SYNTHESIS, CHARACTERIZATION AND CYTOTOXICITY ASSESSMENT OF NEW 4-BENZYL-1,3-OXAZOLE DERIVATIVES INCORPORATING 4-[(4-BROMOPHENYL)SULFONYL]PHENYL FRAGMENT

THEODORA VENERA APOSTOL1,*, CONSTANTIN DRĂGHICI2, LAURA ILEANA SOCEA1, OCTAVIAN TUDOREL OLRU3, GABRIEL ȘARAMET4, MĂDĂLINA HRUBARU2, ȘTEFANIA FELICIA BĂRBEANU1

1“Carol Davila” University of Medicine and Pharmacy, Faculty of Pharmacy, Organic Chemistry Department, 6 Traian Văcărescu Street, 020956, Bucharest, Romania
2“Costin D. Nenitescu” Institute of Organic Chemistry, Romanian Academy, 202B Splaiul Independenței Street, 060023, Bucharest, Romania
3“Carol Davila” University of Medicine and Pharmacy, Faculty of Pharmacy, Pharmaceutical Botany and Cell Biology Department, 6 Traian Văcărescu Street, 020956, Bucharest, Romania
4“Carol Davila” University of Medicine and Pharmacy, Faculty of Pharmacy, Pharmaceutical Technology and Biopharmacy Department, 6 Traian Văcărescu Street, 020956, Bucharest, Romania

*corresponding author: theodora.apostol@umfcd.ro

Abstract

Herein we present the design, synthesis, characterization, and cytotoxicity assessment of seven compounds derived from phenylalanine that incorporate a 4-[(4-bromophenyl)sulfonfonyl]phenyl fragment: four open-chain products (one N-acyl-α-amino acid, one N-acyl-α-amino acyl chloride, two N-acyl-α-amino ketones), and three five-membered heterocycles with two heteroatoms (O and N): one 2-aryl-4-benzyl-1,3-oxazol-5(4H)-one, and two 2-aryl-4-benzyl-1,3-oxazoles with p-toly, and m-xyl substituent, respectively, in position 5. Structures of new derivatives were assigned by elemental analysis, NMR spectroscopy, and other spectral methods (FT-IR, MS, UV-Vis). Evaluation of the purity of the compounds was realized by reversed-phase high-performance liquid chromatography. Daphnia magna toxicological test was used to assess the cytotoxicity of new compounds.

Rezumat

Studiul prezintă proiectarea, sinteza, caracterizarea și evaluarea citotoxicității a şapte compuși derivați de la fenilalanină, care încorporează un fragment 4-[(4-bromofenil)sulfonil]fenil: patru produse cu catenă deschisă (N-acyl-α-aminoacid, o clorură de N-acyl-α-aminoacil, două N-acyl-α-aminoacetone) și trei heterocicluri pentaatomice cu doi heteroatomi (O și N): o 2-ariil-4-benzil-1,3-oxazol-5(4H)-onă și 2-ariil-1,3-oxazol cu substituentul p-tolil și respectiv, m-xilil, în poziția 5. Structurile noilor derivați au fost atribuite prin analiză elementară, spectroscopică RMN și alte metode spectrale (FT-IR, MS, UV-Vis). Evaluarea purității compușilor a fost realizată prin cromatografie de lichid de înaltă performanță cu fază inversă. Testul toxicologic Daphnia magna a fost utilizat pentru a se evalua citotoxicitatea noilor compuși.

Keywords: N-acyl-α-amino acid, 4-benzyl-1,3-oxazol-5(4H)-one, 4-benzyl-1,3-oxazole, cytotoxic effect

Introduction

1,3-Oxazoles have increasing importance in heterocyclic chemistry and drew the researchers’ attention due to their biological and medicinal applications. Though parent 1,3-oxazole does not occur naturally, numerous natural derivatives containing a 1,3-oxazole core have been isolated, especially from marine invertebrates and microorganisms, some of them exhibiting remarkable therapeutic effects [1-4]. Additionally, a series of synthetic pharmaceutical molecules bearing 1,3-oxazole scaffold were reported to have antimicrobial [5], anti-diabetic [6], analgesic, anti-inflammatory [7], anticancer activity [8], etc. 5-Hydroxy-1,3-oxazoles exist in their corresponding keto tautomer: 1,3-oxazol-5(4H)-ones which present cytotoxic [9, 10], antiviral [11], plant growth regulating properties [12]. Some representants of N-acyl-α-amino acids class show biological activities, such as anti-hypertensive [13], anticancer [14], mucolytic [15], antianemic [16], anti-ulcer effect [17], and are specific antidotes in acute intoxications [18, 19]. N-Acyl-α-amino-ketones display anti-inflammatory [20], antiviral [21, 22] and antithrombotic action [23]. Besides, is known that numerous diaryl sulfones are used in therapy for their properties [24-26]. In an attempt to find new effective drugs, diaryl sulfone pharmacophore has been incorporated into a large number of derivatives with potential biological value [9, 10, 12, 27-29].
Given the scientific relevance of compounds from these classes and our previous researches [9, 10, 12, 30], in this article, we report the design, synthesis, and characterization of new N-acyl-α-amino acids, 1,3-oxazol-5(4H)-ones, N-acyl-α-amino acyl chlorides, N-acyl-α-amino ketones, and 1,3-oxazoles analogues derived from phenylalanine containing a 4-[(4-bromophenyl)sulfonyl]phenyl substituent into their structure with the aim of obtaining new bioactive products and to explore their biological action. For this purpose, the cytotoxic effect of compounds was assessed on Daphnia magna crustacean. Amidst the various methods of screening, this assay is fast, reproducible, cost-efficient, simple and can predict biological activity [27].

Materials and Methods

Chemistry

General: Chemicals and reagents were acquired from common commercial suppliers. Dichloromethane was anhydried over anhydrous calcium chloride. Uncorrected melting points were measured on a Boëtius apparatus. UV-Vis spectra were registered for methanolic solutions (≈ 0.025 mM) on an Analytik Jena AG Specord 40 spectrophotometer. FT-IR spectra were recorded in KBr pellets on a Bruker Vertex 70 spectrometer, absorption peaks being described as very strong, vs; strong, s; medium, m; weak, w. NMR spectra were acquired on a Varian Gemini 300 BB instrument at 300 MHz for 1H and 75 MHz for 13C. Combined 2D spectra (COSY, HETCOR) were also recorded. DMSO-d6 or CDCl3 as deuterated solvents were used. Chemical shifts, δ, are in parts per million (ppm), relative to the reference standard tetramethylsilane (TMS) signal. Coupling constants, J, are expressed in hertz (Hz). In the 1H-NMR spectra, signal multiplicity was assigned as singlet (s), doublet (d), doublet of doublets (dd), doublet of doublets of doublets (ddd), triplet (t), multiplet (m); broad is abbreviated b. GC-MS analysis was registered on a Fisons Instruments GC 8000 with an electron impact quadrupole, MD 800 mass spectrometer detector, and a fused-silica capillary column coated with poly(5% diphenyl – 95% dimethylsiloxane) (SLB-5ms, 30 m × 0.32 mm, δi. 0.25 μm). Dichloromethane was used as solvent and the helium flow rate was 2 ml/min. RP-HPLC chromatograms were performed on a Beckman System Gold 126 liquid chromatograph, with a System Gold 166 UV-Vis detector, a non-polar chromatography column (LiChrosorb RP-18, 25 cm × 4 mm, 5 μm particle size), and a Rheodyne injection system. New compounds’ purity and retention time, tR, in minutes (min) are indicated. Elemental analysis was carried out on a Costech ECS 4010 instrument.

Synthesis and characterization of compounds

Synthesis of 4-[(4-bromophenyl)sulfonyl]benzoyl chloride 2

4-[(4-Bromophenyl)sulfonyl]benzoic acid 1 [31] (6.82 g, 20 mmol) was heated under reflux with an excess of thionyl dichloride (35 mL, 57.10 g, 480 mmol). Unreacted SOCl2 was distilled off the reaction mixture under vacuum. Obtained colourless crystals were used crude in the following reaction; yield = 99% (7.12 g), m.p. = 154 - 155°C (lit. [32] 154°C).

FT-IR (KBr, ν cm–1): 3079s; 3040m; 1779vs; 1740vs; 1591m; 1572vs; 1470m; 1333vs; 1302s; 1287s; 1126v; 888vs; 851s; 753vs; 730vs; 647v; 575vs.

Synthesis of 2-[(4-bromophenyl)sulfonyl]benzamido]-3-phenylpropanoic acid 3

Phenylalanine (3.30 g, 20 mmol) was dissolved in 1 N NaOH solution (20 mL). To this solution cooled to 0 - 5°C, a solution of raw 4-[(4-bromophenyl)sulfonyl]benzoyl chloride 2 (7.19 g, 20 mmol) in anhydrous dichloromethane (45 mL), and a 2 N NaOH solution (10 mL), respectively were added simultaneously, dropwise, under stirring, for 30 min. Reaction mixture stirring was continued for 1 h at room temperature. The separated aqueous layer was acidified with 2 N HCl. Precipitated solid was isolated by filtration, washed with water, dried, and recrystallized as white acicular crystals; yield = 92% (8.98 g); m.p. = 179 - 180°C (water).

Figure 1.

Atoms numbering of compound 3 used for the assignment of NMR signals

UV-Vis (CH3OH, λ nm) (lg ε): 202.6 (4.47); 250.9 (4.17).

FT-IR (KBr, ν cm–1): 3356s; 3085m; 3058m; 3027m; 2978m; 2938m; 2869m; 2703w; 2640w; 2590w; 2530w; 1734vs; 1622vs; 1574s; 1547vs; 1492m; 1456m; 1447m; 1323vs; 1310s; 1295vs; 1161vs; 852m; 621s; 570s.

1H-NMR (DMSO-d6, δ ppm, J Hz): 3.04 (dd, 13.7, 10.4, 1H, H-18); 3.20 (dd, 13.7, 4.4, 1H, H-18); 4.64 (m, 1H, H-4); 7.10-7.30 (m, 5H, H-20 – H-24); 7.84 (d, 8.8, 2H, H-14, H-16); 7.91 (d, 8.8, 2H, H-13, H-17); 7.96 (d, 8.5, 2H, H-8, H-10); 8.06 (d, 8.5, 2H, H-7, H-11); 8.99 (d, 8.0, 1H, H-3).

13C-NMR (DMSO-d6, δ ppm): 36.18 (C-18); 54.21 (C-4); 126.32 (C-22); 127.54 (C-8, C-10); 128.13 (C-20, C-24); 128.35 (C-15); 128.65 (C-7, C-11); 128.94
(C-21, C-23); 129.42 (C-13, C-17); 132.88 (C-14, C-16); 137.88 (C-19); 138.63 (C-6); 139.82 (C-9); 142.74 (C-12); 164.95 (C-2); 172.68 (C-5).

RP-HPLC (methanol: water 30:70, v/v; 1 mL/min; 250 nm): purity = 99.63%; t_R = 4.57 min.

Anal. (%): Calcd. for C_{16}H_{14}BrNO_S (488.35 g/mol): C, 54.11; H, 3.72; N, 2.87; S, 6.57. Found: C, 54.06; H, 3.71; N, 2.86; S, 6.57.

**Synthesis of 4-benzyl-2-[(4-bromophenyl)sulfonyl]phenyl]-1,3-oxazol-5-(4H)-one 4**

2-[(4-Bromophenyl)sulfonyl]benzamide]-3-phenylpropanoic acid 3 (5.13 g, 10.5 mmol) was suspended with stirring in anhydrous CH_2Cl_2 (50 mL) and an equimolar quantity of 4-methylmorpholine (1.15 mL) was added. Then, 1 mL (10.5 mmol) of ethyl chloroformate was slowly added to the reaction mixture. The solution was stirred for another 30 min and then poured over a mixture of ice and water (100 mL). The organic phase was isolated, washed with 5% NaHCO_3 solution, with water, and dried over anhydrous MgSO_4. After in vacuo concentration, solid product 4 was recrystallized from cyclohexane as white crystals; yield = 93% (4.59 g); m.p. = 161 - 162°C.

UV-Vis (CH_3OH, λ nm) (lg ε): 202.6 (4.48); 252.9 (4.21).

FT-IR (KBr, v cm⁻¹): 3086m; 3062m; 3031m; 2988w; 2930w; 2851w; 1822vs; 1651vs; 1600m; 1574s; 1496m; 1473m; 1455m; 1327vs; 1297vs; 1278s; 1159vs; 1044vs; 852s; 614vs; 570vs.

^1H-NMR (CDCl_3, δ ppm, J Hz): 3.19 (dd, 14.0, 6.6, 1H, H-1); 3.38 (dd, 14.0, 4.9, 1H, H-8); 4.72 (dd, 6.6, 4.9, 1H, H-4); 7.15-7.24 (m, 5H, H-20 - H-24); 7.67 (d, 8.8, 2H, H-14, H-16); 7.81 (d, 8.8, 2H, H-13, H-17); 7.99 (d, 8.5, 2H, H-8, H-10); 8.04 (d, 8.5, 2H, H-7, H-11).

^13C-NMR (CDCl_3, δ ppm): 37.24 (C-18); 66.84 (C-4); 127.44 (C-22); 128.11 (C-8, C-10); 128.58 (C-7, C-11); 128.92 (C-20, C-24); 129.25 (C-15); 129.50 (C-13, C-17); 129.62 (C-21, C-23); 130.37 (C-6); 132.93 (C-14, C-16); 134.89 (C-19); 139.82 (C-9); 144.90 (C-12); 160.34 (C-2); 176.78 (C-5).

**Figure 2.**

Atoms numbering of compound 4 used for the assignment of NMR signals

A 3-fold molar excess of anhydrous aluminium trichloride (2.00 g, 15 mmol) was gradually added under magnetic stirring, at room temperature, to crude 4-benzyl-2-[(4-bromophenyl)sulfonyl]phenyl]-1,3-oxazol-5-(4H)-one 4 (2.35 g, 5 mmol) in 25 mL of anhydrous aromatic hydrocarbon (toluene or m-xylene). The reaction mixture was further stirred for 20 h until the release of hydrochloric acid ceased and then poured over 100 mL of ice-water mixture acidulated with 5 mL of 37% HCl. The precipitated solid was filtered off, washed with cold water, and then with a cold ethanol-water mixture (1:1, v/v). The

A 3-fold molar excess of anhydrous aluminium trichloride (2.00 g, 15 mmol) was gradually added under magnetic stirring, at room temperature, to crude 4-benzyl-2-[(4-bromophenyl)sulfonyl]phenyl]-1,3-oxazol-5-(4H)-one 4 (2.35 g, 5 mmol) in 25 mL of anhydrous aromatic hydrocarbon (toluene or m-xylene). The reaction mixture was further stirred for 20 h until the release of hydrochloric acid ceased and then poured over 100 mL of ice-water mixture acidulated with 5 mL of 37% HCl. The precipitated solid was filtered off, washed with cold water, and then with a cold ethanol-water mixture (1:1, v/v). The
separated aqueous layer was extracted twice with 15 mL of CH₂Cl₂. Combined organic phases were washed with water, dried (Na₂SO₄), and concentrated by vacuum distillation, leaving the second fraction of the raw product. Purification by recrystallization from ethanol gives 6 as colourless crystals.

4-[(4-Bromophenyl)sulfonyl]-N-[1-oxo-3-phenyl-1-(3-tolylopropan-2-yl)benzamide 6a, obtained by reaction of 4 with toluene (21.63 g, 234.75 mmol); yield = 87% (2.45 g); m.p. = 222 - 224°C.

UV-Vis (CH₃OH, λ nm) (lg ε): 203.5 (4.49); 255.5 (4.26).

FT-IR (KBr, ν cm⁻¹): 3392s; 3084w; 3060w; 3028m; 2970w; 2930m; 2863w; 1682vs; 1650vs; 1604s; 1573s; 1513vs; 1483s; 1455m; 1329vs; 1299s; 1293s; 1162vs; 855m; 617vs; 578vs.

¹H-NMR (DMSO-d₆, δ ppm, J Hz): 2.35 (s, 3H, CH₃); 3.01 (dd, 13.9, 8.9, 1H, H-18); 3.19 (dd, 13.9, 4.4, 1H, H-18); 5.66 (m, 1H, H-4); 7.15 (t, 7.4, 1H, H-22); 7.29-7.37 (m, 4H, H-20, H-21, H-23, H-24); 7.32 (d, 8.2, 2H, H-27, H-29); 7.84 (d, 8.5, 2H, H-14, H-16); 7.90 (d, 8.5, 2H, H-13, H-17); 7.93 (d, 8.5, 4H, H-8, H-10, H-26, H-30); 8.04 (d, 8.5, 2H, H-7, H-11); 9.21 (d, 8.0, 1H, H-3).

¹³C-NMR (DMSO-d₆, δ ppm): 21.25 (CH₃); 36.11 (C-18); 55.84 (C-4); 126.46 (C-22); 127.71 (C-8, C-10); 128.33 (C-15); 128.48 (C-21, C-23, C-27, C-29); 128.80 (C-7, C-11); 129.31 (C-20, C-24); 124.6 (C-13, C-17); 129.58 (C-26, C-30); 132.62 (C-25); 133.04 (C-14, C-16); 137.97 (C-19); 138.6 (C-6); 139.92 (C-9); 142.94 (C-12); 144.02 (C-28); 164.81 (C-2); 197.61 (C-5).

RP-HPLC (methanol:water 60:40, v/v; 1 mL/min; 250 nm): purity = 94.53%; tR = 6.82 min.

Anal. (%): Calcd. for C₃₉H₂₃BrN₂O₅S (562.47 g/mol): C, 61.92; H, 4.30; N, 2.49; S, 5.70. Found: C, 61.97; H, 4.29; N, 2.49; S, 5.72.

4-[(4-Bromophenyl)sulfonyl]-N-[1-(4,4-dimethyl-phenyl)-1-oxo-3-phenylpropan-2-yl]benzamide 6b, obtained by reaction of 4 with m-xylene (21.70 g, 204.39 mmol); yield = 90% (2.59 g); m.p. = 209 - 210°C.

UV-Vis (CH₃OH, λ nm) (lg ε): 203.5 (4.48); 254.6 (4.19).

FT-IR (KBr, ν cm⁻¹): 3394vs; 3084m; 3063m; 3035m; 3033m; 2962m; 2927m; 2863w; 1682vs; 1654vs; 1610s; 1572s; 1513s; 1481vs; 1454s; 1325vs; 1294vs; 1162vs; 835m; 615vs; 578vs.

¹H-NMR (DMSO-d₆, δ ppm, J Hz): 2.27 (s, 3H, CH₃); 2.36 (s, 3H, CH₃); 2.99 (dd, 14.0, 10.0, 1H, H-18); 3.16 (dd, 14.0, 4.4, 1H, H-18); 5.45 (dd, 10.0, 8.0, 4.4, 1H, H-4); 7.00-7.30 (m, 6H, H-20 - H-24, H-29); 7.09 (bs, 1H, H-27); 7.77 (d, 8.5, 1H, H-30); 7.85 (d, 8.8, 2H, H-14, H-16); 7.90 (d, 8.8, 2H, H-13, H-17); 7.90 (d, 8.5, 2H, H-8, H-10); 8.04 (d, 8.5, 2H, H-7, H-11); 9.15 (d, 8.0, 1H, H-3).

¹³C-NMR (DMSO-d₆, δ ppm): 20.30 (CH₃); 20.84 (CH₃); 35.36 (C-18); 58.10 (C-4); 126.12 (C-29); 126.26 (C-22); 127.57 (C-8, C-10); 128.12 (C-21, C-23); 128.13 (C-7, C-11); 128.18 (C-30); 128.51 (C-15); 129.02 (C-20, C-24); 129.43 (C-13, C-17); 132.26 (C-27); 132.89 (C-14, C-16); 133.35 (C-25); 137.89 (C-26); 137.97 (C-19); 138.49 (C-6, C-28); 139.77 (C-9); 141.36 (C-12); 164.80 (C-2); 201.33 (C-5).

Synthesis of 5-aryl-4-benzyl-2-[(4-bromophenyl)sulfonyl]phenyl]-1,3-oxazoles 7a,b

Figure 4.

Atoms numbering of compounds 7a,b used for the assignment of NMR signals

Raw N-[1-aryl-1-oxo-3-phenylpropan-2-yl]-4-[(4-bromophenyl)sulfonyl]benzamide 6 (10 mmol) in phosphoryl trichloride (20 mL, 217.83 mmol) was heated at reflux temperature for 4 h. The excess of POCl₃ was subsequently distilled off in vacuo. The oily residue was poured over an ice-water mixture and extracted with CH₂Cl₂ (2 × 20 mL). Combined organic phases were washed with 5% NaHCO₃ solution, then with water and dried over anhydrous Na₂SO₄. The solvent was removed under vacuum and the obtained crude solid 7 was recrystallized from ethanol as colourless crystals.

4-Benzyl-2-[(4-bromophenyl)sulfonyl]phenyl]-5-(p-tolyl)-1,3-oxazole 7a, obtained from 5.62 g of 6a; yield = 87% (4.74 g); m.p. = 211 - 213°C.

UV-Vis (CH₃OH, λ nm) (lg ε): 203.5 (4.49); 252.0 (4.24); 340.1 (4.24).

FT-IR (KBr, ν cm⁻¹): 3086m; 3076m; 2919m; 2860w; 1597s; 1574s; 1542m; 1509s; 1495m; 1473m; 1453m; 1326vs; 1293s; 1158vs; 1087vs; 844s; 618vs; 566vs.

¹H-NMR (CDCl₃, δ ppm, J Hz): 2.38 (s, 3H, CH₃); 4.18 (s, 2H, H-18); 7.20-7.30 (m, 5H, H-20 - H-24); 7.27 (d, 8.2, 2H, H-27, H-29); 7.54 (d, 8.2, 2H, H-26, H-30); 7.65 (d, 8.6, 2H, H-14, H-16); 7.82 (d, 8.6, 2H, H-13, H-17); 7.99 (d, 8.8, 2H, H-8, H-10); 8.21 (d, 8.8, 2H, H-7, H-11).
Results and Discussion

Chemistry

Synthetic transformations into title derivatives started from the following raw materials: 4-(4-bromophenyl)-sulfonyl]benzoic acid 1 and corresponding acyl chloride 2 which are literature-known compounds [31, 32]. Carboxylic acid 1 was obtained from commercially available 4-methylbenzene-1-sulfoniy chloride and bromobenzene by two successive reactions [31]. Then, compound 1 was converted into 4-(4-bromophenyl)-sulfonyl]benzoyl chloride 2, the method being adapted based on previously described procedures [12, 30]. Further, phenylalanine was acylated with raw material 2 to new 2-[4-(4-bromophenyl)sulfonyl]benzamido]-3-phenylpropanoic acid 3. Subsequently, N-acetyl-ω-amino acid 3 was cyclodehydrated, under the action of ethyl carbonochloridate in presence of 4-methyl- morpholine to 4-benzyl-2-[4-(4-bromophenyl)sulfonyl]phenyl]benzamido]-3-phenylpropanoic acid 5. Was prepared by reacting N-acetylated phenylalanine 3 with thiophen dichloride. By AlCl3-catalysed acylation of arenes (toluene, m-xylene) with 2-aryl-4-benzyl-3-xooazol-5(4H)-one 4, the 4-aryl-2-aza-3-benzyl-1-[(4-bromophenyl) sulfonyl]phenyl]-1,4-butanediones 6a,b were obtained. Further, the intramolecular condensation and dehydration of N-(1-aryl-1-oxoo-3-phenylpropan-2-yl)-4-[4-bromophenyl)sulfonyl]benzamides 6a,b under the action of phosphoryl trichloride, known as Robinson-Gabriel reaction, yielded 5-aryl-4-benzyl-1,3-oxazoles containing 4-[4-bromophenyl]sulfonyl]phenyl moiety in position 7a,b.

Depicted chemical structures of new derivatives were established based on NMR spectroscopy (1H, 13C, COSY, HETCOR), UV-Vis, IR, MS data and elemental analysis.

The UV-Vis spectra of the synthesised compounds presented E band at max = 202.6 or 203.5 nm, and B band in 249.3 - 255.5 nm range. In addition, 1,3-oxazoles 7 spectra showed a third absorption maximum at longer wavelengths (322.5 or 340.1 nm), due to the appearance of the heterocyclic chromophore, which determines the extent of π electrons conjugation.

The FT-IR spectra of the new compounds presented all the expected characteristic absorption bands. In the case of acyclic precursors 3 and 6a,b, main peaks were due to N-H valence vibration, ν(N-H), in 3356 - 3394 cm⁻¹ range, ketonic carbonyl valence vibration, ν(C=O–C–C), at 1682 or 1734 cm⁻¹, and stretching vibration of amidic carbonyl, ν(C=O–N), in 1622 - 1654 cm⁻¹ interval. Representative peaks for the hydrogen-bonded dimer of N-acetyl phenylalanine 3 were also recorded: a broad absorption band due to O-H stretch, ν(O-H), between 2500 and 3300 cm⁻¹, and four broad bands in 2530 - 2703 cm⁻¹ interval. The IR spectrum of acyl chloride 5 showed a fundamental band due to...
to ν(ν=C-Cl) at 1823 cm⁻¹. Fermi resonance peak at 1794 cm⁻¹, and band due to C-Cl valence vibration, ν(ν=Cl-C-Cl), at 891 cm⁻¹. The IR spectral data of five-membered-ring systems 4 and 7a,b demonstrated that cyclcondensation of open-chain intermediates 3 and 6a,b, respectively occurred. In compound 4 IR spectrum, carbonyl valence vibration, ν(C=O), was shifted at higher wavenumber (1822 cm⁻¹); besides, ν(N-H), ν(O-H), and ν(ν=C=N) peaks were not recorded. Also, absorption bands were not observed in the N-H and C=O regions of 1,3-oxazoles 7 spectra. The IR spectra of pentaatomic heterocycles 4 and 7a,b showed C=N valence vibration, ν(C=N), at 1651 cm⁻¹ (4), and 1597 or 1602 cm⁻¹ (7a,b). Furthermore, C-O-C symmetrical stretching vibration, νsymp(C-O-C), appeared at 1044 cm⁻¹ in saturated azlactone 4 spectrum, and at 1089 cm⁻¹ or 1101 cm⁻¹ in 1,3-oxazoles 7a,b spectra, and C-O-C asymmetrical stretching vibration, νasmp(C-O-C), was registered at 1278 cm⁻¹ (4), and 1280 cm⁻¹ (7b).

The 1H-NMR spectra of the new compounds confirmed proposed chemical structures. Moreover, 1H-1H COSY spectra facilitated assigning the signals. In 1H-NMR spectra of compounds 3 and 6a,b, H-3 signal was recorded as a doublet at δ values of 8.99 ppm (3) and 9.15 or 9.21 ppm (6a,b), due to the coupling with H-4 (J = 8.0 Hz). As proof that cyclization of acyclic intermediates 3 and 6a,b have taken place, in 1H-NMR spectra of heterocycles 4 and 7a,b, the signal assigned to NH proton (observed in the structure of the two raw materials) was absent. In 1H-NMR spectra of 3 and 6a,b, the H-4 signal appeared as a doublet of doublets of doublet of multiplet at δ = 4.64 ppm (3) and 5.45 or 5.66 ppm (6a,b), due to H-4 coupling with the two nonequivalent H-18 protons and one NH proton. For azlactone 4, the H-4 signal was registered at δ = 4.72 ppm as a doublet of doublets due to coupling with the nonequivalent H-18 protons. The signal of H-4 (that appeared in 1H-NMR spectra of 6a,b), was not observed in spectra of heterocyclic analogues 7a,b as proof that cyclcondensation occurred. The 1H-NMR spectrum of 4 displayed two signals due to nonequivalent H-18 protons as a doublet of doublets, which showed discernible downfield shifts of 0.15 and 0.18 ppm, compared to corresponding protons signals in the spectrum of intermediate 3. The 1H-NMR spectra of 1,3-oxazoles 7a,b highlighted a downfield shift of signal assigned to two magnetically equivalent H-18 protons as a singlet at δ = 3.94 or 4.18 ppm relative to the two signals registered for N-acyl-α-amino ketones 6a,b as a doublet of doubles (due to geminal coupling between methylene nonequivalent protons and vicinal coupling between H-18 protons and H-4 proton) at 3.01 and 3.19 ppm (6a) and 2.99 and 3.16 ppm (6b).

The 13C-NMR spectra also proved the syntheses of the compounds took place. Besides, 1H-13C COSY spectra allowed the assignments of 13C peaks. The C-4 signal, which was recorded at δ = 54.21 ppm in the 13C-NMR spectrum of N-acylated phenylalanine 3, was shifted downfield with 12.63 ppm after cyclization to 4. Moreover, in the case of 1,3-oxazol-5(4H)-one 4, the C-2 resonated at 160.34 ppm (being shifted upfield with 4.61 ppm than C-2 of intermediate 3), and the C-5 at 176.78 ppm (being deshielded with 4.10 ppm than C-5 of precursor 3). The signal assigned to C-2 of 2,4,5-trisubstituted 1,3-oxazoles 7a,b was registered at 157.71 or 158.43 ppm, while the peak attributed to C-2 from -CONH- group of N-acyl-α-amino ketones 6a,b (from δ = 164.80 or 164.81 ppm) was absent in aromatic heterocycles 7a,b spectra. C-5 of 1,3-oxazoles 7a,b resonated at δ = 148.20 or 148.60 ppm, whereas the corresponding atom of 6a,b at δ = 197.61 or 201.33 ppm, revealing a high-field shift for this carbon from cyclization products 7a,b structure, as a confirmation that the reaction took place.

The mass spectrum of 1,3-oxazol-5(4H)-one 4 obtained by GC-El-MS analysis played an important role in elucidating the structure of this compound. The two very unstable molecular ions of 4 that correspond to bromine isotopes ([79Br/81Br]Br) did not show peaks in the mass spectrum. They began to fragment at the level of the heterocyclic nucleus by removing a molecule of carbon dioxide with the obtaining of two cation-radicals corresponding to isotopes of bromine with m/z = 425/427 (with relative abundances: 13.36% and 15.27%, respectively). The base peak (PB) [79BrC3H5SO]⁺ with m/z = 203 and corresponding cation [81BrC3H5SO]⁺ with m/z = 205 (with relative abundance = 93.13%) were formed in accordance with 79Br/81Br isotopic ratio of about 1:1. Other main fragments of 4 corresponding to 79Br and 81Br isotopes are indicated in section Materials and Methods.

Cytotoxicity assessment

Daphnia magna bioassay results are presented in Table I. At 24 h, at the highest concentrations, with the exception of 6a which exhibited at 24 h an L% of 45%, all tested compounds did not exceed 20%. At 48 h, the highest toxicity was induced by compound 7a. However, this toxicity wasn’t dependent on the concentrations. For this compound, at all concentrations was observed an L% between 30 and 60%, and thus, lower goodness of fit. For all other compounds, the correlation between the concentrations and the L% was satisfactory (for compound 4 and phenylalanine between 0.6 and 0.69 and for other compounds over 0.7) (Figure 5). The cytotoxicity on D. magna decrease in the following order: 7a, 6a, 7b, 6b, 3, 1, and 4. Phenylalanine induced a maximum of 30% lethality at 48 h. The 95% CI values for compounds 6a, 7b and 6b indicate similar toxicities of these three compounds. The values obtained experimentally were significantly higher than those predicted using GUSAR software.
Figure 5.
Lethality curves on *Daphnia magna* for selected compounds:

- a – 3, b – 4, c – 6a, d – 6b, e – 7a, f – 7b, g – phenylalanine, h – 1
Conclusions

Seven new compounds, derived from phenylalanine, which incorporate in the structure the 4-[(4-bromo-phenyl)sulfonyl]phenyl fragment, were designed, synthesized, and physicochemically characterized. 1,3-Oxazol-5-(4H)-one 4 was produced by Steiger acylation of phenylalanine with acyl chloride 2, followed by intramolecular cyclodehydration of acyclic precursor 3. N-Acyl phenylalanyl chloride 5 was obtained by reaction of 3 with SOCl₂. N-Acyl-α-amino ketones 6a,b were generated by the reaction of 1,3-oxazol-5(4H)-one 4 with aromatic hydrocarbons in presence of aluminium trichloride. The 2,4,5-trisubstituted 1,3-oxazoles 7a,b were synthesized by cyclization of open-chain intermediates 6a,b under the action of phosphoryl trichloride. Compounds structure was elucidated through spectral methods and elemental analysis.

*Daphnia magna* bioassay revealed that the newly synthesized compounds 6a, 6b, 7a, and 7b exhibited high cytotoxicity being promising candidates for future biological investigations.

Conflict of interest

The authors declare no conflict of interest.

References


