski je izračunata kao clog *P* u smjesi *n*-oktanola i pufera CLOGP programom (Biobyte Corp).[125] Koncentracije: [b] 5×10^{-5} mol L^{-1}; [c] 1×10^{-4} mol L^{-1}; [d] IC_{50} (μmol L^{-1}); [e] n.a. – nema aktivnosti; [f] n.i. – IC_{50} nije mogla biti određena, postotak inhibicije bio je 12, odnosno 27,5% pri koncentrcijama 100, odnosno 500 μmol L^{-1}; [g] n.o. – nije određeno.

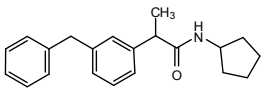
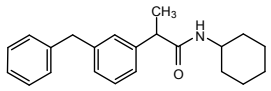
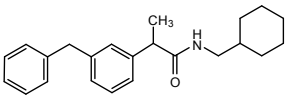
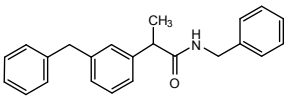
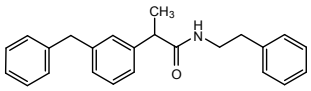
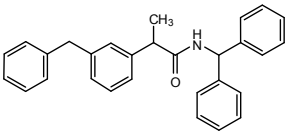
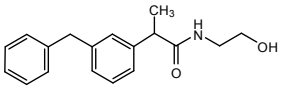
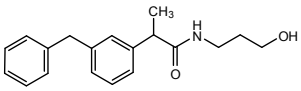
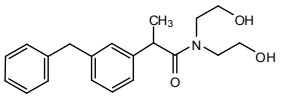
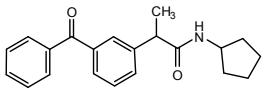
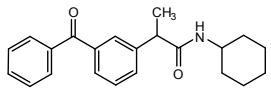
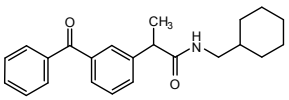
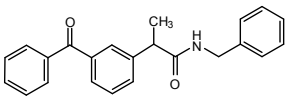
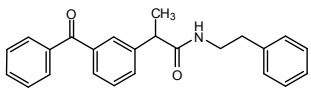
5.2.5.2. Derivati ketoprofena 9–11

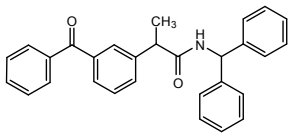
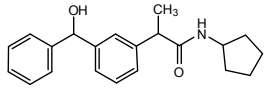
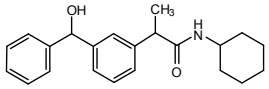
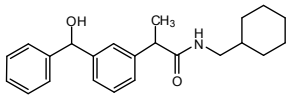
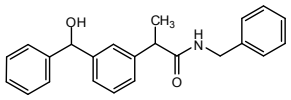
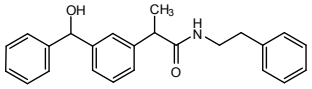
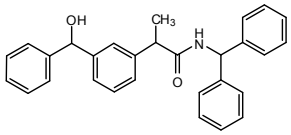
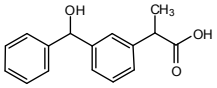
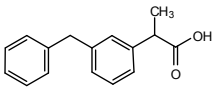
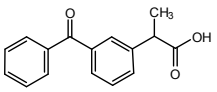
Interakcije derivata ketoprofena **9**–**11** s DPPH promatrana je kod dvije koncentracije spojeva (0,05 i 0,1 mmol L^{-1}, Tablica 40). Rezultati su pokazali da ketoprofen, početni spoj serija **9**–**11**, kao i njegovi reducirani derivati **6a**,**b**, stupaju u vrlo slabu interakciju s DPPH. Nije bilo značajnih promjena u interakciji spojeva s DPPH ovisnih o vremenu. Najaktivniji spojevi pri koncentraciji 0,1 mmol L^{-1} bili su derivati **10f,a,d**,**e** (38,5–35,2%). Nereducirani amidi ketoprofena **10** imali su snažnije djelovanje od reduciranih (npr. **10a > 9a**, **10b > 9b, 10d > 9d**). Najslabiju antioksidacijsku aktivnost imali su derivati ketoprofena s hidroksilnom skupinom **11**. Lipofilnost spojeva ne utječe na njihovu interakciju s DPPH. interakcija svih ispitanih spojeva s DPPH puno je slabija od interakcije NDGA s DPPH kod obje promatrane koncentracije.

Rezultati ispitivanja inhibicije lipidne peroksidacije linolne kiseline pokazali su da su spojevi **9e**, **10d**, **9f**, **9i**, **10f**, **9d**, **10e** i **10c** izvrsni inhibitori lipidne peroksidacije (99,9–92,2%), značajno jači od ketoprofena (69,3%) i njegovih reduciranih derivata **6a** i **6b** (51,8 i 31,1%) (Tabica 40). Općenito, metilenski derivati **9** i keto derivati **10** snažniji su inhibitori lipidne peroksidacije od hidroksi derivata **11** (**9a-g** i **10b-f** snažnji su inhibitori lipidne peroksidacije od troloksa). Usporedba amidnih supstituenata pokazala je da u seriji **9** aktivnost pada na sljedeći način: aromatski > cikloalkilni > alifatski supstituent. U seriji **10** aromatski amidi također su imali jače inhibitorno djelovanje od cikloalkilnih. Utvrđeno je da je lipofilnost molekule glavni parametar koji utječe na inhibiciju lipidne peroksidacije.

Sposobnost inhibicije LO derivatima ketoprofena **9**–**11** ispitana je enzimskim testom temeljenim na mjerenju UV apsorpcije (Tablica 40).[115,117] Svi spojevi bolji su inhibitori LO od poredbene supstancije (kavena kiselina). Usporedba IC_{50} vrijednosti pokazuje da je spoj **11f** najaktivniji od svih ostalih, slijede ga spojevi **9b**, **9e**, **9f**, **10c**, **10a**, **10e** ≈ **10f**, **9d, 9c** i **9a** (IC_{50} = 20,5–82 μmol L^{-1}). Derivati ketoprofena s hidroksilnom skupinom **11**, osim **11f**, imaju puno slabije djelovanje. Općenito, amidi s aromatskim i cikloalkilnim supstituentima snažniji su inhibitori lipooksigenaze (IC_{50} = 37,5–120 μmol L^{-1}) od derivata s funkcionalnom skupinom karboksilnih kiselina (IC_{50} ketoprofena, **6a** i **b** su 130, 200 i 415 μmol L^{-1}). Spoj **11f**, koji je imao najjače inhibitorno djelovanje na LO, nema najveću vrijednost clog *P* te se općenito može reći da lipofilnost molekula nije glavni parametar koji pozitivno utječe na inhibitornu aktivnost.

Tablica 40. Teorijski izračunate clog *P* vrijednosti, interakcija s DPPH, inhibicija lipidne peroksidacije linolne kiseline i *in vitro* inhibicija lipooksigenaze derivata ketoprofena **9–11**.

Spoj	Struktura	clog *P*[a]	DPPH 20 min[b] (%)	DPPH 60 min[b] (%)	DPPH 20 min[c] (%)	DPPH 60 min[c] (%)	Inhibicija LP[c] (%)	LO[d]
9a		4,54	6,0	4,0	28,5	27,6	63,2	82
9b		5,10	5,7	4,9	30,9	29,5	80,4	37,5
9c		5,72	3,1	4,3	31,4	33,9	87,9	80
9d		4,63	6,0	1,1	19,9	26,6	97,0	74,5
9e		5,01	6,6	3,4	32,9	21,7	99,9	43
9f		5,98	4,9	4,5	34,0	28,5	98,8	50
9g		2,69	n.a.[e]	n.a.	33,0	22,5	66,7	270
9h		2,14	2,1	n.a.	32,2	27,7	32,6	280
9i		2,83	2,9	n.a.	32,7	23,5	22,8	400
10a		3,51	2,0	n.a.	38,1	33,8	55,8	61,5
10b		4,07	0,8	n.a.	36,1	31,6	69,7	120
10c		4,69	2,2	n.a.	29,4	35,4	92,2	53
10d		3,61	2,5	n.a.	36,8	31,2	99,0	87,5
10e		3,98	1,5	n.a.	35,2	30,8	92,2	65

10f		4,95	2,6	n.a.	38,5	32,5	98,6	64,5
11a		2,78	3,4	4,9	1,9	2,7	23,6	230
11b		3,34	4,4	5,6	2,9	2,6	43,0	425
11c		3,96	1,5	6,9	2,9	3,4	28,1	295
11d		2,88	2,9	4,5	3,9	2,6	31,2	200
11e		3,26	1,3	0,9	1,2	5,4	35,1	120
11f		4,22	3,2	3,8	3,8	2,1	n.a.	20,5
6a		2,03	n.a.	2,6	n.a.	5,5	51,8	200
6b		3,79	5,6	4,0	6,9	4,8	31,1	415
Ket		2,76	6,4	3,1	8,1	7,2	69,3	130
	NDGA	n.o.[f]	81	83	93	97	n.o.	n.o.
	Troloks	n.o.	n.o.	n.o.	n.o.	n.o.	63	n.o.
	Kavena kiselina	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	600

[a] Lipofilnost molekule teorijski je izračunata kao clog *P* u smjesi *n*-oktanola i pufera CLOGP programom (Biobyte Corp).[125] Koncentracije: [b] 5×10^{-5} mol L^{-1}; [c] 1×10^{-4} mol L^{-1}; [d] IC_{50} (μmol L^{-1}); [e] n.a. – nema aktivnosti; [f] n.o. – nije određeno.

6. ZAKLJUČCI

1. U ovom doktorskom radu ukupno je sintetizirano 85 spojeva, od čega je 48 novih, do sada nepoznatih spojeva. Strukture novih spojeva potvrđene su uobičajenim analitičkim i spektroskopskim metodama (talište, elementarna analiza, IR, ^{1}H i ^{13}C NMR).

2. Sintetizirani su derivati aminokiselina: *N*-Btc-aminokiseline **2**, njihovi kloridi **3**, amidi **4** i 3,5-disupstituirani hidantoin **5**. Serija od 8 strukturnih analoga 3,5-disupstituiranih hidantoina **5a-h** s lipofilnim supstituentima na C-5, N-3 ili na oba atoma pripravljena je intramolekulskom ciklizacijom amida *N*-Btc-aminokiselina **4a-h**. Novi spojevi su **4a** i svi hidantoini, osim **5c** i **5f**.

3. Sintetizirani su derivati NSAID **6–12** (60 spojeva):
   - 3.1. Reducirani derivati ketoprofena **6a**,**b** sintetizirani su katalitičkim hidrogeniranjem ketoprofena uz Pd/C ili Pd/C(en).
   - 3.2. Sintetizirano je 8 NSAID benzotriazolida **7a-h** sljedećih lijekova: ibuprofena, fenoprofena, diklofenaka, indometacina, ketoprofena, **6a**,**b**. Novi spojevi su **7f-h**.
   - 3.3. NSAID hidroksamske kiseline i njihovi derivati **8a-y** (24 spoja) sintetizirani su novim sintetskim postupkom, iz NSAID benzotriazolida **7a-f**, u reakciji nukleofilne supstitucije u kojoj je benzotriazol zamijenjen odgovarajućim hidroksilaminom (hidroksilamin, *N*-metilhidroksilamin, *O*-metilhidroksilamin, *O*-etilhidroksilamin i *O*-benzilhidroksilamin) ili katalitičkim hidrogeniranjem *O*-benzilnih derivata NSAID hidroksamskih kiselina. Novih spojeva je 16 (**8a**,**c**,**e**,**i**,**j**,**m-x**).
   - 3.4. Sintetizirani su derivati ketoprofena **9a-i**, **10a-f** i **11a-f** (21 spoj). Amidi **9a-i** i **10a-f** sintetizirani su reakcijom nukleofilne supstitucije, u kojoj je benzotriazol u NSAID benzotriazolidima **7e** i **g** zamijenjen odgovarajućim aminom. Spojevi **11a-f** sintetizirani su katalitičkim hidrogeniranjem amida ketoprofena **10a-f** uz Pd/C(en). Svi spojevi su novi, osim **10b**,**d**.
   - 3.5. 3-Hidroksipropilamidi NSAID **12a-e** sintetizirani su reakcijom nukleofilne supstitucije, u kojoj je benzotriazol u NSAID benzotriazolidima **7a-e** zamijenjen 3-hidroksipropilaminom. Novi spojevi su **12a**,**c**.

4. Provedena su sljedeća biološka ispitivanja: antitumorsko, antimikrobno, antivirusno i antioksidacijsko djelovanje, inhibicija ureaze, lipooksigenaze i lipidne peroksidacije.
   4.1. Derivati NSAID **8**–**11** pokazali su snažnije antiproliferativno djelovanje od derivata hidantoina **5**. Najbolje antitumorsko djelovanje pokazala je hidroksamska kiselina MeKet **8c**, koja je na rast stanične linije MiaPaCa-2 djelovala diferencijalno i selektivno (IC_{50} = 2,2 μmol L^{-1}).
   4.2. Antimikrobno djelovanje bilo je slabo. Ibuprofen i fenoprofen hidroksamske kiseline (**8b**,**d**) pokazale su široki spektar antimikrobnog djelovanja protiv ispitanih Gram-pozitivnih i Gram-negativnih bakterijskih vrsta, s najvišim vrijednostima *MIC*/*MMcC* (0,116/0,232 mg mL^{-1}) prema *B. subtilis* i *E. coli*.
   4.3. Antivirusno djelovanje svih ispitanih spojeva bilo je slabo (najbolje djelovanje pokazali su hidantoin **5a** i *O*-benzilni derivat oksametacina **8y**).
   4.4. Najjači inhibitor ureaze bila je diklofenak hidroksamska kiselina **8f** (IC_{50} = 37,4 μmol L^{-1}).
   4.5. Najbolji inhibitor LO bio je amid HyKet **11f** (IC_{50} = 20,5 μmol L^{-1}).
   4.5. Najbolje antioksidacijsko djelovanje pokazao je **8o**, *O*-etilni derivat fenoprofen hidroksamske kiseline (interakcija s DPPH 77, odnosno 86%).
   4.6. Najsnažniji inhibitor lipidne peroksidacije linolne kiseline bio je amid MeKet **9e** (99,9% inhibicije).

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8. SAŽETAK

Sintetizirana je serija novih strukturnih analoga 3,5-disupstituiranih hidantoina **5a-h** s lipofilnim supstituentima na C-5, N-3 ili na oba atoma intramolekulskom ciklizacijom amida *N*-(1-benzotriazolkarbonil)aminokiselina **4a-h**.

Sintetizirani su derivati NSAID **6**–**12**: reducirani derivati ketoprofena **6a**,**b**, NSAID benzotriazolidi **7a-h**, NSAID hidroksamske kiseline i njihovi derivati **8a-y**, derivati ketoprofena **9a-i**, **10a-f**, **11a-f** i 3-hidroksipropilamidi NSAID **12a-e**. Reducirani derivati ketoprofena **6** pripravljeni su katalitičkim hidrogeniranjem ketoprofena uz Pd/C ili Pd/C(en). NSAID s karboksilnom funkcionalnom skupinom (ibuprofen, fenoprofen, diklofenak, indometacin, ketoprofen, **6a**,**b**) u reakciji s kloridom 1-benzotriazol-karboksilne kiseline (**1**) dali su odgovarajuće NSAID benzotriazolide **7**, koji su bili polazni spojevi u sintezi derivata NSAID **8**–**12**. Razvijena je nova sintetska metoda za pripravu NSAID hidroksamskih kiselina i derivata **8**, reakcijom NSAID benzotriazolida **7** i odgovarajućih hidroksilamina (hidroksilamin, *N*-metilhidroksilamin, *O*-metilhidroksilamin, *O*-etilhidroksilamin i *O*-benzilhidroksilamin) ili katalitičkim hidrogeniranjem *O*-benzilnih derivata NSAID hidroksamskih kiselina. Derivati ketoprofena **9**, **10** i 3-hidroksipropilamidi NSAID **12** sintetizirani su iz NSAID benzotriazolida **7** i odgovarajućih amina, dok su derivati ketoprofena **11** sintetizirani katalitičkim hidrogeniranjem amida ketoprofena **10** uz Pd/C(en).

Provedena su sljedeća biološka ispitivanja: antitumorsko, antimikrobno, antivirusno i antioksidacijsko djelovanje, inhibicija ureaze, lipooksigenaze i lipidne peroksidacije. Najbolje antitumorsko djelovanje pokazao je **8c**, najbolje antimikrobno **8b**,**d**, najbolje antivirusno **5a** i **8y**, a najbolje antioksidacijsko djelovanje **8o**. Najjači inhibitor ureaze bio je **8f**, a lipooksigenaze **11f**. Najbolji inhibitor lipidne peroksidacije bio je **9e**.

9. SUMMARY

3,5-Disubstituted hydantoin derivatives **5a-h** with lipophilic substituents at C-5, N-3 or both atoms were prepared by the intramolecular cyclization of the corresponding *N*-(1-benzotriazolecarbonyl)amino acid amides **4a-h**.

The following NSAID derivatives **6**–**12** were prepared: the reduced ketoprofen derivatives **6a**,**b**, NSAID benzotriazolides **7a-h**, NSAID hydroxamic acids and their derivatives **8a-y**, ketoprofen derivatives **9a-i**, **10a-f**, **11a-f** and NSAID 3-hydroxypropylamides **12a-e**. The reduced ketoprofen derivatives **6** were prepared by the catalytic hydrogenation of ketoprofen with Pd/C or Pd/C(en) catalyst. NSAIDs bearing carboxylic group (ibuprofen, fenoprofen, diclofenac, indomethacin, ketoprofen, **6a**,**b**) reacted with 1-benzotriazole carboxylic acid chloride (**1**) affording benzotriazolides **7**, which were the starting compounds in the synthesis of NSAID derivatives **8**–**12**. A new synthetic method for the synthesis of NSAID hydroxamic acids and their derivatives was developed. They were obtained in the reaction of NSAID benzotriazolides **7** with an appropriate hydroxylamine (hydroxylamine, *N*-methylhydroxylamine, *O*-methylhydroxylamine, *O*-ethylhydroxylamine or *O*-benzylhydroxylamine) or by the catalytic hydrogenation of *O*-benzyl derivatives of NSAID hydroxamic acids. Ketoprofen derivatives **9**,**10** and NSAID 3-hydroxypropylamides **12** were prepared from NSAID benzotriazolides **7**, in the reaction with an appropriate amine, while compounds of the series **11** were obtained by the catalytic hydrogenation of ketoprofen amides **10** with Pd/C(en).

The compounds were evaluated for their biological activity: antitumor, antimicrobial, antiviral and antioxidant activity, inhibition of urease, lipoxygenase and linoleic acid lipid peroxidation. The best antitumor activity was exerted by **8c**, antimicrobial by **8b**,**d**, antiviral by **5a** and **8y**, and antioxidant by **8o**. The strongest inhibitor of urease was **8f**, while **11f** was the strongest inhibitor of lipoxygenase. Compoung **9e** showed the highest inhibition of linoleic acid lipid peroxidation.

10. ŽIVOTOPIS

Zrinka Rajić rođena je u Požegi, gdje je završila osnovnu školu i gimnaziju, prirodoslovno-matematički smjer. Od 1999. do 2004. studirala je na Farmaceutsko-biokemijskom fakultetu Sveučilišta u Zagrebu. Tijekom studija primala je stipendiju Ministarstva znanosti i tehnologije RH kao najbolji student na godini. Diplomski rad pod nazivom *Sinteza i karakterizacija hidroksamskih kiselina i hidroksiurea* izradila je na Zavodu za farmaceutsku kemiju Farmaceutsko-biokemijskog fakulteta pod vodstvom prof. dr. sc. Branke Zorc te je diplomirala među prvima iz generacije.

Od 1.6.2004. zaposlena je na Farmaceutsko-biokemijskom fakultetu kao znanstveni novak-asistent te sudjeluje u izvođenju nastave na kolegijima Farmaceutska kemija i Biokemija lijekova. 2005. upisala je poslijediplomski doktorski studij Farmaceutske znanosti na Farmaceutsko-biokemijskom fakultetu. Stručni ispit za magistre farmacije položila je u listopadu 2005. i dobila licencu za samostalan rad. Član je Hrvatskog farmaceutskog društva i Hrvatske ljekarničke komore.

U ožujku 2006. boravila je na Tehnološkom sveučilištu u Grazu, Institutu za analitičku, radio- i mikrokemiju, a od rujna 2007. do ožujka 2008. na ETH, Zürich, Laboratoriju za organsku kemiju, u grupi prof. Vaselle, gdje je sudjelovala u sintetskim istraživanjima oligonukleozida.

Objavila je dva znanstvena rada (tri rada poslana su u znanstvene časopise za objavljivanje) i tri stručna rada, sudjelovala s priopćenjima na više domaćih i inozemnih kongresa te održala dva predavanja.

Znanstveni radovi:

1. Z. Rajić, B. Zorc, S. Raić-Malić, K. Ester, M. Kralj, K. Pavelić, J. Balzarini, E. De Clercq, M. Mintas, Hydantoin derivatives of L-and D- amino acids: synthesis, antiviral and antitumoral activity evaluations, *Molecules* **11** (2006) 837–848.

2. K. Wittine, K. Benci, Z. Rajić, B. Zorc, M. Kralj, M. Marjanović, K. Pavelić, E. De Clercq, G. Andrei, R. Snoeck, J. Balzarini, M. Mintas, The novel phosphoramidate derivatives of NSAID 3-hydroxypropylamides: Synthesis, cytostatic and antiviral activity evaluations, *Eur. J. Med. Chem.* (2008), doi:10.1016/j.ejmech.2008.03.037.

3. Z. Rajić, I. Perković, I. Butula, B. Zorc, D. Hadjipavlou-Litina, E. Pontiki, S. Pepeljnjak, I. Kosalec, Synthesis and biological evaluation of *O*-methyl and *O*-ethyl NSAID hydroxamic acids, *J*. *Enzym*. *Inhib*. *Med*. *Ch*. (prihvaćeno).

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5. Z. Rajić, G. Džimbeg, B. Zorc, P. P. Singh, S. Singh, Macromolecular prodrugs. XII. Primaquine conjugates: synthesis and antimalarial evaluation, poslano u *Acta Pharm*.

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2. Z. Rajić, B. Zorc, Dvojni lijekovi, Treći hrvatski kongres farmacije, Cavtat, 2005.

3. Z. Rajić, B. Zorc, Synthesis and characterization of halogenhydroxamic acids, Treći hrvatski kongres farmacije, Cavtat, 2005.

4. Z. Rajić, I. Butula, M. Przyborowska, T. Ossowski, B. Zorc, NSAID-hidroksamske kiseline: sinteza i određivanje pK_a vrijednosti, XX. hrvatski skup kemičara i kemijskih inženjera, Zagreb, 2007.

5. Z. Rajić, B. Zorc, S. Raić-Malić, K. Ester, M. Kralj, K. Pavelić, J. Balzarini, E. De Clercq, M. Mintas, Hidantoinski derivati L- i D-aminokiselina: sinteza i ispitivanje virustatskog i citostatskog djelovanja, XX. hrvatski skup kemičara i kemijskih inženjera, Zagreb, 2007.

6. Z. Rajić, G. Džimbeg, B. Zorc, M. Kralj, K. Ester, K. Pavelić, J. Balzarini, E. De Clercq, M. Mintas, The novel primaquine derivatives of *N*-alkyl, cycloalkyl or aryl urea: synthesis, cytostatic and antiviral activity evaluations, 3rd Pharmaceutical Sciences World Congress, Amsterdam, 2007.

7. Z. Rajić, B. Zorc, M. Jadrijević-Mladar Takač, M. Vinković, V. Takač, A study of H-bond in a group of NSAID hydroxamic acid derivatives by FTIR and NMR spectroscopy, 3rd Pharmaceutical Sciences World Congress, Amsterdam, 2007.

8. Z. Rajić, I. Butula, B. Zorc, S. Kraljević Pavelić, K. Hock, K. Pavelić, L. Naesens, E. De Clercq, J. Balzarini, M. Przyborowska, T. Ossowski, NSAID hydroxamic acids: Synthesis, cytostatic and antiviral activity evaluations, 5th Joint Meeting on Medicinal Chemistry, Portorož, 2007.

9. K. Wittine, Z. Rajić, B. Zorc, M. Kralj, K. Pavelić, E. De Clercq, J. Balzarini, S. Raić-Malić, M. Mintas, The novel 3-hydroxypropylamide derivatives of nonsteroidal antiinflammatory drugs and their phosphoroamidate prodrugs: synthesis, cytostatic and antiviral activity evaluations, 15th European Symposium on Organic Chemistry, Dublin, 2007.

10. Z. Rajić, I. Perković, I. Butula, B. Zorc, I. Kosalec, S. Pepeljnjak, Antibacterial and antiurease evaluation of NSAID hydroxamic acids, *Drugs Fut*. **33** (Suppl. A)(2008): XXth Int. Symp. Med. Chem., Beč, 2008.

11. I. Perković, I. Butula, Z. Rajić, B. Zorc, Preparation of hydrophilic ureido amides, *Drugs Fut*. **33** (Suppl. A)(2008): XXth Int. Symp. Med. Chem., Beč, 2008.

12. Z. Rajić, B. Zorc, Novi amidni derivati ketoprofena, XII. Ružičkini dani, Vukovar, 2008.

13. Z. Rajić, G. Džimbeg, B. Zorc, I. Perković, P. P. Singh, S. Singh, Konjugati primakina: sinteza i antimalarijsko djelovanje, XII. Ružičkini dani, Vukovar, 2008.

Stručni radovi:

1. Z. Rajić, Estrogeni i gestageni u transdermalnim terapijskim sustavima, *Farm*. *Glas*. **63** (2007), 505–517.

2. I. Vuk, Z. Rajić, B. Zorc, Malarija i antimalarici, *Farm*. *Glas*. **64** (2008), 51–60.

3. M. Huzjak, Z. Rajić, B. Zorc, Dvojni lijekovi s identičnim i neidentičnim podjedinicama, *Kem*. *Ind*. **57** (2008) 511–519.

Javna predavanja:

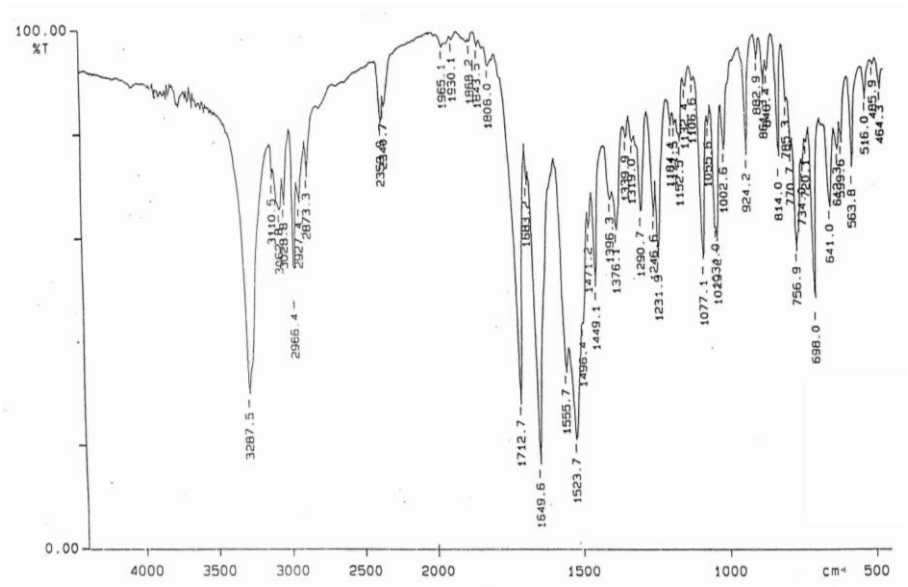
1. Sinteza i studija povezivanja amidno i hidrazidno povezanih analoga nukleozida, Kolokvij zavoda za organsku kemiju i biokemiju i Sekcije za organsku kemiju Hrvatskog kemijskog društva, 12.5.2008., Zagreb.

2. Znanstveni pristupi u istraživanju estrogena i gestagena za transdermalnu primjenu, Hrvatsko farmaceutsko društvo, 30.6.2008., Zagreb.

Ostali radovi:

1. M. Jadrijević-Mladar Takač, N. Mirošević, I. Janković, Z. Rajić, V. Takač, I. Perković, Optimiranje terapije lijekovima – imperativ za svjetsko zdravlje, *Farm*. *Glas*. **64** (2007) 411–419.

11. PRILOZI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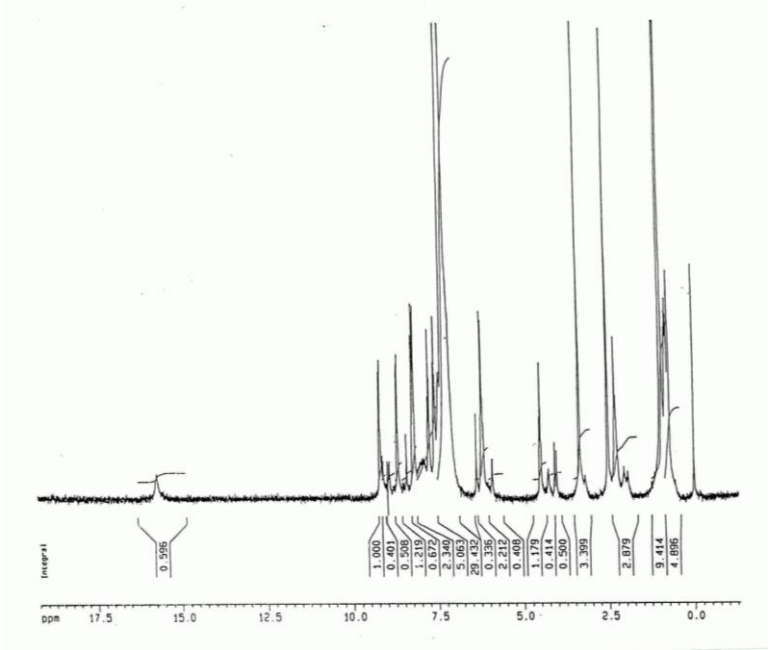
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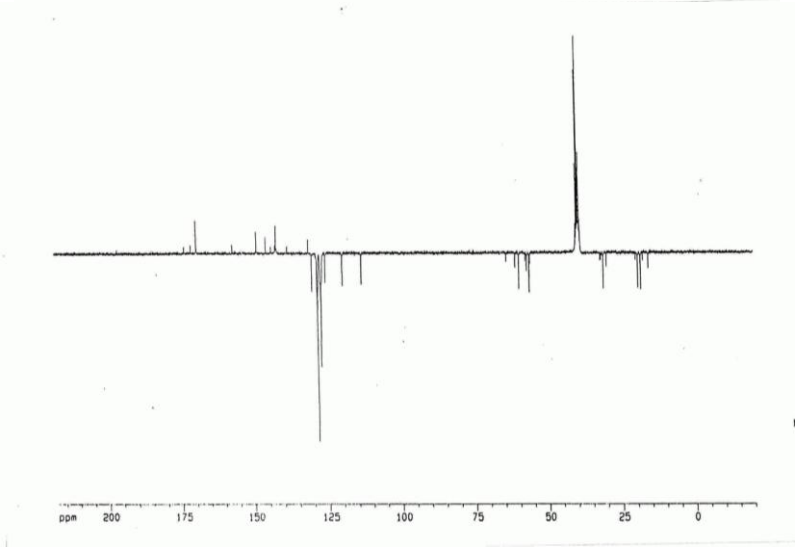
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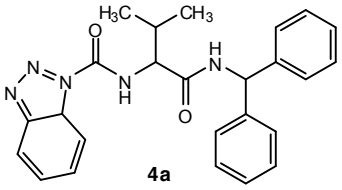

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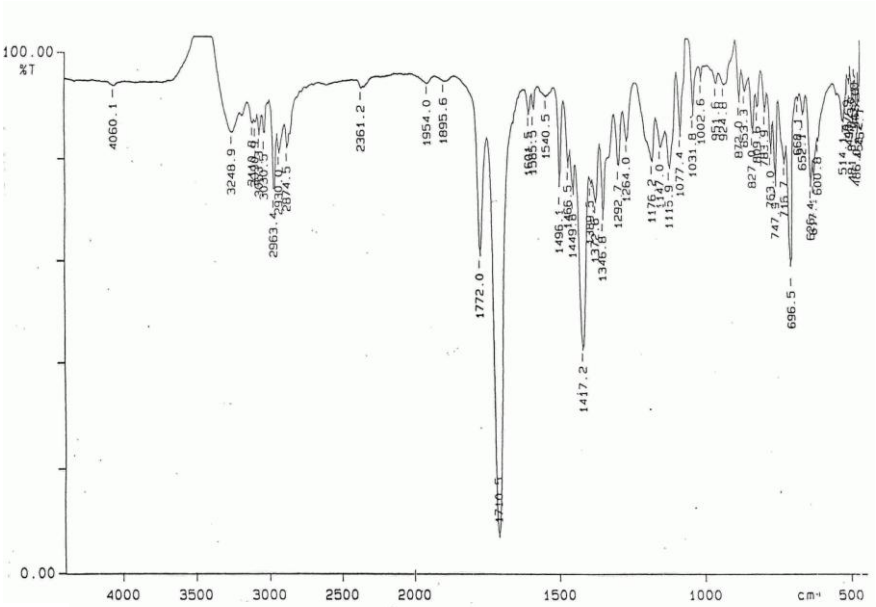
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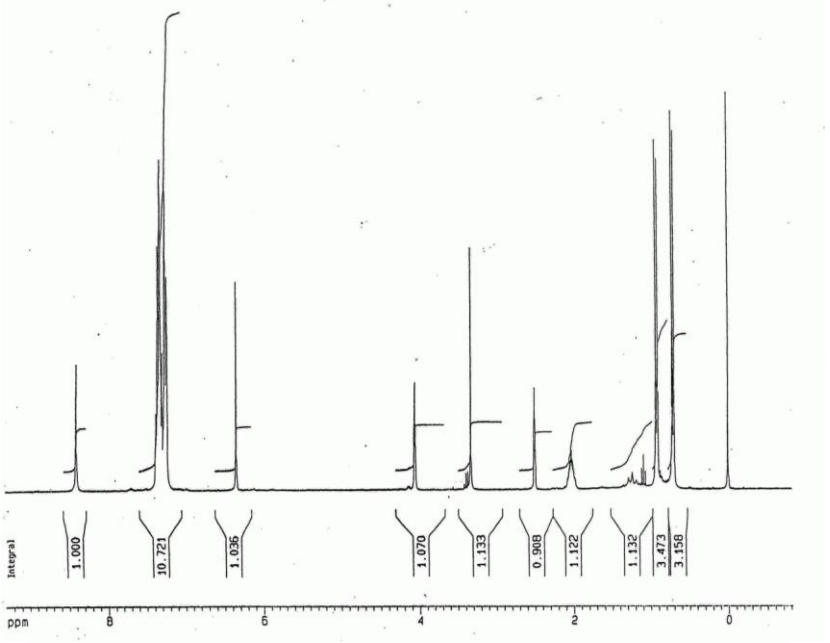
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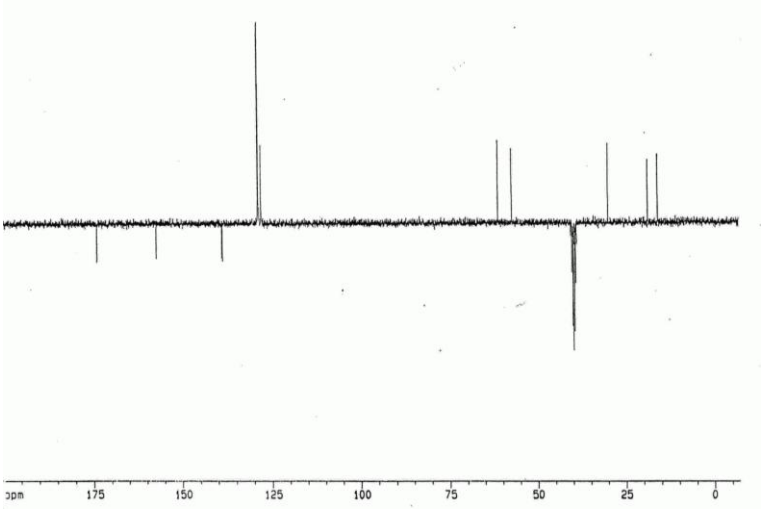
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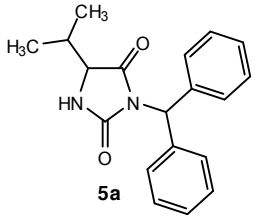
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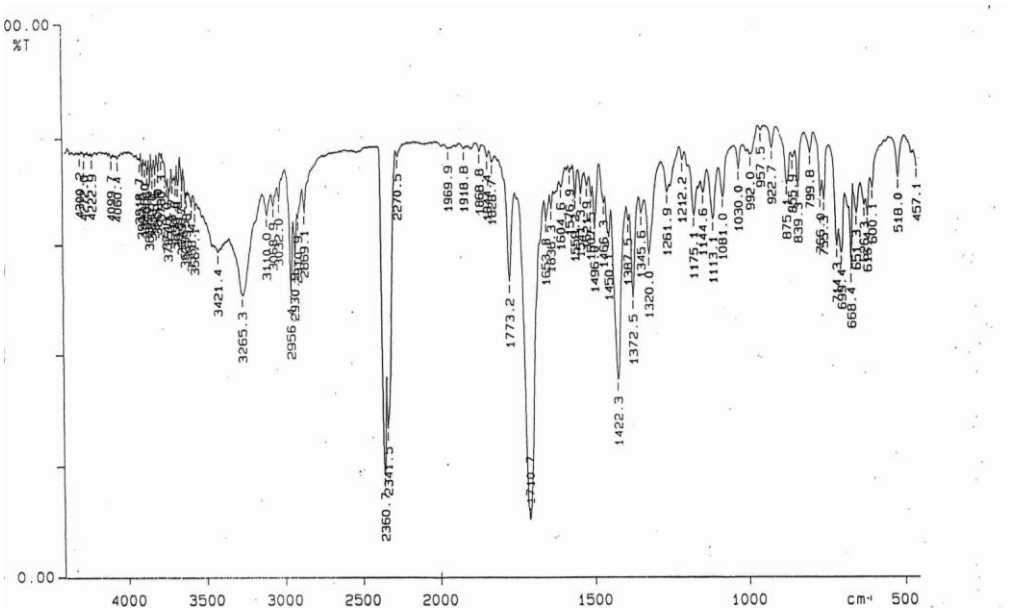
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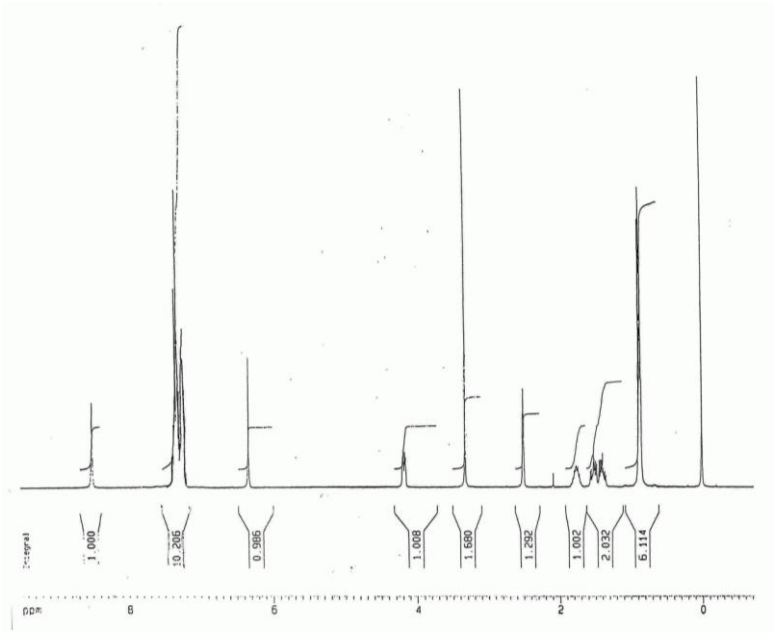
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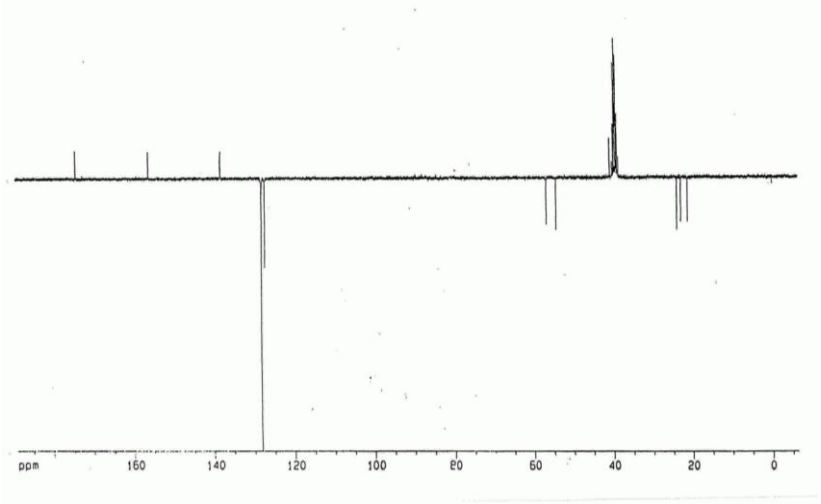
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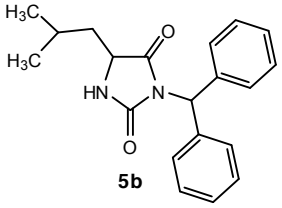
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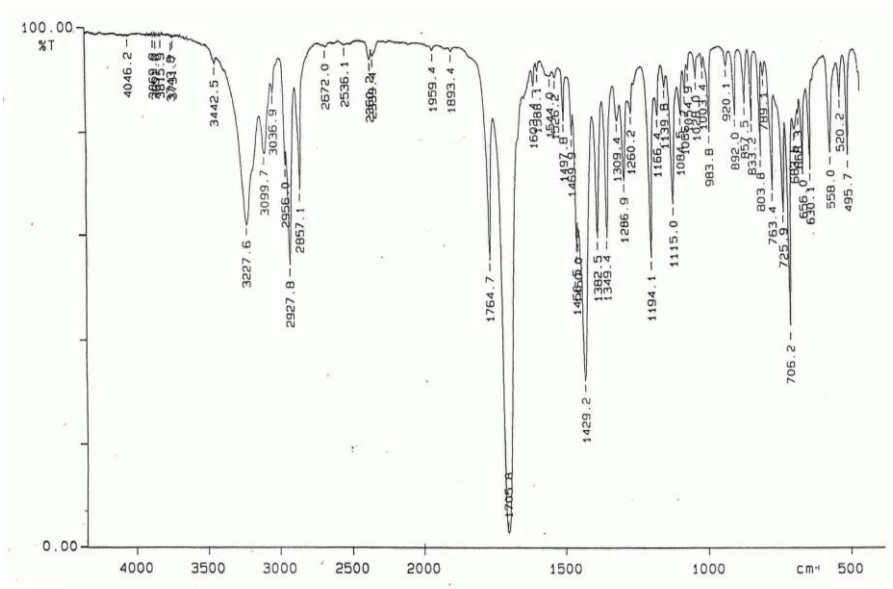
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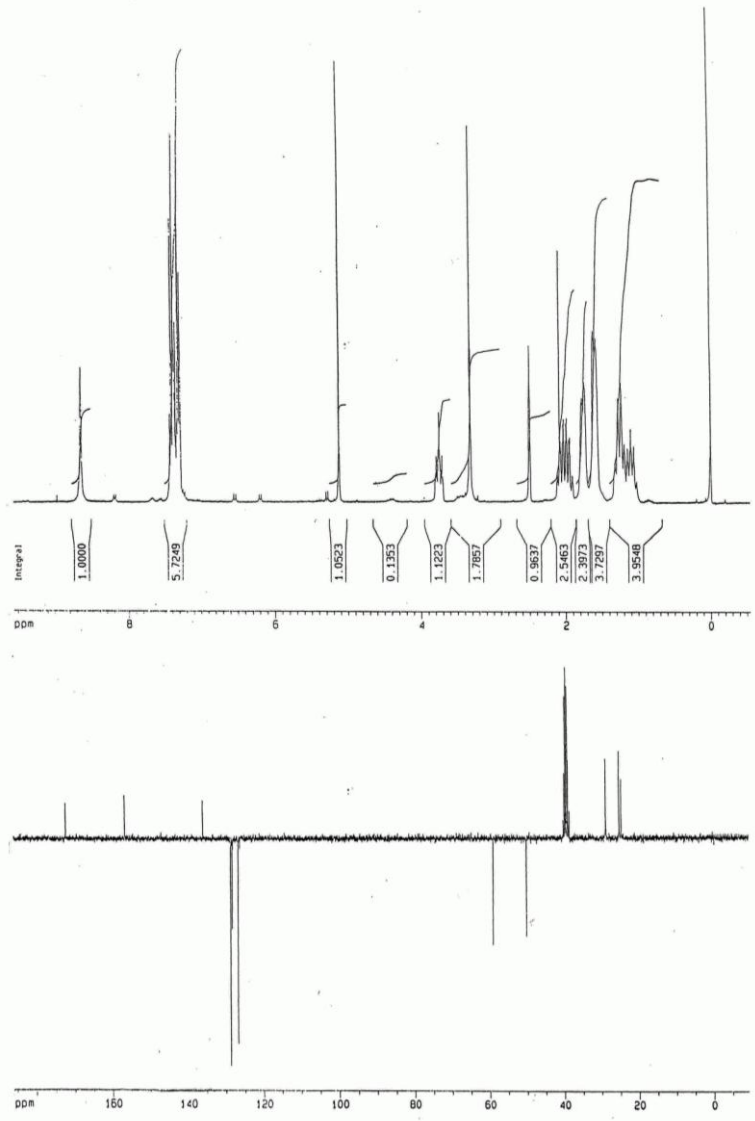
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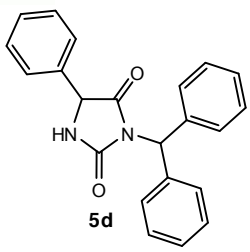
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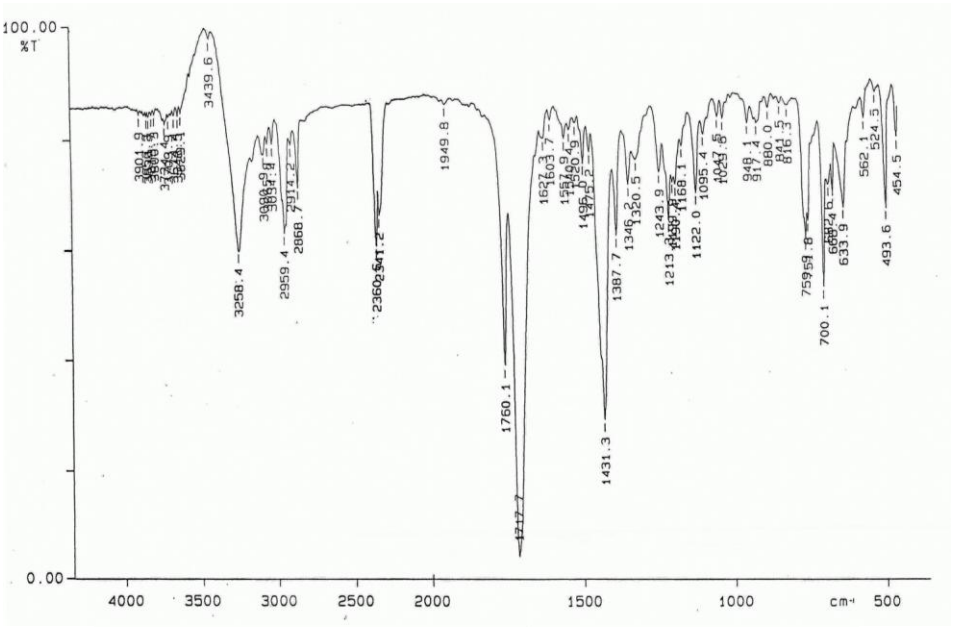
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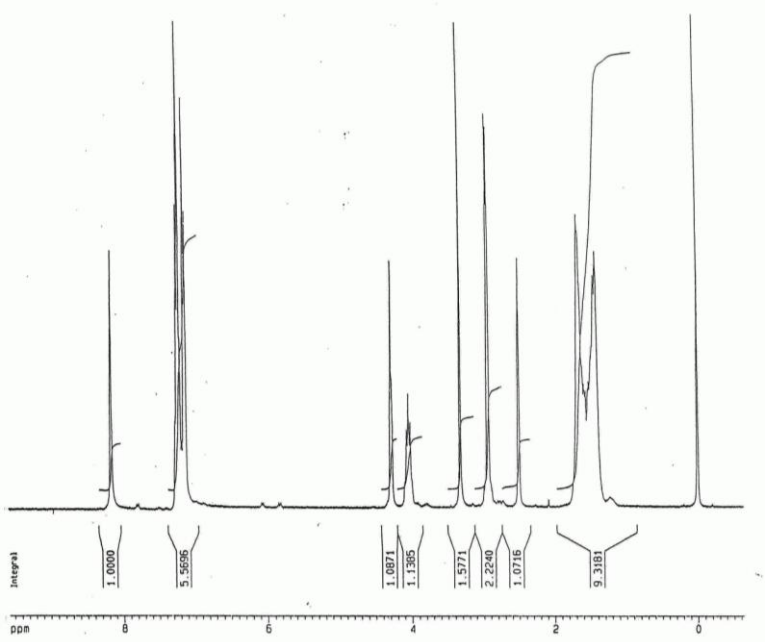
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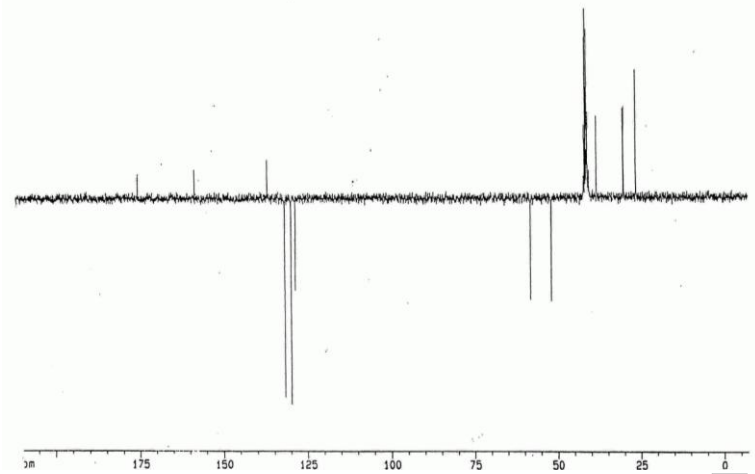
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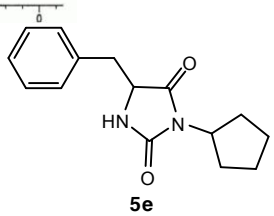
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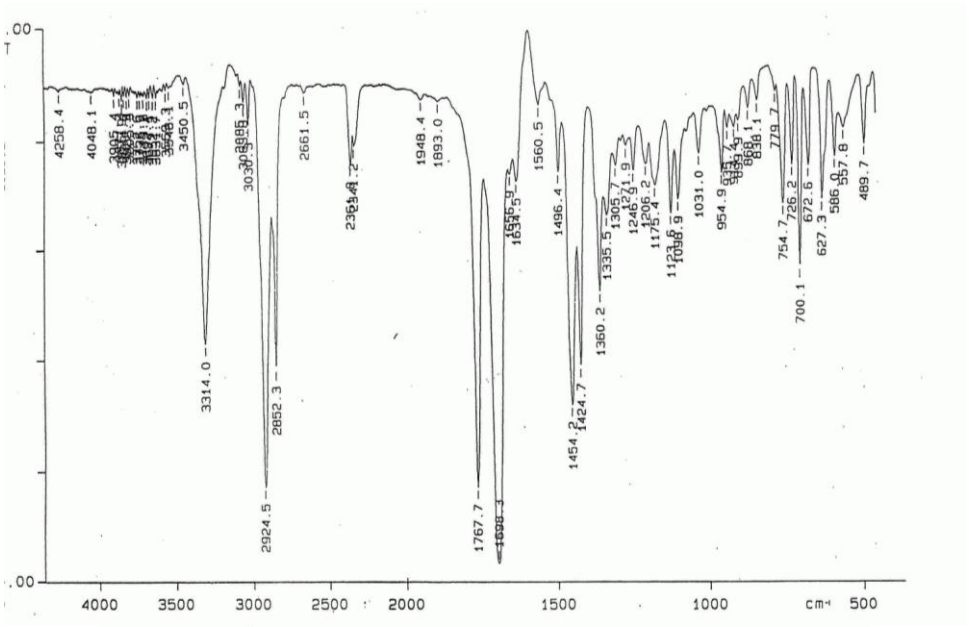
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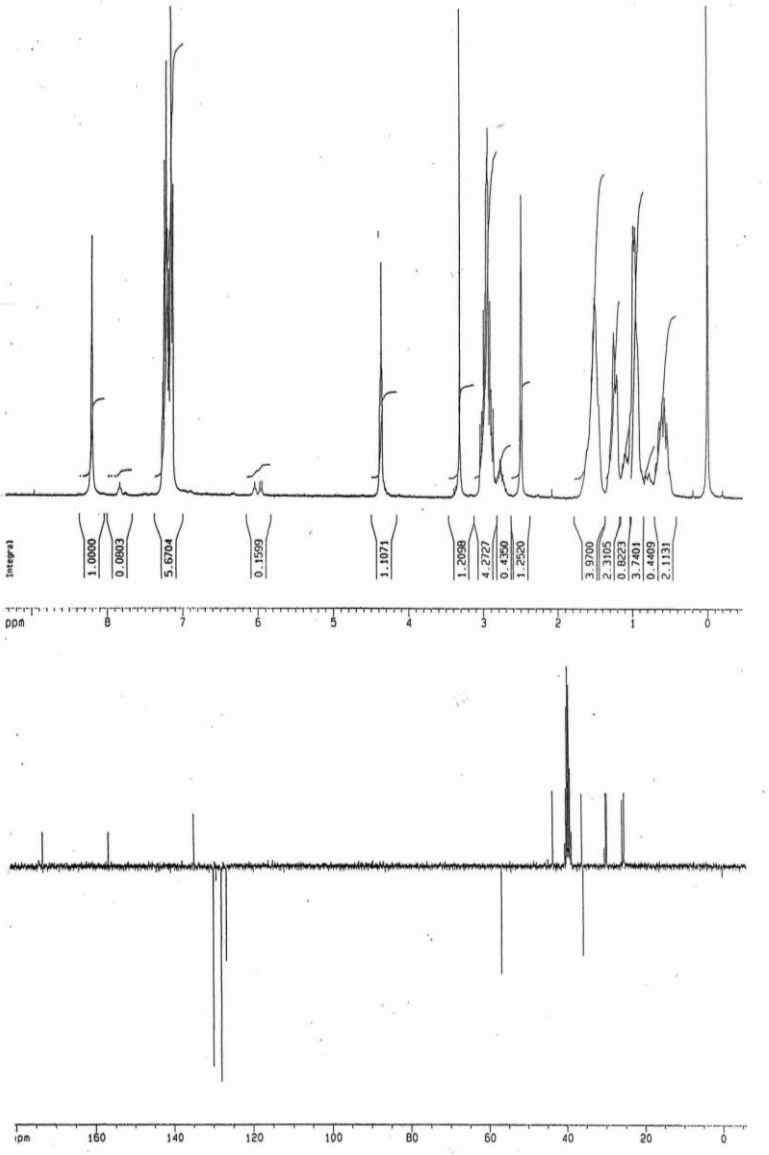
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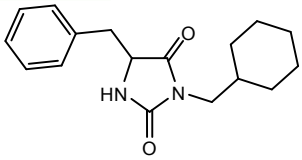
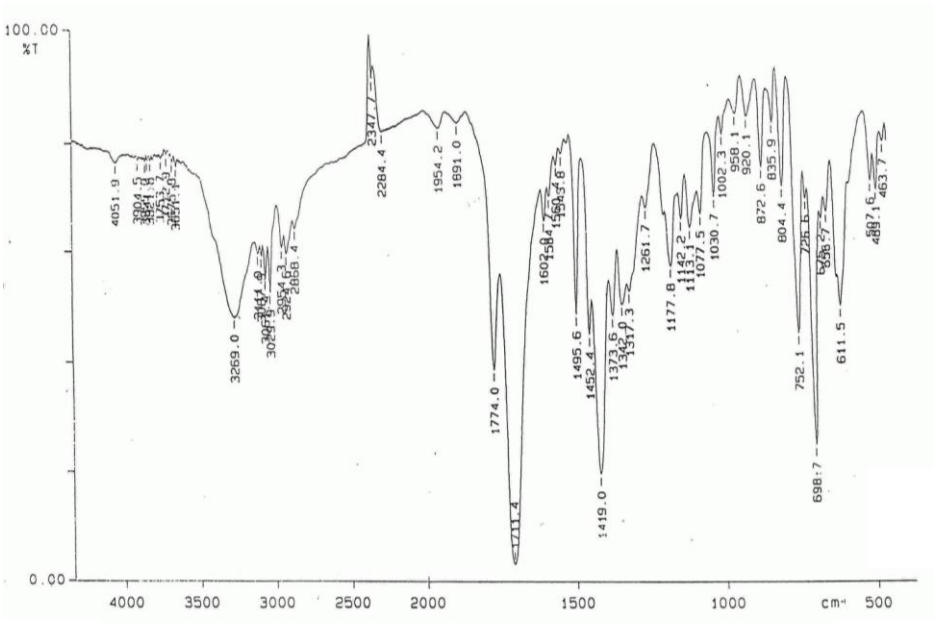
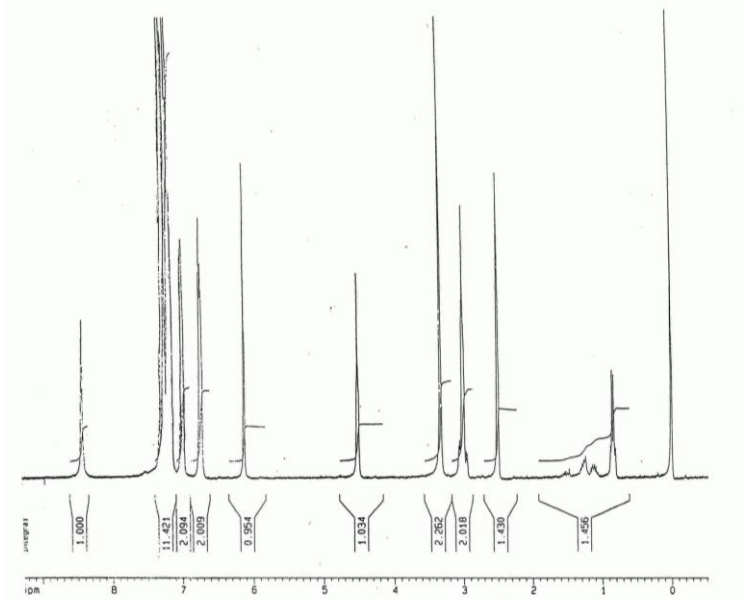
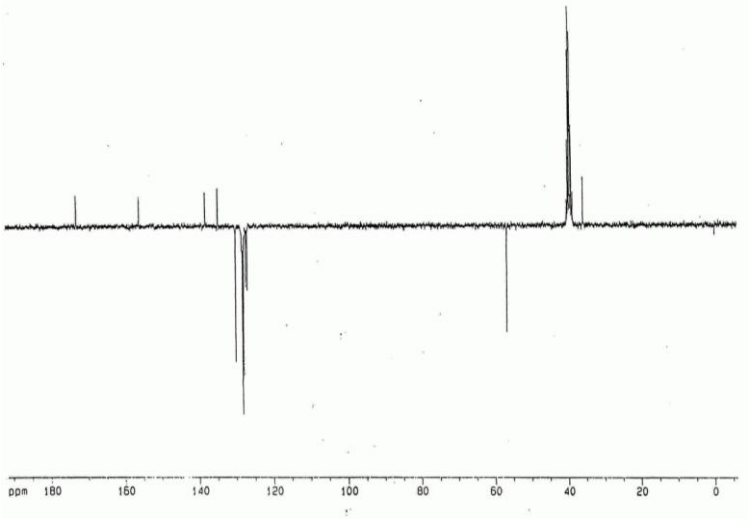
0.0803
5.6704
0.1599
1.1071
1.2098
4.2727
0.4350
1.2520
3.9700
2.3105
0.8223
3.7401
0.4409
2.1131
ppm
8
7
6
5
4
3
2
1
0
160
140
120
100
80
60
40
20
0

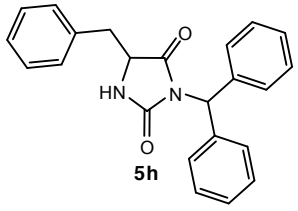

O
HN
N
O

5g

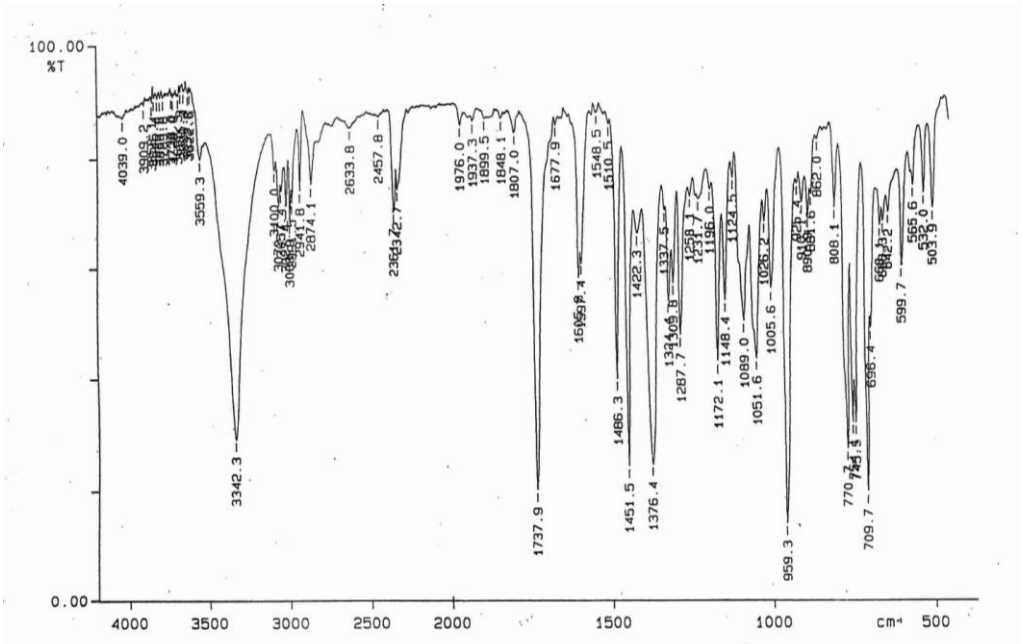

100.00
%T
0.00
4000
3500
3000
2500
2000
1500
1000
cm⁻¹
500
4051.9
3269.0
2347.7
2284.4
1954.2
1891.0
1774.0
1711.4
1495.6
1452.4
1419.0
1261.7
1177.8
1142.2
1077.5
1030.7
1002.3
958.1
920.1
872.6
835.9
804.4
752.1
698.7
611.5
463.7


1.000
11.421
2.094
2.009
0.954
1.034
2.262
2.018
1.430
1.456
ppm
8
7
6
5
4
3
2
1
0


ppm
180
160
140
120
100
80
60
40
20
0


O
HN
N
O

5h


100.00
%T
0.00
4000
3500
3000
2500
2000
1500
1000
cm-1
500
4039.0
3559.3
3342.3
2633.8
2457.8
2342.7
1976.0
1937.3
1899.5
1848.1
1807.0
1737.9
1677.9
1548.5
1510.5
1486.3
1451.5
1422.3
1376.4
1337.5
1287.7
1196.0
1172.1
1148.4
1124.5
1089.0
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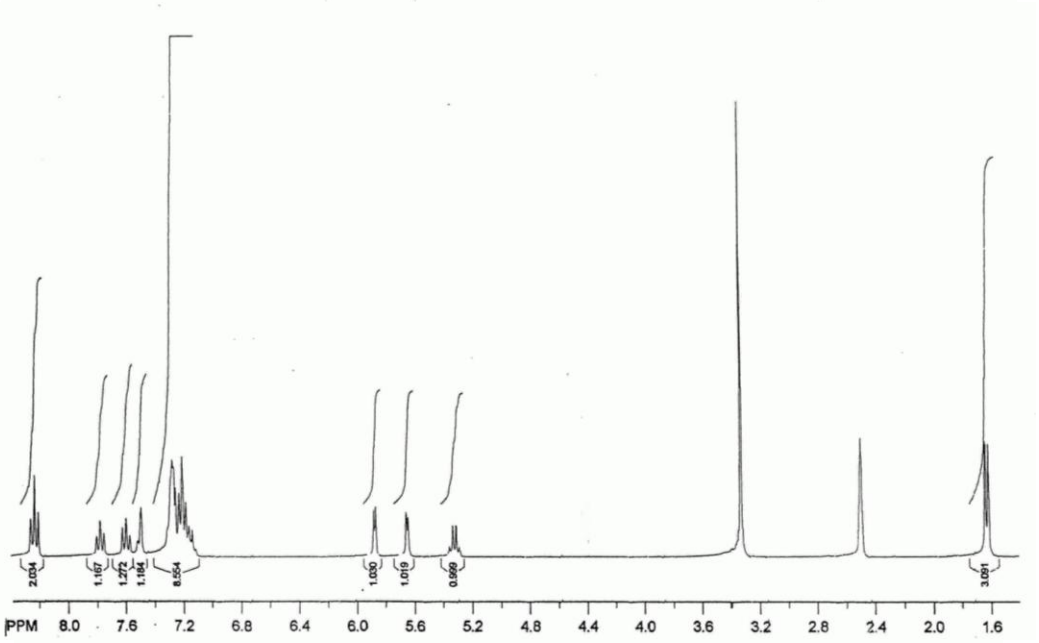
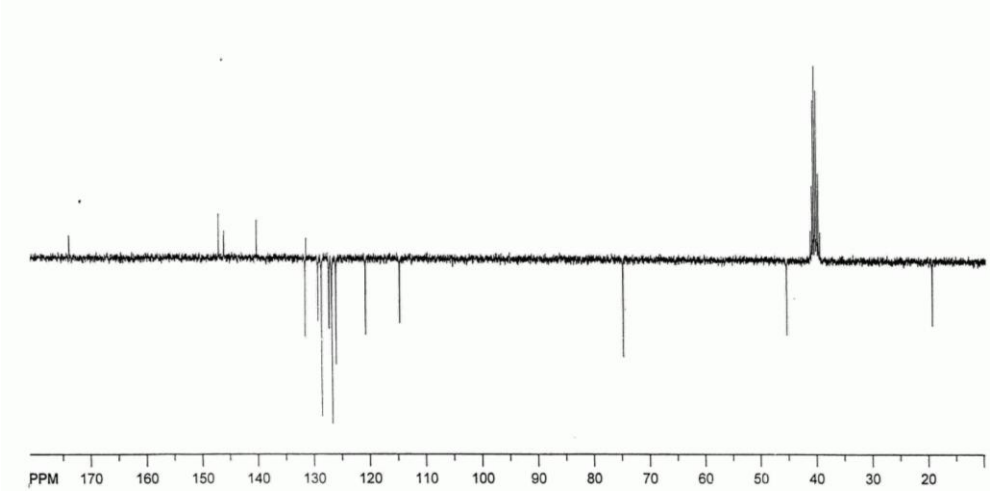
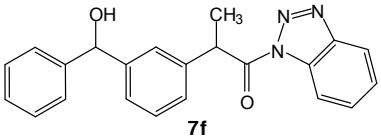
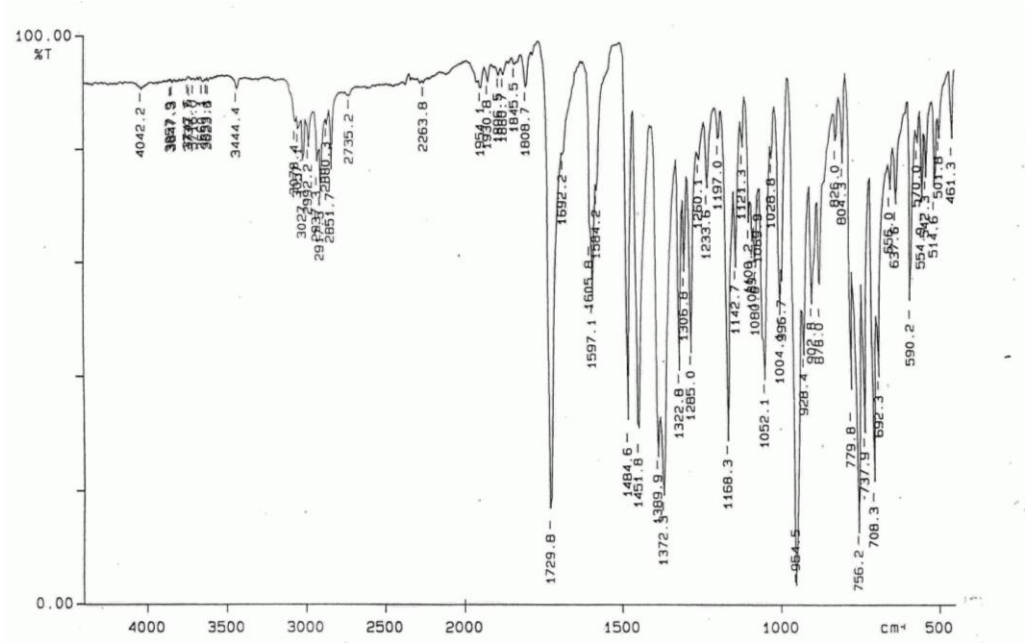
1005.6
959.3
862.0
808.1
770.7
709.7
599.7
565.6
532.0
503.9

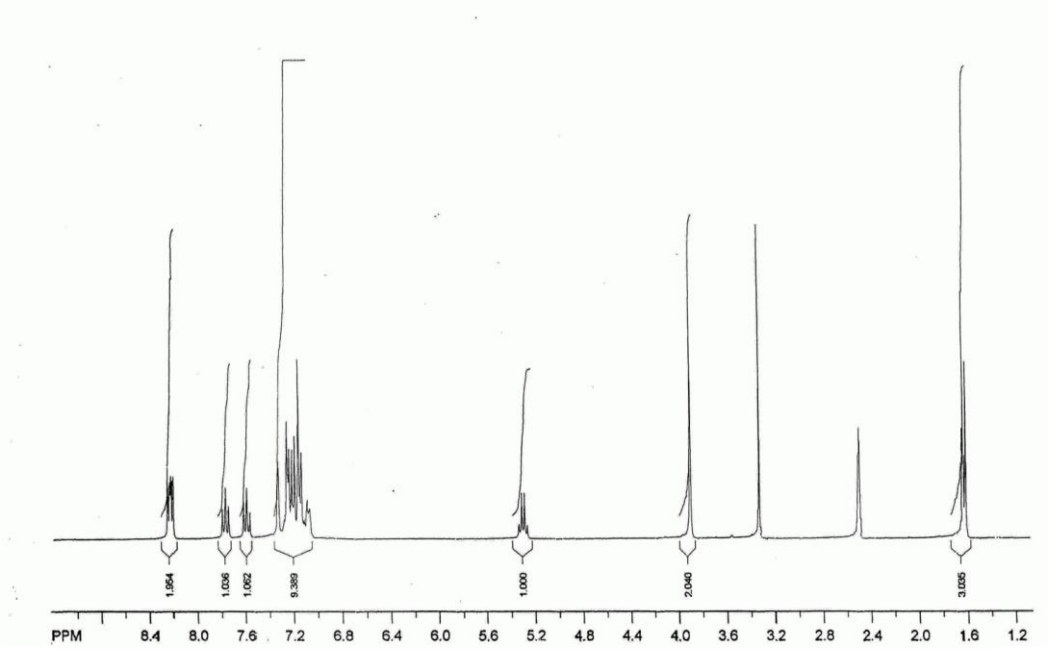

PPM
8.0
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6

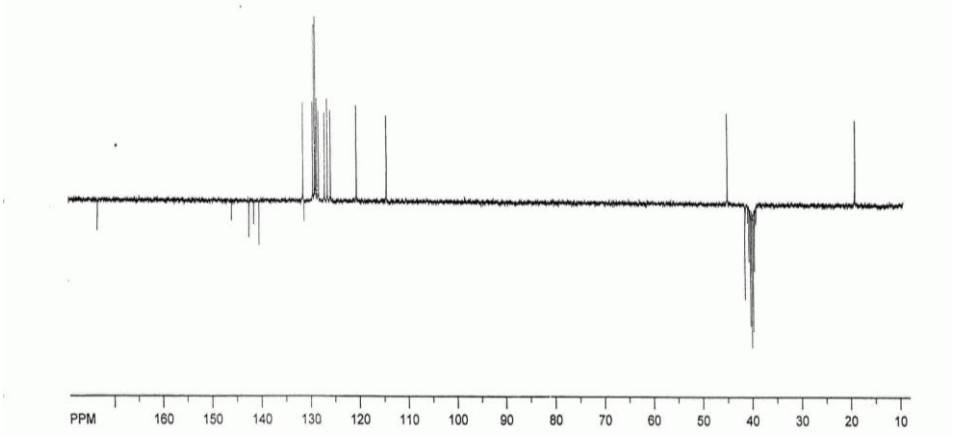

PPM
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20

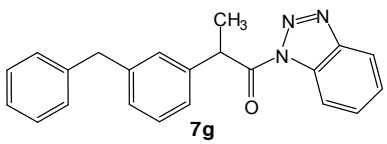

OH
CH3
N
N
N
O

7f

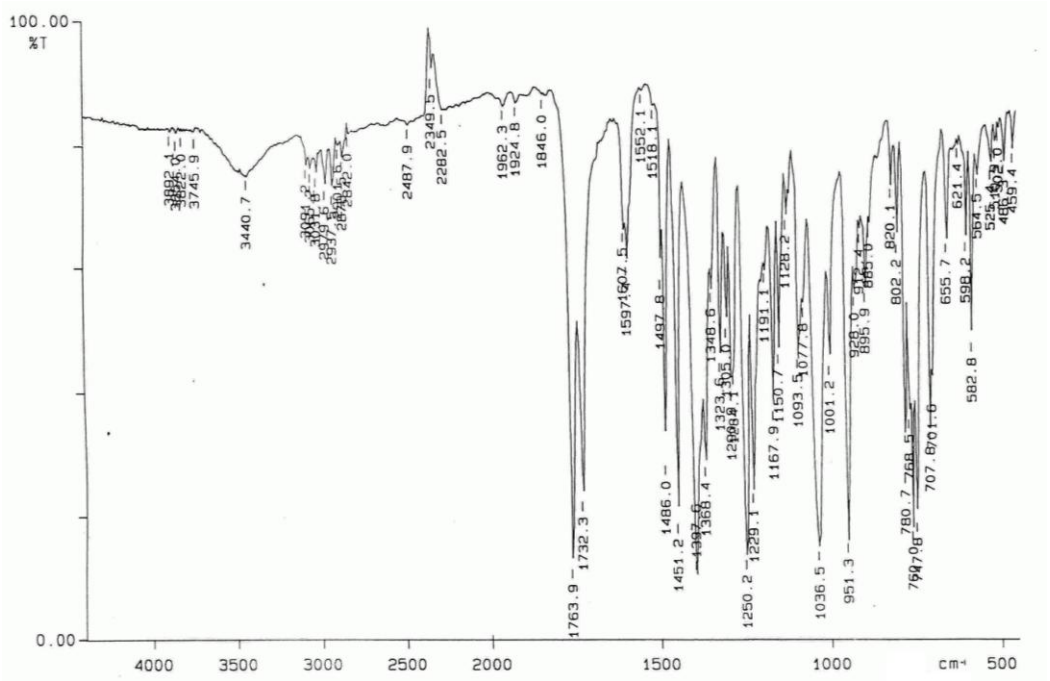

100.00
%T
0.00
4000
3500
3000
2500
2000
1500
1000
cm-1
500

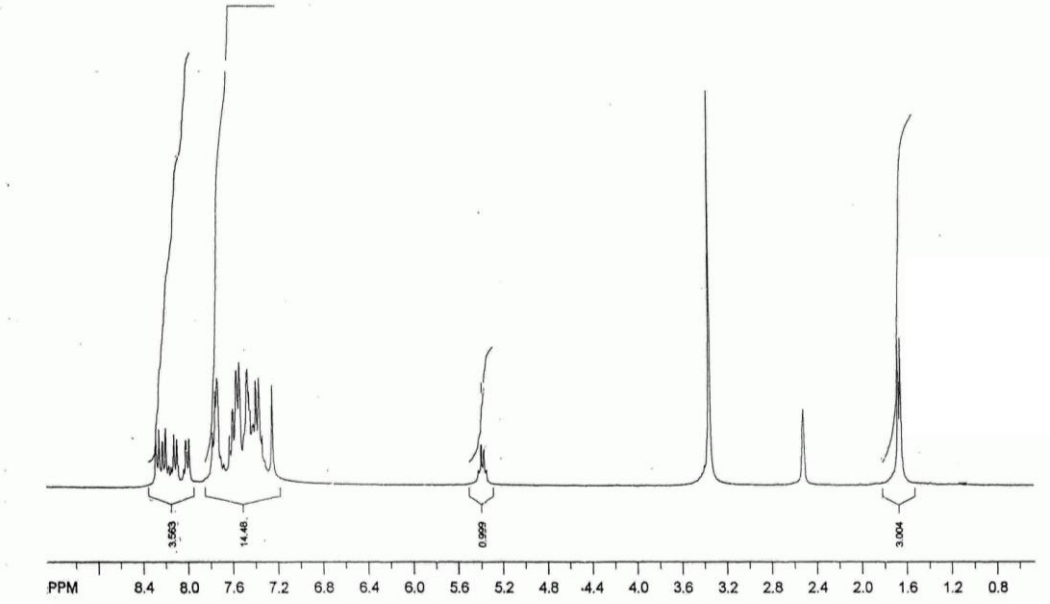

PPM
8.4
8.0
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2

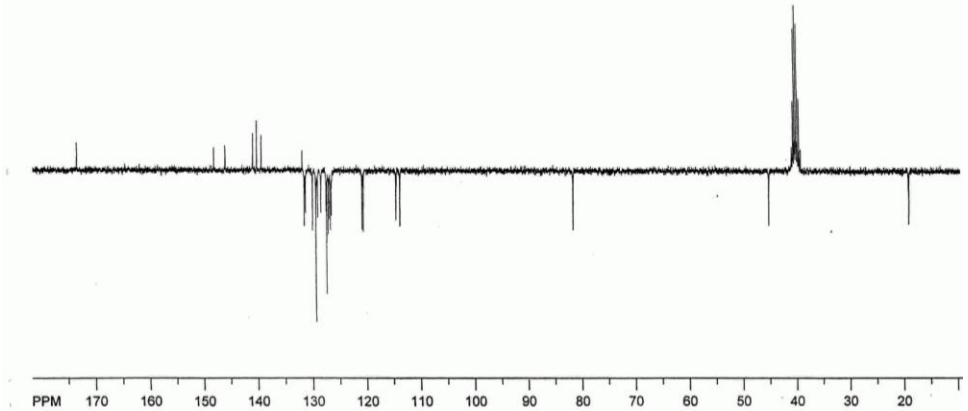

PPM
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

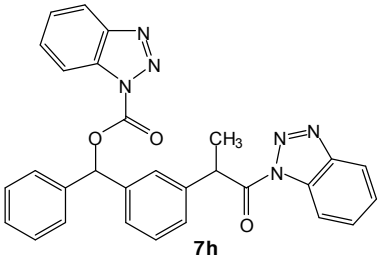

CH3
N
N
N
O

7g

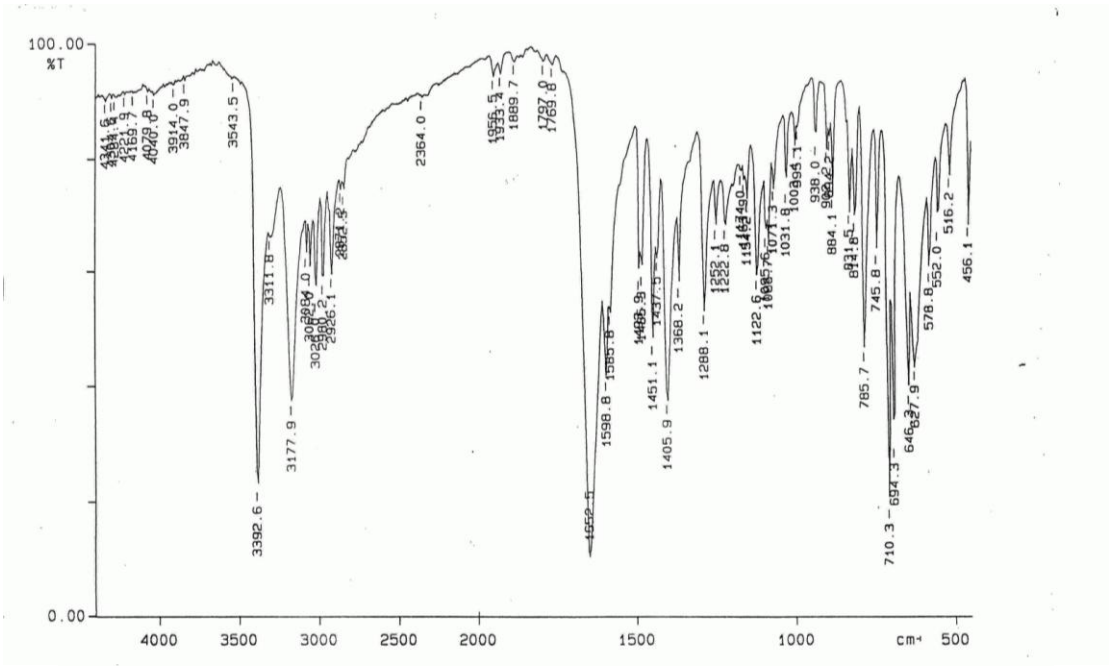

100.00
%T
0.00
4000
3500
3000
2500
2000
1500
1000
cm-1
500

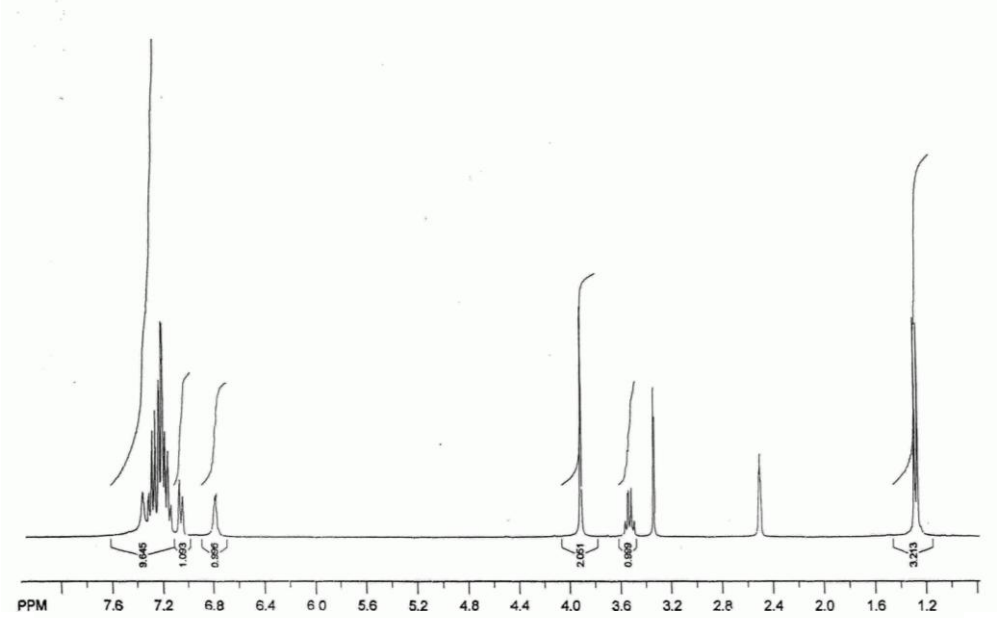

3.563
14.48
0.999
3.004
PPM
8.4
8.0
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8

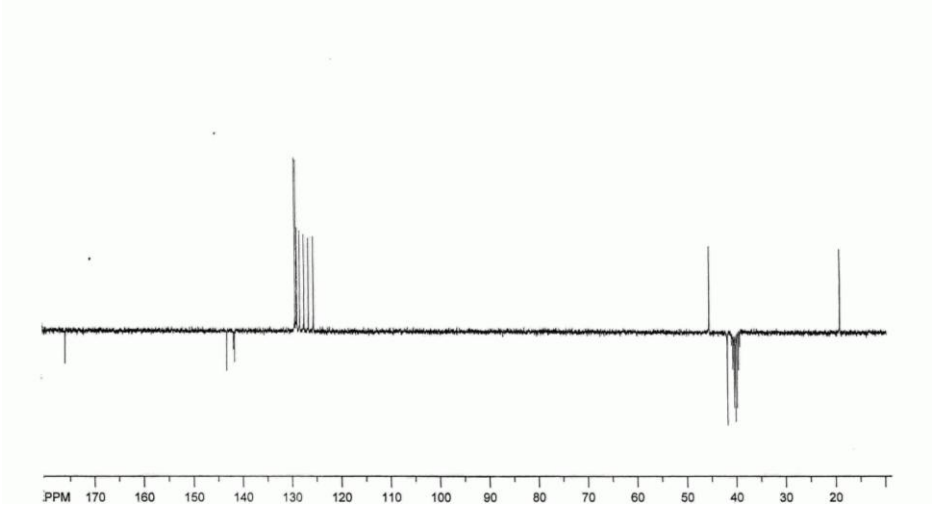

PPM
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20

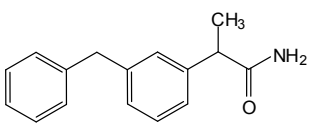

N
N
N
O
O
CH3
N
N
N
O

7h

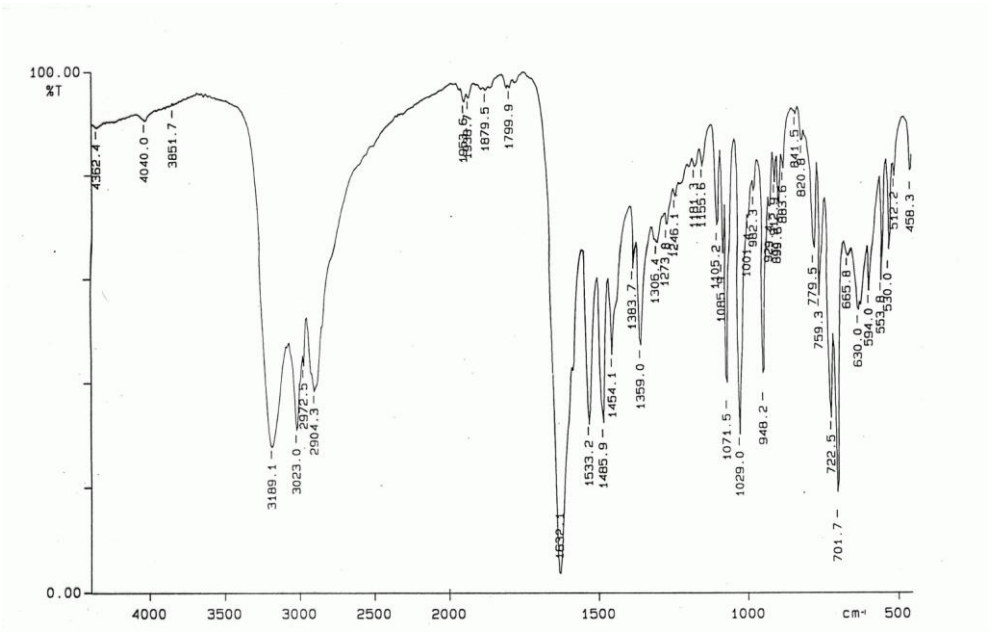

100.00
%T
0.00
4000
3500
3000
2500
2000
1500
1000
cm-1
500
3543.5
3392.6
3311.8
3177.9
2364.0
1598.8
1585.8
1451.1
1405.9
1368.2
1288.1
1122.6
1031.8
938.0
884.1
785.7
745.8
710.3
694.3
646.3
627.9
578.8
552.0
516.2
456.1


PPM
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2


PPM
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20


CH3
NH2
O

8a


100.00
%T
0.00
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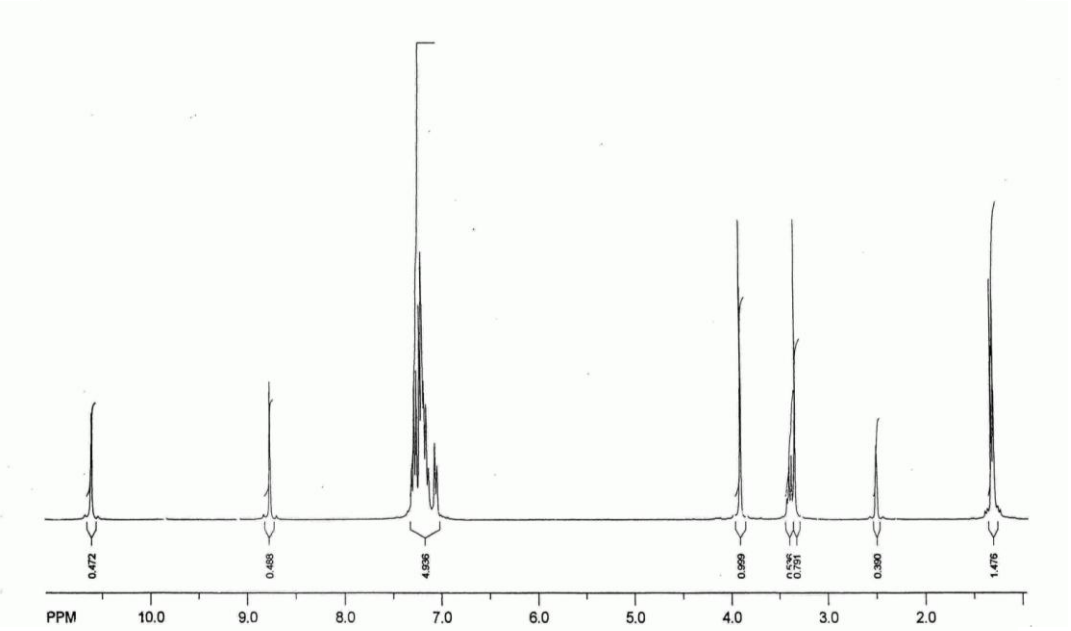
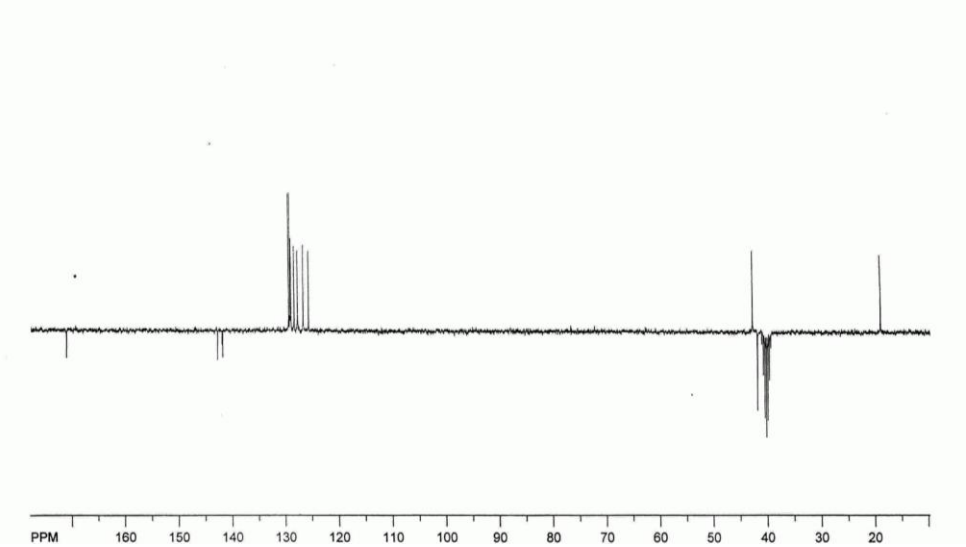
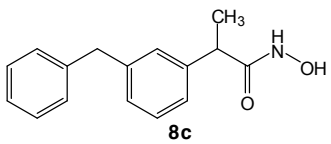
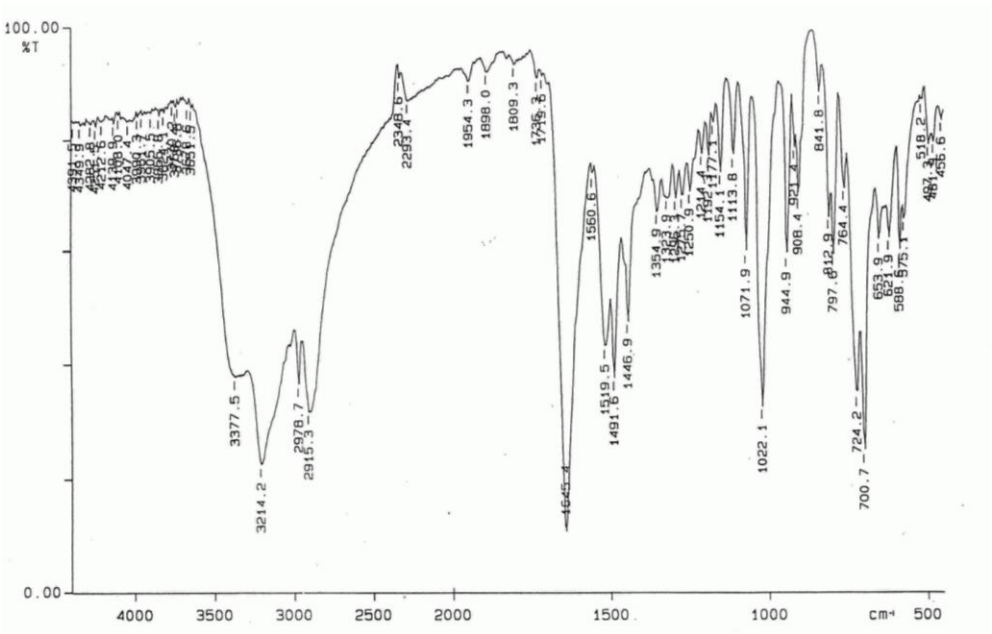
4040.0
3851.7
3189.1
3023.0
2972.5
2904.3
1879.5
1799.9
1632.1
1533.2
1485.9
1454.1
1383.7
1359.0
1306.4
1273.8
1246.1
1155.8
1105.2
1071.5
1029.0
982.3
948.2
841.5
820.8
779.5
759.3
722.5
701.7
665.8
630.0
594.0
553.8
530.0
512.2
458.3
4000
3500
3000
2500
2000
1500
1000
cm⁻¹
500

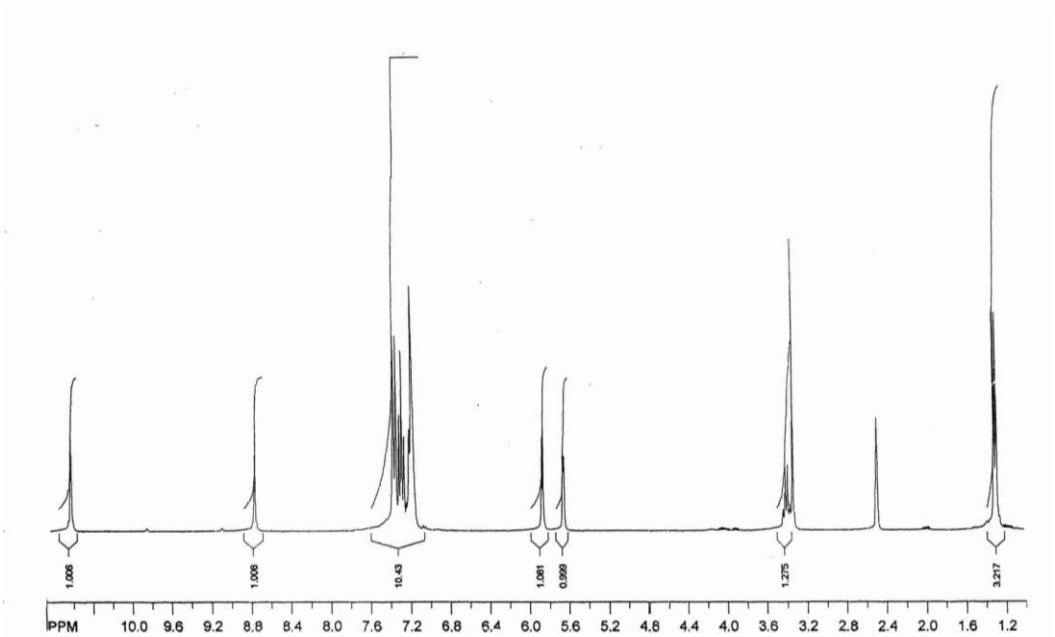

0.472
0.488
4.936
0.999
0.536
0.791
0.390
1.476
PPM
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0

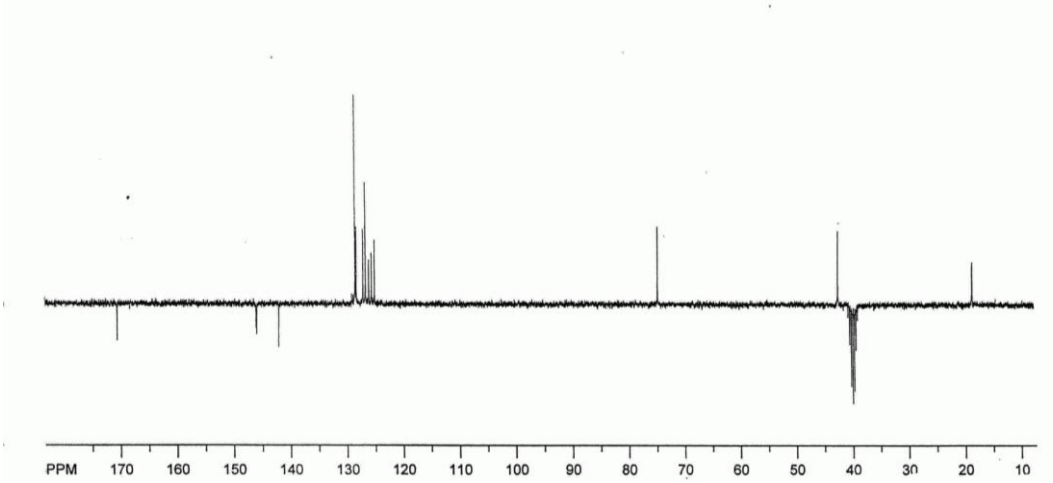

PPM
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20


CH3
H
N
OH
O

8c


100.00
%T
0.00
4000
3500
3000
2500
2000
1500
1000
cm-1
500
3377.5
3214.2
2978.7
2915.3
1645.4
1560.6
1519.5
1491.6
1446.9
1071.9
1022.1
944.9
908.4
841.8
797.8
764.4
724.2
700.7


1.000
1.008
10.43
1.081
0.999
1.275
3.217
PPM
10.0
9.6
9.2
8.8
8.4
8.0
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2


PPM
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160
150
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130
120
110
100
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80
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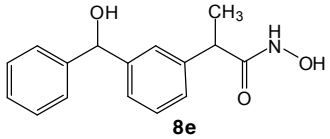
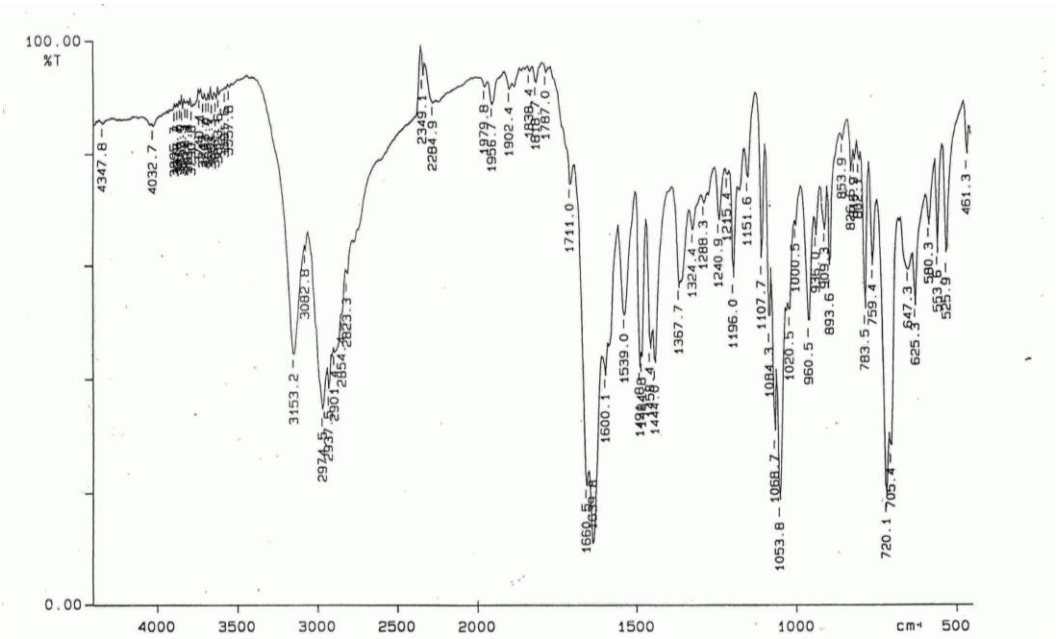
40
30
20
10


OH
CH3
H
N
OH
O

8e


100.00
%T
0.00
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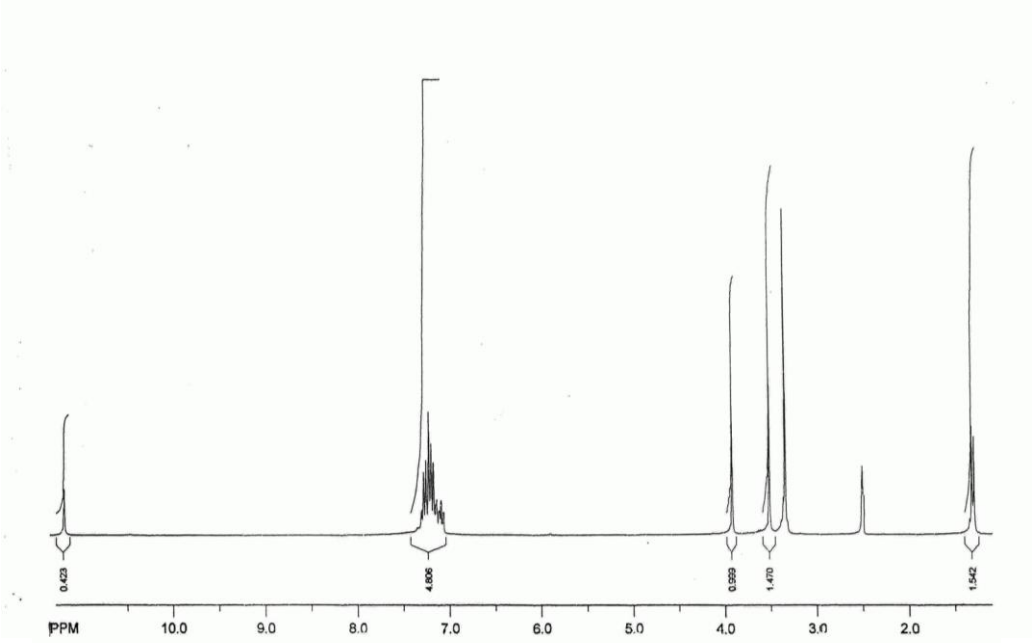
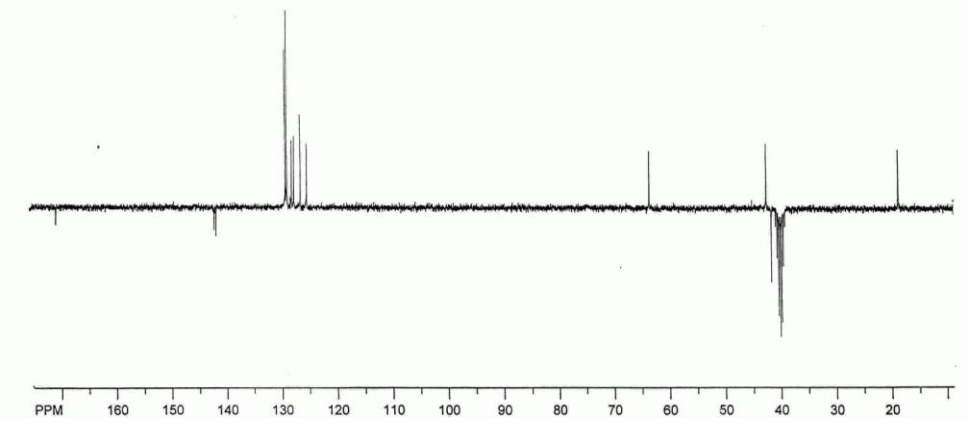
3500
3000
2500
2000
1500
1000
cm-1
500


PPM
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0


PPM
160
150
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130
120
110
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90
80
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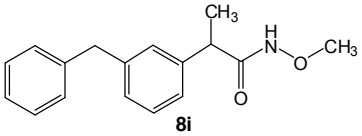
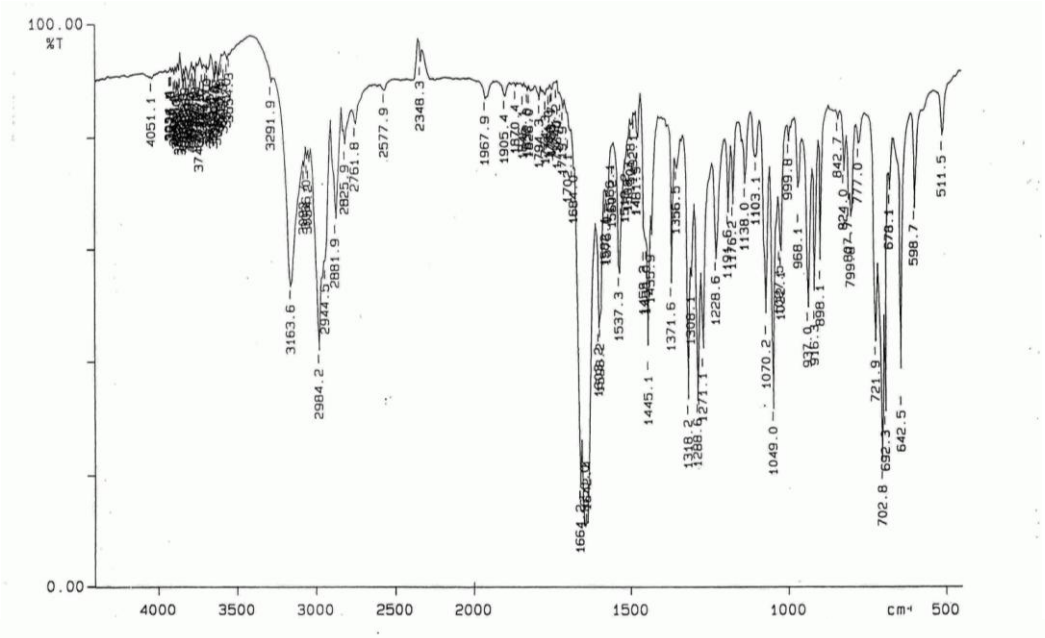
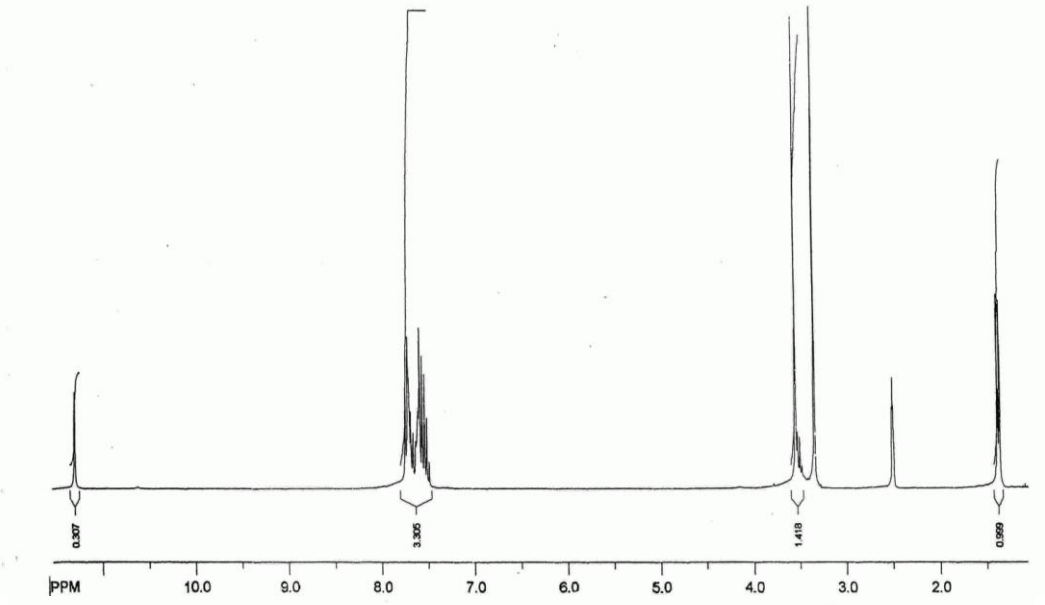
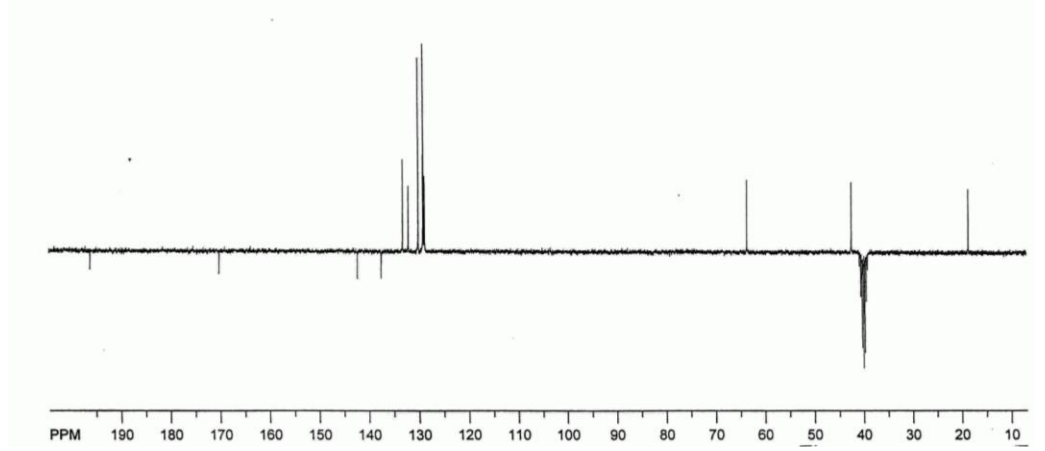
50
40
30
20

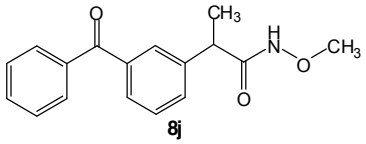

CH3
H
N
O
CH3
O

8i

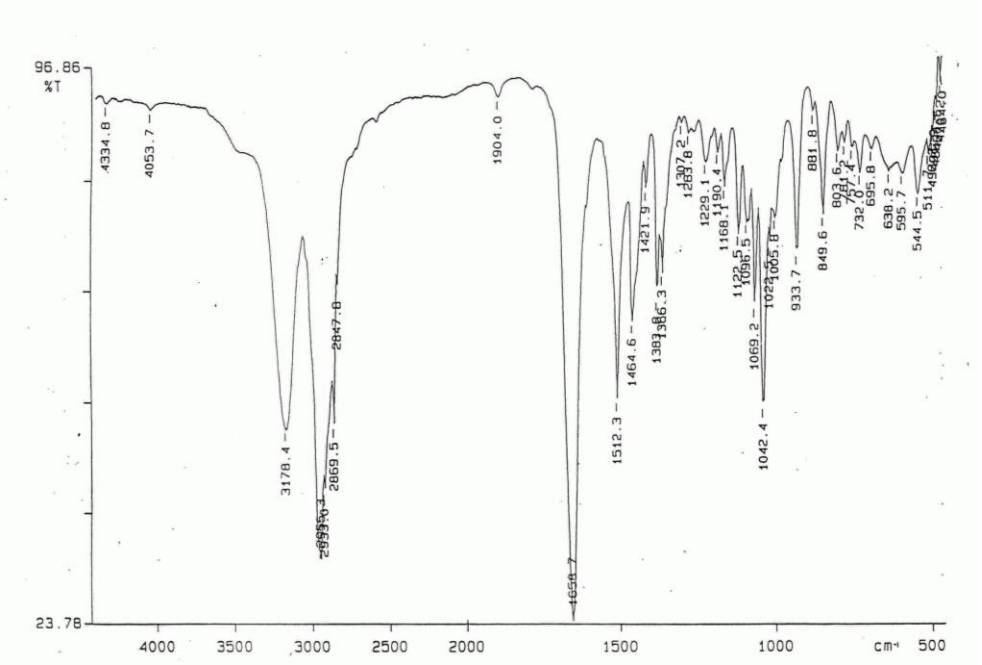

100.00
%T
0.00
4000
3500
3000
2500
2000
1500
1000
cm-1
500


PPM
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0


PPM
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10



8j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2869.5
1904.0
1512.3
1464.6
1421.9
1229.1
1190.4
1069.2
1042.4
933.7
881.8
849.6
695.8
638.2
595.7
544.5
4000
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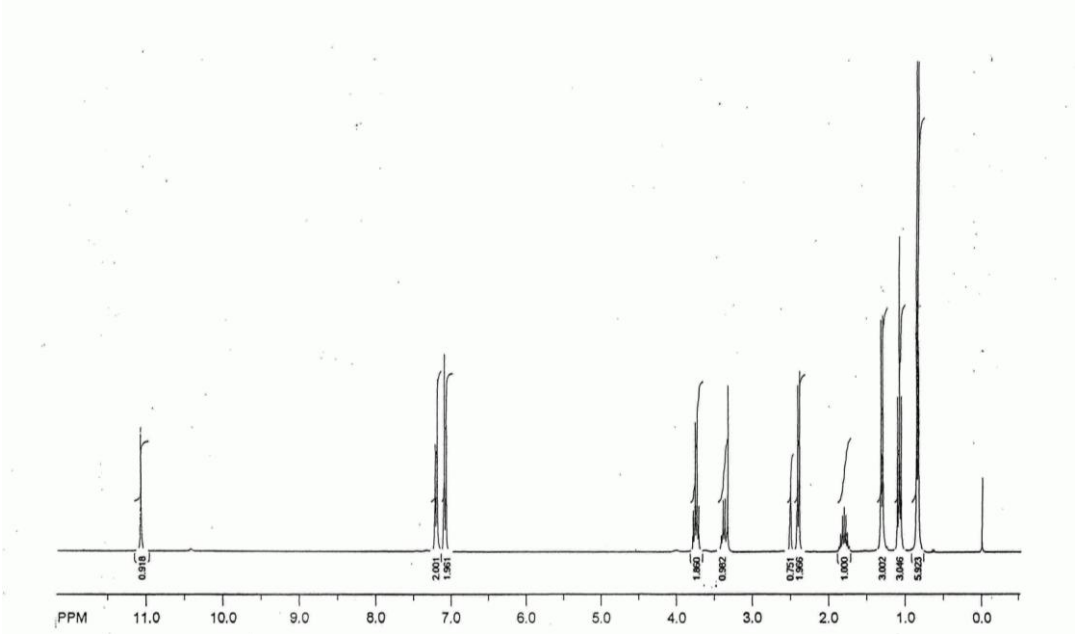
3000
2500
2000
1500
1000
cm⁻¹
500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6.0
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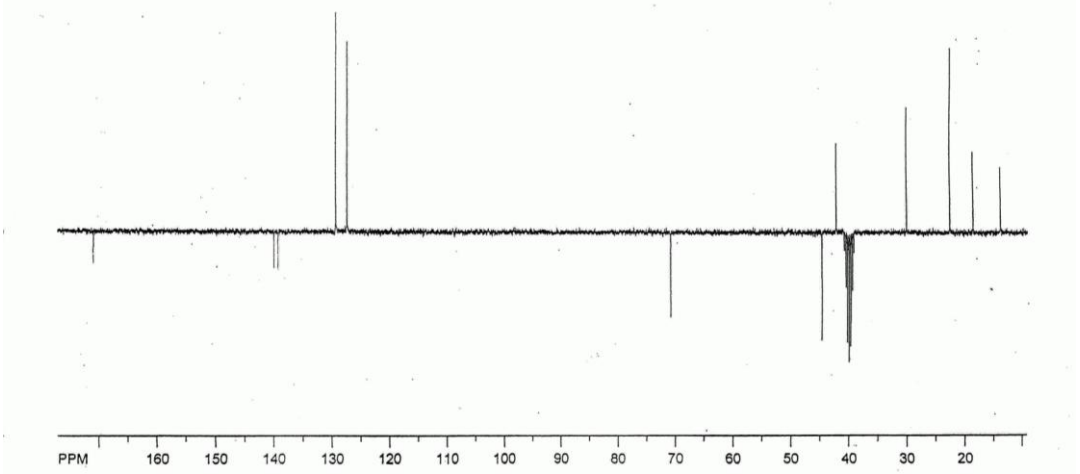
1.0
0.0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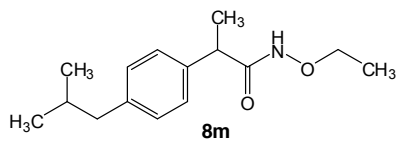
CH3
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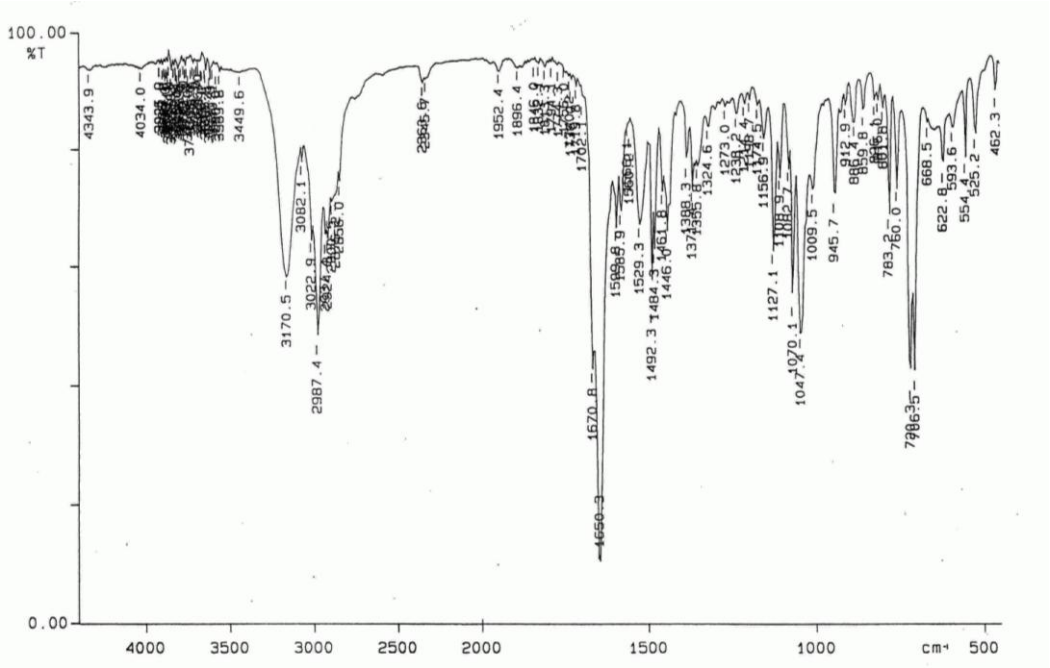
O
CH3
CH3
O
H3C

8m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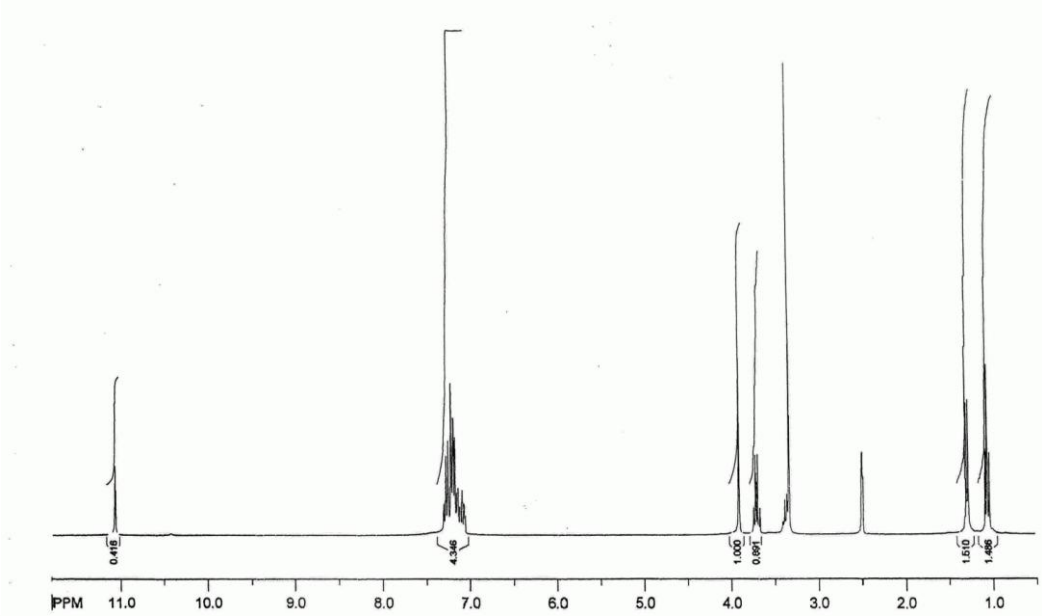
cm-1
500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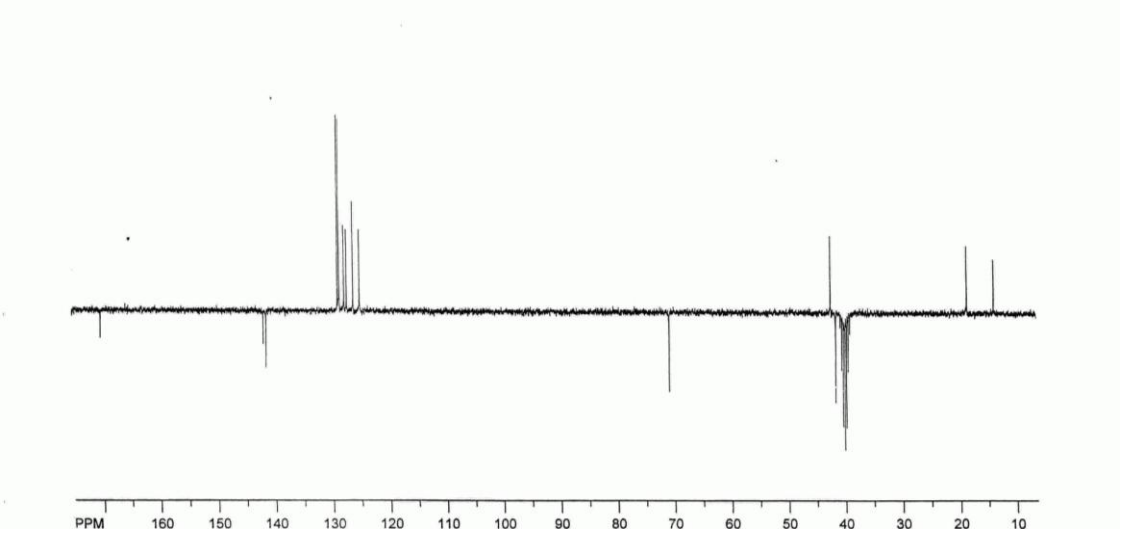
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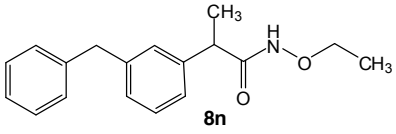
CH3
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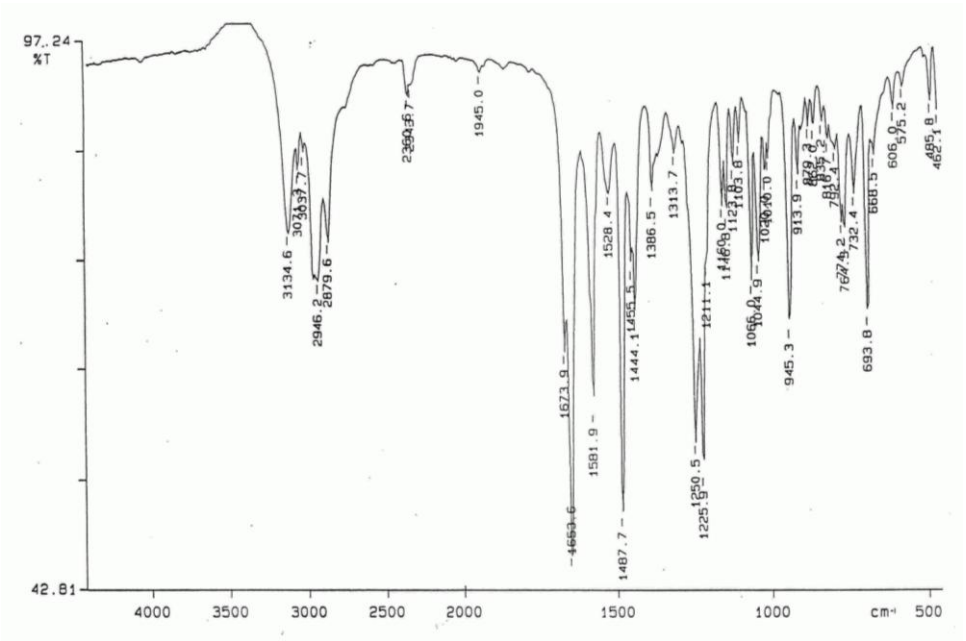
N
O
CH3
O

8n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2500
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1500
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cm-1
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3134.6
2946.2
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1945.0
1673.9
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1487.7
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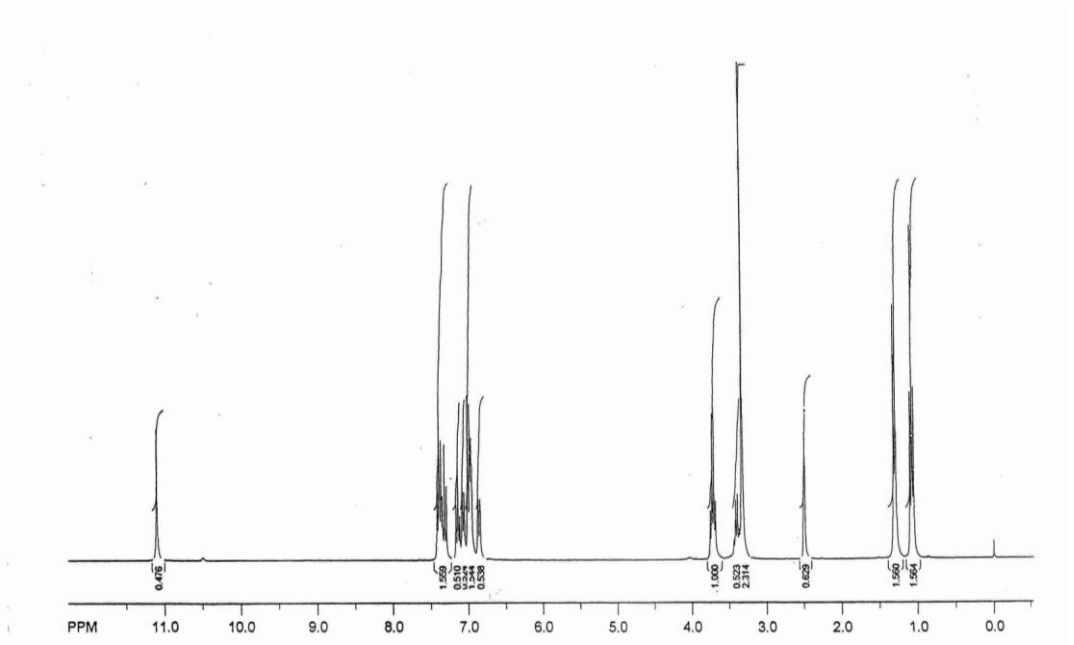
1386.5
1313.7
1250.5
1225.9
945.3
693.8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5.0
4.0
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1.0
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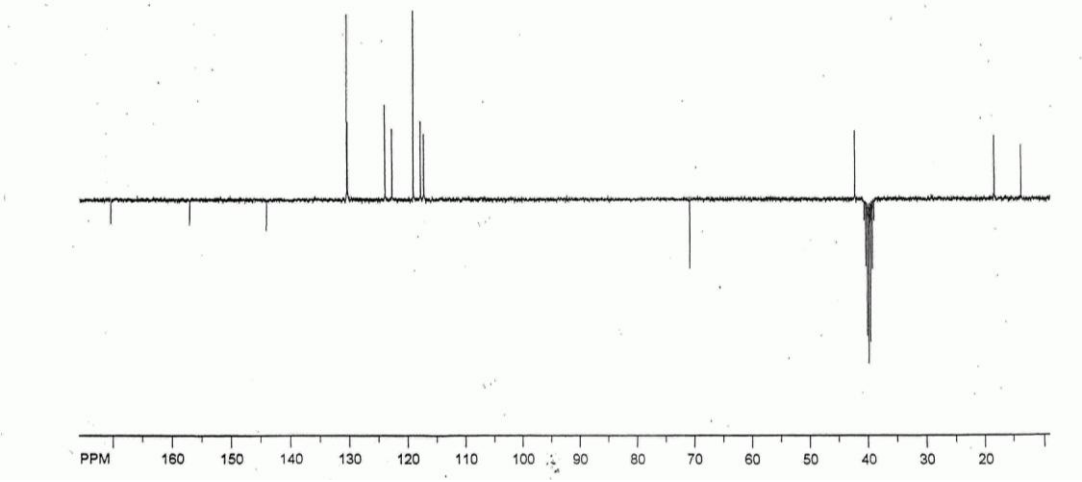
PPM
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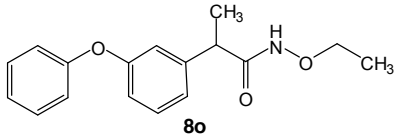
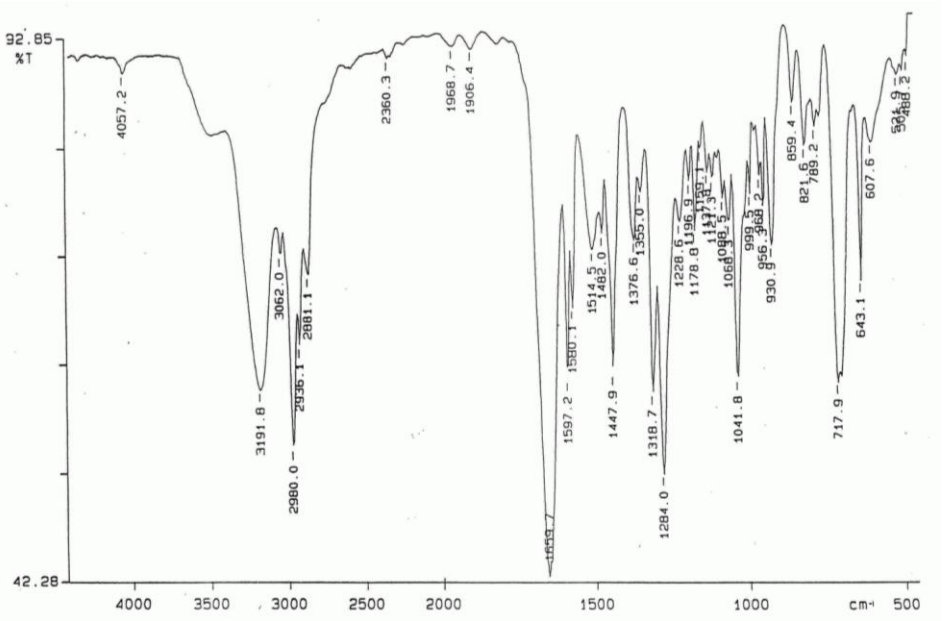

CH3
H
N
O
O
O
CH3

8o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2936.1
2881.1
2360.3
1968.7
1906.4
1659.7
1597.2
1580.1
1514.5
1482.0
1447.9
1376.6
1355.0
1318.7
1284.0
1228.6
1196.9
1178.8
1041.8
999.2
968.2
956.3
930.9
859.4
821.6
789.2
717.9
643.1
607.6
4000
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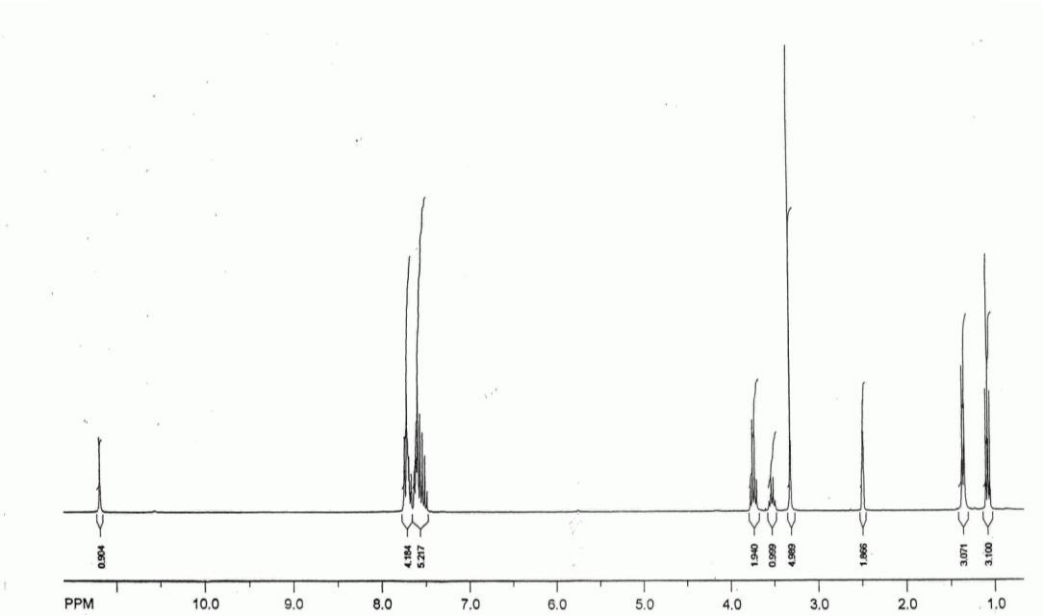
3000
2500
2000
1500
1000
cm⁻¹
500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4.184
5.217
1.940
0.999
4.989
1.866
3.071
3.100
PPM
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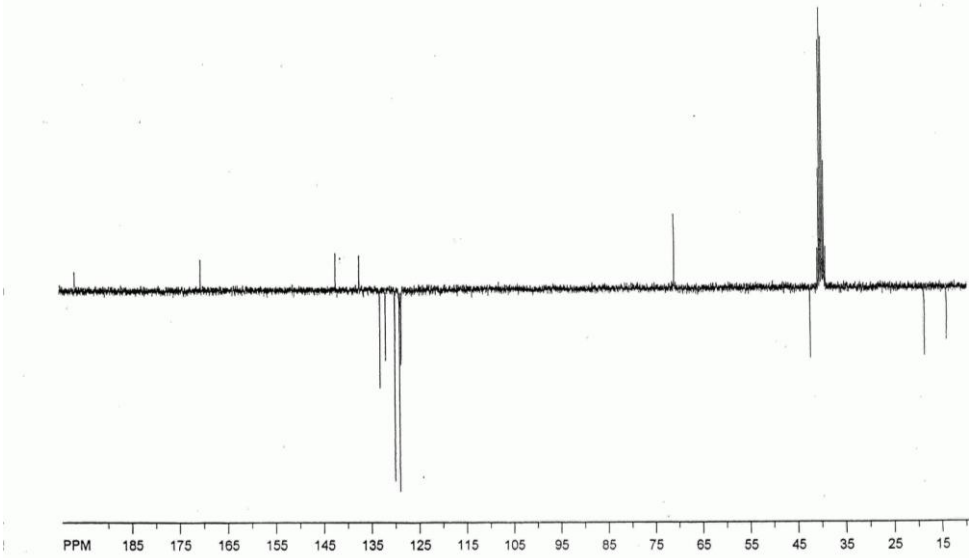
3.0
2.0
1.0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95
85
75
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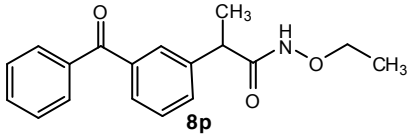
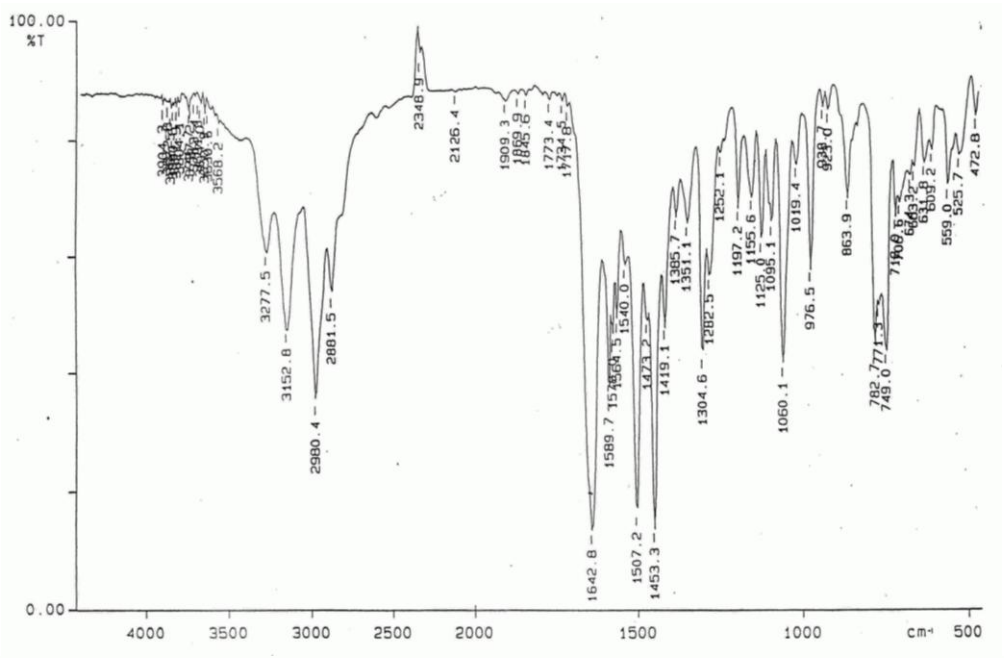
45
35
25
15


O
CH3
H
N
O
CH3
O

8p


100.00
%T
0.00
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3500
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2500
2000
1500
1000
cm-1
500
3568.2
3277.5
3152.8
2980.4
2881.5
2348.9
2126.4
1909.3
1773.4
1642.8
1589.7
1540.0
1507.2
1453.3
1419.1
1385.7
1351.1
1304.6
1282.5
1252.1
1197.2
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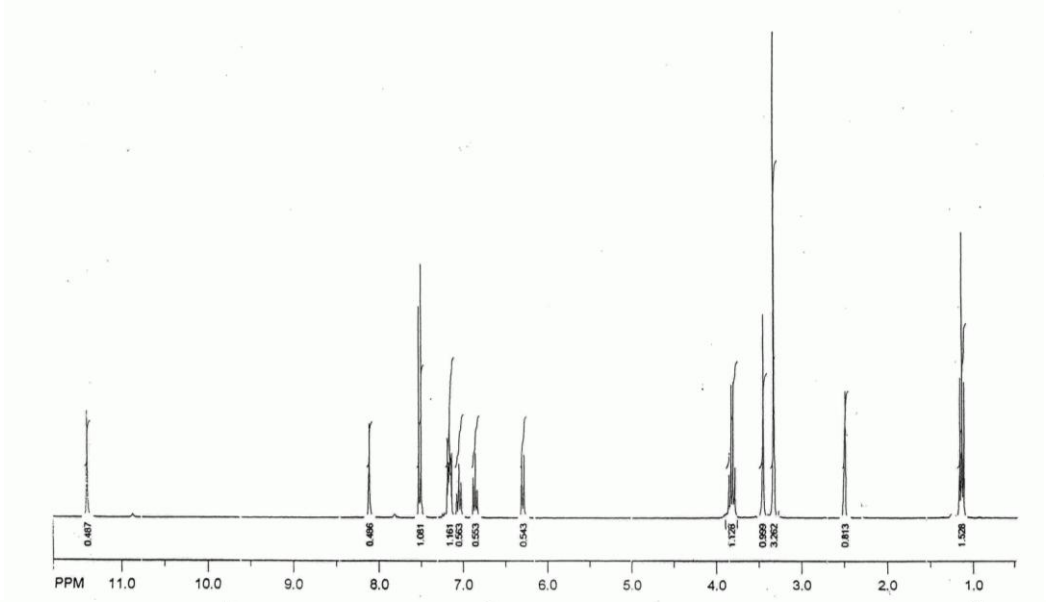
472.8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8.0
7.0
6.0
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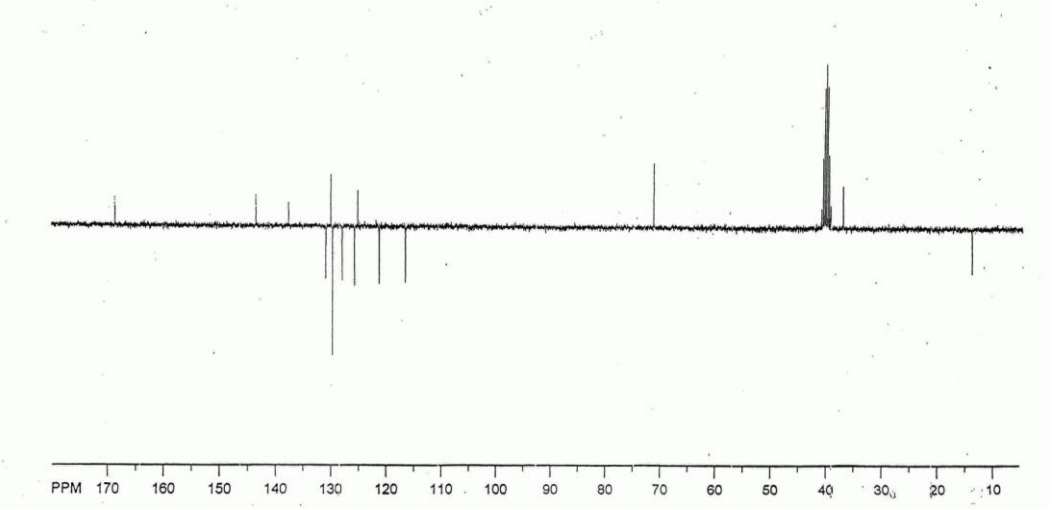
PPM
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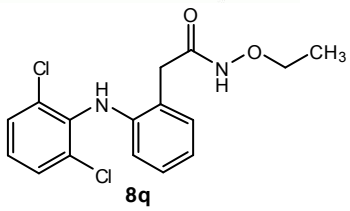
20
10


O
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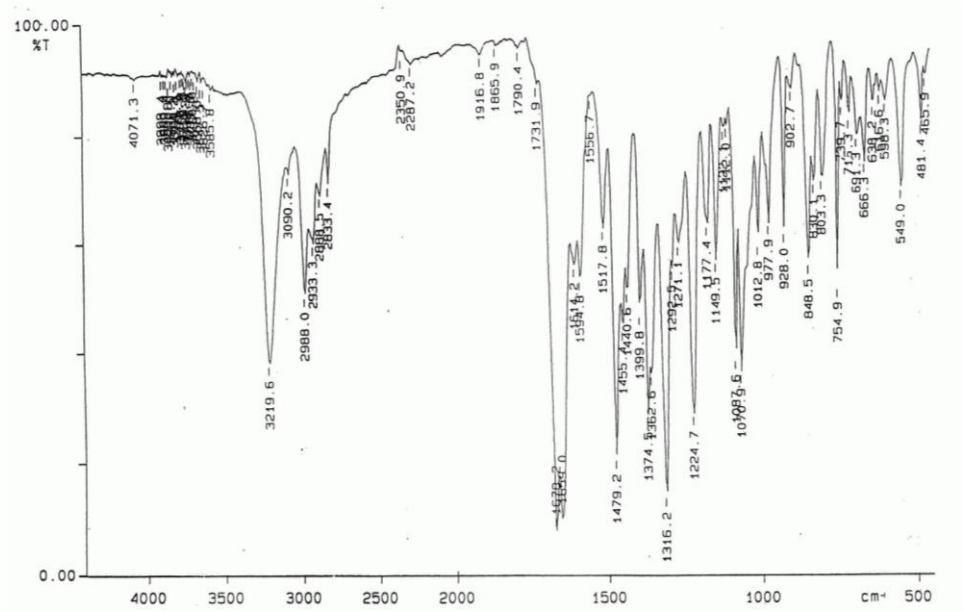
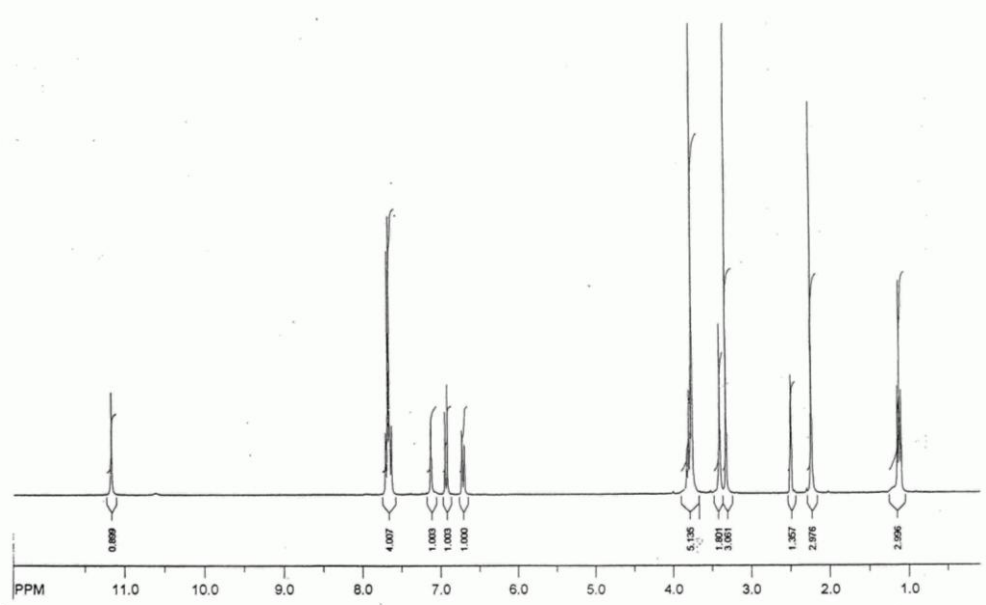
H
O
CH3
Cl
H
N
Cl

8q


100.00
%T
0.00
4000
3500
3000
2500
2000
1500
1000
cm-1
500


PPM
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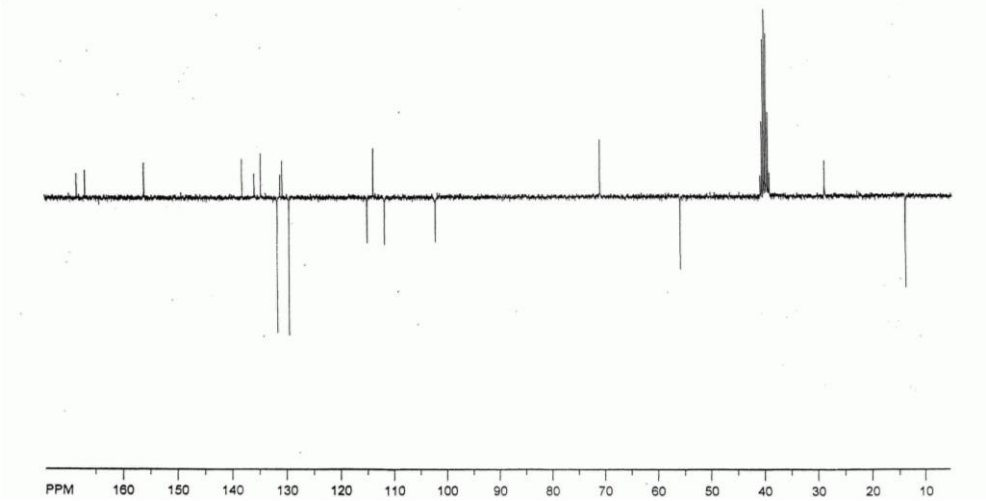
7.0
6.0
5.0
4.0
3.0
2.0
1.0


PPM
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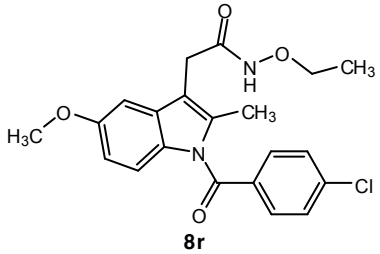
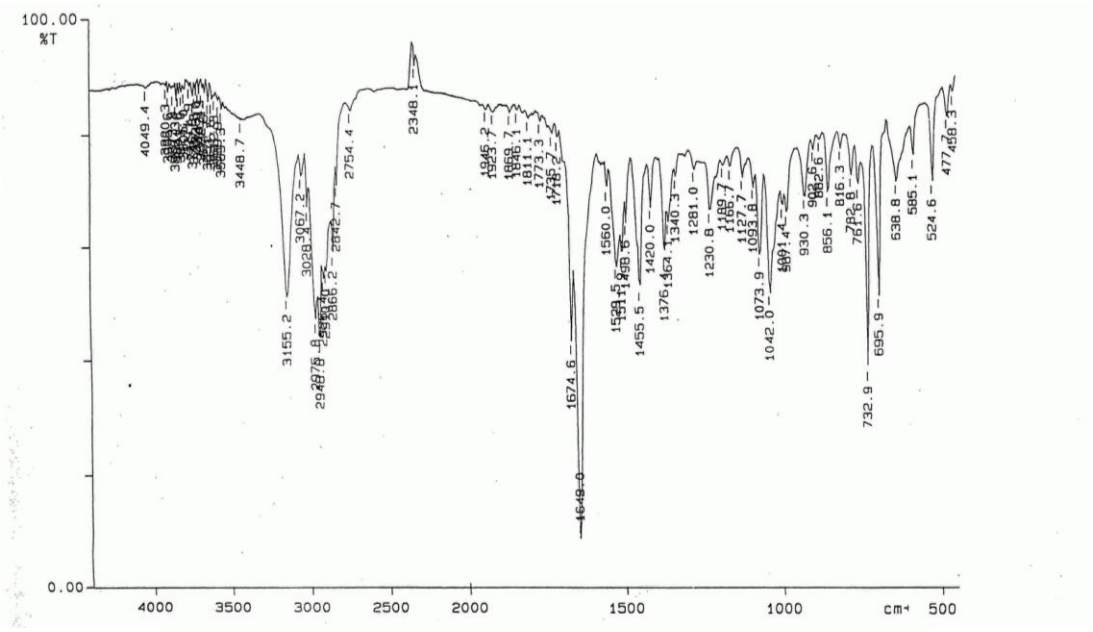

O
N
H
O
CH3
CH3
H3C
O
N
Cl
O

8r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%T
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3000
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cm-1
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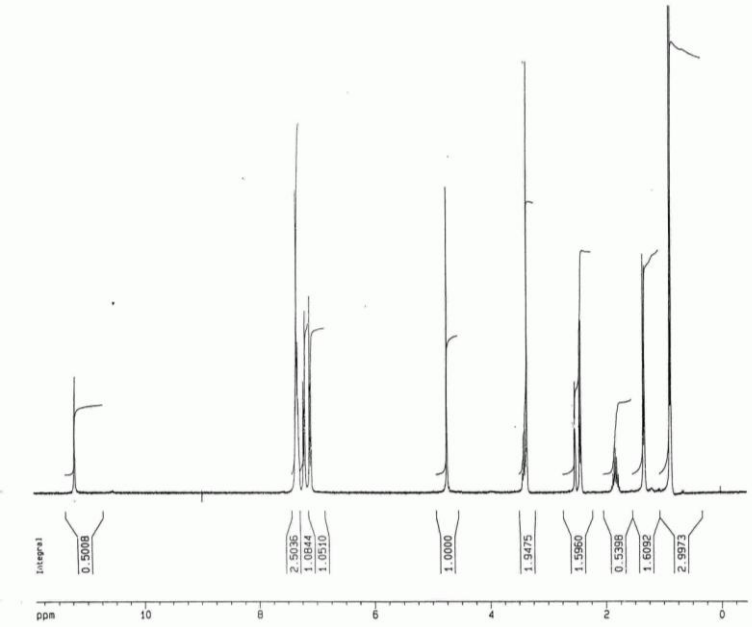
Integral
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1.0000
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0.5398
1.6092
2.9973
ppm
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8
6
4
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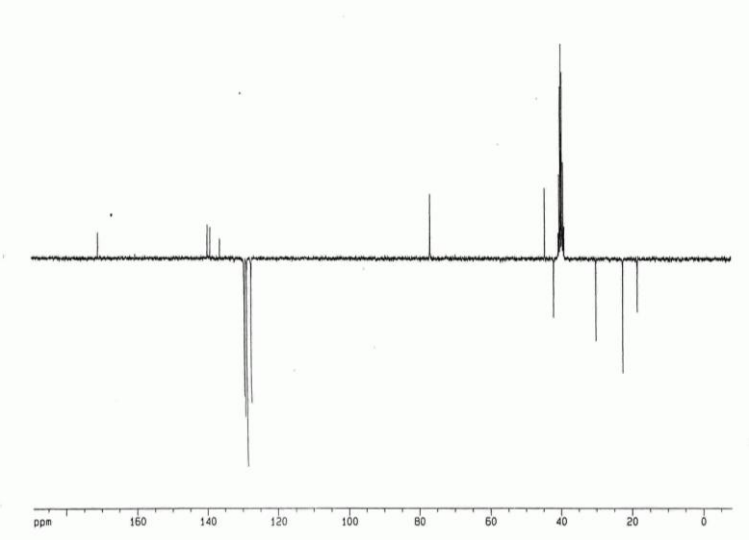
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100
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20
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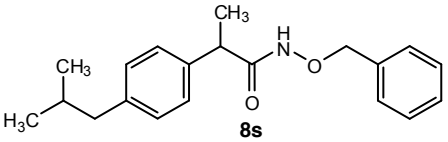
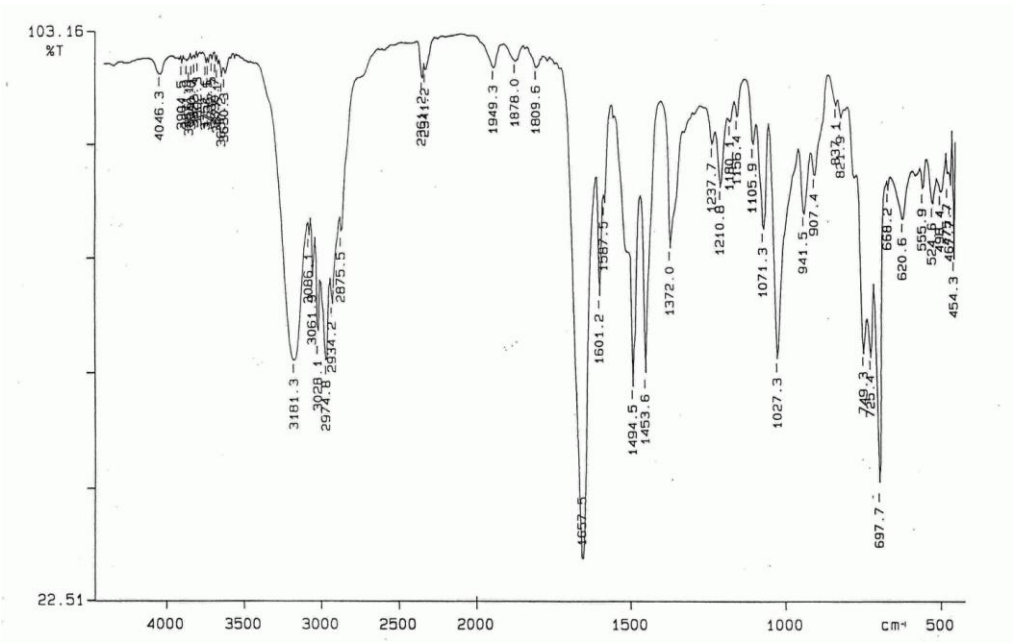

CH3
H
N
O
CH3
H3C
O

8s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1000
cm-1
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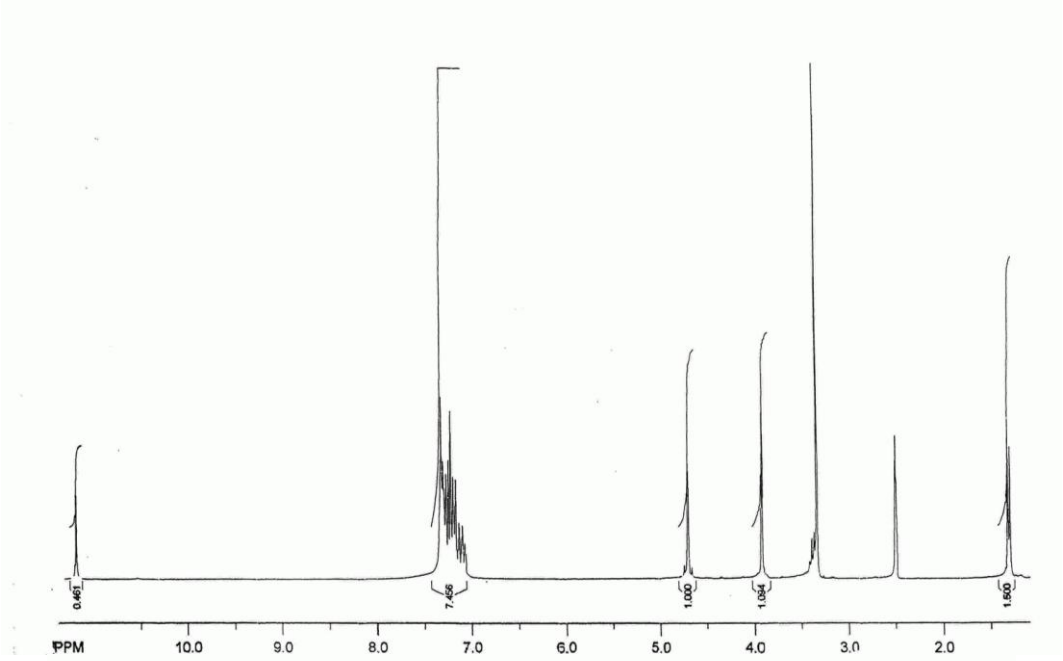
PPM
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7.0
6.0
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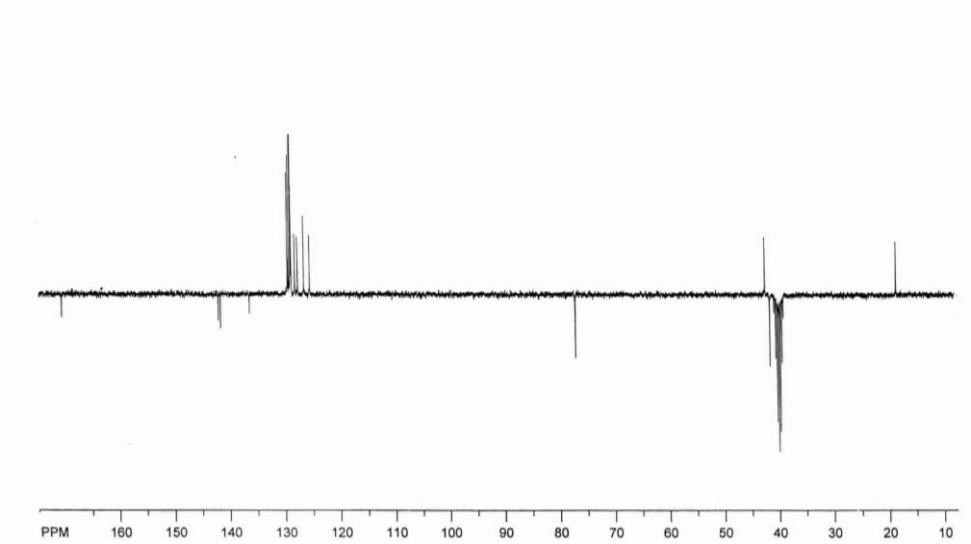
4.0
3.0
2.0


PPM
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120
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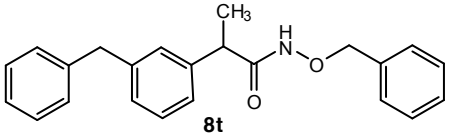
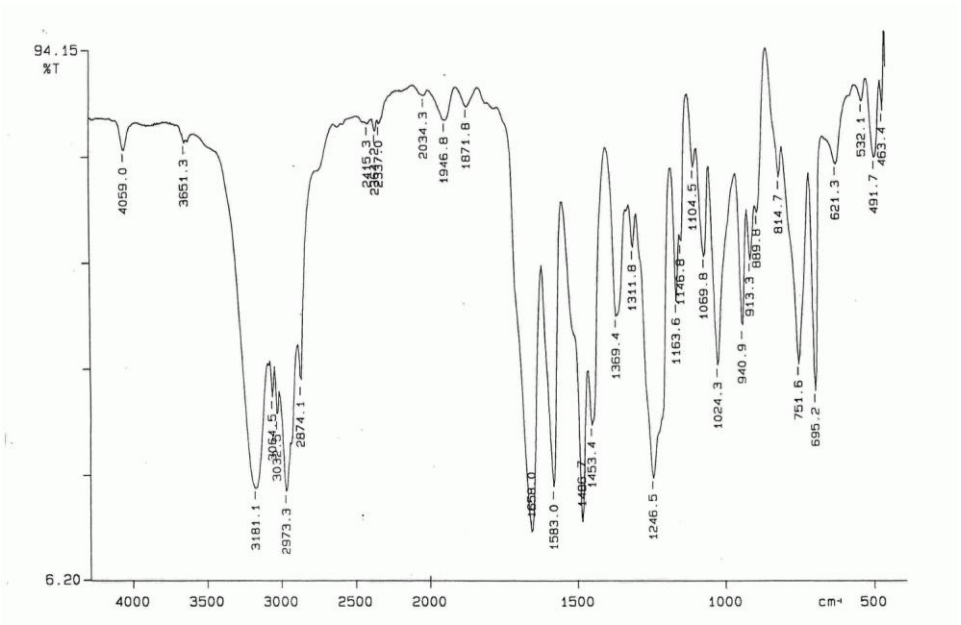
20
10


CH3
H
N
O
O

8t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3032.5
2973.3
2874.1
2034.3
1946.8
1871.8
1583.0
1496.7
1453.4
1369.4
1311.8
1246.5
1163.6
1146.8
1104.5
1069.8
1024.3
940.9
913.3
889.8
814.7
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532.1
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463.4
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3000
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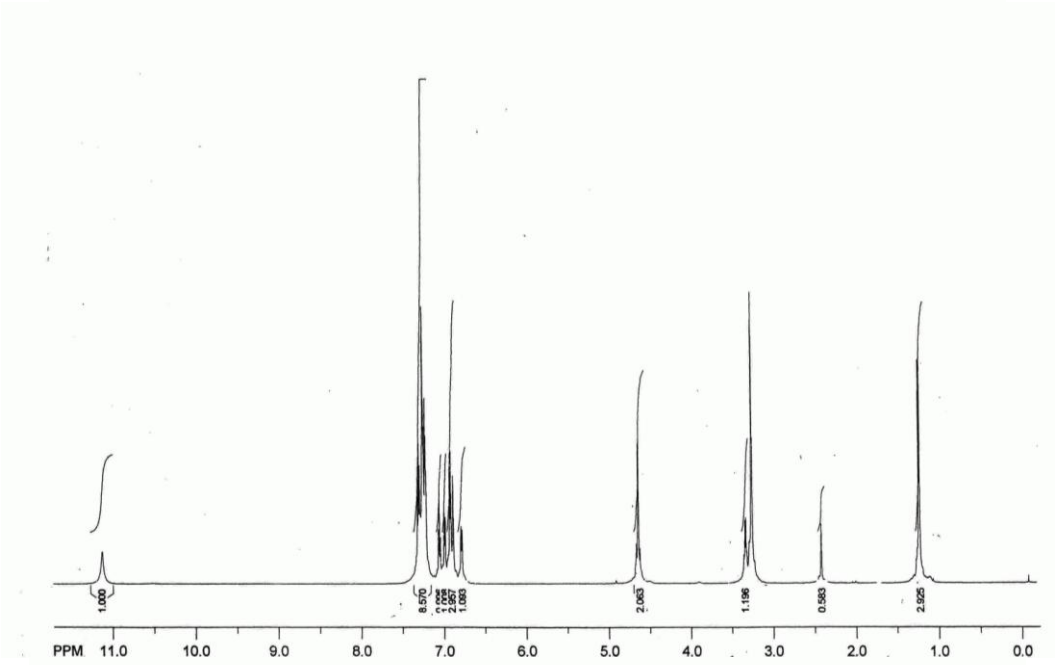
1500
1000
cm-1
500


PPM
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4.0
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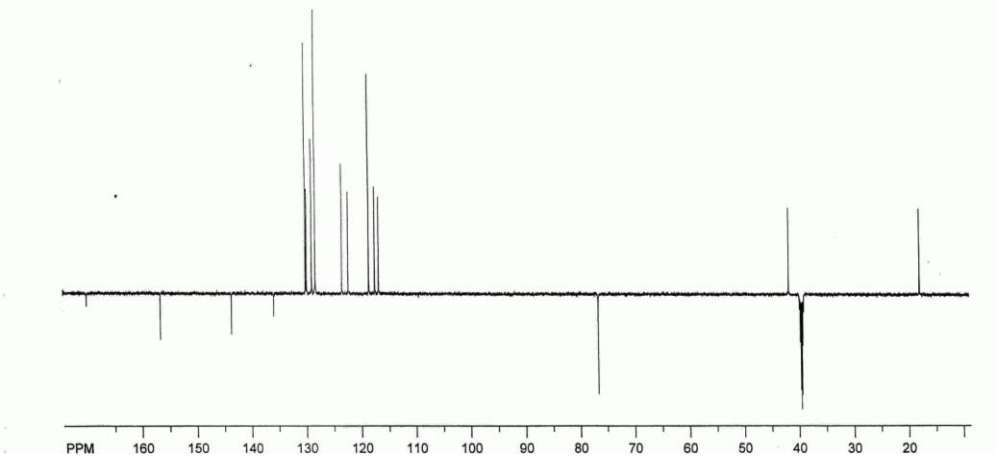
1.0
0.0


PPM
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50
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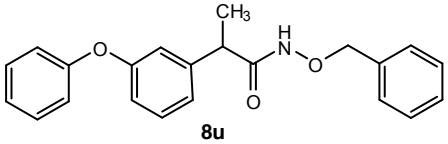
CH3
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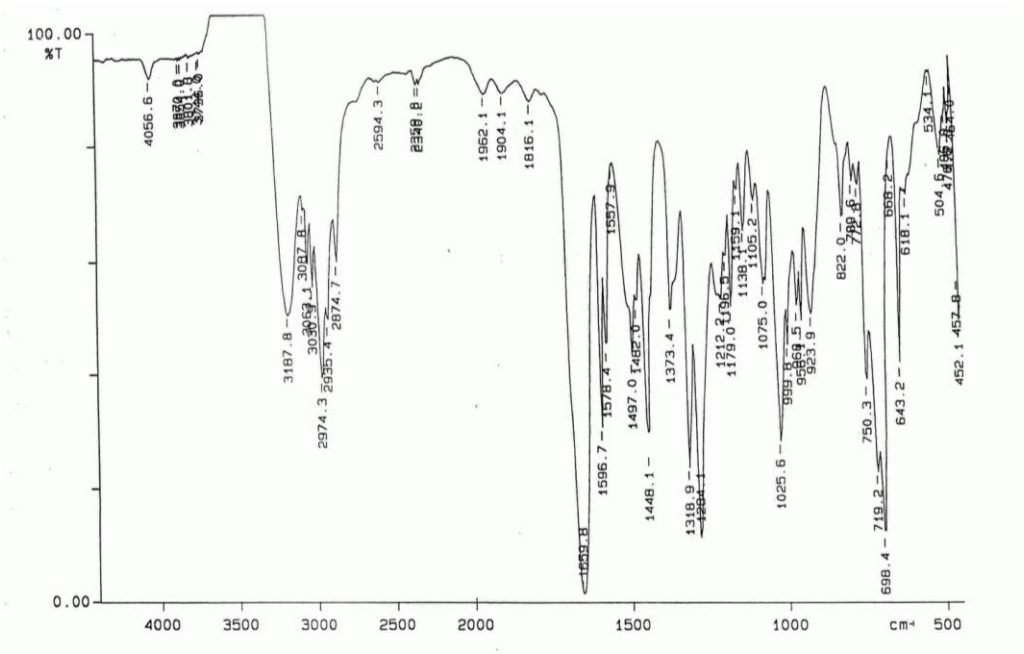
O
O
O

8u


100.00
%T
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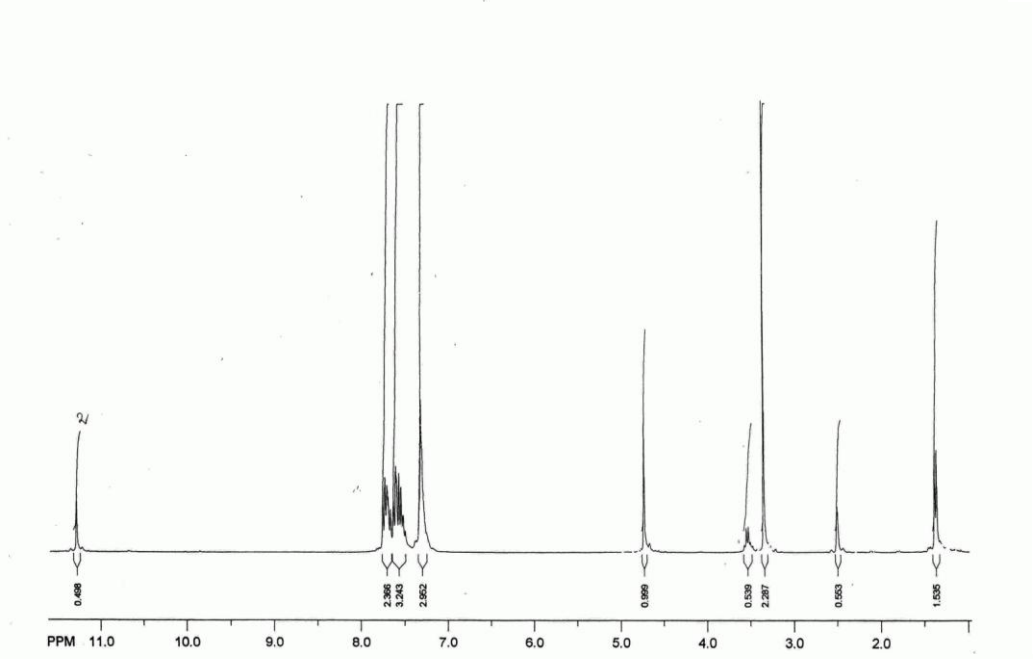
3187.8
3087.8
3063.1
3030.2
2974.3
2935.4
2874.7
2594.3
2358.8
1962.1
1904.1
1816.1
1659.8
1596.7
1578.4
1557.3
1497.0
1482.6
1448.1
1373.4
1318.9
1212.2
1179.0
1159.1
1138.1
1105.2
1075.0
1025.6
999.8
923.9
822.0
750.3
719.2
698.4
668.2
643.2
618.1
534.1
504.6
452.1
4000
3500
3000
2500
2000
1500
1000
cm-1
500


2/
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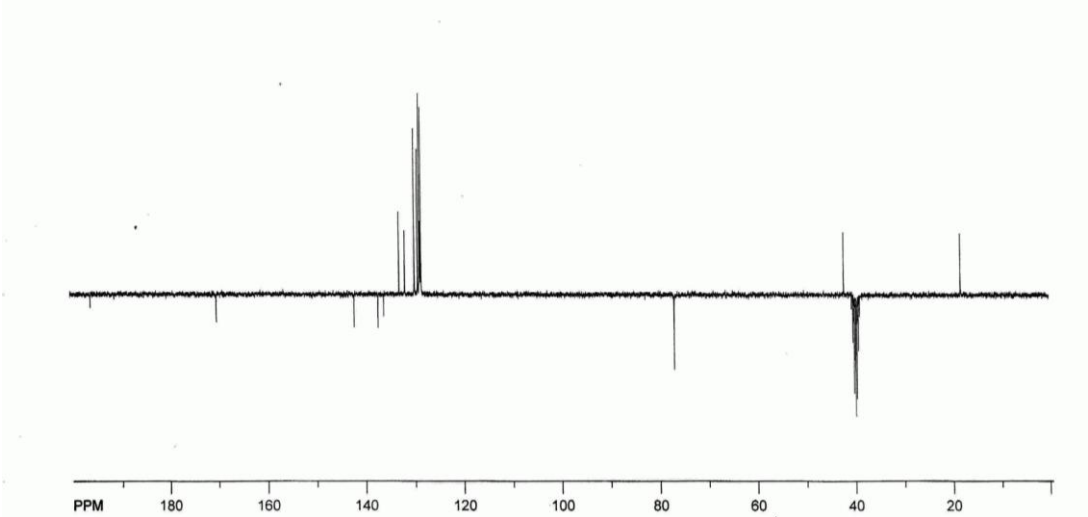
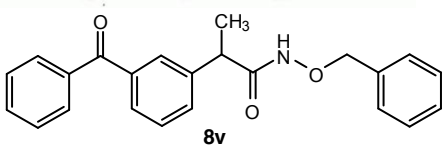
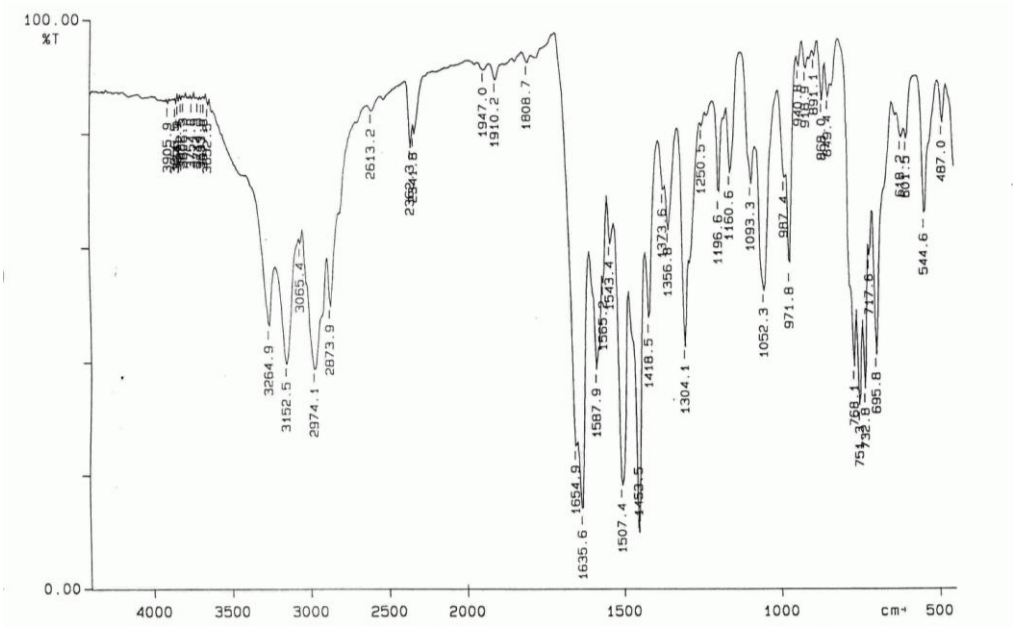
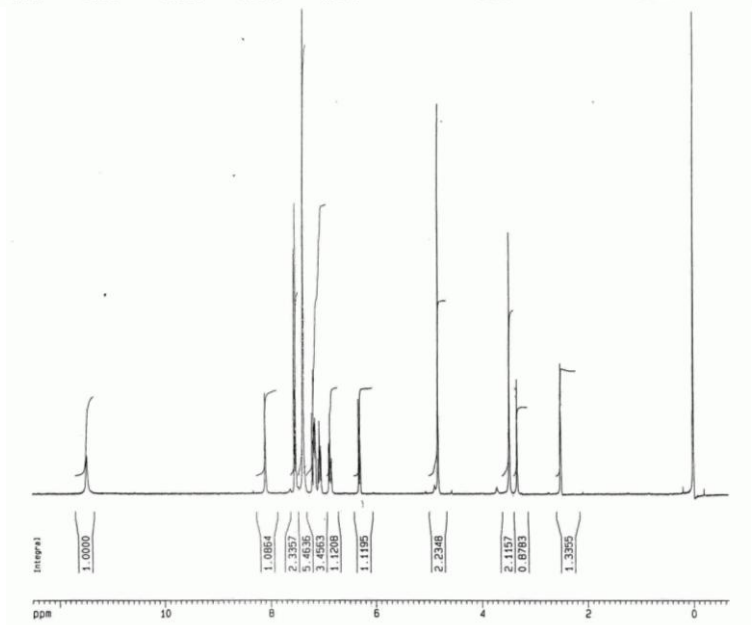
2.366
3.243
2.952
0.999
0.539
2.267
0.553
1.535
PPM
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0

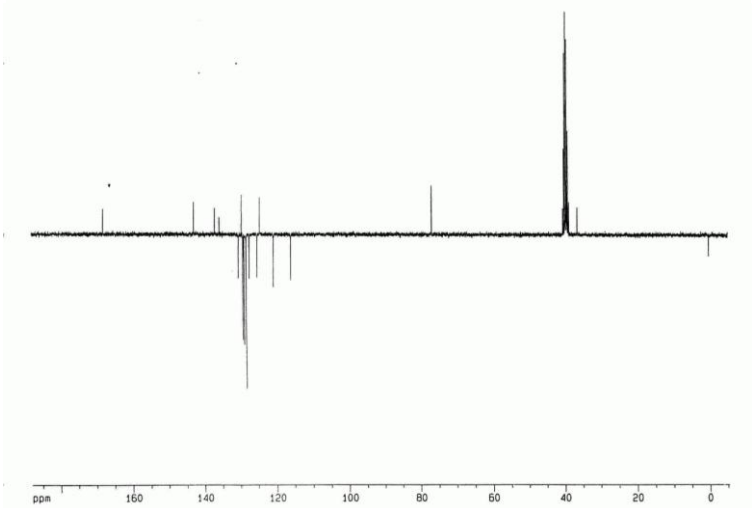

PPM
180
160
140
120
100
80
60
40
20

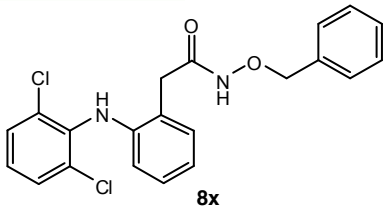

O
CH3
H
N
O
O

8v

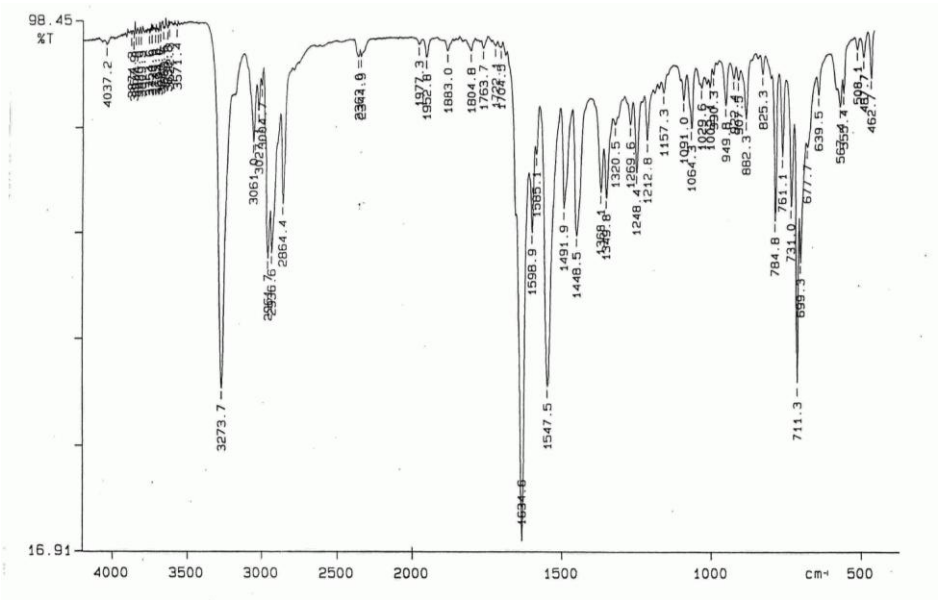

100.00
%T
0.00
4000
3500
3000
2500
2000
1500
1000
cm-1
500
3264.9
3152.5
3065.4
2974.1
2873.9
2613.2
1947.0
1910.2
1808.7
1635.6
1654.9
1587.9
1565.2
1543.4
1507.4
1453.5
1418.5
1373.6
1356.8
1304.1
1250.5
1196.6
1160.6
1093.3
1052.3
987.4
971.8
768.1
751.3
732.8
717.6
695.8
544.6
487.0

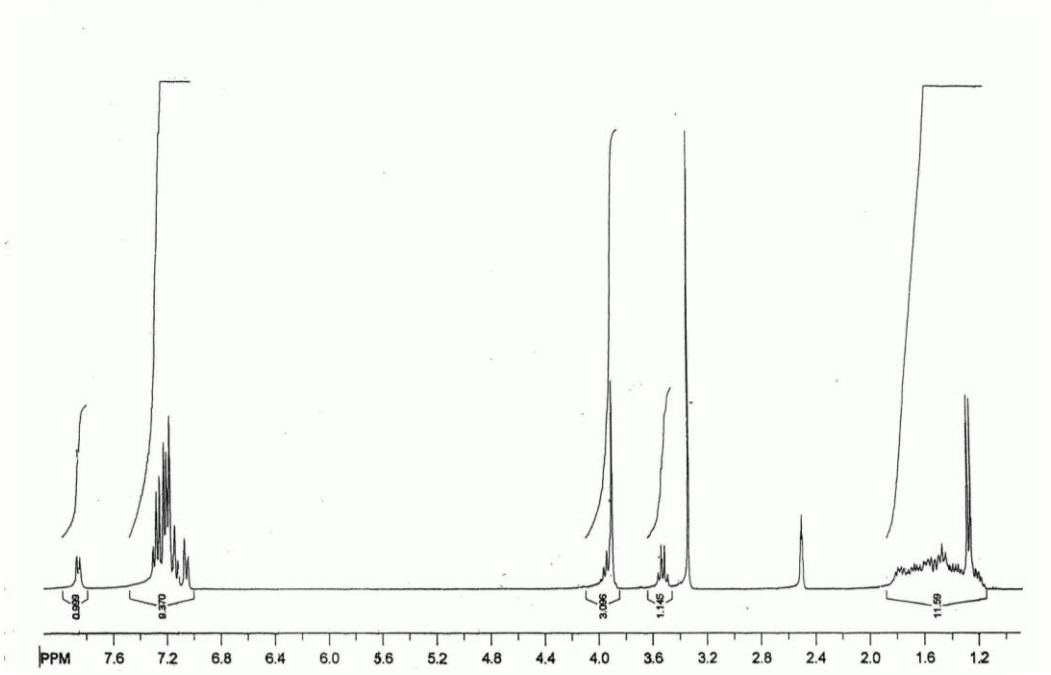

Integral
1.0000
1.0864
2.3357
5.4636
3.4563
1.1208
1.1195
2.2348
2.1157
0.8783
1.3355
ppm
10
8
6
4
2
0

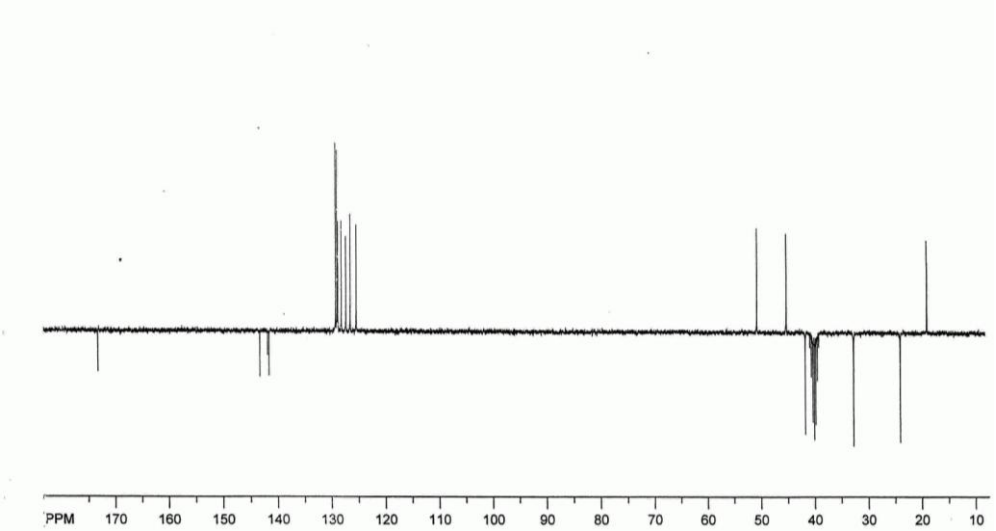

ppm
160
140
120
100
80
60
40
20
0

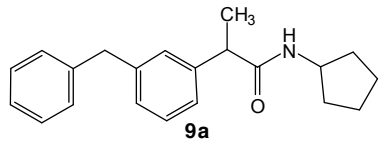

Cl
H
N
Cl
O
N
H
O

8x

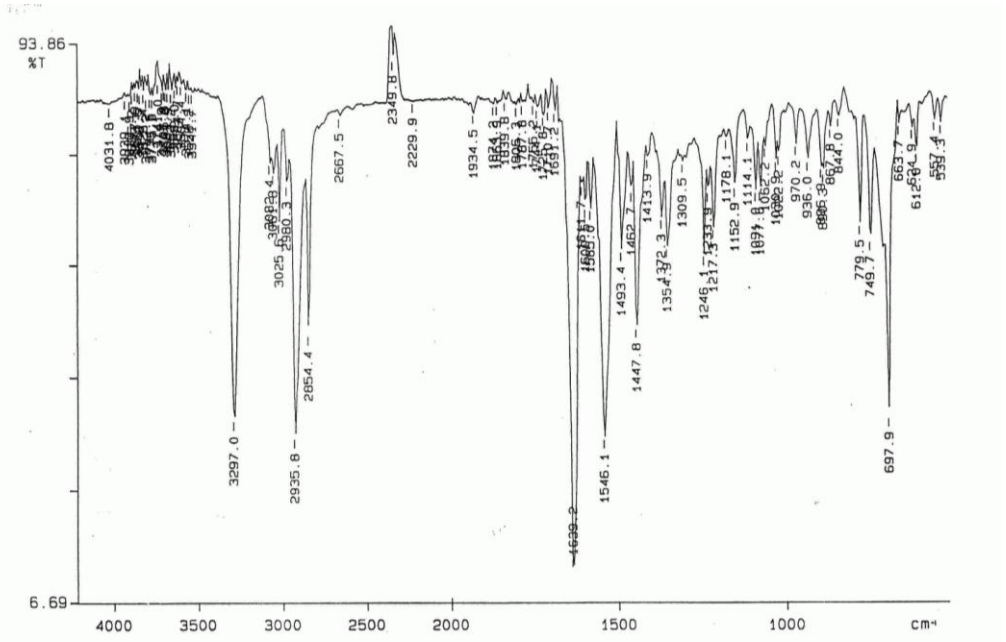

98.45
%T
16.91
4000
3500
3000
2500
2000
1500
1000
cm-1
500
4037.2
3273.7
3061.0
2864.4
1634.6
1598.9
1585.1
1547.5
1491.9
1448.5
1320.5
1269.6
1248.4
1212.8
1157.3
1091.0
1064.3
949.8
882.3
825.3
784.8
761.1
731.0
711.3
699.3
677.7
639.5
462.7

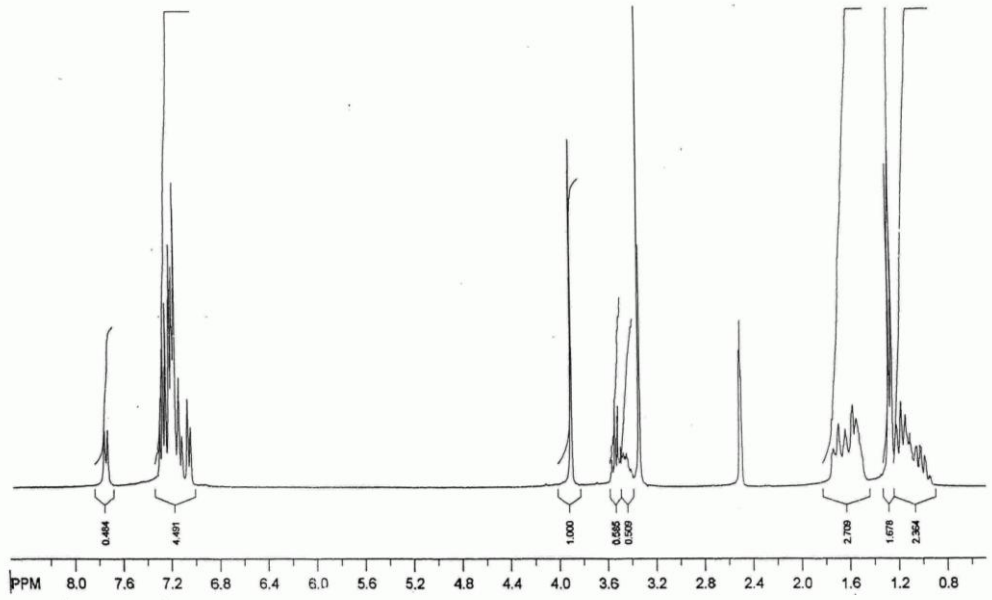

PPM
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.999
9.370
3.096
1.145
11.59

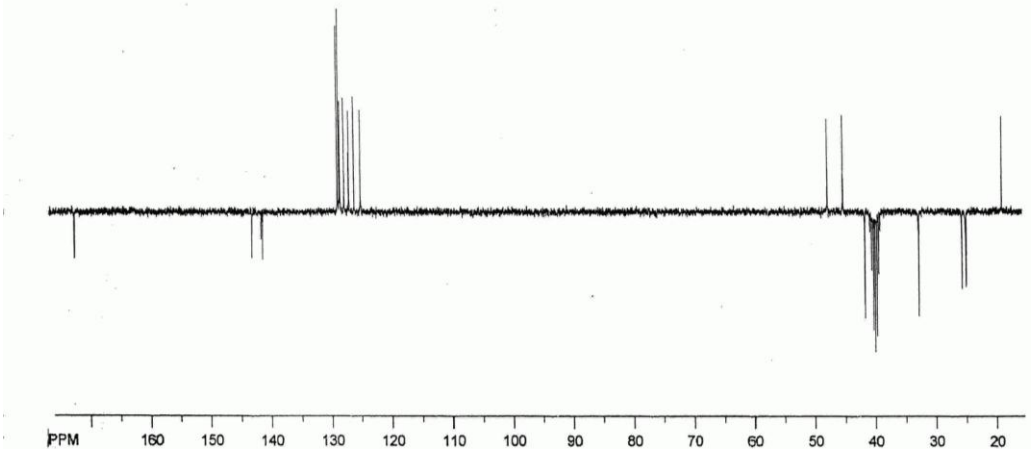

PPM
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

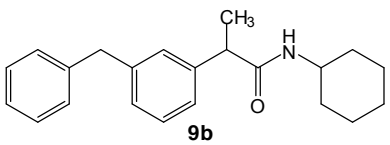

CH3
H
N
O

9a

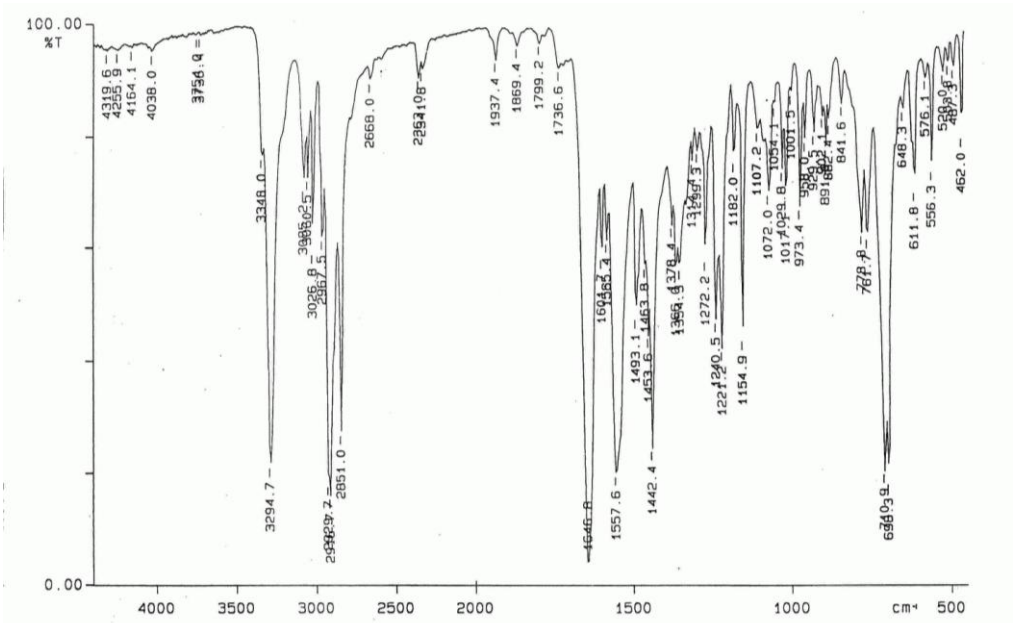

93.86
%T
6.69
4031.8
3297.0
3025.4
2935.8
2854.4
2667.5
2349.8
2229.9
1934.5
1639.2
1546.1
1493.4
1447.8
1413.9
1372.3
1309.5
1246.1
1217.3
1178.1
1152.9
1114.1
970.2
844.0
779.5
749.7
697.9
663.7
4000
3500
3000
2500
2000
1500
1000
cm-1

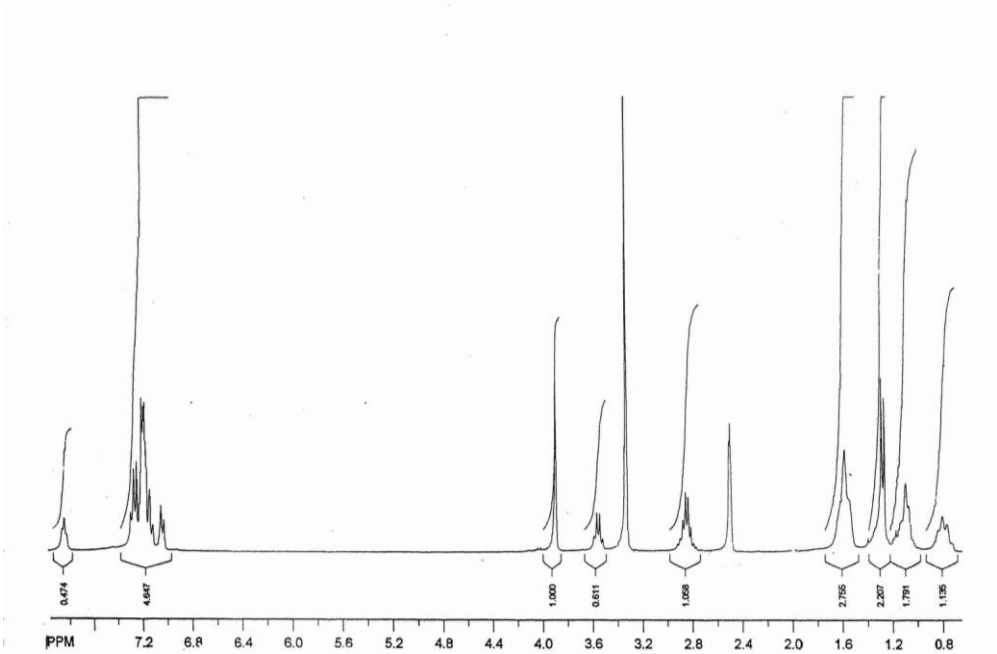

0.484
4.491
1.000
0.585
0.509
2.709
1.678
2.364
PPM
8.0
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8

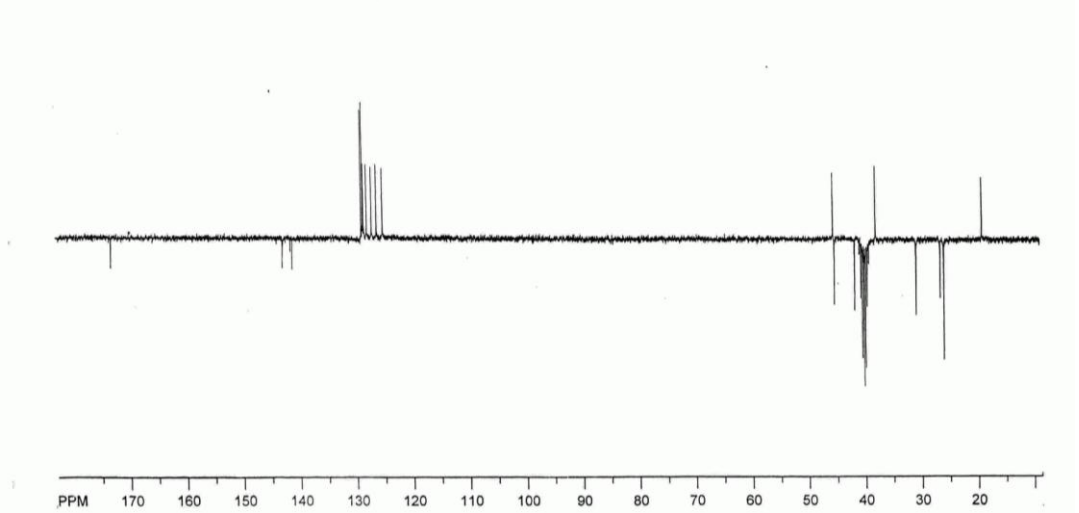

PPM
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20

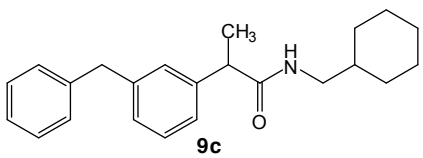

CH3
H
N
O

9b

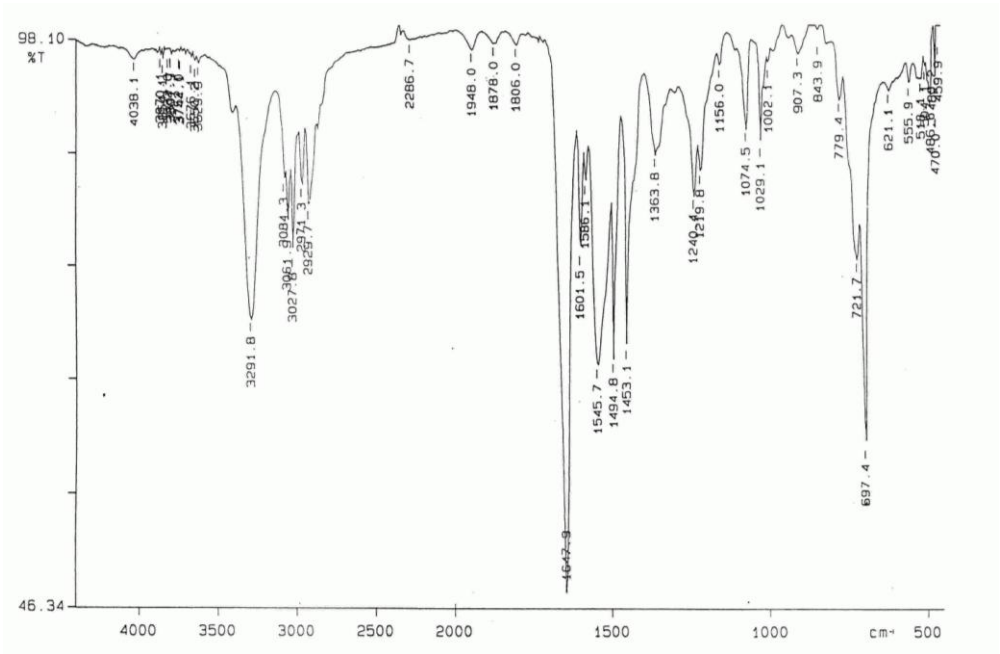

100.00
%T
0.00
4000
3500
3000
2500
2000
1500
1000
cm⁻¹
500

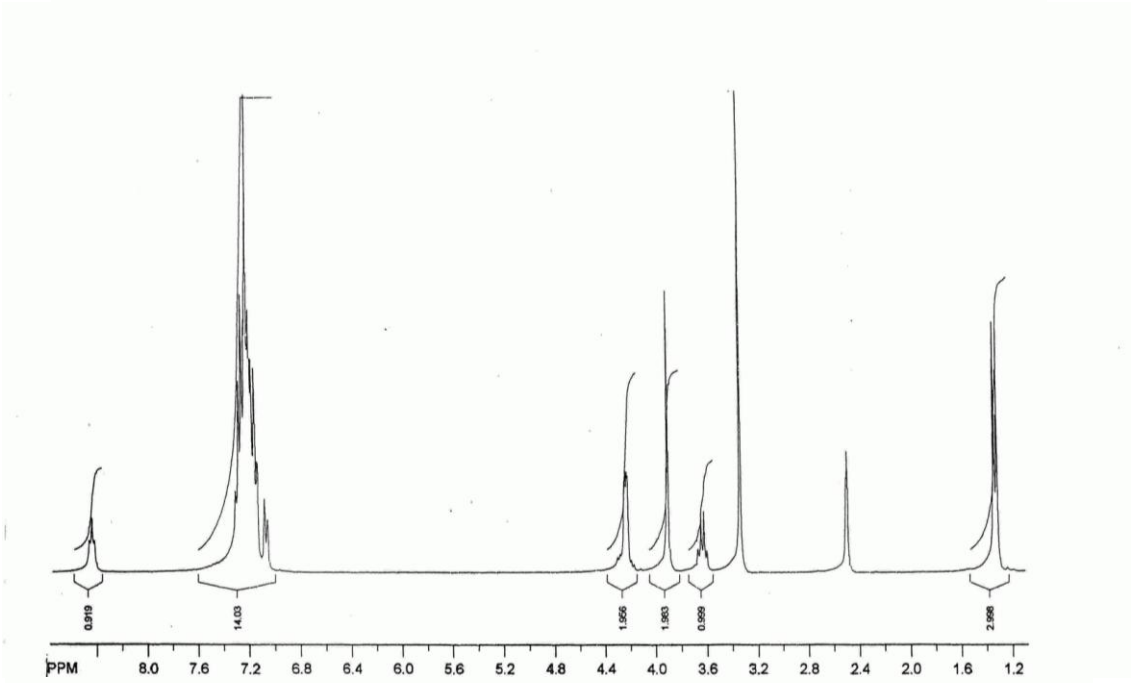

PPM
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8

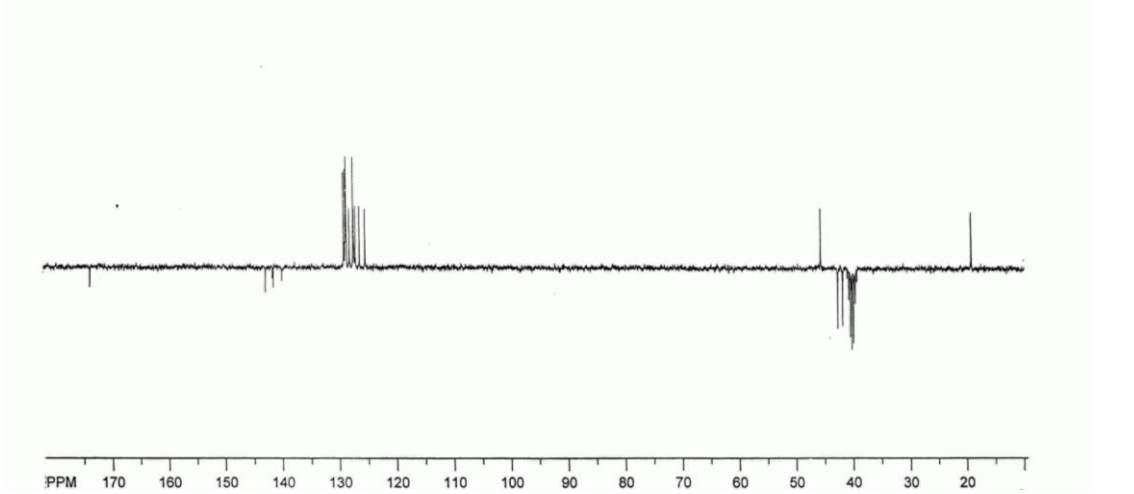

PPM
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20

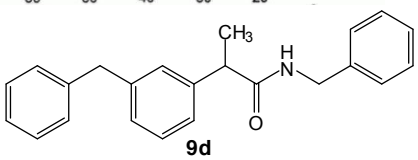

CH3
H
N
O

9c

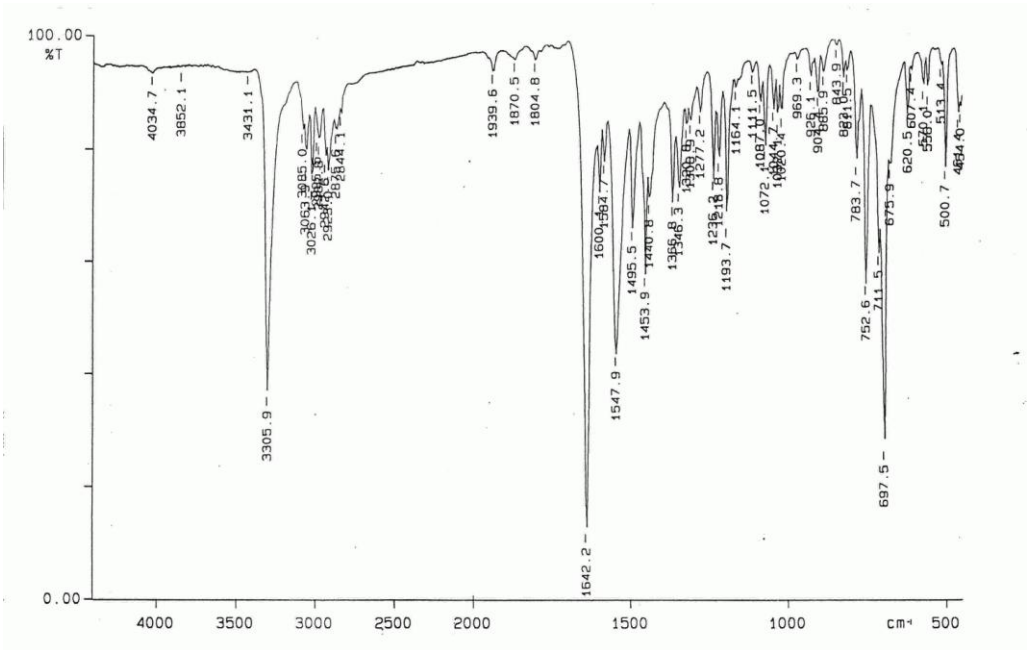

98.10
%T
46.34
4038.1
3291.8
3027.8
3061.3
3084.3
2971.3
2929.7
2286.7
1948.0
1878.0
1806.0
1647.9
1601.5
1586.1
1545.7
1494.8
1453.1
1363.8
1240.4
1219.8
1156.0
1074.5
1029.1
1002.1
907.3
843.9
779.4
721.7
697.4
621.1
555.9
4000
3500
3000
2500
2000
1500
1000
cm-1
500

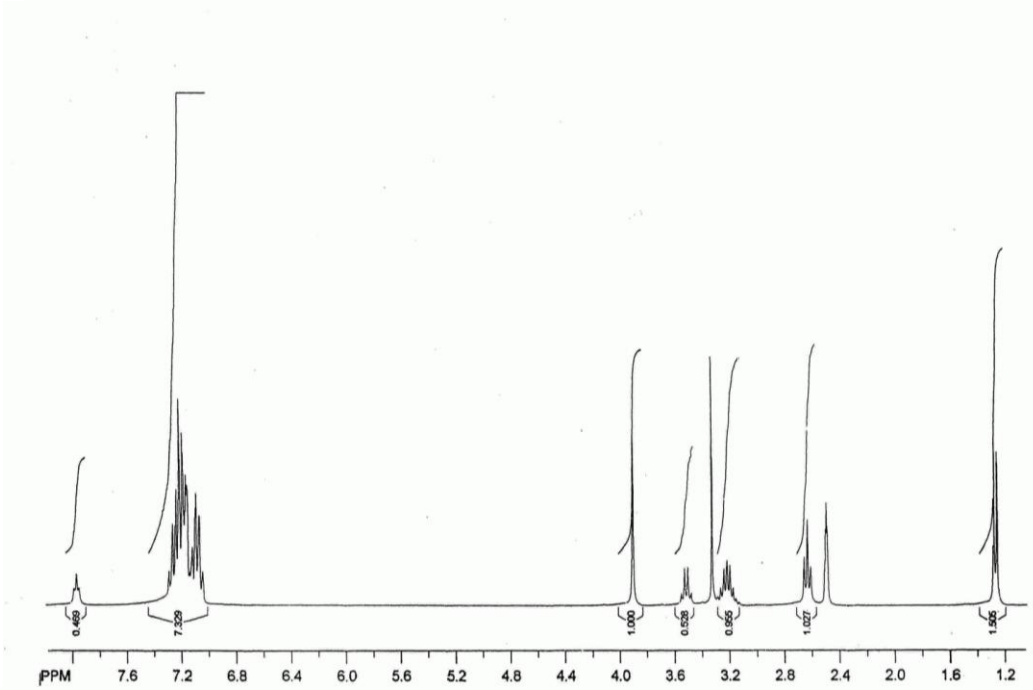

0.919
14.03
1.956
1.983
0.999
2.998
PPM
8.0
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2

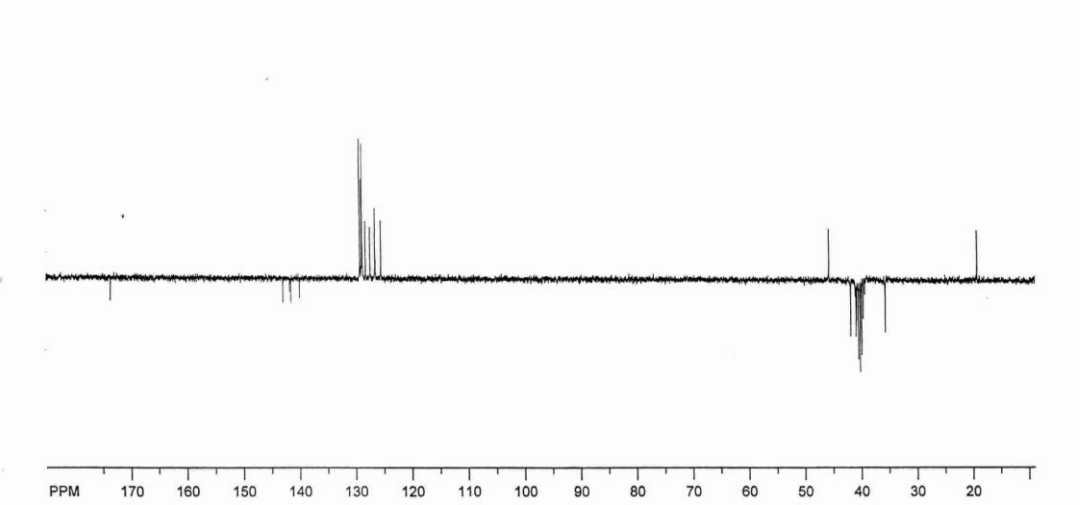

PPM
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20

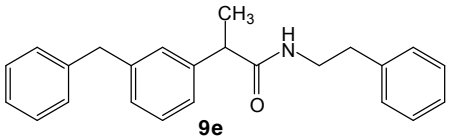

CH3
H
N
O

9d

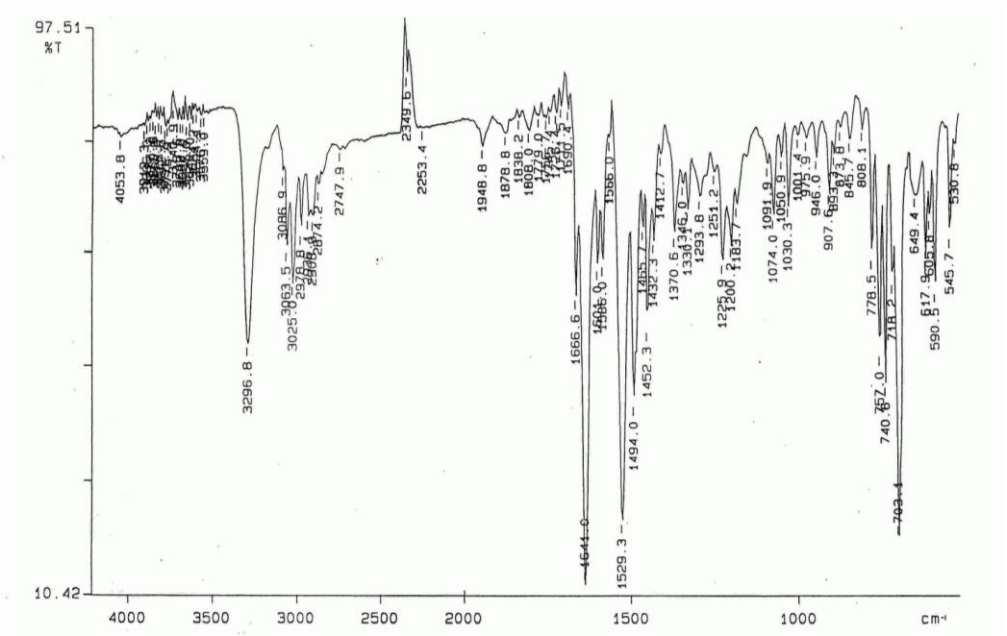

100.00
%T
0.00
4034.7
3852.1
3431.1
3305.9
1939.6
1870.5
1804.8
1642.2
1547.9
1495.5
1453.9
1193.7
1164.1
969.3
783.7
752.6
711.5
697.5
675.9
620.5
500.7
4000
3500
3000
2500
2000
1500
1000
cm⁻¹
500

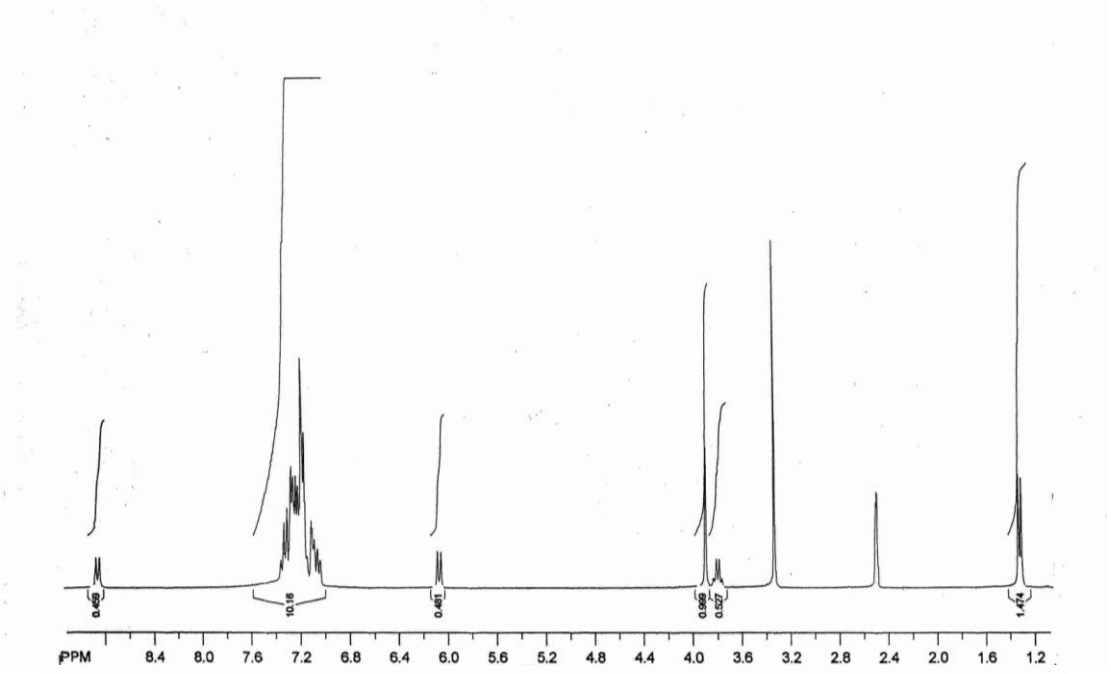

PPM
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2

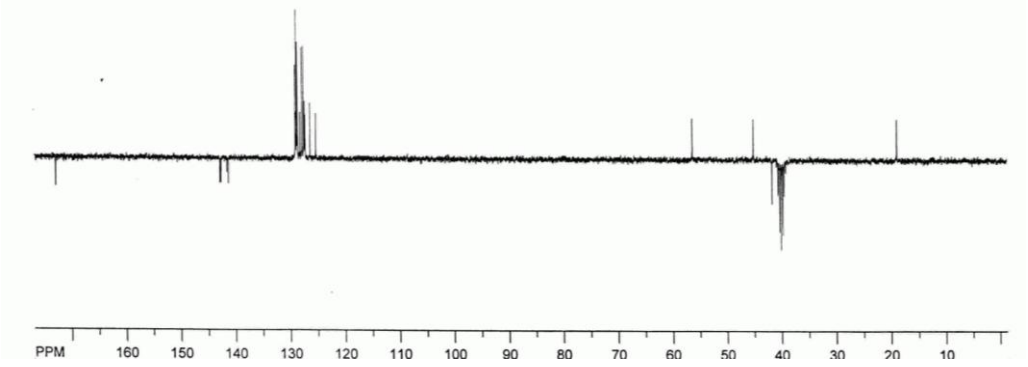

PPM
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20

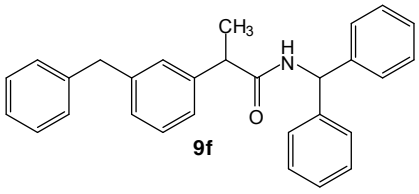

CH3
H
N
O

9e

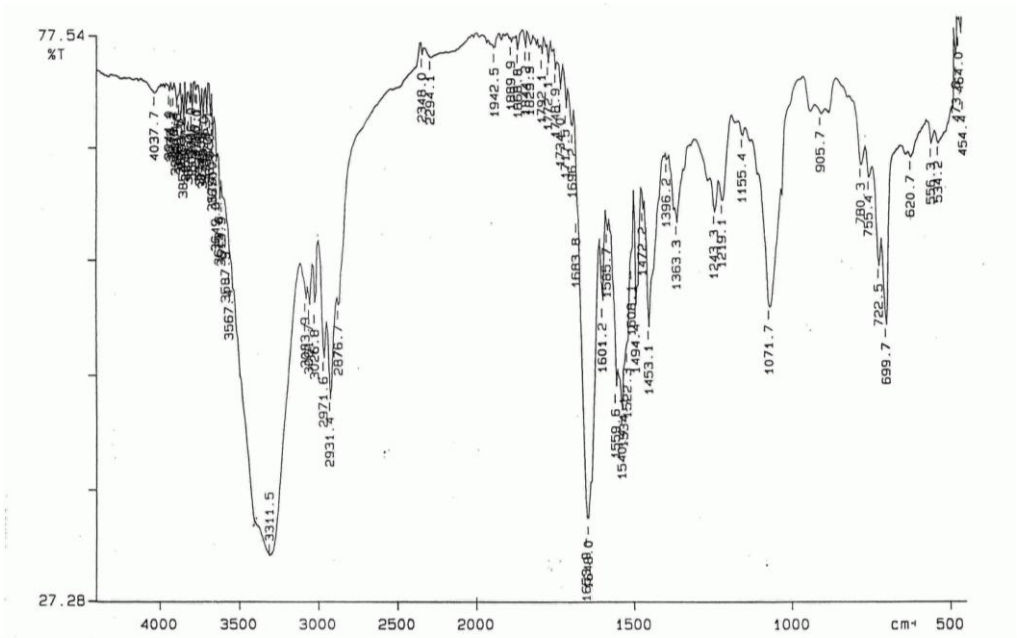

97.51
%T
10.42
4000
3500
3000
2500
2000
1500
1000
cm-1

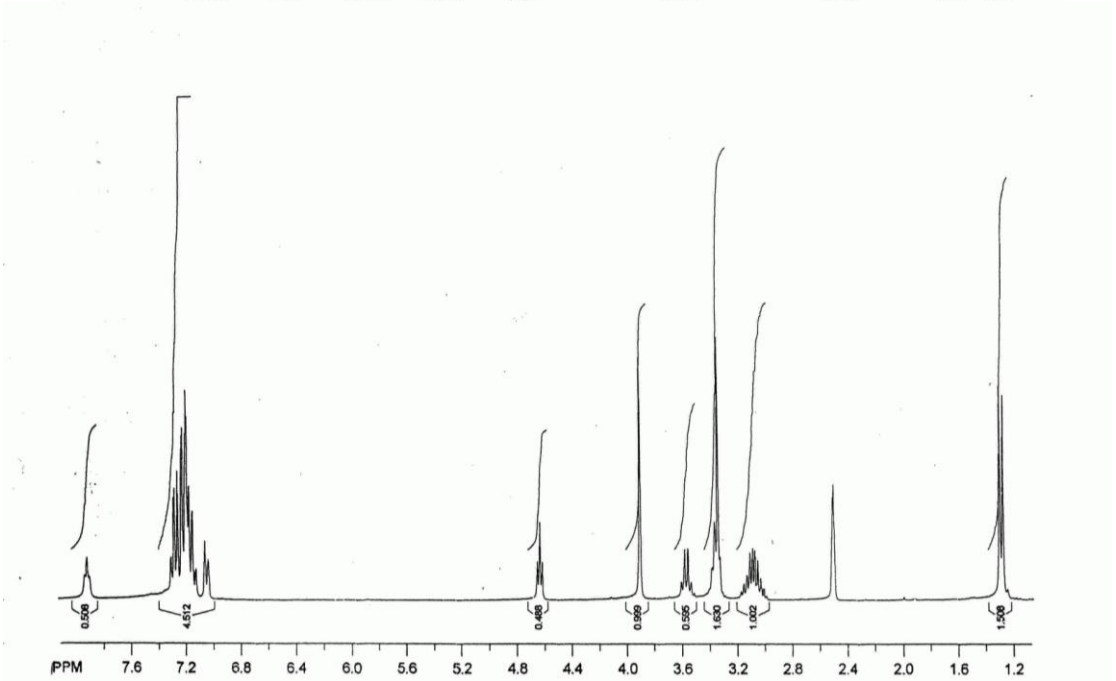

PPM
8.4
8.0
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2

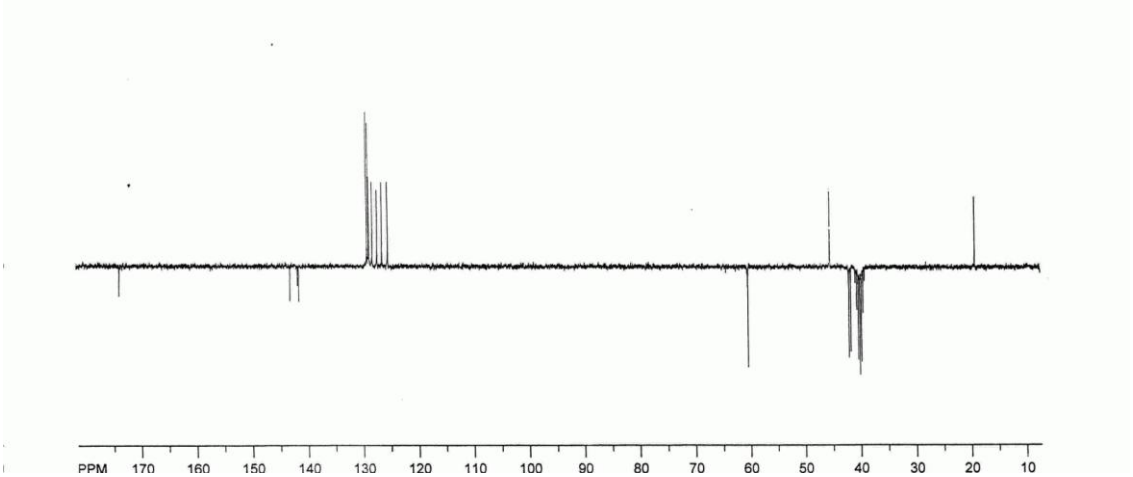

PPM
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

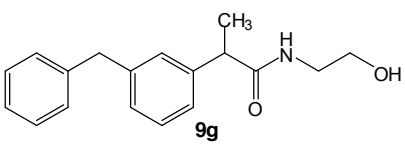

CH3
H
N
O

9f

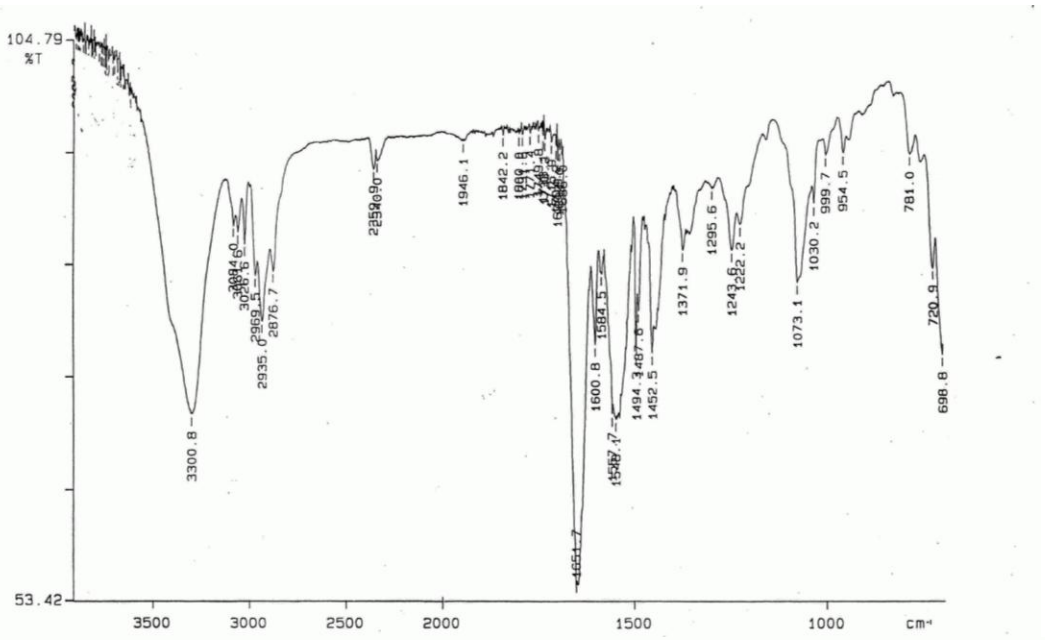

77.54
%T
27.28
4000
3500
3000
2500
2000
1500
1000
cm-1
500
3311.5
2931.4
2876.7
1683.8
1601.2
1453.1
1396.2
1363.3
1155.4
1071.7
905.7
755.4
722.5
699.7
620.7

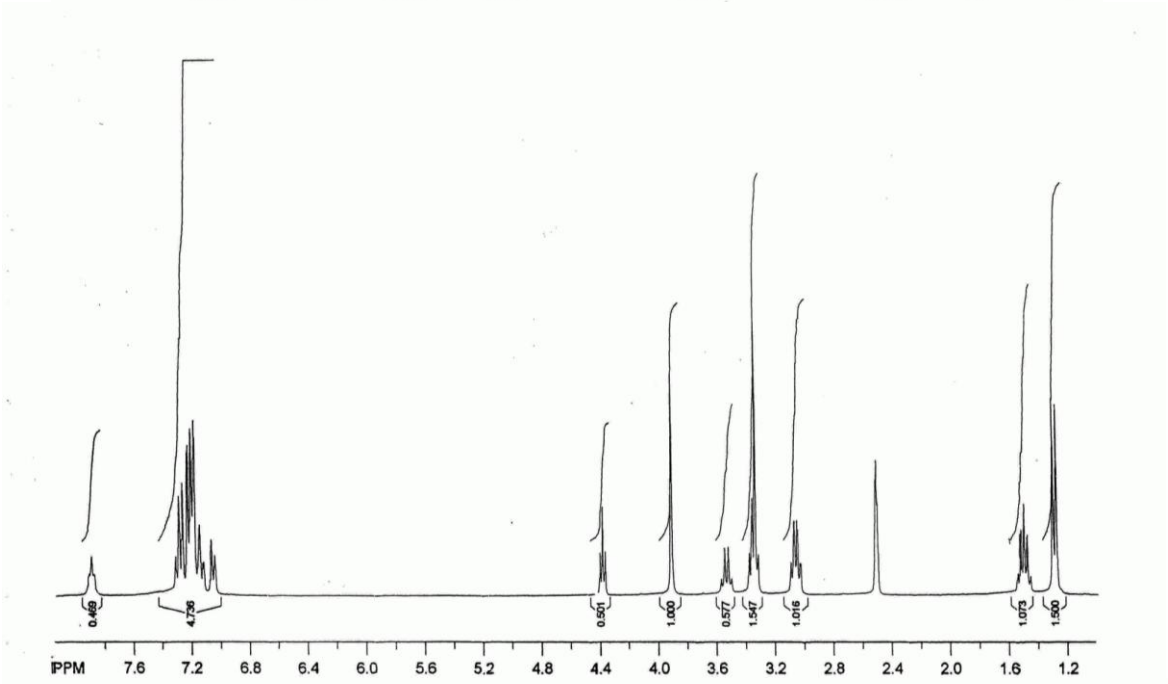

PPM
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2

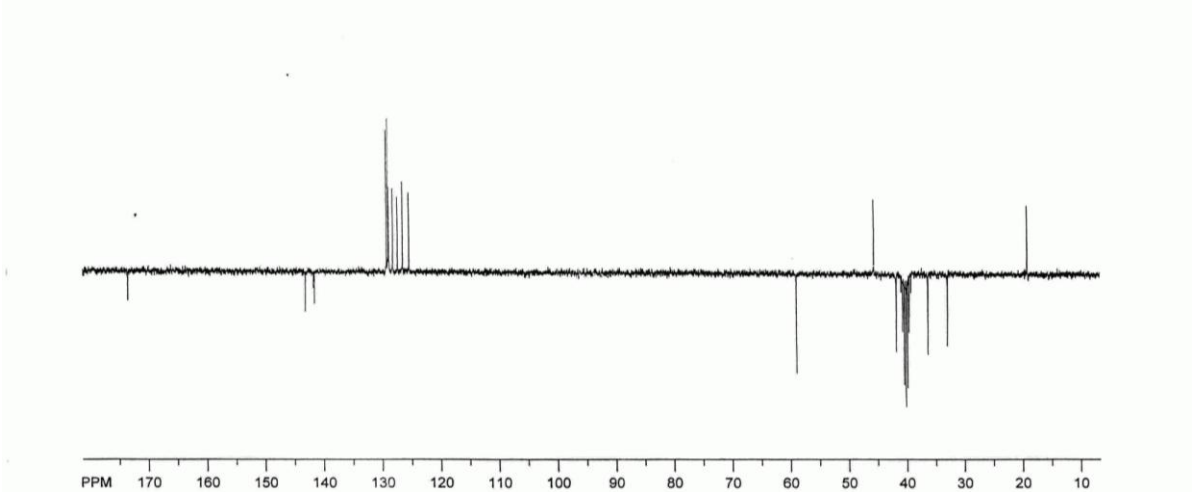

PPM
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

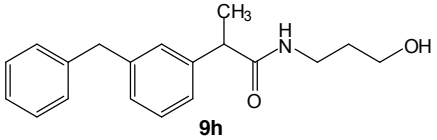

CH3
H
N
OH
O

9g

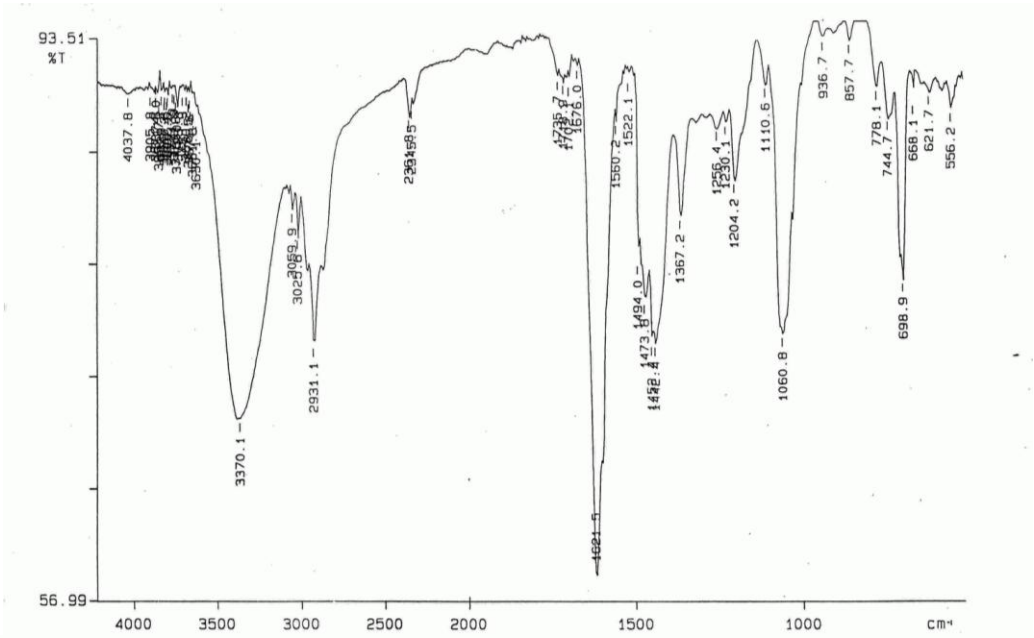

104.79
%T
53.42
3300.8
2935.0
2876.7
1946.1
1661.7
1600.8
1584.5
1494.3
1487.6
1452.5
1371.9
1295.6
1243.6
1222.2
1073.1
1030.2
999.7
954.5
781.0
720.9
698.8
3500
3000
2500
2000
1500
1000
cm⁻¹

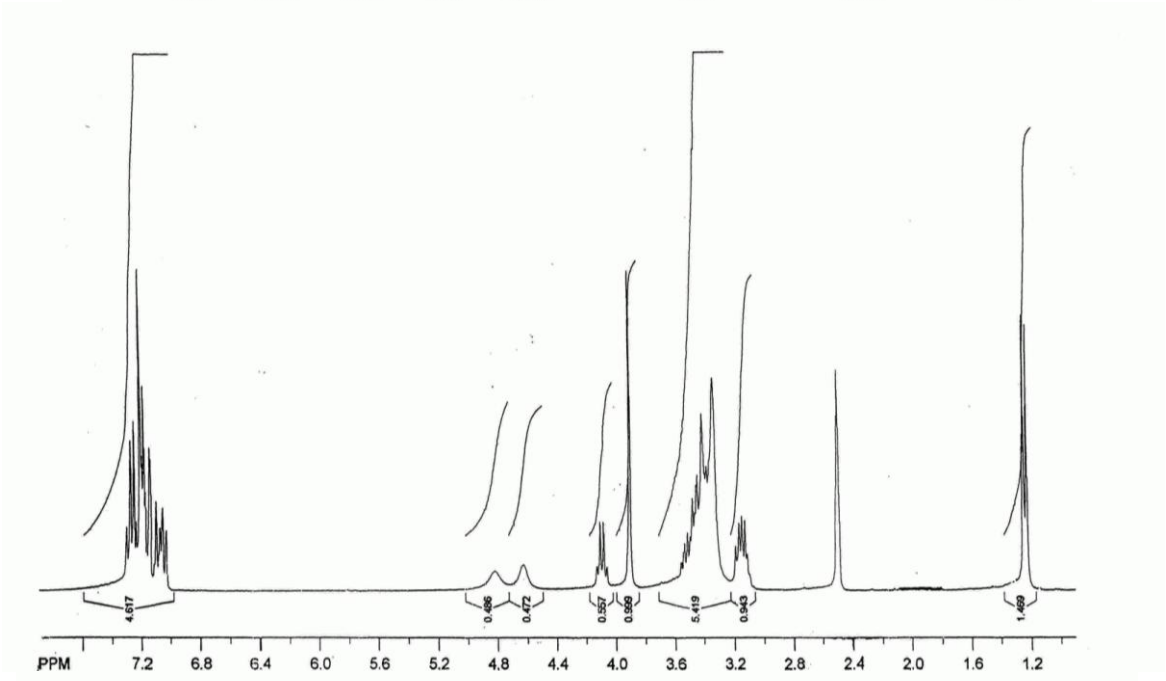

PPM
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2

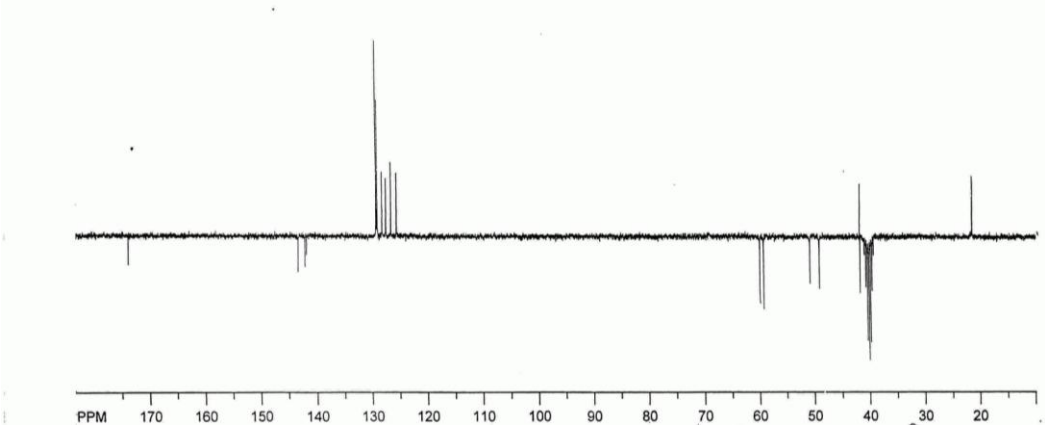

PPM
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

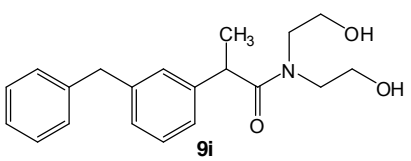

CH3
H
N
OH
O

9h

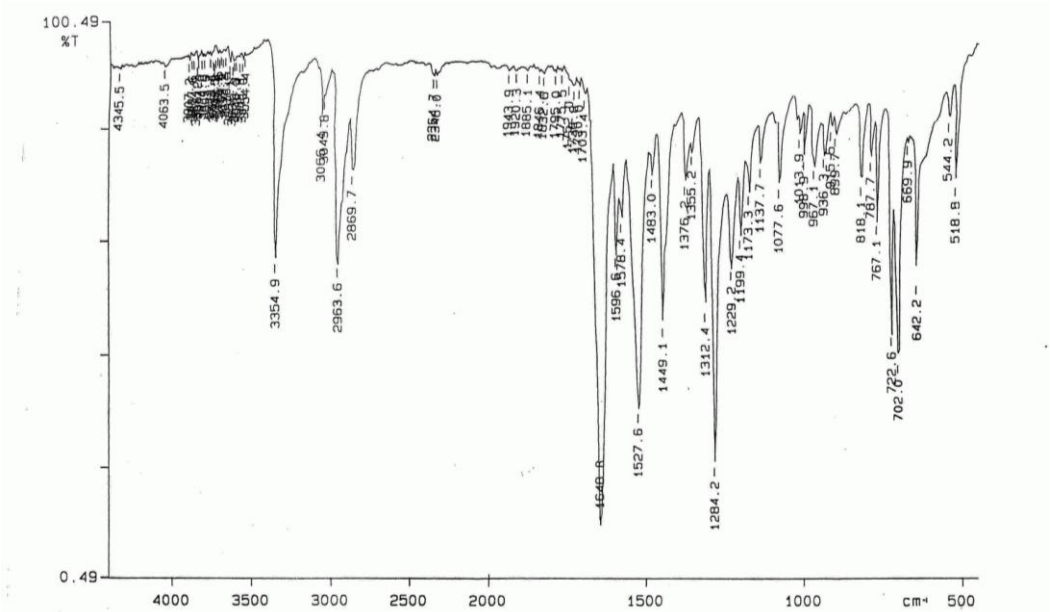

93.51
%T
56.99
4037.8
3370.1
3069.9
2931.1
1735.7
1676.0
1560.2
1522.1
1494.0
1473.1
1367.2
1256.4
1230.1
1204.2
1110.6
1060.8
936.7
857.7
778.1
744.7
698.9
668.1
621.7
556.2
4000
3500
3000
2500
2000
1500
1000
cm⁻¹

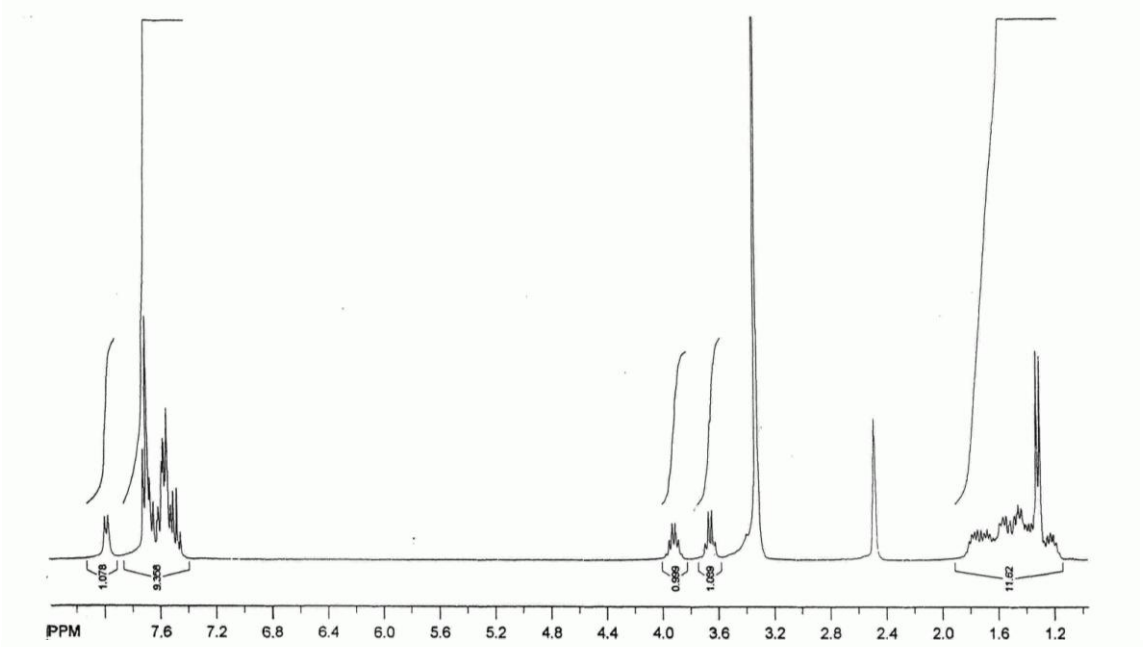

PPM
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2

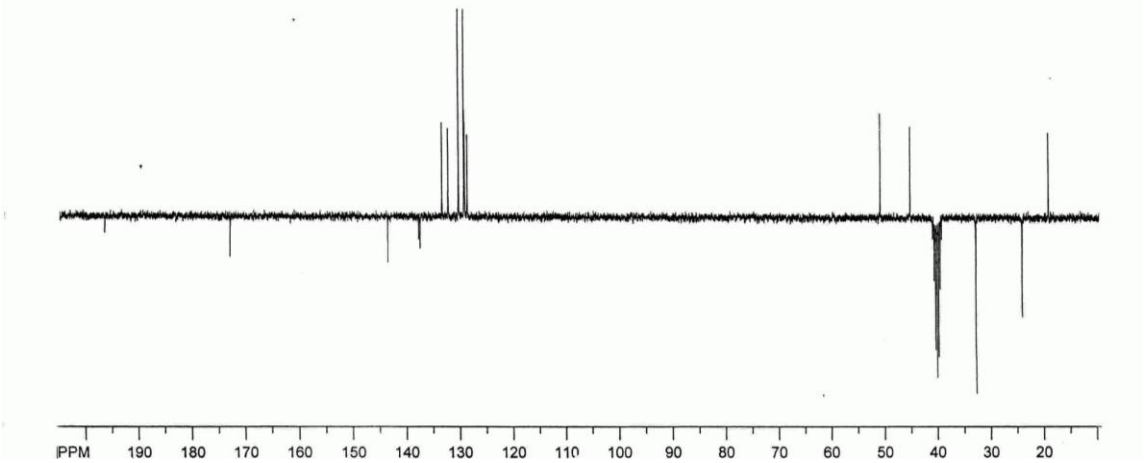

PPM
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20

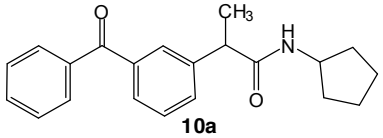

CH3
OH
N
OH
O

9i

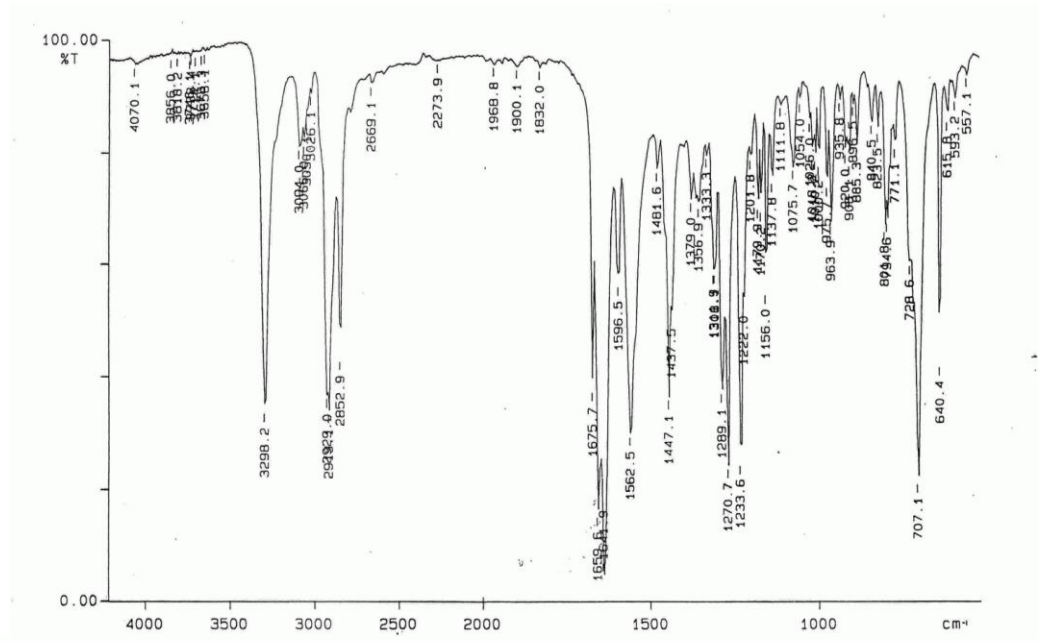

100.49
%T
0.49
4345.5
4063.5
3354.9
3056.8
2963.6
2869.7
1640.8
1596.1
1578.4
1527.6
1483.0
1449.1
1376.2
1355.2
1312.4
1284.2
1229.2
1199.4
1173.3
1137.7
1077.6
818.1
767.1
722.6
702.8
669.9
642.2
544.2
518.8
4000
3500
3000
2500
2000
1500
1000
cm-1
500

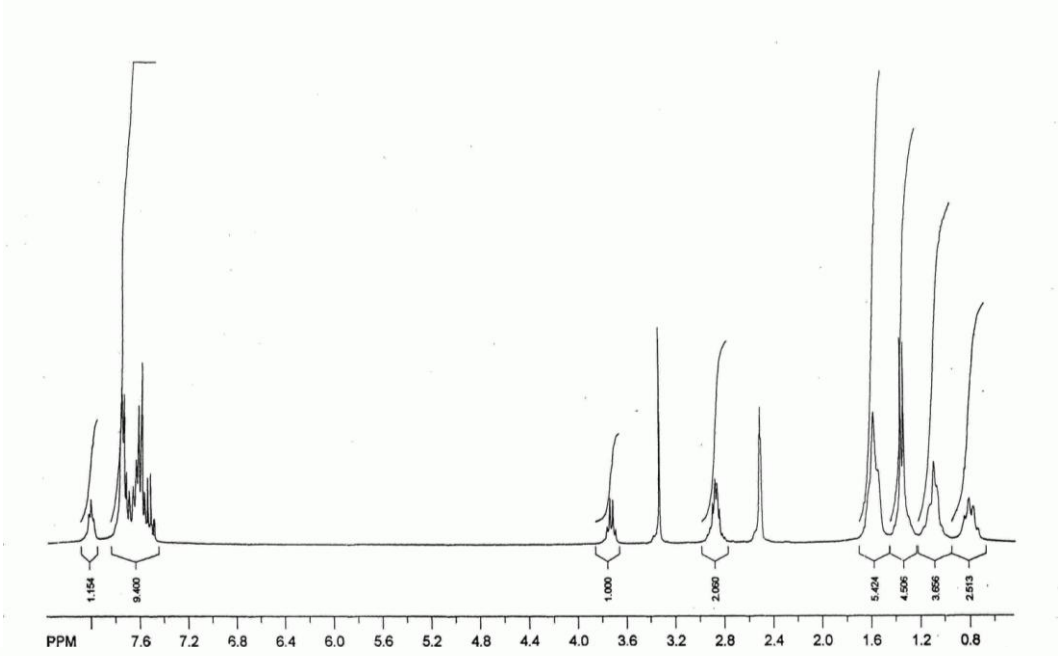

1.078
9.358
0.969
1.088
11.62
PPM
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2

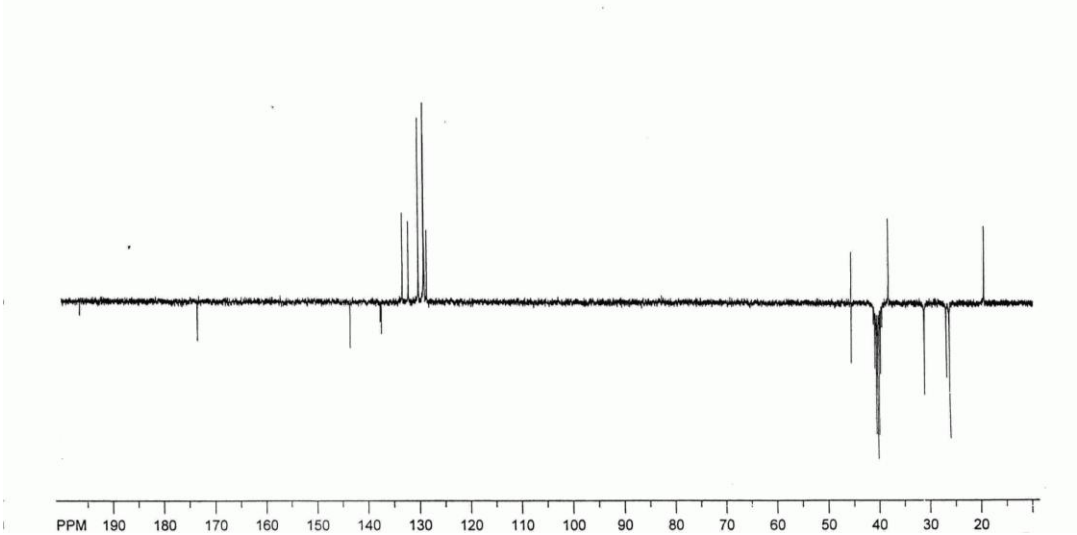

PPM
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20

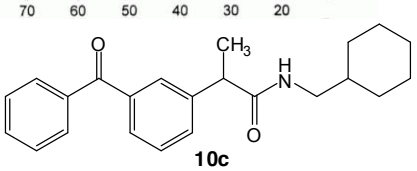

O
CH3
H
N
O

10a

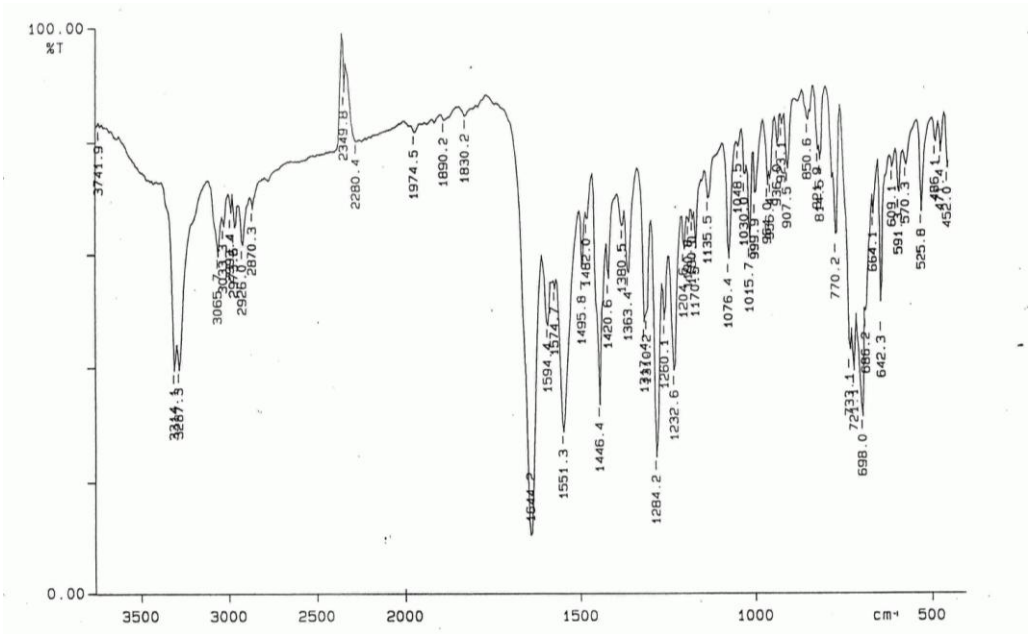

100.00
%T
0.00
4000
3500
3000
2500
2000
1500
1000
cm⁻¹
4070.1
3298.2
2852.9
2669.1
2273.9
1968.8
1900.1
1832.0
1675.7
1596.5
1562.5
1481.6
1447.1
1289.1
1270.7
1233.6
1156.0
1111.8
1075.7
707.1
640.4
557.1

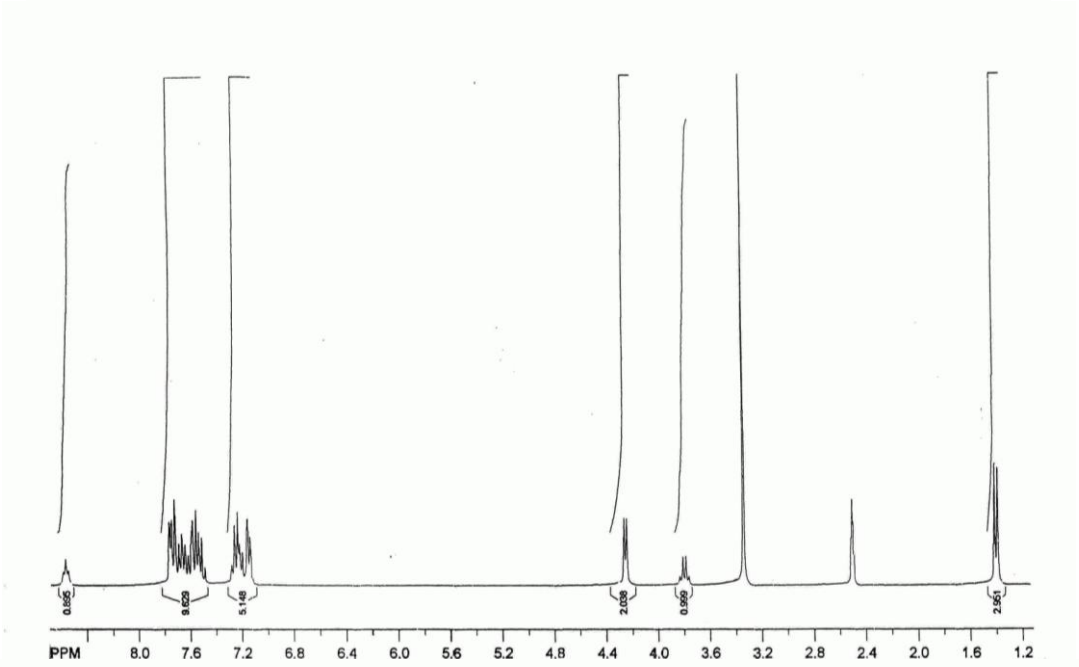

1.154
9.400
1.000
2.060
5.424
4.506
3.656
2.513
PPM
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8

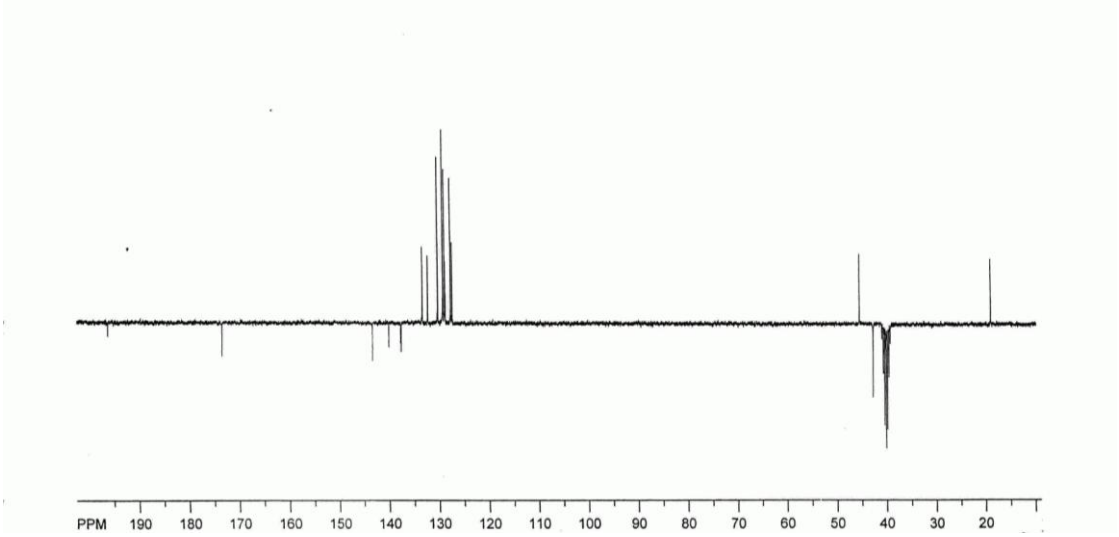

PPM
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20

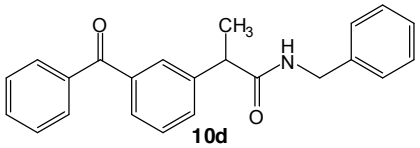

O
CH3
H
N
O

10c

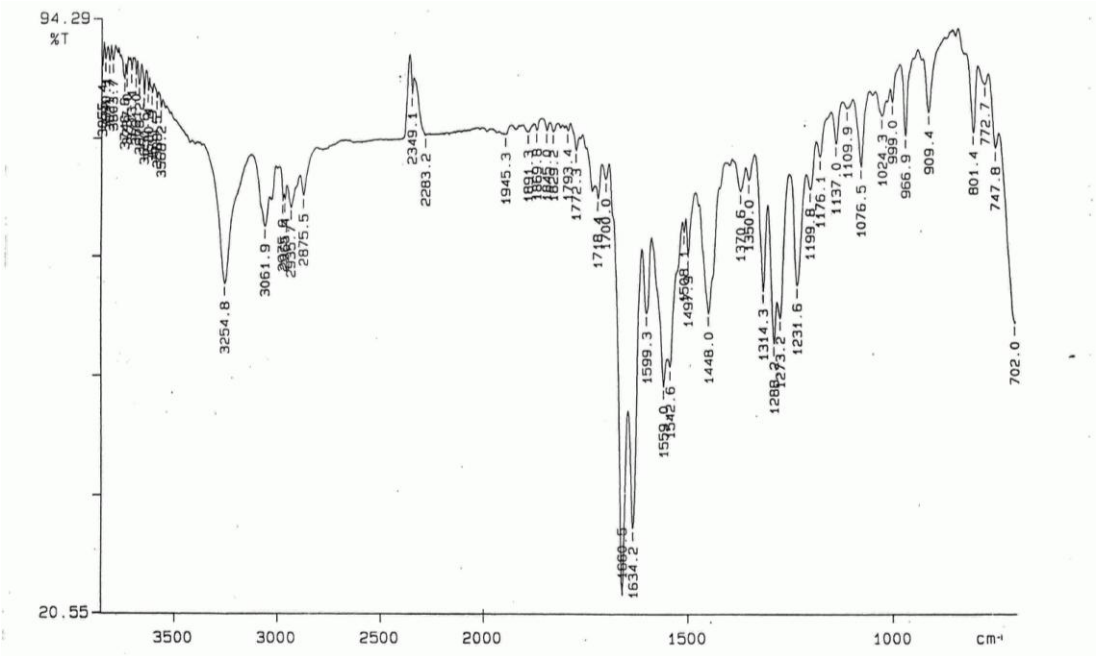

100.00
%T
0.00
3500
3000
2500
2000
1500
1000
cm-1
500

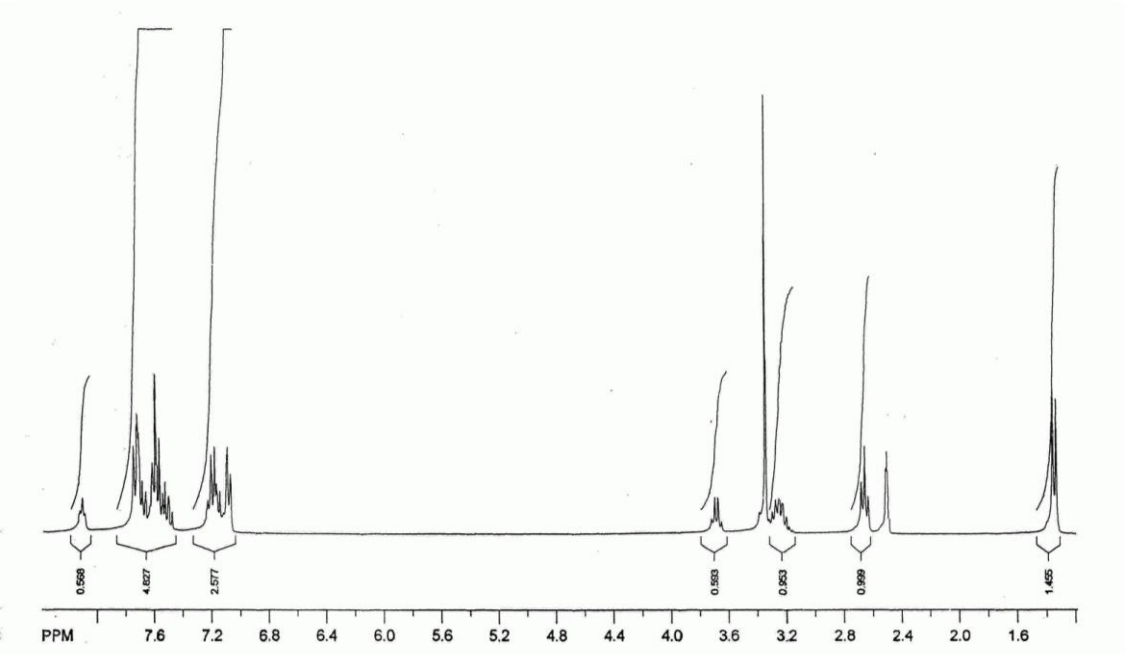

PPM
8.0
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2

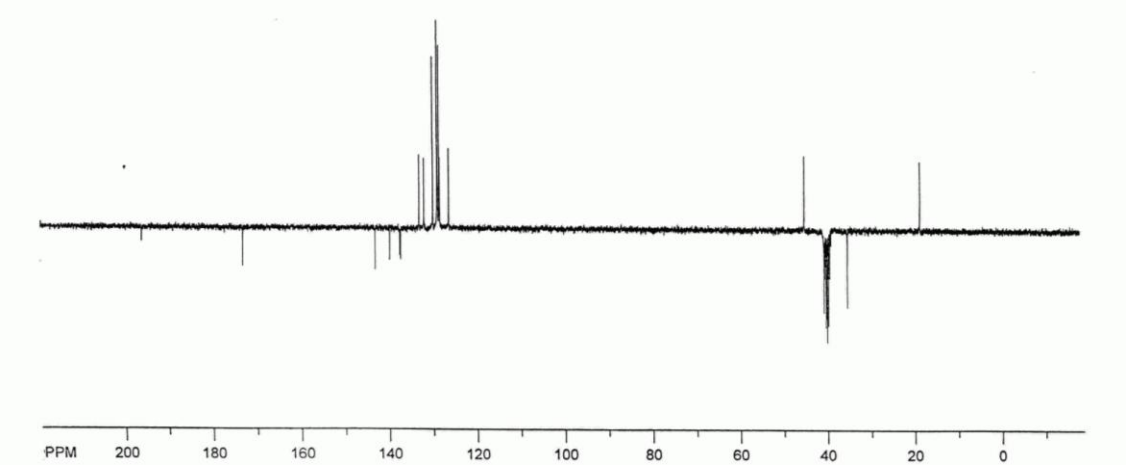

PPM
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20

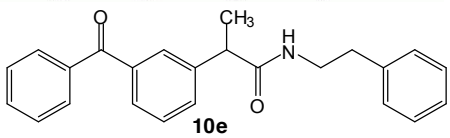

O
CH3
H
N
O

10d

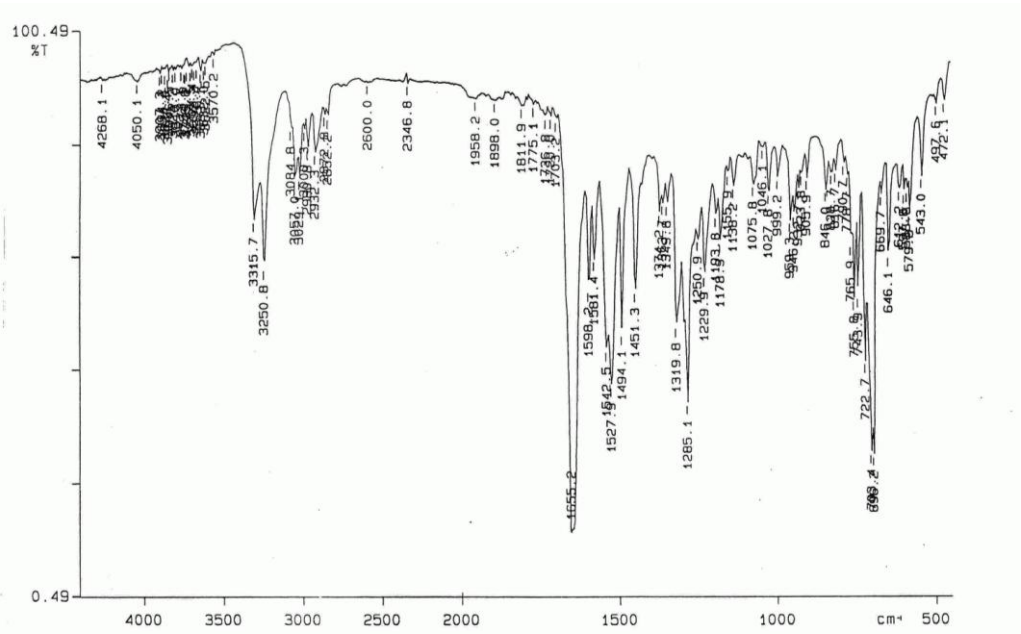

94.29
%T
20.55
3500
3000
2500
2000
1500
1000
cm-1
3254.8
3061.9
2875.5
2349.1
2283.2
1945.3
1772.3
1718.4
1700.0
1634.2
1599.3
1558.0
1448.0
1370.6
1350.0
1314.3
1231.6
1199.8
1176.1
1137.0
1109.9
1076.5
1024.3
999.0
966.9
909.4
801.4
772.7
747.8
702.0

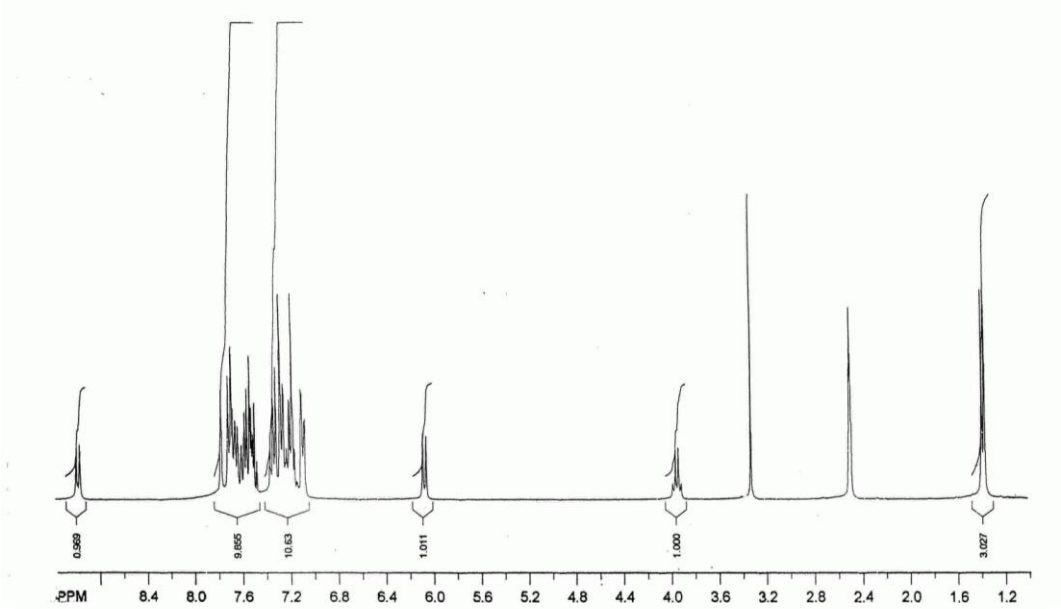

0.568
4.627
2.577
0.593
0.953
0.999
1.455
PPM
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6

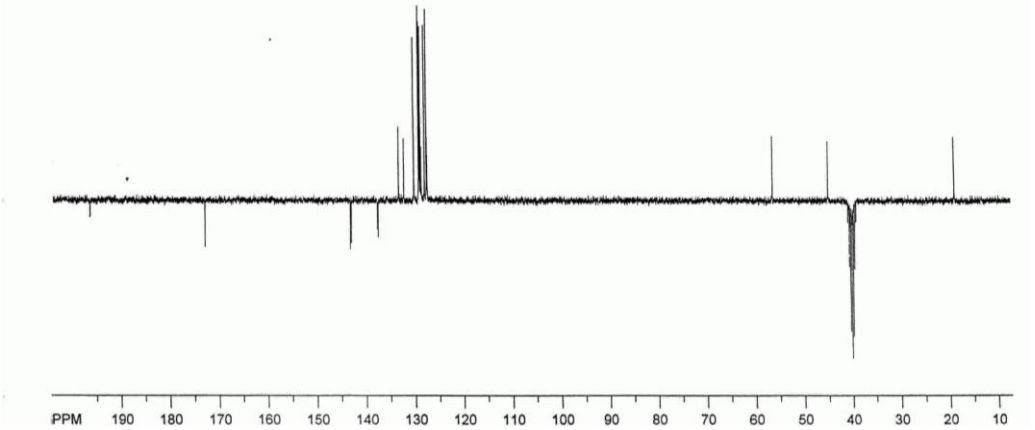

PPM
200
180
160
140
120
100
80
60
40
20
0

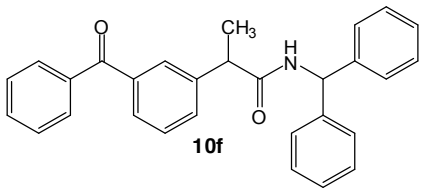

O
CH3
H
N
O

10e

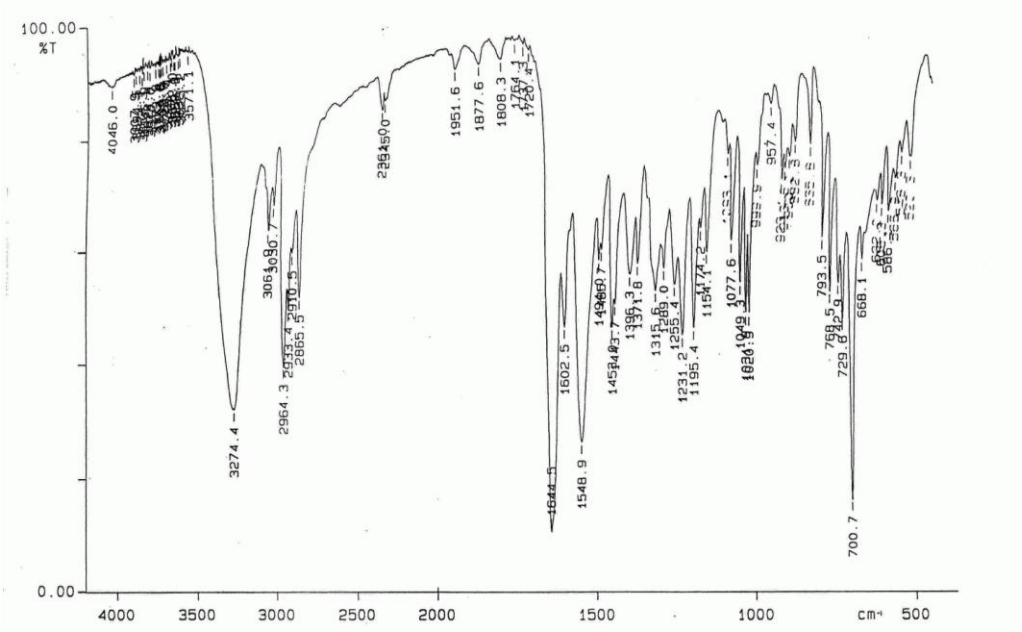

100.49
%T
0.49
4000
3500
3000
2500
2000
1500
1000
cm-1
500

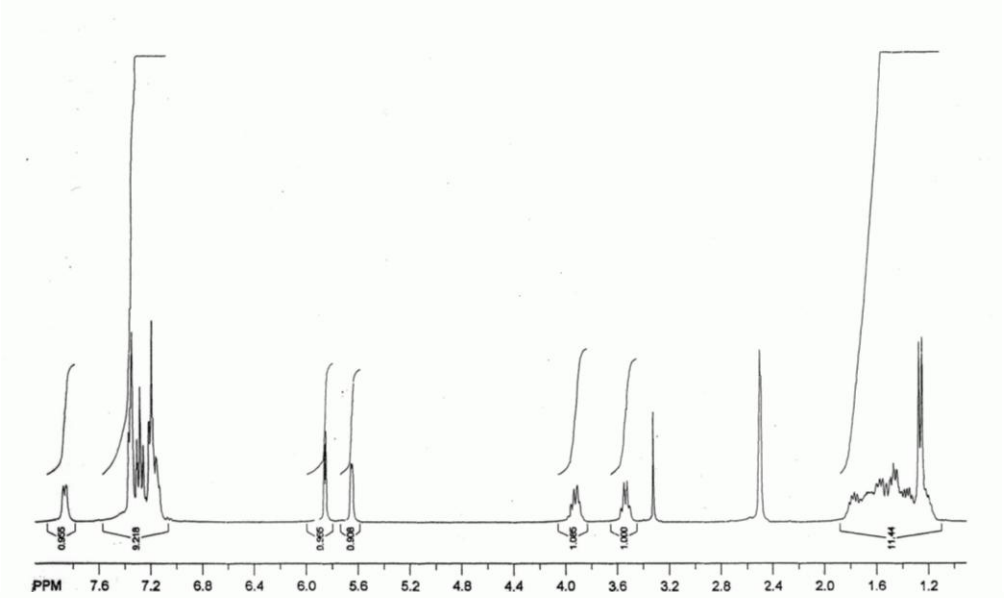

0.969
9.855
10.63
1.011
1.000
3.027
PPM
8.4
8.0
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2

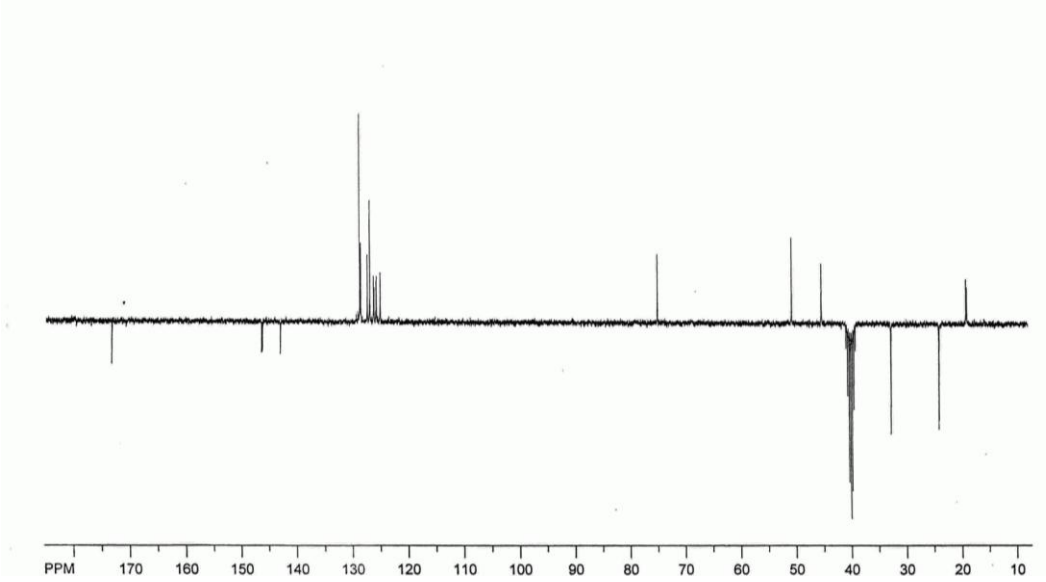

PPM
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

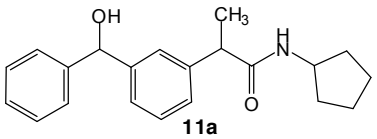

O
CH3
H
N
O

10f

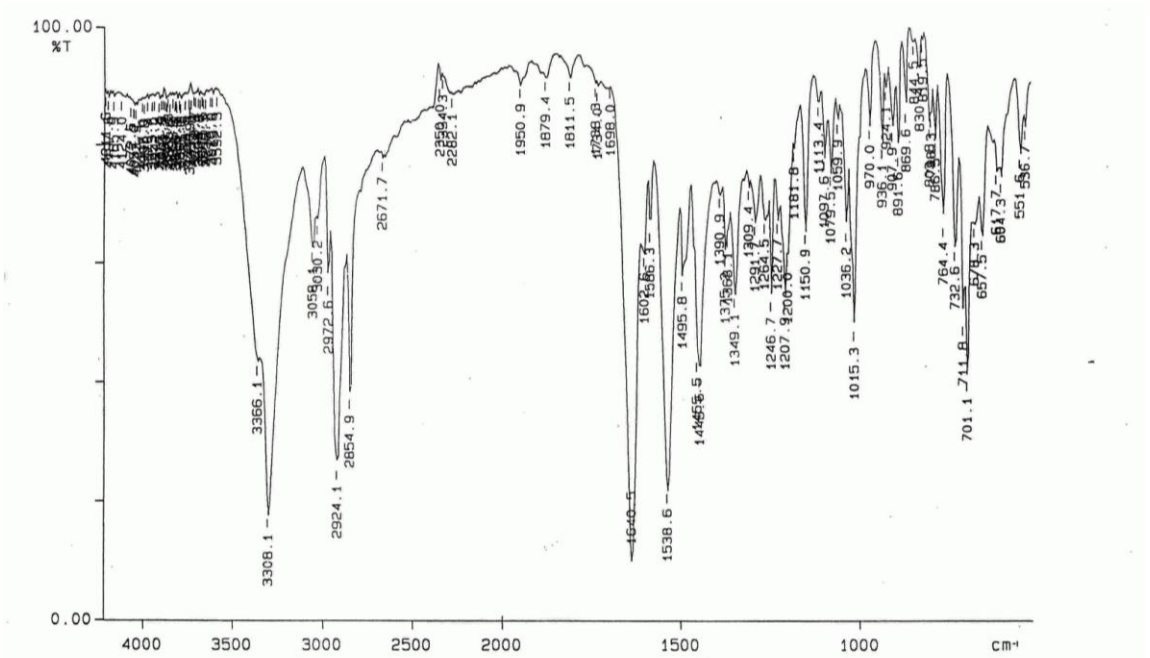

100.00
%T
0.00
4000
3500
3000
2500
2000
1500
1000
cm-1
500
3274.4
1548.9
700.7

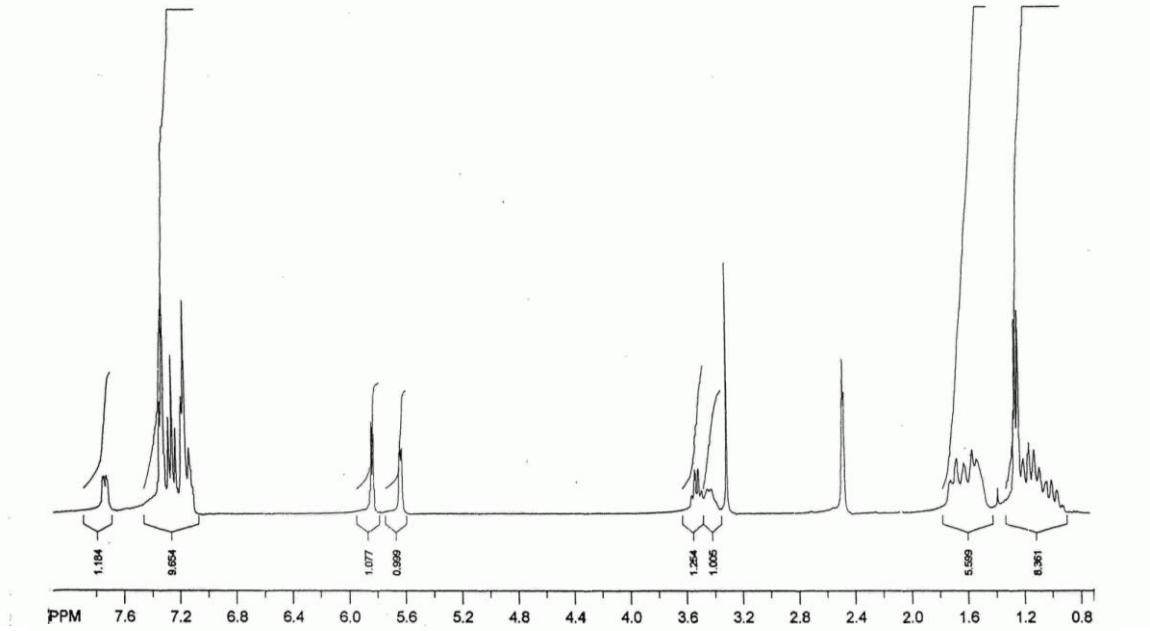

PPM
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2

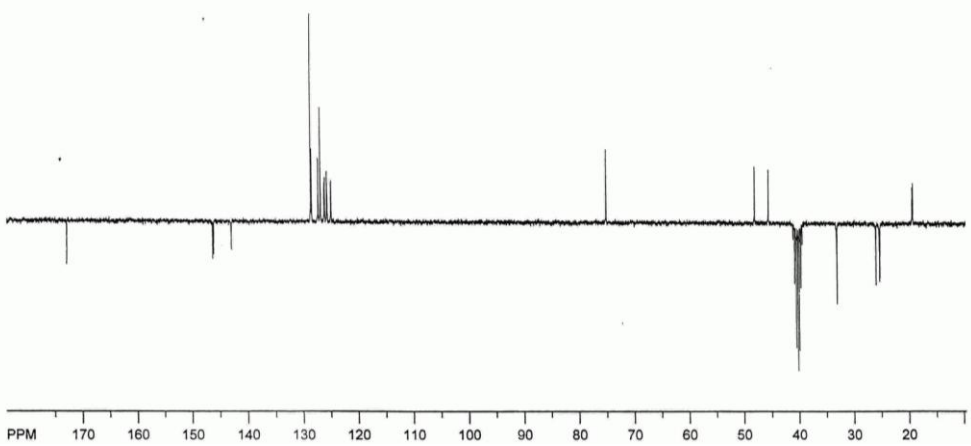

PPM
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10

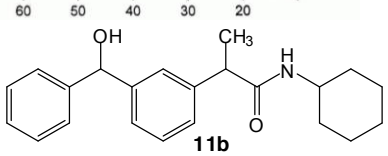

OH
CH3
H
N
O

11a

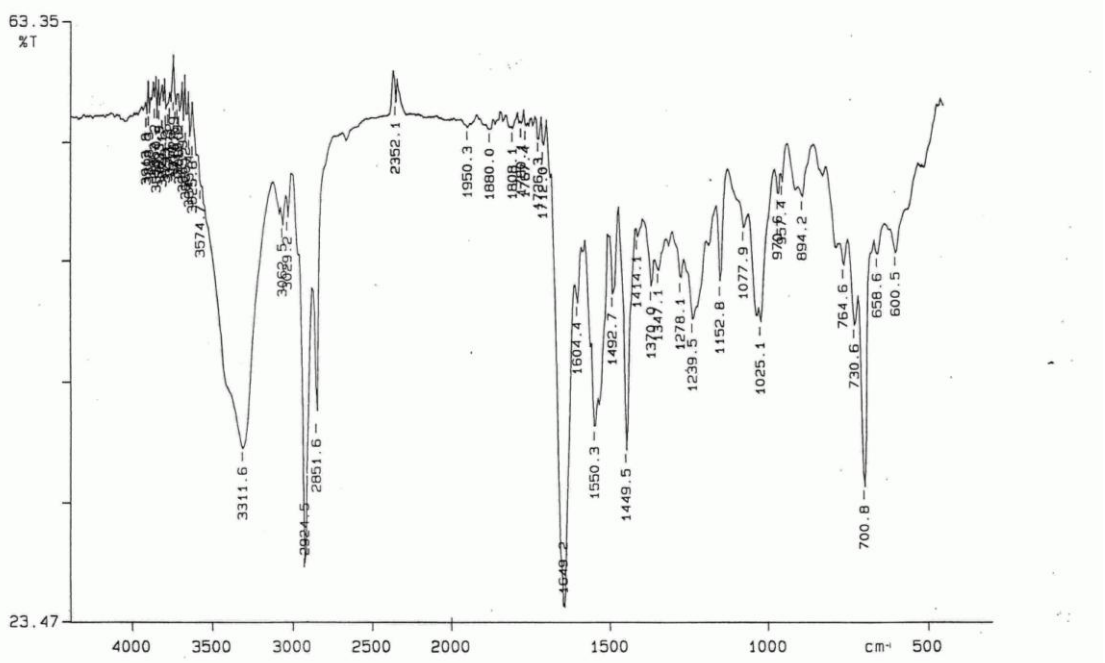

100.00
%T
0.00
4000
3500
3000
2500
2000
1500
1000
cm⁻¹
3366.1
3308.1
2924.1
2854.9
2671.7
1950.9
1879.4
1811.5
1538.6
1495.8
1015.3
1036.2
970.0
764.4
732.6
701.1

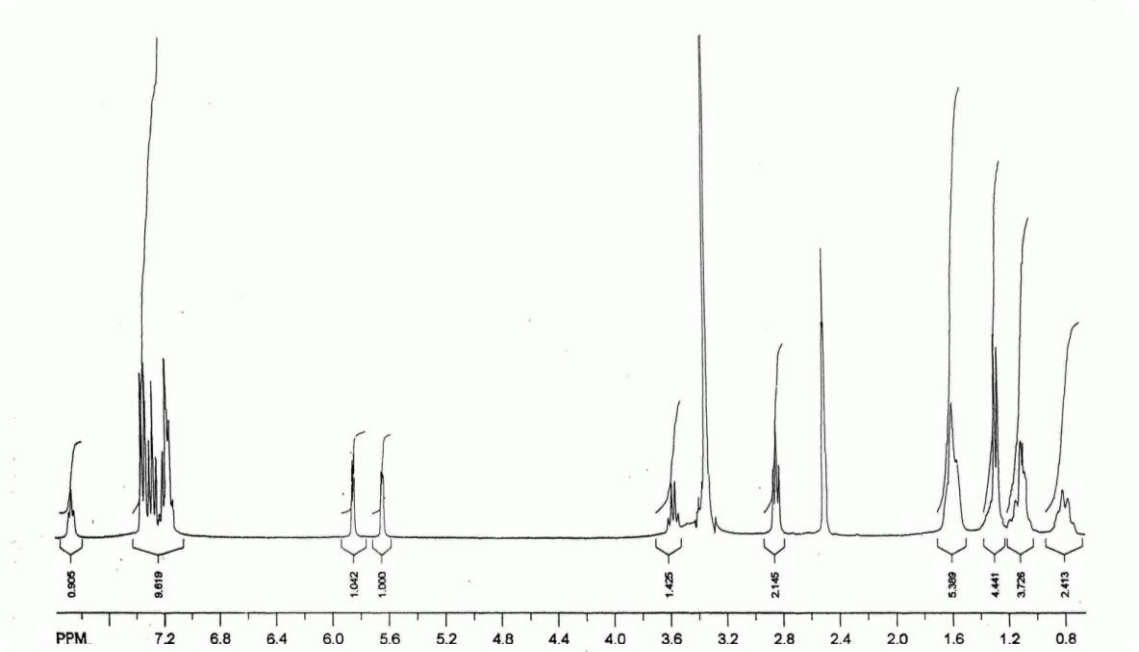

1.184
9.654
1.077
0.999
1.254
1.005
5.599
8.361
PPM
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8

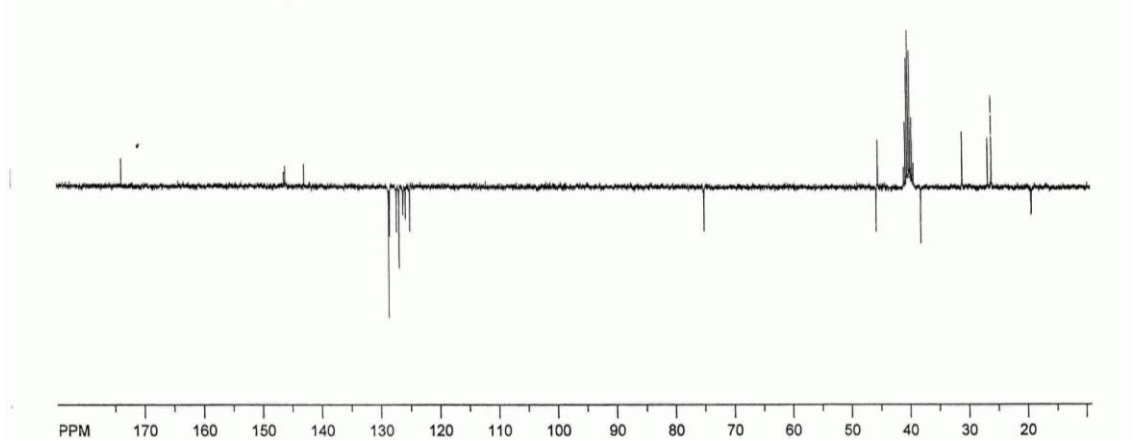

PPM
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20

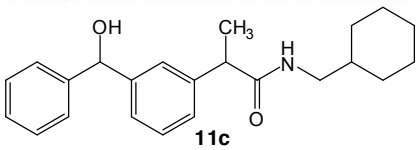

OH
CH3
H
N
O

11b

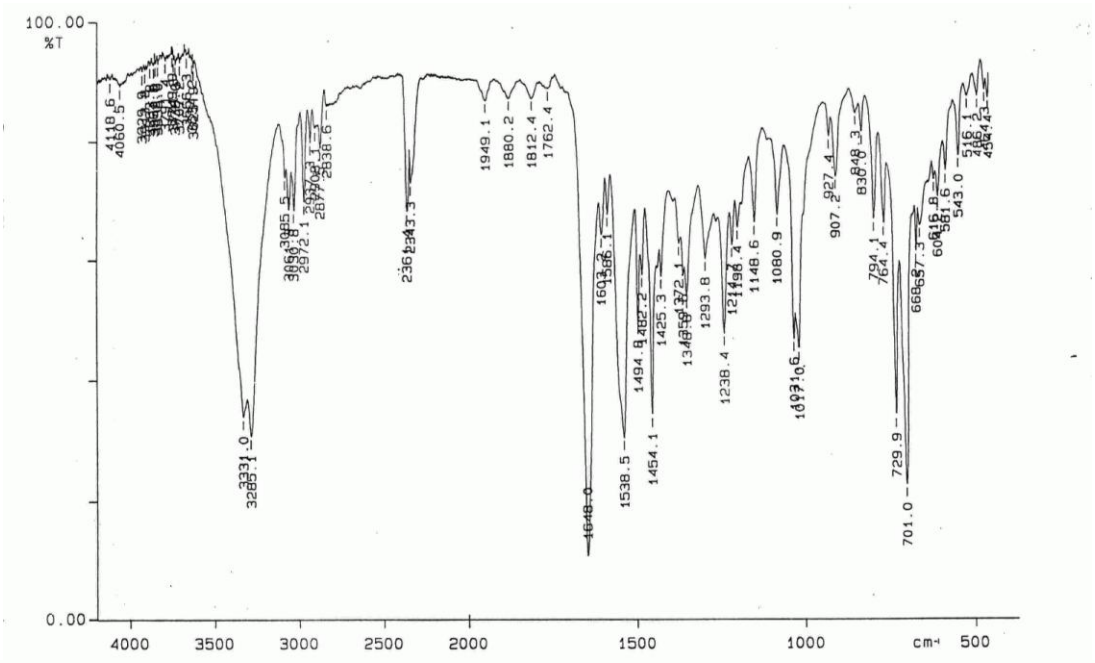

63.35
%T
23.47
4000
3500
3000
2500
2000
1500
1000
cm-1
500
3574.7
3311.6
3063.5
3029.5
2924.5
2851.6
2352.1
1950.3
1880.0
1649.2
1604.4
1550.3
1492.7
1449.5
1414.1
1379.1
1349.1
1278.1
1239.5
1152.8
1077.9
1025.1
894.2
764.6
730.6
700.8
658.6
600.5


0.905
9.619
1.042
1.000
1.425
2.145
5.389
4.441
3.726
2.413
PPM
7.2
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8


PPM
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20


OH
CH3
H
N
O

11c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cm-1
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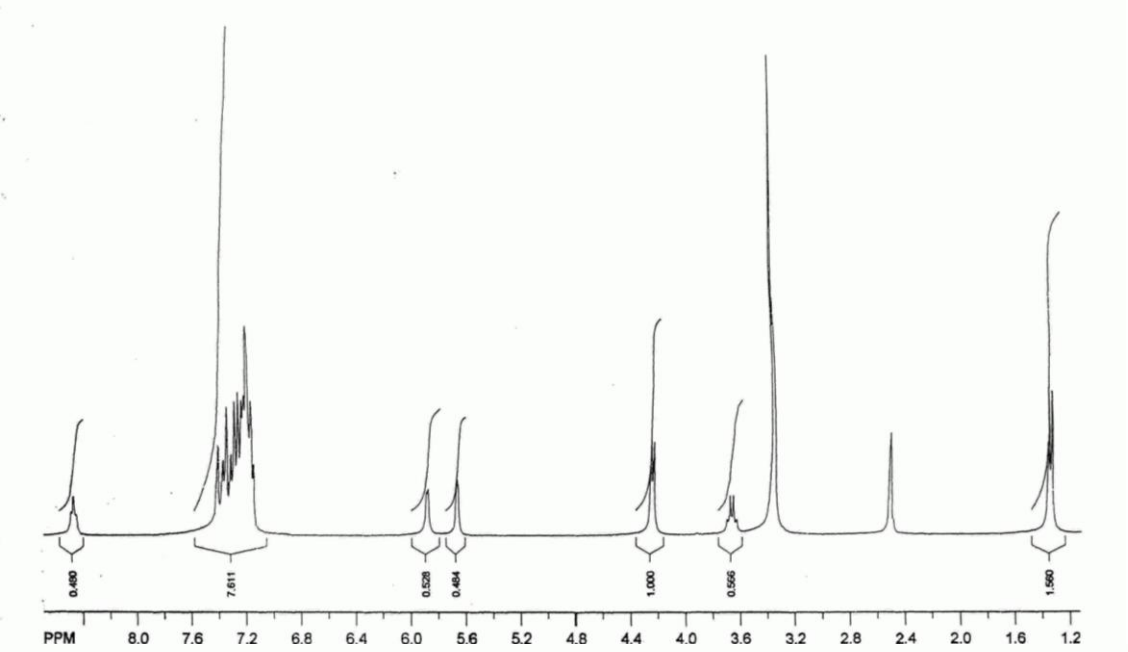
701.0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PPM
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7.6
7.2
6.8
6.4
6.0
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5.2
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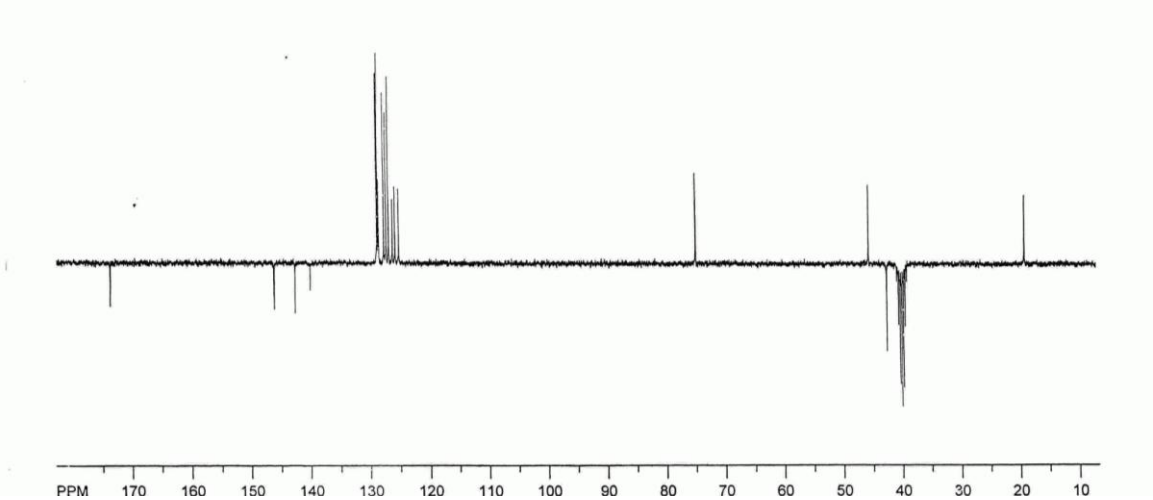
3.2
2.8
2.4
2.0
1.6
1.2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120
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80
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60
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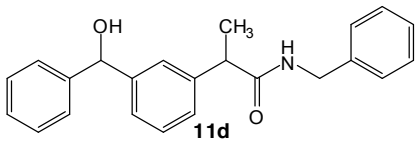
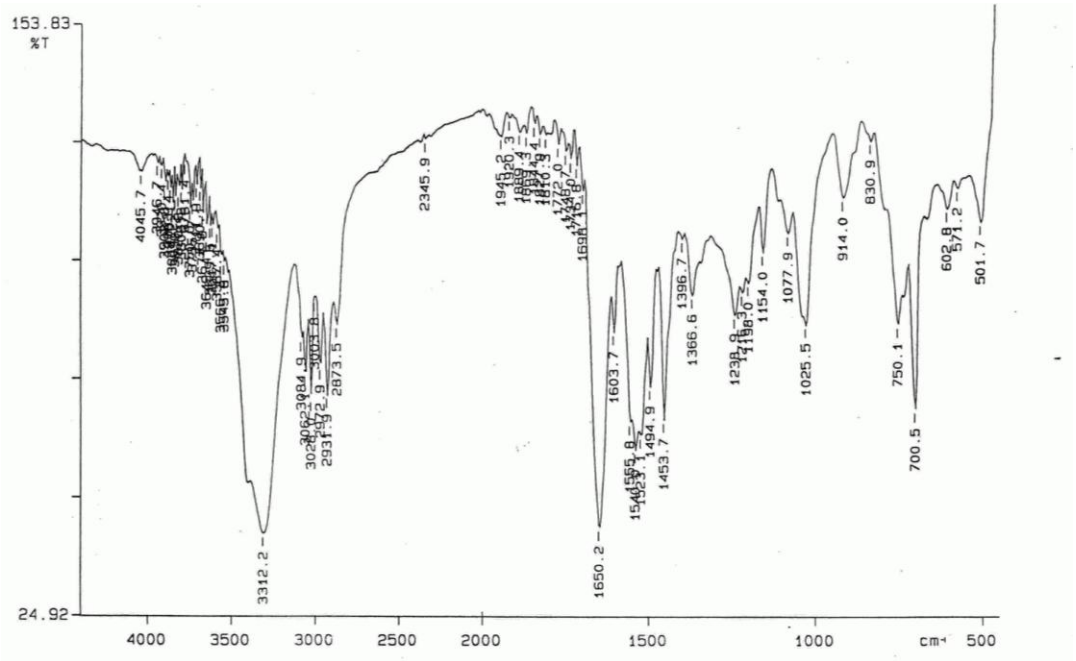
40
30
20
10


OH
CH3
H
N
O

11d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cm
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2345.9
2873.5
1650.2
1603.7
1494.9
1453.7
1396.7
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571.2
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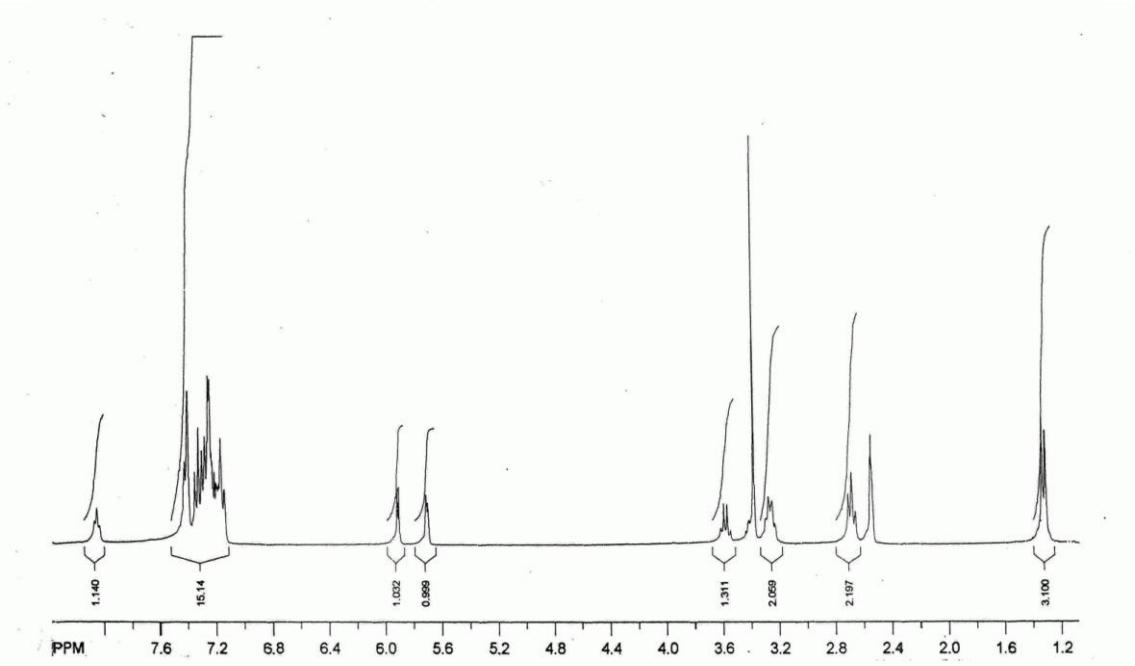
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2.197
3.100
PPM
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7.2
6.8
6.4
6.0
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3.2
2.8
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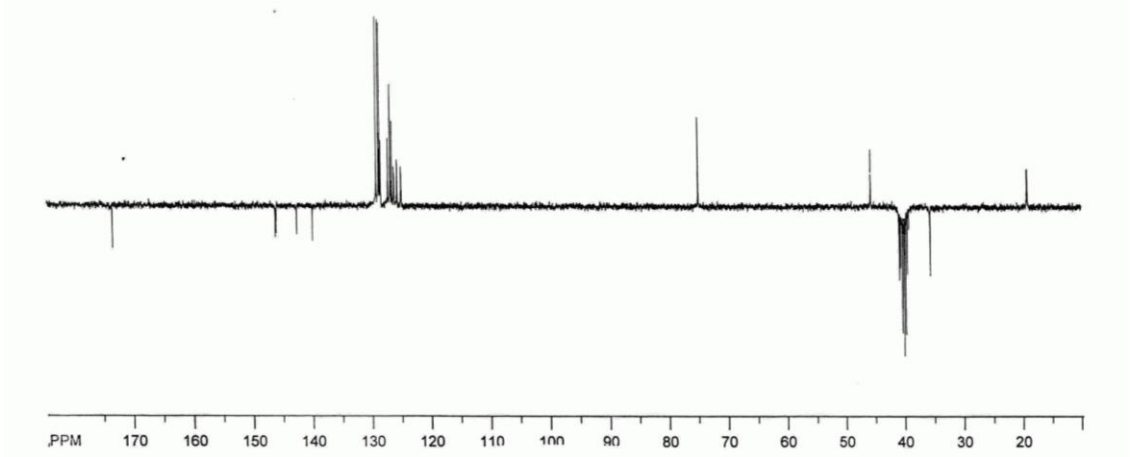
1.6
1.2


PPM
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160
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100
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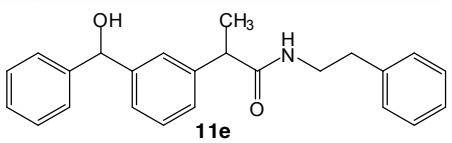
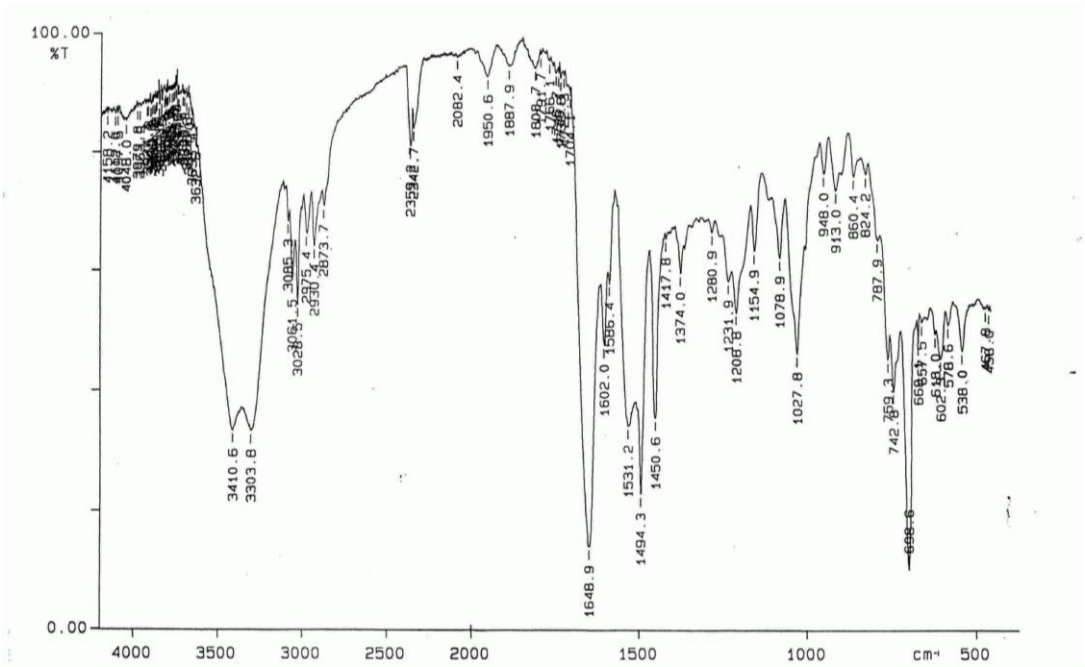
70
60
50
40
30
20


OH
CH3
H
N
O

11e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cm⁻¹
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3303.8
3085.3
3028.5
2975.4
2930.4
2873.7
2082.4
1950.6
1887.9
1648.9
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1417.8
1374.0
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1231.9
1208.8
1154.9
1078.9
1027.8
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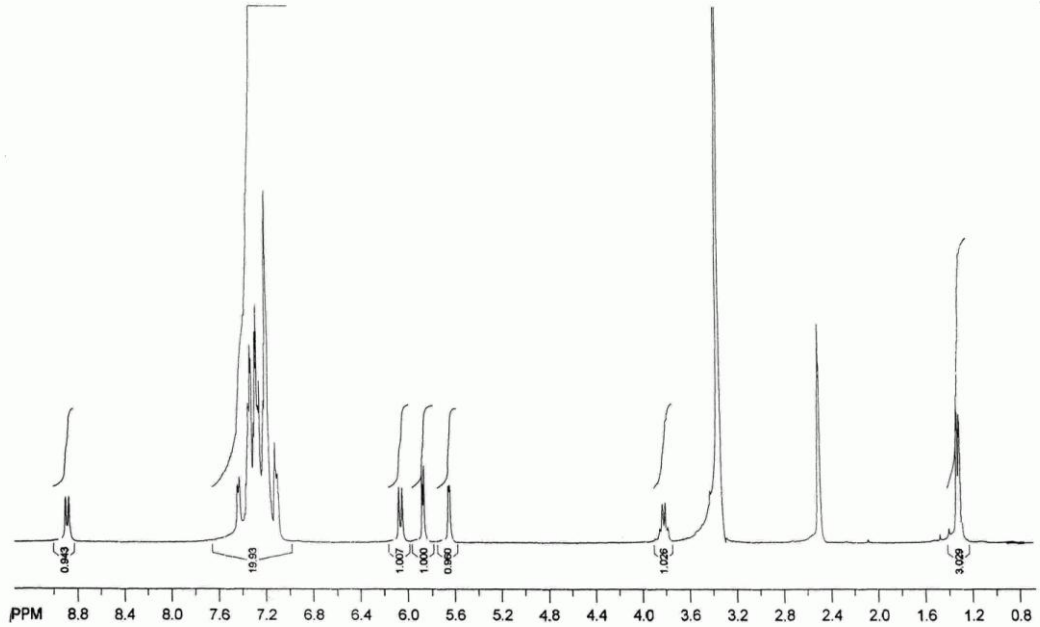
602.5
578.6
538.0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7.2
6.8
6.4
6.0
5.6
5.2
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4.4
4.0
3.6
3.2
2.8
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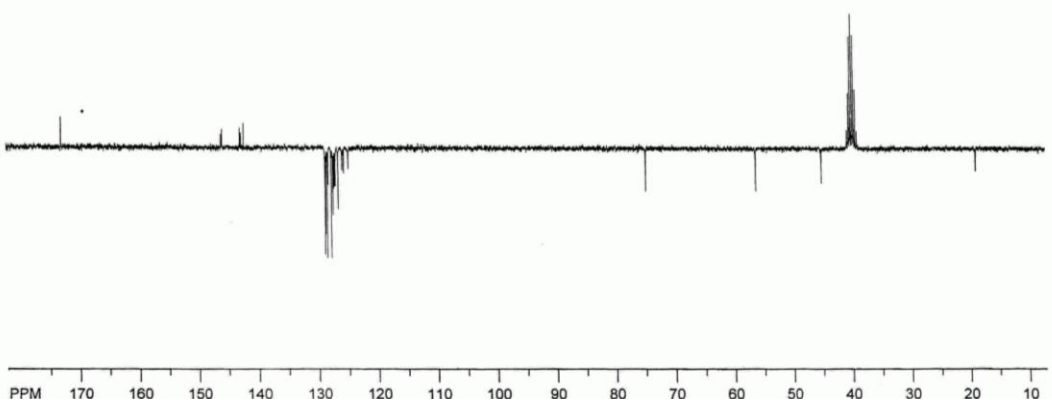
3.029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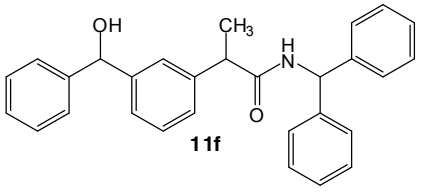
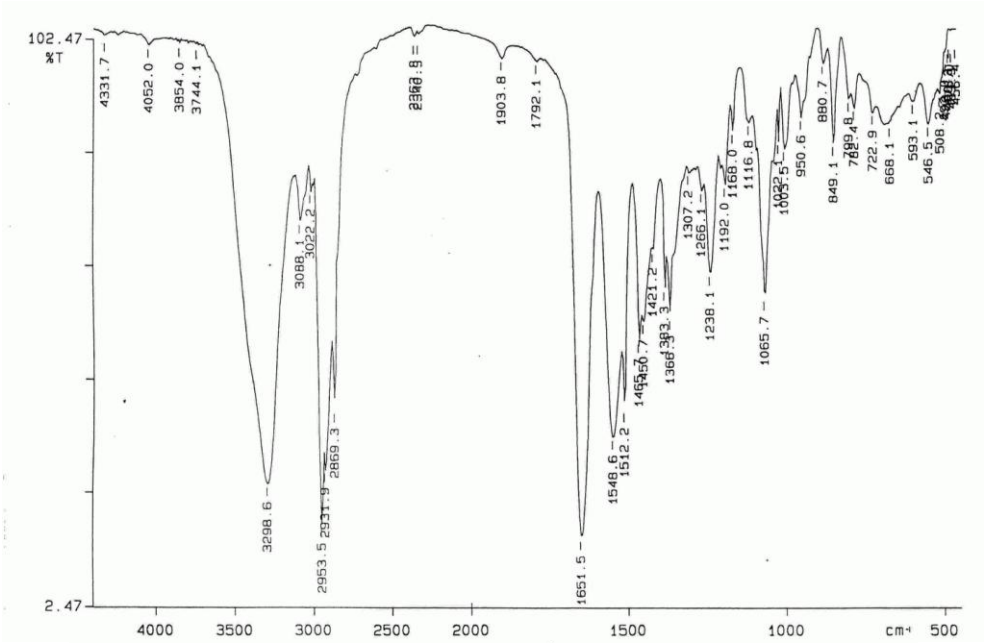

OH
CH3
H
N
O

11f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2.47
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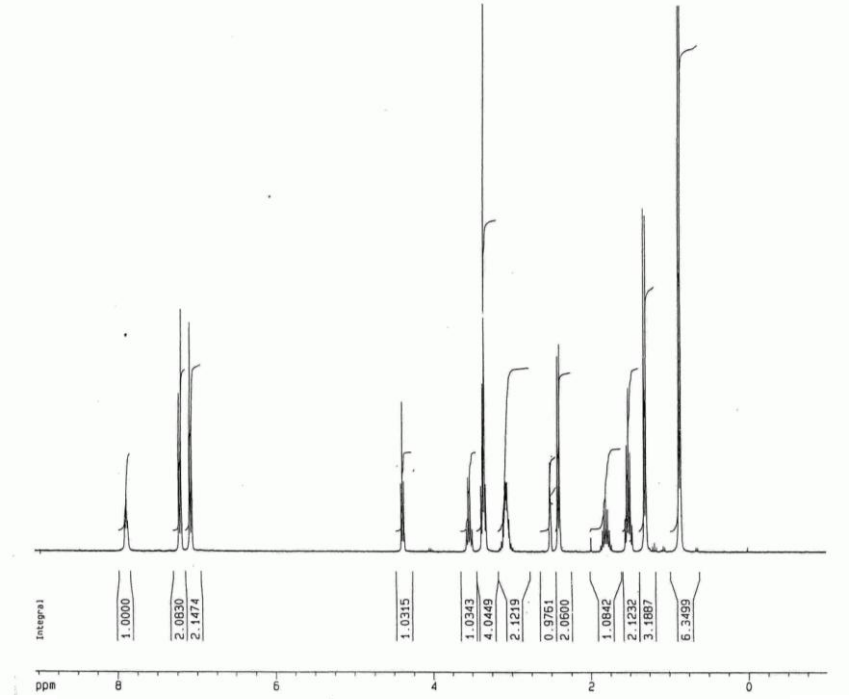
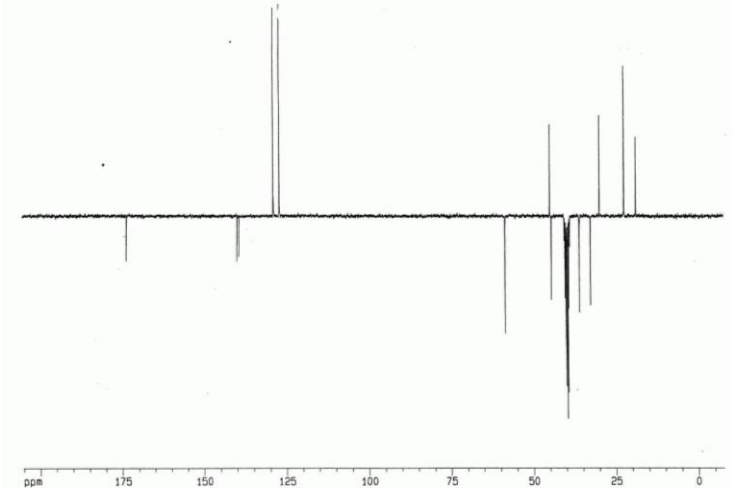
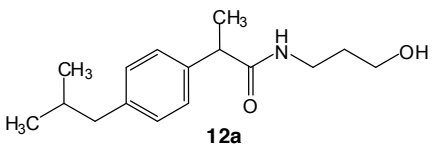
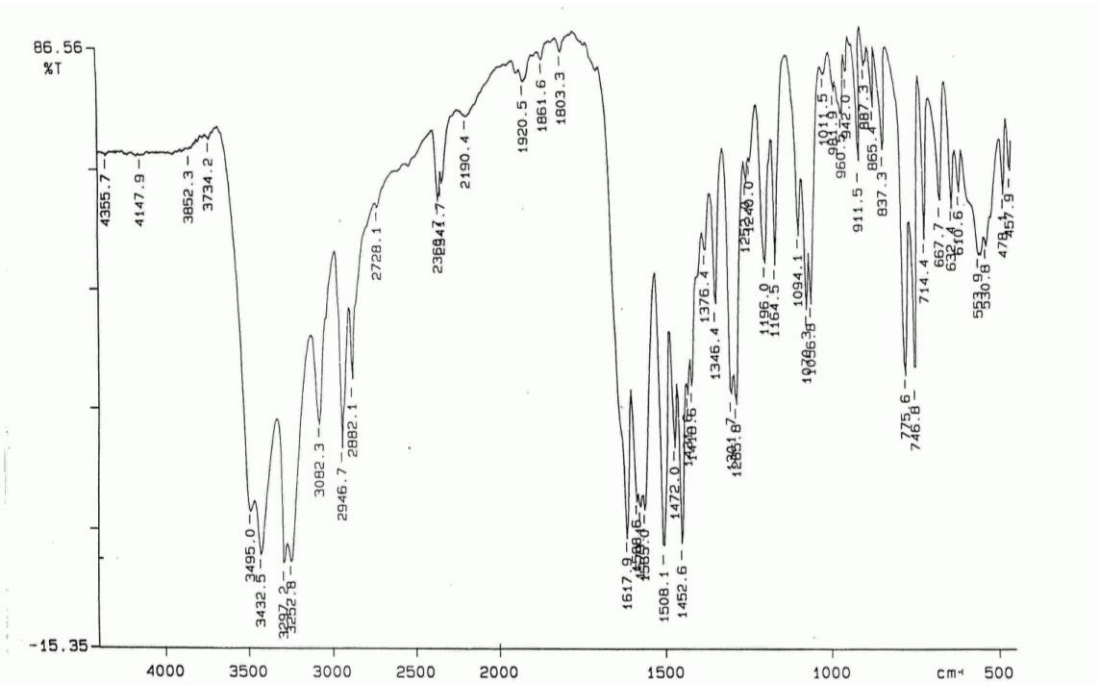
1000
cm-1
500


Integral
ppm


ppm



12a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cm-1
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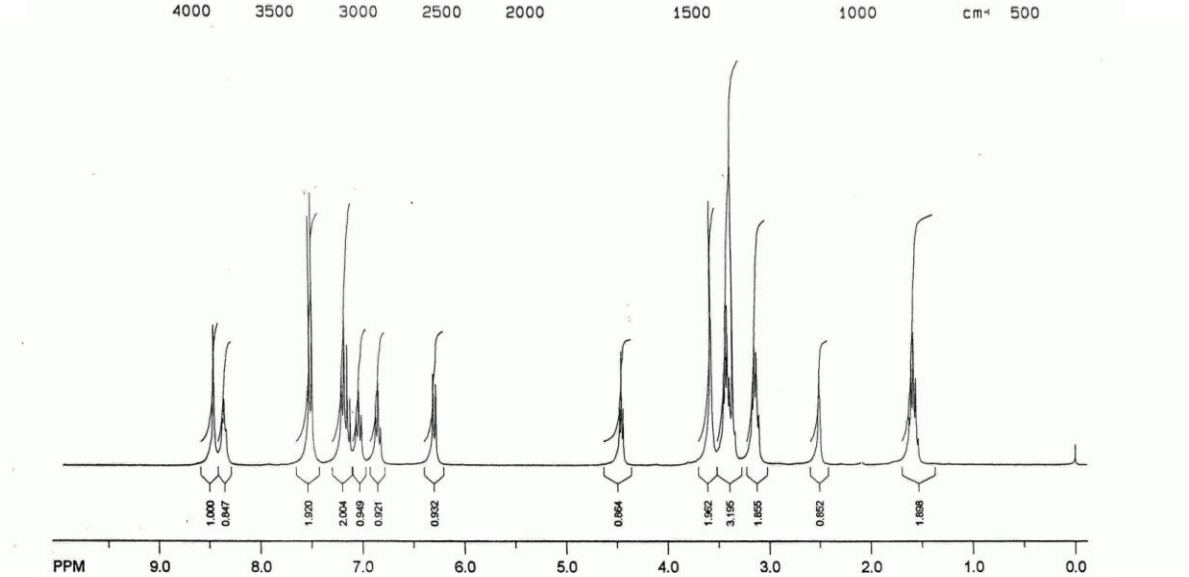
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6.0
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4.0
3.0
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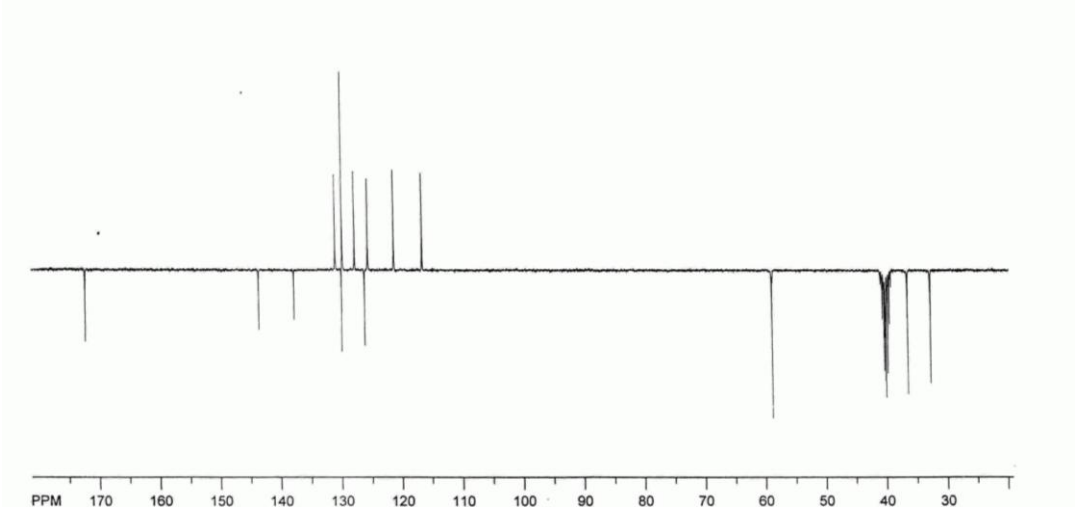
0.0


PPM
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50
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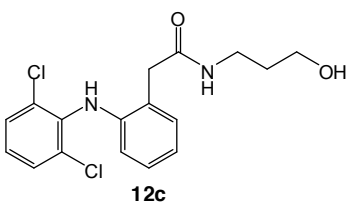
O
N
H
OH
H
N
Cl
Cl

12c

TEMELJNA DOKUMENTACIJSKA KARTICA

Sveučilište u Zagrebu
Farmaceutsko-biokemijski fakultet **Doktorski rad**
A. Kovačića 1, 10000 Zagreb, Hrvatska

DERIVATI NESTEROIDNIH PROTUUPALNIH LIJEKOVA I AMINOKISELINA – SINTEZA, KARAKTERIZACIJA I BIOLOŠKO DJELOVANJE

Zrinka Rajić

SAŽETAK

Sintetizirana je serija novih strukturnih analoga 3,5-disupstituiranih hidantoina **5a-h** s lipofilnim supstituentima na C-5, N-3 ili na oba atoma intramolekulskom ciklizacijom amida *N*-(1-benzotriazolkarbonil)aminokiselina **4a-h**. Sintetizirani su derivati NSAID **6**–**12**: reducirani derivati ketoprofena **6a**,**b**, NSAID benzotriazolidi **7a-h**, NSAID hidroksamske kiseline i njihovi derivati **8a-y**, derivati ketoprofena **9a-i**, **10a-f**, **11a-f** i 3-hidroksipropilamidi NSAID **12a-e**. Reducirani derivati ketoprofena **6** pripravljeni su katalitičkim hidrogeniranjem ketoprofena uz Pd/C ili Pd/C(en). NSAID s karboksilnom funkcionalnom (ibuprofen, fenoprofen, diklofenak, indometacin, ketoprofen, **6a**,**b**) u reakciji s kloridom 1-benzotriazol-karboksilne kiseline (**1**) dali su odgovarajuće NSAID benzotriazolide **7**, koji su bili polazni spojevi u sintezi derivata NSAID **8**–**12**. Razvijena je nova sintetska metoda za pripravu NSAID hidroksamskih kiselina i derivata **8**, reakcijom NSAID benzotriazolida **7** i odgovarajućih hidroksilamina (hidroksilamin, *N*-metilhidroksilamin, *O*-metilhidroksilamin, *O*-etilhidroksilamin i *O*-benzilhidroksilamin) ili katalitičkim hidrogeniranjem *O*-benzilnih derivata NSAID hidroksamskih kiselina. Derivati ketoprofena **9**, **10** i 3-hidroksipropilamidi NSAID **12** sintetizirani su iz NSAID benzotriazolida **7** i odgovarajućih amina, dok su derivati ketoprofena **11** sintetizirani katalitičkim hidrogeniranjem amida ketoprofena **10** uz Pd/C(en).

Provedena su sljedeća biološka ispitivanja: antitumorsko, antimikrobno, antivirusno i antioksidacijsko djelovanje, inhibicija ureaze, lipooksigenaze i lipidne peroksidacije. Najbolje antitumorsko djelovanje pokazao je **8c**, najbolje antimikrobno **8b**,**d**, najbolje antivirusno **5a** i **8y**, a najbolje antioksidacijsko djelovanje **8o**. Najjači inhibitor ureaze bio je **8f**, a lipooksigenaze **11f**. Najbolji inhibitor lipidne peroksidacije bio je **9e**.

Rad je pohranjen u Središnjoj knjižnici Farmaceutsko-biokemijskog fakulteta Sveučilišta u Zagrebu.

Rad sadrži: 214 stranica, 6 slika, 44 sheme, 40 tablica i 125 literaturnih navoda. Izvornik je na hrvatskom jeziku.

Ključne riječi: aminokiselina, hidantoin, nesteroidni protuupalni lijekovi, hidroksamska kiselina, amid, sinteza, biološko djelovanje

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Rad prihvaćen: 10.12.2008.

BASIC DOCUMENTATION CARD

University of Zagreb
Faculty of Pharmacy and Biochemistry **Ph.D. Thesis**
A. Kovačića 1, 10000 Zagreb, Croatia

DERIVATIVES OF NONSTEROIDAL ANTI-INFLAMMATORY DRUGS AND AMINO ACIDS – SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY

Zrinka Rajić

SUMMARY

3,5-Disubstituted hydantoin derivatives **5a-h** with lipophilic substituents at C-5, N-3 or both atoms were prepared by the intramolecular cyclization of the corresponding *N*-(1-benzotriazolecarbonyl)amino acid amides **4a-h**. The following NSAID derivatives **6–12** were prepared: the reduced ketoprofen derivatives **6a**,**b**, NSAID benzotriazolides **7a-h**, NSAID hydroxamic acids and their derivatives **8a-y**, ketoprofen derivatives **9a-i**, **10a-f**, **11a-f** and NSAID 3-hydroxypropylamides **12a-e**. The reduced ketoprofen derivatives **6** were prepared by the catalytic hydrogenation of ketoprofen with Pd/C or Pd/C(en) catalyst. NSAIDs bearing carboxylic group (ibuprofen, fenoprofen, diclofenac, indomethacin, ketoprofen, **6a**,**b**) reacted with 1-benzotriazole carboxylic acid chloride (**1**) affording benzotriazolides **7**, which were the starting compounds in the synthesis of NSAID derivatives **8–12**. A new synthetic method for the synthesis of NSAID hydroxamic acids and their derivatives was developed. They were obtained in the reaction of NSAID benzotriazolides **7** with an appropriate hydroxylamine (hydroxylamine, *N*-methylhydroxylamine, *O*-methylhydroxylamine, *O*-ethylhydroxylamine or *O*-benzylhydroxylamine) or by the catalytic hydrogenation of *O*-benzyl derivatives of NSAID hydroxamic acids. Ketoprofen derivatives **9**, **10** and NSAID 3-hydroxypropylamides **12** were prepared from NSAID benzotriazolides **7**, in the reaction with an appropriate amine, while compounds of the series **11** were obtained by the catalytic hydrogenation of ketoprofen amides **10** with Pd/C(en).

The compounds were evaluated for their biological activity: antitumor, antimicrobial, antiviral and antioxidant activity, inhibition of urease, lipoxygenase and linoleic acid lipid peroxidation. The best antitumor activity was exerted by **8c**, antimicrobial by **8b**,**d**, antiviral by **5a** and **8y**, and antioxidant by **8o**. The strongest inhibitor of urease was **8f**, while **11f** was the strongest inhibitor of lipoxygenase. Compoung **9e** showed the highest inhibition of linoleic acid lipid peroxidation.

Thesis is deposited in the Central library of the Faculty of Pharmacy and Biochemistry, University of Zagreb.

Thesis includes: 214 pages, 6 figures, 44 schemes, 40 tables and 125 references. Original is in Croatian language.

Keywords: amino acid, hydantoin, nonsteroidal anti-inflammatory drugs, hydroxamic acid, amide, synthesis, biological activity

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The thesis accepted: December 10th, 2008.