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Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

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Download date / Datum preuzimanja: 2020-10-02

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Novel Ureidoamides Derived from Amino Acids: Synthesis and Preliminary Biological Screening

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RECEIVED JULY 17, 2009; REVISED OCTOBER 12, 2009; ACCEPTED OCTOBER 13, 2009

Abstract. A series of novel amino acid ureidoamides 4a–o were prepared from N-(1-benzotriazole-carbonyl)-amino acid chloride 3, derived from L-alanine, L-valine, L-leucine, D-phenylglycine and L-phenylalanine, and the corresponding aminoalcohols (2-amino-1-ethanol, 3-amino-1-propanol and 5-amino-1-pentanol). The compounds were fully characterized by standard spectroscopic methods (IR, 1H and 13C NMR) and their structure was confirmed by elemental analysis. Antioxidant screenings (interaction with 1,1-diphenyl-2-picrylhydrazyl, soybean lipoxgenase inhibition activity, inhibition of linoleic acid lipid peroxidation) revealed that the prepared compounds possessed only modest activity. On the other hand, no significant antiproliferative activity against five human cell lines and very weak antimicrobial activity against several bacteria and fungi were detected.

Keywords: urea, amide, amino acid, benzotriazole, hydantoin, hydroxylamine, antioxidant activity

INTRODUCTION

Benzotriazole is a very useful synthetic auxiliary with versatile applications in organic chemistry. Since 1985, Katritzky and collaborators have published more than 300 papers and several reviews dealing with benzotriazole.1–7 Our group started benzotriazole chemistry research in 1977 and the results have been published in approximately 30 papers.8–11 We have used benzotriazole in the synthesis of various heterocyclic compounds (benzoxazine, quinazoline, triazinetrione, hydantoin and oxadiazine derivatives), amino acid derivatives, polymer-drug and thiomert-drug conjugates, carbamates, ureas, semicarbazides, carbazides, sulfonylureas, sulfonylcarbazides, nitroalkanic acid esters, hydantoic acids, etc. This paper is a continuation of two previous papers dealing with hydroxyurea derived from amino acid amides bearing benzotriazole moiety.12,13 Some of the synthesized hydroxyureas showed significant cytostatic activity and we found it worth preparing a series of analogue compounds with hydroxyl group detached from urea nitrogen with two or more methylene spacers. Their synthesis and preliminary biological screening are reported herein.

EXPERIMENTAL

Materials and Methods

Melting points were determined on a Stuart SMP 3 melting apparatus and were uncorrected. IR spectra (ν\text{max}/cm\text{–1}) were recorded on a FTIR Perkin Elmer Paragon 500 spectrometer and UV/VIS spectra on a Varian Cary 100Bio UV-visible spectrometer. 1H and 13C NMR spectra were taken on a Varian Gemini 300 spectrometer, operating at 300 and 75.5 MHz for the 1H and 13C nuclei, respectively. Samples were measured in DMSO-d6 solutions at 20°C in 5 mm NMR tubes. Chemical shifts (δ ppm) were referred to TMS. Precoated silica gel 60 F254 plates were used for thin-layer chromatography. Solvent systems were CH2Cl2/MeOH = 9:1 and 4:1. Spots were visualized by phosphomolybdic acid. Column chromatography was performed on silica gel (0.063–0.200 mm) with CH2Cl2/MeOH (9:1 and 8.5:1.5 and 4:1) and CHCl3/MeOH = 9.5:0.5 as eluents.

Amino acids (L-alanine, L-valine, L-leucine, D-phenylglycine and L-phenylalanine) were purchased from Kemika (Croatia), and amines (2-amino-1-ethanol, 3-amino-1-propanol, 5-amino-1-pentanol, hydroxylamine, O-benzylhydroxylamine and triethylamine...
The following text contains a detailed procedure for the preparation and characterization of novel ureidoamides derived from amino acids. The text describes the reaction conditions, yields, and spectroscopic data for various compounds. The document outlines a general procedure for the synthesis of these compounds, followed by specific examples of the preparation of several derivatives.

**Ureido Derivatives 4a-o. General Procedure**

A mixture of N-(1-benzotriazolocarbonyl)-amino acid chloride (3a-e) (1 mmol) and an appropriate amine (3 mmol) in 10 mL of dry dioxane was stirred at room temperature for 24 h and evaporated. Crude product 4a-o was purified by column chromatography and then triturated or recrystallized using the solvents given below.

1-((3-hydroxypropyl)urea)→1-(3-hydroxypropyl)urea

Crude product 4a was purified by column chromatography (eluent CH\(_2\)Cl\(_2\)/MeOH = 8.5:1.5→4:1) and triturated with Et\(_2\)O. Yield: 0.138 g (53 %); m.p. 178–181 °C; IR (KBr) \(\nu_{\text{max}}/\text{cm}^{-1}\): 3349, 3290, 3098, 2928, 2872, 1625, 1569, 1059, 1059.

**Crude product 4b**

Crude product 4b was purified by column chromatography (eluent CH\(_2\)Cl\(_2\)/MeOH = 4:1) and triturated with Et\(_2\)O. Yield: 0.180 g (59 %); m.p. 142–146 °C; IR (KBr) \(\nu_{\text{max}}/\text{cm}^{-1}\): 3360, 3282, 3111, 2936, 2862, 1627, 1561, 1056.

**Crude product 4c**

Crude product 4c was purified by column chromatography (eluent CH\(_2\)Cl\(_2\)/MeOH = 4:1) and triturated with Et\(_2\)O. Yield: 0.180 g (59 %); m.p. 142–146 °C; IR (KBr) \(\nu_{\text{max}}/\text{cm}^{-1}\): 3360, 3282, 3111, 2936, 2862, 1627, 1561, 1056.

**Crude product 4d**

Crude product 4d was purified by column chromatography (eluent CH\(_2\)Cl\(_2\)/MeOH = 8.5:1.5→4:1) and recrystallized from MeOH/Et\(_2\)O. Yield: 0.140 g (56 %); m.p. 194–198 °C; IR (KBr) \(\nu_{\text{max}}/\text{cm}^{-1}\): 3351, 3280, 3102, 2969, 2874, 1626, 1571, 1063.

**Crude product 4e**

Crude product 4e was purified by column chromatography (elu\(\text{ent CH}\(_2\)Cl\(_2\)/MeOH = 9:1→4:1) and recrystallized from MeOH/Et\(_2\)O. Yield: 0.154 g (56 %); m.p. 178–181 °C; IR (KBr) \(\nu_{\text{max}}/\text{cm}^{-1}\): 3350, 3279, 3102, 2963, 2874, 1630, 1566, 1234, 1078, 1044.

**Anal. Calcd. mass fractions of elements, w/%, for C\(_{12}\)H\(_{23}\)N\(_3\)O\(_4\) (M\(_r\) = 247.29) are: C 57.98, H 8.87, N 16.08; found: C 57.56, H 8.87, N 16.08.

**Crude product 4f**

Crude product 4f was purified by column chromatography (elu\(\text{ent CH}\(_2\)Cl\(_2\)/MeOH = 8.5:1.5) and triturated with Et\(_2\)O. Yield: 0.138 g (53 %); m.p. 157–161 °C; IR (KBr) \(\nu_{\text{max}}/\text{cm}^{-1}\): 3354, 3290, 3098, 2954, 2928, 2872, 1625, 1569, 1059.

**Anal. Calcd. mass fractions of elements, w/%, for C\(_{10}\)H\(_{17}\)N\(_3\)O\(_4\) (M\(_r\) = 303.40) are: C 55.62, H 9.47, N 14.02; found: C 55.62, H 9.47, N 14.02.

**Crude product 4g**

Crude product 4g was purified by column chromatography (elu\(\text{ent CH}\(_2\)Cl\(_2\)/MeOH = 8.5:1.5) and triturated with Et\(_2\)O. Yield: 0.150 g (52 %); m.p. 109–111 °C; IR (KBr) \(\nu_{\text{max}}/\text{cm}^{-1}\): 3351, 3308, 3098, 2957, 2872, 1620, 1572, 1252, 1073.

**Anal. Calcd. mass fractions of elements, w/%, for C\(_{11}\)H\(_{19}\)N\(_3\)O\(_4\) (M\(_r\) = 289.37) are: C 53.96, H 9.40, N 14.52; found: C 53.69, H 9.23, N 14.79.

**Crude product 4h**

Crude product 4h was purified by column chromatography (elu\(\text{ent CH}\(_2\)Cl\(_2\)/MeOH = 8.5:1.5) and triturated with Et\(_2\)O. Yield: 0.138 g (53 %); m.p. 157–161 °C; IR (KBr) \(\nu_{\text{max}}/\text{cm}^{-1}\): 3354, 3290, 3098, 2954, 2928, 2872, 1625, 1569, 1059.

**Anal. Calcd. mass fractions of elements, w/%, for C\(_{11}\)H\(_{17}\)N\(_3\)O\(_4\) (M\(_r\) = 303.40) are: C 55.62, H 9.47, N 14.02; found: C 55.62, H 9.47, N 14.02.

**Crude product 4i**

Crude product 4i was purified by column chromatography (elu\(\text{ent CH}\(_2\)Cl\(_2\)/MeOH = 8.5:1.5) and triturated with Et\(_2\)O. Yield: 0.270 g (78 %); m.p. 83–86 °C; IR (KBr) \(\nu_{\text{max}}/\text{cm}^{-1}\): 3354, 3290, 3098, 2954, 2928, 2872, 1625, 1569, 1059.

**Crude product 4j**

Crude product 4j was purified by column chromatography (elu\(\text{ent CH}\(_2\)Cl\(_2\)/MeOH = 8.5:1.5→4:1) and tritu-
Crude product 4k was purified by column chromatography (elucent CH2Cl2/MeOH = 8.5:1.5) and recrystallized from MeOH/Et2O. Yield: 0.222 g (61 %); m.p. 178–180 °C; IR (KBr) νmax/cm–1: 3362, 3286, 3092, 2942, 2883, 1626, 1561, 1059.

Anal. Calcd. mass fractions of elements, w/%, for C15H23N3O4 (Mr = 295.33) are: C 58.69, H 7.32, N 14.44.

1-(1-(2-Hydroxyethylcarbamoyl)-2-phenylethyl)-3-(2-hydroxyethyl)urea 4m

Crude product 4m was purified by column chromatography (elucent CH2Cl2/MeOH = 8.5:1.5). Yield: 0.230 g (78 %); m.p. 140–143 °C; IR (KBr) νmax/cm–1: 3377, 3310, 3105, 2937, 2876, 1626, 1569, 1496, 1231, 1056.

Anal. Calcd. mass fractions of elements, w/%, for C14H21N3O4 (Mr = 293.33) are: C 56.94, H 7.17, N 14.23; found: C 56.69, H 7.81, N 14.44.

1-(1-(2-Hydroxyethylcarbamoyl)-2-phenylethyl)-3-(2-hydroxyethyl)urea 4n

Crude product 4n was purified by column chromatography (elucent CH2Cl2/MeOH = 8.5:1.5). Yield: 0.243 g (75 %); m.p. 110–113 °C; IR (KBr) νmax/cm–1: 3358, 3280, 3106, 2942, 2878, 1628, 1564, 1235, 1050.


1-(1-(5-Hydroxypentylcarbamoyl)(phenyl)methyl)-3-(5-hydroxypropyl)urea 4o

Crude product 4o was purified by column chromatography (elucent CH2Cl2/MeOH = 9:1) and trituration with Et2O. Yield: 0.197 g (52 %); m.p. 89–93 °C; IR (KBr) νmax/cm–1: 3362, 3282, 3106, 2931, 2859, 1626, 1559, 1236, 1060.

Anal. Calcd. mass fractions of elements, w/%, for C20H33N3O4 (Mr = 365.47) are: C 62.44, H 8.55, N 11.50; found: C 62.65, H 8.71, N 11.69.

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N-(1-benzotriazolocarbonyl)-L-alanine O-benzylxoyamid e 8a

Crude product 8a was triturated with Et2O and filtered off. Yield: 0.474 g (56 %). The sample for analysis was recrystallized from toluene. IR (KBr) νmax/cm–1: 3414, 3357, 3224, 1719, 1671, 1530, 1449, 1231, 1058, 756, 696. Anal. Calcd. mass fractions of elements, w/%, for C17H16N2O3 (M = 296.32) are: C 68.91, H 5.44, N 9.45; found: C 68.55, H 5.62, N 9.31. 1H NMR (DMSO-d6) δ 8.44 (s, 1H, NH), 7.40–7.19 (m, 10H, arom.), 2.99–2.97 (d, 2H, CH2); 13C NMR (DMSO-d6) δ 168.13 (CO), 152.96 (CO), 135.27–127.37 (arom.), 78.85 (OCH2), 55.38 (CH), 36.73 (CH3).

N-(1-benzotriazolocarbonyl)-L-phenylalanine O-benzylxoyamid e 8b

Crude product 8b was purified by column chromatography (eluent CHCl3/MEOH = 9:5.0:5) and recrystallized from Et2O/petrol ether. Yield: 0.353 g (34 %); IR (KBr) νmax/cm–1: 3370, 3173, 2971, 1743, 1674, 1508, 1448, 1084, 756, 701. Anal. Calcd. mass fractions of elements, w/%, for C17H17N5O3 (M = 339.13) are: C 60.17, H 5.05, N 20.64; found: C 60.38, H 5.32, N 20.31. 13C NMR (DMSO-d6) δ 167.03 (CONH), 152.28 (NCONH), 134.06 (arom.), 79.17 (OCH2), 55.71 (CH), 37.10 (CH3).

3-Benzoxyhydantoins 9. General Procedure

A solution of 2 mmol N-(1-benzotriazolocarbonyl)-L-amino acid 2 in 10 mL SOCl2 was stirred at room temperature and evaporated after 24 h. Crude chloride 3 was dissolved in 20 mL toluene. A mixture of 0.246 g (2 mmol) O-benzylhydroxylamine and 0.202 g (2 mmol) TEA in 20 mL toluene was added. The reaction mixture was stirred at r.t. for 5 h, extracted several times with 5 % NaOH solution, then with water, dried over sodium sulphate and evaporated.

3-Benzoyloxy-5-i-butylhydantoin 9a

Crude product 9a was triturated with Et2O and filtered off. Yield: 0.428 g (82 %). The sample for analysis was recrystallized from toluene. Analytical data of compound 9a are consistent with the literature data.19

3-Benzoyloxy-5-benzylhydantoin 9b

Crude product 9b was triturated with Et2O, filtered off and recrystallized from toluene. Yield: 0.255 g (43 %). IR (KBr) νmax/cm–1: 3244, 1783, 1728, 1425, 1214, 748, 724, 696. Anal. Calcd. mass fractions of elements, w/%, for C17H16N2O3 (M = 296.32) are: C 68.91, H 5.44, N 9.45; found: C 68.55 H 5.62, N 9.31. 1H NMR (DMSO-d6) δ 8.44 (s, 1H, NH), 7.40–7.19 (m, 10H, arnom.), 4.73–4.64 (dd, 2H, OCH3), 4.42 (t, 1H, CH), 2.99–2.97 (d, 2H, CH2); 13C NMR (DMSO-d6) δ 168.13 (CO), 152.96 (CO), 135.27–127.37 (arom.), 78.85 (OCH2), 55.38 (CH), 36.73 (CH3).

Biological Evaluation

General Experimental Details

Each experiment in vitro was performed at least in triplicate and the standard deviation of absorbance was less than 10 % of the mean. 1,1-Diphenyl-picyryl-hydrazyl (DPPH), 2,2’-azobis(2-aminopropane) dihydrochloride (AAPH), nordihydroguaiaretic acid (NDGA), sodium linolate, soybean liperoxidase (LO), caffeic acid and Trolox were purchased from Aldrich-Sigma (USA). All the tested compounds were dissolved in DMSO.

Interaction with DPPH Activity20

To a solution of DPPH (c = 0.05 mmol dm–3) in absolute ethanol, an equal volume of ethanolic solution of the tested compound (c = 0.1 or 0.05 mmol dm–3) was added. After 20 and 60 min, the absorbance was recorded at 517 nm and compared with the appropriate standard NDGA. Ethanol was used as a control.

Soybean Lipoxigenase Inhibition Activity

DMSO solution of the tested compound was incubated with sodium linolate (c = 0.1 mmol dm–3) and 0.2 mL of soybean lipoxigenase solution (1/9 × 10–4 w/v in saline) at room temperature. Conversion of sodium linolate to 13-hydroperoxylinoleic acid was recorded at 234 nm and compared with the standard inhibitor caffeic acid, according to the procedure previously reported.20

Inhibition of Linoleic Acid Lipid Peroxidation21

Peroxidation of linoleic acid to conjugated diene hydroperoxide in an aqueous dispersion was monitored at 234 nm. AAPH was used as a free radical initiator. Ten micromolars of linoleic acid dispersion (c = 16 mmol dm–3) was added to the UV cuvette containing 0.93 mL phosphate buffer (c = 0.05 mmol dm–3), pH = 7.4, prethermostated at 37 °C. The oxidation reaction was initiated at 37 °C under air by adding 50 μL of AAPH solution (c = 40 mmol dm–3). Oxidation was carried out in the presence of the tested compounds (10 μL, final concentration 0.1 mmol dm–3). In the assay with no antioxidant, lipid peroxidation was measured in the presence of the same level of DMSO. The rate of oxidation was monitored at 37 °C by recording the increase of absorption at 234 nm caused by conjugated diene hydroperoxides. The results were compared to the standard inhibitor Trolox.

Cytostatic Activity Assays

Cytostatic activity against five human cell lines, derived from 4 cancer types, was measured as described previously.17 The following cell lines were used: MCF-7 (breast carcinoma), SW 620 (colon carcinoma), HCT 116 (colon carcinoma), MOLT-4 (acute lymphoblastic leukaemia) and H 460 (lung carcinoma).


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Antimicrobial Activity

Microbial species (Bacillus subtilis ATCC 6633, Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 10536, Pseudomonas aeruginosa ATCC 27853, Candida albicans ATCC 10231 and Aspergillus niger ATCC 16404) used in the study were obtained from the American Type Culture Collection, LGC Promochem, UK. Trypticasa soy agar and Müller-Hinton agar were purchased from Merck (Germany), Sabouraud 2% (m/v)-glucose agar from BBL (Germany), amphotericin B from Sigma and norfloxacin from Krka (Slovenia).

RESULTS AND DISCUSSION

Chemistry

New ureidoamides 4a-o, derivatives of amino acids, were prepared from N-(1-benzotriazolecarbonyl)-amino acid chlorides 3a-e and the corresponding aminoalcohols. Synthesis of analogous ureidoamides with various monofunctional amines or N-hydroxyurea amides was previously described by us, and the synthesis of the starting compounds 1-benzotriazolcarboxylic acid chloride (1) and N-(1-benzotriazolcarboxyl)-

Scheme 1. Synthesis of ureidoamides 4a-o.

Scheme 2. Reactions with hydroxylamines.
Table 1. Spectroscopic data and atom enumeration for ureidoamides 4a-o

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>$^1$H and $^1$C NMR (DMSO-d$_6$, δppm)</th>
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</thead>
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<tr>
<td>4a</td>
<td><img src="image" alt="Structure" /></td>
<td>7.87 (t, 1H, 1' (amide), J = 5.4 Hz), 6.21 (d, 1H, 3, J = 7.9 Hz), 6.14 (t, 1H, 1' (ureido), J = 5.5 Hz), 4.69, 4.67 (2t, 2H, 4'), J = 5.6 Hz, J = 5.2 Hz), 4.16–4.06 (m, 1H, 2), 3.38–3.32 (m, 4H, 3'), 3.13–3.00 (m, 4H, 2'), 1.12 (d, 3H, 5, J = 7.4 Hz) 173.78 (1), 157.98 (4), 61.19, 60.21 (3'), 49.07 (2), 42.46, 41.86 (2'), 20.11 (5)</td>
</tr>
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<td>4b</td>
<td><img src="image" alt="Structure" /></td>
<td>7.85 (t, 1H, 1' (amide), J = 5.5 Hz), 6.05–6.02 (d, 1H, 3, 1' (ureido)), 4.43, 4.42 (2t, 2H, 5'), J = 4.8 Hz, J = 5.1 Hz), 4.14–4.05 (m, 1H, 2), 3.39 (q, 4H, 4'), J = 6.1 Hz), 3.12–3.00 (m, 4H, 2'), 1.58–1.44 (m, 4H, 3'), 1.12 (d, 3H, 5, J = 6.8 Hz) 173.63 (1), 157.97 (4), 58.83, 58.82 (4'), 49.02 (2), 36.70, 36.12 (2'), 33.66, 32.79 (3'), 20.23 (5)</td>
</tr>
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<td>4c</td>
<td><img src="image" alt="Structure" /></td>
<td>7.84 (t, 1H, 1' (amide), J = 5.3 Hz), 6.03 (t, 1H, 1' (ureido), J = 5.5 Hz), 5.96 (d, 1H, 3, J = 7.8 Hz), 4.34 (t, 2H, 7, J = 5.1 Hz), 4.15–4.05 (m, 1H, 2), 3.37 (q, 4H, 6'), J = 5.8 Hz), 3.05–2.92 (m, 4H, 2'), 1.45–1.29 (m, 12H, 3'–5'), 1.12 (d, 3H, 5) 173.44 (1), 157.77 (4), 61.13, 61.08 (6'), 48.95 (2), 39.63, 38.89 (2'), 32.72, 32.63 (5'), 30.35, 29.39 (3'), 23.39, 23.30 (4'), 20.36 (5)</td>
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<td>4d</td>
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<td>7.87 (t, 1H, 1' (amide), J = 5.4 Hz), 6.16 (t, 1H, 1' (ureido), J = 5.5 Hz), 6.11 (d, 1H, 3, J = 9.1 Hz), 4.65, 4.64 (2t, 2H, 4'), J = 4.8 Hz, J = 5.1 Hz), 3.97 (dd, 1H, 2, J = 6.0 Hz, J = 2.9 Hz), 3.45–3.33 (m, 4H, 3'), 3.18–3.03 (m, 4H, 2'), 1.91–1.80 (m, 1H, 5), 0.82 (d, 3H, 6, J = 6.8 Hz), 0.78 (d, 3H, 7, J = 6.8 Hz) 172.60 (1), 158.44 (4), 61.25, 60.28 (3'), 58.34 (2), 42.49, 41.76 (2'), 31.59 (5), 19.73, 18.25 (6, 7)</td>
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<td><img src="image" alt="Structure" /></td>
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</tr>
</tbody>
</table>
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7.87 (t, 1H, l' (amide), $J = 5.3$ Hz), 6.11 (d, 1H, 3, $J = 8.9$ Hz), 6.04 (t, 1H, l' (ureido), $J = 5.4$ Hz), 4.65–4.64 (m, 2H, 4'), 4.15–4.07 (m, 1H, 2), 3.40–3.34 (m, 4H, 3'), 3.13–3.01 (m, 4H, 2'), 1.60–1.52 (m, 1H, 6), 1.42–1.27 (m, 2H, 5), 0.88–0.84 (m, 6H, 7, 8)

173.69 (1), 158.18 (4), 61.21, 60.24 (3'), 51.95 (2), 42.84, 42.49 (2'), 41.83 (5), 24.69 (6), 23.51, 22.39 (7, 8)

7.89 (t, 1H, l' (amide), $J = 5.5$ Hz), 5.97–5.94 (m, 2H, 3, 3' (amide)), 4.42, 4.40 (2t, 2H, 5, $J = 5.3$ Hz), 4.13–4.05 (m, 1H, 2), 3.42–3.35 (m, 4H, 4), 3.15–2.99 (m, 4H, 2'), 1.59–1.44 (m, 5H, 6, 3'), 1.39–1.30 (m, 2H, 5), 0.87 (d, 3H, 7, $J = 6.2$ Hz), 0.85 (d, 3H, 8, $J = 6.2$ Hz)

173.50 (1), 158.19 (4), 58.90, 58.85 (4'), 51.95 (2), 42.92 (5), 36.73, 36.11 (2'), 33.68, 32.81 (3'), 24.74 (6), 23.43, 22.49 (7, 8)

7.88 (t, 1H, l' (amide), $J = 5.4$ Hz), 5.94 (t, 1H, l' (ureido), $J = 5.7$ Hz), 5.88 (d, 1H, 3, $J = 9.0$ Hz), 4.33 (t, 2H, 7, $J = 5.1$ Hz), 4.13–4.06 (m, 1H, 2), 3.39–3.33 (m, 4H, 6'), 3.08–2.92 (m, 4H, 2'), 1.58–1.22 (m, 15H, 6, 5, 3'–5'), 1.39–1.30 (m, 2H, 5), 0.87 (d, 3H, 7, $J = 6.1$ Hz) 0.85 (d, 3H, 8, $J = 6.1$ Hz)

173.29 (1), 157.98 (4), 61.13, 60.99 (6'), 51.90 (2), 43.03 (5), 39.70, 38.87 (2'), 32.73, 32.64 (5'), 30.35, 29.37 (3'), 24.74 (6), 23.41, 22.57 (7, 8), 23.38, 23.31 (4')

8.31 (t, 1H, l' (amide), $J = 5.5$ Hz), 7.37–7.21 (m, 5H, 6–10), 6.78 (d, 1H, 3, $J = 8.4$ Hz), 6.29 (t, 1H, l' (ureido), $J = 5.5$ Hz), 5.31 (d, 1H, 2, $J = 8.4$ Hz), 4.66, 4.64 (2t, 2H, 4', $J = 5.3$ Hz), 4.20–3.54 (m, 4H, 3'), 3.23–2.97 (m, 4H, 2')

171.25 (1), 157.63 (4), 141.05 (5), 128.61 (6, 10), 127.57 (8), 127.10 (7, 9), 61.18, 60.11 (3'), 56.93 (2), 42.46, 42.01 (2')

8.27 (t, 1H, l' (amide), $J = 5.5$ Hz), 7.36–7.22 (m, 5H, 6–10), 6.65 (d, 1H, 3, $J = 8.3$ Hz), 6.21 (t, 1H, l' (ureido), $J = 5.5$ Hz), 5.26 (d, 1H, 2, $J = 8.5$ Hz), 4.41, 4.39 (2t, 2H, 5', $J = 5.2$ Hz), 3.42–3.32 (m, 4H, 4'), 3.12–2.99 (m, 4H, 2'), 1.56–1.44 (m, 4H, 3')

171.06 (1), 157.67 (4), 141.10 (5), 128.63 (6, 10), 127.59 (8), 126.96 (7, 9), 58.79, 58.72 (4'), 56.98 (2), 39.15, 36.69 (2'), 36.33, 36.28 (3')
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8.26 (t, 1H, l' (amide), J = 5.5 Hz), 7.36–7.21 (m, 5H, 6–10), 6.60 (d, 1H, 3, J = 8.4 Hz), 6.21 (t, 1H, l' (ureido), J = 5.5 Hz), 5.26 (d, 1H, 2, J = 8.4 Hz), 4.35, 4.31 (2t, 2H, 7'), J = 5.3 Hz), 3.40–3.30 (m, 4H, 6'), 3.05–2.92 (m, 4H, 2'), 1.44–1.16 (m, 12H, 3'–5') 170.90 (1), 157.49 (4), 141.21 (5), 128.59 (6, 10), 127.54 (8), 126.93 (7, 9), 61.13, 61.06 (6'), 56.94 (2), 39.63, 39.04 (2'), 32.71, 32.58 (5'), 30.31, 29.24 (3'), 23.38, 23.25 (4')

7.90 (t, 1H, l' (amide), J = 5.1 Hz), 7.28–7.17 (m, 5H, 6–10), 6.16 (d, 1H, 3, J = 8.6 Hz), 6.10 (t, 1H, l' (ureido), J = 5.1 Hz), 4.64–4.59 (m, 2H, 4'), 4.37–4.30 (m, 1H, 2), 3.33–3.29 (m, 4H, 3'), 3.12–2.67 (m, 6H, 2', 5) 172.51 (1), 158.00 (4), 138.41 (6), 129.71 (6, 10), 128.43 (7, 9), 126.58 (8), 61.16, 60.18 (3'), 54.80 (2), 42.42, 41.81 (2'), 39.42 (5)

7.88 (t, 1H, l' (amide), J = 4.6 Hz), 7.27–7.14 (m, 5H, 6–10), 6.04–6.02 (d, 1H, 2, 3, l' (ureido)), 4.42–4.28 (m, 3H, 2, 5'), 3.39–3.34 (m, 4H, 4'), 3.12–2.69 (m, 6H, 2', 5), 1.50–1.43 (m, 4H, 3') 172.28 (1), 158.00 (4), 138.35 (5), 129.68 (8, 10), 128.43 (7, 11), 126.58 (9), 58.93 (4'), 54.79 (2), 39.46 (5), 36.68, 36.14 (2'), 33.61, 32.72 (3')

7.86 (t, 1H, l' (amide), J = 5.5 Hz), 7.27–7.14 (m, 5H, 6–10), 6.01 (t, 1H, l' (ureido), J = 5.5 Hz), 5.96 (d, 1H, 3, J = 8.7 Hz), 4.35–4.28 (m, 3H, 2, 7'), 3.37–3.33 (m, 4H, 6'), 3.07–2.69 (m, 6H, 2', 5), 1.43–1.16 (m, 12H, 3'–5') 172.06 (1), 157.79 (4), 138.34 (5), 129.70 (8, 10), 128.40 (7, 11), 126.55 (9), 61.13, 61.07 (6'), 54.72 (2), 39.62, 39.55 (2'), 38.91 (5), 32.72, 32.67 (5'), 30.30, 29.33 (3'), 23.36, 23.29 (4')
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Table 2. Interaction with DPPH, in vitro inhibition of soybean lipoygenase (LO) and lipid peroxidation (LP). All values are expressed in percents

<table>
<thead>
<tr>
<th>Compound</th>
<th>DPPH 20 min(a)</th>
<th>DPPH 60 min(a)</th>
<th>DPPH 20 min(b)</th>
<th>DPPH 60 min(b)</th>
<th>LO inhibition(b)</th>
<th>LP inhibition(b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>1.7</td>
<td>0.5</td>
<td>n.a.(c)</td>
<td>n.a.</td>
<td>28.1</td>
<td>n.a.</td>
</tr>
<tr>
<td>4b</td>
<td>n.a.</td>
<td>0.9</td>
<td>0.3</td>
<td>1</td>
<td>49.5(d)</td>
<td>32.9</td>
</tr>
<tr>
<td>4c</td>
<td>3.2</td>
<td>3.9</td>
<td>1.4</td>
<td>4.8</td>
<td>22.6</td>
<td>94.6</td>
</tr>
<tr>
<td>4d</td>
<td>3.4</td>
<td>6.3</td>
<td>2.1</td>
<td>3.5</td>
<td>55.7(d)</td>
<td>24.2</td>
</tr>
<tr>
<td>4e</td>
<td>2.7</td>
<td>6.3</td>
<td>5.0</td>
<td>4.7</td>
<td>37.8</td>
<td>15.0</td>
</tr>
<tr>
<td>4f</td>
<td>n.a.</td>
<td>1.2</td>
<td>2.3</td>
<td>5.2</td>
<td>36.1</td>
<td>43.7</td>
</tr>
<tr>
<td>4g</td>
<td>1.2</td>
<td>0.9</td>
<td>n.a.</td>
<td>1.5</td>
<td>52.8(d)</td>
<td>59.6</td>
</tr>
<tr>
<td>4h</td>
<td>n.a.</td>
<td>3.2</td>
<td>0.9</td>
<td>3.6</td>
<td>42.6</td>
<td>16.1</td>
</tr>
<tr>
<td>4i</td>
<td>1.2</td>
<td>1.1</td>
<td>2.7</td>
<td>2.8</td>
<td>42.5</td>
<td>11.1</td>
</tr>
<tr>
<td>4j</td>
<td>2.3</td>
<td>3.8</td>
<td>5.7</td>
<td>7.5</td>
<td>50.1(d)</td>
<td>63.1</td>
</tr>
<tr>
<td>4k</td>
<td>n.a.</td>
<td>2.2</td>
<td>4.9</td>
<td>1.3</td>
<td>55.6(d)</td>
<td>47.9</td>
</tr>
<tr>
<td>4l</td>
<td>1.6</td>
<td>2.4</td>
<td>0.9</td>
<td>3.7</td>
<td>36.9</td>
<td>3.9</td>
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<tr>
<td>4m</td>
<td>0.5</td>
<td>3.0</td>
<td>n.a.</td>
<td>1.1</td>
<td>49.2(d)</td>
<td>87.4</td>
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<tr>
<td>4n</td>
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<td>n.a.</td>
<td>0.9</td>
<td>39.7</td>
<td>19.9</td>
</tr>
<tr>
<td>4o</td>
<td>1.8</td>
<td>3.3</td>
<td>2.5</td>
<td>3.7</td>
<td>27.6</td>
<td>16.6</td>
</tr>
<tr>
<td>NDGA</td>
<td>81</td>
<td>83</td>
<td>93</td>
<td>97</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

Concentrations of the tested compounds:

(a) $5 \times 10^{-5}$ mol dm$^{-3}$.

(b) $1 \times 10^{-4}$ mol dm$^{-3}$.

(c) n.a. – no activity under the reported experimental conditions.

(d) IC50 value was also determined: 100 (4b), 92.5 (4d), 98 (4j), 100 (4j), 88 (4k), 100 (4m) μmol dm$^{-3}$.

(e) n.d. – not determined.

amino acids (2). The optimal molar ratio of aminoalcohol to chloride 3 was 3:1. Amino groups selectively reacted with 2-amino-1-ethanol, 3-amino-1-propanol and 5-amino-1-pentanol and the reaction products were ureidoamides 4 (Scheme 1).

However, when hydroxylamine was used as aminoalcohol, a mixture of products was obtained. N-hydroxyureido hydroxyamic acids of the general formula 7 were obtained only in traces, while the main products were 3-hydroxyhydantoins 6, obtained by cyclization of $N$-(1-benzotriazolecarbonyl)-L-amino acid N-hydroxyamides 5. Compounds 5 could be obtained in quantitative yield by catalytic hydrogenation of N-(1-benzotriazolecarbonyl)-L-amino acid O-benzoxoyamidates 8. L-alanine and L-phenylalanine derivatives 8a and 8b were isolated in the reaction of the corresponding chlorides 3 with O-benzoylhydroxylamine, while L-leucine derivative 8c spontaneously cyclized to 3-benzoyloxyhydantoin 9a. Phenylalanine hydantoin 9b was also prepared directly from chloride 3, without isolation of amide 8b, when the reaction time was prolonged. Hydantoins 9 were readily hydrogenated to 3-hydroxyhydantoins 6. Compounds 6 could be obtained by cyclization of amide 5 in alkaline medium as well.

In our previous research, reactions of chlorides 3 with $N$-phenylhydroxylamine afforded hydroxamic acids 10, which readily cyclized to 1,2,5-oxadiazine derivatives 11 under basic conditions. In that case, hydroxamic acids 10 could be successfully isolated. Scheme II depicts the reactions of amino acid derivatives with hydroxylamines.

To obtain compounds with different physicochemical properties, amino acids with both aliphatic (L-alanine, L-valine and L-leucine) and aromatic (D-phenylglycine and L-phenylalanine) residues were used. Structures of compounds 4a-o were deduced from analyses of their IR, 1H and 13C NMR spectra and were confirmed by elemental analysis. The chemical shifts were consistent with the proposed structures of the novel compounds (Table 1). IR spectra of ureidoamides 4a-o showed characteristic bands at 3422–3345 (OH), 3310–3100 (NH), 1644–1625 (amide and urea carbonyls) and 1572–1559 (amide II) cm$^{-1}$. In 1H NMR spectra, amide NH (1') in all aliphatic amino acid and phenylalanine derivatives showed as triplets between 7.90 and 7.84 ppm, while in phenylglycine derivatives 4j-l it was slightly shifted downfield (8.31–8.26 ppm). Ureido NH (3) showed as doublets between 6.21


NDGA 81 83 93 97 n.d. n.d.

and 5.88 ppm, except in phenylglycine derivatives (6.78–6.60 ppm). Ureido NH (1') appeared as triplets between 6.14 and 6.01 ppm. Signals from phenylglycine derivatives were again slightly shifted to 6.29–6.21. Hydroxyl groups appeared as one or two superimposed triplets between 4.68 and 4.52 (ppm values decreased with the length of the chain). All three NH groups and OH were exchangeable with D_2O. Amide carbonyl (triplets between 4.68 and 4.32 (ppm values decreased with the length of the chain). All three NH groups and OH were exchangeable with D_2O. Amide carbonyl (triplets between 4.68 and 4.32 (ppm values decreased with the length of the chain).

**Biological Activity**

Newly synthesized ureidoamides 4a-o were screened for antioxidative, antimicrobial and cytostatic activities. The results have shown that biological activity of the tested compounds was rather modest.

Interaction of the tested compounds with the free radical DPPH was very weak (Table 2). The results were below 10% and no increase was observed with the increase of ureidoamide concentration (0.05 mmol dm⁻³ and 0.1 mmol dm⁻³) and the time of interaction (20 min and 60 min). Soybean lipoxygenase inhibition activity assay showed that the tested ureidoamides were modest LO inhibitors (Table 2). IC₅₀ values for compounds 4b, 4d, 4g, 4j, 4k, 4m were between 92.5 and 100 µmol dm⁻³, while the percent of inhibition for the other compounds ranged from 22.6 to 42.6% at the concentration 10⁻² M. Most of the tested compounds exerted no significant inhibition of lipid peroxidation, with the exception of compounds 4c and 4m, followed by 4g and 4j (Table 2). From the results it was not possible to delineate the influence of certain structural characteristics and physicochemical properties in terms of QSAR.

Evaluation of cytostatic activities against malignant tumor cell lines has shown that ureidoamides 4a-o possess very weak antiproliferative activity (data not shown), significantly lower than the urea amides with hydroxyl group directly attached to urea nitrogen.¹²,¹³

Results of microbiological screening using the hole-plate diffusion method revealed that ureidoamides 4a-o showed no growth inhibition zones, and were considered inactive at the concentration used (data not shown). The minimum inhibitory (MIC) and minimum microbicidal concentrations (MMcC) were determined by the microdilution broth method.²⁴ MIC/MMcC values were high (data not shown). The strongest activity was exerted towards *Pseudomonas aeruginosa* (1.25/2.5 mg dm⁻³).

**Acknowledgements.** Support for this study was provided by the Ministry of Science, Education and Sports of the Republic of Croatia (Project 006-0000000-3216). The authors thank Marijeta Kralj, PhD, Division of Molecular Medicine, Ruder Bošković Institute, Zagreb, for the cytostatic activity assay and Professor Stjepan Pepešnjak and Ivan Kosalec, PhD, Department of Microbiology, Faculty of Pharmacy, University of Zagreb, for microbiological screening.

**Abbreviations.** AAPH, 2,2′-azobis(2-aminopropane) dihydrochloride; BtCl, 1-benzotriazole carboxylic acid chloride; BH, benzotriazole; DPPH, 1,1-diphenyl-2-picrylhydrazyl radical; LO, soybean lipoxygenase; LP, lipid peroxidation; MCC, minimal inhibitory concentration; MMcC, minimal microbicidal concentration; NDGA, nordihydroguaiaretic acid; TEA, triethylamine.

**REFERENCES**

Novi ureidoamidi iz aminokiselina: Sinteza i biološko djelovanje

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U radu je opisana sinteza novih aminokiselinskih ureidoamida 4a-o polazeći iz klorida N-(1-benzotriazolkarbonil)-aminokiselina 3, derivata L-alanina, L-valina, L-leucina, D-fenilglicina i L-fenilalanina, i odgovarajućih aminoalkohola (2-amino-etan-1-ol, 3-amino-propan-1-ol i 5-amino-pentan-1-ol). Struktura spojeva potvrđena je uobičajenim spektroskopskim metodama (IR, 1H i 13C NMR) i elementarnom analizom. Antioksidativna ispitivanja pokazuju da sintetizirani spojevi posjeduju blago djelovanje (interakcija s 1,1-difenil-pikrilhidrazil radikalom, inhibicija lipoksigenaze iz soje, inhibicija peroksidacije linolne kiseline). Preliminarna citostatska ispitivanja na pet humanih staničnih linija i antimikrobna ispitivanja na nekoliko mikroorganizama pokazuju da sintetizirani ureidoamidi imaju slabo antiproliferativno, odnosno antimikrobno djelovanje.