

Dizajn i sinteza novih malih molekula kao inhibitora Chikungunya virusa

Pejaković, Tajana Iva

Master's thesis / Diplomski rad

2018

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:163:459130>

Rights / Prava: [In copyright](#)/[Zaštićeno autorskim pravom.](#)

Download date / Datum preuzimanja: **2025-01-10**



Repository / Repozitorij:

[Repository of Faculty of Pharmacy and Biochemistry University of Zagreb](#)



Tajana Iva Pejaković

**Design and synthesis of new small molecules as
Chikungunya virus inhibitors**

DIPLOMA THESIS

Submitted to University of Zagreb Faculty of Pharmacy and Biochemistry

Zagreb, 2018

This thesis has been registered in the course Medicinal Chemistry of University of Zagreb Faculty of Pharmacy and Biochemistry. The experimental work was performed at the Department of Chemistry and Technology of Drug of Sapienza University of Rome Faculty of Pharmacy and Medicine, Italy under the expert guidance of Prof. Roberto Di Santo, PhD and co-supervision of Assist. Prof. Mirza Bojić, PhD.

I would like to thank Prof. Di Santo for giving me an opportunity to work at Sapienza University of Rome and for all his help and expert advice during my stay in Rome and while writing this thesis.

I would also like to thank Valentina Noemi Madia, PhD, and Francesco Saccoliti, PhD, together with all the other colleagues that I have met during my laboratory work, for helping me expand my knowledge with the experiments, with writing this thesis, and for making my stay in Rome an amazing and unforgettable experience.

I would especially like to thank Assist. Prof. Mirza Bojić for his continued support during my mobility, as well as his expert care and advices during the writing of this thesis. Hvala Vam!

My gratitude goes to all the professors, assistants and employees of University of Zagreb Faculty of Pharmacy and Biochemistry, for equipping me with the necessary skills and knowledge that have greatly helped me while working on this thesis.

I am also thankful to all my friends and my colleagues for sharing this journey with me - for all their support and understanding, and our shared memories, travels, volunteer work and organised projects. Without you, I would not be who I am today. Hvala vam svima!

Na kraju, najviše zahvaljujem svojim roditeljima i obitelji, bez čije nesebične podrške ne bih došla ovoliko daleko. Bili ste mi najveći oslonac u svemu tijekom ovih godina i zbog vas je sve ovo bilo ostvarivo - hvala vam što ste mi omogućili da u potpunosti uživam u svom studiranju. Once more, thank you all from the bottom of my heart! / Još jednom, hvala vam svima od srca!

LIST OF ABBREVIATIONS

¹ H NMR	proton nuclear magnetic resonance
AAI	arthritogenic <i>Alphaviruses</i> infection
BHK	baby hamster kidney
CDC	Centers for Disease Control and Prevention
CHIKF	Chikungunya fever
CHIKV	Chikungunya virus
CIR	chronic inflammatory rheumatism
COX	cyclooxygenase
CPE	cytopathogenic effect
CRP	C-reactive protein
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
ECSA	East/Central/South American lineage
ELISA	enzyme-linked immunosorbent assay
<i>IC</i> ₅₀	half maximal inhibitory concentration
IFN	interferon
Ig	immunoglobulin
IL-6	interleukin-6
IMPDH	inosine-5'-monophosphate dehydrogenase
IOL	Indian Ocean Lineage
IR	infrared spectroscopy
IRES	internal ribosome entry site
IRF	interferon regulatory factor
ISG	interferon-stimulated gene
MCP-1	monocyte chemotactic protein-1
mRNA	messenger RNA
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)- <i>2H</i> -tetrazolium
MTX	methotrexate
MVA	modified vaccinia Ankara
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate

NSAID	nonsteroidal anti-inflammatory drugs
nsP	non-structural proteins
OAS3	2',5'-oligoadenylate synthetase 3
ORF	open-reading frame
PBMCs	peripheral blood mononuclear cells
PMS	phenazine methosulfate
RNA	ribonucleic acid
RNAi	RNA interference
RNase	ribonuclease
RT-PCR	reverse-transcriptase polymerase chain reaction
shRNA	small hairpin RNA
siRNA	small interfering RNA
TLC	thin-layer chromatography
TMS	tetramethylsilane
UTR	untranslated region
UV	ultraviolet light
Vero cells	African green monkey kidney cells
VLP	virus-like particles
WHO	World Health Organisation

CONTENTS

1. INTRODUCTION.....	1
1.1 CHIKUNGUNYA VIRUS.....	2
1.1.1 VIROLOGY.....	2
1.1.2 EPIDEMIOLOGY	2
1.1.3 TRANSMISSION	3
1.1.4 MECHANISMS OF INFECTION.....	5
1.1.4.1 VIRAL REPLICATION	5
1.1.4.2 PATHOGENESIS.....	6
1.1.5 CLINICAL PRESENTATION	7
1.1.5.1 ACUTE INFECTION.....	7
1.1.5.2 ACUTE SEVERE INFECTION.....	8
1.1.5.3 CHRONIC INFECTION	8
1.2 DIAGNOSIS OF CHIKUNGUNYA FEVER.....	9
1.3 CURRENT THERAPY OF CHIKUNGUNYA VIRUS	10
1.4 EVALUATION OF ANTIVIRAL COMPOUNDS.....	11
1.5 STATE OF THE ART	12
1.5.1 ANTIVIRAL COMPOUNDS	12
1.5.2 VACCINES	15
2. RESEARCH OBJECTIVES	16
3. MATERIALS AND METHODS	19
3.1. Synthesis of 1-(4-chloro-3-nitrophenyl)ethan-1-one (4).....	21
3.2. Synthesis of 1-(2-nitro-[1,1'-biphenyl]-4-yl)ethan-1-one (3)	21
3.3. Synthesis of 1-(9H-carbazol-2-yl)ethan-1-one (2).....	22
3.4. Synthesis of 3-(3-bromo-4-methoxyphenyl)-1-(9H-carbazol-2-yl)prop-2-en-1-one (1a).....	22

3.5. Synthesis of 1-(9 <i>H</i> -carbazol-2-yl)-3-phenylprop-2-en-1-one (1b)	23
4. RESULTS AND DISCUSSION	24
5. CONCLUSION	31
6. REFERENCES.....	33
7. SUMMARY	47
8. STRUKTURIRANI SAŽETAK	49
8.1. UVOD	50
8.2. OBRAZLOŽENJE TEME.....	50
8.3. MATERIJALI I METODE	51
8.4. REZULTATI, RASPRAVA I ZAKLJUČAK.....	52
9. APPENDIX.....	53
10. BASIC DOCUMENTATION CARD/TEMELJNA DOKUMENTACIJSKA KARTICA	

1. INTRODUCTION

1.1 CHIKUNGUNYA VIRUS

1.1.1 VIROLOGY

Chikungunya virus (CHIKV) is an arthritogenic *Alphavirus* and a member of the *Togaviridae* family, typically maintained in natural cycles involving transmission by an arthropod vector. CHIKV and other *Alphaviruses*, Ross River virus, Barmah Forest virus, Mayaro virus, O'nyong-nyong virus, Semliki Forest virus and Sindbis virus, are maintained under diverse ecological conditions and can have a widespread distribution and cause similar disease symptoms (Powers et al., 2001).

CHIKV is an enveloped virus that is approximately 70 nm in diameter in neutral pH and contains a 11.8 kb single-stranded, positive-sense RNA genome. The genome consists of a 5' methylated terminal cap untranslated region (UTR), followed by RNA coding for 4 non-structural proteins (nsP 1-4) and 5 structural proteins (C-E3-E2-6K-E1), and a 3' terminal poly-A tail (Strauss and Strauss, 1994). The non-structural proteins and structural proteins, governed by 2 separate open-reading frames (ORFs), contribute to the propagation of new virions (www.ncbi.nlm.nih.gov; Lum and Ng, 2015).

1.1.2 EPIDEMIOLOGY

The name „chikungunya“ derives from a word in the Kimakonde language, meaning „to become contorted“, and describes the stooped appearance of sufferers with joint pain or arthralgia (www.who.int).

The first epidemic was recognized in July 1952 in present day Tanzania. Chikungunya fever (CHIKF) was described as a „very sharp onset of crippling joint pains, severe fever, and eventually the conspicuous rash“. The first direct evidence of CHIKF outside of Africa came from Bangkok, Thailand, in 1958 during an outbreak associated with *Aedes aegypti* transmission. CHIKV was also discovered in Cambodia and India from 1961 to 1963 (Weaver and Forrester, 2015). In 2005, CHIKV spread into islands in the Indian Ocean, namely the Comoros, Madagascar, Mayotte, Mauritius, Réunion Island and the Seychelles, where major outbreaks with high attack rates occurred (Weaver and Forrester, 2015; www.who.int). Between January 2001 and April 2007, Indonesia reported 15207 CHIKV cases. Imported cases of CHIKF were recorded in the Caribbean in 2006, occurring in travellers from the Indian Ocean islands, and 35 cases were reported in the United States, 2 from Réunion and 33 from India (www.who.int). Other cases were reported in Europe (UK, Belgium, Germany,

Czech Republic, Norway, Spain, France), Hong Kong, Canada, Taiwan, Sri Lanka and the USA (Figure 1) (Lo Presti et al., 2014; www.cdc.gov).

As of 26 September 2017, 183 cases have been notified to the Lazio Region of Italy; 109 are confirmed and 74 additional cases are being investigated. Three more confirmed cases have also been notified from other areas with a travel history to Lazio Region. There is a risk for further transmission, due to the area being highly populated by tourists in summer months and *Aedes albopictus* being established throughout the Mediterranean basin (www.who.int).

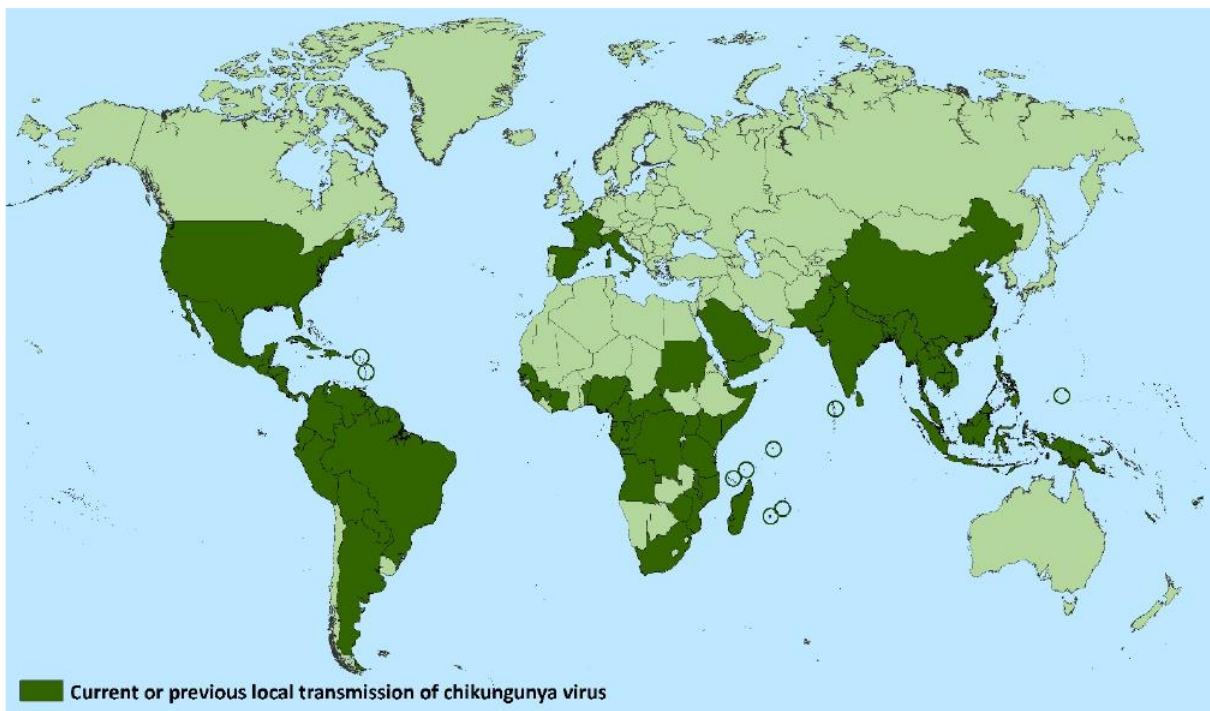


Figure 1. Countries and territories where CHIKV cases have been reported (as of 29 May 2018) (www.cdc.gov)

1.1.3 TRANSMISSION

It is believed that the epidemics of CHIKV are the result from spillover enzootic infections to persons working in forested habitats or living in nearby villages, followed by transport to urban centers populated by the epidemic vectors, *Aedes aegypti* and/or *Aedes albopictus* (Table 1; Figure 2) (Weaver, 2013).

Table 1. Ecological and behavioral characteristics of *Aedes aegypti* and *Aedes albopictus*.

<i>Aedes aegypti</i>	<i>Aedes albopictus</i>
<ul style="list-style-type: none"> • Tropical, subtropical • Feeds almost exclusively on humans and takes multiple blood meals within one gonotrophic cycle • Larvae habitats can be found in artificial water containers near houses • Adult females can be found inside houses • Is moderately susceptible to CHIKV 	<ul style="list-style-type: none"> • Tropical • Feeds opportunistically and takes a single meal within one gonotrophic cycle • Habitats can be found in natural and artificial water containers • Varied levels of anthrophily and endophily • Is moderately to highly susceptible to CHIKV



Figure 2. *Aedes aegypti* (left) and *Aedes albopictus* (right) (www.vectorbase.org)

Longitudinal studies of enzootic CHIKV in eastern Senegal revealed a pattern of infections by enzootic vectors, in 1975, 1979, 1983, and 1992 (Diallo et al., 2012). CHIKV isolates from these and a few other studies in the region were sequenced and shown to comprise an enzootic clade termed the West African lineage. Recently, sequencing and phylogenetic studies placed together representative CHIKV strains from sub-Saharan African locations into one clade termed the East/Central/South American (ECSA) lineage. Moreover, they have demonstrated that CHIKV strains isolated during the Asian outbreaks from 1958 to 1973 comprise a monophyletic group, now termed the Asian epidemic lineage (Volk et al., 2011). During the Réunion epidemic, genomic sequencing of CHIKV isolates revealed a transition in the predominant amino acid from alanine to valine at position 226 of the E1 envelope glycoprotein, and therefore the emergence of new epidemic strain from the ECSA

enzootic lineage, named Indian Ocean Lineage (IOL) (Schuffenecker et al., 2006). Experimental infections supported that this substitution increased the ability of Réunion CHIKV strains to infect *Aedes albopictus*, which was more common for transmission there, with little effect on infection of *Aedes aegypti* (Tsetsarkin et al., 2007; Vazeille et al., 2007; Weaver and Forrester, 2015).

CHIKV epidemics display secular, cyclical and seasonal trends. There is an inter-epidemic period of 4-8 years, but sometimes it can be as long as 20 years. Outbreaks are most likely to occur when the vector density is very high, for example, in the post-monsoon period. During epidemic periods, human beings serve as the CHIKV reservoir, while during inter-epidemic periods, a number of vertebrates (monkeys, rodents, birds, etc.) have been implied as the reservoir (www.who.int).

1.1.4 MECHANISMS OF INFECTION

1.1.4.1 VIRAL REPLICATION

After the CHIKV entry into target cells (Figure 3), acidic endosomal environment causes conformational changes in the virus envelope. The E1 glycoprotein is therefore exposed, which mediates fusion of the CHIKV membrane with the host endosomal membrane, and the nucleocapsid is released into the cytoplasm. The free viral RNA gets translated into a polyprotein that gets cleaved into the nsPs 1-4. These nsPs form a viral replication complex, which generates the full-length negative sense RNA intermediate. This RNA intermediate is used as a template for downstream synthesis of the 49S genomic RNA and the 26S subgenomic mRNA. The 26S subgenomic mRNA is translated to give the structural proteins (C-pE2-6K-E1), which are processed by serine proteases in order to release the capsid protein (C) into the cytoplasm. Remaining proteins are directed to the endoplasmic reticulum, where they undergo post-translational modifications. After the cleavage in the Golgi apparatus, pE2 gives E2 and E3 glycoproteins. E1 and E2 glycoproteins will heterodimerize and get transported to the host cell plasma membrane, where they would be incorporated onto the virion surface in the form of trimeric spikes. Capsid protein associates in the cytoplasm, forming the icosahedral nucleocapsid that contains the 49S genomic RNA. Mature virions are assembled at the plasma membrane, where they will acquire a membrane bilayer from the host cell plasma membrane (Lum and Ng, 2015; Strauss and Strauss, 1994; www.ncbi.nlm.nih.gov).

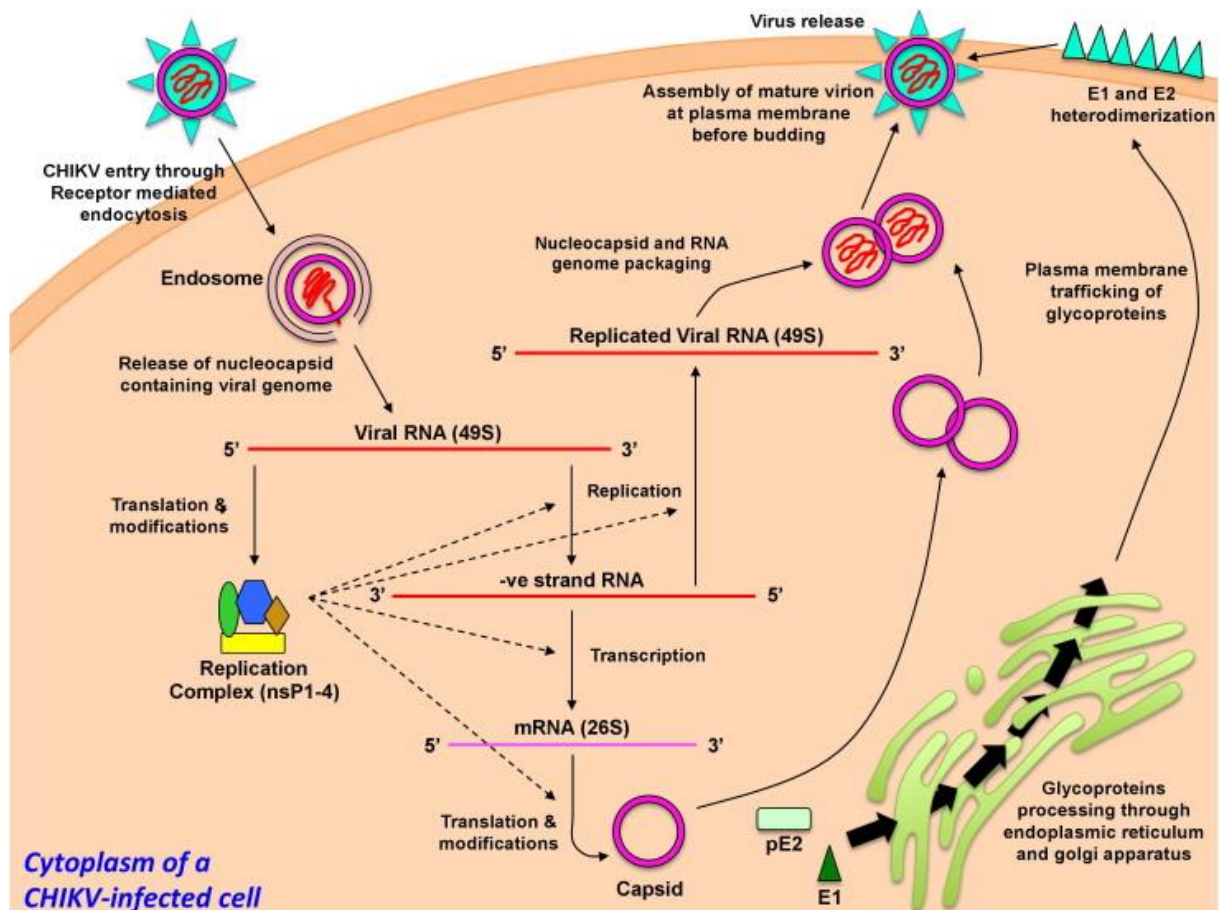


Figure 3. CHIKV replication cycle (Lum and Ng, 2015)

CHIKV infects the host through inoculation at the skin via bites from infected mosquitoes. It can infect a variety of cell lines and adherent cells, such as endothelial, epithelial and fibroblastic primary skin cells (Couderc et al., 2008; Salvador et al., 2009; Sourisseau et al., 2007; Wikan et al., 2012), and human muscle satellite cells (Ozden et al., 2007). Various research reported contrasting results about the infectability of peripheral blood mononuclear cells (PBMCs) (Lum and Ng, 2015; Sourisseau et al., 2007).

1.1.4.2 PATHOGENESIS

CHIKV and other *Alphaviruses* are strong inducers of type-I interferon (IFN) and are sensitive to type-I IFN responses (Couderc and Lecuit, 2015). Particularly IFN α and IFN β have been reported to play crucial anti-viral roles in both CHIKV infected humans and mice (Couderc et al., 2008; Lum and Ng, 2015; Schilte et al., 2012; Sourisseau et al., 2007). Expression of interferon regulatory factors (IRF), most notably IRF-3 or IRF-7, was shown to be inducing an antiviral response in either hematopoietic or nonhematopoietic cell compartments (Schilte et al., 2012). Interferon-stimulated genes (ISGs), activated by Type-I

IFN, have been found to exert an antiviral role against CHIKV *in vitro* and/or *in vivo*. Some of those ISGs include ISG15, ISG20, P56, ZAP, OAS3 and Viperin (Couderc and Lecuit, 2015; Lenschow et al., 2007; MacDonald et al., 2007; Werneke et al., 2011).

Pro-inflammatory cytokines such as interleukin-6 (IL-6), monocyte chemotactic protein-1 (MCP-1) and IFN γ were found to be elevated during the acute phase of the disease. (Hoarau et al., 2010; Kelvin et al., 2011; Lum and Ng, 2015; Wauquier et al., 2011).

Viral replication in joint tissues was shown to lead to the recruitment of monocytes, macrophages, and natural killer cells in studies with non-human primate and mouse models (Labadie et al., 2010). Autophagy has been shown to be an innate mechanism involved in host response to CHIKV infection (Couderc and Lecuit, 2015; Joubert et al., 2012). CHIKV also infects human osteoblasts and causes cytopathic effects, which can contribute to the joint pathology and erosive disease (Burt et al., 2017).

CD8⁺ T cells dominate the early stages of the disease, while CD4⁺ T cells appear at a later time to aid in the production of CHIKV-specific humoral response (Wauquier et al., 2011). B cells and CHIKV-specific antibodies have also been extensively characterized (Lum et al., 2013; Poo et al., 2014). Viral load CHIKV infection induces a robust anti-CHIKV IgG antibody response dominated by the IgG3 isotype (Lum and Ng, 2015). Since neutralizing antibodies persist for at least 21 months, and even for years, a strong antiviral immunity is developed that can prevent clinical symptoms in the event of a second infection with CHIKV (Burt et al., 2017).

1.1.5 CLINICAL PRESENTATION

1.1.5.1 ACUTE INFECTION

The incubation period of CHIKV infection ends with a sudden onset of high fever (>38.5 °C), which coincides with viremia. Blood viral load can rapidly reach up to 10⁹ viral genome copies per milliliter (Couderc and Lecuit, 2015; Parola et al., 2006; Staikowsky et al., 2009). In addition, the most common biological abnormality is leukopenia, particularly lymphopenia (Staikowsky et al., 2009). Joint pain affects the extremities (ankles, wrists, phalanges) and larger joints (shoulders, elbows, knees), and is typically polyarticular, bilateral, and symmetrical (Couderc and Lecuit, 2015; Sissoko et al., 2010; Thiberville et al., 2013). Swelling may also occur, as well as pain along ligament insertions, notably in children (Couderc and Lecuit, 2015). Rash occurs in 10% to 40% of cases for 2-3 days, and is

characterized by transient macular or maculopapular rash that involves mainly the extremities (Couderc and Lecuit, 2015; Economopoulou et al., 2009).

1.1.5.2 ACUTE SEVERE INFECTION

Severe CHIKV disease in otherwise healthy individuals occurs mainly in elderly patients and young children, while adults with severe disease usually display underlying conditions, such as diabetes, alcoholic hepatopathy, stroke, epilepsy, hypertension, or impaired renal function (Economopoulou et al., 2009). Severe disease of CHIKV can manifest as encephalopathy and encephalitis, cardiovascular and respiratory disorders, renal failure, hepatitis and myocarditis (Das et al., 2010; Economopoulou et al., 2009; Staikowsky et al., 2009).

Mother-to-child transmission of CHIKV infection was first reported during the Réunion outbreak, as a cause of severe neonatal disease, associated with neurological acute symptoms (Couderc and Lecuit, 2015; Gérardin et al., 2008; Gupta et al., 2015). Nevertheless, the overall prevalence of maternal-fetal transmission is actually low (0.25% after 22 weeks) (Gérardin et al., 2008), and vertical transmission is observed exclusively in near-term deliveries (Couderc and Lecuit, 2015; Gérardin et al., 2008).

1.1.5.3 CHRONIC INFECTION

CHIKV chronic disease can affect a large proportion of patients who can exhibit persistent or relapsing arthralgia in the following months or years, which may mimic rheumatoid arthritis (Couderc and Lecuit, 2015; Javelle et al., 2015; Messaoudi et al., 2013; Schilte et al., 2013; Sissoko et al., 2009; Teo et al., 2015).

In a 6-year retrospective study on a cohort of patients in Réunion, two main categories of post-CHIKV persisting rheumatic and musculoskeletal disorders were distinguished: patients without previously defined arthritis, but with current musculoskeletal disorders (loco-regional or diffuse), who represent 27% of patients, and patients with non-crystalline polyarthritis who represent 70% of patients fulfilling the diagnostic criteria of rheumatoid arthritis, spondyloarthritis or undifferentiated polyarthritis. The latter were referred to as patients with chronic inflammatory rheumatism (CIR). Among them, a minority (16%) had pre-existing CIR that immediately exacerbated after CHIKV infection, while all the other developed CIR after CHIKV infection (Javelle et al., 2015).

1.2 DIAGNOSIS OF CHIKUNGUNYA FEVER

Usually the diagnosis of CHIKF is clinical, because the association of acute fever and arthralgia is highly predictive in areas where the disease is endemic, but laboratory confirmation is crucial in distinguishing CHIKV from various disorders that have similar clinical manifestations, such as dengue fever and other *Alphaviruses* (Table 2) (Burt et al., 2012; Staikowsky et al., 2009; Weaver and Lecuit, 2015).

The main laboratory finding is lymphopenia, which, when the lymphocyte count is less than 1000 per cubic millimeter, is closely associated with viremia. Other laboratory abnormalities include thrombocytopenia, increased levels of aspartate aminotransferase and alanine aminotransferase in blood, and hypocalcemia (Weaver and Lecuit, 2015).

The acute phase of CHIKV illness can be confirmed with detection of viral nucleic acid in serum samples by reverse-transcriptase polymerase chain reaction (RT-PCR), isolation of the virus, or detection of an antibody response (Burt et al., 2012). Virus isolation has been successful largely in antibody-negative samples obtained on or before day 2 of illness (Panning et al., 2008). RT-PCR can detect viral nucleic acid in samples obtained from 1 day before onset of symptoms up to and including day 7 (Leo et al., 2009; Panning et al., 2008). Antigen capture enzyme-linked immunosorbent assay (ELISA) has been described for detection of antigen in serum samples and cerebrospinal fluid obtained as early as day 2 after onset, while indirect immunofluorescence and ELISA can distinguish between IgG and IgM antibodies (Burt et al., 2012; Litzba et al., 2008; Panning et al., 2008; Shukla et al., 2009; Yap et al., 2010).

Elevated levels of C-reactive protein (CRP) and proinflammatory cytokines correlate with disease activity, but there is no specific assay for assessing chronic signs and symptoms of CHIKF (Schilte et al., 2013). Although persistence of specific IgM antibodies within 24 months after infection has been reported, persistence of high antibody titers may indicate delayed antigen clearance rather than viral persistence (Burt et al., 2012; Malvy et al., 2009; Panning et al., 2008; Weaver and Lecuit, 2015).

Serological tests can be used to distinguish CHIKV infections from *Flavivirus* infections and other *Alphaviruses*, such as Mayaro and Ross River viruses, with the exception of O'nyong-nyong virus (Burt et al., 2012).

Table 2. CHIKF is diagnosed on the basis of clinical, epidemiological, and laboratory criteria.

Criteria	Description	Diagnosis of CHIKF
Clinical criteria	Acute onset of fever >38.5 °C and severe arthralgia or arthritis	Possible case when not explained by other medical condition (dengue or alphaviral infection, arthritic disease, endemic malaria)
Epidemiological criteria	Residing in or visited epidemic area within 15 days before onset of symptoms	Probable case if clinical and epidemiological criteria are met
Laboratory criteria	Virus isolation, presence of viral RNA, specific IgM antibodies, four-fold increase in IgG titres in paired samples	Confirmed case if a patient tests positive for one of the laboratory criteria, irrespective of clinical manifestations

1.3 CURRENT THERAPY OF CHIKUNGUNYA VIRUS

Current therapy of CHIKV consists of the use of analgesics, antipyretics and anti-inflammatory agents (Abdelnabi et al., 2015).

During the 2005-2006 Réunion outbreak, a number of patients who experienced chronic rheumatic symptoms were successfully treated with methotrexate (MTX) (Parashar and Cherian, 2014). Despite this, other findings of MTX effectiveness seem to be contradictive, thus its effect on acute viral induced arthritis remains unclear (Taylor et al., 2013). The widespread use of these nonsteroidal anti-inflammatory drugs (NSAIDs) has resulted in serious gastrointestinal complications, thus prompting the combined therapy with gastroprotective agents. Selective COX-II inhibitors (rofecoxib, celecoxib, parecoxib) given alone have shown comparable efficacy with a significantly reduced propensity to cause gastrointestinal toxicity. Therefore, they are being considered as a first-line therapy in patients requiring long-term pain control (Lazzaroni and Bianchi Porro, 2004).

Paracetamol was the most used analgesic in Réunion patients (in 95.4% of treatments), but this also led to the emergence of severe liver afflictions diagnosed during the epidemic, particularly when doses >3 g/day were taken (Staikowsky et al., 2008). Morphine was

reported to be effective during early treatments of CHIKV (Parashar and Cherian, 2014), and acetaminophen, another analgesic, was shown to modulate the transcriptional response to recombinant IFN β (Farnsworth et al., 2010).

Some plant species, such as *Fernelia spp.*, are also known for their antiviral properties, and were mainly used during the outbreaks in Réunion to treat fever, pain, and inflammation. However, the efficacy of these plants has yet to be studied (Staikowsky et al., 2008).

Additionally, corticosteroids were prescribed to treat arthralgia (27.7% of cases) during the CHIKV epidemic in the Réunion (Staikowsky et al., 2008). A study carried out in South India during the CHIKV epidemic in 2007 recommended coadministration of low-dose systemic corticosteroids with NSAIDs as the best regimen in treating acute CHIKV cases with arthralgia (Padmakumar et al., 2009). However, because of the risk of immunosuppression causing disease exacerbation, corticosteroid use during acute viral arthritis is considered to be contraindicated (Parashar and Cherian, 2014).

1.4 EVALUATION OF ANTIVIRAL COMPOUNDS

After the worldwide re-emergence of CHIKV (Weaver and Forrester, 2015), it became apparent that the development of potent antiviral drugs is urgently needed (Abdelnabi et al., 2015).

Bio-safe surrogates for CHIKV, such as BHK replicon cell lines containing a persistently replicating CHIKV replicon and a Semliki Forest virus strain with *Renilla luciferase* (Pohjala et al., 2011), can be used to identify antiviral molecules. The downside with using a replicon model in which only viral replication occurs is that there is a risk of not identifying molecules that inhibit other steps of the viral life cycle. Using infectious viruses that have a complete life cycle is the best option when identifying molecules with anti-CHIKV activity and inhibitors of CHIKV replication (Abdelnabi et al., 2015), such as CHIKV-0708 strain (Kaur and Chu, 2013; Lam et al., 2012), DRDE-06 strain (Khan et al., 2011) and DMERI09/08 strain (Rathore et al., 2014).

Cell viability or cytopathogenic effect (CPE) reduction assays are usually used for the initial identification of molecules with activity against CHIKV (Abdelnabi et al., 2015; Bassetto et al., 2013; Bourjot et al., 2014; Cruz et al., 2013; Jadav et al., 2015). Their advantage is the possibility of discovering or identifying new antiviral targets and the evaluation of the cytotoxic effect of putative antiviral molecules. The most commonly used

cells in these assays are the African green monkey kidney (Vero) cells (Abdelnabi et al., 2015).

To assess the antiviral efficacy of small molecules and monoclonal antibodies against CHIKV-induced death, lethal infection models using adult immunodeficient mice can be used (Abdelnabi et al., 2015). However, immunocompetent mice such as C57BL/6 (Goha et al., 2013; Lam et al., 2012; Parashar et al., 2013; Selvarajah et al., 2013) and Swiss albino mice (Parashar et al., 2013) can be used to assess the efficacy of drug therapy against CHIKV-induced arthritis and inflammation (Abdelnabi et al., 2015).

In addition, discovery of antiviral compounds is encouraged by the availability of the crystal structures of several proteins of the CHIKV RNA genome and other related alphaviruses, with the aim to use target structure-based pharmacophore modeling, virtual library screening, and drug docking approaches (Bissantz et al., 2000). Identified structural models are further used to optimize the compound's activity (Parashar and Cherian, 2014).

1.5 STATE OF THE ART

1.5.1 ANTIVIRAL COMPOUNDS

Some antivirals that have been effective against CHIKV in humans, and are therefore promising, include broad-spectrum antivirals like chloroquine and ribavirin (in combination with interferon). Additionally, research has shown that cellular factors could be used in the future as a basis for immune-based control strategies (Table 3) (Parashar and Cherian, 2014).

Inhibition of viral replication by RNA interference (RNAi) has emerged as a promising antiviral strategy. Stopping of the viral replication can be achieved by using the post-transcriptional process RNAi, where the shutdown of the protein expression is triggered by targeting specific viral proteins. The small interfering RNA (siRNA) and small hairpin RNA (shRNA) molecules are central to RNA interference (Parashar and Cherian, 2014). Moreover, the *in vitro* use of siRNA or plasmid based shRNA has shown promising results in the cell based infection model of CHIKV (Lam et al., 2012; Parashar and Cherian, 2014; Parashar et al., 2013).

Nevertheless, further clinical testing and studies in *in vivo* systems are essential for establishing the effectiveness of the potential compounds (Parashar and Cherian, 2014).

Table 3. Specific chemical compounds and cellular factors tested against CHIKV.

Products	Hypothesized target	Pros	Cons	References
Chloroquine	Disrupted endosome-mediated CHIKV internalization	Blocks the production of proinflammatory cytokines and the proliferation of monocytes, macrophages, and lymphocytes	<i>In vivo</i> study required	Parashar and Cherian, 2014
Ribavirine	Can interact with the intracellular viral RNA production	Faster resolution of joint and soft tissue manifestations	Involvement of a small number of patients and lack of planning	Ravichandran and Manian, 2008
6-Azauridine	Inhibition of orotidine monophosphate decarboxylase	Showed a significant inhibition of CHIKV at a low concentration	The antiviral activity has been difficult to replicate <i>in vivo</i>	Briolant et al., 2004
Arbidol	Inhibition of virus mediated fusion and blocking of the viral entry into the target cells	Well-tolerated with minimal side effects	Not tested in <i>in vivo</i> system	Parashar and Cherian, 2014

Harringtonine	Affects CHIKV RNA production inside the infected cell and viral protein expression	Minimal cytotoxicity	Not tested in <i>in vivo</i> system	Kaur et al., 2013
Furin inhibitors	Intracellular furin-mediated cleavage of viral envelope glycoproteins	Able to induce a stronger inhibition of viral infection	Not tested in <i>in vivo</i> system	Ozden et al., 2008
2',5'-Oligoadenylate synthetase 3 (OAS3)	Affects CHIKV replication through RNase L-dependent pathway	Ability of OAS3 to inhibit alphavirus growth	Cannot rule out the possibility that OAS3-mediated inhibition of CHIKV is also due to a block early in virus life cycle	Bréhin et al., 2009
Cellular IMPDH enzyme	Depletion of intracellular guanosine pool	Potential and effective target to prevent CHIKV infection	It would be useful to explore similar findings by targeting IMPDH in case of other <i>Alphaviruses</i>	Khan et al., 2011
Viperine	Endoplasmic reticulum	Provides a preclinical basis for the design of effective control strategies against CHIKV	Large gaps in our understanding of the precise mechanisms at play for viperin	Teng et al., 2012

1.5.2 VACCINES

There is still no effective vaccine to prevent CHIKV, and some of the problems related to their development is further *in vivo* evaluation, the need for clinical trials, cost and scalability issues (Parashar and Cherian, 2014).

Over the years, various number of preclinical CHIKV vaccines have been described. Some of them include live-attenuated virus vaccines (Parashar and Cherian, 2014; Plante et al., 2011), inactivated virus formulations (Gardner et al., 2010; Harrison et al, 1971; Tiwari et al, 2009), chimeric virus vaccines (Wang et al., 2008), DNA vaccines (Mallilankaraman et al., 2011; Muthumani et al., 2008), T cell based peptide vaccine (www.link.springer.com), a recombinant adenovirus vaccine (Wang et al., 2011), subunit protein vaccines (Kumar et al., 2012; Parashar and Cherian, 2014; www.link.springer.com), and a virus-like particle (VLP) formulation (Akahata et al., 2010; Parashar and Cherian, 2014).

Recently, a live CHIKV vaccine (CHIKV/IRES), that is highly attenuated yet immunogenic in mouse models, was shown to be incapable of replicating in mosquito cells (Chu et al., 2013). The mechanism is based on the insertion of a picornavirus internal ribosome entry site (IRES) sequence into the genome of CHIKV, and these CHIKV/IRES vaccine candidates appear to be safe and efficacious (Roy et al., 2014).

A novel CHIKV vaccine candidate was generated, termed MVA-CHIKV, based on the highly attenuated poxvirus vector, modified vaccinia Ankara (MVA), expressing the CHIKV structural genes C, E3, E2, 6K, and E1 (García-Arriaza et al., 2014). The immunogenicity profile and the efficacy of a MVA-CHIKV was generated and it was found to be an effective vaccine against CHIKV infection. It induced strong, broad, highly polyfunctional and longlasting CHIKV-specific CD8⁺ T cell responses, together with neutralizing antibodies against CHIKV (Parashar and Cherian, 2014).

2. RESEARCH OBJECTIVES

The estimation that 1.4-6.5 million people were infected with CHIKV in India during the 2005-2007 epidemic, and the emergence of CHIKV in Europe and Americas clearly shows that arthritogenic *Alphaviruses* infections (AAIs) epidemics pose a serious public health issue worldwide (Lo Presti et al., 2014; www.digital.library.adelaide.edu.au). Therefore, there is a need for the development of vaccine and antiviral therapy of CHIKV.

To identify structures as potential CHKV inhibitors, MTS assay using Vero cell lines was performed at Rega Institute for Medical Research, KU Leuven, Belgium. MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium) assay is based on the reduction of the MTS tetrazolium compound in the presence of phenazine methosulfate (PMS) by viable cells to generate a colored formazan dye that is soluble in cell culture media. This conversion is thought to be carried out by NAD(P)H-dependent dehydrogenase enzymes in metabolically active cells. The formazan dye is quantified by measuring the absorbance at 490 nm (Riss et al., 2013).

The structure with the most suitable properties showed to be 3-(3-bromo-4-hydroxyphenyl)-1-(9*H*-carbazol-2-yl)prop-2-en-1-one (**5**) (Figure 4), with the half maximal inhibitory concentration (IC_{50}) of 4.3 $\mu\text{g/ml}$ (Figure 5).

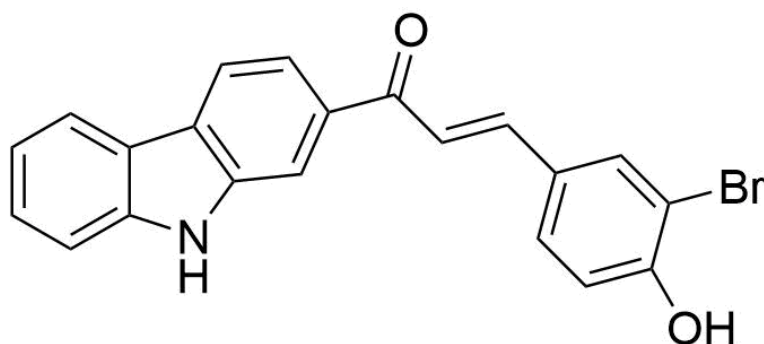


Figure 4. Structure of compound **5**

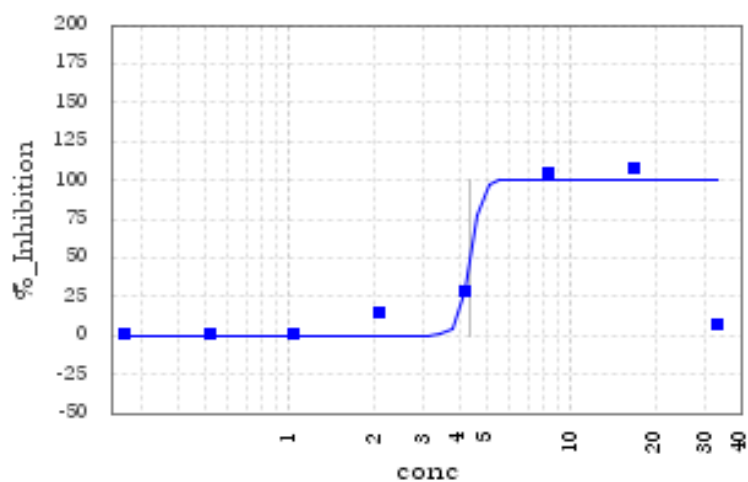


Figure 5. Graph of performed MTS assay of compound **5**

To better understand the relationship between the structure of the molecule and its activity, the goal of this thesis was to synthesize and characterize two new derivatives of the structure **5**. In the first derivative, 3-(3-bromo-4-methoxyphenyl)-1-(9*H*-carbazol-2-yl)prop-2-en-1-one (**1a**), with the formation of the methoxy group, the aim was to observe the importance of the hydroxy group in the activity of the structure, since it is prone to undergo various metabolic reactions. In the second one, 1-(9*H*-carbazol-2-yl)-3-phenylprop-2-en-1-one (**1b**), a molecule without functional groups was synthesized to assess if their presence plays a role in metabolic reactions as well.

In this thesis the synthesis of the derivatives **1a** and **1b** is described, and their analytical and spectroscopic data are shown. In further research, which is beyond the scope of this thesis, the inhibition of CHIKV by these molecules will be tested.

3. MATERIALS AND METHODS

Melting points were determined on a Bobby Stuart Scientific SMP1 melting point apparatus and are uncorrected.

Infrared (IR) spectra were recorded on a PerkinElmer Spectrum-One spectrophotometer. ¹H NMR spectra were recorded at 400 MHz on a Bruker AC 400 Ultrashield 10 spectrophotometer (400 MHz). Dimethyl sulfoxide-d₆ 99.9% (code 44,139-2) of isotopic purity (Aldrich) was used. Chemical shifts (δ) in ppm were measured in reference to tetramethylsilane (TMS).

Column chromatographies were performed on silica gel (Merck; 70-230 mesh) column and chloroform/*n*-hexane (7:3), chloroform/ethanol (99:1), chloroform/acetone (95:5), chloroform/ethyl acetate (85:15), dichloromethane/*n*-hexane/ethanol (98:2:1), dichloromethane/petroleum ether or *n*-hexane (6:4), petroleum ether/ethyl acetate (7:3) and *n*-hexane/ethyl acetate (6:4) were used as mobile phases.

All compounds were routinely checked on TLC by using aluminumbaked silica gel plates (Fluka DC-Alufolien Kieselgel 60 F254) and chloroform/methanol (96:4), chloroform/acetone (95:5) and dichloromethane/petroleum ether (6:4) as mobile phases. Developed plates were visualized by UV light.

Concentration of solutions after reactions and extractions involved the use of rotary evaporator (Büchi) operating at a reduced pressure (~20 Torr).

2 molar solution of K₂CO₃ was prepared with dissolution of K₂CO₃ in distilled water, and was used in following reaction step without additional purification. Other solvents were purchased by Sigma-Aldrich company, they were reagent grade and, when necessary, were purified and dried by standard methods. Organic solutions were dried over anhydrous sodium sulfate (Merck). All reactions were carried out under argon.

3.1. Synthesis of 1-(4-chloro-3-nitrophenyl)ethan-1-one (4)

4'-Chloroacetophenone (0.4 ml; 3.234×10^{-3} mol) was suspended in conc. H_2SO_4 (0.8 ml) at the temperature of $-10\text{ }^\circ\text{C}$. A mixture of HNO_3 (0.6 ml) and conc. H_2SO_4 (1.2 ml) (1:2 v/v) was added dropwise with constant stirring. The temperature was kept below $-10\text{ }^\circ\text{C}$ using ice-based cooling bath. Stirring was continued for further 30 min, during which nitration product separated out. Crushed ice was added to the solid suspension and the obtained solid was recrystallized from ice cold methanol with vacuum filtration to give colourless crystals of compound **4** (0.451 g).

Yield: 70%

m.p. = $99\text{-}101\text{ }^\circ\text{C}$

IR (KBr, $\nu_{\text{max}}/\text{cm}^{-1}$): 1685 (C=O), 1532 (NO_2), 1345 (NO_2)

^1H NMR (DMSO- d_6 , δ/ppm): 2.64 (s, 3H, 1'), 7.94-7.96 (d, 1H, 3, $J_o = 8\text{ Hz}$), 8.19-8.22 (dd, 1H, 2, $J_o = 8\text{ Hz}$), 8.54-8.55 (d, 1H, 1, $J_m = 4\text{ Hz}$)

3.2. Synthesis of 1-(2-nitro-[1,1'-biphenyl]-4-yl)ethan-1-one (3)

Compound **4** (2.505×10^{-3} mol; 0.500 g) and 1,1 equiv. of phenylboronic acid (2.756×10^{-3} mol; 0.336 g) was suspended in toluen (1.4 ml per mmol of halid; 3.5 ml). K_2CO_3 (2.5 ml) was added and sparged with bubbling N_2 for 5 min. At that time, $\text{Pd}(\text{PPh}_3)_4$ (0.289 g) was added and sparging continued for an additional 10 minutes before the flask was closed and the contents heated to reflux temperature ($111\text{ }^\circ\text{C}$) using heated oil-based bath. Complete consumption of the halid starting material took ~ 18 h. Afterwards, the reaction was cooled, filtered, and washed with Et_2O (~ 150 ml). The organic mixture was washed with H_2O (2×50 ml) and brine, dried over Na_2SO_4 , and concentrated in vacuum. Solid compound **3** (0.370 g) was purified with column chromatography (stationary phase: silica gel, mobile phase: dichloromethane/petroleum ether or *n*-hexane 6:4). Afterwards, the mixed fraction of compound **3** was purified with another column chromatography (stationary phase: silica gel, mobile phase: chloroform/*n*-hexane 7:3).

Yield: 62%

m.p. = $95\text{-}97\text{ }^\circ\text{C}$

IR (KBr, $\nu_{\text{max}}/\text{cm}^{-1}$): 1683 (C=O), 1525 (NO_2), 1357 (NO_2)

^1H NMR (DMSO- d_6 , δ/ppm): 2.69 (s, 3H, 1"), 7.38-7.40 (m, 2H, 2', 4'), 7.48-7.51 (m, 3H, 1', 3', 5'), 7.73-7.75 (d, 1H, 3, $J_o = 8\text{ Hz}$), 8.26-8.29 (dd, 1H, 2), 8.46 (d, 1H, 1, $J_m = 4\text{ Hz}$)

3.3. Synthesis of 1-(9*H*-carbazol-2-yl)ethan-1-one (2)

Compound **3** (1.244×10^{-4} mol; 0.300 g) and triphenylphosphine (2.5 equiv.; 3.109×10^{-3} mol; 0.815 g) were added to 1,2-dichlorobenzene (2 ml per mmol of nitro; 2.48 ml). The reaction was run under argon, reflux temperature (178-180 °C), with rigorous stirring, using oil-based heating bath. The reaction was stopped upon complete consumption of the starting material of compound **3**. The reaction was cooled and *n*-hexane was added to the mixture to assist in the formation of the compound **2** as a powdery precipitate. The precipitate was filtered under high vacuum. Solid was transferred from the flask using the minimum quantity of ethyl acetate, which was precipitated again using *n*-hexane. A mixture of compound **2** as a crystal and a powdery solid was attained. The product was purified with column chromatography, using two separate systems. Stationary phase was silica gel and mobile phases were petroleum ether/ethyl acetate 7:3 and *n*-hexane/ethyl acetate 6:4, which showed better results.

Yield: 40%

m.p. = 232-234 °C

¹H NMR (DMSO-*d*₆, δ/ppm): 2.69 (s, 3H, 1'), 7.22-7.24 (t, 1H, 5, $J_o = 8$ Hz), 7.48-7.50 (m, 1H, 6, $J_o = 8$ Hz), 7.55-7.58 (d, 1H, 7, $J_o = 8$ Hz), 7.79-7.81 (dd, 2, 1H), 8.09-8.10 (ds, 1H, 1, $J_m = 4$ Hz), 8.20-8.25 (m, 2H, 3, 4), 11.54 (s, 1H, 8)

3.4. Synthesis of 3-(3-bromo-4-methoxyphenyl)-1-(9*H*-carbazol-2-yl)prop-2-en-1-one (1a)

Compound **2** was suspended (0.200 g; 9.558×10^{-4} mol) in methanol (8.8 ml). Afterwards, barium hydroxide octahydrate (0.603 g; 1.912×10^{-4} mol) and 3-bromo-4-hydroxybenzaldehyde (0.205 g; 9.558×10^{-4} mol) were added and the system was adjusted to the reflux temperature of the methanol (65 °C). Upon completion, water was added to the reaction system and the precipitate was filtered under vacuum. A solid powder of compound **1a** was attained and purified using column chromatography (stationary phase: silica gel, mobile phase: chloroform/ethanol 99:1).

Yield: 18%

m.p. = 194-206 °C

IR (KBr, ν_{max}/cm^{-1}): 3275 (NH), 1651 (C=O), 1627 (C=O)

¹H NMR (DMSO-*d*₆, δ/ppm): 3.93 (s, 3H, 1"), 7.20-7.24 (m, 2H, 5, 4'), 7.46-7.50 (t, 1H, 6, $J_o = 8$ Hz), 7.56-7.58 (d, 1H, 3', $J_o = 8$ Hz), 7.70-7.74 (d, 1H, 1', $J_{trans} = 16$ Hz), 7.88-7.91 (dd, 1H, 7), 8.02-8.06 (m, 2H, 2', 4), 8.22-8.31 (m, 4H, 1, 2, 3, 5'), 11.54 (s, 1H, 8)

3.5. Synthesis of 1-(9*H*-carbazol-2-yl)-3-phenylprop-2-en-1-one (**1b**)

Compound **2** was suspended (0.200 g; 9.558×10^{-4}) in methanol (8.8 ml). Afterwards, barium hydroxide octahydrate (0.603 g; 1.912×10^{-4} mol) and benzaldehyde (0.101 g; 9.558×10^{-4} mol) were added and the system was adjusted to the reflux temperature of the methanol (65 °C). Upon completion, water was added to the reaction system and the precipitate was filtered under vacuum. A solid powder of compound **1b** was attained and purified using column chromatography (stationary phase: silica gel, mobile phase: chloroform/acetone 95:5).

Yield: 23%

m.p. = 273-284 °C

IR (KBr, ν_{max}/cm^{-1}): 3291 (NH), 1652 (C=O), 1627 (C=O)

^1H NMR (DMSO- d_6 , δ/ppm): 7.21-7.24 (t, 1H, 5, $J_o = 8$ Hz), 7.47-7.50 (m, 4H, 4', 5', 6', 6), 7.57-7.59 (d, 1H, 7, $J_o = 8$ Hz), 7.77-7.81 (d, 1H, 1', $J_{trans} = 16$ Hz), 7.92-7.94 (m, 2H, 3', 7'), 8.01-8.03 (d, 1H, 2, $J_o = 8$ Hz), 8.06-8.10 (d, 1H, 2', $J_{trans} = 16$ Hz), 8.23-8.31 (m, 3H, 1, 3, 4), 11.56 (s, 1H, 8)

4. RESULTS AND DISCUSSION

Starting compound in the reaction mechanism is 4'-chloroacetophenone, which is commercially available.

The synthesis of derivative of 2-halonitrobenzene (**4**) was the electrophilic substitution reaction between benzene ring of 4'-chloroacetophenone and nitric acid (Figure 6). The mixture of concentrated nitric acid and concentrated sulphuric acid was added, while the temperature was kept below -10 °C, because of the exothermic nature of the reaction and the greater chance of getting more than one nitro group substituted onto the ring if the temperature increased. The electrophile is the nitronium ion or the nitryl cation, NO_2^+ . It was formed by the reaction between the nitric acid and the sulphuric acid, which is acting as a catalyst (Joshi et al., 2012).

2-Nitrobiphenyl derivative (**3**) was synthesized via Suzuki-Miyaura cross coupling. The carbon-carbon single bond was formed by coupling phenylboronic acid and 2-halonitrobenzen (**4**) using $\text{Pd}(\text{PPh}_3)_4$ as a catalyst and K_2CO_3 as a base. Toluene was used as a solvent. The solid product (**3**) was purified using column chromatography (Freeman et al., 2005; Xu et al., 2016).

The carbazole derivative (**2**) was synthesized using PPh_3 -mediated reductive cyclization of 2-nitrobiphenyl (**3**) in 1,2-dichlorobenzene (Figure 7). These solvents were selected because of their similar polarity, high solvent power, high boiling points, and availability. The primary byproduct was 2 equivalents of PPh_3O . The purification of the carbazole derivative (**2**) was achieved with the combination of the precipitation of the PPh_3O from the product using *n*-hexane, and column chromatography (Freeman et al., 2005; Xu et al., 2016).

Our desired products (**1a**, **1b**) were synthesized using the base catalyzed aldol reaction, followed by the base catalyzed dehydration of the water molecule. $\text{Ba}(\text{OH})_2$ was added first after carbazole derivative (**2**) in order to deprotonate it. That way, it was achieved that the carbazole derivative (**2**) forms an enolate, which reacts more easily with benzaldehyde and 3-bromo-4-methoxybenzaldehyde, which are commercially available. Solid products (**1a**, **1b**) were attained using column chromatography.

All synthesized products were characterized with usual spectroscopic methods (IR and ^1H NMR), and melting points were defined for solid products. Analytical and spectroscopic data for compounds **1-4** are shown in the Tables 4, 5 and 6.

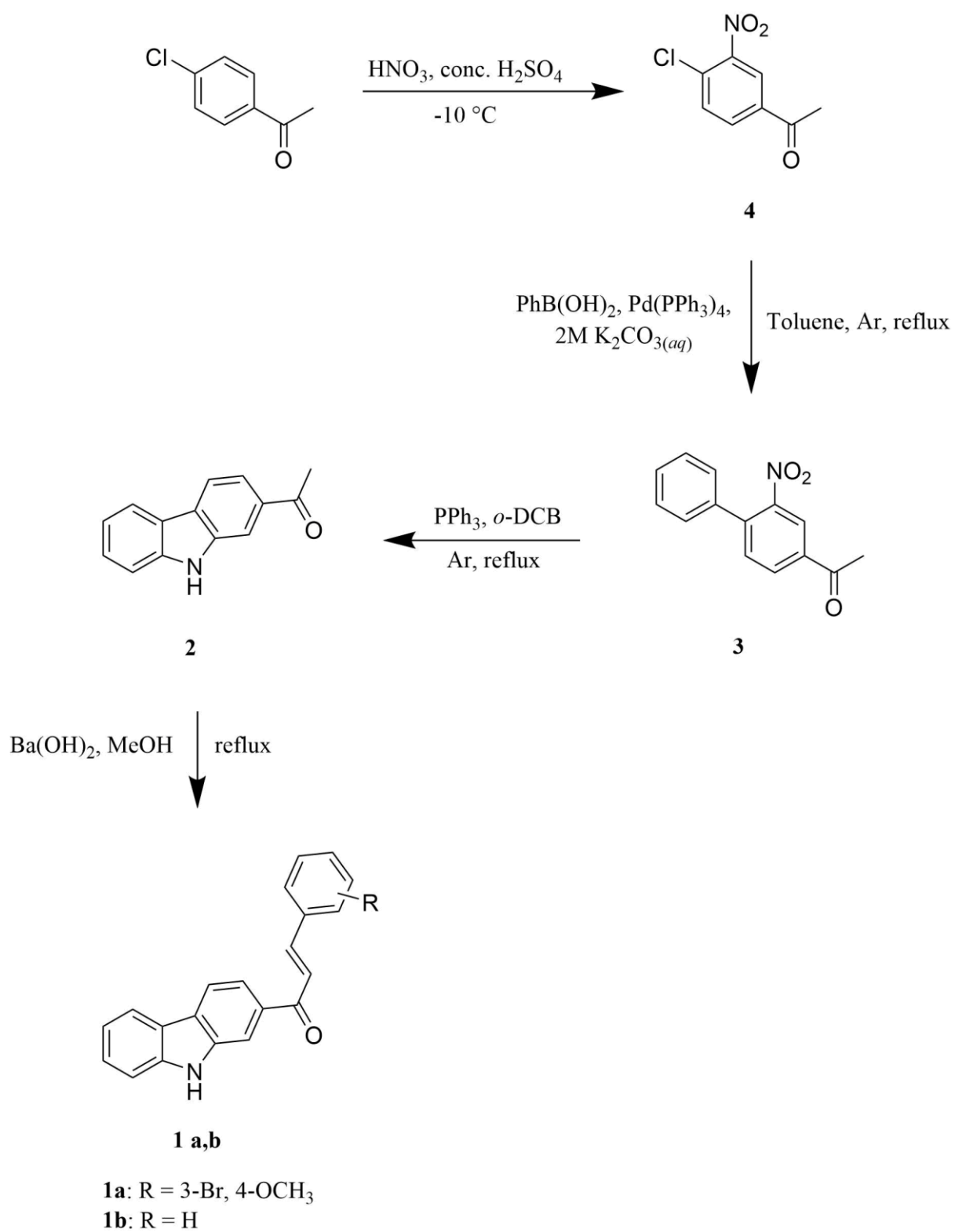


Figure 6. Synthesis of carbonyl derivatives

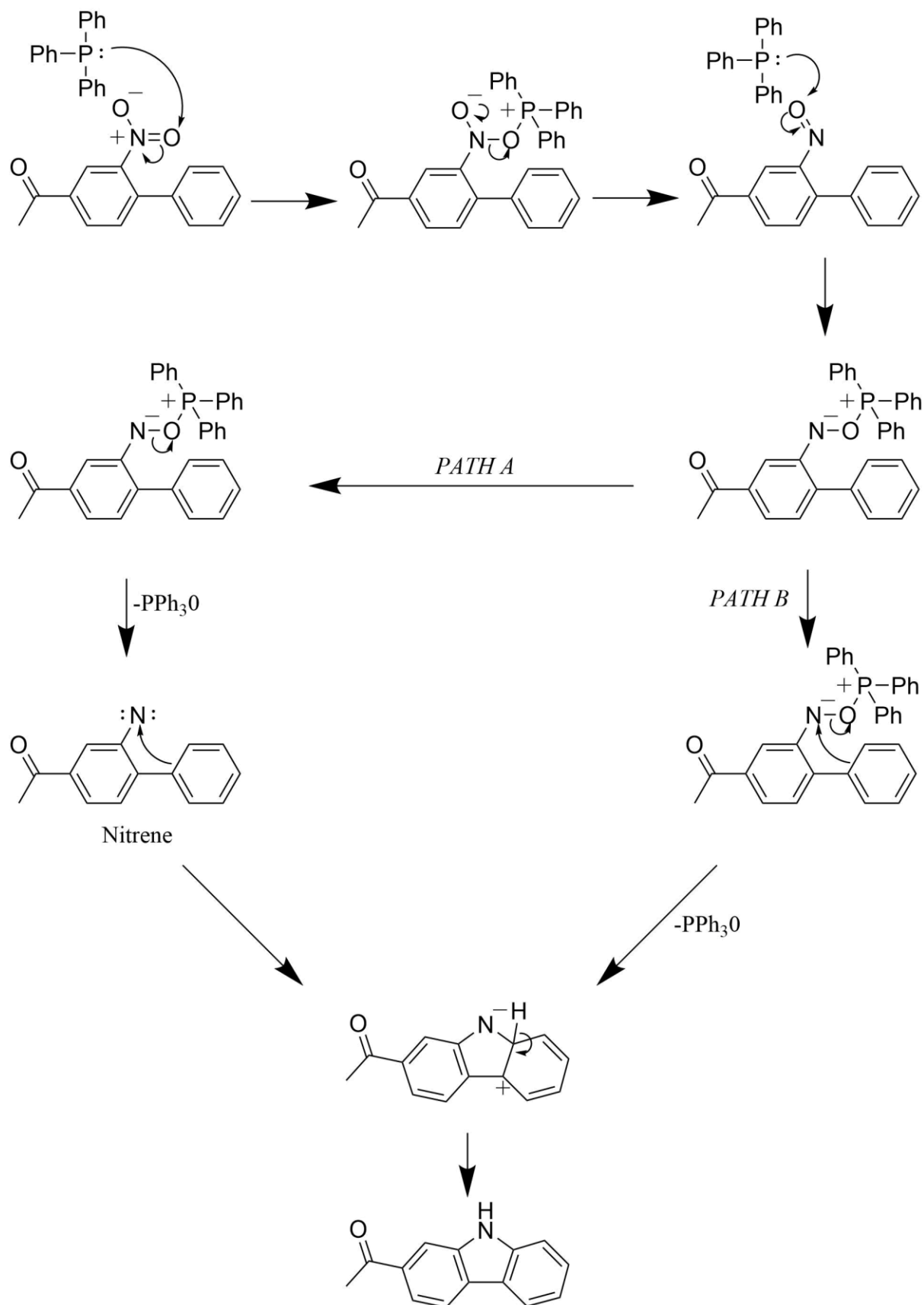


Figure 7. Proposed reaction mechanism for reductive cyclization

Table 4. Analytical data for compounds 1-4.

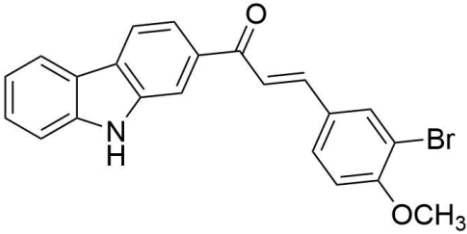
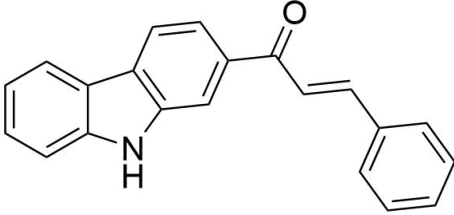
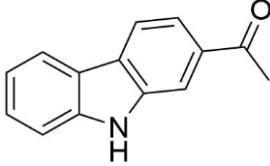
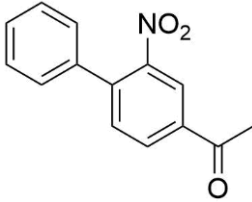
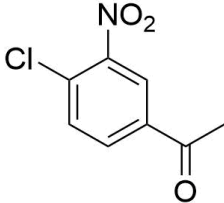
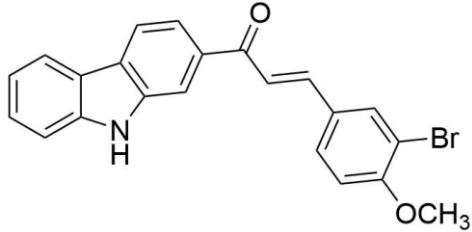
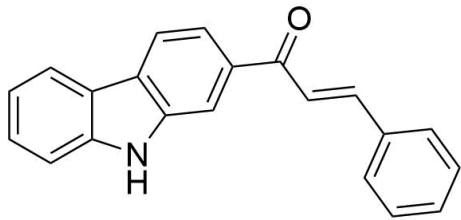
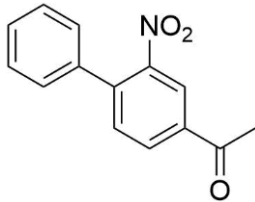
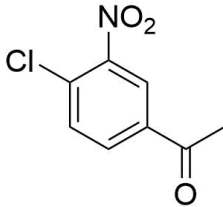
Compound	Structure	Molecular formula (M _r)	Yield (%)	m.p. (°C)
1a		C ₂₂ H ₁₆ BrNO ₂ (406.28)	18	194-206
1b		C ₂₁ H ₁₅ NO (297.36)	23	273-284
2		C ₁₄ H ₁₁ NO (209.25)	40	232-234
3		C ₁₄ H ₁₁ NO ₃ (241.25)	62	95-97
4		C ₈ H ₆ ClNO ₃ (199.59)	70	99-101

Table 5. ^1H NMR spectroscopic data for compounds 1-4.

Compound	Structure	^1H NMR (DMSO- d_6 , δ ppm, J/Hz)
1a		3.93 (s, 3H, 1"), 7.20-7.24 (m, 2H, 5, 4'), 7.46-7.50 (t, 1H, 6, $J_o = 8$ Hz), 7.56-7.58 (d, 1H, 3', $J_o = 8$ Hz), 7.70-7.74 (d, 1H, 1', $J_{trans} = 16$ Hz), 7.88-7.91 (dd, 1H, 7), 8.02-8.06 (m, 2H, 2', 4), 8.22-8.31 (m, 4H, 1, 2, 3, 5'), 11.54 (s, 1H, 8)
1b		7.21-7.24 (t, 1H, 5, $J_o = 8$ Hz), 7.47-7.50 (m, 4H, 4', 5', 6', 6), 7.57-7.59 (d, 1H, 7, $J_o = 8$ Hz), 7.77-7.81 (d, 1H, 1', $J_{trans} = 16$ Hz), 7.92-7.94 (m, 2H, 3', 7'), 8.01-8.03 (d, 1H, 2, $J_o = 8$ Hz), 8.06-8.10 (d, 1H, 2', $J_{trans} = 16$ Hz), 8.23-8.31 (m, 3H, 1, 3, 4), 11.56 (s, 1H, 8)
2		2.69 (s, 3H, 1'), 7.22-7.24 (t, 1H, 5, $J_o = 8$ Hz), 7.48-7.50 (m, 1H, 6, $J_o = 8$ Hz), 7.55-7.58 (d, 1H, 7, $J_o = 8$ Hz), 7.79-7.81 (dd, 2, 1H), 8.09-8.10 (ds, 1H, 1, $J_m = 4$ Hz), 8.20-8.25 (m, 2H, 3, 4), 11.54 (s, 1H, 8)
3		2.69 (s, 3H, 1"), 7.38-7.40 (m, 2H, 2', 4'), 7.48-7.51 (m, 3H, 1', 3', 5'), 7.73-7.75 (d, 1H, 3, $J_o = 8$ Hz), 8.26-8.29 (dd, 1H, 2), 8.46 (d, 1H, 1, $J_m = 4$ Hz)
4		2.64 (s, 3H, 1'), 7.94-7.96 (d, 1H, 3, $J_o = 8$ Hz), 8.19-8.22 (dd, 1H, 2, $J_o = 8$ Hz), 8.54-8.55 (d, 1H, 1, $J_m = 4$ Hz)

Table 6. IR Spectroscopic data for compounds **1-4**.

Compound	Structure	IR (KBr) $\nu_{max}(\text{cm}^{-1})$
1a		3275 (NH), 1651 (C=O), 1627 (C=O)
1b		3291 (NH), 1652 (C=O), 1627 (C=O)
3		1683 (C=O), 1525 (NO ₂), 1357 (NO ₂)
4		1685 (C=O), 1532 (NO ₂), 1345 (NO ₂)

5. CONCLUSION

In this thesis, the research into carbonyl derivatives as potential CHIKV inhibitors, which is being carried out at the Sapienza University of Rome Faculty of Pharmacy and Medicine, was continued. The synthesis of two unsaturated carbonyls, attained by condensation of carbazole and benzaldehyde derivatives, was described. Structures of every compound was confirmed by spectroscopic methods (IR and ^1H NMR). In further research, which is beyond the scope of this thesis, synthesized carbonyl derivatives will be tested for their anti-CHIKV activity.

6. REFERENCES

Abdelnabi R, Neyts J, Delang L. Towards antivirals against chikungunya virus. *Antiviral Research*, 2015, 121, 59-68.

Akahata W, Yang Z, Andersen H, Sun S, Holdaway HA, Kong W-P, Lewis MG, Higgs S, Rosmann MG, Rao S, Nabel GJ. A VLP vaccine for epidemic Chikungunya virus protects non-human primates against infection. *Nature medicine*, 2010, 16, 334-338.

Bassetto M, De Burghgraeve T, Delang L, Massarotti A, Coluccia A, Zonta N, Gatti V, Colombano G, Sorba G, Silvestri R, Tron GC, Neyts J, Leyssen P, Brancale A. Computer-aided identification, design and synthesis of a novel series of compounds with selective antiviral activity against chikungunya virus. *Antiviral Research*, 2013, 98, 12-18.

Bissantz C, Folkers G, Rognan D. Protein-Based Virtual Screening of Chemical Databases. 1. Evaluation of Different Docking/Scoring Combinations. *Journal of Medical Chemistry*, 2000, 43, 4759-4767.

Bourjot M, Leyssen P, Neyts J, Dumontet V, Litaudon M. Trigocherrierin A, a Potent Inhibitor of Chikungunya Virus Replication. *Molecules*, 2014, 19, 3617-3627.

Bréhin AC, Casadémont I, Frenkiel MP, Julier C, Sakuntabhai A, Desprès P. The large form of human 2',5'-Oligoadenylate Synthetase (OAS3) exerts antiviral effect against Chikungunya virus. *Virology*, 2009, 384, 216-222.

Briolant S, Garin D, Scaramozzino N, Jouan A, Crance JM. In vitro inhibition of Chikungunya and Semliki Forest viruses replication by antiviral compounds: synergistic effect of interferon- α and ribavirin combination. *Antiviral Research*, 2004, 61, 111-117.

Burt FJ, Chen W, Miner JJ, Lenschow DJ, Merits A, Schnettler E, Kohl A, Rudd PA, Taylor A, Herrero LJ, Zaid A, Ng LFP, Mahalingam S. Chikungunya virus: an update on the biology and pathogenesis of this emerging pathogen. *The Lancet Infectious Diseases*, 2017, 17, e107-e117.

Burt FJ, Rolph MS, Rulli NE, Mahalingam S, Heise MT. Chikungunya: a re-emerging virus. *Lancet*, 2012, 379, 662-671.

CDC. Chikungunya virus, 2018, <https://www.cdc.gov>, accessed 18 June 2018.

Chikungunya virus: Evidence for global policy, practice and research in disease management, surveillance, and mosquito control, 2014, <https://www.digital.library.adelaide.edu.au>, accessed 12 June 2018.

Chu H, Das SC, Fuchs JF, Suresh M, Weaver SC, Stinchcomb DT, Partidos CD, Osorio JE. Deciphering the Protective Role of Adaptive Immunity to CHIKV/IRES a Novel Candidate Vaccine against Chikungunya in the A129 Mouse Model. *Vaccine*, 2013, 31, 3353-3360.

Couderc T, Chrétien F, Schilte C, Disson O, Brigitte M, Guivel-Benhassine F, Touret Y, Barau G, Cayet N, Schuffenecker I, Desprès P, Arenzana-Seisdedos F, Michault A, Albert ML, Lecuit M. A Mouse Model for Chikungunya: Young Age and Inefficient Type-I Interferon Signaling Are Risk Factors for Severe Disease. *PLoS Pathogens*, 2008, 4, e29.

Couderc T, Lecuit M. Chikungunya virus pathogenesis: From bedside to bench. *Antiviral Research*, 2015, 121, 120-131.

Critical role of glycosylation in determining the length and structure of T cell epitopes, 2009, <http://www.link.springer.com>, accessed 12 June 2018.

Cruz DJM, Bonotto RM, Gomes RG, da Silva CT, Taniguchi JB, No JH, Lombardot B, Schwartz O, Hansen MA, Freitas-Junior LH. Identification of Novel Compounds Inhibiting Chikungunya Virus-Induced Cell Death by High Throughput Screening of a Kinase Inhibitor Library. *PLoS Neglected Tropical Diseases*, 2013, 7, e2471.

Das T, Jaffar-Bandjee MC, Hoarau JJ, Krejbich Trotot P, Denizot M, Lee-Pat-Yuen G, Sahoo R, Guiraud P, Ramful D, Robin S, Alessandri JL, Gauzere BA, Gasque P. Chikungunya fever: CNS infection and pathologies of a re-emerging arbovirus. *Progress in Neurobiology*, 2010, 91, 121-129.

Diallo D, Chen R, Diagne CT, Baa Y, Diaa I, Sallc AA, Weaverb SC, Dialloa M. Bloodfeeding patterns of sylvatic arbovirus vectors in southeastern Senegal. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2013, 107, 200-203.

Economopoulou A, Dominguez M, Helynck B, Sissoko D, Wichmann O, Quenel P, Germonneau P, Quatresous I. Atypical Chikungunya virus infections: clinical manifestations, mortality and risk factors for severe disease during the 2005-2006 outbreak on Réunion. *Epidemiology & Infection*, 2009, 137, 534-541.

Farnsworth A, Flaman AS, Prasad SS, Gravel C, Williams A, Yauk CL, Li X. Acetaminophen Modulates the Transcriptional Response to Recombinant Interferon- β . *PLoS ONE*, 2010, 5, e11031.

Fok-Moon Lum, Lisa F.P. Ng. Cellular and molecular mechanisms of chikungunya pathogenesis. *Antiviral Research*, 2015, 120, 165-174.

Freeman AW, Urvoy M, Criswell ME. Tryphenylphosphine-mediated reductive cyclization of 2-nitrophenyls: a practical and convenient synthesis of carbazoles. *Journal of Organic Chemistry*, 2005, 70, 5014-5019.

Functional processing and secretion of Chikungunya virus E1 and E2 glycoproteins in insect cells, 2011, <https://www.link.springer.com>, accessed 12 June 2018.

García-Arriaza J, Cepeda V, Hallengård D, Sorzano COS, Kümmerer BM, Liljeström P, Estebana M. A Novel Poxvirus-Based Vaccine, MVA-CHIKV, Is Highly Immunogenic and Protects Mice against Chikungunya Infection. Perlman S, ed. *Journal of Virology*, 2014, 88, 3527-3547.

Gardner J, Anraku I, Le TT, Larcher T, Major L, Roques P, Schroder WA, Higgs S, Suhrbier A. Chikungunya Virus Arthritis in Adult Wild-Type Mice. *Journal of Virology*, 2010, 84, 8021-8032.

Gérardin P, Barau G, Michault A, Bintner M, Randrianaivo H, Choker G, Lenglet Y, Touret Y, Bouvert A, Grivard P, Le Roux K, Blanc S, Schuffenecker I, Couderc T, Arenzana-Seisdedos F, Lecuit M, Robillard P-Y. Multidisciplinary Prospective Study of Mother-to-Child Chikungunya Virus Infections on the Island of La Réunion. *PLoS Medicine*, 2008, 5, e60.

Goha LYH, Hobson-Peters J, Prow NA, Gardner J, Bielefeldt-Ohmann H, Pyke AT, Suhrbier A, Hall RA. Neutralizing monoclonal antibodies to the E2 protein of chikungunya virus protects against disease in a mouse model. *Clinical Immunology*, 2013, 149, 487-497.

Gupta D, Bose A, Rose W. Acquired Neonatal Chikungunya Encephalopathy. *The Indian Journal of Pediatrics*, 2015, 82, 1065-1066.

Harrison VR, Eckels KH, Bartelloni PJ, Hampton C. Production and evaluation of a formalin-killed Chikungunya vaccine. *The Journal of Immunology*, 1971, 107, 643-647.

Hoarau JJ, Jaffar Bandjee MC, Krejbich Trotot P, Das T, Li-Pat-Yuen G, Dassa B, Denizot M, Guichard E, Ribera A, Henni T, Tallet F, Moiton MP, Gauzère BA, Bruniquet S, Jaffar Bandjee Z, Morbidelli P, Martigny G, Jolivet M, Gay F, Grandadam M, Tolou H, Vieillard V, Debré P, Autran B, Gasque P. Persistent Chronic Inflammation and Infection by Chikungunya Arthritogenic Alphavirus in Spite of a Robust Host Immune Response. *The Journal of Immunology*, 2010, 184, 5914-5927.

Jadav SS, Sinha BN, Hilgenfeld R, Pastorino B, de Lamballerie X, Jayaprakash V. Thiazolidone derivatives as inhibitors of chikungunya virus. *European Journal of Medicinal Chemistry*, 2015, 89, 172-178.

Javelle E, Ribera A, Degasne I, Gaüzère B-A, Marimoutou C, Simon F. Specific Management of Post-Chikungunya Rheumatic Disorders: A Retrospective Study of 159 Cases in Reunion Island from 2006-2012. *PLoS Neglected Tropical Diseases*, 2015, 9, e0003603.

Joshi A, Hussain N, Sain D, Talesara GL. Synthetic studies on N-methylpiperazine containing pirazoles and their ethoxyphthalimide derivatives. *Journal of Chemistry*, 2012, 24, 5933-5936.

Joubert P-E, Werneke SW, de la Calle C, Guivel-Benhassine F, Giodini A, Peduto L, Levine B, Schwartz O, Lenschow DJ, Albert ML. Chikungunya virus-induced autophagy delays caspase-dependent cell death. *The Journal of Experimental Medicine*, 2012, 209, 1029-1047.

Kaur P, Chu JJ. Chikungunya virus: an update on antiviral development and challenges. *Drug Discovery Today*, 2013, 18, 969-983.

Kaur P, Thiruchelvan M, Lee RC, Chen H, Chen KC, Ng ML, Chu JJ. Inhibition of Chikungunya Virus Replication by Harringtonine, a Novel Antiviral That Suppresses Viral Protein Expression. *Antimicrobial Agents and Chemotherapy*, 2013, 57, 155-167.

Kelvin AA, Banner D, Silvi G, Moro ML, Spataro N, Gaibani P, Cavrini F, Pierro A, Rossini G, Cameron MJ, Bermejo-Martin JF, Paquette SG, Xu L, Danesh A, Farooqui A, Borghetto I, Kelvin DJ, Sambri V, Rubino S. Inflammatory Cytokine Expression Is Associated with Chikungunya Virus Resolution and Symptom Severity. *PLoS Neglected Tropical Diseases*, 2011, 5, e1279.

Khan M, Dhanwania R, Patro IK, Rao PVL, Parida MM. Cellular IMPDH enzyme activity is a potential target for the inhibition of Chikungunya virus replication and virus induced apoptosis in cultured mammalian cells. *Antiviral Research*, 2011, 89, 1-8.

Kumar M, Sudeep AB, Arankalle VA. Evaluation of recombinant E2 protein-based and whole-virus inactivated candidate vaccines against chikungunya virus. *Vaccine*, 2012, 30, 6142-6149.

Labadie K, Larcher T, Joubert C, Mannioui A, Delache B, Brochard P, Guigand L, Dubreil L, Lebon P, Verrier B, de Lamballerie X, Suhrbier A, Cherel Y, Le Grand R, Roques P. Chikungunya disease in nonhuman primates involves long-term viral persistence in macrophages. *The Journal of Clinical Investigation*, 2010, 120, 894-906.

Lam S, Chen KC, Ng MM-L, Chu JJH. Expression of Plasmid-Based shRNA against the E1 and nsP1 Genes Effectively Silenced Chikungunya Virus Replication. *PLoS ONE*, 2012, 7, e46396.

Lazzaroni M, Bianchi Porro G. Gastrointestinal side-effects of traditional non-steroidal anti-inflammatory drugs and new formulations. *Alimentary Pharmacology & Therapeutics*, 2004, 20, 48-58.

Lenschow DJ, Lai C, Frias-Staheli N, Giannakopoulos NV, Lutz A, Wolff T, Osiak A, Levine B, Schmidt RE, García-Sastre A, Leib DA, Pekosz A, Knobeloch KP, Horak I, Virgin HW. 4th. IFN-stimulated gene 15 functions as a critical antiviral molecule against influenza, herpes, and Sindbis viruses. *Proceedings of the National Academy of Sciences of the United States of America*, 2007, 104, 1371-1376.

Leo YS, Chow ALP, Tan LK, Lye DC, Lin L, Ng LC. Chikungunya Outbreak, Singapore, 2008. *Emerging Infectious Diseases*, 2009, 15, 836-837.

Litzba N, Schuffenecker I, Zeller H, Drosten C, Emmerich P, Charrel R, Kreher P, Niedrig M. Evaluation of the first commercial chikungunya virus indirect immunofluorescence test. *Journal of Virological Methods*, 2008, 149, 175-179.

Lo Presti A, Lai A, Cella E, Zehender G, Ciccozzi M. Chikungunya virus, epidemiology, clinics and phylogenesis: A review. *Asian Pacific Journal of Tropical Medicine*, 2014, 7, 925-932.

Lum F-M, Ng LFP. Cellular and molecular mechanisms of chikungunya pathogenesis. *Antiviral Research*, 2015, 165-174.

Lum F-M, Teo T-H, Lee WWL, Kam Y-W, Rénia L, Ng LFP. An Essential Role of Antibodies in the Control of Chikungunya Virus Infection. *The Journal of Immunology*, 2013, 190, 6295-6302.

MacDonald MR, Machlin ES, Albin OR, Levy DE. The Zinc Finger Antiviral Protein Acts Synergistically with an Interferon-Induced Factor for Maximal Activity against Alphaviruses. *Journal of Virology*, 2007, 81, 13509-13518.

Mallilankaraman K, Shedlock DJ, Bao H, Kawalekar OU, Fagone P, Ramanathan AA, Ferraro B, Stabenow J, Vijayachari P, Sundaram SG, Muruganandam N, Sarangan G, Srikanth P, Khan AS, Lewis MG, Kim JJ, Sardesai NY, Muthumani K, Weiner DB. A DNA Vaccine against Chikungunya Virus Is Protective in Mice and Induces Neutralizing Antibodies in Mice and Nonhuman Primates. *PLoS Neglected Tropical Diseases*, 2011, 5, e928.

Malvy D, Ezzedine K, Mamani-Matsuda M, Autran B, Tolou H, Receveur M-C, Pistone T, Rambert J, Moynet D, Mossalayi D. Destructive arthritis in a patient with chikungunya virus infection with persistent specific IgM antibodies. *BMC Infectious Diseases*, 2009, 9, 1471-2334.

Messaoudi I, Vomasse J, Totonchy T, Kreklywich CN, Haberthur K, Springgay L, Brien JD, Diamond MS, Defilippis VR, Streblow DN. Chikungunya Virus Infection Results in Higher and Persistent Viral Replication in Aged Rhesus Macaques Due to Defects in Anti-Viral Immunity. *PLoS Neglected Tropical Diseases*, 2013, 7, e2343.

Muthumani K, Lankaraman KM, Laddy DJ, Sundaram SG, Chung CW, Sako E, Wu L, Khan A, Sardesai N, Kim JJ, Vijayachari P, Weiner DB. Immunogenicity of novel consensus-based DNA vaccines against Chikungunya virus. *Vaccine*, 2008, 26, 5128-5134.

Ozden S, Huerre M, Riviere JP, Coffey LL, Afonso PV, Mouly V, de Monredon J, Roger JC, El Amrani M, Yvin JL, Jaffar MC, Frenkiel MP, Sourisseau M, Schwartz O, Butler-Browne G, Desprès P, Gessain A, Ceccaldi PE. Human Muscle Satellite Cells as Targets of Chikungunya Virus Infection. *PLoS ONE*, 2007, 2, e527.

Ozden S, Lucas-Hourani M, Ceccaldi PE, Basak A, Valentine M, Benjannet S, Hamelin J, Jacob Y, Mamchaoui K, Mouly V, Desprès P, Gessain A, Butler-Browne G, Chrétien M, Tangy F, Vidalain PO, Seidah NG. Inhibition of Chikungunya virus infection in cultured human muscle cells by furin inhibitors: impairment of the maturation of the E2 surface glycoprotein. *Journal of Biological Chemistry*, 2008, 283, 21899-21908.

Padmakumar B, Jayan JB, Menon RMR, Krishnankutty B, Payippallil R, Nisha RS. Comparative evaluation of four therapeutic regimes in chikungunya arthritis: a prospective randomized parallel-group study. *Indian Journal of Rheumatology*, 2009, 4, 94-101.

Panning M, Grywna K, van Esbroeck M, Emmerich P, Drosten C. Chikungunya Fever in Travelers Returning to Europe from the Indian Ocean Region, 2006. *Emerging Infectious Diseases*, 2008, 14, 416-422.

Parashar D, Cherian S. Antiviral Perspectives for Chikungunya Virus. *Hindawi Publishing Corporation, BioMed Research International*, 2014, 2014, e631642.

Parashar D, Paingankar MS, Kumar S, Gokhale MD, Sudeep AB, Shinde SB, Arankalle VA. Administration of E2 and NS1 siRNAs Inhibit Chikungunya Virus Replication In Vitro and Protects Mice Infected with the Virus. *PLoS Neglected Tropical Diseases*, 2013, 7, e2405.

Parola P, de Lamballerie X, Jourdan J, Rovey C, Vaillant V, Minodier P, Brouqui P, Flahault A, Raoult D, Charrel RM. Novel Chikungunya Virus Variant in Travelers Returning from Indian Ocean Islands. *Emerging Infectious Diseases*, 2006, 12, 1493-1499.

Plante K, Wang E, Partidos CD, Weger J, Gorchakov R, Tsetsarkin K, Borland EM, Powers AM, Seymour R, Stinchcomb DT, Osorio JE, Frolov I, Weaver SC. Novel Chikungunya Vaccine Candidate with an IRES-Based Attenuation and Host Range Alteration Mechanism. *PLoS Pathogens*, 2011, 7, e1002142.

Pohjala L, Utt A, Varjak M, Lulla A, Merits A, Ahola T, Tammela P. Inhibitors of Alphavirus Entry and Replication Identified with a Stable Chikungunya Replicon Cell Line and Virus-Based Assays. *PLoS ONE*, 2011, 6, e28923.

Poo YS, Rudd PA, Gardner J, Wilson JA, Larcher T, Colle MA, Le TT, Nakaya HI, Warrilow D, Allcock R, Bielefeldt-Ohmann H, Schroder WA, Khromykh AA, Lopez JA, Suhrbier A. Multiple Immune Factors Are Involved in Controlling Acute and Chronic Chikungunya Virus Infection. *PLoS Neglected Tropical Diseases*, 2014, 8, e3354.

Powers AM, Brault AC, Shirako Y, Strauss EG, Kang W, Strauss JH, Weaver SC. Evolutionary Relationships and Systematics of the Alphaviruses. *Journal of Virology*, 2001, 75, 10118-10131.

Rathore APS, Haystead T, Das PK, Merits A, Ng ML, Vasudevan SG. Chikungunya virus nsP3 & nsP4 interacts with HSP-90 to promote virus replication: HSP-90 inhibitors reduce CHIKV infection and inflammation in vivo. *Antiviral Research*, 2014, 103, 7-16.

Ravichandran R, Manian M. Ribavirin therapy for Chikungunya arthritis. *The Journal of Infection in Developing Countries*, 2008 , 2, 140-142.

Replication of Alphaviruses: A Review on the Entry Process of Alphaviruses into Cells, 2011, <https://www.ncbi.nlm.nih.gov>, accessed 12 June 2018.

Riss TL, Moravec RA, Niles AL, Duellman S, Benink HA, Worzella TJ, Minor L. Assay Guidance Manual. Bethesda (MD), Eli Lilly & Company and the National Center for Advancing Translational Sciences, 2013, 355-387.

Roy CJ, Adams AP, Wang E, Adams AP, Wang E, Plante K, Gorchakov R, Seymour RL, Vinet-Oliphant H, Weaver SC. Chikungunya Vaccine Candidate Is Highly Attenuated and Protects Nonhuman Primates Against Telemetrically Monitored Disease Following a Single Dose. *The Journal of Infectious Diseases*, 2014, 209, 1891-1899.

Salvador B, Zhou Y, Michault A, Muench MO, Simmons G. Characterization of Chikungunya pseudotyped viruses: Identification of refractory cell lines and demonstration of cellular tropism differences mediated by mutations in E1 glycoprotein. *Virology*, 2009, 393, 33-41.

Schilte C, Buckwalter MR, Laird ME, Diamond MS, Schwartz O, Albert ML. Independent roles for IRF-3 and IRF-7 in hematopoietic and nonhematopoietic cells during host response to Chikungunya infection. *The Journal of Immunology*, 2012, 188, 2967-2971.

Schilte C, Staikowsky F, Couderc T, Madec Y, Carpentier F, Kassab S, Albert ML, Lecuit M, Michault A. Chikungunya Virus-associated Long-term Arthralgia: A 36-month Prospective Longitudinal Study. *PLoS Neglected Tropical Diseases*, 2013, 7, e2137.

Schuffenecker I, Iteman I, Michault A, Murri S, Frangeul L, Vaney M-C, Lavenir R, Pardigon N, Reynes J-M, Pettinelli F, Biscornet L, Diancourt L, Michel S, Duquerroy S, Guigon G, Frenkiel M-P, Bréhin A-C, Cubito N, Desprès P, Kunst F, Rey FA, Zeller H, Brisse S. Genome Microevolution of Chikungunya Viruses Causing the Indian Ocean Outbreak. *PLoS Med*, 2016, 3, e263.

Selvarajah S, Sexton NR, Kahle KM, Fong RH, Mattia K-A, Gardner J, Lu K, Liss NM, Salvador B, Tucker DF, Barnes T, Mabila M, Zhou X, Rossini G, Rucker JB, Sanders DA, Suhrbier A, Sambri V, Michault A, Muench MO, Doranz BJ, Simmons G. A Neutralizing Monoclonal Antibody Targeting the Acid-Sensitive Region in Chikungunya Virus E2 Protects from Disease. *PLoS Neglected Tropical Diseases*, 2013, 7, e2423.

Shukla J, Khan M, Tiwari M, Sannarangaiah S, Sharma S, Rao PVL, Parida M. Development and evaluation of antigen capture ELISA for early clinical diagnosis of chikungunya. *Diagnostic Microbiology & Infectious Disease*, 2009, 65, 142-149.

Sissoko D, Malvy D, Ezzedine K, Renault P, Moscetti F, Martine Ledrans M, Vincent Pierre V. Post-Epidemic Chikungunya Disease on Reunion Island: Course of Rheumatic Manifestations and Associated Factors over a 15-Month Period. *PLoS Neglected Tropical Diseases*, 2009, 3, e389.

Sourisseau M, Schilte C, Casartelli N, Trouillet C, Guivel-Benhassine F, Rudnicka D, Sol-Foulon N, Le Roux K, Prevost MC, Fsihi H, Frenkiel MP, Blanchet F, Afonso PV, Ceccaldi PE, Ozden S, Gessain A, Schuffenecker I, Verhasselt B, Zamborlini A, Saïb A, Rey FA, Arenzana-Seisdedos F, Desprès P, Michault A, Albert ML, Schwartz O. Characterization of Reemerging Chikungunya Virus. *PLoS Pathogens*, 2007, 3, e89.

Staikowsky F, Le Roux K, Schuffenecker I, P. Laurent, P. Grivard, A. Develay, A. Michault. Retrospective Survey Of Chikungunya disease in Réunion Island hospital staff. *Epidemiology and Infection*, 2008, 136, 196-206.

Staikowsky F, Talarmin F, Grivard P, Souab A, Schuffenecker I, Le Roux K, Lecuit M, Michault A. Prospective Study of Chikungunya Virus Acute Infection in the Island of La Réunion during the 2005–2006 Outbreak. *PLoS ONE*, 2009, 4, e7603.

Strauss JH, Strauss EG. The alphaviruses: gene expression, replication, and evolution. *Microbiological Reviews*, 1994, 58, 491-562.

Taylor A, Sheng K-C, Herrero LJ, Chen W, Rulli NE, Mahalingam S. Methotrexate Treatment Causes Early Onset of Disease in a Mouse Model of Ross River Virus-Induced Inflammatory Disease through Increased Monocyte Production. *PLoS ONE*, 2013, 8, e71146.

Teng T-S, Foo S-S, Simamarta D, Lum F-M, Teo T-H, Lulla A, Yeo NKW, Koh EGL, Chow A, Leo Y-S, Merits A, Chin K-C, Ng LFP. Viperin restricts chikungunya virus replication and pathology. *The Journal of Clinical Investigation*, 2012, 122, 4447-4460.

Teo T-H, Her Z, Tan JJ, Lum FM, Lee WW, Chan YH, Ong RY, Kam YW, Leparc-Goffart I, Gallian P, Rénia L, de Lamballerie X, Ng LF. Caribbean and La Réunion Chikungunya Virus Isolates Differ in Their Capacity To Induce Proinflammatory Th1 and NK Cell Responses and Acute Joint Pathology. *Journal of Virology*, 2015, 89, 7955-7969.

Thiberville SD, Moyen N, Dupuis-Maguiraga L, Nougairede A, Gould EA, Roques P, de Lamballerie X. Chikungunya fever: epidemiology, clinical syndrome, pathogenesis and therapy. *Antiviral Research*, 2013, 99, 345-370.

Tiwari M, Parida M, Santhosh SR, Khan M, Dash PK, Rao PV. Assessment of immunogenic potential of Vero adapted formalin inactivated vaccine derived from novel ECSA genotype of Chikungunya virus. *Vaccine*, 2009, 27, 2513-2522.

Tsetsarkin KA, Chen R, Leal G, Forrester N, Higgs S, Huang J, Weaver SC. Chikungunya virus emergence is constrained in Asia by lineage-specific adaptive landscapes. *Proceedings of the National Academy of Sciences of the United States of America*, 2011, 108, 7872-7877.

Vazeille M, Moutailler S, Coudrier D, Rousseaux C, Khun H, Huerre M, Thiria J, Dehecq JS, Fontenille D, Schuffenecker I, Despres P, Failloux AB. Two Chikungunya Isolates from the Outbreak of La Reunion (Indian Ocean) Exhibit Different Patterns of Infection in the Mosquito, *Aedes albopictus*. *PLoS ONE*, 2007, 2, e1168.

VectorBase, 2017, <https://www.vectorbase.org>, accessed 7 May, 2018.

Volk SM, Chen R, Tsetsarkin KA, Adams AP, Garcia TI, Sall AA, Nasar F, Schuh AJ, Holmes EC, Higgs S, Maharaj PD, Brault AC, Weaver SC. Genome-Scale Phylogenetic

Analyses of Chikungunya Virus Reveal Independent Emergences of Recent Epidemics and Various Evolutionary Rates . *Journal of Virology*, 2010, 84, 6497-6504.

Wang D, Suhrbier A, Penn-Nicholson A, Woraratanadharm J, Gardner J, Luo M, Le TT, Anraku I, Sakalian M, Einfeld D, Dong JY. A complex adenovirus vaccine against chikungunya virus provides complete protection against viraemia and arthritis. *Vaccine*, 2011, 29, 2803-2809.

Wang E, Volkova E, Adams Ap, Forrester N, Xiao S-Y, Frolov I, Weaver Sc. Chimeric Alphavirus Vaccine Candidates For Chikungunya. *Vaccine*, 2008, 26, 5030-5039.

Wauquier N, Becquart P, Nkoghe D, Padilla C, Ndjoyi-Mbiguino A, Leroy EM. The Acute Phase of Chikungunya Virus Infection in Humans Is Associated With Strong Innate Immunity and T CD8 Cell Activation. *The Journal of Infectious Diseases*, 2011, 204, 115-123.

Weaver SC, Forrester NL. Chikungunya: Evolutionary history and recent epidemic spread. *Antiviral Research*, 2015, 120, 32-39.

Weaver SC, Lecuit M. Chikungunya virus and the global spread of a mosquito-borne disease. *The New England Journal of Medicine*, 2015 , 26, 1231-1239.

Weaver SC. Urbanization and geographic expansion of zoonotic arboviral diseases: mechanisms and potential strategies for prevention. *Trends in microbiology*, 2013, 21, 360-363.

Werneke SW, Schilte C, Rohatgi A, Monte KJ, Michault A, Arenzana-Seisdedos F, Vanlandingham DL, Higgs S, Fontanet A, Albert ML, Lenschow DJ. ISG15 Is Critical in the Control of Chikungunya Virus Infection Independent of UbE1L Mediated Conjugation. *PLoS Pathogens*, 2011, 7, e1002322.

Wikan N, Sakoonwatanyoo P, Ubol S, Yoksan S, Smith DR. Chikungunya Virus Infection of Cell Lines: Analysis of the East, Central and South African Lineage. *PLoS ONE*, 2012, 7, e31102.

World Health Organisation, 2017, <http://www.who.int/>, accessed 18 June, 2018.

World Health Organization, Regional Office for South-East Asia. Guidelines for prevention and control of chikungunya fever, 2009, <http://www.who.int>, accessed 5 May, 2018.

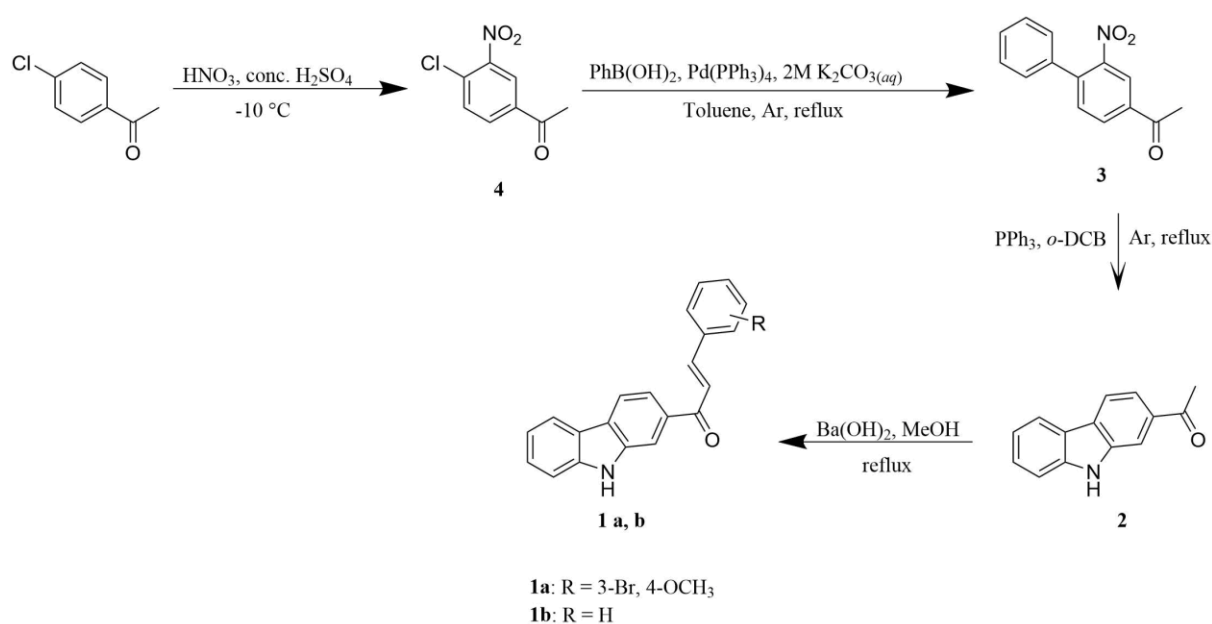
Xu Z, Li J, Wu Y, Sun Z, Luo L, Hu Z, He S, Zheng J, Zhang H, Zhang X. Design, synthesis, and evaluation of potent Wnt signaling inhibitors featuring a fused 3-ring system. *European Journal of Medicinal Chemistry*, 2016, 108, 154-165.

Yap G, Pok K-Y, Lai Y-L, Hapuarachchi H-C, Chow A, Leo Y-S, Tan L-K, Ng L-C. Evaluation of Chikungunya Diagnostic Assays: Differences in Sensitivity of Serology Assays in Two Independent Outbreaks. *PLoS Neglected Tropical Diseases*, 2010, 4, e753.

7. SUMMARY

The worldwide impact of Chikungunya virus (CHIKV) and the emergence of new transmission strains in Europe and Americas shows that arthritogenic *Alphavirus* infection epidemics pose a serious public health issue. There are no vaccines or antivirals available for the prevention or treatment of CHIKV infection, and most of the treatment regimens are symptomatic and based on the clinical manifestations. The purpose of this thesis was to synthesize new carbonyl compounds that could act as CHIKV inhibitors.

Compound **4** was prepared by nitration with concentrated sulfuric acid using the commercially available 4'-chloroacetophenone. Compound **3** was prepared using Suzuki-Miyaura cross coupling reaction, while compound **2** was prepared using PPh₃-mediated reductive cyclization. Both of the compounds **1a** and **1b** were prepared with base catalyzed aldol reaction, using the previously synthesized compound **2** and commercially available benzaldehyde and 3-bromo-4-methoxybenzaldehyde. The structures of the compounds were confirmed by usual analytical and spectroscopic methods (IR and ¹H NMR spectroscopy).



Key words: chikungunya virus, carbonyl, inhibitor, synthesis

8. STRUKTURIRANI SAŽETAK

8.1. UVOD

Chikungunya virus (CHIKV) je artritogeni *Alphavirus* iz obitelji *Togoviridae*, koji se uobičajeno prenosi putem artropodnog vektora. Prva epidemija CHIKV prepoznata je u današnjoj Tanzaniji, 1952., no u 2005., CHIKV se proširila na otoke u Indijskom oceanu, a između siječnja 2001. i travnja 2007., Indonezija je zabilježila 15207 CHIKV slučajeva. Slučajevi Chikungunya groznice su se počeli bilježiti unutar Europe (Italija, UK, Belgija, Njemačka, Češka Republika, Norveška, Španjolska, Francuska), ali i u Hong Kongu, Kanadi, Tajvanu, Šri Lanci i SAD-u. Do 26. rujna 2017., zabilježeno je 183 slučaja u Lazio regiji Italije; 109 su potvrđeni slučajevi Chikungunya groznice, a dodatnih 74 se još uvijek istražuje. Vjeruje se da su epidemije CHIKV uzrokovane prijelazom enzootskih infekcija na osobe naseljene u šumskim prostorima ili u neposrednim selima. Tome prijelazu slijedi transport u urbane centre koji su nastanjeni vektorima, *Aedes aegypti* i/ili *Aedes albopictus*. Infekcija CHIKV se događa putem kože, inokulacijom od zaraženih komarca. Može zahvatiti razne stanične linije, poput endotelnih, epitelnih i fibroblastnih primarnih stanica. CHIKV epidemije pokazuju cikličke i sezonske trendove. Postoji inter-epidemički period od 4 do 8 godina, koji ponekad zna trajati i 20 godina. Klinička prezentacija bolesti se može podijeliti na akutnu infekciju, tešku akutnu infekciju te kroničnu infekciju. CHIKV se obično dijagnosticira putem kliničkih, epidemioloških i laboratorijskih kriterija. Trenutna terapija CHIKV se sastoji od upotrebe analgetika, antipiretika te protu-upalnih lijekova, a cjepiva i antivirusici za prevenciju ili liječenje CHIKV još uvijek nisu razvijena. Za evaluaciju antiviralnih spojeva, možemo koristiti virusne vektore, testove citopatogenog učinka, ili modele imunodeficientnih miševa. Neki antivirusici koji su pokazali obećavajuće rezultate protiv CHIKV u ljudi uključuju širokospektralne antivirusike poput klorokina te ribavirina (u kombinaciji s interferonom). Također, istraživanja staničnih faktora su pokazala da se mogu koristiti kao baza za budući razvoj strategija za kontrolu imunološkog odgovora. Glavni problemi kod nedostatka razvoja cjepiva su potreba za dodatnom *in vivo* i kliničkim istraživanjima, te reduciranje troškova.

8.2. OBRAZLOŽENJE TEME

MTS test koristeći Vero staničnu liniju je proveden na Rega Institutu za medicinsko istraživanje, KU Leuven u Belgiji, kako bi se identificirale strukture potencijalnih inhibitora CHIKV. MTS test se temelji na redukciji MTS tetrazolium supstrata u obojeni formazan u prisutnosti fenazin metosulfata (PMS) pomoću NAD(P)H-ovisnih dehidrogenaza u

metabolički aktivnim stanicama. Kvantifikacija obojenog formazana se vrši mjerenjem apsorpcije na valnoj duljini od 490 nm. Spoj 3-(3-bromo-4-hidroksifenil)-1-(9*H*-karbazol-2-il)prop-2-en-1-on (**5**) je pokazao najprikladnija svojstva, prema maksimalnoj inhibitornoj koncentraciji (*IC*₅₀) od 4,3 µg/ml. Cilj ovog rada je bio sintetizirati dva nova derivata spoja **5**. Kod prvog derivata, 3-(2-bromo-4-metoksifenil)-1-(9*H*-karbazol-2-il)prop-2-en-1-on (**1a**), s uvođenjem metoksi grupe se željela ispitati važnost učinka hidroksi grupe u aktivnosti cijele molekule, budući da je ona sklona mnogim metaboličkim reakcijama. Kod drugog derivata, 1-(9*H*-karbazol-2-il)-3-fenilprop-2-en-1-on (**1b**), sintetizirana je molekula bez funkcionalnih grupa na benzenskom prstenu, kako bi se istražilo utječe li nedostatak tih grupa na aktivnost molekule u metabolizmu.

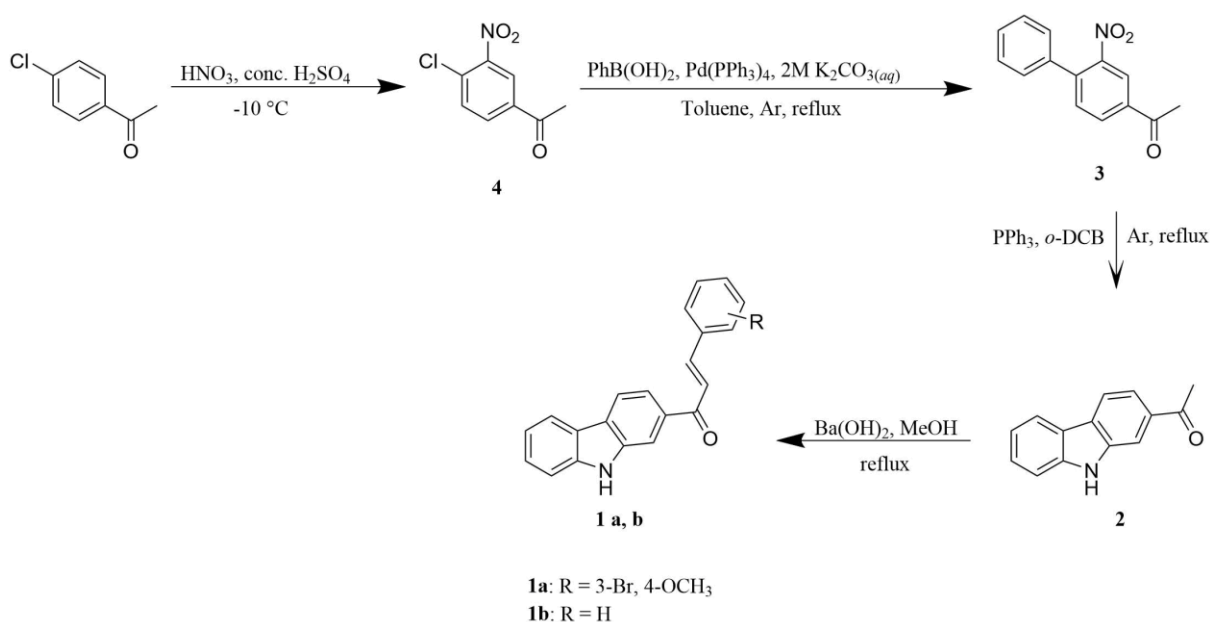
8.3. MATERIJALI I METODE

Tališta su određena na Bobby Stuart Scientific (SMP1) uređaju i nisu korigirana. IR spektri su snimljeni na PerkinElmer Spectrum-One spektrometru. ¹H NMR spektri su snimljeni na Bruker AC 400 Ultrashield 10 spektrometru (400 MHz). Uzorci su mjereni u DMSO-*d*₆ (kod 44, 139-2) otopinama izotopne čistoće (Aldrich). Kemijski pomaci (δ) dani su u ppm u odnosu na tetrametilsilan (TMS) kao unutarnji standard. Kromatografije na koloni su izvedene na silikagel (Merck; 70-230 mesh) koloni, a kloroform/*n*-heksan (7:3), kloroform/etanol (99:1), kloroform/acetona (95:5), kloroform/etil-acetat (85:15), diklormetan/*n*-heksan/etanol (98:2:1), diklormetan/petroleter ili *n*-heksan (6:4), petroleter/etil-acetat (7:3) and *n*-heksan/etil-acetat (6:4) su korištene kao mobilne faze. Svi spojevi su rutinski kontrolirani na TLC-u, uz upotrebu silikagel ploča (Fluka DC-Alufohlen Kieselgel 60 F254), uz kloroform/metanol (96:4), kloroform/acetona (95:5) i diklormetan/petroleter (6:4) kao mobilne faze. Tvari su vizualizirane pod UV svjetom. Koncentracija otopina nakon reakcija i ekstrakcija je uključivala korištenje rotavapora (Büchi), uz sniženi tlak (~20 Torr). 2 molarna otopina K₂CO₃ je pripravljena otapanjem K₂CO₃ u destiliranoj vodi, te je bez daljnjeg čišćenja upotrijebljena u sljedećem reakcijskom koraku. Ostale kemikalije nabavljene su od tvrtke Sigma-Aldrich. Kemikalije su bile *p.a.* čistoće te su bile pročišćene i osušene standardnim metodama, po potrebi. Organska otapala su osušena pomoću bezvodnog natrijevog sulfata (Merck). Sve reakcije su provedene u inertnoj atmosferi argona.

8.4. REZULTATI, RASPRAVA I ZAKLJUČAK

Početni spoj u reakcijskom mehanizmu je 4'-kloracetofenon, koji je komercijalno dostupan. Sinteza derivata 2-halonitrobenzena (**4**) je elektrofilna supstitucija nitracije između benzenskog prstena 4'-kloracetofenona i dušične kiseline, koristeći koncentriranu sumpornu kiselinu kao katalizator. Derivat 2-nitrobifenila (**3**) je sintetiziran pomoću Suzuki-Miyaura reakcije. Nastajanje jednostruke veze između dva atoma ugljika je postignuto spajanjem fenilboronične kiseline i 2-halonitrobenzena (**4**) koristeći $\text{Pd}(\text{PPh}_3)_4$ kao katalizator i K_2CO_3 kao bazu, dok je toluen korišten kao otapalo. Derivat karbazola (**2**) je sintetiziran koristeći PPh_3 -potpomognutu reduktivnu ciklizaciju 2-nitrobifenila (**3**) u 1,2-diklorbenzenu. Željeni produkti (**1a**, **1b**) su dobiveni bazičnom aldolnom kondenzacijom s benzaldehidom i 3-bromo-4-metoksibenzaldehidom, koji su komercijalno dostupni.

Strukture svih sintetiziranih spojeva potvrđene su uobičajenim spektroskopskim metodama (IR, ^1H NMR) te je svim čvrstim produktima određeno talište. Sintetiziranim derivatima karbonila u daljnjim će istraživanjima biti ispitano anti-CHIKV djelovanje.



Ključne riječi: chikungunya virus, karbonil, inhibitor, sinteza

9. APPENDIX

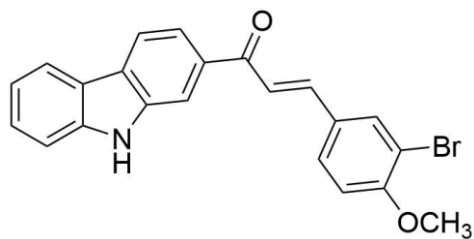


Figure 8. Structure of 3-(3-bromo-4-methoxyphenyl)-1-(9*H*-carbazol-2-yl)prop-2-en-1-one (**1a**)

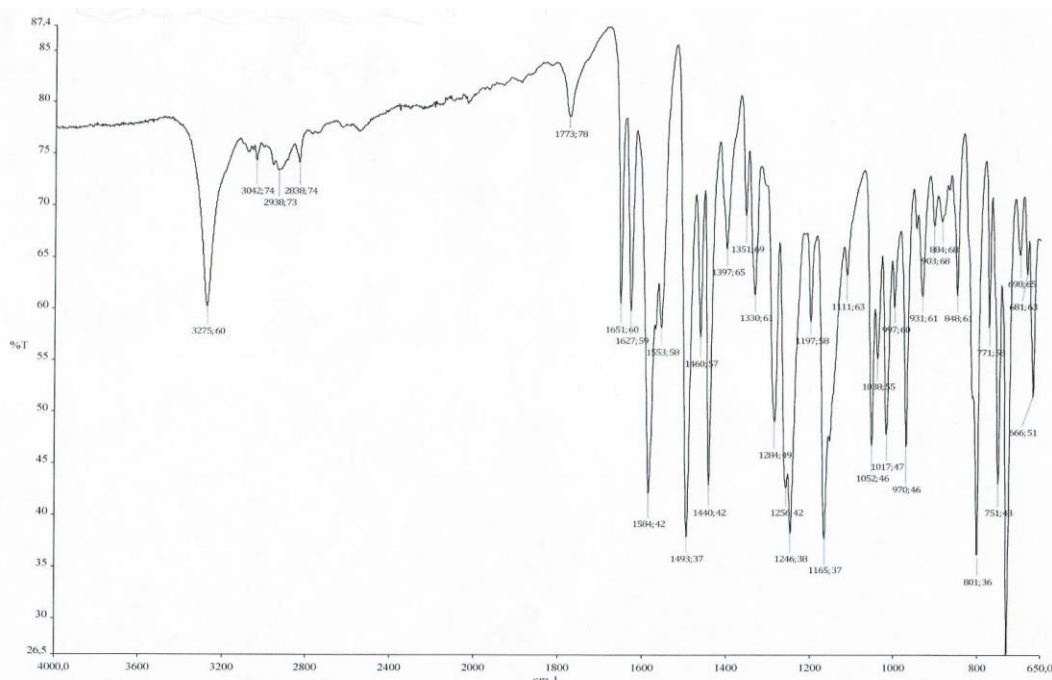


Figure 9. IR spectrum of compound **1a**

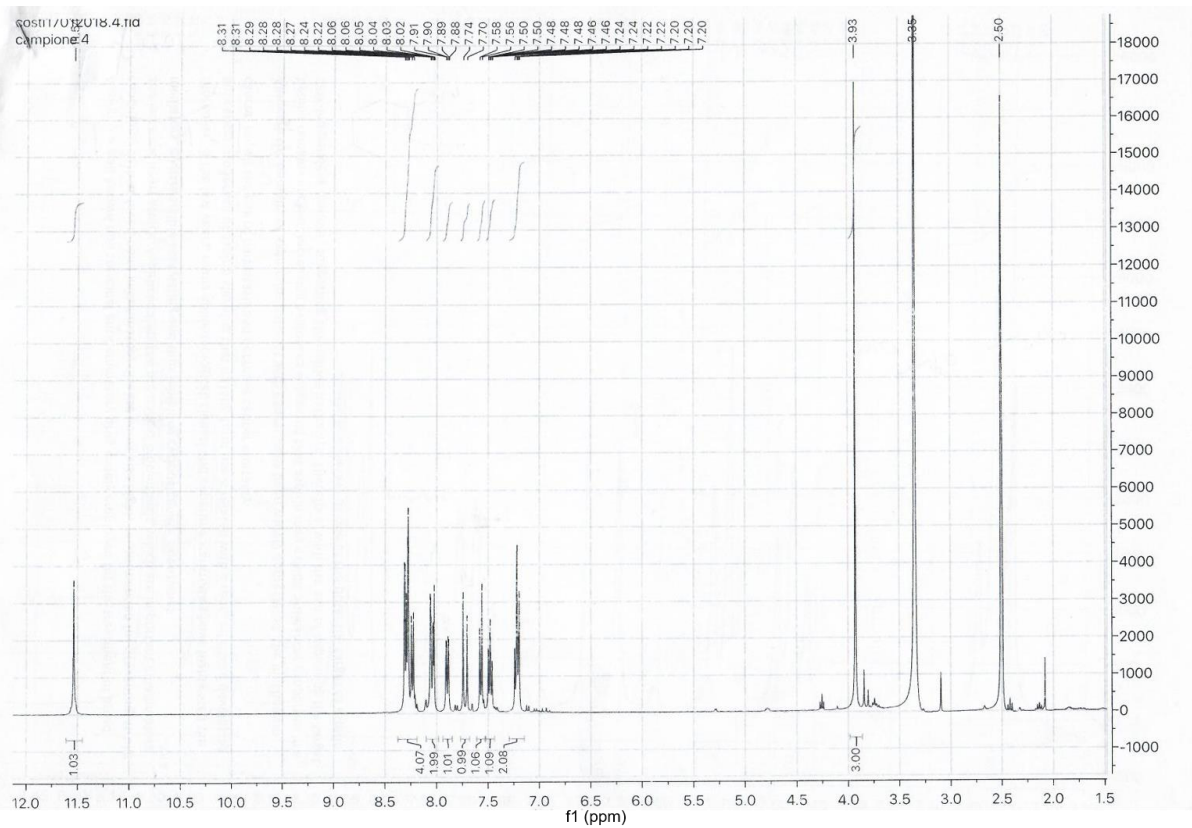


Figure 10. ^1H NMR spectrum of compound **1a**

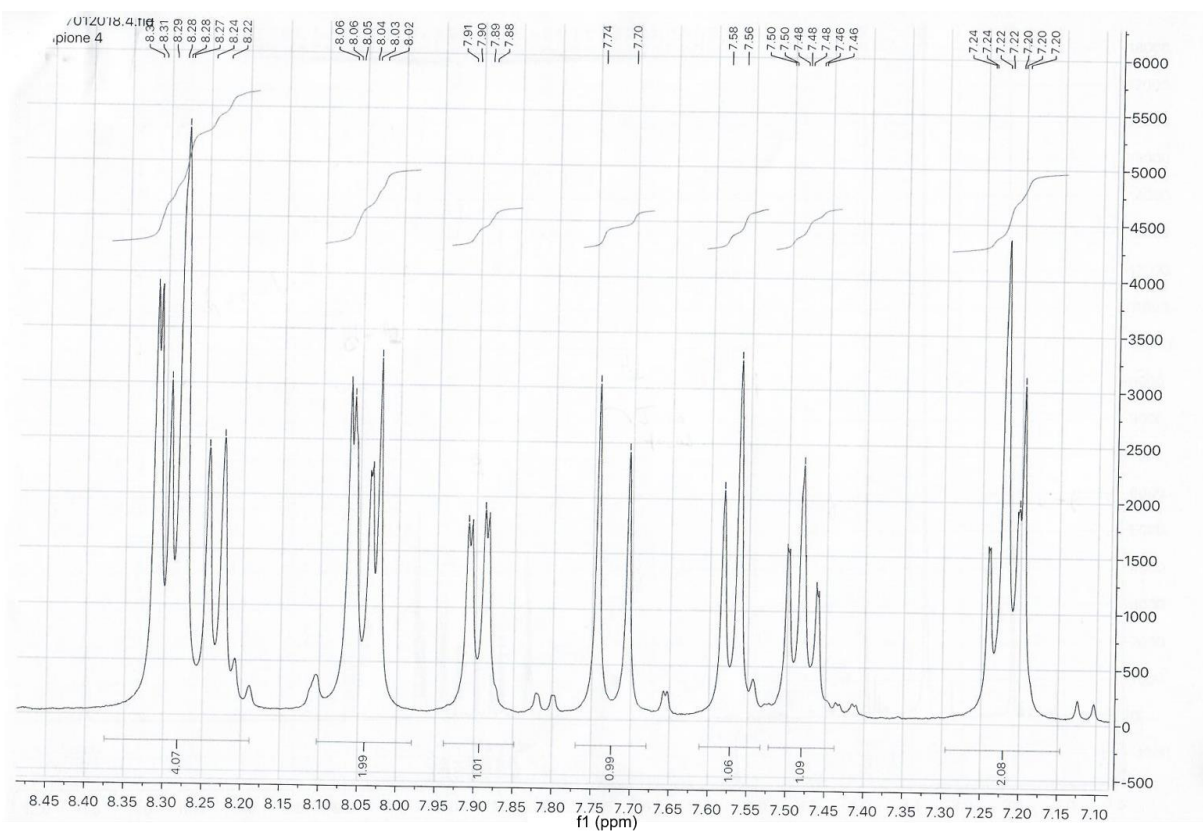


Figure 11. ^1H NMR spectrum of compound **1a**

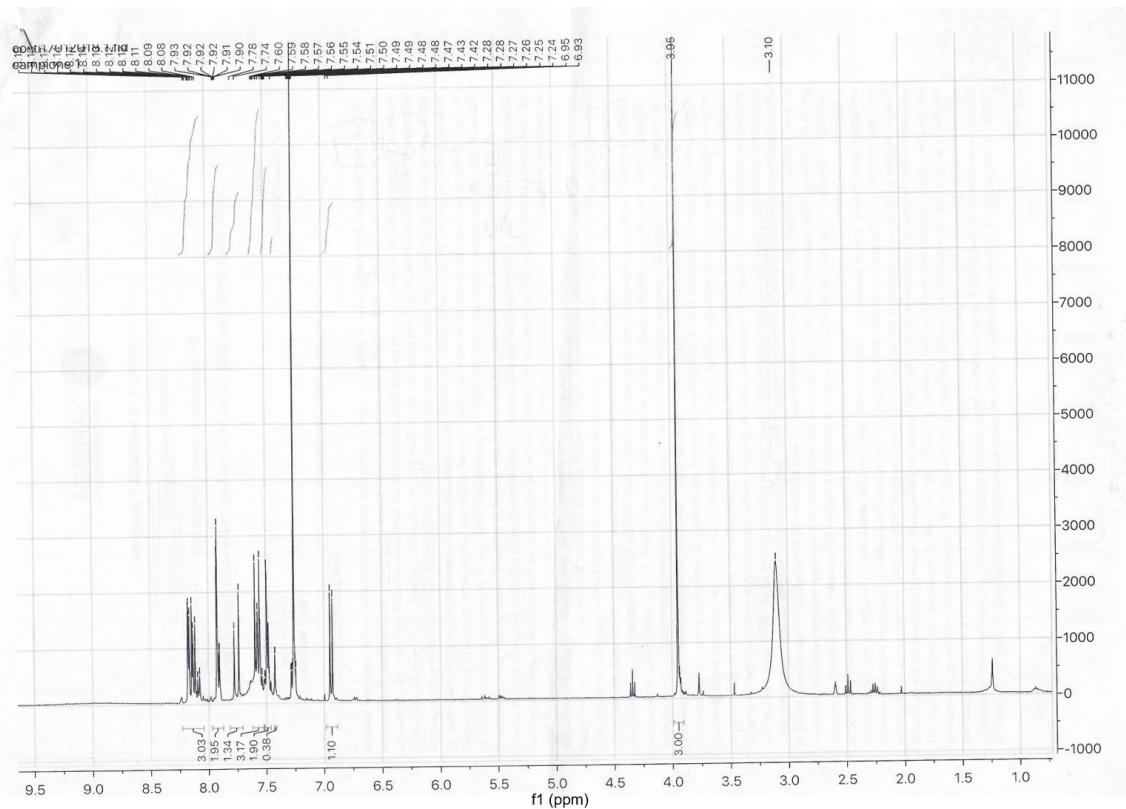


Figure 12. ^1H NMR spectrum of compound **1a**

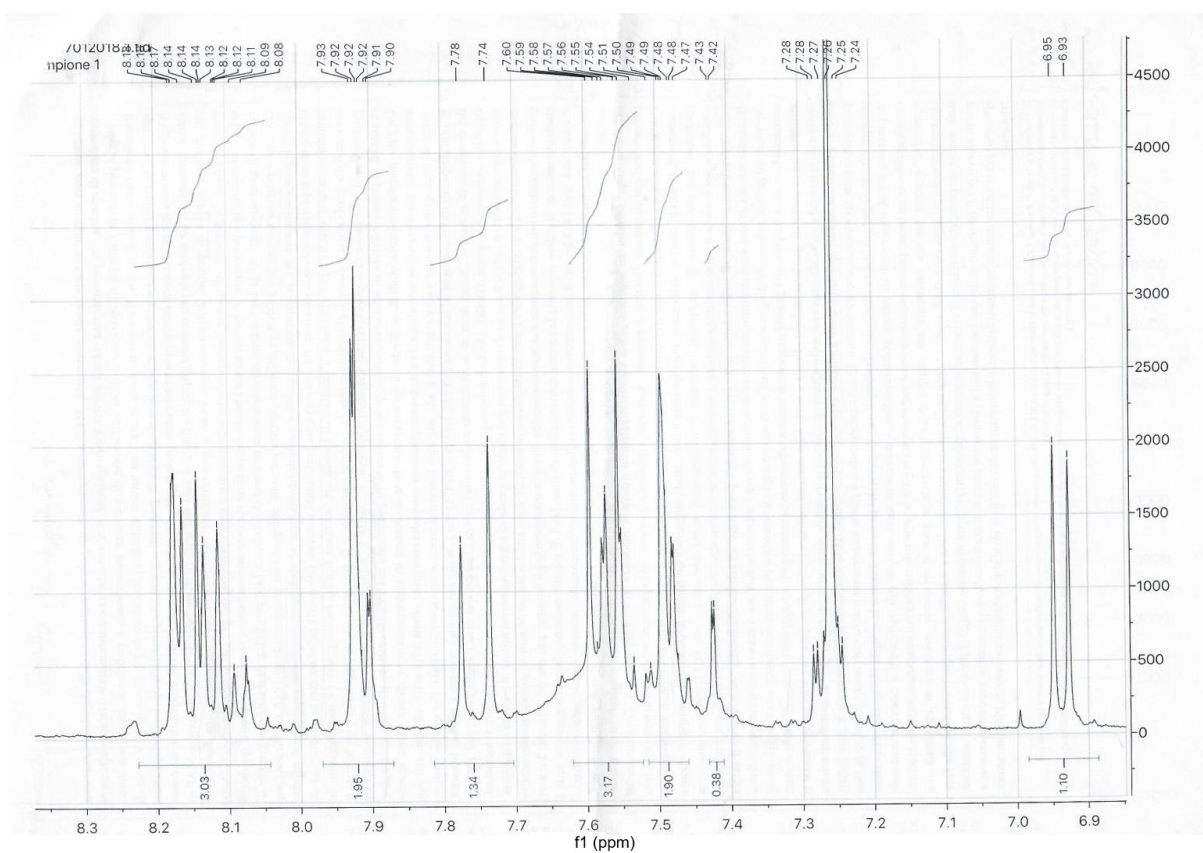


Figure 13. ^1H NMR spectrum of compound **1a**

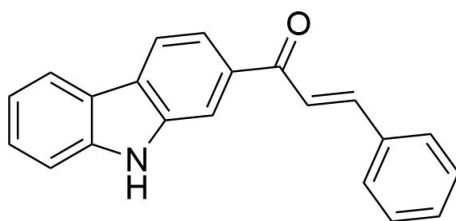


Figure 14. Structure of 1-(9*H*-carbazol-2-yl)-3-phenylprop-2-en-1-one (**1b**)

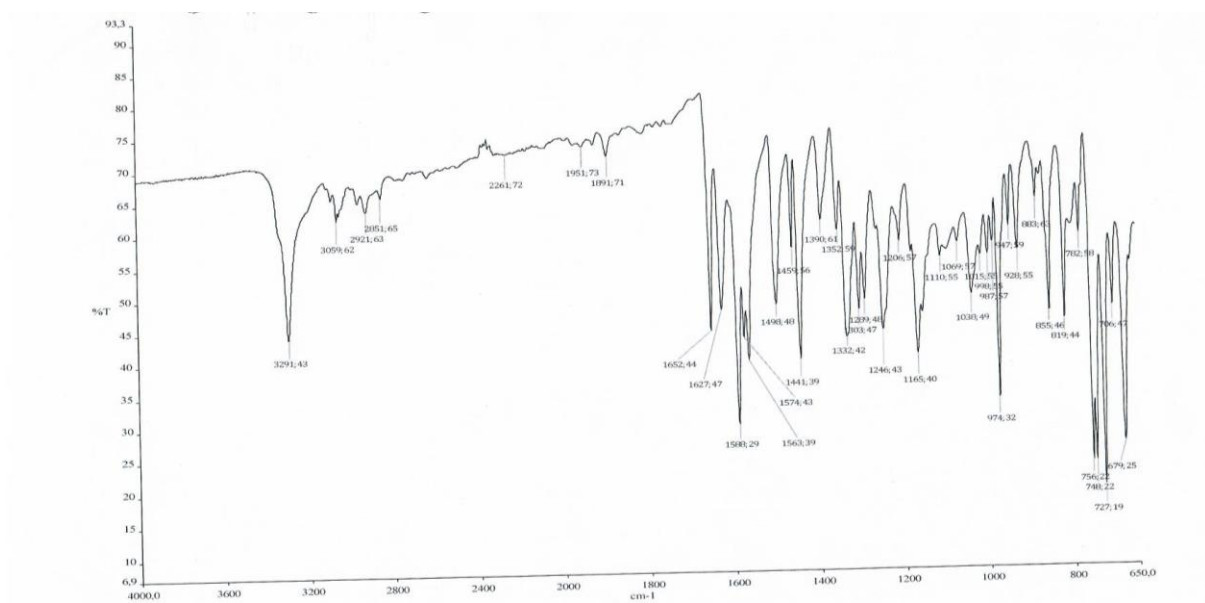


Figure 15. IR spectrum of compound **1b**

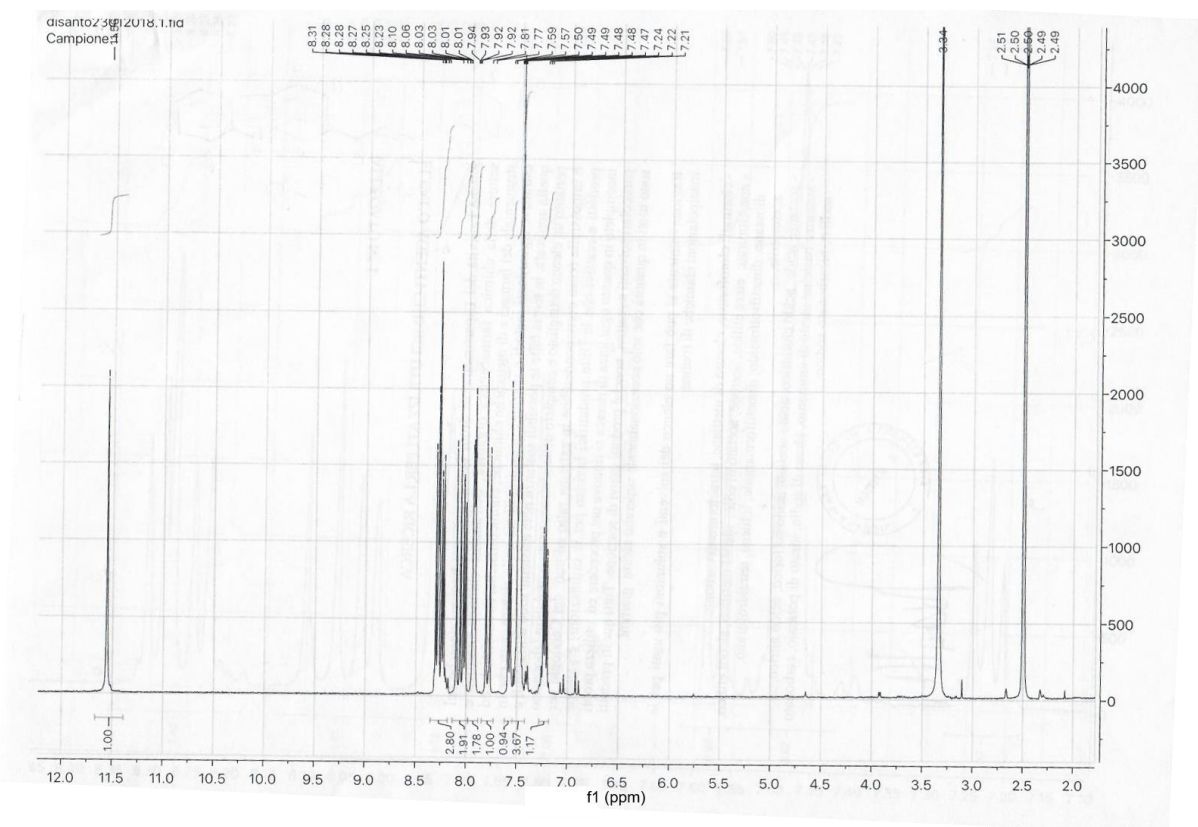


Figure 16. ^1H NMR spectrum of compound **1b**

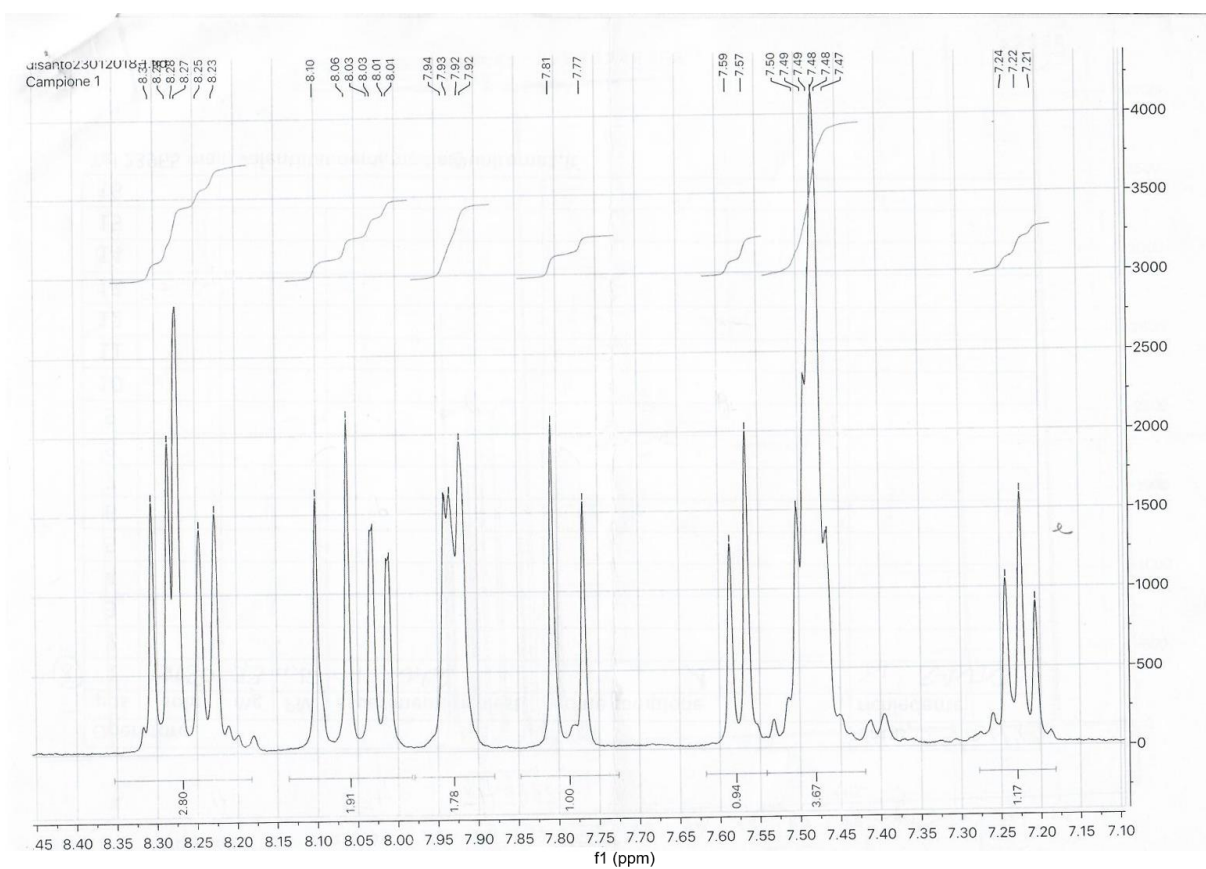


Figure 17. ^1H NMR spectrum of compound **1b**

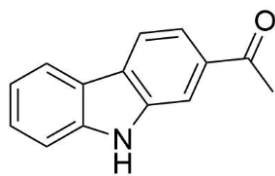


Figure 18. Structure of 1-(9*H*-carbazol-2-yl)ethan-1-one (**2**)

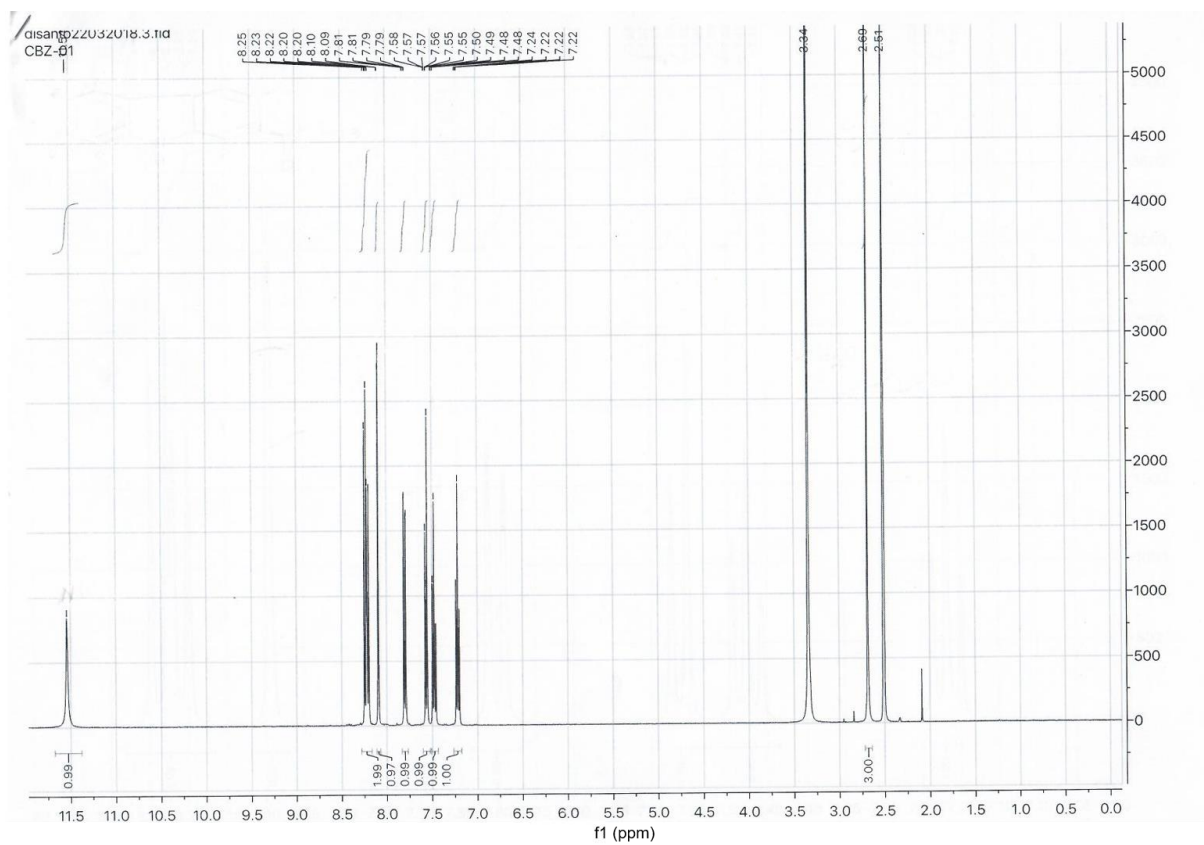


Figure 19. ¹H NMR spectrum of compound **2**

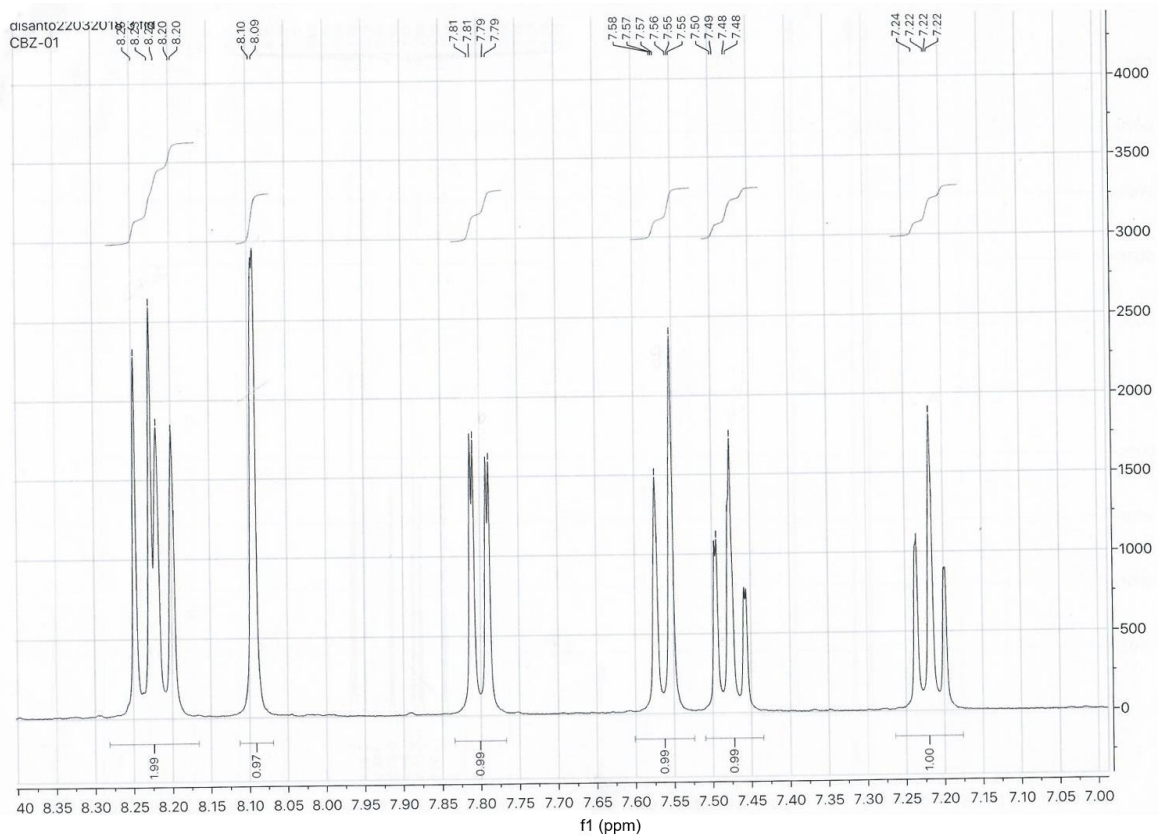


Figure 20. ¹H NMR spectrum of compound 2

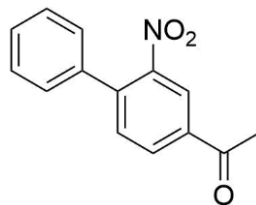


Figure 21. Structure of 1-(2-nitro-[1,1'-biphenyl]-4-yl)ethan-1-one (**3**)

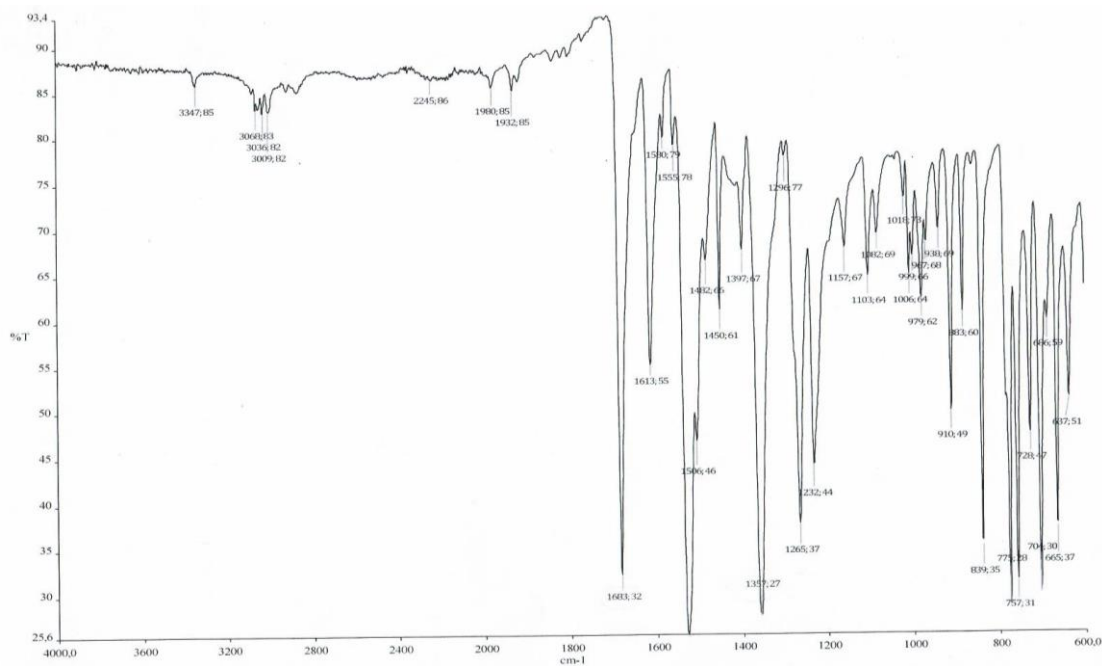


Figure 22. IR spectrum of compound **3**

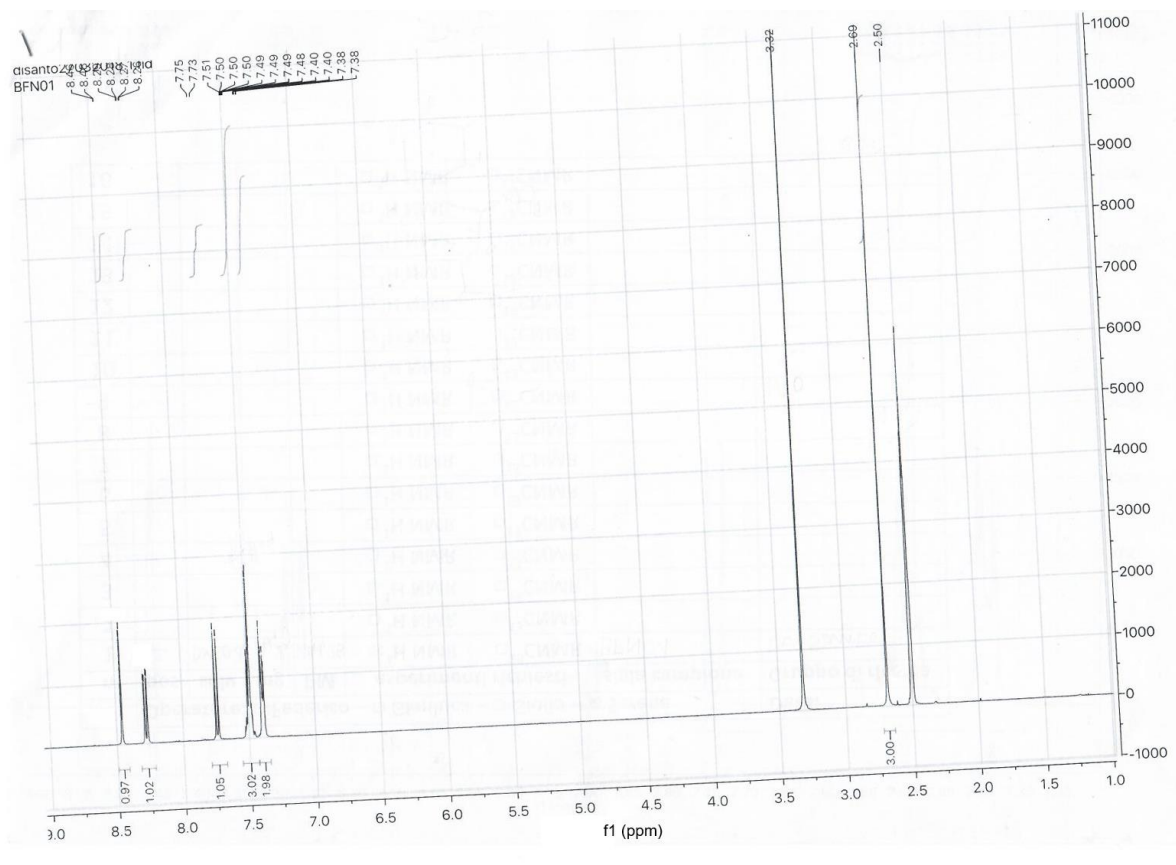


Figure 23. ¹H NMR spectrum of compound 3

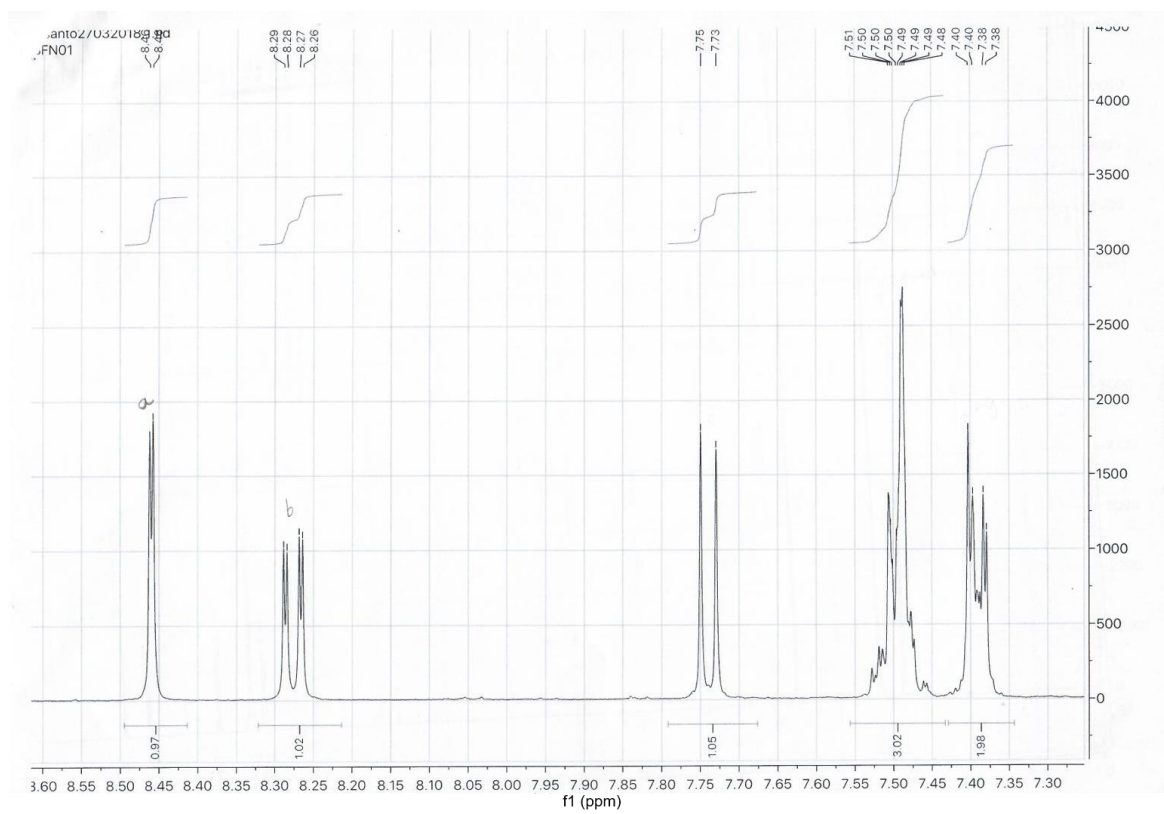


Figure 24. ¹H NMR spectrum of compound 3

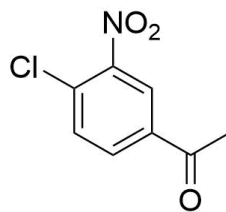


Figure 25. Structure of 1-(4-chloro-3-nitrophenyl)ethan-1-one (**4**)

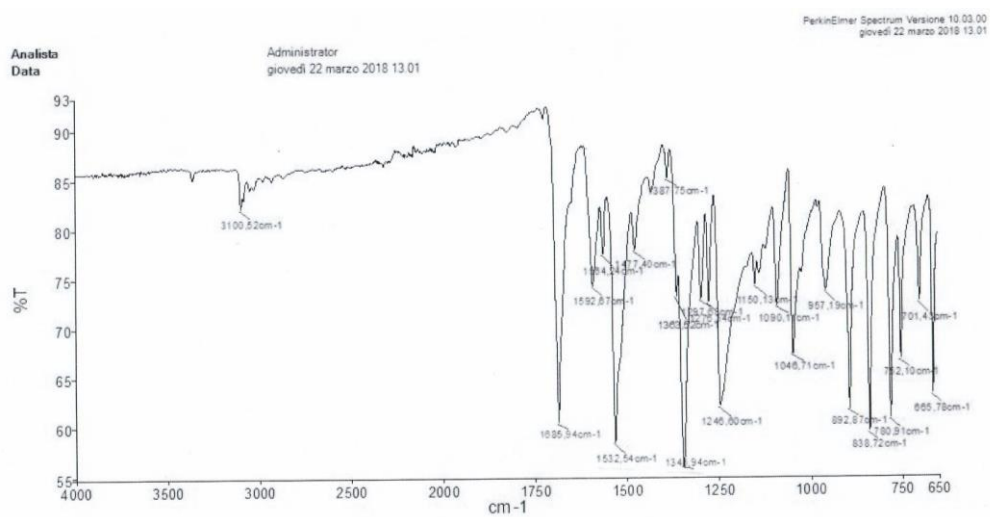


Figure 26. IR spectrum of compound **4**

Basic documentation card

University of Zagreb
Faculty of Pharmacy and Biochemistry
Study: Master of Pharmacy
Department of Medicinal Chemistry
A. Kovačića 1, 10000 Zagreb, Croatia

Diploma thesis

DESIGN AND SYNTHESIS OF NEW SMALL MOLECULES AS CHIKUNGUNYA VIRUS INHIBITORS

Tajana Iva Pejaković

SUMMARY

The worldwide impact of Chikungunya virus (CHIKV) and the emergence of new transmission strains in Europe and Americas shows that arthritogenic *Alphaviruses* infections pose a serious public health issue. There are no vaccines or antivirals available for the prevention or treatment of CHIKV infection, and most of the treatment regimens are symptomatic and based on the clinical manifestations. The purpose of this thesis was to synthesize new carbonyl compounds that could act as CHIKV inhibitors. Compound **4** was prepared by nitration with concentrated sulfuric acid using the commercially available 4'-chloroacetophenone. Compound **3** was prepared using Suzuki-Miyaura cross coupling reaction, while compound **2** was prepared using PPh₃-mediated reductive cyclization. Both of the compounds **1a** and **1b** were prepared with base catalyzed aldol reaction, using the previously synthesized compound **2** and commercially available benzaldehyde and 3-bromo-4-methoxybenzaldehyde. The structures of the compounds were confirmed by usual analytical and spectroscopic methods (IR and ¹H NMR spectroscopy).

The thesis is deposited in the Central Library of the University of Zagreb Faculty of Pharmacy and Biochemistry.

Thesis includes: 64 pages, 28 figures, 6 tables and 101 references. Original is in English language.

Keywords: chikungunya virus, carbonyl, inhibitor, synthesis

Mentor: **Mirza Bojić, Ph.D.** *Assistant Professor*, University of Zagreb Faculty of Pharmacy and Biochemistry

Reviewers: **Mirza Bojić, Ph.D.** *Assistant Professor*, University of Zagreb Faculty of Pharmacy and Biochemistry

Željko Maleš, Ph.D. *Full Professor*, University of Zagreb Faculty of Pharmacy and Biochemistry

Mirela Matić, Ph.D. *Assistant Professor*, University of Zagreb Faculty of Pharmacy and Biochemistry

The thesis was accepted: July 2018

Temeljna dokumentacijska kartica

Sveučilište u Zagrebu
Farmaceutsko-biokemijski fakultet
Studij: Farmacija
Zavod za farmaceutsku kemiju
A. Kovačića 1, 10000 Zagreb, Hrvatska

Diplomski rad

DIZAJN I SINTEZA NOVIH MALIH MOLEKULA KAO INHIBITORA CHIKUNGUNYA VIRUSA

Tajana Iva Pejaković

SAŽETAK

Utjecaj Chikungunya virusa (CHIKV) širom svijeta te pojavljivanje novih prijenosnih sojeva u Europi i Americi pokazuje koliko ozbiljni javnozdravstveni problem predstavljaju infekcije artritogenim *Alphavirus*-ima. Ne postoji cjepivo niti antivirusik na tržištu koji bi se koristio u prevenciji ili liječenju CHIKV infekcije, a većina terapije je simptomatska i temelji se na kliničkim manifestacijama. Cilj ovog rada je bio sintetizirati nove derivate karbonila koji bi mogli djelovati kao CHIKV inhibitori. Spoj **4** je pripremljen nitracijom komercijalno dostupnog 4'-kloracetofenona koristeći koncentriranu sumpornu kiselinu. Spoj **3** pripremljen je Suzuki-Miyaura reakcijskim mehanizmom, dok je spoj **2** pripremljen uz PPh_3 -potpomognutu reduktivnu ciklizaciju. Spojevi **1a** i **1b** su sintetizirani bazičnom aldolnom kondenzacijom, koristeći prethodno pripremljeni spoj **2** i komercijalno dostupni benzaldehid i 3-bromo-4-metoksibenzaldehid. Strukture svih sintetiziranih spojeva potvrđene su uobičajenim spektroskopskim metodama (IR, ^1H NMR).

Rad je pohranjen u Središnjoj knjižnici Sveučilišta u Zagrebu Farmaceutsko-biokemijskog fakulteta.

Rad sadrži: 64 stranica, 28 grafičkih prikaza, 6 tablica i 101 literaturni navod. Izvornik je na engleskom jeziku.

Ključne riječi: chikungunya virus, karbonil, inhibitor, sinteza

Mentor: **Dr. sc. Mirza Bojić**, *docent Sveučilišta u Zagrebu Farmaceutsko-biokemijskog fakulteta.*

Ocjenjivači: **Dr. sc. Mirza Bojić**, *docent Sveučilišta u Zagrebu Farmaceutsko-biokemijskog fakulteta.*

Dr. sc. Željko Maleš, *redoviti profesor Sveučilišta u Zagrebu Farmaceutsko-biokemijskog fakulteta.*

Dr. sc. Mirela Matić, *docentica Sveučilišta u Zagrebu Farmaceutsko-biokemijskog fakulteta.*

Rad prihvaćen: srpanj 2018.