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Original Scientific Article

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

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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, ^{1}H and ^{13}C NMR) and their structure was confirmed by elemental analysis. Antioxidant screenings (interaction with 1,1-diphenyl-2-picrylhydrazyl, soybean lipoxygenase 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 thiomer-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_{max}/cm^{-1}) were recorded on a FTIR Perkin Elmer Paragon 500 spectrometer and UV/VIS spectra on a Varian Cary 100Bio UV-visible spectrometer. ^{1}H and ^{13}C NMR spectra were taken on a Varian Gemini 300 spectrometer, operating at 300 and 75.5 MHz for the ^{1}H and ^{13}C nuclei, respectively. Samples were measured in DMSO-d_6 solutions at 20 °C in 5 mm NMR tubes. Chemical shifts (δ/ppm) were referred to TMS. Precoated silica gel 60 F_{254} plates were used for thin-layer chromatography. Solvent systems were CH_2Cl_2/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 CH_2Cl_2/MeOH (9:1, 8.5:1.5 and 4:1) and $CHCl_3$/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

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(TEA)) from Sigma-Aldrich (USA). 1-Benzotriazole carboxylic acid chloride (**1**), *N*-(1-benzotriazolecarbonyl)-amino acids **2** and chlorides **3** were prepared according to previously published procedures.[8,14–16]

Ureido Derivatives 4a-o. General Procedure

A mixture of *N*-(1-benzotriazolecarbonyl)-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-(1-(2-Hydroxyethylcarbamoyl)ethyl)-3-(2-hydroxyethyl)urea **4a**
Crude product **4a** was purified by column chromatography (eluent CH_2Cl_2/MeOH = 8.5:1.5→4:1) and triturated with Et_2O. Yield: 0.132 g (61 %); m.p. 150–154 ºC; IR (KBr) ν_{max}/cm^{-1}: 3422, 3274, 2969, 2937, 2879, 1644, 1570, 1056.
Anal. Calcd. mass fractions of elements, *w*/%, for $C_8H_{17}N_3O_4$ (M_r = 219.24) are: C 43.83, H 7.82, N 19.17; found: C 43.93, H 7.73, N 19.05.

1-(1-(3-Hydroxypropylcarbamoyl)ethyl)-3-(3-hydroxypropyl)urea **4b**
Crude product **4b** was purified by column chromatography (eluent CH_2Cl_2/MeOH = 4:1) and triturated with Et_2O. Yield: 0.139 g (56 %); m.p. 152–156 ºC; IR (KBr) ν_{max}/cm^{-1}: 3345, 3275, 2941, 2875, 1638, 1560, 1060.
Anal. Calcd. mass fractions of elements, *w*/%, for $C_{10}H_{21}N_3O_4$ (M_r = 247.29) are: C 48.57, H 8.56, N 16.99; found: C 48.35, H 8.77, N 16.84.

1-(1-(5-Hydroxypentylcarbamoyl)ethyl)-3-(5-hydroxypentyl)urea **4c**
Crude product **4c** was purified by column chromatography (eluent CH_2Cl_2/MeOH = 4:1) and triturated with Et_2O. Yield: 0.180 g (59 %); m.p. 142–146 ºC; IR (KBr) ν_{max}/cm^{-1}: 3360, 3282, 3111, 2936, 2862, 1627, 1561, 1056.
Anal. Calcd. mass fractions of elements, *w*/%, for $C_{14}H_{29}N_3O_4$ (M_r = 303.40) are: C 55.42, H 9.63, N 13.8; found: C 55.62, H 9.47, N 14.02.

1-(1-(2-Hydroxyethylcarbamoyl)-2-methylpropyl)-3-(2-hydroxyethyl)urea **4d**
Crude product **4d** was purified by column chromatography (eluent CH_2Cl_2/MeOH = 8.5:1.5→4:1) and recrystallized from MeOH/Et_2O. Yield: 0.140 g (57 %); m.p. 194–198 ºC; IR (KBr) ν_{max}/cm^{-1}: 3351, 3280, 3102, 2969, 2874, 1626, 1571, 1063.
Anal. Calcd. mass fractions of elements, *w*/%, for $C_{10}H_{21}N_3O_4$ (M_r = 247.29) are: C 48.57, H 8.56, N 16.99; found: C 48.88, H 8.53, N 16.61.

1-(1-(3-Hydroxypropylcarbamoyl)-2-methylpropyl)-3-(3-hydroxypropyl)urea **4e**
Crude product **4e** was purified by column chromatography (eluent CH_2Cl_2/MeOH = 9:1→4:1) and recrystallized from MeOH/Et_2O. Yield: 0.154 g (56 %); m.p. 178–181 ºC; IR (KBr) ν_{max}/cm^{-1}: 3350, 3279, 3102, 2963, 2874, 1630, 1566, 1234, 1078, 1044.
Anal. Calcd. mass fractions of elements, *w*/%, for $C_{12}H_{25}N_3O_4$ (M_r = 275.34) are: C 52.34, H 79.15, N 15.26; found: C 52.53, H 9.01, N 15.44.

1-(1-(5-Hydroxypentylcarbamoyl)-2-methylpropyl)-3-(5-hydroxypentyl)urea **4f**
Crude product **4f** was purified by column chromatography (eluent CH_2Cl_2/MeOH = 9:1→4:1) and triturated with Et_2O. Yield: 0.171 g (52 %); m.p. 159–162 ºC; IR (KBr) ν_{max}/cm^{-1}: 3349, 3278, 3107, 2935, 2861, 1628, 1572, 1236, 1059.
Anal. Calcd. mass fractions of elements, *w*/%, for $C_{16}H_{33}N_3O$ (M_r = 331.45) are: C 57.98, H 10.04, N 12.68; found: C 57.75, H 10.17, N 12.93.

1-(1-(2-Hydroxyethylcarbamoyl)-3-methylbutyl)-3-(2-hydroxyethyl)urea **4g**
Crude product **4g** was purified by column chromatography (eluent CH_2Cl_2/MeOH = 8.5:1.5). Yield: 0.138 g (53 %); m.p. 157–161 ºC; IR (KBr) ν_{max}/cm^{-1}: 3354, 3290, 3098, 2954, 2928, 2872, 1625, 1569, 1059.
Anal. Calcd. mass fractions of elements, *w*/%, for $C_{11}H_{23}N_3O_4$ (M_r = 261.32) are: C 50.56, H 8.87, N 16.08; found: C 50.56, H 8.87, N 16.08.

1-(1-(3-Hydroxypropylcarbamoyl)-3-methylbutyl)-3-(3-hydroxypropyl)urea **4h**
Crude product **4h** was purified by column chromatography (eluent CH_2Cl_2/MeOH = 8.5:1.5) and triturated with Et_2O. Yield: 0.150 g (52 %); m.p. 109–111 ºC; IR (KBr) ν_{max}/cm^{-1}: 3351, 3308, 3098, 2957, 2872, 1630, 1572, 1252, 1073.
Anal. Calcd. mass fractions of elements, *w*/%, for $C_{13}H_{27}N_3O_4$ (M_r = 289.37) are: C 53.96, H 9.40, N 14.52; found: C 53.69, H 9.23, N 14.79.

1-(1-(5-Hydroxypentylcarbamoyl)-3-methylbutyl)-3-(5-hydroxypentyl)urea **4i**
Crude product **4i** was purified by column chromatography (eluent CH_2Cl_2/MeOH = 9:1→8.5:1.5) and triturated with Et_2O. Yield: 0.270 g (78 %); m.p. 83–86 ºC; IR (KBr) ν_{max}/cm^{-1}: 3349, 3280, 3100, 2937, 2862, 1626, 1562, 1262, 1059.
Anal. Calcd. mass fractions of elements, *w*/%, for $C_{17}H_{35}N_3O_4$ (M_r = 345.48) are: C 59.10, H 10.21, N 12.16; found: C 59.30, H 10.11, N 12.19.

1-((2-Hydroxyethylcarbamoyl)(phenyl)methyl)-3-(2-hydroxyethyl)urea **4j**
Crude product **4j** was purified by column chromatography (eluent CH_2Cl_2/MeOH = 8.5:1.5→4:1) and tritu-

rated with Et_2O. Yield: 0.171 g (61 %); m.p. 206−208 ºC; IR (KBr) ν_{max}/cm^{-1}: 3422, 3274, 2969, 2937, 2879, 1644, 1570, 1056.
Anal. Calcd. mass fractions of elements, *w*/%, for $C_{13}H_{19}N_3O_4$ (M_r = 281.31) are: C 55.50, H 6.81, N 14.94; found: C 55.78, H 6.57, N 14.77.

1-((3-Hydroxypropylcarbamoyl)(phenyl)methyl)-3-(3-hydroxypropyl)urea **4k**
Crude product **4k** was purified by column chromatography (eluent CH_2Cl_2/MeOH = 8.5:1.5) and recrystallized from MeOH/Et_2O. Yield: 0.170 g (55 %); m.p. 182−185 ºC; IR (KBr) ν_{max}/cm^{-1}: 3354, 3289, 3092, 2942, 2883, 1626, 1561, 1059.
Anal. Calcd. mass fractions of elements, *w*/%, for $C_{15}H_{23}N_3O_4$ (M_r = 309.36) are: C 58.24, H 7.49, N 13.58; found: C 58.37, H 7.26, N 13.73.

1-((5-Hydroxypentylcarbamoyl)(phenyl)methyl)-3-(5-hydroxypentyl)urea **4l**
Crude product **4l** was purified by column chromatography (eluent CH_2Cl_2/MeOH = 8.5:1.5) and recrystallized from MeOH/Et_2O. Yield: 0.222 g (61 %); m.p. 178−180 ºC; IR (KBr) ν_{max}/cm^{-1}: 3362, 3286, 3093, 2937, 2861, 1628, 1564, 1352, 1061.
Anal. Calcd. mass fractions of elements, *w*/%, for $C_{19}H_{31}N_3O_4$ (M_r = 365.47) are: C 62.44, H 8.55, N 11.50; found: C 62.65, H 8.71, N 11.69.

1-(1-(2-Hydroxyethylcarbamoyl)-2-phenylethyl)-3-(2-hydroxyethyl)urea **4m**
Crude product **4m** was purified by column chromatography (eluent CH_2Cl_2/MeOH = 8.5:1.5). Yield: 0.230 g (78 %); m.p. 140−143 ºC; IR (KBr) ν_{max}/cm^{-1}: 3377, 3310, 3105, 2937, 2876, 1626, 1569, 1496, 1231, 1056.
Anal. Calcd. mass fractions of elements, *w*/%, for $C_{14}H_{21}N_3O_4$ (M_r = 295.33) are: C 56.94, H 7.17, N 14.23; found: C 56.69, H 7.32, N 14.44.

1-(1-(3-Hydroxypropylcarbamoyl)-2-phenylethyl)-3-(3-hydroxypropyl)urea **4n**
Crude product **4n** was purified by column chromatography (eluent CH_2Cl_2/MeOH = 8.5:1.5). Yield: 0.243 g (75 %); m.p. 110−113 ºC; IR (KBr) ν_{max}/cm^{-1}: 3358, 3280, 3106, 2942, 2878, 1628, 1564, 1235, 1050.
Anal. Calcd. mass fractions of elements, *w*/%, for $C_{16}H_{25}N_3O_4$ (M_r = 323.39) are: C 59.42, H 7.79, N 12.99; found: C 59.38, H 7.88, N 12.75.

1-(1-(5-Hydroxypentylcarbamoyl)-2-phenylethyl)-3-(5-hydroxypentyl)urea **4o**
Crude product **4o** was purified by column chromatography (eluent CH_2Cl_2/MeOH = 9:1) and trituration with Et_2O. Yield: 0.197 g (52 %); m.p. 89−93 ºC; IR (KBr) ν_{max}/cm^{-1}: 3362, 3282, 3106, 2931, 2859, 1626, 1559, 1236, 1060.
Anal. Calcd. mass fractions of elements, *w*/%, for $C_{20}H_{33}N_3O_4$ (M_r = 379.49) are: C 63.30, H 8.76, N 11.07; found: C 63.45, H 8.92, N 11.31.

N-*(1-benzotriazolecarbonyl)-L-alanine* N-*hydroxyamide* **5a**
A solution of 0.339 g (1 mmol) *N*-(1-benzotriazolecarbonyl)-L-alanine *O*-benzyloxyamide (**8a**) in 20 mL MeOH was hydrogenolyzed using 50 mg Pd/C as a catalyst. After 2.5 h the reaction mixture was filtered, evaporated and the thus obtained crude product was triturated with Et_2O and filtered off. Yield: 0.231 g (93 %). IR (KBr) ν_{max}/cm^{-1}: 3613, 3225, 3079, 1714, 1694, 1657, 1523, 1068, 843, 772.
Anal. Calcd. mass fractions of elements, *w*/%, for $C_{10}H_{11}N_5O_3$ (M_r = 249.23) are: C 48.19, H 4.45, N 28.10; found: C 48.45, H 4.72, N 28.46; ^{1}H NMR (DMSO-d_6) δ 10.81 (s, 1H, OH), 9.09−9.06 (d, 1H, NCONH), 8.94 (s, 1H, NHO), 8.23−7.53 (m, 4H, arom.), 4.46−4.37 (q, 1H, CH), 1.48−1.45 (d, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 168.58 (CO amide), 149.04 (CO urea), 145.90−113.99 (arom.), 48.56 (CH), 18.39 (CH_3).

3-Hydroxyhydantoins 6

3-Hydroxy-5-methylhydantoin **6a**
To a solution of 0.249 g (1 mmol) *N*-(1-benzotriazolecarbonyl)-L-alanine *N*-hydroxyamide (**5a**) in acetone (40 mL), 5 % Na_2CO_3 solution (4 mL) was added.[17] The reaction mixture was stirred at r.t. for 2 h. Acetone was evaporated *in vacuo* and the precipitated product **6a** was filtered off, washed with water and recrystallized from acetone and water. Analytical data of compound **6a** are consistent with the literature data.[18]

3-Hydroxy-5-benzylhydantoin **6b**
A solution of 0.207 g (0.7 mmol) of 3-benzyloxy-5-benzylhydantoin (**9b**) in 30 mL MeOH was hydrogenolyzed using 50 mg Pd/C as a catalyst. After 2.5 h the reaction mixture was filtered, methanol was evaporated and the thus obtained crude product was triturated with Et_2O. Yield: 0.086 g (60 %). Analytical data of compound **6b** are consistent with the literature data.[18]

N-(1-benzotriazolecarbonyl)-L-amino acid *O*-benzyloxyamides 8. General Procedure

A solution of 2.5 mmol *N*-(1-benzotriazolecarbonyl)-L-amino acid **2** in 10 mL $SOCl_2$ was stirred at room temperature and evaporated after 24 h. Crude chloride **3** was dissolved in 20 mL toluene and cooled to 0 °C. A mixture of 0.308 g (2.5 mmol) *O*-benzylhydroxylamine and 0.253 g (2.5 mmol) TEA in 25 mL toluene was added dropwise. The reaction mixture was stirred on an ice bath for 1 h, extracted several times with water, dried over sodium sulphate and evaporated.

N-*(1-benzotriazolecarbonyl)-L-alanine* O-*benzyloxyamide* **8a**

Crude product **8a** was triturated with Et_2O and filtered off. Yield: 0.474 g (56 %). The sample for analysis was recrystallized from toluene. IR (KBr) ν_{max}/cm^{-1}: 3414, 3357, 3224, 1719, 1671, 1530, 1449, 1231, 1058, 756, 696.

Anal. Calcd. mass fractions of elements, *w*/%, for $C_{17}H_{17}N_5O_3$ (M_r = 339.13) are: C 60.17, H 5.05, N 20.64; found: C 60.38, H 5.32, N 20.31; ^{13}C NMR (DMSO-d_6) δ 169.73 (CONH), 153.06 (NCONH), 134.06−125.66 (arom.), 78.81 (OCH_2), 50.39 (CH), 17.38 (CH_3).

N-*(1-benzotriazolecarbonyl)-L-phenylalanine* O-*benzyloxyamide* **8b**

Crude product **8b** was purified by column chromatography (eluent $CHCl_3$/MeOH = 9.5:0.5) and recrystallized from Et_2O/petrolether. Yield: 0.353 g (34 %); IR (KBr) ν_{max}/cm^{-1}: 3370, 3173, 2971, 1743, 1697, 1674, 1508, 1448, 1048, 756, 701.

Anal. Calcd. mass fractions of elements, *w*/%, for $C_{23}H_{21}N_5O_3$ (M_r = 415.44) are: C 66.49, H 5.09, N 16.86; found: C 66.45, H 4.92, N 16.51; ^{13}C NMR (DMSO-d_6) δ 168.44 (CONH), 153.28 (NCONH), 135.64−127.65 (arom.), 79.17 (OCH_2), 55.71 (CH), 37.10 (CH_2).

3-Benzyloxyhydantoins 9. General Procedure

A solution of 2 mmol *N*-(1-benzotriazolecarbonyl)-L-amino acid **2** in 10 mL $SOCl_2$ 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-Benzyloxy-5-*i*-butylhydantoin* **9a**

Crude product **9a** was triturated with Et_2O 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-Benzyloxy-5-benzylhydantoin **9b**

Crude product **9b** was triturated with Et_2O, filtered off and recrystallized from toluene. Yield: 0.255 g (43 %). IR (KBr) ν_{max}/cm^{-1}: 3244, 1783, 1728, 1425, 1214, 748, 724, 696.

Anal. Calcd. mass fractions of elements, *w*/%, for $C_{17}H_{16}N_2O_3$ (M_r = 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-d_6) δ 8.44 (s, 1H, NH), 7.40−7.19 (m, 10H, arom.), 4.73−4.64 (dd, 2H, OCH_2), 4.42 (t, 1H, CH), 2.99−2.97 (d, 2H, CH_2); ^{13}C NMR (DMSO-d_6) δ 168.13 (CO), 152.96 (CO), 135.27−127.37 (arom.), 78.85 (OCH_2), 55.38 (CH), 36.73 (CH_2).

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-picrylhydrazyl (DPPH), 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH), nordihydroguaiaretic acid (NDGA), sodium linoleate, soybean lipoxygenase (LO), caffeic acid and Trolox were purchased from Aldrich-Sigma (USA). All the tested compounds were dissolved in DMSO.

Interaction with DPPH Activity[20]

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 Lipoxygenase Inhibition Activity

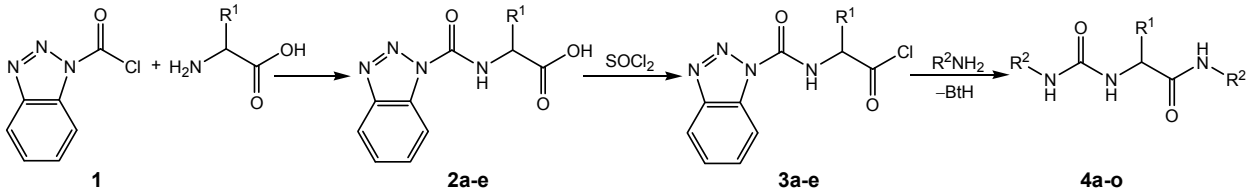
DMSO solution of the tested compound was incubated with sodium linoleate (c = 0.1 mmol dm^{-3}) and 0.2 mL of soybean lipoxygenase solution ($1/9 \times 10^{-4}$ *w*/*v* in saline) at room temperature. Conversion of sodium linoleate to 13-hydroperoxylinoleic acid was recorded at 234 nm and compared with the standard inhibitor caffeic acid, according to the procedure previously reported.[20]

Inhibition of Linoleic Acid Lipid Peroxidation[21]

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 microliters 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).



	4a	4b	4c	4d	4e	4f	4g	4h
R^1	CH_3	CH_3	CH_3	$CH(CH_3)_2$	$CH(CH_3)_2$	$CH(CH_3)_2$	$CH_2CH(CH_3)_2$	$CH_2CH(CH_3)_2$
R^2	$(CH_2)_2OH$	$(CH_2)_3OH$	$(CH_2)_5OH$	$(CH_2)_2OH$	$(CH_2)_3OH$	$(CH_2)_5OH$	$(CH_2)_2OH$	$(CH_2)_3OH$

	4i	4j	4k	4l	4m	4n	4o
R^1	$CH_2CH(CH_3)_2$	Ph	Ph	Ph	Bn	Bn	Bn
R^2	$(CH_2)_5OH$	$(CH_2)_2OH$	$(CH_2)_3OH$	$(CH_2)_5OH$	$(CH_2)_2OH$	$(CH_2)_3OH$	$(CH_2)_5OH$

Scheme 1. Synthesis of ureidoamides **4a-o**.

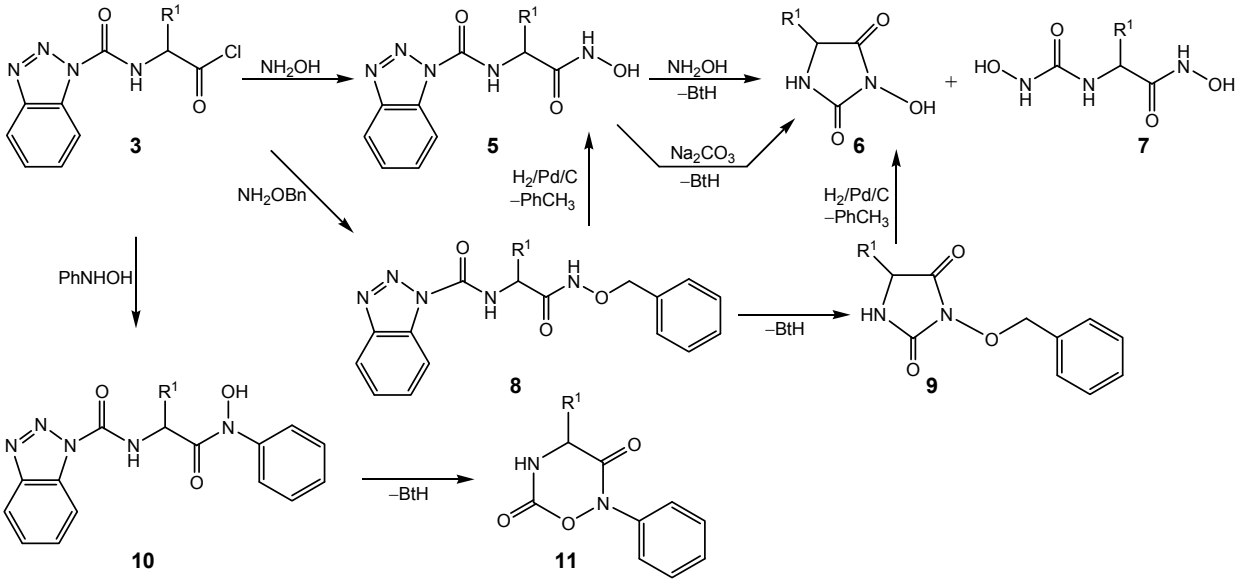
Antimicrobial Activity

Microbial species (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 10536, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 10231 and *Aspergilus 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

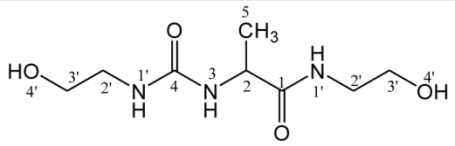
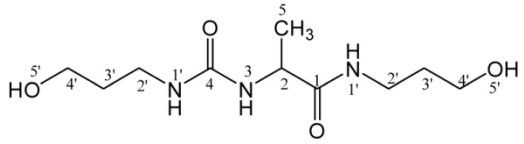
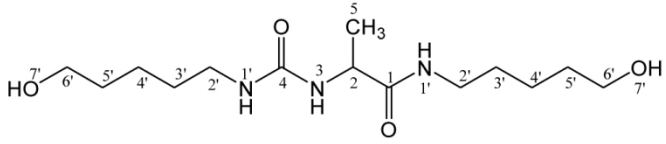
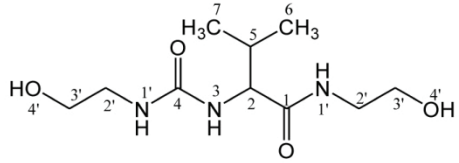
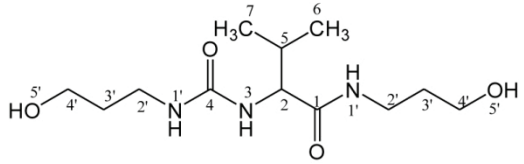
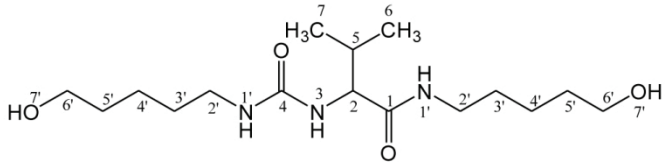
Chemistry

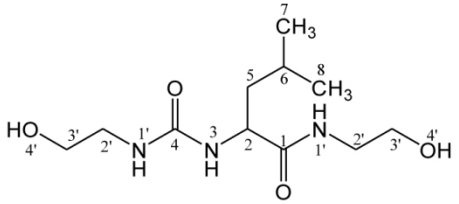
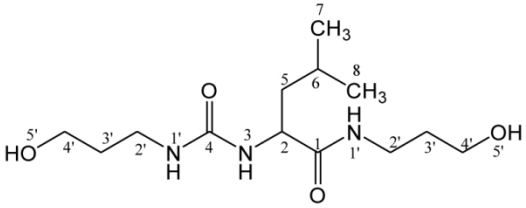
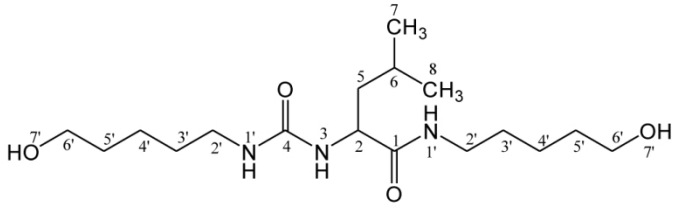
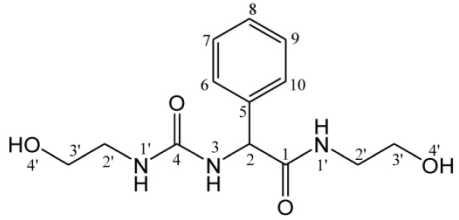
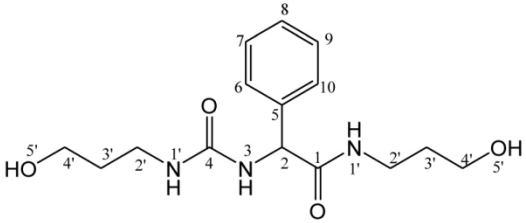
New ureidoamides **4a-o**, derivatives of amino acids, were prepared from *N*-(1-benzotriazolecarbonyl)-amino acid chlorides **3a-e** and the corresponding aminoalcohols. Synthesis of analogous ureidoamides with various monofunctional amines or *N*-hydroxyurea amides was previously described by us,[12,13,16] as well as the synthesis of the starting compounds 1-benzotriazolecarboxylic acid chloride (**1**)[8,14] and *N*-(1-benzotriazolecarbonyl)-



Scheme 2. Reactions with hydroxylamines.

Table 1. Spectroscopic data and atom enumeration for ureidoamides **4a-o**

Compound	Structure	^{1}H and ^{13}C NMR (DMSO-d_6, δ/ppm)
4a		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)
4b		7.85 (t, 1H, 1' (amide), *J* = 5.5 Hz), 6.05–6.02 (d, t, 2H, 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)
4c		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)
4d		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)
4e		7.90 (t, 1H, 1' (amide), *J* = 5.6 Hz), 6.06 (t, 1H, 1' (ureido), *J* = 5.7 Hz), 5.96 (d, 1H, 3, *J* = 9.1 Hz), 4.42, 4.41 (2t, 2H, 5', *J* = 5.4 Hz), 3.95 (dd, 1H, 2, *J* = 6.1 Hz, *J* = 3.0 Hz), 3.39 (q, 4H, 4', *J* = 6.0 Hz), 3.19–3.00 (m, 4H, 2'), 1.89–1.78 (m, 1H, 5), 1.58–1.45 (m, 4H, 3'), 0.82 (d, 3H, 6, *J* = 6.8 Hz), 0.79 (d, 3H, 7, *J* = 7.0 Hz) 172.40 (1), 158.45 (4), 58.89, 58.79 (4'), 58.28 (2), 36.68, 36.07 (2'), 33.71, 32.86 (3'), 31.72 (5), 19.71, 18.35 (6, 7)
4f		7.89 (t, 1H, 1' (amide), *J* = 5.5 Hz), 6.05 (t, 1H, 1' (ureido), *J* = 5.4 Hz), 5.90 (d, 1H, 3, *J* = 9.0 Hz), 4.35–4.32 (m, 2H, 7'), 3.95 (dd, 1H, 2, *J* = 6.2 Hz, *J* = 2.7 Hz), 3.38–3.35 (m, 4H, 6'), 3.12–2.93 (m, 4H, 2'), 1.88–1.77 (m, 1H, 5), 1.44–1.25 (m, 12H, 3'–5'), 0.81 (d, 3H, 6, *J* = 6.8 Hz), 0.78 (d, 3H, 7, *J* = 6.9) 172.23 (1), 158.25 (4), 61.13, 61.08 (6'), 58.21 (2), 39.67, 38.83 (2'), 32.72, 32.62 (5'), 31.78 (5), 30.35, 29.41 (3'), 23.39, 23.35 (4'), 19.70, 18.33 (6,7)

4g		7.87 (t, 1H, 1' (amide), *J* = 5.3 Hz), 6.11 (d, 1H, 3, *J* = 8.9 Hz), 6.04 (t, 1H, 1' (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)
4h		7.89 (t, 1H, 1' (amide), *J* = 5.5 Hz), 5.97–5.94 (m, 2H, 3, 1' (ureido)), 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)
4i		7.88 (t, 1H, 1' (amide), *J* = 5.4 Hz), 5.94 (t, 1H, 1' (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, 61.09 (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')
4j		8.31 (t, 1H, 1' (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, 1' (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, *J* = 5.1 Hz), 3.40–3.34 (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')
4k		8.27 (t, 1H, 1' (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, 1' (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')

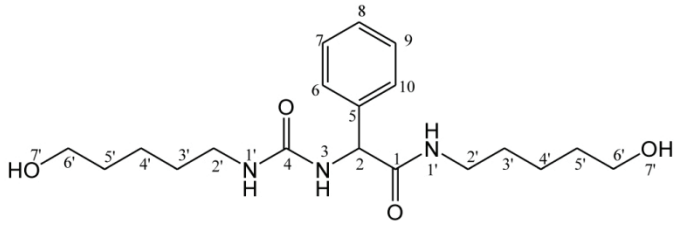
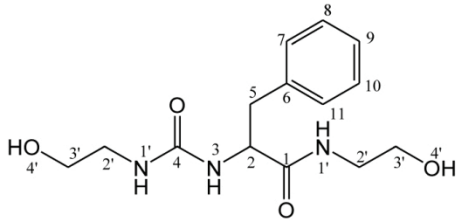
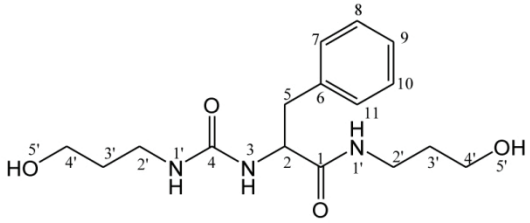
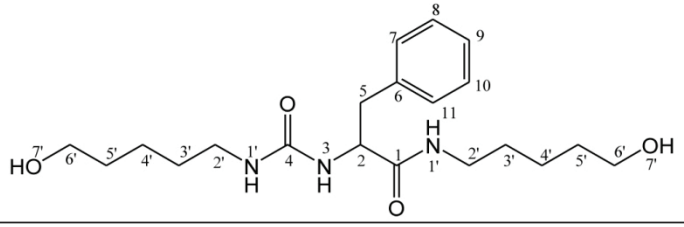
4l		8.26 (t, 1H, 1' (amide), *J* = 5.5 Hz), 7.36–7.21 (m, 5H, 6–10), 6.60 (d, 1H, 3, *J* = 8.4 Hz), 6.21 (t, 1H, 1' (ureido), *J* = 5.5 Hz), 5.26 (d, 1H, 2, *J* = 8.4 Hz), 4.35, 4.31 (2t, 2H, 7', *J* = 5.3 Hz), 3.40–3.30 (m, 4H, 6'), 3.05–2.92 (m, 4H, 2'), 1.44–1.16 (m, 12H, 3'–5') 170.90 (1), 157.49 (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')
4m		7.90 (t, 1H, 1' (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, 1' (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)
4n		7.88 (t, 1H, 1' (amide), *J* = 4.6 Hz), 7.27–7.14 (m, 5H, 6–10), 6.04–6.02 (d, t, 2H, 3, 1' (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')
4o		7.86 (t, 1H, 1' (amide), *J* = 5,5 Hz), 7.27–7.14 (m, 5H, 6–10), 6.01 (t, 1H, 1' (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')

Table 2. Interaction with DPPH, *in vitro* inhibition of soybean lipoxygenase (LO) and lipid peroxidation (LP). All values are expressed in percents

Compound	DPPH 20 min[(a)]	DPPH 60 min[(a)]	DPPH 20 min[(b)]	DPPH 60 min[(b)]	LO inhibition[(b)]	LP inhibition[(b)]
4a	1.7	0.5	n.a.[(c)]	n.a.	28.1	n.a.
4b	n.a.	0.9	0.3	1	49.5[(d)]	32.9
4c	3.2	3.9	1.4	4.8	22.6	94.6
4d	3.4	6.3	2.1	3.5	55.7[(d)]	24.2
4e	2.7	6.3	5.0	4.7	37.8	15.0
4f	n.a.	1.2	2.3	5.2	36.1	43.7
4g	1.2	0.9	n.a.	1.5	52.8[(d)]	59.6
4h	n.a.	3.2	0.9	3.6	42.6	16.1
4i	1.2	1.1	2.7	2.8	42.5	11.1
4j	2.3	3.8	5.7	7.5	50.1[(d)]	63.1
4k	n.a.	2.2	4.9	1.3	55.6[(d)]	47.9
4l	1.6	2.4	0.9	3.7	36.9	3.9
4m	0.5	3.0	n.a.	1.1	49.2[(d)]	87.4
4n	0.9	1.8	n.a.	0.9	39.7	19.9
4o	1.8	3.3	2.5	3.7	27.6	16.6
Caffeic acid	n.d.[(e)]	n.d.	n.d.	n.d.	600	n.d.
NDGA	81	83	93	97	n.d.	n.d.
Trolox	n.d.	n.d.	n.d.	n.d.	n.d.	63

Concentrations of the tested compounds:
[(a)] 5×10^{-5} mol dm^{-3}.
[(b)] 1×10^{-4} mol dm^{-3}.
[(c)] n.a. – no activity under the reported experimental conditions.
[(d)] IC_{50} value was also determined: 100 (**4b**), 92.5 (**4d**), 98 (**4g**), 100 (**4j**), 88 (**4k**), 100 (**4m**) μmol dm^{-3}.
[(e)] n.d. – not determined.

amino acids (**2**).[15] 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 hydroxamic 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*-benzyloxyamides **8**. L-alanine and L-phenylalanine derivatives **8a** and **8b** were isolated in the reaction of the corresponding chlorides **3** with *O*-benzylhydroxylamine, while L-leucine derivative **8c** spontaneously cyclized to 3-benzyloxyhydantoin **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.[17]

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.[22] 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, ^{1}H and ^{13}C NMR spectra and were confirmed by elemental analysis. The chemical shifts were consistent with the proposed structures of the novel compounds (Table1). 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 ^{1}H 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

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 4.32 (ppm values decreased with the length of the chain). All three NH groups and OH were exchangeable with D_2O. Amide carbonyl group (1) in ^{13}C NMR spectra appeared between 173.78 and 172.06, except in phenylglycine derivatives (171.25−170.96), while ureido carbonyl was uniformly located between 158.45 and 157.49 ppm.

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). IC_{50} 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 $c = 1 \times 10^{-4}$ mol dm^{-3}. 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 (Q)SAR.

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[23] 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}).

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Abbreviations. AAPH, 2,2'-azobis(2-amidinopropane) dihydrochloride; BtcCl, 1-benzotriazole carboxylic acid chloride; BtH, benzotriazole, DPPH, 1,1-diphenyl-2-picrylhydrazyl radical; LO, soybean lipoxygenase; LP, lipid peroxidation; MIC, minimal inhibitory concentration; MMcC, minimal microbicidal concentration; NDGA, nordihydroguaiaretic acid; TEA, triethylamine.

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SAŽETAK

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, ^{1}H i ^{13}C 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.