https://doi.org/10.31925/farmacia.2021.4.5 **ORIGINAL ARTICLE**

SYNTHESIS, CHARACTERIZATION AND CYTOTOXICITY EVALUATION OF NEW DIPHENYL SULFONE DERIVATIVES

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Abstract

Taking into account that the substituted diphenyl sulfone is an important pharmacophore in medicinal chemistry, in this paper, we report the synthesis, characterization, and cytotoxicity evaluation of seven new compounds: six ethyl 1,3-oxazol-5-yl carbonates and an acyclic product (ethyl ester of an *N*-acyl-α-amino acid), containing the arylsulfonylphenyl moiety. The synthesized compounds were characterized by elemental analysis and spectral techniques (UV-Vis, FT-IR, MS, ^{1}H-NMR, ^{13}C-NMR). Their purity was checked by reversed phase HPLC. The cytotoxic effect of new compounds was assessed using *Daphnia magna* bioassay.

Rezumat

Luând în considerare faptul că difenilsulfona substituită este un farmacofor important în chimia medicală, în această lucrare este descrisă sinteza, caracterizarea și evaluarea citotoxicității a șapte compuși noi: șase carbonați de etil 1,3-oxazol-5-il și un produs aciclic (esterul etilic al unui *N*-acil-α-aminoacid), care conțin fragmentul arilsulfonilfenil. Compușii sintetizați au fost caracterizați prin analiză elementală și tehnici spectrale (UV-Viz, FT-IR, SM, ^{1}H-RMN, ^{13}C-RMN). Puritatea lor a fost verificată prin HPLC cu fază inversă. Efectul citotoxic al noilor compuși a fost evaluat utilizând testul biologic *Daphnia magna*.

Keywords: diaryl sulfone, ethyl 1,3-oxazol-5-yl carbonate, *N*-acyl-α-amino acid ester, cytotoxicity

Introduction

Biological research in the field of compounds from the diaryl sulfones class has shown that they have various therapeutic actions, including antimicrobial, antimalarial, antimycobacterial, antioxidant, anticancer, anti-inflammatory effects, being used in the treatment of leprosy, malaria, tuberculosis, pneumonia and other infectious diseases [1-8]. Furthermore, it was found that diverse heterocyclic moieties linked to a diaryl sulfone pharmacophore, are potential bioactive compounds [9-15].

In addition, the 1,3-oxazole pharmacophore is present in the structures of numerous biologically active natural products, including those used as antibiotics and antiproliferatives [16-20]. Also, 1,3-oxazoles are substructures in many synthetic bioactive substances with a wide spectrum of pharmacological properties, like anticancer, anti-inflammatory, analgesic, antibacterial, antifungal, antiviral, antidiabetic, antitubercular, antiparasitic, anti-obesity, cytotoxic and antioxidant actions [21-30].

Besides, the esters of *N*-acyl-α-amino acids were reported to have monoamine oxidase (MAO) inhibitory, antibacterial, antitumor, anti-depressant and anti-leishmanial activities [31-36].

Due to their biological importance, 5-membered heterocyclic compounds with the 1,3-oxazole ring, have been obtained over time by a variety of chemical synthesis methods [37-41]. To date, the most widely used methodologies for preparation of these compounds include the condensation of cyanohydrins (formed from aromatic aldehydes) and aromatic aldehydes in the dry ether in the presence of anhydrous hydrogen chloride (Fischer synthesis) [42], Cornforth rearrangement [43], the reaction of aldehydes with *p*-toluene-

sulfonylmethyl isocyanide (van Leusen synthesis) [37, 44], Doyle reaction of nitriles with diazocarbonyl compounds [45], Robinson-Gabriel synthesis which involves cyclodehydration of *N*-acyl-α-amino ketones [46, 47]. Other synthetic methods are the coupled Ugi and Robinson-Gabriel reaction between primary amines, carbonyl compounds, carboxylic acids, and isocyanides to afford the corresponding functionalized α-acylamino amides followed by their cyclodehydration [48], Friedel-Crafts/Robinson-Gabriel synthesis using 1,3-oxazol-5(4*H*)-one templates in presence of $AlCl_3$ as the Friedel-Crafts catalyst and trifluoromethanesulfonic acid as the Robinson-Gabriel cyclodehydrating agent [49], Dakin-West reaction of *N*-acyl-α-amino acids with carboxylic anhydrides in the presence of a weak base, such as pyridine [50], reaction of *N*-acyl-α-amino acids with di-*tert*-butyl dicarbonate [51], Blümlein-Lewy method which consists in the condensation of amides with α-halo or α-hydroxy ketones [52], etc.

With the purpose of obtaining biologically active substances, in this work, we synthesized new ethyl 1,3-oxazol-5-yl carbonates, having the 4-(4-X-phenylsulfonyl)phenyl moiety (X = H, Cl or Br) into their structures, by reaction of *N*-acyl-α-amino acids with ethyl chloroformate in the presence of 4-methylmorpholine. A new *N*-acyl-α-alanine ester bearing a fragment derived from diphenyl sulfone was also obtained. All newly synthesized compounds were physicochemically characterized and evaluated for cytotoxic effect using the *Daphnia magna* test, a rapid and simple method used widely to predict the biological activity [53].

Materials and Methods

Chemistry

General: Chemicals and reagents were obtained from commercial suppliers. Dichloromethane was dried over anhydrous calcium chloride. The melting points were determined with a Boëtius instrument (VEB Wägetechnik Rapido, PHMK 81/3026, Germany) and are reported uncorrected. The UV-Vis spectra were acquired for ≈ 0.025 mM solutions in methanol on an Analytik Jena AG Specord 40 spectrophotometer (Analytik Jena AG, Jena, Germany). FT-IR spectra were measured with a Bruker Vertex 70 spectrometer (Bruker Optik GmbH, Ettlingen, Germany) as KBr pellets. The relative intensity of the selected IR absorption bands is reported as very strong, vs; strong, s; medium, m; weak, w. The NMR spectra were recorded on a Varian Gemini 300 BB spectrometer (Varian, Inc., Palo Alto, CA, USA) at 300 MHz for ^{1}H and 75 MHz for ^{13}C using tetramethylsilane (TMS) as internal standard and $CDCl_3$ as the solvent. Also, combined 2D spectra (COSY, HETCOR) were registered. Chemical shifts, δ, in parts *per* million (ppm), and coupling constants, *J*, expressed in hertz (Hz) are reported. The multiplicity of the ^{1}H-NMR signals was assigned as singlet, s; doublet, d; doublet of doublets, dd; triplet, t; triplet of triplets, tt; quartet, q; quintet, quint; multiplet, m. Mass spectra of two 1,3-oxazoles were recorded on a Varian 1200 L-MS/MS triple quadrupole mass spectrometer (Varian, Inc., Walnut Creek, CA, USA) with an electrospray interface, in positive ion mode. A solution (in 90% methanol and the rest water with 100 mM ammonium formate) of around 1 ppm of these compounds was directly infused with a Prostar 240 SDM at 70 µL/min flow rate, using a Rheodyne manual injector. Pseudo-molecular ions (protonated molecules) were selected with the first quadrupole. Fragments were obtained by collision with argon at different energies up to 50 eV. GC-EI-MS analysis was performed on a Fisons Instruments GC 8000 (Fisons Instruments SpA, Rodano, Milano, Italy), with an electron impact quadrupole, and coupled to an MD 800 mass spectrometer detector, using an SLB-5ms capillary column (L × I.D. 30 m × 0.32 mm, d_f = 0.25 µm), dichloromethane as the solvent and a helium carrier gas flow rate of 2 mL/min. RP-HPLC chromatograms were registered on a Beckman System Gold 126 liquid chromatograph (Beckman Coulter, Inc., Fullerton, CA, USA), with a System Gold 166 UV-Vis detector, a LiChrosorb RP-18 column (L × I.D. 25 cm × 4.6 mm, d_p = 5 µm), and a Rheodyne injection system. Purity (%) and retention time, t_R, in minutes (min) of analysed compounds are reported. Quantitative elemental analysis was determined with a Costech ECS 4010 instrument (Costech Analytical Technologies Inc., Valencia, CA, USA).

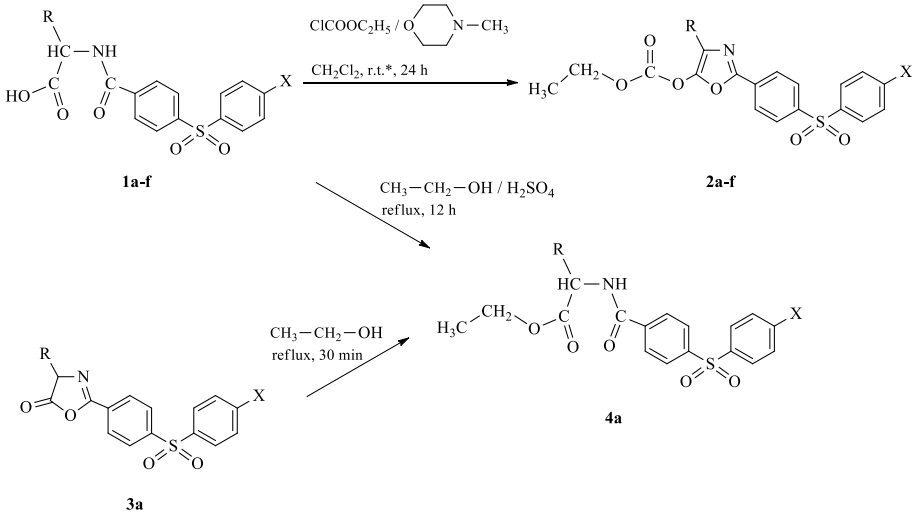
Synthesis and characterization of compounds

The synthesis of the new diphenyl sulfone derivatives **2a-f** and **4a** was achieved employing the strategy illustrated in Figure 1, using as starting materials *N*-acyl-α-amino acids **1a-f** and 1,3-oxazol-5(4*H*)-one **3a** whose obtaining was formerly published [10, 22, 30, 40, 47, 54].

*General synthesis of {4-(benzyl/methyl)-2-[4-(4-X-phenyl-sulfonyl)phenyl]-1,3-oxazol-5-yl} ethyl carbonates **2a-f***

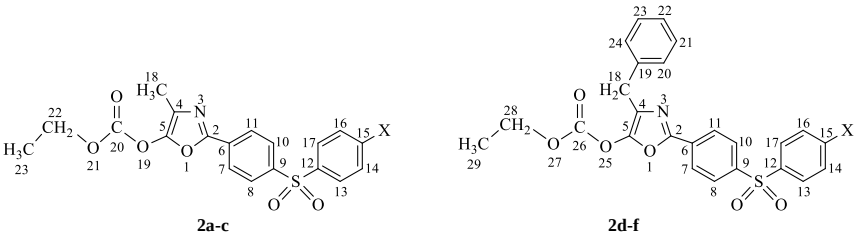
N-Acyl-α-amino acid **1** (10 mmol) was suspended, under magnetically stirring, at room temperature in 50 mL of anhydrous dichloromethane. The mass reaction was stirred for a few minutes, then 1.66 mL (1.52 g, 15 mmol) of 4-methylmorpholine was slowly added and continued stirring until all the crystals were dissolved. Then, 1.43 mL (1.63 g, 15 mmol) of ethyl chloroformate was added gradually, in drops, and with stirring. The resulting reaction mixture was stirred for a further 24 h at room temperature, then poured over 100 mL of cold water. The separated organic phase was washed with 5% sodium bicarbonate solution and then with water. After drying over anhydrous magnesium sulfate, di-

chloromethane was removed by *vacuum* distillation. The crude product **2** was recrystallized from ethanol when white crystals were obtained.



1a, **2a, 3a**, **4a**: X = H, R = CH_3
1b, **2b**: X = Cl, R = CH_3
1c, **2c**: X = Br, R = CH_3
1d, **2d**: X = H, R = $CH_2C_6H_5$
1e, **2e**: X = Cl, R = $CH_2C_6H_5$
1f, **2f**: X = Br, R = $CH_2C_6H_5$

Figure 1.
General synthesis of new diphenyl sulfone derivatives **2a-f** and **4a**
(* r.t. = room temperature)



2a, 2d: X = H
2b, 2e: X = Cl
2c, 2f: X = Br

Figure 2.
Structures of compounds **2a-c** and **2d-f** with the numbering of atoms (used for NMR assignments)

*Ethyl {4-methyl-2-[4-(phenylsulfonyl)phenyl]-1,3-oxazol-5-yl} carbonate **2a*** (obtained from 3.33 g of 2-[4-(phenylsulfonyl)benzamido]propanoic acid **1a** [47]); yield = 90% (3.49 g); m.p. = 154 - 156ºC.

UV-Vis (CH_3OH, λ nm) (lg ε): 202.6 (4.47); 302.2 (4.28).

FT-IR (KBr, ν cm^{-1}): 3097m; 3073w; 3050w; 2982m; 2928w; 2875w; 2858w; 1788vs; 1660s; 1600m; 1581m; 1547m; 1470m; 1443s; 1326s; 1291s; 1244vs; 1216vs; 1163vs; 1077s; 844m.

^{1}H-NMR ($CDCl_3$, δ ppm, J Hz): 1.42 (t, 7.2, 3H, H-23); 2.15 (s, 3H, H-18); 4.39 (q, 7.2, 2H, H-22); 7.50-7.60 (m, 7.4, 5H, H-13, H-14, H-15, H-16, H-17); 8.01 (d, 9.1, 2H, H-8, H-10); 8.07 (d, 9.1, 2H, H-7, H-11).

^{13}C-NMR ($CDCl_3$, δ ppm): 10.39 (C-23); 14.16 (C-18); 66.75 (C-22); 121.64 (C-6); 126.58 (C-8, C-10); 127.85 (C-13, C-17); 128.32 (C-7, C-11); 129.52 (C-14, C-16); 131.47 (C-4); 133.54 (C-15); 141.42 (C-12); 142.83 (C-9); 147.04 (C-5); 151.35 (C-2); 152.96 (C-20).

GC-EI-MS (*m/z*, rel. abund. %): 343 (5.65) [M-C_2H_4O]$^{+\cdot}$; 315 (32.01) [M-C_2H_4O- CO]$^{+\cdot}$; 245 (100, BP) [$C_6H_5SO_2C_6H_4CH{=}NH$]$^{+\cdot}$; 218 (24.48) [$C_6H_5SO_2C_6H_5$]$^{+\cdot}$; 152 (7.32); 141 (4.81) [$C_6H_5SO_2$]$^+$; 125 (32.22) [C_6H_5SO]$^+$; 104 (15.06) [$C_6H_4CH{=}NH$]$^+$; 97 (5.65); 77 (14.64) [C_6H_5]$^+$; 51 (3.97) [C_4H_3]$^+$; t_R = 29.85 min.

RP-HPLC (methanol-water 60:40, v/v; 1 mL/min; 303 nm): purity = 99.44%; t_R = 4.02 min.

Elemental analysis (%): calculated for $C_{19}H_{17}NO_6S$ (387.41 g/mol): C, 58.91; H, 4.42; N, 3.62; S, 8.28 and found: C, 58.97; H, 4.41; N, 3.63; S, 8.25.

*{2-{4-[(4-Chlorophenyl)sulfonyl]phenyl}-4-methyl-1,3-oxazol-5-yl} ethyl carbonate **2b*** (obtained from 3.68 g of 2-{4-[(4-chlorophenyl)sulfonyl]benzamido}propanoic

acid **1b** [54]); yield = 91% (3.84 g); m.p. = 153 - 155ºC.

UV-Vis (CH_3OH, λ nm) (lg ε): 202.6 (4.48); 223.8 (4.18); 246.7 (4.24); 303.1 (4.18).

FT-IR (KBr, ν cm^{-1}): 3094m; 3043w; 2986m; 2933w; 2872w; 2848w; 1778vs; 1670s; 1602m; 1581m; 1548m; 1475s; 1447m; 1327vs; 1286s; 1255vs; 1225vs; 1157vs; 1076s; 839m; 767vs.

^{1}H-NMR ($CDCl_3$, δ ppm, J Hz): 1.43 (t, 7.1, 3H, H-23); 2.16 (s, 3H, H-18); 4.40 (q, 7.1, 2H, H-22); 7.50 (d, 8.5, 2H, H-14, H-16); 7.90 (d, 8.5, 2H, H-13, H-17); 7.99 (d, 8.5, 2H, H-8, H-10); 8.08 (d, 8.5, 2H, H-7, H-11).

^{13}C-NMR ($CDCl_3$, δ ppm): 10.35 (C-23); 14.13 (C-18); 66.75 (C-22); 121.68 (C-6); 126.63 (C-8, C-10); 128.27 (C-7, C-11); 128.93 (C-13, C-17); 129.84 (C-14, C-16); 131.60 (C-4); 139.81 (C-15); 140.29 (C-12); 142.28 (C-9); 147.00 (C-5); 151.30 (C-2); 152.80 (C-20).

GC-EI-MS (*m/z*, rel. abund. %): 377 (^{35}Cl)/379 (^{37}Cl) (7.53/3.35) $[M\text{-}C_2H_4O]^{+\cdot}$; 349 (^{35}Cl)/351 (^{37}Cl) (29.29/10.04) $[M\text{-}C_2H_4O\text{-}CO]^{+\cdot}$; 279 (^{35}Cl)/281 (^{37}Cl) (100, BP/35.56) $[^{35}ClC_6H_4SO_2C_6H_4CH{=}NH]^{+\cdot}$/ $[^{37}ClC_6H_4SO_2\,C_6H_4CH{=}NH]^{+\cdot}$; 252 (^{35}Cl)/254 (^{37}Cl) (18.83/.28) $[^{35}ClC_6H_4SO_2C_6H_5]^{+\cdot}$/$[^{37}ClC_6H_4SO_2C_6H_5]^{+\cdot}$; 175 (3.76); 168 (7.32); 159 (^{35}Cl)/161 (^{37}Cl) (41.42/17.15) $[^{35}ClC_6H_4SO]^{+}$/$[^{37}ClC_6H_4SO]^{+}$; 152 (8.16); 146 (5.86); 131 (4.60) $[C_6H_5CH{=}CHCO]^{+}$; 124 (2.51); 111 (^{35}Cl)/113 (^{37}Cl) (14.85/4.60) $[^{35}ClC_6H_4]^{+}$/ $[^{37}ClC_6H_4]^{+}$; 104 (16.11) $[C_6H_4CH{=}NH]^{+}$; 76 (12.55) $[C_6H_4]^{+\cdot}$; 50 (2.93) $[C_4H_2]^{+\cdot}$; 42 (5.44) $[C_3H_6]^{+\cdot}$; t_R = 36.04 min.

RP-HPLC (methanol-water 60:40, v/v; 1 mL/min; 303 nm): purity = 93.29%; t_R = 4.13 min.

Elemental analysis (%): calculated for $C_{19}H_{16}ClNO_6S$ (421.85 g/mol): C, 54.10; H, 3.82; N, 3.32; S, 7.60 and found: C, 54.14; H, 3.81; N, 3.33; S, 7.60.

{2-{4-[(4-Bromophenyl)sulfonyl]phenyl}-4-methyl-1,3-oxazol-5-yl} ethyl carbonate ***2c*** (obtained from 4.12 g of 2-{4-[(4-bromophenyl)sulfonyl]benzamido}propanoic acid **1c** [40]); yield = 91% (4.24 g); m.p. = 158 - 160ºC.

UV-Vis (CH_3OH, λ nm) (lg ε): 202.6 (4.49); 227.3 (4.18); 250.2 (4.30); 304.0 (4.19).

FT-IR (KBr, ν cm^{-1}): 3092m; 3063w; 2984m; 2932m; 2869w; 2843w; 1778vs; 1669s; 1602m; 1573s; 1548m; 1470m; 1446m; 1327vs; 1288s; 1253vs; 1224vs; 1158vs; 1074s; 839m; 616s; 566s.

^{1}H-NMR ($CDCl_3$, δ ppm, J Hz): 1.43 (t, 7.1, 3H, H-23); 2.16 (s, 3H, H-18); 4.40 (q, 7.1, 2H, H-22); 7.67 (d, 8.8, 2H, H-14, H-16); 7.82 (d, 8.8, 2H, H-13, H-17); 7.99 (d, 8.5, 2H, H-8, H-10); 8.08 (d, 8.5, 2H, H-7, H-11).

^{13}C-NMR ($CDCl_3$, δ ppm): 10.40 (C-23); 14.18 (C-18); 66.79 (C-22); 121.73 (C-6); 126.67 (C-8, C-10); 128.32 (C-7, C-11); 128.92 (C-15); 129.36 (C-13, C-17); 131.64 (C-4); 132.87 (C-14, C-16); 140.36 (C-12); 142.23 (C-9); 147.01 (C-5); 151.34 (C-2); 152.83 (C-20).

+ESI-MS/MS (*m/z*, rel. abund. %): 466 (^{79}Br)/468 (^{81}Br) $[M+H]^+$; 422 (^{79}Br)/424 (^{81}Br) (23.8/20.8) $[M+H\text{-}C_2H_4O]^+$; 394 (^{79}Br)/396 (^{81}Br) (100, BP) $[M+H\text{-}C_2H_4O\text{-}CO]^+$; 323/325 (5.4/4.8) $[^{79}BrC_6H_4SO_2C_6H_4CO]^+$/ $[^{81}BrC_6H_4SO_2C_6H_4CO]^+$.

GC-EI-MS (*m/z*, rel. abund. %): 421 (^{79}Br)/423 (^{81}Br) (10.78/6.37) $[M\text{-}C_2H_4O]^{+\cdot}$; 393/395 (31.85/36.36) $[M\text{-}C_2H_4O\text{-}CO]^{+\cdot}$; 323/325 (100, BP/74.31) $[^{79}BrC_6H_4SO_2C_6H_4CH{=}NH]^{+\cdot}$/$[^{81}BrC_6H_4SO_2C_6H_4CH{=}NH]^{+\cdot}$; 296/298 (18.18/16.56) $[^{79}BrC_6H_4SO_2C_6H_5]^{+\cdot}$/ $[^{81}BrC_6H_4SO_2C_6H_5]^{+\cdot}$; 245 (3.59); 203/205 (43.55/ 37.79) $[^{79}BrC_6H_4SO]^{+}$/$[^{81}BrC_6H_4SO]^{+}$; 187 (5.07); 173 (5.49); 168 (15.22); 155/157 (23.68/21.23) $[^{79}BrC_6H_4]^{+}$/$[^{81}BrC_6H_4]^{+}$; 152 (13.11); 133 (4.65); 124 (4.23); 104 (51.58) $[C_6H_4CH{=}NH]^{+}$; 96 (7.61); 87 (3.38); 76 (58.35) $[C_6H_4]^{+\cdot}$; 70 (5.71); 50 (9.09) $[C_4H_2]^{+\cdot}$; t_R = 43.20 min.

RP-HPLC (methanol-water 60:40, v/v; 1 mL/min; 303 nm): purity = 97.80%; t_R = 4.48 min.

Elemental analysis (%): calculated for $C_{19}H_{16}BrNO_6S$ (466.30 g/mol): C, 48.94; H, 3.46; N, 3.00; S, 6.88 and found: C, 48.99; H, 3.45; N, 3.01; S, 6.90.

{4-Benzyl-2-[4-(phenylsulfonyl)phenyl]-1,3-oxazol-5-yl} ethyl carbonate ***2d*** (obtained from 4.09 g of 3-phenyl-2-[4-(phenylsulfonyl)benzamido]propanoic acid **1d** [22]); yield = 85% (3.94 g); m.p. = 130 - 132ºC.

UV-Vis (CH_3OH, λ nm) (lg ε): 202.6 (4.49); 300.4 (4.11).

FT-IR (KBr, ν cm^{-1}): 3089w; 3072m; 3055m; 3026w; 2990m; 2944w; 2873w; 2836w; 1781vs; 1665s; 1602s; 1583w; 1549m; 1498m; 1476s; 1446s; 1320s; 1307vs; 1289vs; 1222vs; 1159vs; 1074vs; 845m.

^{1}H-NMR ($CDCl_3$, δ ppm, J Hz): 1.35 (t, 7.1, 3H, H-29); 3.87 (s, 2H, H-18); 4.29 (q, 7.1, 2H, H-28); 7.20-7.33 (m, 5H, H-20, H-21, H-22, H-23, H-24); 7.71 (t, 7.4, 2H, H-14, H-16); 7.78 (tt, 7.4, 1.4, 1H, H-15); 7.95 (dd, 7.4, 1.4, 2H, H-13, H-17); 7.97 (d, 8.8, 2H, H-8, H-10); 8.06 (d, 8.8, 2H, H-7, H-11).

^{13}C-NMR ($CDCl_3$, δ ppm): 14.12 (C-29); 31.51 (C-18); 66.73 (C-28); 124.59 (C-6); 126.73 (C-22); 126.75 (C-8, C-10); 127.81 (C-13, C-17); 128.27 (C-7, C-11); 128.63 (C-21, C-23); 128.94 (C-20, C-24); 129.53 (C-14, C-16); 131.39 (C-4); 133.56 (C-15); 137.31 (C-19); 141.33 (C-12); 142.81 (C-9); 147.23 (C-5); 151.22 (C-2); 153.31 (C-26).

RP-HPLC (methanol-water 70:30, v/v; 1 mL/min; 303 nm): purity = 98.98%; t_R = 3.83 min.

Elemental analysis (%): calculated for $C_{25}H_{21}NO_6S$ (463.50 g/mol): C, 64.78; H, 4.57; N, 3.02; S, 6.92 and found: C, 64.73; H, 4.56; N, 3.02; S, 6.94.

{4-Benzyl-2-{4-[(4-chlorophenyl)sulfonyl]phenyl}-1,3-oxazol-5-yl} ethyl carbonate ***2e*** (obtained from 4.44 g of 2-{4-[(4-chlorophenyl)sulfonyl]benzamido}-3-

phenylpropanoic acid **1e** [30]); yield = 89% (4.43 g); m.p. = 149 - 151ºC.

UV-Vis (CH_3OH, λ nm) (lg ε): 202.6 (4.48); 246.7 (4.18); 303.1 (4.13).

FT-IR (KBr, ν cm^{-1}): 3089m; 3065m; 3030m; 2983m; 2938w; 2872w; 2842w; 1776vs; 1654vs; 1603s; 1582s; 1553m; 1496m; 1476s; 1454m; 1325vs; 1290vs; 1255vs; 1230vs; 1156vs; 1076vs; 839s; 767vs.

^{1}H-NMR ($CDCl_3$, δ ppm, J Hz): 1.36 (t, 7.1, 3H, H-29); 3.87 (s, 2H, H-18); 4.30 (q, 7.1, 2H, H-28); 7.20-7.35 (m, 5H, H-20, H-21, H-22, H-23, H-24); 7.49 (d, 8.8, 2H, H-14, H-16); 7.88 (d, 8.8, 2H, H-13, H-17); 7.97 (d, 8.8, 2H, H-8, H-10); 8.08 (d, 8.8, 2H, H-7, H-11).

^{13}C-NMR ($CDCl_3$, δ ppm): 14.13 (C-29); 31.52 (C-18); 66.75 (C-28); 124.68 (C-6); 126.78 (C-22); 126.84 (C-8, C-10); 128.28 (C-7, C-11); 128.65 (C-21, C-23); 128.95 (C-20, C-24); 129.29 (C-13, C-17); 129.88 (C-14, C-16); 131.64 (C-4); 137.28 (C-19); 139.85 (C-15); 140.34 (C-12); 142.35 (C-9); 147.28 (C-5); 151.22 (C-2); 153.20 (C-26).

+ESI-MS/MS (*m/z*, rel. abund. %): 498 (^{35}Cl)/500 (^{37}Cl) $[M+H]^+$; 454 (^{35}Cl)/456 (^{37}Cl) (16.7/22.4) $[M+H-C_2H_4O]^+$; 426 (^{35}Cl)/428 (^{37}Cl) (100, BP) $[M+H-C_2H_4O-CO]^+$; 348 (^{35}Cl)/350 (^{37}Cl) (15.3/16.6) $[M+H-C_2H_4O-CO-C_6H_6]^+$; 279/281 (88.4/73.2) $[^{35}ClC_6H_4SO_2C_6H_4CO]^+$/$[^{37}ClC_6H_4SO_2C_6H_4CO]^+$.

RP-HPLC (methanol-water 70:30, v/v; 1 mL/min; 303 nm): purity = 99.36%; t_R = 4.30 min.

Elemental analysis (%): calculated for $C_{25}H_{20}ClNO_6S$ (497.95 g/mol): C, 60.30; H, 4.05; N, 2.81; S, 6.44 and found: C, 60.37; H, 4.04; N, 2.82; S, 6.44.

{4-Benzyl-2-{4-[(4-bromophenyl)sulfonyl]phenyl}-1,3-oxazol-5-yl} ethyl carbonate ***2f*** (obtained from 4.88 g of 2-{4-[(4-bromophenyl)sulfonyl]benzamido}-3-phenylpropanoic acid **1f** [10]); yield = 90% (4.88 g); m.p. = 140 - 142ºC.

UV-Vis (CH_3OH, λ nm) (lg ε): 203.5 (4.48); 250.2 (4.18); 304.8 (4.19).

FT-IR (KBr, ν cm^{-1}): 3087m; 3064m; 3029m; 2983m; 2937w; 2871w; 2849w; 1776vs; 1654s; 1603s; 1573s; 1551m; 1496m; 1472s; 1454m; 1325vs; 1290vs; 1255vs; 1228vs; 1157vs; 1070vs; 846m; 615vs; 573vs.

^{1}H-NMR ($CDCl_3$, δ ppm, J Hz): 1.36 (t, 7.1, 3H, H-29); 3.87 (s, 2H, H-18); 4.30 (q, 7.1, 2H, H-28); 7.20-7.35 (m, 5H, H-20, H-21, H-22, H-23, H-24); 7.65 (d, 8.8, 2H, H-14, H-16); 7.80 (d, 8.8, 2H, H-13, H-17); 7.97 (d, 8.5, 2H, H-8, H-10); 8.08 (d, 8.5, 2H, H-7, H-11).

^{13}C-NMR ($CDCl_3$, δ ppm): 14.12 (C-29); 31.51 (C-18); 66.74 (C-28); 124.67 (C-6); 126.71 (C-22); 126.83 (C-8, C-10); 128.27 (C-7, C-11); 128.64 (C-21, C-23); 128.89 (C-15); 128.94 (C-20, C-24); 129.34 (C-13, C-17); 131.65 (C-4); 132.85 (C-14, C-16); 137.28 (C-19); 140.42 (C-12); 142.30 (C-9); 147.28 (C-5); 151.20 (C-2); 153.18 (C-26).

RP-HPLC (methanol-water 70:30, v/v; 1 mL/min; 303 nm): purity = 98.81%; t_R = 5.00 min.

Elemental analysis (%): calculated for $C_{25}H_{20}BrNO_6S$ (542.40 g/mol): C, 55.36; H, 3.72; N, 2.58; S, 5.91 and found: C, 55.41; H, 3.71; N, 2.59; S, 5.89.

Synthesis of ethyl 2-[4-(phenylsulfonyl)benzamido] propanoate ***4a***

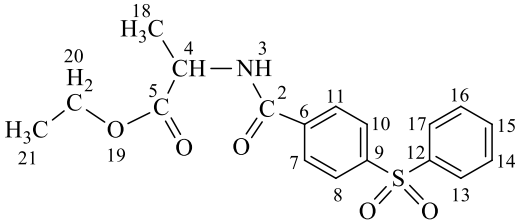


Figure 3.
Structure of compound **4a** with the numbering of atoms (used for NMR assignments)

Method 1. To a mixture of 2-[4-(phenylsulfonyl) benzamido]propanoic acid **1a** [47] (1.67 g, 5 mmol) in 17 mL (13.41 g, 291.08 mmol) of absolute ethanol, 0.85 mL (1.56, 16 mmol) of 98% sulfuric acid was added, and the mass reaction was refluxed for 12 h until a solution was obtained. The hot reaction mixture was filtered off, cooled, then poured into 30 mL of ice water, and brought to a slightly alkaline pH with 8% sodium carbonate solution. The resulting precipitate was filtered off, washed with water, dried, and purified by recrystallization from toluene when colourless crystals were obtained; yield = 84% (1.52 g).

Method 2. 4-Methyl-2-[4-(phenylsulfonyl)phenyl]-1,3-oxazol-5(4*H*)-one **3a** [47] (1.58 g, 5 mmol) was refluxed under stirring with 25 mL (19.73 g, 428.26 mmol) of absolute ethanol for 30 min until the reaction mass was dissolved. The hot reaction mixture was then filtered off and the filtrate was concentrated to dryness under reduced pressure. The solid crude product was recrystallized from toluene to give colourless crystals; yield = 90% (1.63 g); m.p. = 135 - 137ºC.

UV-Vis (CH_3OH, λ nm) (lg ε): 203.5 (4.48); 244.9 (4.42).

FT-IR (KBr, ν cm^{-1}): 3297s; 3088m; 3071m; 2989m; 2941m; 2911m; 2872w; 2820w; 1747vs; 1647vs; 1599m; 1547vs; 1486m; 1447s; 1320vs; 1309vs; 1293vs; 1211vs; 1177vs; 1157vs; 853m.

^{1}H-NMR ($CDCl_3$, δ ppm, J Hz): 1.32 (t, 7.0, 3H, H-21); 1.53 (d, 7.1, 3H, H-18); 4.26 (q, 7.0, 2H, H-20); 4.76 (quint, 7.1, 1H, H-4); 7.62 (tt, 7.3, 1.7, 1H, H-15); 7.54 (t, 7.3, 2H, H-14, H-16); 7.90 (d, 8.5, 2H, H-8, H-10); 7.97 (dd, 7.3, 1.7, 2H, H-13, H-17); 7.98 (d, 8.5, 2H, H-7, H-11); 9.11 (d, 7.1, 1H, H-3).

^{13}C-NMR ($CDCl_3$, δ ppm): 14.11 (C-21); 18.30 (C-18); 48.78 (C-4); 61.82 (C-20); 127.74 (C-8, C-10); 127.89 (C-7, C-11); 128.11 (C-14, C-16); 129.47 (C-

13, C-17); 133.63 (C-15); 138.25 (C-6); 140.81 (C-12); 144.20 (C-9); 165.30 (C-2); 172.99 (C-5).

GC-EI-MS (70 eV) (*m/z*, rel. abund. %): 316 (1.48) $[M\text{-}C_2H_5O\cdot]^+$; 315 (5.50); 288 (26.64) $[M\text{-}C_2H_5O\cdot\text{-}CO]^+$; 272 (2.33) $[C_6H_5SO_2C_6H_4CON{=}CH]^+$; 271 (13.53); 245 (100, BP) $[C_6H_5SO_2C_6H_4C{=}O]^+$; 125 (47.15) $[C_6H_5S{=}O]^+$; 104 (13.32) $[C_6H_4C{\equiv}O]^{+\cdot}$; 77 (25.16) $[C_6H_5]^+$; 51 (7.61) $[C_4H_3]^+$; purity = 96.54%; t_R = 32.52 min.

RP-HPLC (methanol-water 60:40, v/v; 1 mL/min; 250 nm): purity = 94.41%; t_R = 3.37 min.

Elemental analysis (%): calculated for $C_{18}H_{19}NO_5S$ (361.41 g/mol): C, 59.82; H, 5.30; N, 3.88; S, 8.87 and found: C, 59.87; H, 5.28; N, 3.87; S, 8.84.

Cytotoxicity assessment

Freshwater cladoceran *Daphnia magna* was used for cytotoxicity evaluation. The crustaceans were selected according to their size from a parthenogenetic culture since 2012 (Department of Pharmaceutical Botany and Cell Biology, Faculty of Pharmacy, Bucharest, Romania). The culture is maintained at 25°C with a photoperiod of 16 h light/8 h dark in an MLR-351 H climatic chamber (Sanyo, San Diego, CA, USA). The determinations were carried out in duplicate, against 1% DMSO (negative control). The experiment was performed according to the previously described protocol [55, 56]. From each compound, six concentrations between 0.4 and 40 μg/mL were tested in the conditions mentioned above. The lethality curves were plotted against the logarithm of concentrations and lethality (L%) were recorded at 24 h and 48 h. The calculations were made using GraphPad Prism version 5.01 software (GraphPad Software, Inc., La Jolla, CA, USA). The predictions were performed using the freely available online GUSAR software application (Institute of Biomedical Chemistry, Moscow, Russia) [57].

Results and Discussion

Chemistry

Open-chain precursors **1a-f** were prepared from α-alanine or phenylalanine and 4-(phenylsulfonyl]benzoyl chloride or 4-[(4-X-phenyl)sulfonyl]benzoyl chloride (X = Cl, Br) by Steiger *N*-acylation reaction, and 1,3-oxazol-5(4*H*)-one **3a** was obtained by cyclo-dehydration of **1a**, according to our previously reported literature [10, 22, 30, 40, 47, 54].

Then, efficient one-pot synthesis to obtain ethyl 1,3-oxazol-5-yl carbonates **2a-f** has been developed through the reaction of *N*-acyl-α-amino acids **1a-f** with ethyl chloroformate in the presence of *N*-methyl-morpholine (NMM), in anhydrous methylene chloride, at room temperature (Figure 1). Thus, when the molar ratio of *N*-acyl-α-amino acids (**1a-f**): $ClCO_2C_2H_5$:NMM was 1:1.5:1.5 and the reaction time was increased to 24 h, new heterocyclic compounds of the ethyl 1,3-oxazol-5-yl carbonates class **2a-f** were obtained and not corresponding saturated 1,3-oxazol-5(4*H*)-ones (which were isolated at molar ratio of **1a-f**:$ClCO_2C_2H_5$:NMM = 1:1:1; reaction time = 30 min [10, 22, 30, 40, 47, 54]), as expected.

The proposed mechanism for obtaining 4-(benzyl/methyl)-5-(ethoxycarbonyloxy)-2-[4-(4-X-phenyl-sulfonyl)phenyl]-1,3-oxazoles **2a-f** from *N*-acyl-α-amino acids **1a-f** by reaction with ethyl chloroformate in the basic medium occurs through mixed anhydride **I**, formed by a nucleophilic substitution reaction, and which is in tautomeric equilibrium with dienolic form **II**. Then, in the presence of excess NMM, **II** passes into corresponding anion **III**. Finally, the obtaining of the new heterocyclic compounds **2a-f** from intermediate anion-enol **III** can be explained by a nucleophilic addition mechanism, followed by elimination (Figure 4).

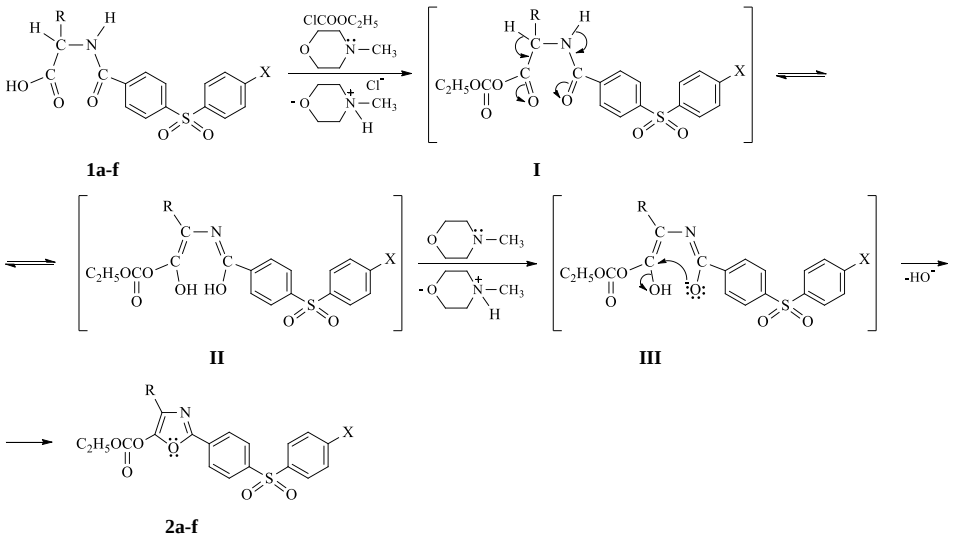


Figure 4.
Proposed reaction mechanism for obtaining ethyl 1,3-oxazol-5-yl carbonates **2a-f** from *N*-acyl-α-amino acids **1a-f** through mixed anhydride **I**

Another possible reaction mechanism (Figure 5) involves the intermediate formation of saturated azlactones **IV**, which undergo *in situ* keto-enol tautomerism to the corresponding 1,3-oxazol-5-ols **V**. These through a nucleophilic substitution reaction lead to ethyl {2-[4-(4-X-phenylsulfonyl)phenyl]-1,3-oxazol-5-yl} carbonates **2a-f**, through the tetrahedral intermediate **VI**.

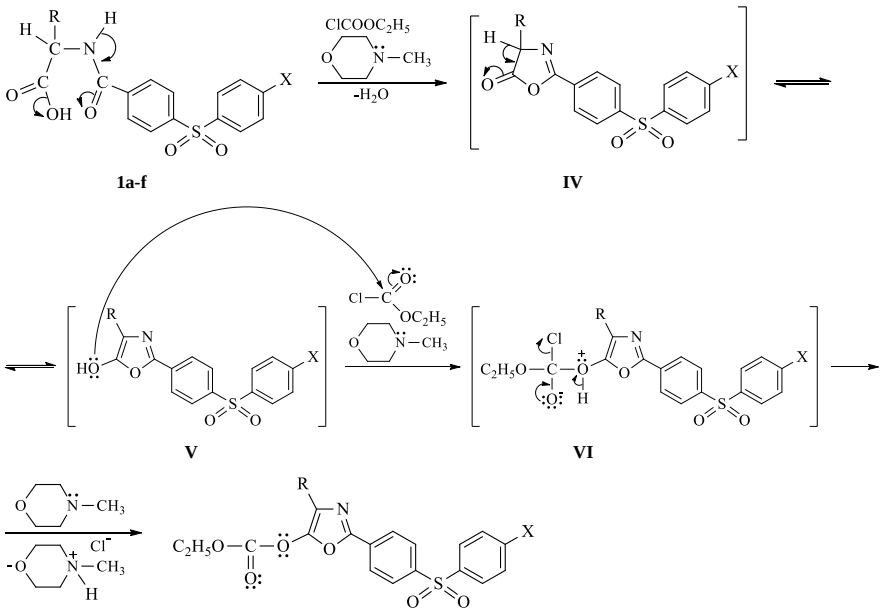


Figure 5.
Proposed reaction mechanism for obtaining ethyl 1,3-oxazol-5-yl carbonates **2a-f** from *N*-acyl-α-amino acids **1a-f** *via* non-isolable 1,3-oxazol-5(4*H*)-ones **IV**

The synthesis of ethyl 2-[4-(phenylsulfonyl)benzamido] propanoate **4a** was performed by two methods. Thus, the esterification of *N*-acyl-α-alanine **1a** with ethanol was carried out under vigorous conditions, namely, a long reaction time, in acid catalysis (concentrated H_2SO_4). In the second method, by brief refluxing of 1,3-oxazol-5(4*H*)-one **3a** in the presence of ethanol, the nucleophilic opening of the 5(4*H*)-oxazolone ring and the formation of the corresponding ester **4a** take place, because the saturated azlactones are very strong acylating agents (Figure 1).

O-Acylation of ethanol with 4-methyl-2-[4-(phenylsulfonyl)phenyl]-1,3-oxazol-5(4*H*)-one **3a** occurs through a nucleophilic substitution mechanism. In the first step, the nucleophilic attack of the alcoholic oxygen atom on the carbon atom at position 5 of the 1,3-oxazol-5(4*H*)-one ring (which has an increased reactivity) takes place. Because the protonated alcohol group is a strong acid, in the second step, the amphionic intermediate **VII** loses a proton. This is fixed to the nitrogen atom from position 3, which functions as a Lewis base, with the formation of the acyclic product of ethanolysis **4a** (Figure 6).

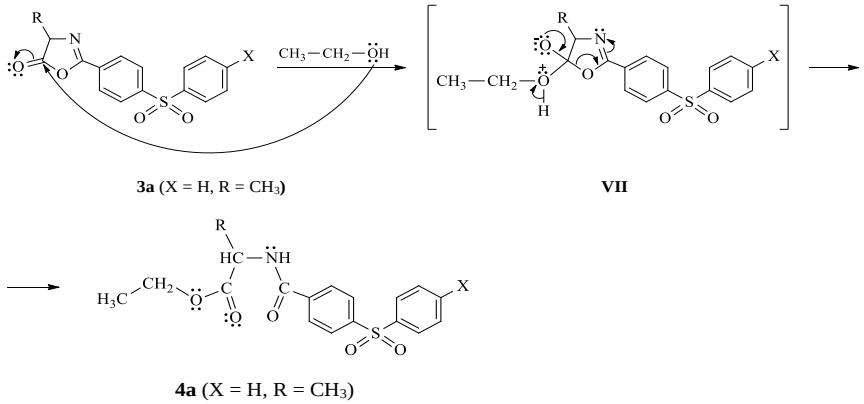


Figure 6.
Proposed reaction mechanism for obtaining ester **4a** from saturated azlactone **3a**

All newly synthesized compounds were physico-chemically characterized by NMR (^{1}H, ^{13}C, COSY, HETCOR), IR, UV-Vis spectroscopy, MS, and technique of elemental analysis.

UV-Vis spectra of ethyl 1,3-oxazol-5-yl carbonates **2a-f** showed two, three or four absorption maxima at 202.6 or 203.5 nm (E band) (**2a-f**), 223.8 or 227.3 nm (K band) (**2b,c**), 246.7 or 250.2 nm (B band) (**2b,c,e,f**), and 300.4 - 304.8 nm (**2a-f**). These absorption bands were due to the $\pi \rightarrow \pi^*$ transitions from the chromophore systems existing in the molecules of the new carbonates. In the case of compounds **2a,d**, the second maximum was weak, had a "shoulder" appearance, and the wavelength value could not be determined. Comparing the electronic spectra of ethyl 1,3-oxazol-5-yl carbonates **2a-f** with those of the acyclic precursors **1a-f** was found the appearance of a new absorption maximum at high wavelength values between 300.4 - 304.8 nm, due to the extension of the conjugation of π electrons by the formation of the 1,3-oxazole chromophore.

The UV spectrum of ester **4a** presented the E band at λ_{max} = 203.5 nm and the B band at λ_{max} = 244.9 nm.

FT-IR spectra of newly obtained compounds showed the characteristic absorption bands of the functional groups. The structures of the new ethyl 1,3-oxazol-5-yl carbonates **2a-f** were confirmed by the absence in IR spectra of absorption bands due to the stretching vibrations: ν(N-H), ν(O-H), ν(O=C-O), and ν(O=C-N), which were recorded in the acyclic precursors **1a-f** spectra. Characteristic for the newly synthesized 5-(ethoxycarbonyloxy)-1,3-oxazoles **2a-f** were: the very intense absorption band due to the valence vibration of the C=O bond of the carbonate group, ν(C=O), which appeared in 1776 - 1788 cm^{-1} region, and the absorption peak due to the C=N stretching vibration, ν(C=N), from the 1,3-oxazole nucleus, displayed in 1654 - 1670 cm^{-1} range. Spectra of heterocyclic compounds **2a-f** presented also intense absorption bands due to the two symmetrical and antisymmetric stretching vibrations of the cyclic ether group, which also confirmed the formation of the 1,3-oxazole ring. The absorption band due to the symmetrical stretching vibration, ν_{sym}(C-O-C), appeared in the region: 1070 - 1077 cm^{-1}, and the absorption peak corresponding to the antisymmetric stretching vibration, ν_{as}(C-O-C), was registered in the range of 1222 - 1255 cm^{-1}.

In the IR spectrum of ester **4a**, a very intense absorption band was recorded, due to the stretching vibration of the ester-type carbonyl group, ν(C=O), at 1747 cm^{-1}, which was shifted compared to the corresponding absorption band in the *N*-acyl-α-amino acid **1a** spectrum from 1731 cm^{-1} and compared to that due to the C=O group at position 5 of 1,3-oxazol-5(4*H*)-one **3a** from 1828 cm^{-1}. The main evidence that the *O*-acylation reactions of ethanol with **1a** and **3a**, respectively took place in IR consisted in the presence of the two absorption peaks due to symmetrical and antisymmetric stretching vibrations of the C-O group from the ethoxycarbonyl fragment, which occurred at 1177 cm^{-1} and 1211 cm^{-1}, respectively (ester band). Characteristic band for compound **4a** was also that due to the valence vibration of the N-H bond, ν(N-H), observed at 3297 cm^{-1}, and the very intense amidic carbonyl absorption, ν(O=C-N), from 1647 cm^{-1} (amide I peak). The amide II band, attributed to the N-H bond deformation vibration, δ(N-H), was recorded at 1547 cm^{-1}. Additional proof of obtaining ester **4a** was the absence in its spectrum of the strong and very broad band attributed to the stretching vibration of the associated O-H group, ν(O-H), which was observed in the precursor **1a** spectrum.

The *^{1}H-NMR spectra* of new compounds proved the chemical structures; ^{1}H-^{1}H COSY spectra facilitated assigning the signals. In the **2a-f** spectra, both the doublet signal from 8.93 - 9.00 ppm attributed to the proton of the NH group and that due to the proton of the methine group from position 4 of *N*-acyl-α-amino acids **1a-f** were not registered, and these proved that reaction took place. The formation of the 1,3-oxazole ring was also confirmed by the presence in the ^{1}H-NMR spectra of compounds **2a-f** of the signal of hydrogen atoms from the radical bounded to the C-4. In the ^{1}H-NMR spectra of ethyl {4-methyl-2-[4-(X-phenylsulfonyl)phenyl]-1,3-oxazol-5-yl} carbonates **2a-c**, methyl radical protons (H-18) appeared as a singlet at δ_H = 2.15 or 2.16 ppm, more de-shielded compared to the corresponding protons from acyclic precursors **1a-c**, for which a doublet (due to the vicinal coupling to H-4) at δ_H = 1.38 or 1.39 ppm was observed. Magnetically equivalent protons of the methylene group from benzyl substituent linked to C-4 of {4-benzyl-2-[4-(X-phenylsulfonyl)phenyl]-1,3-oxazol-5-yl} ethyl carbonates **2d-f** resonated at δ_H = 3.87 ppm as a singlet, compared to the corresponding diastereotopic protons from the **1d-f**, which showed two signals as a doublet of doublets at δ_H = 3.20 or 3.21 ppm and δ_H = 3.04 ppm, respectively, due to the germinal and vicinal coupling to H-4 (Figure 2). An argument for confirming the structures of ethyl 1,3-oxazol-5-yl carbonates **2a-f** was the presence in the ^{1}H-NMR spectra of the characteristic signals for the ethyl group from the C-5-linked ethoxy-carbonyloxy moiety. For the CH_2 group, quartet signal appeared at δ_H = 4.39 or 4.40 ppm for compounds **2a-c** and 4.29 or 4.30 ppm for **2d-f**, and for the CH_3 group, triplet signal was recorded at 1,42 or 1,43 ppm for **2a-c** and 1.35 or 1.36 ppm for **2d-f** (with ^3J = 7.1 or 7.2 ppm).

The presence in the ^{1}H-NMR spectrum of *N*-acyl-α-alanine ethyl ester **4a** of the signal as a doublet assigned to the proton of the NH group, at δ_H = 9.11 ppm, coupled to H-4 (^3J = 7.1 Hz), was the main proof that the sequence of nucleophilic substitution and decyclization of **3a** took place. The H-4 signal

appeared in the **4a** spectrum at 4.76 ppm as a quintet, due to the coupling to the NH proton and with the protons of the methyl radical bounded to the C-4 atom. In the **4a** spectrum, the signal attributed to the protons of the C-4-linked methyl group appeared as a doublet at a chemical shift of 1.53 ppm due to H-4 coupling (Figure 3). For the ethyl group from the ethoxycarbonyl fragment, a quartet signal due to the protons of the methylene group (H-20) appeared at 4.26 ppm, and a high field triplet signal (δ_H = 1.32 ppm) assigned to the methyl group protons (H-21) was observed.

^{13}C-NMR spectra confirmed the obtaining of the newly synthesized compounds and ^{1}H-^{13}C HETCOR spectra allowed the assignment of ^{13}C signals. In the ^{13}C-NMR spectra of 1,3-oxazoles **2a-f**, the C-4 peak was strongly deshielded (with 77.01 - 83.28 ppm), compared to the corresponding carbon atom signal from the acyclic intermediates **1a-f** and this confirmed that the reaction took place. Another proof of obtaining the new heterocyclic compounds **2a-f** was the presence of the signal attributed to the C-2 atom from the 1,3-oxazole ring, which appeared in the region of 151.20 - 151.35 ppm, while the corresponding C atom signal from -CONH- group of acyclic intermediates **1a-f** (from 164.88 - 165.20 ppm region) was not observed. The C-5 atom resonated in the interval: 147.00 - 147.28 ppm, compared to the corresponding carbonyl carbon of precursors **1a-f**, which resonated in the 172.68 - 175.88 ppm range, this being an additional argument that the formation of the 1,3-oxazole core took place. Characteristic for the **2a-f** was also the signal of the carbonyl carbon (C-20 in **2a-c** or C-26 in **2d-f**) from the ethoxy-carbonyloxy group observed in the region: 152.80 - 153.31 ppm.

In the ^{13}C-NMR spectrum of **4a**, the presence of the C-2 signal at δ_C = 165.30 ppm, confirmed the ethanolysis reaction of **3a**. In addition, the ester carbon atom (C-5), being strongly deshielded, resonated at 172.99 ppm. Ester **4a** also showed a signal at 48.78 ppm for the C-4 atom, displaced to the high field (with 12.61 ppm) compared to the signal of the corresponding carbon of precursor **3a**. For the strongly shielded carbon atom of the C-4-linked methyl group, a signal appeared in the spectrum at δ_C = 18.30 ppm. The ethyl ester group was evidenced by the presence of the carbon atom of the CH_2 group for which a signal appeared at 61.82 ppm and the C atom of the CH_3 group, which showed a signal at δ_C = 14.11 ppm.

Mass spectra of ethyl {4-methyl-2-[4-(4-X-phenylsulfonyl)phenyl]-1,3-oxazol-5-yl} carbonates **2a-c** and ester **4a** were registered by gas chromatography coupled to mass spectrometry (GC/MS), due to their increased volatility compared to **2d-f**. The molecular ion, $[M]^{+\cdot}$, of these compounds was not evidenced, but the fragments resulting from the removal of ethylene oxide (**2a-c**) or $C_2H_5O\cdot$radical (**4a**).

For further confirmation of the molecular structures of the compounds from 1,3-oxazoles class, the mass spectra of **2c** and **2e** were recorded by direct introduction of the sample into an ionic interface at atmospheric pressure, coupled to tandem mass spectrometry (ESI/MS/MS). The electrospray ionization (ESI) is a soft ionization technique whose main result is the pseudo-molecular ion of a molecule, which in the case of positive ionization is the protonated molecular ion. Isotopic contributions from halogens can be traced. If there is one chlorine atom in the molecule (**2e**), in full scan mode, two protonated molecular ions, $[^{35}ClM+H]^+$ and $[^{37}ClM+H]^+$, were obtained with a difference of two *m/z* units and an abundance ratio of 3/1 corresponding to isotopes of chlorine. The same is right for **2c**, but $[^{79}BrM+H]^+$ and $[^{81}BrM+H]^+$ corresponding to bromine isotopes gave a 1/1 abundance ratio. The presence of isotopes does not affect the fragmentation pathway. Therefore, the fragmentations of the protonated molecular ions of **2c** and **2e** were identical.

Cytotoxicity assessment

Daphnia magna bioassay results are presented in Table I and the lethality curves in Figure 7.

Table I

Daphnia magna bioassay results

Compounds	Predicted $LC50_{48\ h}$ (µg/mL)	Determined $LC50_{48\ h}$ (µg/mL)	95% CI of $LC50_{48\ h}$ (µg/mL)
2a	0.52	9.02	4.15 - 19.58
2b	0.8	NC*	NC*
2c	0.09	142.1	NC**
2d	0.1	NC*	NC*
2e	0.04	NC*	NC*
2f	0.02	295	NC**
4a	0.005	23.25	11.05 - 48.90

LC50 – 50% lethal concentration; 95% CI – 95% confidence interval; NC – not calculated due to obtained results;
* L% < 10%; ** 95% CI is very wide

LC50 could not be calculated for any of the tested compounds at 24 h due to an L% below 10%. At 48 h, the highest toxicity was induced by compound **2a**, followed by **4a** and **2c**. Compounds **2b**, **2d**, and **2e** induced a maximum L% of 35%, however, the effect not being dependent on the concentration. For all tested compounds, the predicted LC50 values were significantly lower than those obtained experimentally. The precursors' LC50 values were the following: $LC50_{48h}$ of **1a** = 20.19 µg/mL; $LC50_{48h}$ of **1b** = 37.66 µg/mL; $LC50_{48h}$ of **1c** > 200 µg/mL; $LC50_{48h}$ of **1d** = 34.01 µg/mL; $LC50_{48h}$ of **1e** > 46 µg/mL; $LC50_{48h}$ of **1f** = 105 µg/mL; $LC50_{48h}$ of **3a** = 106.00 µg/mL [10, 22, 30, 40]. Compounds **2a** and **2c** induced higher toxicity than their precursors, whereas compounds **2b**, **2d**, **2e**, and **2f** were less toxic. The toxicity of compound **4a** was comparable with **1a** and higher than **3a**.

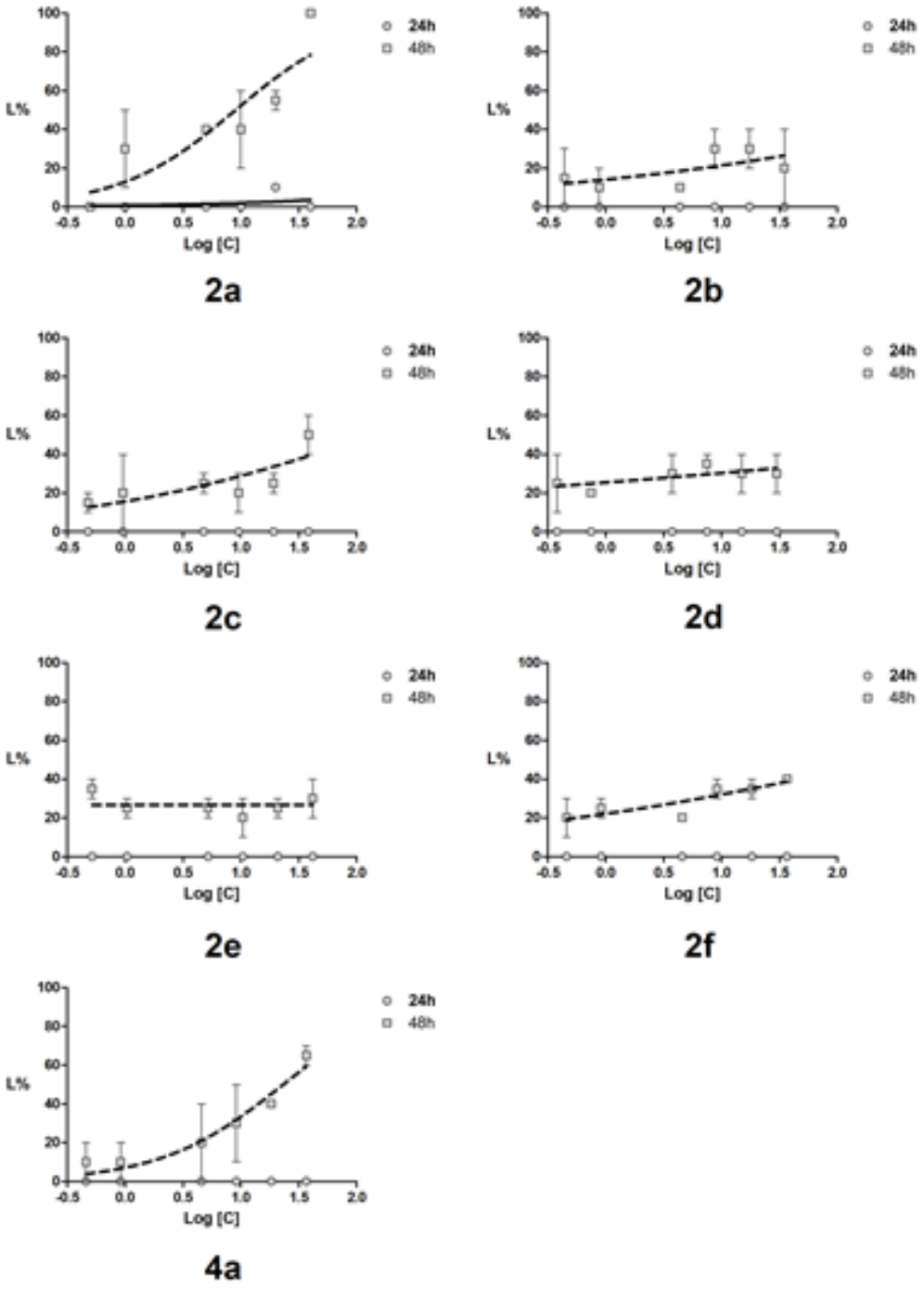


Figure 7.
Daphnia magna bioassay: lethality curves for tested compounds: **2a-f** and **4a**

Conclusions

Seven new analogues from 1,3-oxazoles and *N*-acyl-α-amino acid esters classes, that incorporate into their structures the 4-(4-X-phenylsulfonyl)phenyl substituent, were synthesized and physicochemically characterized, in order to assess their biological activity. The structures of compounds were confirmed by the elemental analysis and spectral methods. The newly synthesized compounds have been investigated for their biological activity on *Daphnia magna*. The compounds **2a**, **2c** and **4a** showed high biological activity, being good candidates for future investigations.

Conflict of interest

The authors declare no conflict of interest.

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