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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, ¹H and ¹³C 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 thiomers-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 ($\nu_{\max}/\text{cm}^{-1}$) 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, 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

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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 CH₂Cl₂/MeOH = 8.5:1.5 → 4:1) and triturated with Et₂O. Yield: 0.132 g (61 %); m.p. 150–154 °C; IR (KBr) $\nu_{\max}/\text{cm}^{-1}$: 3422, 3274, 2969, 2937, 2879, 1644, 1570, 1056.

Anal. Calcd. mass fractions of elements, *w*%, for C₈H₁₇N₃O₄ (*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 CH₂Cl₂/MeOH = 4:1) and triturated with Et₂O. Yield: 0.139 g (56 %); m.p. 152–156 °C; IR (KBr) $\nu_{\max}/\text{cm}^{-1}$: 3345, 3275, 2941, 2875, 1638, 1560, 1060.

Anal. Calcd. mass fractions of elements, *w*%, for C₁₀H₂₁N₃O₄ (*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 CH₂Cl₂/MeOH = 4:1) and triturated with Et₂O. Yield: 0.180 g (59 %); m.p. 142–146 °C; IR (KBr) $\nu_{\max}/\text{cm}^{-1}$: 3360, 3282, 3111, 2936, 2862, 1627, 1561, 1056.

Anal. Calcd. mass fractions of elements, *w*%, for C₁₄H₂₉N₃O₄ (*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 CH₂Cl₂/MeOH = 8.5:1.5 → 4:1) and recrystallized from MeOH/Et₂O. Yield: 0.140 g (57 %); m.p. 194–198 °C; IR (KBr) $\nu_{\max}/\text{cm}^{-1}$: 3351, 3280, 3102, 2969, 2874, 1626, 1571, 1063.

Anal. Calcd. mass fractions of elements, *w*%, for C₁₀H₂₁N₃O₄ (*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 CH₂Cl₂/MeOH = 9:1 → 4:1) and recrystallized from MeOH/Et₂O. Yield: 0.154 g (56 %); m.p. 178–181 °C; IR (KBr) $\nu_{\max}/\text{cm}^{-1}$: 3350, 3279, 3102, 2963, 2874, 1630, 1566, 1234, 1078, 1044.

Anal. Calcd. mass fractions of elements, *w*%, for C₁₂H₂₅N₃O₄ (*M_r* = 275.34) are: C 52.34, H 7.91, N 15.26; found: C 52.53, H 7.91, N 15.44.

1-(1-(5-Hydroxypentylcarbamoyl)-2-methylpropyl)-3-(5-hydroxypentyl)urea **4f**

Crude product **4f** was purified by column chromatography (eluent CH₂Cl₂/MeOH = 9:1 → 4:1) and triturated with Et₂O. Yield: 0.171 g (52 %); m.p. 159–162 °C; IR (KBr) $\nu_{\max}/\text{cm}^{-1}$: 3349, 3278, 3107, 2935, 2861, 1628, 1572, 1236, 1059.

Anal. Calcd. mass fractions of elements, *w*%, for C₁₆H₃₃N₃O₄ (*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 CH₂Cl₂/MeOH = 8.5:1.5). Yield: 0.138 g (53 %); m.p. 157–161 °C; IR (KBr) $\nu_{\max}/\text{cm}^{-1}$: 3354, 3290, 3098, 2954, 2928, 2872, 1625, 1569, 1059.

Anal. Calcd. mass fractions of elements, *w*%, for C₁₁H₂₃N₃O₄ (*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 CH₂Cl₂/MeOH = 8.5:1.5) and triturated with Et₂O. Yield: 0.150 g (52 %); m.p. 109–111 °C; IR (KBr) $\nu_{\max}/\text{cm}^{-1}$: 3351, 3308, 3098, 2957, 2872, 1630, 1572, 1252, 1073.

Anal. Calcd. mass fractions of elements, *w*%, for C₁₃H₂₇N₃O₄ (*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 CH₂Cl₂/MeOH = 9:1 → 8.5:1.5) and triturated with Et₂O. Yield: 0.270 g (78 %); m.p. 83–86 °C; IR (KBr) $\nu_{\max}/\text{cm}^{-1}$: 3349, 3280, 3100, 2937, 2862, 1626, 1562, 1262, 1059.

Anal. Calcd. mass fractions of elements, *w*%, for C₁₇H₃₅N₃O₄ (*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 CH₂Cl₂/MeOH = 8.5:1.5 → 4:1) and tritu-

rated with Et₂O. Yield: 0.171 g (61 %); m.p. 206–208 °C; IR (KBr) $\nu_{\max}/\text{cm}^{-1}$: 3422, 3274, 2969, 2937, 2879, 1644, 1570, 1056.

Anal. Calcd. mass fractions of elements, w/%, for C₁₃H₁₉N₃O₄ ($M_r = 281.31$) 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 CH₂Cl₂/MeOH = 8.5:1.5) and recrystallized from MeOH/Et₂O. Yield: 0.170 g (55 %); m.p. 182–185 °C; IR (KBr) $\nu_{\max}/\text{cm}^{-1}$: 3354, 3289, 3092, 2942, 2883, 1626, 1561, 1059.

Anal. Calcd. mass fractions of elements, w/%, for C₁₅H₂₃N₃O₄ ($M_r = 309.36$) 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 CH₂Cl₂/MeOH = 8.5:1.5) and recrystallized from MeOH/Et₂O. Yield: 0.222 g (61 %); m.p. 178–180 °C; IR (KBr) $\nu_{\max}/\text{cm}^{-1}$: 3362, 3286, 3093, 2937, 2861, 1628, 1564, 1352, 1061.

Anal. Calcd. mass fractions of elements, w/%, for C₁₉H₃₁N₃O₄ ($M_r = 365.47$) 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 CH₂Cl₂/MeOH = 8.5:1.5). Yield: 0.230 g (78 %); m.p. 140–143 °C; IR (KBr) $\nu_{\max}/\text{cm}^{-1}$: 3377, 3310, 3105, 2937, 2876, 1626, 1569, 1496, 1231, 1056.

Anal. Calcd. mass fractions of elements, w/%, for C₁₄H₂₁N₃O₄ ($M_r = 295.33$) 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 CH₂Cl₂/MeOH = 8.5:1.5). Yield: 0.243 g (75 %); m.p. 110–113 °C; IR (KBr) $\nu_{\max}/\text{cm}^{-1}$: 3358, 3280, 3106, 2942, 2878, 1628, 1564, 1235, 1050.

Anal. Calcd. mass fractions of elements, w/%, for C₁₆H₂₅N₃O₄ ($M_r = 323.39$) 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 CH₂Cl₂/MeOH = 9:1) and trituration with Et₂O. Yield: 0.197 g (52 %); m.p. 89–93 °C; IR (KBr) $\nu_{\max}/\text{cm}^{-1}$: 3362, 3282, 3106, 2931, 2859, 1626, 1559, 1236, 1060.

Anal. Calcd. mass fractions of elements, w/%, for C₂₀H₃₃N₃O₄ ($M_r = 379.49$) 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 Et₂O and filtered off. Yield: 0.231 g (93 %). IR (KBr) $\nu_{\max}/\text{cm}^{-1}$: 3613, 3225, 3079, 1714, 1694, 1657, 1523, 1068, 843, 772.

Anal. Calcd. mass fractions of elements, w/%, for C₁₀H₁₁N₅O₃ ($M_r = 249.23$) are: C 48.19, H 4.45, N 28.10; found: C 48.45, H 4.72, N 28.46; ¹H NMR (DMSO-d₆) 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, CH₃); ¹³C NMR (DMSO-d₆) δ 168.58 (CO amide), 149.04 (CO urea), 145.90–113.99 (arom.), 48.56 (CH), 18.39 (CH₃).

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 % Na₂CO₃ 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 Et₂O. Yield: 0.086 g (60 %). Analytical data of compound **6b** are consistent with the literature data.¹⁸

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

A solution of 2.5 mmol *N*-(1-benzotriazolecarbonyl)-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 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 Et₂O and filtered off. Yield: 0.474 g (56 %). The sample for analysis was recrystallized from toluene. IR (KBr) $\nu_{\max}/\text{cm}^{-1}$: 3414, 3357, 3224, 1719, 1671, 1530, 1449, 1231, 1058, 756, 696.

Anal. Calcd. mass fractions of elements, *w*/%, for C₁₇H₁₇N₅O₃ (*M_r* = 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 169.73 (CONH), 153.06 (NCONH), 134.06–125.66 (arom.), 78.81 (OCH₂), 50.39 (CH), 17.38 (CH₃).

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

Crude product **8b** was purified by column chromatography (eluent CHCl₃/MeOH = 9.5:0.5) and recrystallized from Et₂O/petrolether. Yield: 0.353 g (34 %); IR (KBr) $\nu_{\max}/\text{cm}^{-1}$: 3370, 3173, 2971, 1743, 1697, 1674, 1508, 1448, 1048, 756, 701.

Anal. Calcd. mass fractions of elements, *w*/%, for C₂₃H₂₁N₅O₃ (*M_r* = 415.44) are: C 66.49, H 5.09, N 16.86; found: C 66.45, H 4.92, N 16.51; ¹³C NMR (DMSO-d₆) δ 168.44 (CONH), 153.28 (NCONH), 135.64–127.65 (arom.), 79.17 (OCH₂), 55.71 (CH), 37.10 (CH₂).

3-Benzoyloxyhydantoin **9. General Procedure**

A solution of 2 mmol *N*-(1-benzotriazolecarbonyl)-*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*-Benzyloxy-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*-Benzyloxy-5-*benzyl*hydantoin **9b*

Crude product **9b** was triturated with Et₂O, filtered off and recrystallized from toluene. Yield: 0.255 g (43 %). IR (KBr) $\nu_{\max}/\text{cm}^{-1}$: 3244, 1783, 1728, 1425, 1214, 748, 724, 696.

Anal. Calcd. mass fractions of elements, *w*/%, for C₁₇H₁₆N₂O₃ (*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-d₆) δ 8.44 (s, 1H, NH), 7.40–7.19 (m, 10H, arom.), 4.73–4.64 (dd, 2H, OCH₂), 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 (CH₂).

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 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 Lipoxygenase Inhibition Activity

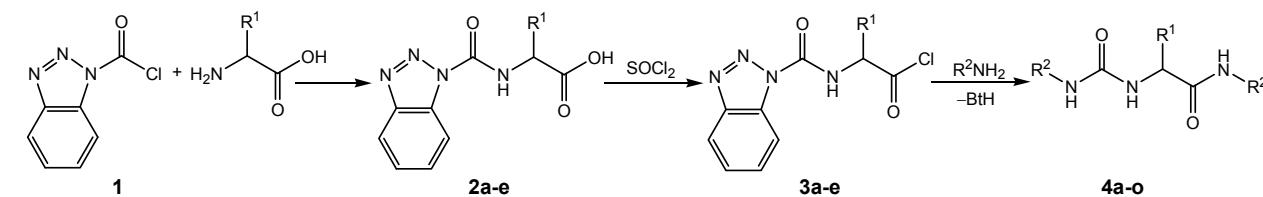
DMSO solution of the tested compound was incubated with sodium linoleate (*c* = 0.1 mmol dm⁻³) and 0.2 mL of soybean lipoxygenase solution (1/9 × 10⁻⁴ *w/v* in saline) at room temperature. Conversion of sodium linoleate to 13-hydroperoxylinoleic acid was recorded at 234 nm and compared with the standard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

Inhibition of Linoleic Acid Lipid 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 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 ₂ CH(CH ₃) ₂	CH ₂ CH(CH ₃) ₂
R ²	(CH ₂) ₂ OH	(CH ₂) ₃ OH	(CH ₂) ₅ OH	(CH ₂) ₂ OH	(CH ₂) ₃ OH	(CH ₂) ₅ OH	(CH ₂) ₂ OH	(CH ₂) ₃ OH

	4i	4j	4k	4l	4m	4n	4o
R ¹	CH ₂ CH(CH ₃) ₂	Ph	Ph	Ph	Bn	Bn	Bn
R ²	(CH ₂) ₅ OH	(CH ₂) ₂ OH	(CH ₂) ₃ OH	(CH ₂) ₅ OH	(CH ₂) ₂ OH	(CH ₂) ₃ OH	(CH ₂) ₅ OH

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

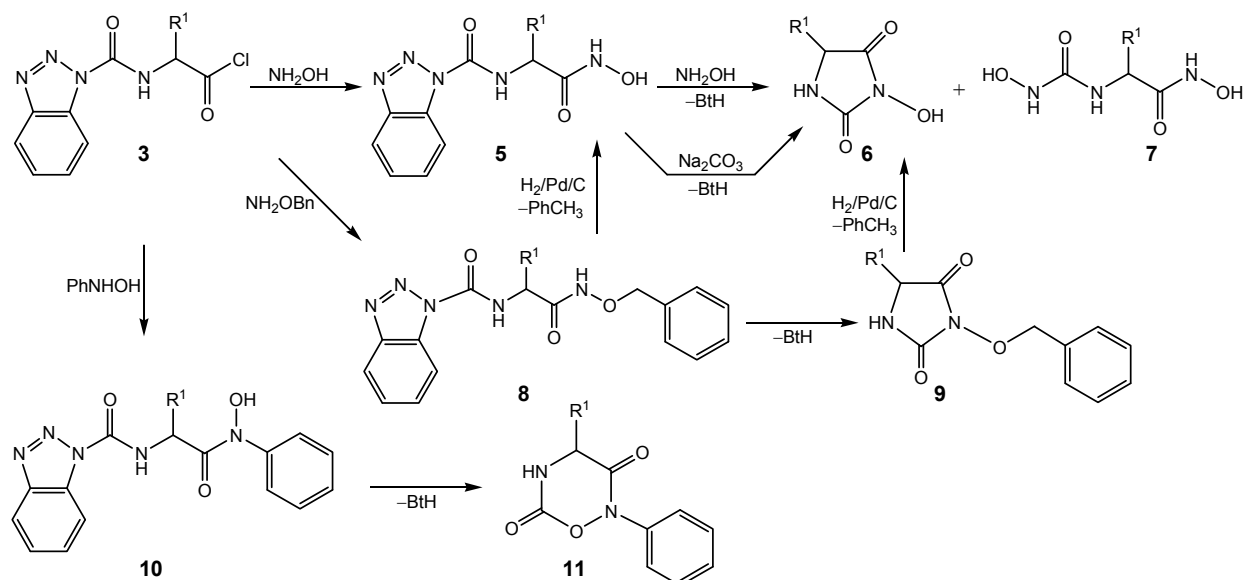
Antimicrobial Activity

Microbial species (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 10536, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404) used in the study were obtained from the American Type Culture Collection, LGC Promochem, UK. Trypticase 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)-

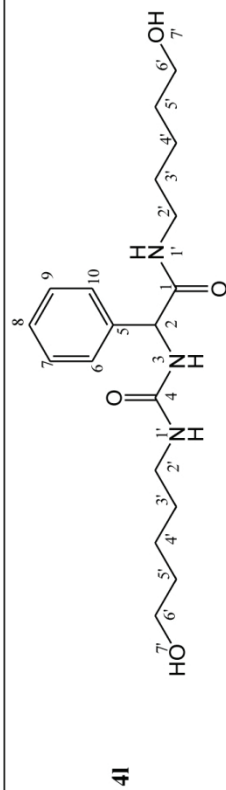


Scheme 2. Reactions with hydroxylamines.

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

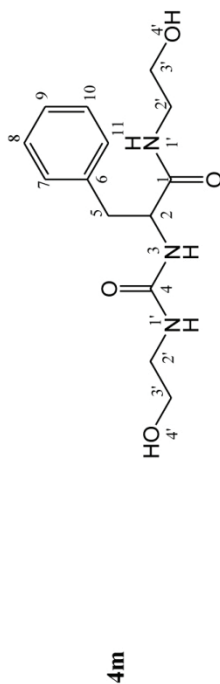
Compound	Structure	¹ H and ¹³ C NMR (DMSO- <i>d</i> ₆ , δ/ppm)
4a		7.87 (t, 1H, 1' (amide), <i>J</i> = 5.4 Hz), 6.21 (d, 1H, 3, <i>J</i> = 7.9 Hz), 6.14 (t, 1H, 1' (ureido), <i>J</i> = 5.5 Hz), 4.69, 4.67 (2t, 2H, 4', <i>J</i> = 5.6 Hz, <i>J</i> = 5.2 Hz), 4.16–4.06 (m, 1H, 2), 3.38–3.32 (m, 4H, 3'), 3.13–3.00 (m, 4H, 2'), 1.12 (d, 3H, 5, <i>J</i> = 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), <i>J</i> = 5.5 Hz), 6.05–6.02 (d, t, 2H, 3, 1' (ureido)), 4.43, 4.42 (2t, 2H, 5', <i>J</i> = 4.8 Hz, <i>J</i> = 5.1 Hz), 4.14–4.05 (m, 1H, 2), 3.39 (q, 4H, 4', <i>J</i> = 6.1 Hz), 3.12–3.00 (m, 4H, 2'), 1.58–1.44 (m, 4H, 3'), 1.12 (d, 3H, 5, <i>J</i> = 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), <i>J</i> = 5.3 Hz), 6.03 (t, 1H, 1' (ureido), <i>J</i> = 5.5 Hz), 5.96 (d, 1H, 3, <i>J</i> = 7.8 Hz), 4.34 (t, 2H, 7', <i>J</i> = 5.1 Hz), 4.15–4.05 (m, 1H, 2), 3.37 (q, 4H, 6', <i>J</i> = 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), <i>J</i> = 5.4 Hz), 6.16 (t, 1H, 1' (ureido), <i>J</i> = 5.5 Hz), 6.11 (d, 1H, 3, <i>J</i> = 9.1 Hz), 4.65, 4.64 (2t, 2H, 4', <i>J</i> = 4.8 Hz, <i>J</i> = 5.1 Hz), 3.97 (dd, 1H, 2, <i>J</i> = 6.0 Hz, <i>J</i> = 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, <i>J</i> = 6.8 Hz), 0.78 (d, 3H, 7, <i>J</i> = 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), <i>J</i> = 5.6 Hz), 6.06 (t, 1H, 1' (ureido), <i>J</i> = 5.7 Hz), 5.96 (d, 1H, 3, <i>J</i> = 9.1 Hz), 4.42, 4.41 (2t, 2H, 5', <i>J</i> = 5.4 Hz), 3.95 (dd, 1H, 2, <i>J</i> = 6.1 Hz, <i>J</i> = 3.0 Hz), 3.39 (q, 4H, 4', <i>J</i> = 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, <i>J</i> = 6.8 Hz), 0.79 (d, 3H, 7, <i>J</i> = 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), <i>J</i> = 5.5 Hz), 6.05 (t, 1H, 1' (ureido), <i>J</i> = 5.4 Hz), 5.90 (d, 1H, 3, <i>J</i> = 9.0 Hz), 4.35–4.32 (m, 2H, 7'), 3.95 (dd, 1H, 2, <i>J</i> = 6.2 Hz, <i>J</i> = 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, <i>J</i> = 6.8 Hz), 0.78 (d, 3H, 7, <i>J</i> = 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		<p>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)</p>
4h		<p>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)</p>
4i		<p>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')</p>
4j		<p>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')</p>
4k		<p>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')</p>



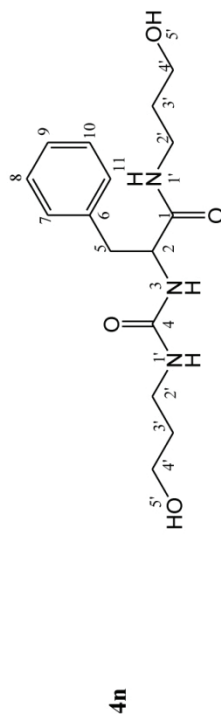
8.26 (t, 1H, 1' (amide), $J = 5.5$ Hz), 7.36–7.21 (m, 5H, 6–10), 6.60 (d, 1H, 3, $J = 8.4$ Hz), 6.21 (t, 1H, 1' (ureido), $J = 5.5$ Hz), 5.26 (d, 1H, 2, $J = 8.4$ Hz), 4.35, 4.31 (2t, 2H, 7, $J = 5.3$ Hz), 3.40–3.30 (m, 4H, 6'), 3.05–2.92 (m, 4H, 2'), 1.44–1.16 (m, 12H, 3'–5')

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')



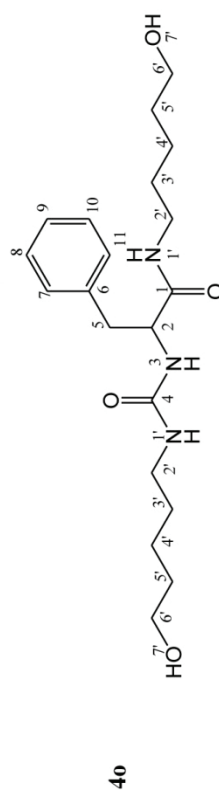
7.90 (t, 1H, 1' (amide), $J = 5.1$ Hz), 7.28–7.17 (m, 5H, 6–10), 6.16 (d, 1H, 3, $J = 8.6$ Hz), 6.10 (t, 1H, 1' (ureido), $J = 5.1$ Hz), 4.64–4.59 (m, 2H, 4'), 4.37–4.30 (m, 1H, 2), 3.33–3.29 (m, 4H, 3'), 3.12–2.67 (m, 6H, 2', 5)

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)



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')



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) IC₅₀ value was also determined: 100 (**4b**), 92.5 (**4d**), 98 (**4g**), 100 (**4j**), 88 (**4k**), 100 (**4m**) μ mol 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-hydroxyhydantoin **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. Hydantoin **9** were readily hydrogenated to 3-hydroxyhydantoin **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 physico-chemical 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

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 D₂O. Amide carbonyl group (1) in ¹³C 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). 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 $c = 1 \times 10^{-4}$ 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 method²³ 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, 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.