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-acetyl-α-amino acid, one N-acetyl-α-amino acyl chloride, and two N-acetyl-α-amino ketones), and four cyclization products: one 1,3-oxazol-5(4H)-one, and three 1,3-oxazoles substituted in 5-position with phenyl, p-tolyl, and m-xylol group, respectively. Structures of synthesized compounds were confirmed by elemental analysis and spectral methods (UV-Vis, FT-IR, MS, 1H- and 13C-NMR). The compounds purity determination was performed by RP-HPLC. Cytotoxic effect of synthesized compounds was evaluated on Daphnia magna crustacean.

Resumat

Lucrarea prezintă proiectarea, sinteza, caracterizarea și evaluarea citotoxicității a opt compuși organici care au în moleculele lor fragmentul 4-(4-clorofenilsulfonil)fenil: patru precursori acicli derivați de la fenilalanină (un N-acil-α-aminoacid, o clorură de N-acil-α-aminoacil și două N-acil-α-aminoacetone) și patru produși de ciclizare: o 1,3-oxazol-5(4H)-onă și trei 1,3-oxazoli substituți 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-Vis, FT-IR, SM, 1H- și 13C-RMN). Determinarea purității compușilor a fost realizată prin RP-HPLC. Efectul citotic al compușilor sintetizați a fost evaluat pe crustaceul Daphnia magna.

Keywords: N-acetyl-α-amino acid, 1,3-oxazol-5(4H)-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(4H)-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-acetyl-α-amino acids class are known to present mucolytic [14], antihypertensive [15], anticancer [16], antinamic [17], anti-ulcer [18], vasocostructor action [19]. In addition, some N-acylated α-amino acids are specific antities in acute intoxications with antifolates [20], paracetamol [21] etc. Open-chain precursors from N-acetyl-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(4H)-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 \( \times \) \( 10^{-5} \) 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: \( \text{vs} = \) very strong, \( s = \) strong, \( m = \) medium and \( w = \) weak. NMR spectra were registered on a Varian Gemini 300BB spectrometer at 300 MHz for \(^1\text{H}-\text{NMR}\) and 75 MHz for \(^13\text{C}-\text{NMR}\) in DMSO-\(d_6\) or CDCl\(3\) as solvents. Chemical shifts (\( \delta \)) 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 \(^1\text{H}-\text{NMR}\) 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-El-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% dipheny/95% dimethylsiloxane) (SLB-5ms, L \( \times \) I.D. 30 m \( \times \) 0.32 mm, d 0.25 \( \mu \)m) was used. A 1 \( \mu \)L 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 \( \mu \)m particle size, L \( \times \) I.D. 25 cm \( \times \) 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(4H)-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 3 was refluxed 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\text{H}-\text{and} \(^{13}\text{C}-\text{NMR}\)) and elemental analysis data.
**Figure 1.**
Multistep reaction sequence for compounds synthesis

**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$_3$OH, $\lambda$ nm) ($lg \varepsilon$): 202.6 (4.48); 250.2 (4.16).

FT-IR (KBr, $\nu$ 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$_6$, $\delta$ 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,
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Method 1. 4-Methylmorpholine (1.15 mL, 10.5 mmol) was added under agitation into a suspension of 2-[4-(4-chlorophenylsulfonyl)benzamido]-3-phenylpropanoic 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 (MgSO4). 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-Acetyl-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 140°C 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 (CH3OH, λ nm) (lg ε): 202.6 (4.48); 250.2 (4.16).

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

1H-NMR (CDCl3, 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).

13C-NMR (CDCl3, 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).

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

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(4H)-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 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 (Na2SO4) 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 AlCl3 (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 hydroxide carbonate solution, then with water and dried (Na2SO4). 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 (CH3OH, λ nm) (lg ε): 203.5 (4.49); 253.7 (4.22).

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

1H-NMR (DMSO-d6, δ ppm, J Hz): 2.34 (s, 3H, CH3); 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-12); 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).

13C-NMR (DMSO-d6, δ ppm): 21.11 (CH3); 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 [43Cl]520 [Cl]+; 500/502 (16.2/50.9) [M+H]+; 296/298 (26.2/39.0) [Cl]53Cl52H5SO2C6H4CONH2 + H][Cl]53Cl52H5SO2C6H4CONH2 + H]; 279/281 (55.0/63.4) [Cl]53Cl52H5SO2C6H4CO; 223 (100, BP) [C18H18CH2CHOCOC6H4CH2]; 195 (55.0/52.1) [C18H18CH2CHOCOC6H4CH2]; 131 [C18H18CH2C=CO]; 91 [78/163.2] [CH3Cl, H3].

RP-HPLC (CH3OH:H2O = 60:40, 1 mL/min, 250 nm): purity = 98.30%; tR = 6.40 min.

Anal. (%) : Calcd. for C22H21ClINO(S)2 (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°C (ethanol).

UV-Vis (CH3OH, λ nm) (lg ε): 203.5 (4.48); 252.9 (4.17).

FT-IR (KBr, v cm⁻¹): 3365s; 3087m; 3065w; 3032w; 2963w; 2926w; 2863w; 1682s; 1654s; 1519m; 1494m; 1326s; 1294s; 1162s; 834m; 753s.

1H-NMR (DMSO-d6, δ ppm, J Hz): 2.28 (s, 3H, CH3); 2.36 (s, 3H, CH3); 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).

13C-NMR (DMSO-d6, δ ppm): 20.32 (CH3); 20.86 (CH3); 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); 127.39 (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 (CH₃OH:H₂O = 60:40, 1 mL/min, 250 nm): purity = 90.83%; tₑ = 7.63 min.

Anal. (%): Calcd. for C₆H₅ClNO₅S (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-e*

**Figure 5.**

General structure of compounds 7 with atomic numbering (for NMR assignments)

*General method 1 for obtaining 7a-e.* Crude N-(1-aryl-1-oxo-3-phenylpropan-2-yl)-(4-chlorophenylsulfonyl)benzamides 6a-e (10 mmol) in phosphoric trichloride (20 mL, 217.83 mmol) were refluxed for 4 h. Unreacted POCl₃ 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% NaHCO₃ solution, then with water and dried (Na₂SO₄). After solvent removal, crude products 7a-e 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 (5.44 g, 10.51 mmol) was dissolved in ethyl acetate (40 mL). Acetic anhydride (3 mL, 32.2 g, 315 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) dilute 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 (Na₂SO₄) 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-phenoxazolone 7a

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

UV-Vis (CH₃OH, 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; 1327s; 1290s; 1281s; 1158vs; 1088vs; 845s; 765vs.

1H-NMR (CDCl₃, 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).

13C-NMR (CDCl₃, 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 (CH₃OH: H₂O = 70:30, 1 mL/min, 335 nm): purity = 95.1%; tₑ = 4.68 min.

Anal. (%): Calcd. for C₇H₅ClNO₅S (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-toloxazole 7b

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

UV-Vis (CH₃OH, 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.

1H-NMR (CDCl₃, δ ppm, J Hz): 2.34 (s, 3H, CH₃); 4.13 (s, 2H, H-18); 7.15-7.28 (m, 7H, H-7, 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).

13C-NMR (CDCl₃, δ ppm): 21.43 (CH₃); 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-24, C-28); 127.82 (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 [13Cl]⁺/502 [13Cl]+[H⁺]; 422/424 [M+H]+; 279/281 (38.0/37.1) [13ClC₆H₅SO₂C₆H₄CO]+/[12ClC₆H₅SO₂C₆H₄CO⁺]; 159/161 [13ClC₆H₅SO₂]+/[12ClC₆H₅SO₂⁺]; 117 (100, BP) [C₂H₅C₆H₅C=CH₃]⁺.

RP-HPLC (CH₃OH:H₂O = 70:30, 1 mL/min, 335 nm): purity = 97.57%; tₑ = 4.85 min.

Anal. (%): Calcd. for C₇H₅ClNO₅S (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 (CH₃OH, 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; 2932m; 2860w; 1602s; 1582s; 1494s; 1475m; 1453m; 1327vs; 1285s; 1268s; 1246s; 1192s; 1176s; 1157s; 1137s; 1125s; 1088s; 843m; 767vs.

1H-NMR (CDCl₃, δ ppm, J Hz): 2.31 (s, 3H, CH₃); 2.37 (s, 3H, CH₃) 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).

13C-NMR (CDCl₃, δ ppm): 20.45 (CH₃); 21.41 (CH₃); 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 (CH₃OH:H₂O = 70:30, 1 mL/min, 335 nm): purity = 99.15%; tᵣ = 4.87 min.

Anal. (%): Calcd. for C₃₅H₅₇ClN₀₂S (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 LC₅₀₄₆₈ 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-methylbenzenesulfonfyl chloride in presence of AlCl₃, 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-chlorophenylsulfonyl)benzamido]-3-phenylpropanoic acid 3.

Intramolecular cyclodehydration of compound 3, using ethyl chloroformate in presence of N-methylmorpholine in CH₂Cl₂ at ambient temperature or acetic anhydride at reflux, led to 4-benzyl-2-[4-(4-chlorophenylsulfonyl)-phenyl]oxazol-5(4H)-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(4H)-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-Acetyl-α-amino acid 3 is in equilibrium with its enol tautomer 1, 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 pentamontic heterocyclic compound 4.

Also, N-acetylated phenylalanine 3 was reacted with SOCl₂ at reflux to 2-[4-(4-chlorophenylsulfonyl)-benzamido]-3-phenylpropanoic chloride 5 by a nucleophilic substitution mechanism.

Friedel-Crafts acylation reaction of arenes (benzene, toluene, m-xylene) with 1,3-oxazol-5(4H)-one 4, catalysed by AlCl₃, 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-Acetyl-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 H₂SO₄/(CH₃CO)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 POCl₃ and H₂SO₄, respectively are described in our recent literature [10].

Spectral analysis methods (UV-Vis, IR, MS, ¹H- and ¹³C-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 λmax = 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.
The absorption band at 1650 cm⁻¹ peaks were presented. In the case of 1,3 are characteristic for acyclic intermediate (H), ν(O=C) stretching vibration and 1793 cm⁻¹ to ν(C=O) due to carbonyl group stretching, ν(O=O) at 1234 cm⁻¹ and 1283 cm⁻¹. Main absorption bands presented in IR spectrum of acyl chloride 5 (namely, two strong absorption bands due to O-H stretching vibration, ν(O-H), 1682 - 1734 cm⁻¹ due to carbonyl stretching vibration, ν(O=O=O), 1623 - 1654 cm⁻¹ due to amide carbonyl group absorption, ν(C=O-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=O=O) at 1824 cm⁻¹ assigned to fundamental vibration and 1793 cm⁻¹ named Fermi resonance band, and a medium absorption band due to ν(C=O=O) at 891 cm⁻¹) confirmed that reaction of N-acyl-α-amino acid 3 with SOCl₂ 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=O=O) 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_s(C=O-C). Absorption band due to asymmetrical stretching vibration of the C-O-C system, ν_a(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 1H-NMR spectra of new compounds are also consistent with proposed chemical structures. Besides, 2D 1H-1H COSY experiments were performed, which facilitated unambiguous assignments. It can be noticed presence in 1H-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 (J = 8.0 Hz). In 1H-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 1H-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 CH₂ group and one proton of NH group. For 1,3-oxazol-5(4H)-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 CH₂ group from benzyl fragment linked in position 4. Signal presented by H-4 proton in 1H-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-$\alpha$-amino acid 3 spectrum, due to stronger deshielding effect of 1,3-oxazol-5(4H)-one ring, compared to that of carbonyl and N-mono-substituted 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 $^3$J = 14.0 Hz and of vicinal coupling of these protons with H-4 with $^3$J = 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-$\alpha$-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-mono-substituted 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-$\alpha$-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(4H)-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$_2$H$_5$SO]$^+$ with m/z = 159 and corresponding fragment [$^{35}$ClC$_2$H$_5$SO]$^+$ 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                       |
| 7c              | 0.094                    | 33.35                    | 23.58 - 47.16            |
| 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
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(4H)-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(4H)-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(4H)-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$_2$SO$_4$-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, 6bc, 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.

References

1. Zhang HZ, Zhao ZL, Zhou CH, Recent advance in oxazole-based medicinal chemistry. Eur J Med Chem., 2018; 144: 444-492.
2. Ansari A, Ali A, Asif M, Rauf MA, Owais M, Shamsuzzaman, Facile one-pot multicomponent synthesis and molecular docking studies of steroidal oxazole/thiazole derivatives with effective antimicrobial, antibiofilm and hemolytic properties. Steroids, 2018; 134: 22-36.
3. Abhale YK, Sasane AV, Chavan AP, Shekh SH, Deshmukh KK, Bhansali S, Nawale L, Sarkar D, Mhaske PC, Synthesis and antimycobacterial screening of new thiazolyl-oxazole derivatives. Eur J Med Chem., 2017; 132: 333-340.
4. Massaro M, Scoditti E, Pellegrino M, Carluccio MA, Calabrìo N, Wabiitsch M, Storelli C, Wright M, De Caterina R, Therapeutic potential of the dual peroxisome proliferator activated receptor (PPAR)α/γ agonist aleglitazar in attenuating TNF-α-mediated inflammation and insulin resistance in human adipocytes. Pharmacol Res., 2016; 107: 125-136.
