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ORIGINAL ARTICLE

DESIGN, SYNTHESIS, CHARACTERIZATION, AND CYTOTOXICITY EVALUATION OF NEW 4-BENZYL-1,3-OXAZOLE DERIVATIVES BEARING 4-(4-CHLOROPHENYLSULFONYL)PHENYL MOIETY

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Abstract

This paper presents the design, synthesis, characterization, and cytotoxicity evaluation of eight organic compounds that have in their molecules the 4-(4-chlorophenylsulfonyl)phenyl moiety: four acyclic precursors derived from phenylalanine (one *N*-acyl-α-amino acid, one *N*-acyl-α-amino acyl chloride, and two *N*-acyl-α-amino ketones), and four cyclization products: one 1,3-oxazol-5(4*H*)-one, and three 1,3-oxazoles substituted in 5-position with phenyl, *p*-tolyl, and *m*-xylyl group, respectively. Structures of synthesized compounds were confirmed by elemental analysis and spectral methods (UV-Vis, FT-IR, MS, ^{1}H- and ^{13}C-NMR). The compounds purity determination was performed by RP-HPLC. Cytotoxic effect of synthesized compounds was evaluated on *Daphnia magna* crustacean.

Rezumat

Lucrarea prezintă proiectarea, sinteza, caracterizarea și evaluarea citotoxicității a opt compuși organici care au în moleculele lor fragmentul 4-(4-clorofenilsulfonil)fenil: patru precursori aciclici derivați de la fenilalanină (un *N*-acil-α-aminoacid, o clorură de *N*-acil-α-aminoacil și două *N*-acil-α-aminocetone) și patru produși de ciclizare: o 1,3-oxazol-5(4*H*)-onă și trei 1,3-oxazoli substituiți în poziția 5 cu grupa fenil, *p*-tolil și respectiv, *m*-xilil. Structurile compușilor sintetizați au fost confirmate prin analiză elementală și metode spectrale (UV-Viz, FT-IR, SM, ^{1}H- și ^{13}C-RMN). Determinarea purității compușilor a fost realizată prin RP-HPLC. Efectul citotoxic al compușilor sintetizați a fost evaluat pe crustaceul *Daphnia magna*.

Keywords: *N*-acyl-α-amino acid, 1,3-oxazol-5(4*H*)-one, 1,3-oxazole, cytotoxicity

Introduction

Five-membered heterocycles with 1,3-oxazole ring are valuable compounds both for synthetic organic chemistry and medicinal/pharmaceutical chemistry because of the extensive spectrum of biological properties that they present. Numerous representatives of this class are bioactive molecules with analgesic, antiinflammatory [1], antimicrobial [2], antituberculosis [3], antidiabetic [4], anticancer effect [5] etc. Moreover, in nature are present various 1,3-oxazole-based derivatives, which were identified to have significant therapeutic properties, such as analgesic, antiviral, antioxidant, antifungal [6], antibacterial [7], anticancer [8], antimycobacterial action [9] etc. Saturated 1,3-oxazol-5(4*H*)-ones – which are stable, keto form of corresponding 5-hydroxy substituted 1,3-oxazoles – have cytotoxic [10], antiviral [11, 12], plant growth regulating activity [13].

Also, acyclic intermediates from *N*-acyl-α-amino acids class are known to present mucolytic [14], antihypertensive [15], anticancer [16], antianemic [17], anti-ulcer [18], vasoconstrictor action [19]. In addition, some *N*-acylated α-amino acids are specific antidotes in acute intoxications with antifolates [20], paracetamol [21] etc. Open-chain precursors from *N*-acyl-α-amino ketones class display antiviral [22], anti-inflammatory [23] and antithrombotic effect [24].

Besides, the literature study revealed that many diaryl sulfones have several biological properties [25-30]. For example, the structural prototype of this class: Dapsone exhibits remarkable efficient pharmacological

activity against *Mycobacterium leprae*. It is also used as part of multidrug therapy in the treatment of patients with all types of leprosy, dermatitis herpetiformis, and other dermatoses, for prophylaxis of *Pneumocystis jiroveci* pneumonia in immunodeficient patients and prevention of malaria. Notable, compounds resulting from incorporation into the same structure of a diaryl sulfone pharmacophore and of various types of heterocyclic analogues have shown potential biological activities [10, 13, 31, 32].

Taking into consideration these literature data and as a continuation of our research to obtain heterocycles with a 1,3-oxazole ring containing in 2-position a substituent derived from a diaryl sulfone [10, 13, 33], in this study, we present the design, synthesis, and characterization of new organic compounds from *N*-acyl-α-amino acids, 1,3-oxazol-5(4*H*)-ones, *N*-acyl-α-amino acyl chlorides, *N*-acyl-α-amino ketones, and 1,3-oxazoles classes which incorporate a 4-(4-chlorophenylsulfonyl)phenyl moiety into their molecules with the purpose of discovering new active substances and to study the influence of structural changes on biological activity. In this order, newly derivatives were assessed for cytotoxic activity using *Daphnia magna* bioassay. This screening test is reproducible, fast, simple, cost-effective and can predict the biological action [10].

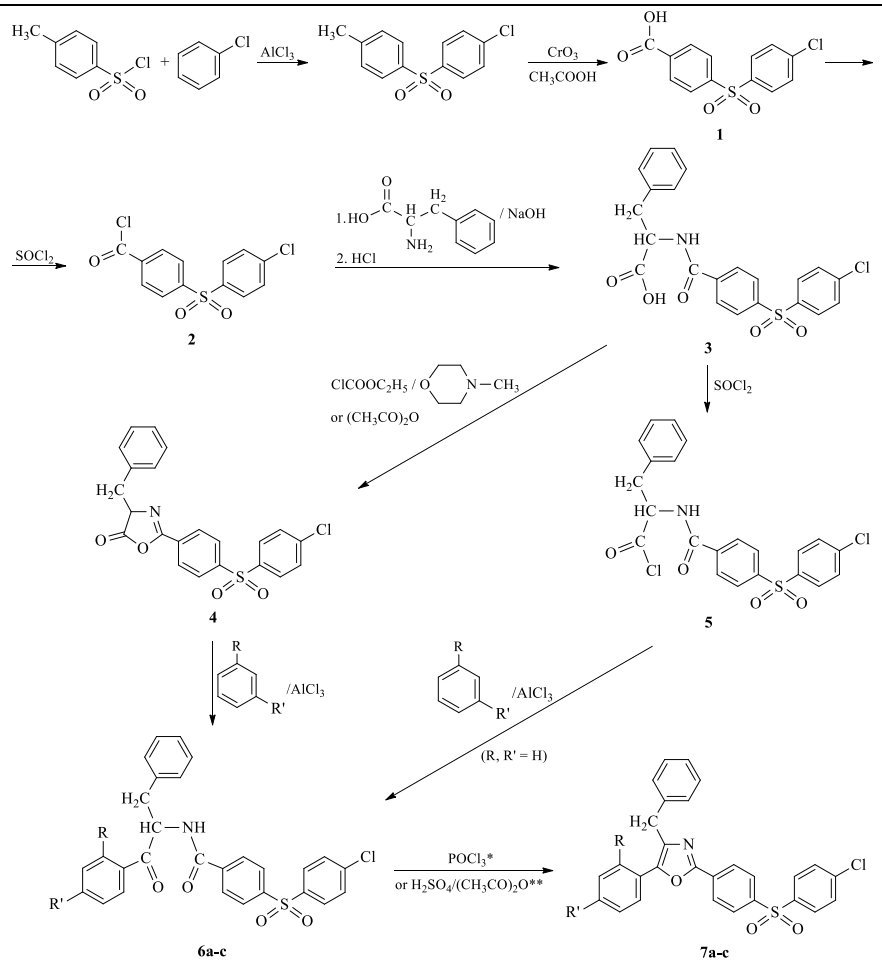
Materials and Methods

Chemistry

General: Melting points (uncorrected) were determined with a Boetius apparatus. UV-Vis spectra were registered for solutions in methanol (in a concentration of about 2.5×10^{-5} M) on an Analytik Jena AG Specord 40 spectrophotometer. FT-IR spectra were recorded on a Bruker Vertex 70 spectrometer (in KBr pellets). IR absorption bands intensity is given as: vs = very strong, s = strong, m = medium and w = weak. NMR spectra were registered on a Varian Gemini 300BB spectrometer at 300 MHz for ^{1}H-NMR and 75 MHz for ^{13}C-NMR in DMSO-d_6 or $CDCl_3$ as solvents. Chemical shifts (δ) are reported in ppm downfield of tetramethylsilane used as an internal standard. Coupling constants (*J*) are expressed in Hz. Abbreviations for multiplicities and descriptors of signals in ^{1}H-NMR spectra are as follows: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, m = multiplet; b = broad signal. LC-ESI-MS/MS spectra were recorded on a Varian 1200 LC-MS/MS high-performance liquid chromatograph coupled with a triple quadrupole mass spectrometer with an electrospray interface (ESI), by positive and/or negative ionization. Fragments were obtained by collision with argon at different energies up to 50 eV. GC-EI-MS analysis was performed using a Fisons Instruments GC 8000 with an electron impact quadrupole, MD 800 mass spectrometer detector. A fused-silica capillary column coated with poly(5% diphenyl/95% dimethylsiloxane) (SLB-5ms, L × I.D. 30 m × 0.32 mm, d_f 0.25 μm) was used. A 1 μL sample solution in dichloromethane was injected. The flow rate of the carrier gas (helium) was 2 mL/min. RP-HPLC chromatograms were registered on a Beckman System Gold 126 liquid chromatograph, with a System Gold 166 UV-Vis detector, a non-polar chromatography column (type LiChrosorb RP-18, 5 μm particle size, L × I.D. 25 cm × 4 mm) and a Rheodyne injection system. The flow rate of the mobile phase (a mixture of methanol-water in various proportions) was 1 mL/min. the purity and retention time (t_R) of the analysed compounds in minutes (min) are reported. Elemental analysis was performed using a Costech ECS 4010 analyser.

Synthesis and characterization of compounds

Successive reactions used to obtain title compounds are shown in Figure 1. Known raw material, acyl chloride **2**, was prepared by reacting corresponding carboxylic acid **1** with thionyl dichloride. Steiger *N*-acylation of phenylalanine with acyl chloride **2** afforded compound **3**. Further, *N*-acyl-α-amino acid **3** was cyclodehydrated in presence of ethyl chloroformate and 4-methylmorpholine or of acetic anhydride to saturated 2,4-disubstituted 1,3-oxazol-5(4*H*)-one **4**. Also, *N*-acylated phenylalanine **3** was refluxed in thionyl dichloride to acyl chloride **5**. Friedel-Crafts reaction of aromatic hydrocarbons (benzene, toluene, *m*-xylene) with saturated azlactone **4**, in presence of aluminium chloride, produced *N*-acyl-α-amino ketones **6**. Also, the reaction of *N*-acyl phenylalanyl chloride **5** with benzene was carried out. Subsequently, acyclic intermediates **6** were converted by Robinson-Gabriel synthesis into 2,4,5-trisubstituted 1,3-oxazoles **7** using phosphoryl trichloride or concentrated sulfuric acid in acetic anhydride as cyclodehydrating agents. Depicted chemical structures of novel compounds **3**-**7** were corroborated by spectral (UV-Vis, FT-IR, MS, ^{1}H- and ^{13}C-NMR) and elemental analysis data.



6a, **7a**: R = H, R' = H
6b, **7b**: R = H, R' = CH_3
6c, **7c**: R, R' = CH_3

* used for obtaining **7a-c**
** used for obtaining **7b**

Figure 1.
Multistep reaction sequence for compounds synthesis

*Synthesis of 2-[4-(4-chlorophenylsulfonyl)benzamido]-3-phenylpropanoic acid **3***

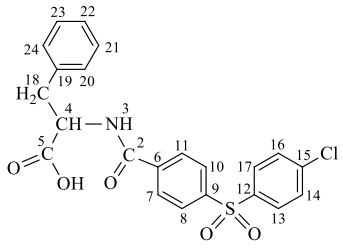


Figure 2.
Compound **3** structure with atomic numbering (for NMR assignments)

Phenylalanine (3.30 g, 20 mmol) was dissolved in 1 N sodium hydroxide solution (20 mL). To this solution cooled in an ice bath, a solution of crude 4-(4-chlorophenylsulfonyl)benzoyl chloride **2** (6.30 g, 20 mmol) in anhydrous dichloromethane (45 mL) and a 2 N sodium hydroxide solution (10 mL), respectively were added simultaneously, gradually, with agitation, for 30 min. The reaction mixture stirring was continued for 1 h at room temperature. Then, the aqueous layer was separated and acidified with 2 N hydrochloric acid. The precipitated solid was separated by filtration, washed with water, dried and purified by recrystallization from water as white acicular crystals; yield = 94% (8.35 g); m.p. = 165 - 166°C.

UV-Vis (CH_3OH, λ nm) (lg ε): 202.6 (4.48); 250.2 (4.16).

FT-IR (KBr, ν cm^{-1}): 3355s; 3086m; 3066m; 3028m; 2977m; 2937m; 2869m; 2704m; 2639m; 2592m; 2532m; 1734vs; 1623vs; 1567s; 1548vs; 1494m; 1478m; 1447m; 1324vs; 1309s; 1295s; 1162vs; 852m; 758s.

^{1}H-NMR (DMSO-d_6, δ ppm, *J* Hz): 3.04 (dd, 14.0, 10.7, 1H, H-18); 3.21 (dd, 14.0, 4.7, 1H, H-18); 4.64 (m, 1H, H-4); 7.16 (bt, 6.6, 1H, H-22); 7.20-7.38 (m, 4H, H-20, H-21, H-23, H-24); 7.70 (d, 8.8, 2H, H-14, H-16); 7.97 (d, 8.5, 2H, H-8, H-10); 7.99 (d, 8.8, 2H,

H-13, H-17); 8.07 (d, 8.5, 2H, H-7, H-11); 9.00 (d, 8.0, 1H, NH).

^{13}C-NMR (DMSO-d_6, δ ppm): 36.34 (C-18); 54.38 (C-4); 126.48 (C-22); 127.71 (C-8, C-10); 128.29 (C-20, C-24); 128.81 (C-7, C-11); 129.10 (C-21, C-23); 129.56 (C-13, C-17); 130.09 (C-14, C-16); 138.04 (C-19); 138.78 (C-6); 139.22 (C-15); 139.55 (C-12); 142.94 (C-9); 165.12 (C-2); 172.86 (C-5).

+ESI-MS/MS (*m/z*, rel. abund. %): 444 (^{35}Cl)/446 (^{37}Cl) $[M+H]^+$; 426/428 (8.3/11.3) $[M+H-H_2O]^+$; 398/400 (100, BP) $[M+H-H_2O-CO]^+$; 279/281 (16.7/23.3) $[^{35}ClC_6H_4SO_2C_6H_4CO]^+/[^{37}ClC_6H_4SO_2C_6H_4CO]^+$.

-ESI-MS/MS (*m/z*, rel. abund. %): 442 (^{35}Cl)/444 (^{37}Cl) $[M-H]^-$; 398/400 (76.7/80.6) $[M-H-CO_2]^-$; 307/309 (12.6/11.7) $[^{35}ClC_6H_4SO_2C_6H_4CONHCH\cdot]^-/[^{37}ClC_6H_4SO_2C_6H_4CONHCH\cdot]^-$; 294/296 (12.9/11.0) $[^{35}ClC_6H_4SO_2C_6H_4CONH]^-/[^{37}ClC_6H_4SO_2C_6H_4CONH]^-$; 279/281 (13.1/12.0) $[^{35}ClC_6H_4SO_2C_6H_4CO]^-/[^{37}ClC_6H_4SO_2C_6H_4CO]^-$; 251/253 (100, BP) $[^{35}ClC_6H_4SO_2C_6H_4]^-/[^{37}ClC_6H_4SO_2C_6H_4]^-$; 175/177 (10.3/10.2) $[^{35}ClC_6H_4SO_2]^-/[^{37}ClC_6H_4SO_2]^-$.

RP-HPLC (CH_3OH:H_2O = 30:70, 1 mL/min, 250 nm): purity = 99.05%; t_R = 4.48 min.

Anal. (%): Calcd. for $C_{22}H_{18}ClNO_5S$ (443.90 g/mol): C, 59.53; H, 4.09; N, 3.16; S, 7.22. Found: C, 59.59; H, 4.08; N, 3.16; S, 7.24.

*Synthesis of 4-benzyl-2-[4-(4-chlorophenylsulfonyl)-phenyl]oxazol-5(4H)-one **4***

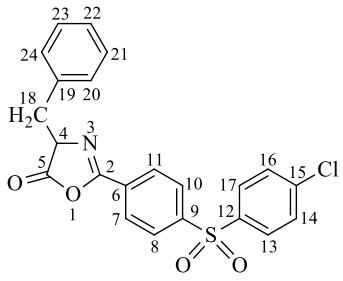


Figure 3.
Compound **4** structure with atomic numbering (for NMR assignments)

Method 1. 4-Methylmorpholine (1.15 mL, 10.5 mmol) was added under agitation into a suspension of 2-[4-(4-chlorophenylsulfonyl)benzamido]-3-phenyl-propanoic acid **3** (4.66 g, 10.5 mmol) in anhydrous dichloromethane (50 mL). Ethyl chloroformate (1 mL, 10.5 mmol) was added slowly to the reaction mixture. The solution obtained was stirred for another 30 min and then poured over ice water (100 mL). The organic phase was isolated, washed with 5% sodium hydrogen carbonate solution, then with water and dried ($MgSO_4$). The solvent was removed under *vacuum* and the solid product was recrystallized from cyclohexane as white crystals; yield = 93% (4.16 g).

Method 2. N-Acyl-α-amino acid **3** (2.23 g, 5 mmol) in an 8-fold molar excess of acetic anhydride (3.8 mL, 40 mmol) was heated under stirring at 140ºC for 1 h until all crystals were dissolved and then for another 30 min until saturated azlactone **4** was crystallized. After cooling, the precipitate was filtered, washed with cold ethanol and dried. White crystals were obtained; yield = 93% (2.06 g).

m.p. = 170 - 171°C (cyclohexane).

UV-Vis (CH_3OH, λ nm) (lg ε): 202.6 (4.48); 250.2 (4.16).

FT-IR (KBr, ν cm^{-1}): 3094m; 3064m; 3024m; 2953m; 2923w; 2851w; 1825vs; 1650vs; 1599m; 1572s; 1493m; 1478m; 1453m; 1320vs; 1296vs; 1234m; 1154vs; 1043vs; 854m; 766vs.

^{1}H-NMR ($CDCl_3$, δ ppm, *J* Hz): 3.19 (dd, 14.0, 6.6, 1H, H-18); 3.38 (dd, 14.0, 5.0, 1H, H-18); 4.72 (dd, 6.6, 5.0, 1H, H-4); 7.15-7.27 (m, 5H, H-20, H-21, H-22, H-23, H-24); 7.50 (d, 8.8, 2H, H-14, H-16); 7.89 (d, 8.8, 2H, H-13, H-17); 7.99 (d, 8.8, 2H, H-8, H-10); 8.05 (d, 8.8, 2H, H-7, H-11).

^{13}C-NMR ($CDCl_3$, δ ppm): 37.26 (C-18); 66.81 (C-4); 127.44 (C-22); 128.09 (C-8, C-10); 128.56 (C-20, C-24); 128.88 (C-7, C-11); 129.43 (C-13, C-17); 129.59 (C-21, C-23); 129.94 (C-14, C-16); 130.40 (C-6); 134.33 (C-19); 139.33 (C-15); 140.65 (C-9); 145.01 (C-12); 160.36 (C-2); 176.70 (C-5).

+ESI-MS/MS (*m/z*, rel. abund. %): 426 (^{35}Cl)/428 (^{37}Cl) $[M+H]^+$; 398/400 (100, BP) $[M+H-CO]^+$; 279/281 (41.5/21.4) $[^{35}ClC_6H_4SO_2C_6H_4CO]^+/[^{37}ClC_6H_4SO_2C_6H_4CO]^+$; 159/161 $[^{35}ClC_6H_4SO]^+/[^{37}ClC_6H_4SO]^+$.

GC-EI-MS (*m/z*, rel. abund. %): 381 (^{35}Cl)/383 (^{37}Cl) (8.51/4.26) $[M-CO_2]^{+\cdot}$; 265/267 (19.15/7.23) $[^{35}ClC_6H_4SO_2C_6H_4CH_2]^+/[^{37}ClC_6H_4SO_2C_6H_4CH_2]^+$; 159/161 (100, BP/30.64) $[^{35}ClC_6H_4SO]^+/[^{37}ClC_6H_4SO]^+$; 131 (11.91) $[C_6H_5CH{=}CHCO]^+$; 116 (23.83) $[C_6H_5C{=}C{=}NH]^+$; 111/113 (8.94/2.55) $[^{35}ClC_6H_4]^+/[^{37}ClC_6H_4]^+$; 91 (1.70) $[C_6H_5CH_2]^+$; 89 (15.32); 75 (3.40); 63 (2.55) $[C_5H_3]^+$; t_R = 46.61 min.

RP-HPLC (CH_3OH:H_2O = 60:40, 1 mL/min, 250 nm): purity = 91.49%; t_R = 5.05 min.

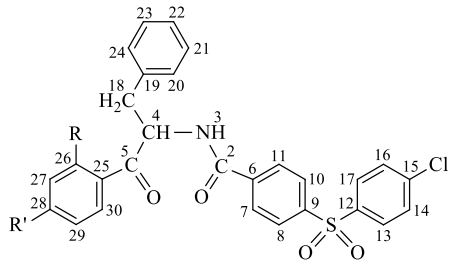
Anal. (%): Calcd. for $C_{22}H_{16}ClNO_4S$ (425.88 g/mol): C, 62.04; H, 3.79; N, 3.29; S, 7.53. Found: C, 62.09; H, 3.78; N, 3.30; S, 7.50.

*Synthesis of 2-[4-(4-chlorophenylsulfonyl)benzamido]-3-phenylpropanoyl chloride **5***

2-[4-(4-Chlorophenylsulfonyl)benzamido]-3-phenyl-propanoic acid **3** (2.44 g, 5.5 mmol) was heated under reflux with a 25-fold molar excess of thionyl dichloride (10 mL) until the emissions of gaseous hydrogen chloride and sulphur dioxide ceased. Excess $SOCl_2$ was distilled under *vacuum*. Crude product in the form of yellow crystals was subsequently used without further purification; yield = 99% (2.52 g); m.p. = 90 - 92°C.

FT-IR (KBr, ν cm^{-1}): 3373m; 3090m; 3065m; 3033m; 2969w; 2949w; 2843w; 1824s; 1793s; 1651s; 1580m; 1519m; 1477s; 1455m; 1327vs; 1292s; 1161vs; 891m; 851m; 765vs.

*Synthesis of N-(1-aryl-1-oxo-3-phenylpropan-2-yl)-4-(4-chlorophenylsulfonyl)benzamides **6a-c***



6a: R, R' = H; 6b: R = H, R' = CH_3; 6c: R, R' = CH_3

Figure 4.
General structure of compounds **6** with atomic numbering (for NMR assignments)

*General method 1 for obtaining **6a-c***. A 3-fold molar excess of anhydrous aluminium chloride (2.00 g, 15 mmol) was gradually added under agitation to crude 4-benzyl-2-[4-(4-chlorophenylsulfonyl)phenyl]-oxazol-5(4*H*)-one **4** (2.13 g, 5 mmol) in 25 mL of anhydrous aromatic hydrocarbons (benzene, toluene or *m*-xylene). The reaction mass was stirred for 20 h until hydrogen acid gas evolution ceased and then poured over 100 mL of ice-water mixture acidulated with 37% hydrochloric acid (5 mL). The precipitate was filtered, washed with cold water and then with a cool mixture in equal volumes of water-ethanol. The aqueous phase was separated and extracted twice with dichloromethane (15 mL). Combined organic layers were washed with water, dried (Na_2SO_4) and concentrated *in vacuo*, leaving the second fraction of crude product. Purification by recrystallization from ethanol led to **6b,c** as colourless crystals; **6a** could not be isolated in the pure state.

*Method 2 for obtaining **6a***. A 3-fold molar excess of anhydrous $AlCl_3$ (2.00 g, 15 mmol) was slowly added under stirring, to crude 2-[4-(4-chlorophenylsulfonyl)-benzamido]-3-phenylpropanoyl chloride **5** (2.31 g, 5 mmol) in anhydrous benzene (25 mL, 280 mmol). The reaction mixture was agitated for 20 h and then poured over ice water (100 mL), acidulated with fuming hydrochloric acid (5 mL). After extraction in dichloromethane, the organic phase was washed with a 5% sodium hydrogen carbonate solution, then with water and dried (Na_2SO_4). The concentration of solvents mixture *in vacuo* resulted in a crude product, which could not be purified.

*4-(4-Chlorophenylsulfonyl)-N-(1-oxo-3-phenyl-1-p-tolylpropan-2-yl)benzamide **6b***, obtained by reaction with toluene; method 1: yield = 89% (2.31 g); m.p. = 207 - 208°C (ethanol).

UV-Vis (CH_3OH, λ nm) (lg ε): 203.5 (4.49); 253.7 (4.22).

FT-IR (KBr, ν cm^{-1}): 3393s; 3085w; 3061w; 3029m; 2970w; 2933m; 2863w; 1682s; 1650vs; 1604s; 1575m; 1513vs; 1476s; 1456m; 1329vs; 1299s; 1162vs; 855m; 753vs.

^{1}H-NMR (DMSO-d_6, δ ppm, *J* Hz): 2.34 (s, 3H, CH_3); 3.02 (dd, 14.0, 9.9, 1H, H-18); 3.20 (dd, 14.0, 4.7, 1H, H-18); 5.67 (m, 1H, H-4); 7.15 (bt, 7.5, 1H, H-22); 7.24 (t, 7.5, 2H, H-21, H-23); 7.25 (m, 2H, H-20, H-24); 7.27 (d, 8.2, 2H, H-27, H-29); 7.69 (d, 8.5, 2H, H-14, H-16); 7.93 (d, 8.2, 4H, H-8, H-10, H-26, H-30); 7.98 (d, 8.5, 2H, H-13, H-17); 8.04 (d, 8.2, 2H, H-7, H-11); 9.21 (d, 8.0, 1H, NH).

^{13}C-NMR (DMSO-d_6, δ ppm): 21.11 (CH_3); 35.97 (C-18); 55.69 (C-4); 126.31 (C-22); 127.57 (C-8, C-10); 128.12 (C-21, C-23); 128.34 (C-7, C-11); 128.66 (C-27, C-29); 129.17 (C-26, C-30); 129.32 (C-20, C-24); 129.41 (C-13, C-17); 129.94 (C-14, C-16); 132.46 (C-25); 137.83 (C-19); 138.44 (C-6); 139.07 (C-15); 139.36 (C-9); 142.84 (C-12); 143.88 (C-28); 164.66 (C-2); 197.47 (C-5).

+ESI-MS/MS (*m/z*, rel. abund. %): 518 (^{35}Cl)/520 (^{37}Cl) $[M+H]^+$; 500/502 (16.2/50.9) $[M+H-H_2O]^+$; 296/298 (26.2/39.0) $[^{35}ClC_6H_4SO_2C_6H_4CONH_2 + H]^+$/$[^{37}ClC_6H_4SO_2C_6H_4CONH_2 + H]^+$; 279/281 (55.0/63.4) $[^{35}ClC_6H_4SO_2C_6H_4CO]^+$/$[^{37}ClC_6H_4SO_2C_6H_4CO]^+$; 223 (100, BP) $[C_6H_5CH_2CHCOC_6H_4CH_3]^+$; 195 (55.0/52.1) $[C_6H_5CH_2CHCOC_6H_4CH_3\text{-}CO]^+$; 131 $[C_6H_5CH_2C{=}CO]^+$; 91 (78.1/63.2) $[CH_3C_6H_4]^+$.

RP-HPLC (CH_3OH:H_2O = 60:40, 1 mL/min, 250 nm): purity = 98.30%; t_R = 6.40 min.

Anal. (%): Calcd. for $C_{29}H_{24}ClNO_4S$ (518.02 g/mol): C, 67.24; H, 4.67; N, 2.70; S, 6.19. Found: C, 67.29; H, 4.67; N, 2.71; S, 6.17.

*4-(4-Chlorophenylsulfonyl)-N-[1-(2,4-dimethylphenyl)-1-oxo-3-phenylpropan-2-yl]benzamide **6c***, obtained by reaction with *m*-xylene; method 1: yield = 91% (2.42 g); m.p. = 208-210 ºC (ethanol).

UV-Vis (CH_3OH, λ nm) (lg ε): 203.5 (4.48); 252.9 (4.17).

FT-IR (KBr, ν cm^{-1}): 3395s; 3087m; 3065w; 3056w; 3032w; 2963w; 2926w; 2863w; 1682s; 1654vs; 1611m; 1568m; 1512s; 1480s; 1454m; 1326vs; 1294s; 1162vs; 834m; 753s.

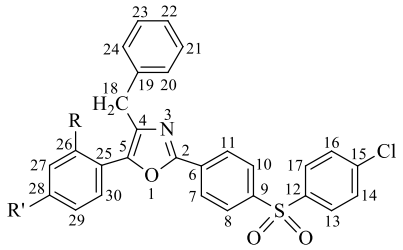
^{1}H-NMR (DMSO-d_6, δ ppm, *J* Hz): 2.28 (s, 3H, CH_3); 2.36 (s, 3H, CH_3); 2.99 (dd, 14.0, 9.9, 1H, H-18); 3.15 (dd, 14.0, 4.7, 1H, H-18); 5.44 (m, 1H, H-4); 7.05 - 7.32 (m, 7H, H-20, H-21, H-22, H-23, H-24, H-27, H-29); 7.70 (d, 8.8, 2H, H-14, H-16); 7.76 (d, 8.5, 1H, H-30); 7.84 (d, 8.8, 2H, H-8, H-10); 7.98 (d, 8.8, 2H, H-13, H-17); 8.04 (d, 8.8, 2H, H-7, H-11); 9.20 (d, 8.0, 1H, NH).

^{13}C-NMR (DMSO-d_6, δ ppm): 20.32 (CH_3); 20.86 (CH_3); 35.37 (C-18); 58.12 (C-4); 126.14 (C-29); 126.29 (C-22); 127.61 (C-8, C-10); 128.15 (C-21, C-23); 128.40 (C-30); 128.60 (C-7, C-11); 129.05 (C-20, C-24); 129.44 (C-13, C-17); 129.97 (C-14, C-16); 132.29 (C-27); 133.36 (C-25); 137.92 (C-26); 138.00 (C-19); 138.50 (C-6); 139.10 (C-15); 139.37 (C-9); 141.39 (C-28); 142.84 (C-12); 164.82 (C-2); 201.35 (C-5).

RP-HPLC (CH_3OH:H_2O = 60:40, 1 mL/min, 250 nm): purity = 90.83%; t_R = 7.63 min.

Anal. (%): Calcd. for $C_{30}H_{26}ClNO_4S$ (532.05 g/mol): C, 67.72; H, 4.93; N, 2.63; S, 6.03. Found: C, 67.70; H, 4.92; N, 2.64; S, 6.05.

*Synthesis of 5-aryl-4-benzyl-2-[4-(4-chlorophenylsulfonyl)phenyl]oxazoles **7a-c***



7a: R, R' = H; **7b**: R = H, R' = CH_3; **7c**: R, R' = CH_3

Figure 5.
General structure of compounds **7** with atomic numbering (for NMR assignments)

*General method 1 for obtaining **7a-c***. Crude *N*-(1-aryl-1-oxo-3-phenylpropan-2-yl)-4-(4-chlorophenylsulfonyl)benzamides **6a-c** (10 mmol) in phosphoryl trichloride (20 mL, 217.83 mmol) were refluxed for 4 h. Unreacted $POCl_3$ was removed by distillation under reduced pressure. After cooling, oily residue was added into ice-water and extracted twice with dichloromethane (20 mL). Combined organic phases were washed several times with 5% $NaHCO_3$ solution, then with water and dried (Na_2SO_4). After solvent removal, crude products **7a-c** were purified by recrystallization from ethanol as colourless crystals.

*Method 2 for obtaining **7b***. 4-(4-Chlorophenylsulfonyl)-*N*-(1-oxo-3-phenyl-1-*p*-tolylpropan-2-yl)benzamide **6b** (5.444 g, 10.51 mmol) was dissolved in ethyl acetate (40 mL). Acetic anhydride (3 mL, 3.22 g, 31.54 mmol) and 98% sulfuric acid (0.17 mL, 0.31 g, 3.16 mmol) in ethyl acetate (2.5 mL) were added. After refluxing for 3 h, a 5 N NaOH solution (12.6 mL, 63 mmol) diluted to 25 mL with water was added at room temperature. The reaction mass was heated for another 30 min and then cooled. The precipitate was filtered, washed with cold 1 N HCl, 10% NaCl solution and water. Layers of the filtrate were separated and the organic phase was dried (Na_2SO_4), and concentrated to dryness *in vacuo*, leaving the second fraction of crude product. High purity colourless crystals of compound **7b** resulted after purification.

*4-Benzyl-2-[4-(4-chlorophenylsulfonyl)phenyl]-5-phenyloxazole **7a***

Method 1: yield = 89% (4.33 g); m.p. = 199 - 201°C (ethanol).

UV-Vis (CH_3OH, λ nm) (lg ε): 203.5 (4.48); 247.6 (4.06); 333.9 (4.11).

FT-IR (KBr, ν cm^{-1}): 3087m; 3061m; 3028m; 2926w; 2845w; 1602s; 1590s; 1547m; 1495s; 1476s; 1453m; 1327vs; 1290s; 1281s; 1158vs; 1088vs; 845s; 765vs.

^{1}H-NMR ($CDCl_3$, δ ppm, *J* Hz): 4.20 (s, 2H, H-18); 7.18 - 7.32 (m, 5H, H-20, H-21, H-22, H-23, H-24); 7.36 (bt, 7.9, 1H, H-28); 7.45 (bt, 7.9, 2H, H-27, H-29); 7.49 (d, 8.8, 2H, H-14, H-16); 7.68 (d, 7.9, 2H, H-26, H-30); 7.85 (d, 8.8, 2H, H-13, H-17); 7.95 (d, 8.8, 2H, H-8, H-10); 8.16 (d, 8.8, 2H, H-7, H-11).

^{13}C-NMR ($CDCl_3$, δ ppm): 33.18 (C-18); 126.00 (C-26, C-30); 126.68 (C-22); 127.18 (C-8, C-10); 128.33 (C-7, C-11); 128.51 (C-20, C-24); 128.60 (C-28); 128.78 (C-21, C-23); 129.11 (C-27, C-29); 129.30 (C-13, C-17); 129.88 (C-14, C-16); 132.00 (C-25); 136.74 (C-6); 138.34 (C-4, C-19); 139.98 (C-9); 140.31 (C-15); 142.17 (C-12); 147.99 (C-5); 158.00 (C-2).

RP-HPLC (CH_3OH:H_2O = 70:30, 1 mL/min, 335 nm): purity = 95.13%; t_R = 4.68 min.

Anal. (%): Calcd. for $C_{28}H_{20}ClNO_3S$ (485.98 g/mol): C, 69.20; H, 4.15; N, 2.88; S, 6.60. Found: C, 69.25; H, 4.14; N, 2.89; S, 6.58.

*4-Benzyl-2-[4-(4-chlorophenylsulfonyl)phenyl]-5-p-tolyloxazole **7b***

Method 1: yield = 95% (4.75 g), method 2: yield = 90% (4.73 g); m.p. = 213 - 214°C (ethanol).

UV-Vis (CH_3OH, λ nm) (lg ε): 203.5 (4.49); 250.2 (4.10); 340.1 (4.12).

FT-IR (KBr, ν cm^{-1}): 3089m; 3063m; 3029m; 2919m; 2861w; 1595s; 1544m; 1509s; 1495s; 1476s; 1454m; 1326vs; 1290s; 1283s; 1155vs; 1087vs; 843s; 767vs.

^{1}H-NMR ($CDCl_3$, δ ppm, *J* Hz): 2.34 (s, 3H, CH_3); 4.13 (s, 2H, H-18); 7.15-7.28 (m, 7H, H-20, H-21, H-22, H-23, H-24, H-27, H-29); 7.50 (d, 7.9, 2H, H-26, H-30); 7.64 (d, 8.8, 2H, H-14, H-16); 7.85 (d, 8.8, 2H, H-13, H-17); 7.95 (d, 8.8, 2H, H-8, H-10); 8.16 (d, 8.8, 2H, H-7, H-11).

^{13}C-NMR ($CDCl_3$, δ ppm): 21.43 (CH_3); 33.17 (C-18); 125.49 (C-6); 125.94 (C-26, C-30); 126.60 (C-22); 127.08 (C-8, C-10); 128.28 (C-7, C-11); 128.50 (C-20, C-24); 128.72 (C-21, C-23); 129.26 (C-13, C-17); 129.77 (C-27, C-29); 129.83 (C-14, C-16); 132.07 (C-25); 136.16 (C-28); 138.48 (C-19); 138.86 (C-4); 140.05 (C-9); 140.24 (C-12); 142.04 (C-15); 148.18 (C-5); 157.70 (C-2).

+ESI-MS/MS (*m/z*, rel. abund. %): 500 (^{35}Cl)/502 (^{37}Cl) $[M+H]^+$; 422/424 $[M+H-C_6H_6]^+$; 279/281 (38.0/37.1) $[^{35}ClC_6H_4SO_2C_6H_4CO]^+$/$[^{37}ClC_6H_4SO_2C_6H_4CO]^+$; 159/161 $[^{35}ClC_6H_4SO]^+$/$[^{37}ClC_6H_4SO]^+$; 117 (100, BP) $[CH_3C_6H_4C{=}CH_2]^+$.

RP-HPLC (CH_3OH:H_2O = 70:30, 1 mL/min, 335 nm): purity = 97.57%; t_R = 4.85 min.

Anal. (%): Calcd. for $C_{29}H_{22}ClNO_3S$ (500.01 g/mol): C, 69.66; H, 4.43; N, 2.80; S, 6.41. Found: C, 69.69; H, 4.42; N, 2.81; S, 6.40.

*4-Benzyl-2-[4-(4-chlorophenylsulfonyl)phenyl]-5-(2,4-dimethylphenyl)oxazole **7c***

Method 1: yield = 90% (4.63 g); m.p. = 158 - 160°C (ethanol).

UV-Vis (CH_3OH, λ nm) (lg ε): 203.5 (4.47); 246.7 (4.02); 322.5 (4.01).

FT-IR (KBr, ν cm^{-1}): 3087w; 3063w; 3027m; 2923m; 2860w; 1602s; 1582s; 1494s; 1475m; 1453m; 1327vs; 1289s; 1281s; 1158vs; 1088vs; 843m; 767vs.

^{1}H-NMR ($CDCl_3$, δ ppm, *J* Hz): 2.31 (s, 3H, CH_3); 2.37 (s, 3H, CH_3); 3.94 (s, 2H, H-18); 7.06 (bd, 7.7, 1H, H-29); 7.10-7.30 (m, 5H, H-20, H-21, H-22, H-23, H-24); 7.14 (bs, 1H, H-27); 7.20 (d, 7.7, 1H, H-30); 7.48 (d, 8.8, 2H, H-14, H-16); 7.88 (d, 8.8, 2H, H-13, H-17); 7.98 (d, 8.6, 2H, H-8, H-10); 8.17 (d, 8.6, 2H, H-7, H-11).

^{13}C-NMR ($CDCl_3$, δ ppm): 20.45 (CH_3); 21.41 (CH_3); 32.45 (C-18); 124.46 (C-6); 126.46 (C-22); 126.77 (C-29); 127.01 (C-8, C-10); 128.30 (C-7, C-11); 128.59 (C-20, C-24); 128.65 (C-21, C-23); 129.27 (C-13, C-17); 129.83 (C-14, C-16); 130.15 (C-30); 131.79 (C-27); 132.32 (C-25); 137.82 (C-26); 138.95 (C-4, C-19); 139.97 (C-9, C-28); 140.24 (C-12); 142.04 (C-15); 148.59 (C-5); 158.42 (C-2).

RP-HPLC (CH_3OH:H_2O = 70:30, 1 mL/min, 335 nm): purity = 99.15%; t_R = 4.87 min.

Anal. (%): Calcd. for $C_{30}H_{24}ClNO_3S$ (514.03 g/mol): C, 70.10; H, 4.71; N, 2.72; S, 6.24. Found: C, 70.15; H, 4.70; N, 2.71; S, 6.22.

Cytotoxicity evaluation

The biological assessment was performed by *Daphnia magna* bioassay. The procedure was described in our previous works [10]. Young daphnids which were exposed to six concentrations from each compound (from 2.2 to 46 µg/mL) for a period of 72 h were used. The bioassay was carried out in duplicate. Phenylalanine and compound **1** were used as positive controls, whereas 1% dimethyl sulfoxide as a negative control. The experiment was performed at 25 ± 1°C in Sanyo MLR-351H, USA climatic chamber.

Lethality was registered at 24, 48 and 72 h. Lethality curves were plotted using the logarithm of concentration against lethality percentage, L (%). Prediction of $LC_{50\,48\,h}$ values was performed using GUSAR software.

Results and Discussion

Chemistry

Synthetic approach to title compounds starts from the key precursor, 4-(4-chlorophenylsulfonyl)-benzoic acid **1**, and corresponding acyl chloride **2** which were previously reported in the literature [34, 35].

Compound **1** was synthesized in two stages: by Friedel-Crafts sulfonylation of chlorobenzene with 4-methyl-benzenesulfonyl chloride in presence of $AlCl_3$, followed by oxidation of 1-(4-chlorophenylsulfonyl)-4-methylbenzene with chromium trioxide in glacial acetic acid, both reactions occurring at reflux [34]. Then, carboxylic acid **1** was transformed by reaction with thionyl dichloride into 4-(4-chlorophenylsulfonyl)-benzoyl chloride **2** [33]. This crude product was used for the *N*-acylation of phenylalanine to 2-[4-(4-chloro-phenylsulfonyl)benzamido]-3-phenylpropanoic acid **3**.

Intramolecular cyclodehydration of compound **3**, using ethyl chloroformate in presence of *N*-methylmorpholine in CH_2Cl_2 at ambient temperature or acetic anhydride at reflux, led to 4-benzyl-2-[4-(4-chlorophenylsulfonyl)-phenyl]oxazol-5(4*H*)-one **4**. Cyclization under basic conditions can be explained by a reaction mechanism similar to that previously indicated by us in literature for another 1,3-oxazol-5(4*H*)-one [13]. Cyclization of **3** to saturated azlactone **4** in the presence of acetic anhydride can take place through the reaction mechanism proposed in Figure 6. *N*-Acyl-α-amino acid **3** is in equilibrium with its enol tautomer **I**, which under the action of acetic anhydride gives a nucleophilic substitution reaction, with the formation of key intermediate, namely unstable acyclic ester **II**, which by an intramolecular nucleophilic addition mechanism with the removal of acetic acid leads to pentatomic heterocyclic compound **4**.

Also, *N*-acylated phenylalanine **3** was reacted with $SOCl_2$ at reflux to 2-[4-(4-chlorophenylsulfonyl)-benzamido]-3-phenylpropanoyl chloride **5** by a nucleophilic substitution mechanism.

Friedel-Crafts acylation reaction of arenes (benzene, toluene, *m*-xylene) with 1,3-oxazol-5(4*H*)-one **4**, catalysed by $AlCl_3$, yielded *N*-(1-aryl-1-oxo-3-phenylpropan-2-yl)-4-(4-chlorophenylsulfonyl)benzamides **6** by an electrophilic aromatic substitution mechanism presented by us in a formerly article [33]. *N*-Acyl-α-amino ketones **6b,c** were isolated as pure colorless crystals and physicochemically characterized, except for 4-(4-chlorophenylsulfonyl)-*N*-(1-oxo-1,3-diphenylpropan-2-yl)benzamide **6a** (obtained by reaction of **4** and **5**, respectively with benzene), which was used in crude form to synthesize 1,3-oxazole **7a**.

4-Aryl-2-aza-3-benzyl-1-[4-(4-chlorophenylsulfonyl)-phenyl]-1,4-butanediones **6a-c** underwent Robinson-Gabriel cyclization using phosphoryl trichloride at reflux with the formation of 5-aryl-4-benzyl-2-[4-(4-chlorophenylsulfonyl)phenyl]oxazoles **7a-c** in very good yields. Also, cyclization of **6a** to **7b** was carried out using H_2SO_4/$(CH_3CO)_2O$ in ethyl acetate, by heating under reflux. Proposed reaction mechanisms for the synthesis of 1,3-oxazole analogs starting from *N*-acyl-α-amino ketones under the action of $POCl_3$ and H_2SO_4, respectively are described in our recent literature [10].

Spectral analysis methods (UV-Vis, IR, MS, ^{1}H- and ^{13}C-NMR) and elemental analysis were used for characterizing synthesized compounds.

UV-Vis Spectral Data

UV-Vis spectra of new compounds show a sharp band (E band) at λ_{max} = 202.6 or 203.5 nm and an absorption maximum (B band) in range 246.7-253.7 nm. Besides, electronic absorption spectra of 1,3-oxazoles **7** presented a third intense band at longer wavelengths (322.5 - 340.1 nm), because of conjugation extension by the formation of the 1,3-oxazole chromophore.

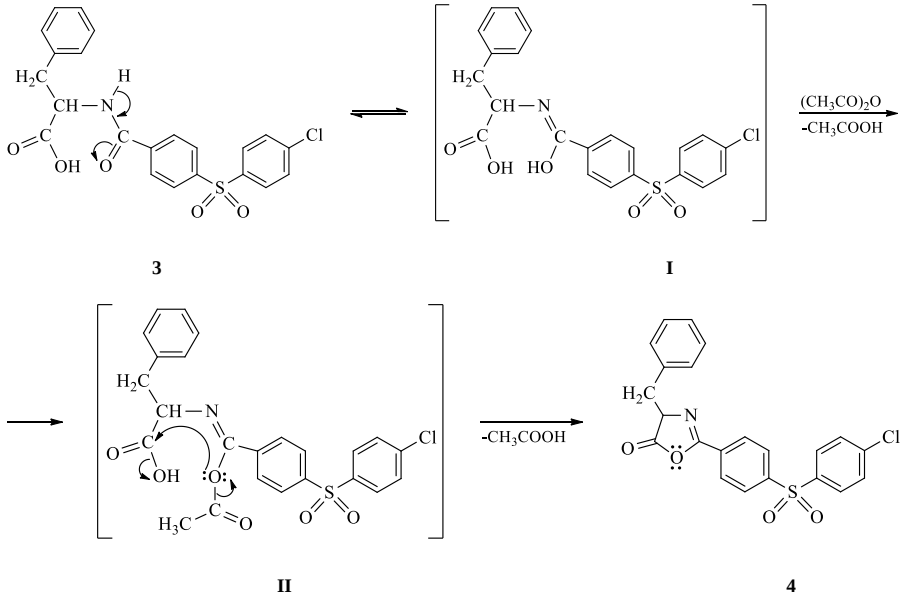


Figure 6.
Proposed mechanism for synthesis of 1,3-oxazol-5(4*H*)-one **4** from *N*-acyl-α-amino acid **3** in presence of $(CH_3CO)_2O$

IR Spectral Data

Infrared absorption spectra of newly synthesized compounds show absorption bands at characteristically wavenumbers of structural fragments present in molecules. Evidence of formation of open-chain intermediates **3** and **6**, is the presence in IR spectra of following characteristic absorption bands in intervals: 3355 - 3395 cm^{-1} due to N-H stretching vibration, ν(N-H), 1682 - 1734 cm^{-1} due to carbonyl stretching vibration, ν(O=C-C), 1623 - 1654 cm^{-1} due to amidic carbonyl group absorption, ν(O=C-N). Furthermore, for the hydrogen-bonded dimer of **3**, a very broad absorption band due to O-H stretching vibration, ν(O-H), is extending from 2500 cm^{-1} to 3200 cm^{-1}. Representative for dimer are also four broad medium intensity bands between 2532 and 2704 cm^{-1}. Main absorption bands presented in IR spectrum of acyl chloride **5** (namely, two strong absorption bands due to ν(O=C-Cl) at 1824 cm^{-1} assigned to fundamental vibration and 1793 cm^{-1} named Fermi resonance band, and a medium absorption band due to ν(Cl-C=O) at 891 cm^{-1}) confirmed that reaction of *N*-acyl-α-amino acid **3** with $SOCl_2$ took place. IR spectra of heterocycles **4** and **7** differ from those of acyclic precursors (**3** and **6**) from which they were obtained, by fact that they have absorption bands at characteristic, different values of wavenumbers, which proves that cyclizations have occurred. In IR spectrum of **4**, very strong absorption band due to carbonyl valence vibration, ν(C=O), is shifted at 1825 cm^{-1} and ν(N-H), ν(O-H) and ν(O=C-N) absorption bands which are characteristic for acyclic intermediate **3** were not presented. In the case of 1,3-oxazoles **7**, absorption peaks were not registered in the N-H and C=O regions. The absorption band at 1650 cm^{-1} (**4**) and 1595 or 1602 cm^{-1} (**7**) was attributed to C=N stretching vibration, ν(C=N), of these five-membered-ring systems. Moreover, IR spectra of **4** and **7** showed supplementary, very strong peaks at 1043 cm^{-1} (**4**) and 1087 or 1088 cm^{-1} (**7**) due to symmetrical C-O-C stretching vibration, ν_{sym}(C-O-C). Absorption band due to asymmetrical stretching vibration of the C-O-C system, ν_{as}(C-O-C), appears in the saturated azlactone **4** spectrum at 1234 cm^{-1} and in 1,3-oxazoles **7a-c** spectra at 1281 or 1283 cm^{-1}.

NMR Spectral Data

Signals in the ^{1}H-NMR spectra of new compounds are also consistent with proposed chemical structures. Besides, 2D ^{1}H-^{1}H COSY experiments were performed, which facilitated unambiguous assignments. It can be noticed presence in ^{1}H-NMR spectra of acyclic intermediates **3** and **6** of a signal as a doublet at δ = 9.00 ppm (**3**) and 9.20 or 9.21 ppm (**6**), assigned to secondary amide proton (H-3), which couples with H-4 (^3J = 8.0 Hz). In ^{1}H-NMR spectra of heterocyclic compounds **4** and **7**, signal attributed to the proton of NH group (H-3) from acyclic precursors **3** and **6** is absent and this demonstrates that cyclization products **4** and **7** have been synthesized. In ^{1}H-NMR spectra of **3** and **6**, the H-4 signal appears as a multiplet at 4.64 ppm (**3**) and 5.44 or 5.67 ppm (**6**), which results from coupling to two nonequivalent protons of CH_2 group and one proton of NH group. For 1,3-oxazol-5(4*H*)-one **4**, the signal of methine proton from C-4 is recorded at 4.72 ppm as a doublet of doublets due to coupling to both nonequivalent protons of CH_2 group from benzyl fragment linked in position 4. Signal presented by H-4 proton in ^{1}H-NMR spectra of compounds **6**, is absent in the case of heterocyclic compounds **7** and this is evidence that cyclization

has taken place. Moreover, the two signals of H-18 nonequivalent protons from CH_2 group as a doublet of doublets, in the ^{1}H-NMR spectrum of saturated azlactone **4**, showed discernible downfield shifts of 0.15 and 0.17 ppm, relative to CH_2 protons signals from *N*-acyl-α-amino acid **3** spectrum, due to stronger deshielding effect of 1,3-oxazol-5(4*H*)-one ring, compared to that of carboxyl and *N*-monosubstituted aminocarbonyl groups from acyclic compound **3**. Further proof for formation of 2,5-diaryl-4-benzyl-1,3-oxazoles **7** is provided by the fact that their ^{1}H-NMR spectra revealed a downfield shift of signal attributed to two H-18 protons of CH_2 group as a singlet (because in this case, the two protons of CH_2 group are magnetically equivalent) in the range 3.94 - 4.20 ppm compared to the two signals as a doublet of doublets (because of germinal coupling between non-equivalent protons of CH_2 group with ^2J = 14.0 Hz and of vicinal coupling of these protons with H-4 with ^3J = 9.9 and 4.7 Hz, respectively) at δ values of 2.99 or 3.02 ppm and 3.15 or 3.20 ppm registered for *N*-acyl-α-amino ketones **6**. This is due to the stronger deshielding effect of the 1,3-oxazole ring (from **7**) in comparison with that of carbonyl and *N*-monosubstituted carboxamide groups from acyclic intermediates **6**.

Syntheses of compounds **3**, **4**, **6** and **7** were also proved by ^{13}C-NMR spectra. Moreover, 2D ^{1}H-^{13}C HETCOR experiments allowed univocal assignments of ^{13}C signals. C-4 peak, which appears at δ = 54.38 ppm in the ^{13}C-NMR spectrum of *N*-acyl phenylalanine **3**, is shifted downfield with 12.43 ppm after cyclization to **4**. Furthermore, in compound **4**, C-2 atom resonated at 160.36 ppm (being shielded with 4.76 ppm than C-2 from -CONH- group of acyclic precursor **3**), and C-5 at 176.70 ppm (being shifted downfield with 3.84 ppm than C-5 from -COOH group of **3**). The upfield signal assigned to C-2 from the structure of 1,3-oxazoles **7** is present in interval 157.70-158.42 ppm, while the signal attributed to C-2 from *N*-mono-substituted carboxamide group of acyclic precursors **6** (from 164.66 or 164.82 ppm) is absent in case of heterocyclic compounds **7**. Furthermore, C-5 from the 1,3-oxazole nucleus (**7**) resonated in range 147.99 - 148.59 ppm, whereas ketonic carbonyl carbon of *N*-acyl-α-amino ketones **6** at δ = 197.47 or 201.35 ppm revealing an upfield shift for this C atom in 1,3-oxazoles **7** structure, which confirm that obtaining of 1,3-oxazole ring occurred.

Mass Spectral Data

A supplementary contribution in the structural elucidation of compounds **3**, **4**, **6b**, and **7b** was achieved by registering mass spectra by LC-ESI-MS/MS technique. Protonated and/or deprotonated molecular ions and main fragments of these compounds corresponding to chloride isotopes (^{35}Cl/^{37}Cl) are reported in the experimental part. Due to lower polarity, higher volatility and stability at high temperatures only saturated azlactone **4** could be analyzed by GC-EI-MS. In this case, the two molecular ions of **4** (corresponding to ^{35}Cl and ^{37}Cl isotopes) being very unstable does not show signals in the mass spectrum. These begin to split at the level of 1,3-oxazol-5(4*H*)-one ring by eliminating a molecule of CO_2 when two cation-radicals corresponding to isotopes of chloride with *m/z* = 381/383 (with relative abundances: 8.51% and 4.26%, respectively) are formed. Base peak (PB), cation $[^{35}ClC_6H_4SO]^+$ with *m/z* = 159 and corresponding fragment $[^{37}ClC_6H_4SO]^+$ with *m/z* = 161 (with relative abundance = 30.64%) were obtained according to ^{35}Cl/^{37}Cl isotopic ratio of approximatively 3:1. The fragmentation pattern is consistent with structure.

Cytotoxicity evaluation

At 24 h tested compounds exhibited a maximum L% of 20%. At 48 h, except for **7a** and **1**, all compounds induced L% lower than 40%. LC_{50} at 48 h for compound **7a** was 100.1 µg/mL (95% CI: 7.24 - 1384 µg/mL), whereas for compound **1** the value couldn't be calculated due poor correlation between concentration and L%. For all other compounds, LC_{50} couldn't be calculated at 24 and 48 h. The LC_{50} values at 72 h are presented in Table I and the lethality curves in Figure 7.

Table I

Results of *Daphnia magna* bioassay

Tested compounds	Predicted $LC_{50\ 48\ h}$ (µg/mL)	Determined $LC_{50\ 72\ h}$ (µg/mL)	95% CI of $LC_{50\ 72\ h}$ (µg/mL)
3	1.25	44.24	37.67 - 51.95
4	1.04	26.58	21.55 - 32.79
6b	0.119	37.27	22.02 - 63.10
6c	0.057	≈ 22.95	NC
7a	0.287	≈ 17.71	NC
7b	0.094	33.35	23.58 - 47.16
7c	0.029	≈ 47.67	NC
phenylalanine	170.79	NC	NC
1	40.68	44.88	28.48 - 70.71

LC_{50} – 50% lethal concentration; 95% CI – 95% confidence interval; NC – not calculated due to obtained results

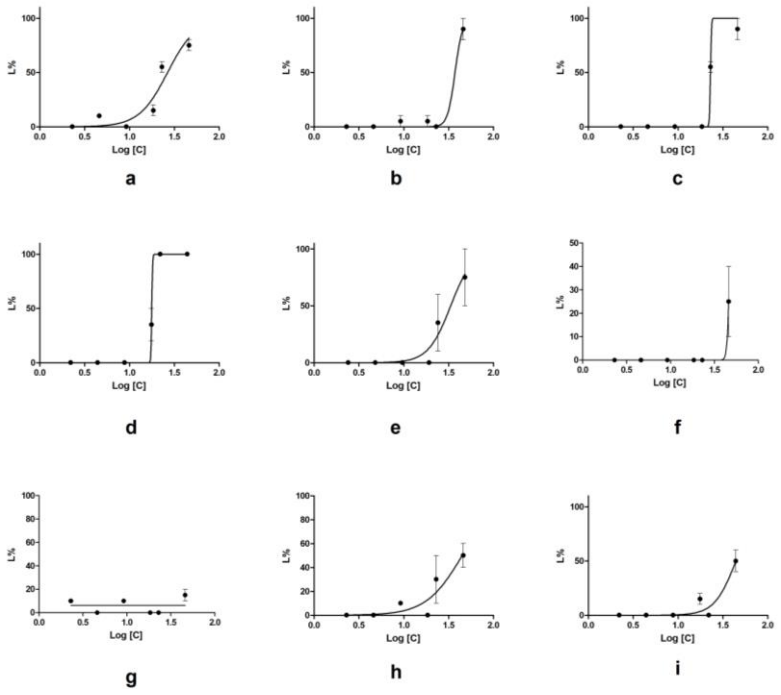


Figure 7.
Lethality curves on *Daphnia magna* at 72 h of exposure for tested compounds;
a - **3**, b - **4**, c - **6b**, d - **6c**, e - **7a**, f - **7b**, g - **7c**, h - phenylalanine, i – **1**

The 72 h decreasing order of toxicity of tested compounds was: **7a**, **6c**, **4**, **7b**, **6b**, **3**, **1** and **7c**. Phenylalanine induced a maximum of 20% lethality on daphnids at 72 h. Values obtained experimentally differs greatly from those predicted with GUSAR software. Not only that at 48 h of exposure, LC_{50} couldn't be calculated for most compounds, but LC_{50} values obtained at 72 h are much lower than those predicted at 48 h. Given the results obtained at 72 h, the toxicity of compounds could be similar due to values obtained for 95% CI which overlap. Compounds **7a** and **1** can be considered most toxic on *D. magna* because they exhibited toxicity on 48 h.

Conclusions

Eight new compounds from *N*-acyl-α-amino acids, *N*-acyl-α-amino acyl chlorides, 1,3-oxazol-5(4*H*)-ones, *N*-acyl-α-amino ketones and 1,3-oxazoles classes, bearing a 4-(4-chlorophenylsulfonyl)phenyl moiety, were synthesized and characterized. 2-Aryl-4-benzyl-1,3-oxazol-5(4*H*)-one **4** was obtained by *N*-acylation of phenylalanine with acyl chloride **2**, followed by intramolecular cyclization of acyclic intermediate **3**. *N*-Acyl-α-amino acyl chloride **5** was also produced from compound **3**. *N*-Acyl-α-amino ketones **6** were synthesized by treatment of saturated 1,3-oxazol-5(4*H*)-one **4** with arenes in the presence of $AlCl_3$. Also, the acylation of benzene with **5** was performed. By $POCl_3$ and/or H_2SO_4-catalysed cyclodehydrations of acyclic precursors **6**, the 2,5-diaryl-4-benzyl-1,3-oxazoles **7** were obtained. Chemical structures of synthesized compounds were confirmed by elemental analysis and different spectral techniques.
New compounds **3**, **4**, **6b,c**, **7a-c** have been investigated for their toxicity and biological activity on *Daphnia magna*. All compounds exhibited cytotoxicity at 72 h of exposure, the effect being dependent on the exposure period. 1,3-Oxazole **7a** showed the highest toxicity.

Conflict of interest

The authors declare no conflict of interest.

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