IN SILICO AND EXPERIMENTAL STUDIES FOR THE DEVELOPMENT OF NOVEL OXAZOL-5(4H)-ONES WITH PHARMACOLOGICAL POTENTIAL

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Abstract

New 2-(4-(4-(X-phenylsulfonyl)phenyl)-4-(3-bromo-3-bromo-4-methoxy-benzylidene)oxazol-5(4H)-ones have been synthesized by reaction of some 2-(4-(4-(X-phenylsulfonyl)benzamido)acetic acids derivatives with 3-bromobenzaldehyde or 3-bromo-4-methoxybenzaldehyde. The structures of the newly obtained heterocycles were elucidated by IR, 1H-NMR, 13C-NMR, mass spectra and elemental analysis. The cytotoxicity of the new oxazolones was evaluated using Artemia salina and Daphnia magna organisms and the in vitro antimicrobial activity was assessed on Gram-positive and Gram-negative bacterial and fungal strains.

Rezumat

Noi 2-(4-(X-fenilsulfonil)fenil)-4-(3-bromo-3-bromo-4-metoxi-benziliden)-oxazol-5(4H)-one au fost sintetizate prin reacția unor derivați ai acidului 2-(4-(4-(X-fenilsulfonil)benzamido)acetic cu 3-bromobenzaldehidă sau 3-bromo-4-metoxybenzaldehidă. Structurile noilor heterocicli au fost elucidate prin spectrometrie în IR, 1H-NMR, 13C-NMR, spectre de măsă și analiză elementară. Citotoxicitatea noilor oxazolone a fost evaluată pe modele experimentale animale reprezentate de Artemia salina and Daphnia magna. Compuși au fost testați in vitro pentru activitatea lor antimicrobiană față de tulpi bacteriene Gram-pozițive și Gram-negative și fungice.

Keywords: oxazol-5(4H)-one, spectral characterization, cytotoxicity, antimicrobial activity

Introduction

The increasing resistance of human pathogenic microorganisms to the great majority of the antibiotics currently available on the market has become an alarming problem at global level. Therefore, considerable efforts are being made worldwide to surpass microbial resistance by developing new generation drugs with increased antimicrobial action and low toxicity [1, 2]. Heterocycles are very important compounds in medicinal chemistry, some of them being used as pharmaceutical ingredients in different drugs with various biological actions [3].

The interest for the heterocyclic compounds with 1,3-oxazolic nucleus increased in the field of medicinal chemistry, both because these are building blocks for the synthesis of many biologically active compounds, but also for their various biological properties such as antibacterial [4-9], antifungal [4, 5, 8, 10], antitubercular [4-6], antitumor [4, 11, 12], anti-inflammatory [4, 13], antiviral [5], antidiabetic [4, 14], anti-obesity [4], etc. The oxazole ring is present in nature, being identified in some natural products with antibiotic or antimicrobial properties [5, 15]. Moreover, the oxazole nucleus and its derivatives are found in the structure of some medicinal compounds, such as: oxaprozin (anti-
inflammatory), mubritinib (antitumoral), aleglitazar (antidiabetic), nilmenidine (antihypertensive), sulfamoxole (antibacterial) [5, 15]. From oxazoles class, 2,4-disubstituted oxazole-5(4H)-ones derivatives are standing out for their biological properties, including: antibacterial [3, 16-19], antifungal [3, 17, 19], antioxidant [17, 20], anti-inflammatory [21] and antitumoral [22, 23] activity.

Regarding the antimicrobial activity, the in vitro study results performed on a series of (4Z)-4-(substituted benzylidene)-2-phenyl-oxazol-5(4H)-ones evaluated against some bacterial cultures (Pseudomonas aeruginosa, Staphylococcus Pyogenes, Klebsiella pneumonia, Methicillin resistant Staphylococcus aureus, Escherichia coli) and fungi (Candida albicans, Aspergillus fumigatus, Penicillium marneffei, Trichophyton mentagrophytes) demonstrated that the compounds have a significant biological activity, depending on the radicals grafted on the benzylidene fragment. Thus, the most active among all compounds in terms of antibacterial as well as antifungal activity, was the derivative containing the methoxy group in the 3rd and 4th positions on the benzylidene moiety, with a MIC value of 12.5 μg/mL, equal with that of standards (ciprofloxacin for bacteria or amphotericin B for fungi), against P. aeruginosa, A. fumigatus and P. marneffei [17].

The results obtained by antibacterial screening of various 4-(substituted benzylidene)-2-(4-substituted phenoxymethyl)-oxazol-5-ones indicated that some of them have antibacterial activity against Escherichia coli and Xanthomonas citri compared to streptomycin used as reference standard. From all compounds, the derivative containing 3-bromophenyl in the molecule stood out for its antibacterial activity against E. coli better than streptomycin, while the compound with the 4-chlorophenyl fragment was more active than the standard against X. citri [16].

Also, new 4-(substituted benzylidene)-2-methyl-oxazol-5(4H)-ones have been tested for their antibacterial activity against some standard or isolated bacteria (Staphylococcus aureus, Bacillus cereus, Streptococcus pneumonia, Enterococcus faecalis, Corynebacterium pyogenes, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumoniae, Escherichia coli, Vibrio cholerae sp.). Some of the tested compounds exhibited a broad antibacterial spectrum for different Gram-positive and Gram-negative bacteria tested comparatively to ampicillin or streptomycin standards. The compounds with methoxy group in 3rd and 4th positions on the benzylidene fragment was found to be the most potent, with better activity than the ampicillin standard drug, against E. coli [18].

Important biological properties, such as antibacterial, antifungal and antitumoral activity have been also reported for the diarylsulfones class, among which, dapsone is well known as an antibacterial and anti-inflammatory agent [24, 25].

In our previous studies we have synthesized and characterized different heterocyclic compounds having a diarylsulfone moiety with the purpose of investigating their biological potential [26-32].

Taking into account all these data, our goal was to synthesize new compounds from the oxazolone class incorporating in their molecule a diarylsulfone fragment in order to obtain new antimicrobial derivatives.

The cytotoxicity of the obtained compounds was evaluated using Artemia salina and Daphnia magna organisms. The antimicrobial activity of the new compounds against bacterial and fungal strains was evaluated using in vitro assays.

Materials and Methods

Chemistry

The melting points of the synthesized compounds were determined using a Boetius apparatus and are uncorrected.

The elemental analysis was registered on a Perkin-Elmer 2400 Series II CHNS/O Elemental Analyzer (Waltham, MA, USA).

The IR spectra were recorded, in KBr pellets, on a Bruker Vertex 70 spectrometer and the IR absorption bands intensity are given as: very strong (vs), strong (s), medium (m), and weak (w).

The 1H-NMR and 13C-NMR spectra were recorded in dimethylsulfoxide (DMSO-d6) on a Varian Gemini 300BB spectrometer operating at 300 MHz for 1H-NMR and at 75 MHz for 13C-NMR. The chemical shifts δ are reported in parts per million (ppm) with tetramethylsilane (TMS) as internal standard and the coupling constants J in Hertz (Hz). The multiplicities were abbreviated as following: s (singlet), d (doublet), dd (double doublets), t (triplet), br (broad). The mass spectra were registered on an APCI mass spectrometer. The compounds solutions in chloroform (0.5 mg/mL) were diluted 10 times with methanol (1% formic acid), to obtain protonated molecular ions [M+H]+. 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 μL/min was used to drive the solutions. The protonated molecular ions were fragmented into the argon collision cell at 1.5 eV.

General procedure for the synthesis of 2-(4-(4-X-phenylsulfonyl)phenyl)-4-(3-bromo/o3-bromo-4-methoxybenzylidene)oxazol-5(4H)-ones 5a-c

An equimolecular mixture of N-acetylated glycine 3a-c (10 mmol) and corresponding aromatic aldehyde (10 mmol) in freshly-distilled acetic anhydride (19 mL) containing fused sodium acetate (0.82 g) was refluxed with continuous stirring for 4 h. To the obtained solution, 2 mL of ethanol was added and allowed it to stand overnight at cold. The solid mass was filtered off, washed with boiling water and cold ethanol. The
crude product was recrystallized from an ethanol-chloroform mixture (1:2, v/v) when a yellow crystalline product was obtained.

4-(3-Bromobenzylidene)-2-(4-(phenylsulfonyl)-phenyl)oxazol-5(4H)-one 4a
m.p. 207 - 209°C; yield 59%; FT-IR (KBr, ν cm⁻¹): 3091m, 3063m, 1805vs, 1665vs, 1460s, 1449s, 1324vs, 1294vs, 1195m, 852s, 603vs, 565s; ¹H-NMR (DMSO-d₆, δ ppm, J Hz): 8.47 (brs, 1H, H-20), 8.32 (d, 7.2, 1H, H-24), 8.30 (d, 8.2, 2H, H-7, H-11), 8.19 (d, 8.2, 2H, H-8, H-10), 8.01 (brd, 7.7, 2H, H-13, H-17), 7.74 (brt, 7.7, 1H, H-15), 7.71 (d, 7.9, 1H, H-22), 7.66 (t, 7.2, 2H, H-14, H-16), 7.50 (t, 7.9, 1H, H-23), 7.44 (s, 1H, H-18); ¹³C-NMR (DMSO-d₆, δ ppm): 166.18 (C-5), 162.38 (C-2), 144.42 (C-9), 139.41 (C-12), 134.42 (C-20), 134.19 (C-19), 134.00 (C-22), 134.32 (C-4), 133.10 (C-23), 133.09 (C-14, C-16), 131.50 (C-24), 130.35 (C-18), 129.64 (7-C, C-11), 129.35 (C-6), 128.52 (C-15), 128.38 (C-13, C-17), 128.31 (C-8, C-10), 122.16 (C-21); Anal. (%): Calcd. for C₂₃H₂₂BrN₂O₅S: 547.22 g/mol; C, 59.11; H, 3.92; N, 3.57. Found: C, 59.06; H, 3.96; N, 3.56. 

4-(3-Bromo-4-methoxybenzylidene)-2-(4-(phenylsulfonyl)phenyl)oxazol-5(4H)-one 5a
m.p. 274 - 276°C; yield 79%; FT-IR (KBr, ν cm⁻¹): 3090w, 3067w, 3024w, 2940w, 2839w, 1793s, 1653s, 1593s, 1557m, 1496s, 1384m, 1290m, 1276w, 1223m, 1164vs, 1051s, 864m, 605vs, 568s; ¹H-NMR (DMSO-d₆, δ ppm, J Hz): 8.57 (brs, 1H, H-20), 8.32 (d, 8.8, 1H, H-24), 8.26 (d, 8.5, 2H, H-7, H-11), 8.20 (d, 8.5, 1H, H-8, H-10), 8.02 (brd, 7.9, 2H, H-13, H-17), 7.74 (brt, 6.6, 1H, H-15), 7.66 (t, 8.2, 2H, H-14, H-16), 7.41 (s, 1H, H-18), 7.29 (d, 8.8, 2H, H-23), 3.96 (OCH₃); ¹³C-NMR (DMSO-d₆, δ ppm): 166.18 (C-5), 161.00 (C-2), 157.80 (C-22), 144.01 (C-9), 139.20 (C-12), 136.40 (C-20), 134.50 (C-15), 134.20 (C-24), 132.80 (C-14, C-16), 131.30 (C-4), 131.00 (C-18), 129.70 (C-7, C-11), 129.40 (C-10), 128.40 (C-19), 128.10 (C-6), 127.15 (C-13, C-17), 112.90 (C-23), 111.00 (C-21), 55.60 (OCH₃); Anal. (%): Calcd. for C₂₃H₂₁BrN₂O₅S: 498.35 g/mol; C, 55.43; H, 3.24; N, 2.81; S, 6.43. Found: C, 55.10; H, 3.41; N, 3.04; S, 6.67; APCl, m/z (%): 498 [¹¹BrM+H]⁺, 500 [¹¹BrM+H]⁺, 245 [C₄H₆SO₂CH₃CO]⁻, 125 [C₆H₅SO]⁻.
4-(3-Bromo-4-methoxybenzylidene)-2-(4-(4-bromo-phenylsulfonyl)phenyl)-oxazol-5(4H)-one 5e

m.p. 285 - 287°C; yield 59%; FT-IR (KBr, v cm⁻¹): 3093m, 3035w, 2943w, 2841w, 1770s, 1649vs, 1592s, 1576s, 1541m, 1491s, 1330s, 1290m, 1275vs, 1222m, 1164vs, 1051s, 857m, 576m; ¹H-NMR (DMSO-d₆, δ ppm, J Hz): 8.58 (d, 2.2, 1H, H-20), 8.32 (dd, 8.5; 2.2, 1H, H-24), 8.27 (d, 8.8, 2H, H-7, H-11), 8.20 (d, 8.8, 2H, H-8, H-10), 7.95 (d, 9.0, 2H, H-13, H-17), 7.88 (d, 9.0, 2H, H-14, H-16), 7.42 (s, 1H, H-18), 7.29 (t, 8.8, 2H, H-23), 3.95 (OCH₃); ¹³C-NMR (DMSO-d₆, δ ppm): 166.28 (C-5), 161.03 (C-2), 157.85 (C-22), 144.00 (C-9), 139.38 (C-12), 136.54 (C-20), 134.28 (C-24), 132.99 (C-14, C-16), 131.39 (C-4), 131.09 (C-18), 129.86 (C-8, C-10), 129.53 (C-7, C-11), 128.92 (C-19), 128.42 (C-15), 128.14 (C-6), 127.23 (C-13, C-17), 112.99 (C-23), 111.19 (C-21), 55.90 (OCH₃); Anal. (%): Calcld. for C₂₀H₁₂Br₂N₀₂S (577.24 g/mol): C, 47.86; H, 2.62; N, 2.43; S, 5.55. Found: C, 47.75; H, 2.83; N, 2.73; S, 5.70; APCI, m/z (%): 576 [⁴¹Br₂Br⁺, Br⁺ M+H⁺], 578 [⁴¹Br₂Br⁺, Br⁺ M+H⁺], 580 [⁴¹Br₂Br⁺, Br⁺ M+H⁺], 323 [⁴¹BrC₆H₅SO₂C₆H₄CO⁺], 325 [⁴¹BrC₆H₄SO₂C₆H₄CO⁺], 203 [³⁹BrC₆H₅SO⁺], 205 [³⁹BrC₆H₄SO⁺].

**Cytotoxicity bioassay**

Both experiments were carried out in a controlled environment using a climatic chamber Sanyo MLR-351H; Sanyo, San Diego, CA, USA (25 ± 1°C, in the dark). Both animal species are widely used in the screening of newly synthesized compounds. These methods are alternatives to the use of vertebrates due to several advantages, among which we mention rapidity, simplicity, reproducibility and cost-efficiency [33-35].

**Artemia salina** bioassay

The biotest was conducted according to the method described by Meyer et al. [35] with some modifications [36]. Brine shrimp cysts were incubated in artificial sea water (40 g/L salinity) for 24 h under continuous aeration. The nauplii were separated from the shells, transferred to fresh sea water and incubated for another 24 h. Each determination was carried out in duplicate in polypropylene (PP) tissue culture with 24-wells (Greiner Bio-One) using 10 organisms in each well in a final volume of 1 mL/sample. A 1% DMSO solution was used as negative control. Each compound was tested at seven different concentrations in the range 5 - 250 μg/mL. The lethality (L%) was recorded over a 48 h period of exposure and 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 (C.I.95%) of LC₅₀ were also calculated using the same method. All the calculations were performed using the software GraphPad Prism v 5.1.

**Daphnia magna bioassay**

*Daphnia magna* bioassay was performed according to the method described in our previous research [37]. Each determination was carried out in duplicate, PP tissue culture wells (Greiner Bio-One) using 10 organisms in each well at a final volume of 4 mL/sample and a DMSO of 1% solution was used as negative control. The compounds were tested at seven concentrations ranging from 1.5 to 104 μg/mL. The concentrations range differs from *A. salina* bioassay and was established taking into account the higher susceptibility of *D. magna* compared to *A. salina* and a pre-screening test. The lethality (L%) was recorded over a 48 h period of exposure at two points: 24 and 48 h. All calculations were performed using as described above. The prediction of LC₅₀₉₅ was performed using the GUSAR software application.

**Antimicrobial activity assay against planktonic and biofilm embedded cells**

The antimicrobial activity of the new compounds was investigated using a broth micro-dilution assay. Serial two-fold dilutions of the samples solubilized in DMSO were done in 96 well plates in Nutrient Broth. Microbial suspensions of Gram-negative (*Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 27853) and Gram-positive bacteria (*Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 6538) as well as *Candida parapsilosis* ATCC22019 with a density of 10⁵ CFU/mL were prepared from 24 h solid cultures. The microbial suspensions were subsequently inoculated on each microtiter well containing the two-fold dilutions of the tested compounds. A sterility control was added with 100 µL broth. Ticarcillin antibiotic and fluconazole antifungal agents were used as positive controls. The experiments were done in triplicate. The wells were incubated for 18 - 24 h in aerobic conditions, at 37°C. After incubation, the minimum inhibitory concentration (MIC) value was determined spectrophotometrically at 620 nm.

To investigate the influence of the tested compounds on the ability of the tested microbial strains to colonize the inert substratum, a microtiter plate method was used. The microplates used for the MIC assay were emptied and further washed three times with phosphate buffered saline. The biofilm formed on the plastic wells wall was fixed for 5 min with ice-cold methanol, coloured by violet crystal solution (15 min) and re-suspended in 33% acetic acid solution. The microbial cell density was measured by reading the optical density of the coloured solution at 490 nm. The minimal biofilm eradication concentration (MBEC) values were considered as the lowest concentration of the tested compounds that inhibited the development of biofilm on the plate wells [38].

**Docking study on 50S ribosomal subunit of S. aureus**

Local docking for the synthesised compounds 4a-c and 5a-c was performed using PyRx 0.8 virtual screening
software. Linezolid was chosen as a control ligand, due to the preference for the same molecular target. 3D coordinates generation and ligand preparation was done using OpenBabel v2.4.1. X-ray diffraction structure of the ribosomal 50S subunit was downloaded from Protein Data Bank (PDB ID: 4WFA), and protein preparation was performed using Chimera v1.12 software. Default-sized gridbox was chosen around binding site of linezolid in 4WFA crystal and local docking was performed with AutoDock Vina algorithm. The free binding energies corresponding to the first nine conformations generated for each ligand were retrieved, from which the first valid one for each compound was considered as result. The 2D and 3D graphic representations were obtained with Biovia Discovery Studio Visualizer v17.2.0.16349 software. The binding site analysis of the docked poses was performed for the six compounds in order to predict some possible protein-ligand interactions that could favour the inhibition of the 50S ribosomal unit of the *S. aureus* [39].

**Results and Discussion**

**Chemistry**

The synthesis of the target compounds is outlined in Figure 1. The 2-(4-(4-X-phenylsulfonyl)benzamido)-acetic acids intermediates were prepared according to procedures described in previous works [40, 41]. The new 2-(4-(4-X-phenylsulfonyl)phenyl)-4-(3-bromo/3-bromo-4-methoxy-benzylidene)oxazol-5(4H)-ones 4,5a-c were obtained by reaction of 2-(4-(4-X-phenylsulfonyl)benzamido)acetic acids 3 with 3-bromobenzaldehyde or 3-bromo-4-methoxybenzaldehyde, at reflux, in presence of sodium acetate, using acetic anhydride as reaction medium. The (4-(4-X-phenylsulfonyl)benzoic acids 1 were treated with thionyl chloride, at reflux, in order to obtain the 4-(4-X-phenylsulfonyl)benzoyl chlorides 2. The corresponding chlorides were treated with glycine according to Steiger’s procedure [40], leading to 2-(4-(4-X-phenylsulfonyl)benzamido)acetic acids 3. The structures of the title compounds were elucidated on the basis of $^1$H-NMR, $^{13}$C-NMR, IR, mass spectra and elemental analysis. The $^1$H-NMR spectra of new compounds 4a-c - 5a-c contain a singlet signal at δ 7.41 - 7.45 ppm corresponding to =CH benzylidene group (H-18), confirming the formation of benzylidene-oxazol-5(4H)-ones by condensation of N-acylated glycine with the corresponding substituted benzaldehydes. Also, as expected, the $^1$H-NMR spectra of these new compounds revealed the disappearance of NH proton from the N-acylated glycine derivatives intermediates [40, 41]. In the case of the derivatives 5a-c, the protons of the OCH$_3$ group appeared as singlet at 3.95 for 5c and 3.96 ppm for 5a,b.

The 13C-NMR spectra of the new compounds have shown the characteristic signal of benzylidene-oxazol-5(4H)-ones. Thus, the C=O resonated at 166.11 - 166.28 ppm, the carbon from the 2nd position at 161.00 - 162.38 ppm and that from 4th position of oxazole nucleus at 131.29 - 133.99 ppm. Also, the benzylidene carbon (C-18), signal appeared at 130.21 - 131.09 ppm. The compounds 5a-c exhibited the
characteristic signal of methoxy carbon at 55.60 - 55.90 ppm.

In the IR spectra of oxazolones, the disappearance of NH stretching vibration from N-acylated glycine [40, 41] confirmed the cyclization reaction. The C=O stretching vibrations bands appeared in the IR spectra of oxazolones in the 1768 - 1829 cm⁻¹ region.

The mass spectra of the compounds 4a-c and 5a-c indicated the molecular ions (containing the ⁷⁹Br/⁸¹Br isotopes and the ³⁵Cl/³⁷Cl isotopes only in case of 4b and 5b) corresponding to the molecular mass which, together with the most important fragments presented at the characterization of the compounds, confirmed the expected structures.

**Biological evaluation**

**Cytotoxicity bioassay**

The cytotoxicity tests indicated that, after a 24 h of exposure, the L% induced in both *A. salina* and *D. magna*, were between 0 and 30% for all but the highest tested concentrations.

The cytotoxicity tests indicated that, after a 24 h of exposure, the L% induced at both, *A. salina* and *D. magna*, were between 0 and 30% at all levels of concentration, except the highest concentration. At the highest concentration level, the L% was between 10 and 80%. However, the data obtained at 24h did not allow the calculation of LC50. The results for the 48 h determination are presented in Table I and Figure 2.

**Table I**

| Tested compound | A. salina | D. magna |
|-----------------|-----------|----------|
|                 | LC50₄₈ₜₜ (µg/mL) | 95% CI of LC50₄₈ₜₜ (µg/mL) | Predicted LC50₄₈ₜₜ (µg/mL) | LC50₄₈ₜₜ (µg/mL) | 95% CI of LC50₄₈ₜₜ (µg/mL) |
| 4a              | 291.7     | 27.6 - 1477 | 0.215 | 23.69 | 13.13 - 42.75 |
| 4b              | 28.92     | 12.74 - 65.63 | 0.291 | NC*   | NC*           |
| 4c              | 21.84     | 9.24 - 51.6  | 0.136 | 40.24 | NC*           |
| 5a              | 372.9     | NC*         | 0.059 | 26.98 | NC*           |
| 5b              | 51.6      | 25.64 - 103.9 | 0.032 | NC*   | NC*           |
| 5c              | 229.3     | NC*         | 0.023 | 72.12 | NC*           |

LC50 - 50% lethal concentration; 95% CI - 95% confidence interval; NC* - LC50 and 95% CI couldn't be calculated due to the obtained results.

**Figure 2.**

*A. salina* (1) and *D. magna* (2) lethality curves at 48 h for some representative compounds. a - 4a, b - 4c, c - 5a, d - 5c.
All tested compounds exhibited toxicity on *A. salina*, the most toxic ones (LC50 value below or equal to 50 µg/mL) being: 4c, 4b and 5b. The compound 5c (LC50 < 250 µg/mL) exhibited moderate toxicity, whereas 4a and 5a exhibited a moderate to low toxicity level, the estimated LC50 being over 250 µg/mL. For these compounds the 95% CI was very wide or could not be calculated. The obtained LC50 values were spread over a wide range. The obtained results using the *D. magna* assay were different from those obtained on *A. salina*, due to the differences between the two species on invertebrates. Oxazolones 4a followed by 5a and 4c were the most toxic on *D. magna*, with a LC50 value below 50 µg/mL. The derivative 5c exhibited moderate to high toxicity, whereas 4b and 5b were practically non-toxic to daphnids, the highest 1% induced being of 40%. The predicted LC50 values using the GUSAR software application were significantly lower than the experimental ones. All predicted values are below 1 µg/mL and suggest that the newly synthesized compounds are all very toxic for daphnids. A possible explanation for the differences between the predicted and the experimental values could be the biotransformation of the compounds.

**Antimicrobial activity assay**

The antimicrobial activity of the novel compounds was tested against reference microbial strains of *S. aureus*, *E. faecalis*, *E. coli*, *P. aeruginosa* and *C. parapsilosis*. The results of the quantitative assay of the antimicrobial activity of the tested compounds are shown in Table II. The quantitative assay revealed that all tested compounds exhibited MIC values in the range of 0.625 - 1.25 mg/mL. The most susceptible microbial strain proved to be *C. parapsilosis*, all tested compounds exhibiting the same MIC value (0.625 mg/mL) against this fungal strain, followed by *E. faecalis* (MIC value of 0.625 mg/mL) against this fungal strain, followed by *E. faecalis* (MIC value of 0.625 mg/mL) against *S. aureus*, *E. coli* and *P. aeruginosa*. The compounds 5a and 5b proved to be the most active, exhibiting lower MIC values against three of the five tested strains, i.e. *E. coli* (Gram-negative bacterial strain), *E. faecalis* (Gram-positive bacterial strain) and *C. parapsilosis* (fungal strain).

### Table II

| Tested compound | *P. aeruginosa* | *S. aureus* | *E. coli* | *E. faecalis* | *C. parapsilosis* |
|-----------------|-----------------|-------------|-----------|---------------|------------------|
| 4a              | 1.25            | 1.25        | 1.25      | 0.625         | 0.625            |
| 4b              | 1.25            | 1.25        | 1.25      | 0.625         | 0.625            |
| 4c              | 1.25            | 1.25        | 1.25      | 0.625         | 0.625            |
| 5a              | 1.25            | 1.25        | 0.625     | 0.625         | 0.625            |
| 5b              | 1.25            | 1.25        | 0.625     | 0.625         | 0.625            |
| 5c              | 1.25            | 1.25        | 0.625     | 0.625         | 0.625            |
| Ticarcillin     | 0.032           | 0.008       | 0.016     | 0.064         | -                |
| Fluconazole     | -               | -           | -         | -             | 0.008            |

Most infections are characterized by biofilm development on natural, intact or damaged tissues as well as on artificial medical devices accompanied by chronic evolution, middle intensity symptoms, and unfortunately resistance to antimicrobial compounds. Hence, tremendous research efforts are made in order to develop new chemical agents acting as inhibitors of biofilm formation that target biofilm cells connection, rendering the microorganisms susceptible to antibiotics [42-45]. To test the antibiofilm activity of the newly synthesized compounds, we used mini volumes and multiple well plastic plates, allowing the simultaneous testing of a large spectrum of concentrations. All compounds exhibited MBEC values ranging from 0.625 to 1.25 mg/mL (Table III).

### Table III

| Tested compound | *P. aeruginosa* | *S. aureus* | *E. coli* | *E. faecalis* | *C. parapsilosis* |
|-----------------|-----------------|-------------|-----------|---------------|------------------|
| 4a              | 1.25            | 1.25        | 1.25      | 1.25          | 0.625            |
| 4b              | 1.25            | 1.25        | 1.25      | 1.25          | 0.625            |
| 4c              | 1.25            | 1.25        | 1.25      | 1.25          | 0.625            |
| 5a              | 1.25            | 1.25        | 1.25      | 1.25          | 0.625            |
| 5b              | 1.25            | 1.25        | 1.25      | 1.25          | 0.625            |
| 5c              | 1.25            | 1.25        | 1.25      | 1.25          | 0.625            |
| Ticarcillin     | 1.024           | 1.024       | 1.024     | 2.048         | -                |
| Fluconazole     | -               | -           | -         | -             | 0.512            |

The compounds 4a, 4b, 5a, 5b and 5c exhibited a MBEC of 0.625 mg/mL against *C. parapsilosis*, confirming the best susceptibility of this fungal strain to the majority of the tested compounds. Regarding the tested Gram positive and Gram negative bacterial strains, the MBEC values of the tested compounds were similar to the corresponding MIC values or two times higher. Taking into account that the microbial biofilms are
much more resistant to antimicrobial agents, as compared to their planktonic counterparts, the MBEC values being up to 1000 times higher than the MIC values [43], our results demonstrate the good antibiofilm potential of the obtained compounds. If in the case of planktonic cells, the tested compounds exhibited MIC values much higher than the control antibiotic and antifungal agent respectively, in case of biofilm cells, the activity of the tested compounds was similar to that of the currently used antimicrobial agents.

**Docking studies**

The docking simulations generated binding poses for the 6 compounds in the same binding site as for linezolid. The affinity of the control for the binding site was due to alkyl and Pi-Alkyl interactions with ALA157 residue, van der Waals interactions with SER153, GLY155 and SER158, all stabilised through a H-bond realised with the MET157 residue. All of the compounds displayed interactions with ALA157 residues, while 4a, 4b and 4c also formed hydrogen bonds with SER158 residues. Binding affinities, presented in Table IV, were similar with the control for all of the docked compounds, with the highest being for 5a and the lowest for 4b and 5b. The similar binding affinities for all tested compounds are in agreement with the identical MIC values obtained on S. aureus.

**Table IV**

| Ligand   | Mode (docking pose) | Binding Affinity (Kcal/mol) | rmsd/upper bound | rmsd/lower bound |
|----------|----------------------|-----------------------------|------------------|------------------|
| LZD      | 0                    | -2.0                        | 0.00             | 0.00             |
| 4a       | 0                    | -2.0                        | 0.00             | 0.00             |
| 4b       | 0                    | -1.8                        | 0.00             | 0.00             |
| 4c       | 0                    | -2.0                        | 0.00             | 0.00             |
| 5a       | 1                    | -2.1                        | 11.27            | 4.84             |
| 5b       | 1                    | -1.8                        | 5.06             | 4.13             |
| 5c       | 0                    | -2.0                        | 0.00             | 0.00             |

Figure 3 presents the binding conformation of linezolid (control), highlighting the hydrophobicity surface area around the ligand and dashes represent interactions with the aminoacid residues. The pink dashes represent Pi-Alkyl and Alkyl interactions, green dashes represent hydrogen bonds and light green residues form van der Waals interactions with the ligand.

**Figure 3.**

a - 3D binding conformation of linezolid (control), b - 2D diagram of protein-ligand interactions between linezolid and the 50S ribosomal subunit of S. aureus

**Figure 4.**

The 3D conformation of the docking pose of 5a

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Figure 4 presents the binding conformation of compound 5a, the compound with the highest binding affinity towards the functional site 50S ribosomal subunit of S. aureus.

Conclusions

A series of six oxazole-5(4H)-ones derivatives containing arylsulfonylphenyl and 3-bromo/3-bromo-4-methoxybenzylidene fragments in their molecules were synthesized, characterized and their toxicity and antimicrobial activities were evaluated. The antimicrobial assays indicated that the new oxazolones, and particularly 5a and 5b compounds, have a good antifungal and anti-biofilm potential. Moreover, all tested compounds showed moderate to high toxicity on at least one invertebrate species, being good candidates for further biological screening tests of their anticancer properties.

Conflict of interest

The authors declare no conflict of interest.

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