

Synthesis, Characterization and Cytotoxicity Evaluation of New Compounds from Oxazol-5(4H)-Ones and 1,2,4-Triazin-6(5H)-Ones Classes

ELENA VALENTINA ROSCA¹, THEODORA VENERA APOSTOL^{1*}, CONSTANTIN DRAGHICI², OCTAVIAN TUDOREL OLARU^{3*}, LAURA ILEANA SOCEA¹, LUCIAN ISCRULESCU¹, GABRIEL SARAME⁴, FLORICA BARBUCEANU⁵, ELENA MIHAELA PAHONTU⁶, STELIAN BARAITAREANU⁷, STEFANIA FELICIA BARBUCEANU¹

¹ Carol Davila University of Medicine and Pharmacy, Faculty of Pharmacy, Organic Chemistry Department, 6 Traian Vuia Str., 020956, Bucharest, Romania

² Military Medical Scientific Research Center, 24-28 Gr. Cobalcescu Str., 010195, Bucharest, Romania

³ Carol Davila University of Medicine and Pharmacy, Faculty of Pharmacy, Pharmaceutical Botany and Cell Biology Department, 6 Traian Vuia Str., 020956, Bucharest, Romania

⁴ Carol Davila University of Medicine and Pharmacy, Faculty of Pharmacy, Pharmaceutical Technology and Biopharmacy Department, 6 Traian Vuia Str., 020956, Bucharest, Romania

⁵ Institute for Diagnosis and Animal Health, 63 Dr. Staicovici Str., 050557, Bucharest, Romania

⁶ Carol Davila University of Medicine and Pharmacy, Faculty of Pharmacy, General and Inorganic Chemistry Department, 6 Traian Vuia Str., 020956, Bucharest, Romania

⁷ University of Agronomical Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine, Clinical Sciences Department, 105 Splaiul Independentei, 050097, Bucharest, Romania

*This paper presents the synthesis of new heterocyclic compounds from oxazoles and triazinones classes. Some 2-(4-(4-X-phenylsulfonyl)benzamido)acetic acids were condensed with 4-iodobenzaldehyde in presence of acetic anhydride and sodium acetate, when the new corresponding unsaturated oxazol-5(4H)-one were obtained. Through the reaction of these oxazol-5(4H)-ones with phenylhydrazine, in presence of the acetic acid and sodium acetate, the new heterocyclic compounds from 1,2,4-triazin-6(5H)-ones class were obtained. The structures of synthesized compounds have been confirmed by spectral methods (FT-IR, ¹H-NMR, ¹³C-NMR and MS) and elemental analysis. The cytotoxicity of the compounds was evaluated using the *Daphnia magna* and *Artemia salina* bioassays.*

Keywords: oxazol-5(4H)-one, 1,2,4-triazin-6(5H)-one, cytotoxicity, *Daphnia magna*, *Artemia salina*

The heterocyclic compounds with oxazole nucleus arouse the interest of many researchers both in terms of their synthesis and physico-chemical properties, as well as of their biological potential, among them, the oxazolones occupying a special place. Oxazol-5(4H)-ones, also known as azlactones, are considered saturated or unsaturated, depending on the nature of the exocyclic bond from the 4th atom of the nucleus. Of these, unsaturated oxazolones present a particular importance both for the organic synthesis, as intermediates in the obtaining of other classes of compounds, as well as for their biological properties [1,2]. A large number of derivatives from this class are reported in the literature as having biological activity, such as antitumoral [3,4], antibacterial [5-10], antifungal [5,8,9], anti-inflammatory [11], antioxidant [5,12], tyrosinase inhibitors [13,14].

Among the heterocyclic compounds obtained from unsaturated oxazolones are the triazinones cited in the literature for different biological properties, including antitumoral [15,16], antibacterial [17-19], antifungal [17], antioxidant [18], anti-inflammatory [11], anticonvulsant [20].

In previous research we have synthesized compounds with biologic potential having a diarylsulfone fragment [21-28] starting from the literature data which indicate that this fragment is present in the structure of various compounds with biological properties, some of them being also used in therapeutics [24,29-31].

Motivated by these literature data, we initiated the synthesis of a new class of heterocyclic compounds from

unsaturated oxazolones and of some triazinones derived from these, which to contain a diarylsulfone fragment in the same molecule, in order to obtain new derivatives with biological potential.

In a first stage, we investigated the cytotoxicity of the new synthesized compounds using *Daphnia magna* and *Artemia salina* organisms. Among other screening methods alternative to vertebrate animals, these bioassays are rapid, simple, reproducible and cost-efficient. Various studies demonstrated the usefulness of this method in testing various biological end-points [32-36].

Experimental part

Chemistry

The reactants and solvents used in the synthesis were purchased commercially with high purity and were used without further purification. The melting points of the compounds were determined with a Böetius apparatus and are uncorrected. The elemental analysis was determined using a ECS-40-10 Costech micro-dosimeter. The FT-IR spectra were registered on a Bruker Vertex 70 spectrometer (in KBr pellets). The intensity of IR absorption bands are given as: very strong (vs), strong (s), medium (m) and weak (w). The NMR spectra were recorded on a Varian Gemini 300BB spectrometer at 300 MHz for ¹H-NMR and 75 MHz for ¹³C-NMR. The DMSO-d₆ was used as solvent. The chemical shifts δ are reported in ppm relative to tetramethylsilane (TMS) internal standard and the coupling constants *J* are expressed in hertz Hz. The abbreviations for multiplicities and descriptors of signals used in the ¹H-

* email: theodoraapostol@yahoo.com, octav_olaru2002@yahoo.com

NMR spectra are the following: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, m = multiplet, br = broad signal. For the acquisition of the mass spectra of compounds a APCI (Atmospheric Pressure Chemical Ionization) mass spectrometer was used. For oxazolones, a solution of about 0.5 mg/mL in chloroform was prepared and then was diluted 10 times with methanol (1% formic acid) for positive ionization, when protonated molecular ions were obtained $[M+H]^+$. For triazinones a 0.5 mg/mL solution was diluted 10 times with methanol (1% ammonium carbonate) for negative ionization $[M-H]^-$. The solutions of the oxazolones series were passed through the APCI interface with positive ionization and the triazinones solutions were passed through the APCI interface with negative ionization. A solution injection system with a loop mounted on a Reodyne 7725 valve and a Varian Prostar 240 SDM pump operating at a flow rate of 50 μ L/min was used to drive the solutions. Protonated molecular ions (in positive ionization) or negative (by removing an acidic proton) were fragmented into the argon collision cell at 1.5 mTorr. The fragments were scanned with the third quadrupole.

Synthesis and characterization of compounds

The reaction stages for the synthesis of the title compounds are presented in scheme 1. For the synthesis of the new heterocycles from oxazolones and triazinones class we started to synthesize the 2-(4-(4-X-phenylsulfonyl)benzamido)acetic acids precursors using a multi-step reaction [37,38]. Thus, by the reaction of benzene or other halobenzenes ($X = \text{Cl}$ or Br) with tosyl chloride, the diarylsulfones **1** were obtained, which by oxidation with chromic anhydride gave the corresponding acids **2**. In the next step, by treating the acids with thionyl chloride, the 4-(4-X-phenylsulfonyl)benzoyl chlorides **3** were obtained [24]. The N-acylation of glycine with chlorides **3** conducted

to the 2-(4-(4-X-phenylsulfonyl)benzamido)acetic acids **4** [37,38]. Further, the condensation of the hippuric acids derivatives **4** with 4-iodobenzaldehyde, in the presence of acetic anhydride and sodium acetate, took place with the obtaining of the new benzylidene-oxazol-5(4H)-ones **5**, namely 2-(4-(4-X-phenylsulfonyl)phenyl)-4-(4-iodobenzylidene)oxazol-5(4H)-ones. Finally, 3-(4-(4-X-phenylsulfonyl)phenyl)-5-(4-iodobenzylidene)-2-phenyl-1,2-dihydro-1,2,4-triazin-6(5H)-ones **6a-c** were obtained by treating of oxazolones **5a-c** with phenylhydrazine, in the presence of acetic acid and sodium acetate.

The chemical structures of the new compounds were confirmed by FT-IR, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, MS spectra and elemental analysis.

Synthesis of 2-(4-(4-X-phenylsulfonyl)phenyl)-4-(4-iodobenzylidene)oxazol-5(4H)-ones **5a-c**

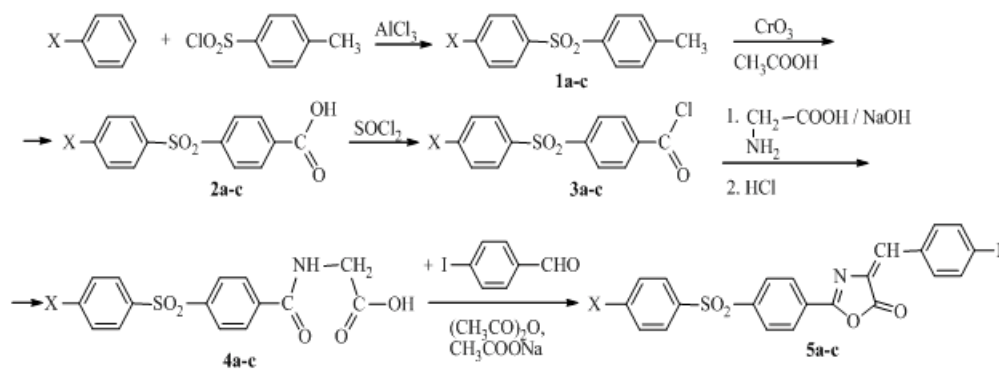
A mixture of N-acylated glycine **4a-c** (5 mmol), 4-iodobenzaldehyde (5 mmol) and sodium acetate (0.41 g) in acetic anhydride (1.8 mL) was refluxed for 4 h with magnetic stirring. To the obtained solution, 2 mL of ethanol was added and allowed it to stand overnight at cold. The obtained precipitate was filtered off, washed with boiling water and cold ethanol, when a yellow coloured crystalline compound was obtained. The compound was recrystallized from an ethanol-chloroform mixture (1:2, v:v).

4-(4-Iodobenzylidene)-2-(4-(phenylsulfonyl) phenyl)oxazol-5(4H)-one **5a**

m.p. = 244-246°C; yield = 78 %

FT-IR (KBr, ν cm^{-1}): 3085m, 3062m, 1793vs, 1772s, 1655s, 1580s, 1556m, 1554m, 1479m, 1399s, 1327vs, 1306s, 1290s, 1160vs, 1098s, 861s, 573s;

$^1\text{H-NMR}$ (DMSO-d_6 , δ ppm, J Hz): 7.41 (s, 1H, H-18), 7.70 (t, 7.3, 1H, H-15), 7.85 (t, 7.3, 2H, H-14, H-16), 7.90 (d,



Scheme 1. Synthetic route of compounds **5a-c** and **6a-c**

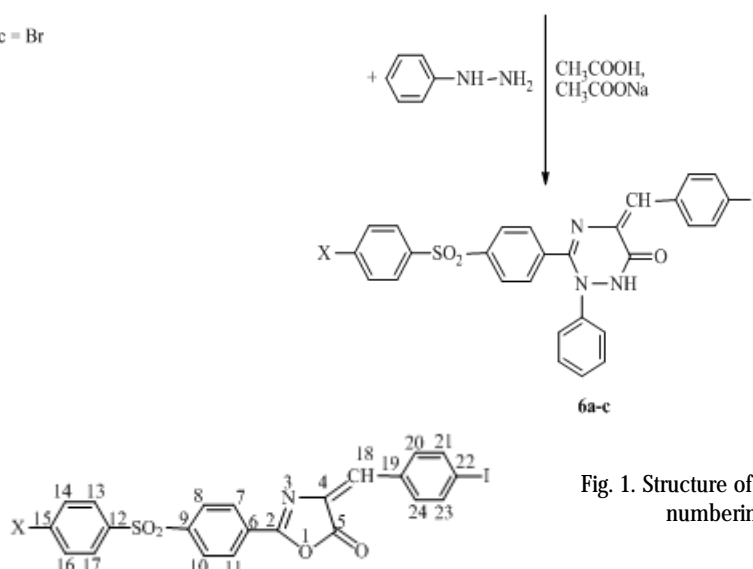


Fig. 1. Structure of oxazol-5(4H)-ones **5a-c** with atom numbering (for NMR assignments)

8.6, 2H, H-20, H-24), 7.92 (d, 8.6, 2H, H-21, H-23), 8.00 (br d, 7.3, 2H, H-13, H-17), 8.19 (d, 8.8, 2H, H-8, H-10), 8.31 (d, 8.8, 2H, H-7, H-11);

$^{13}\text{C-NMR}$ (DMSO- d_6 , δ ppm): 99.81 (C-22), 127.54 (C-13, C-17), 128.16 (C-8, C-10), 129.15 (C-7, C-11), 129.61 (C-6), 134.15 (C-15), 129.89 (C-14, C-16), 131.22 (C-18), 132.50 (C-19), 133.40 (C-4), 134.15 (C-20, C-24), 137.95 (C-21, C-23), 140.13 (C-12), 144.76 (C-9), 161.86 (C-2), 166.25 (C-5);

Anal. (%): Calcd. for $\text{C}_{22}\text{H}_{14}\text{INO}_3\text{S}$ (514.97 g/mol): C, 51.28; H, 2.74; N, 2.72; S, 6.22. Found: C, 51.31; H, 2.69; N, 2.80; S, 6.19;

APCI, m/z (%): 516 $[\text{M}+\text{H}]^+$, 245 $[\text{C}_6\text{H}_5\text{SO}_2\text{C}_6\text{H}_4\text{CO}]^+$, 125 $[\text{C}_6\text{H}_5\text{SO}]^+$

2-(4-(4-Chlorophenylsulfonyl)phenyl)-4-(4-iodobenzylidene)oxazol-5(4H)-one 5b

m.p. = 257-259 °C; yield = 67 %

FT-IR (KBr, ν cm^{-1}): 3096m, 3075m, 1794vs, 1769s, 1653s, 1577s, 1557m, 1545m, 1474m, 1400s, 1327vs, 1291s, 1158vs, 1097s, 856s, 768s, 574s;

$^1\text{H-NMR}$ (DMSO- d_6 , δ ppm, J Hz): 7.40 (s, 1H, H-18), 7.75 (d, 8.8, 2H, H-14, H-16), 7.90 (m, 2H, H-20, H-24), 8.05 (m, 4H, H-13, H-17, H-21, H-23), 8.20 (d, 8.0, 2H, H-8, H-10), 8.30 (d, 8.0, 2H, H-7, H-11);

$^{13}\text{C-NMR}$ (DMSO- d_6 , δ ppm): 99.93 (C-22), 128.35 (C-13, C-17), 129.29 (C-8, C-10), 130.15 (C-7, C-11), 129.88 (C-6), 139.03 (C-15), 129.63 (C-14, C-16), 131.37 (C-18), 129.88 (C-19), 133.47 (C-4), 133.90 (C-20, C-24), 138.05 (C-21, C-23), 139.39 (C-12), 144.33 (C-9), 161.90 (C-2), 166.34 (C-5);

Anal. (%): Calcd. for $\text{C}_{22}\text{H}_{13}\text{ClINO}_3\text{S}$ (549.77 g/mol): C, 48.06; H, 2.38; N, 2.55; S, 5.83. Found: C, 48.13; H, 2.23; N, 2.62; S, 5.79;

APCI, m/z : 550 ^{35}Cl $[\text{M}+\text{H}]^+$, 552 ^{37}Cl $[\text{M}+\text{H}]^+$, 279 $^{35}\text{ClC}_6\text{H}_4\text{SO}_2\text{C}_6\text{H}_4\text{CO}]^+$, 281 $^{37}\text{ClC}_6\text{H}_4\text{SO}_2\text{C}_6\text{H}_4\text{CO}]^+$, 159 $^{35}\text{ClC}_6\text{H}_4\text{SO}]^+$, 161 $^{37}\text{ClC}_6\text{H}_4\text{SO}]^+$

2-(4-(4-Bromophenylsulfonyl)phenyl)-4-(4-iodobenzylidene)oxazol-5(4H)-one 5c

m.p. = 281-283 °C; yield = 69 %

FT-IR (KBr, ν cm^{-1}): 3088m, 3043w, 1796vs, 1772s, 1654s, 1574s, 1555m, 1544m, 1480m, 1399s, 1328vs, 1307s, 1290s, 1160vs, 1097s, 854s, 615s, 575s;

$^1\text{H-NMR}$ (DMSO- d_6 , δ ppm, J Hz): 7.39 (s, 1H, H-18), 7.90-8.04 (m, 6H, H-14, H-16, H-20, H-21, H-23, H-24), 8.05 (d, 8.4, 2H, H-13, H-17), 8.18 (d, 8.4, 2H, H-8, H-10), 8.29 (d, 8.4, 2H, H-7, H-11);

$^{13}\text{C-NMR}$ (DMSO- d_6 , δ ppm): 99.90 (C-22), 128.34 (C-13, C-17), 128.79 (C-15), 129.42 (C-7, C-11), 129.61 (C-8, C-10), 132.24 (C-6), 130.27 (C-19), 132.62 (C-18), 132.92 (C-4), 133.42 (C-14, C-16), 134.30 (C-20, C-24), 138.02 (C-21, C-23), 139.53 (C-12), 144.26 (C-9), 161.62 (C-2), 166.46 (C-5);

Anal. (%): Calcd. for $\text{C}_{22}\text{H}_{13}\text{BrINO}_3\text{S}$ (594.22 g/mol): C, 44.47; H, 2.21; N, 2.36; S, 5.40. Found: C, 44.36; H, 2.30; N, 2.27; S, 5.36;

APCI, m/z : 594 ^{79}Br $[\text{M}+\text{H}]^+$, 596 ^{81}Br $[\text{M}+\text{H}]^+$, 323 $^{79}\text{BrC}_6\text{H}_4\text{SO}_2\text{C}_6\text{H}_4\text{CO}]^+$, 325 $^{81}\text{BrC}_6\text{H}_4\text{SO}_2\text{C}_6\text{H}_4\text{CO}]^+$, 203 $^{79}\text{BrC}_6\text{H}_4\text{SO}]^+$, 205 $^{81}\text{BrC}_6\text{H}_4\text{SO}]^+$

Synthesis of 3-(4-(4-X-phenylsulfonyl)phenyl)-5-(4-iodobenzylidene)-2-phenyl-1,2-dihydro-1,2,4-triazin-6(5H)-ones 6a-c

An equimolar mixture of oxazolone 5 and phenylhydrazine (2 mmol) in acetic acid (5 mL) and in the presence of anhydrous sodium acetate (0.3 mmol) was heated under reflux, with magnetic stirring, for 5 hours.

The reaction mixture was cooled, filtered off and washed with hot water. The obtained product as yellow crystals was recrystallized from ethanol.

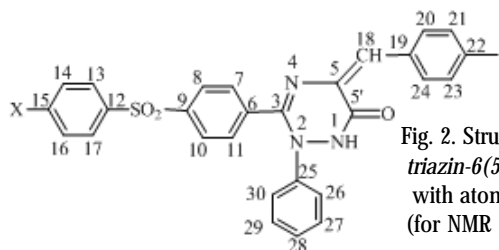


Fig. 2. Structure of 1,2,4-triazin-6(5H)-ones **6a-c** with atom numbering (for NMR assignments)

5-(4-Iodobenzylidene)-2-phenyl-3-(4-(phenylsulfonyl)phenyl)-1,2-dihydro-1,2,4-triazin-6(5H)-one 6a

m.p. = 272-273 °C; yield = 79.5 %

FT-IR (KBr, ν cm^{-1}): 3281m, 3097w, 3065w, 3040w, 1722vs, 1636m, 1603m, 1578m, 1493m, 1482m, 1405m, 1321m, 1308m, 1289s, 1154vs, 1105m, 819m, 566m;

$^1\text{H-NMR}$ (DMSO- d_6 , δ ppm, J Hz): 6.72 (dd, 7.5, 1.1, 2H, H-26, H-30), 6.82 (t, 7.5, 1H, H-28), 7.29 (s, 1H, H-18), 7.62 (t, 7.4, 2H, H-14, H-16), 7.70 (t, 7.4, 1H, H-15), 7.80 (t, 7.6, 2H, H-27, H-29), 7.89 (d, 8.5, 2H, H-21, H-23), 7.98 (dd, 8.2, 1.2, 2H, H-13, H-17), 8.11 (d, 8.5, 4H, H-8, H-10, H-20, H-24), 8.30 (d, 8.5, 2H, H-7, H-11), 8.98 (s, 1H, NH);

$^{13}\text{C-NMR}$ (DMSO- d_6 , δ ppm): 99.13 (C-22), 112.49 (C-26, C-30), 120.45 (C-28), 127.58 (C-8, C-10), 127.65 (C-13, C-17), 128.54 (C-18), 129.86 (C-7, C-11), 129.33 (C-14, C-16), 129.38 (C-27, C-29), 132.07 (C-6), 133.14 (C-19), 134.08 (C-15), 134.29 (C-20, C-24), 136.58 (C-5), 137.86 (C-21, C-23), 140.33 (C-12), 143.83 (C-9), 146.10 (C-25), 159.96 (C-3), 166.70 (C=O);

Anal. (%): Calcd. for $\text{C}_{28}\text{H}_{20}\text{IN}_3\text{O}_3\text{S}$ (605.45 g/mol): C, 55.55; H, 3.33; N, 6.94; S, 5.30. Found: C, 55.61; H, 3.27 N, 6.90; S, 5.32;

APCI, m/z : 604 $[\text{M}-\text{H}]^-$, 513 $[\text{M}-\text{H}-\text{C}_6\text{H}_5\text{N}]^-$, 127 I

3-(4-(4-Chlorophenylsulfonyl)phenyl)-5-(4-iodobenzylidene)-2-phenyl-1,2-dihydro-1,2,4-triazin-6(5H)-one 6b

m.p. = 280-282 °C; yield = 85 %

FT-IR (KBr, ν cm^{-1}): 3281m, 3087w, 3056w, 3040m, 1723vs, 1636s, 1603m, 1580s, 1494m, 1481m, 1404m, 1327s, 1310m, 1291s, 1154vs, 1103m, 1088m, 819m, 760s, 575m

$^1\text{H-NMR}$ (DMSO- d_6 , δ ppm, J Hz): 6.73 (d, 7.8, 2H, H-26, H-30), 6.83 (br t, 7.1, 1H, H-28), 7.30 (s, 1H, H-18), 7.70 (d, 8.5, 2H, H-14, H-16), 7.18 (t, 7.1, 2H, H-27, H-29), 7.89 (d, 8.3, 2H, H-21, H-23), 8.00 (d, 8.5, 2H, H-13, H-17), 8.12 (br d, 8.4, 4H, H-8, H-10, H-20, H-24), 8.30 (d, 8.4, 2H, H-7, H-11), 8.99 (s, 1H, NH);

$^{13}\text{C-NMR}$ (DMSO- d_6 , δ ppm): 99.24 (C-22), 112.53 (C-26, C-30), 120.50 (C-28), 128.67 (C-18), 127.79 (C-8, C-10), 129.63 (C-13, C-17), 130.08 (C-7, C-11), 129.93 (C-14, C-16), 129.38 (C-27, C-29), 132.31 (C-6), 133.19 (C-19), 139.17 (C-15), 134.35 (C-20, C-24), 136.63 (C-5), 137.92 (C-21, C-23), 139.28 (C-12), 143.39 (C-9), 146.16 (C-25), 159.99 (C-3), 168.77 (C=O);

Anal. (%): Calcd. for $\text{C}_{28}\text{H}_{19}\text{ClIN}_3\text{O}_3\text{S}$ (639.89 g/mol): C, 52.56; H, 2.99; N, 6.57; S, 5.01. Found: C, 52.64; H, 2.91; N, 6.62; S, 5.11;

APCI, m/z : 638 ^{35}Cl $[\text{M}-\text{H}]^-$, 640 ^{37}Cl $[\text{M}-\text{H}]^-$, 547 $[\text{M}-\text{H}-\text{C}_6\text{H}_5\text{N}]^-$, 549 $[\text{M}-\text{H}-\text{C}_6\text{H}_5\text{N}]^-$, 127 I

3-(4-(4-Bromophenylsulfonyl)phenyl)-5-(4-iodobenzylidene)-2-phenyl-1,2-dihydro-1,2,4-triazin-6(5H)-one 6c

m.p. = 281-282 °C; yield = 71 %

FT-IR (KBr, ν cm^{-1}): 3282m, 3087w, 3061w, 1722s, 1637s, 1602m, 1575s, 1483s, 1402m, 1325s, 1289s, 1156vs, 1102s, 1068s, 819m, 615s, 575m;

$^1\text{H-NMR}$ (DMSO-d_6 , δ ppm, J Hz): 6.73 (br d, 7.9, 2H, H-26, H-30), 6.82 (br t, 7.3, 1H, H-28), 7.29 (s, 1H, H-18), 7.84 (t, 8.6, 2H, H-14, H-16), 7.19 (br t, 7.9, 2H, H-27, H-29), 7.90 (d, 8.2, 2H, H-21, H-23), 7.91 (d, 8.6, 2H, H-13, H-17), 8.11 (d, 8.2, 4H, H-8, H-10, H-20, H-24), 8.30 (d, 8.6, 2H, H-7, H-11), 8.98 (s, 1H, NH);

$^{13}\text{C-NMR}$ (DMSO-d_6 , δ ppm): 99.15 (C-22), 112.47 (C-26, C-30), 120.41 (C-28), 128.63 (C-18), 127.70 (C-8, C-10), 129.29 (C-13, C-17), 129.35 (C-7, C-11), 132.99 (C-14, C-16), 129.61 (C-27, C-29), 132.30 (C-6), 133.17 (C-19), 128.40 (C-15), 134.37 (C-20, C-24), 136.62 (C-5), 137.89 (C-21, C-23), 139.58 (C-12), 143.34 (C-9), 146.22 (C-25), 159.98 (C-3), 168.75 (C=O);

Anal. (%): Calcd. for $\text{C}_{28}\text{H}_{19}\text{BrIN}_3\text{O}_3\text{S}$ (684.34 g/mol): C, 49.14; H, 2.80; N, 6.14; S, 4.69. Found: C, 49.30; H, 2.71; N, 6.03; S, 4.75;

APCI, m/z : 682 [^{79}Br M-H] $^+$, 684 [^{81}Br M-H] $^+$, 591 [M-H-C $_6\text{H}_5\text{N}$] $^+$, 593 [M-H-C $_6\text{H}_5\text{N}$] $^+$, 127 I

Cytotoxicity evaluation

The cytotoxicity bioassay was performed for compounds **5a-c**, **6a,b** and intermediates **4a-c**.

Both experiments were carried out in controlled environment at $25 \pm 1^\circ\text{C}$, using a 16-h photoperiod and 8 h of darkness using a climatic chamber (Sanyo MLR-351H; Sanyo, San Diego, CA, USA).

Daphnia magna bioassay

The bioassay was performed in duplicate according to the method described in our previous research [41]. Each determination was carried out in PP tissue culture wells (Greiner Bio-One) using 10 organisms in each well at a final volume of 4 mL/sample and a final concentration of DMSO of 1%, used as negative control. Seven compounds were tested at 6 concentration levels ranging from 8 to 198 $\mu\text{g/mL}$. The lethality (L%) was recorded over a 48h period of exposure at two points: 24 and 48h. The 50% lethal concentrations (LC50) were computed for each compound by interpolation on lethality curves plotted between L% values and the logarithm of concentrations using the least square fit method. The LC50 and 95% confidence intervals (CI95%) of LC50 were also calculated using the same method. All calculations were performed using the software GraphPad Prism v 5.1. The prediction of LC50_{48h} was performed using the GUSAR software application.

Artemia salina bioassay

Brine shrimp lethality test was conducted according to the protocol described by Meyer et al. [36] with some modifications [42]. *A. salina* cysts were incubated in

artificial sea water (40 g/L salinity) for 24h under continuous aeration. The newly hatched nauplii were separated from the shells, transferred to fresh sea water and incubated for 24 h. Each determination was carried out in PP tissue culture with 24-wells (Greiner Bio-One) using 10 organisms in each well at a final volume of 1 mL/sample and a final concentration of DMSO of 1%, used as negative control. The compounds were tested at the same levels of concentration as *D. magna*. The lethality (L%) was recorded over a 24h period of exposure and the calculations were made as presented above. No predictions could be performed for brine shrimps.

Results and discussions

Chemistry

The key precursor, 2-(4-(4-X-phenylsulfonyl) benzamido) acetic acids **4**, previously reported in the literature [37,38], were obtained following the Steiger procedure consisting of N-acylation of glycine with 4-(4-X-phenylsulfonyl) benzoyl chlorides **3**. The simultaneous condensation and cyclization reaction of N-acylated glycine derivatives **4** with 4-iodobenzaldehyde, in acetic anhydride and in the presence of sodium acetate, led to the formation of new compounds from 4-iodobenzylidene-oxazole-5(4H)-ones **5**. The 1,2,4-triazin-6(5H)-ones **6** resulted through a ring-opening and recyclization reaction of the oxazolones **5** with phenylhydrazine, in acetic acid and sodium acetate. The heterocyclic compounds were obtained in moderate yields, in a relatively short reaction time. The spectral data validated the structures of these compounds.

IR Spectral data

The reaction between N-acylated glycine **4** and 4-iodobenzaldehyde is firstly confirmed by the disappearance of the NH absorption band from the IR spectra of new compounds **5** that was present at 3416 cm^{-1} (**4a**), 3351 cm^{-1} (**4b**) and 3341 cm^{-1} (**4c**) in precursors **4** [37,38]. The C=O lactone group from oxazolones **5** is highlighted in the IR spectra of these compounds by absorption bands due to stretching vibration, $\nu\text{C=O}$, at $1793\text{--}1796\text{ cm}^{-1}$ and $1769\text{--}1772\text{ cm}^{-1}$ according to the literature for similar derivatives [39,40].

The obtaining of triazinones from reaction of oxazolones with phenylhydrazine is firstly confirmed by appearance in the IR spectra of compounds **6** of a new absorption band at 3281 cm^{-1} in case of derivatives **6a,b** and at 3282 cm^{-1} in **6c**, characteristic to stretching vibration of NH group. The absorption band due to the stretching vibration of the C=O group appears at 1722 cm^{-1} for triazinones **6a,c** and at 1723 cm^{-1} for triazinone **6b**.

NMR Spectra data

The $^1\text{H-NMR}$ spectra of compounds **5** showed a singlet signal characteristic of =CH proton, with δ in the range

No. compound	Predicted LC50 _{48h} ($\mu\text{g/mL}$)	LC50 _{48h} ($\mu\text{g/mL}$)	95% CI of LC50 _{48h} ($\mu\text{g/mL}$)
5a	0.27	56.15	35.37 to 89.13
5b	0.17	355.5	179.3 to 704.7
5c	0.16	111.7	93.49 to 133.4
6a	0.19	178.2	138.7 to 229.0
6b	0.08	159.1	135.2 to 187.1
4a	28.96	219.1	142.1 to 337.9
4b	15.27	NC*	NC*
4c	4.01	NC*	NC*

LC50 - 50% lethal concentration; 95% CI - 95% confidence interval;
NC - not calculated due to the obtained results; * - LC50 and 95% CI couldn't be calculated because no lethality was recorded.

Table 1
LETALITY OF THE TESTED COMPOUNDS ON *DAPHNIA*
MAGNA BIOASSAY

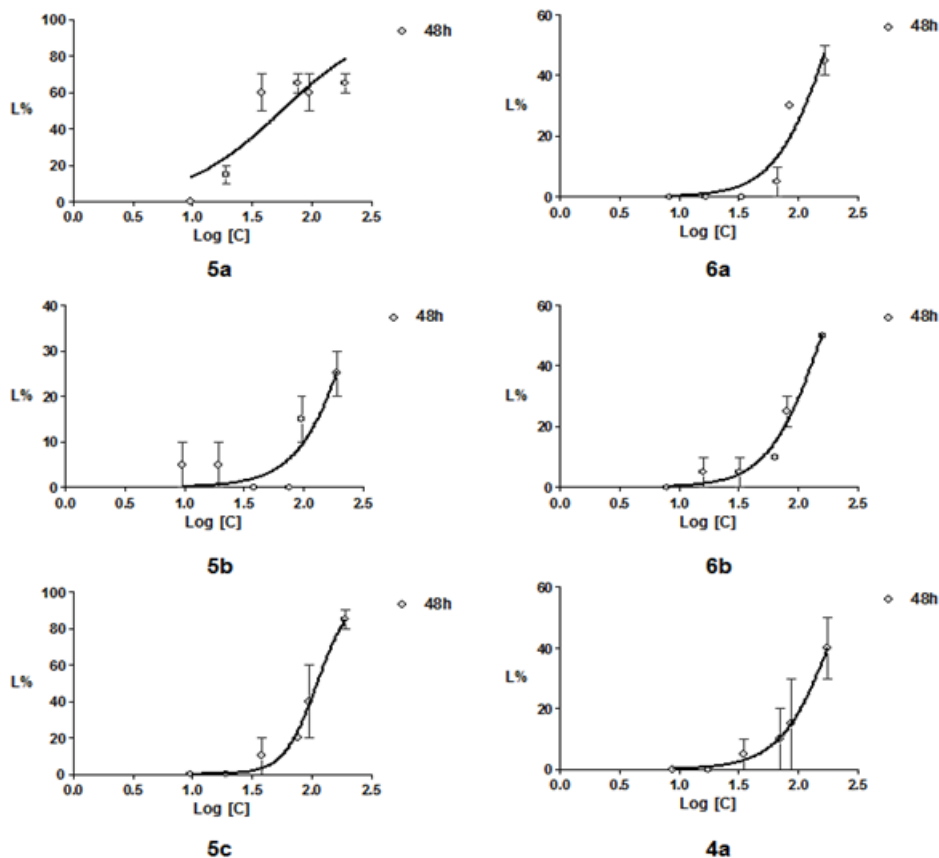


Fig. 3. *Daphnia magna* lethality curves at 48h for tested compounds

7.39-7.41 ppm, which proves that the condensation reaction of N-acylated glycine with 4-iodobenzaldehyde took place. Also, the absence of the triplet signal characteristic of the proton of NH group from compounds **4** ($\delta_{\text{NH}} = 8.22\text{-}9.09$ ppm) [37,38] and of doublet signal of CH_2 protons ($\delta_{\text{CH}_2} = 3.94\text{-}4.37$ ppm), represents another proof that the condensation reaction occurred simultaneously with the intramolecular cyclization.

In the $^1\text{H-NMR}$ spectra of the compounds **6**, the presence of a new signal of NH proton from phenylhydrazine moiety, at ~ 9.00 ppm, is the most important evidence that the reaction of oxazolone with phenylhydrazine occurred.

In the $^{13}\text{C-NMR}$ spectra of the compounds **5**, the carbon atoms from oxazole ring were highlighted by the following signals that appeared at the corresponding chemical shifts: 161.86-161.62 ppm for C-2, 132.92-133.47 ppm for the $>\text{C}=\text{CH}$ (C-4) and 166.25-166.46 ppm for $\text{C}=\text{O}$ (C-5), the last signal being the most deshielded, as expected.

In the $^{13}\text{C-NMR}$ spectra of triazinones **6**, the C-3 atom resonated at $\delta = 159.96\text{-}159.99$ ppm, while the $>\text{C}=\text{CH}$ (C-5) at 136.58-136.63 ppm. The $\text{C}=\text{O}$ signal on the triazinone ring is observed in the range 166.70-168.77 ppm. Also, the methyne carbon from oxazolones **5** resonated at 131.22-132.62 ppm while the same carbon from the same position of triazinones **6** was appeared in region 128.54-128.67 ppm.

Mass spectra

Another support for the assigned structure of new compounds from oxazolones and triazinones classes was obtained by recording the mass spectra.

The mass spectra showed the presence of the molecular ions, thus confirming the structures of these compounds. Fragmentation of oxazolones generally occurs by obtaining a carbonyl ion, stabilized by conjugation. For triazinones, the most characteristic fragmentation is the elimination of a neutral fragment with the formula $\text{C}_6\text{H}_5\text{N}$.

For Cl and Br atoms, isotopic contributions were taken into account.

Cytotoxicity evaluation

Daphnia magna bioassay

The results are presented in table 1 and figure 3. At 24 h, the lethality induced by the tested compounds was below 30% and LC_{50} couldn't be calculated. After 48h of exposure, **4b** and **4c** did not produced any lethal effects and no further calculations were possible. After calculating the LC_{50} , the highest toxicity was exhibited by **5a** and the lowest by **5b**. Among the tested compounds, **5a** had a LC_{50} lower than $100\mu\text{g/mL}$, thus being a potent compound with biological activity on *D. magna*.

No correlation was found between GUSAR prediction values and experimental LC_{50} values. Moreover, **5b**, **4b** and **4c** were found not to be toxic or exhibiting low toxicity, whereas the prediction software indicates them as potent cytotoxic compounds. By applying the t-student test on the two sets of data, predicted and experimentally obtained values, no similarity was found, p value being < 0.0001 which indicate a significant difference. A slightly correlation was found for compounds **4a**, and **4b** regarding the order of magnitude.

Artemia salina bioassay

The toxicity at 24h on *A. salina* was lower than 25% for all compounds at the tested concentrations. The results are comparable to those obtained at *D. magna* bioassay. As showed previously [42], the brine shrimp lethality test is less sensitive than *D. magna*, mainly due to a better resistance of *A. salina*. Further tests will be performed to evaluate the toxicity of the compounds at 48 h.

Conclusions

Six new heterocyclic compounds from oxazol-5(4*H*)-ones and 1,2,4-triazin-6(5*H*)-ones classes containing a diarylsulfone moiety have been synthesized and

characterized by spectral techniques and elemental analysis. The oxazolones have been obtained by the reaction of some 2-(4-(4-X-phenylsulfonyl) benzamido) acetic acids with 4-iodobenzaldehyde. The 1,2,4-triazin-6(5H)-ones were synthesized by treating 1,3-oxazol-5(4H)-ones with phenylhydrazine.

The cytotoxicity bioassays on *Daphnia magna* and *Artemia salina* indicated that among the tested compounds oxazolone **5a** showed moderate to high cytotoxicity on *D. magna*, thus being a good candidate for further biological activity screening, including anticancer and antimicrobial.

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