

SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW N-(2-DIMETHYLAMINOETHYL)-N-((UN)SUBSTITUTED PHENYL)-2-(4-METHYL/METHOXY-PHENOXYMETHYL)BENZAMIDES

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Abstract

New N-(2-dimethylaminoethyl)-N-((un)substituted phenyl)-2-(4-methyl/methoxy-phenoxy-methyl)benzamide hydrochlorides were designed and synthesized from 2-(4-methyl/methoxy-phenoxy-methyl)benzoyl chloride and N-(2-dimethylaminoethyl)-((un)substituted anilines, the resulted benzamides being treated with ethereal hydrochloric acid to obtain the corresponding hydrochlorides. The new compounds have been characterized by their physical constants such as melting point and solubility. The structures of the synthesized compounds were elucidated on the basis of their elemental analysis and spectroscopic data (IR, ¹H-NMR and ¹³C-NMR). The chemical structures of all compounds and intermediaries were in full agreement with the proposed structures. The new benzamides were screened for their *in vitro* antimicrobial and anti-biofilm activities by quantitative assays, allowing to establish the minimum inhibitory concentration and minimal biofilm eradication concentration parameters. Compounds bearing halogenated moieties proved to be the most efficient. They showed increased activity against Gram-positive bacteria, but the activity also extends to Gram-negative bacteria and yeasts. These structures represent promising scaffolds for further development of anti-infective agents.

Rezumat

Noi clorhidrați ai N-(2-dimetilaminoetil)-N-fenil (ne)substituit-2-(4-metil/metoxi-fenoximetil)benzamidelor, au fost proiectați și sintetizați folosind clorură de 2-(4-metil/metoxi-fenoximetil)benzoil și N-(2-dimetilaminoetil)aniline (ne)substituite, benzamidele rezultate fiind apoi tratate cu acid clorhidric în eter etilic pentru a obține clorhidrații corespunzători. Noii compuși au fost caracterizați cu ajutorul constantelor fizice și chimice, cum ar fi temperatura de topire și solubilitatea. Structura acestora a fost determinată pe baza analizei elementale și a datelor spectrale (IR, ¹H-NMR și ¹³C-NMR). Structurile chimice ale tuturor compușilor și intermediarilor au fost în deplină concordanță cu structurile propuse. Noile benzamide au fost testate *in vitro* pentru activitățile lor antimicrobiene și anti-biofilm, prin analize cantitative, permițând stabilirea concentrației minime inhibitorii și a concentrației minime de eradicare a biofilmului. Compușii substituiți cu halogeni s-au dovedit a fi cei mai eficienți. Ei au avut o activitate crescută pe bacterii Gram-pozitive, dar activitatea este extinsă și la bacterii Gram-negative și fungi. Acești compuși reprezintă structuri promițătoare pentru dezvoltarea viitoare de noi agenți antimicrobieni.

Keywords: new benzamides, ¹H-NMR, ¹³C-NMR, IR spectroscopy, antimicrobial activity, anti-biofilm activity

Introduction

The infectious diseases are increasingly affecting the human health at global level and one of the main challenges for the pharmaceutical industry is the synthesis of new anti-infective agents, efficient against viral, bacterial, fungal and parasitic infectious agents, which continuously develop resistance to all current antimicrobials.

Benzamides are well known for their diverse pharmacological effects, such as antimicrobial, anti-parasitic, local anaesthetic, antiarrhythmic, analgesic,

antipsychotic and antidepressant effects. New benzamides have been reported to show anti-infective features, including a potent activity against resistant microorganisms.

The antimicrobial and antifungal activity of the new benzamides was modelled using various descriptors, noticing the predictive ability of the developed QSAR models [5].

The N-((2-hydroxynaphthalen-1-yl)(substituted phenyl)methyl)-4-morpholinobenzamide derivatives can be developed as potent chemotherapeutic agents.

The study of structure-activity relationship showed that the morpholine and β -naphthol moieties are essential for their antibacterial activity. Also the electron donating groups on aromatic ring increase the antimicrobial activity. The correlation of the activity with the structure of newly synthesized derivatives revealed that amide linkage is essential for the antitubercular activity of these molecules [16, 17].

It was also evaluated the anti-*Helicobacter pylori* activity of N-methyl-3-[2-(2-naphtyl)acetylamino]-benzamide and the ability of *H. pylori* to acquire *in vitro* resistance to it. The activity can overcome the drawbacks of the present triple combination of a proton pump inhibitor and two antimicrobial agents favourable for treatment of peptic ulcers and active chronic gastritis [2].

Some substituted N-(2-hydroxy-4-nitrophenyl)benzamides and phenylacetamides, as possible metabolites of active antimicrobial benzoxazoles, have been tested for their antimicrobial activity and the obtained results suggest that they could be expected to give a prolonged antimicrobial activity [20].

New N-[3-(2-oxo-1,2-dihydro-indol-3-ylidenehydrazinocarbonyl)benzyl]benzamides were tested for the *in vitro* antimicrobial activity and the study revealed that the compounds having halogens in the 5th position were the most active [13].

N-[5-(4-methylphenyl)-diazeryl-4-phenyl-1,3-thiazol-2-yl]benzamide derivatives can be used for the development of new drugs used in the treatment of bacterial and fungal diseases [15].

In order to obtain new antimicrobial agents, new 2-aminobenzamide derivatives were synthesized and tested for their biological effect. All compounds showed good activity against the tested strains of bacteria and fungi [10].

We designed a new molecular structure and the preliminary studies revealed that they could act as inhibitors of two bacterial components from the regulatory system, thus interfering with the most diverse process regulated by them [8]. The benzamides can also exhibit biocidal activity at relatively low concentrations. Their mechanism of action appears to be the impairment of the selective permeability of the cytoplasmic membrane by disrupting the proton forces and subsequently, the membrane functions such as the active transport and energy metabolism [12, 19].

Another suggested mechanism of action of antimicrobial benzamides is that they could act as inhibitors of two main bacterial regulatory systems, thus interfering with the very diverse processed regulated by them [1, 11].

On the other hand, it was proven that N-(4-amino-2-methylquinolin-6-yl)-2-(4-ethylphenoxyethyl)-benzamide hydrochloride is a nociceptin-induced allodynia antagonist in mice and also an antalgic in

a hot-plate test using mice and in a formalin test using rats. These results suggest that this compound can be a promising novel type of analgesic that differs from μ -opioid agonists [18].

Taking into account that bacterial infections are often accompanied by pain and inflammation, the availability of drugs exhibiting simultaneously antimicrobial, analgesic and anti-inflammatory effects could represent a significant advantage for their therapeutic management.

In view of the development of new molecules, we report in this paper the design, synthesis, and the biological activity evaluation of some new benzamides, as promising anti-infective agents.

Materials and Methods

Chemistry

All chemicals and solvents were purchased from Merck or Aldrich (Darmstadt and Steinheim, Germany). Acetone and 1,2-dichloroethane were dried over calcium chloride. Ammonium thiocyanate was dried by heating at 100°C and then used in the reactions. Toluene was dried stored over sodium and then distilled. Anhydrous sodium carbonate was prepared by calcination and triethylamine was distilled after drying on potassium hydroxide.

The melting points were determined on an Electro-thermal 9100 capillary melting point apparatus (Bibby Scientific Ltd, Stone, UK) in open capillary tubes and the data are not corrected.

The IR spectra were recorded on a Bruker Vertex 70 FT-IR spectrometer (Bruker Corporation, Billerica, MA, USA).

The ¹H-NMR and ¹³C-NMR spectra were recorded in deuterated chloroform (CDCl₃) on a Varian Gemini 300BB instrument (Varian Medical Systems, Palo Alto, CA, USA) operating at 300.0 MHz for proton (¹H)-NMR, and 75.0 MHz for carbon-13 (¹³C)-NMR.

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

The synthesis of N-(2-dimethylaminoethyl)-(un)-substituted anilines (4a-c)

A mixture of 0.15 mol N-(2-chloroethyl)-N,N-dimethylamine hydrochloride, 0.225 mol of dry sodium carbonate in 200 mL anhydrous toluene was refluxed for 3 h with continuous stirring. 0.225 mol of corresponding anilines (aniline, 3-chloroaniline or 3-(trifluoromethyl)aniline) in 50 mL toluene were slowly added and then the mixture was refluxed 50 h. 0.225 mol of 5 M sodium hydroxide solution were added and the organic layer was separated and dried using sodium sulphate anhydrous and then distilled, resulting an yellow oil (N-(2-dimethylaminoethyl)aniline with b.p. 124°C (6 mmHg), 53% yield; N-(2-dimethylaminoethyl)-3-chloroaniline with

b.p. 134 - 138°C (5 mmHg), 62% yield; N-(2-dimethylaminoethyl)-3-(trifluoromethyl)aniline with b.p. 142 - 144°C (8 mmHg), 48% yield).

The synthesis of N-(2-dimethylaminoethyl)-N-((un)-substituted phenyl-2-(4-methyl/methoxy-phenoxy-methyl)benzamide hydrochlorides (1a-d)

To a stirred solution of 0.025 mol N-(2-dimethylaminoethyl)-(un)substituted aniline and 0.075 mol triethylamine in 40 mL dry toluene was added 0.0275 mol of 2-(4-methyl/methoxy-phenoxy-methyl)-benzoic acid chloride. The resulting mixture was refluxed for 8 h. After removing the triethylamine hydrochloride, the toluene was evaporated *in vacuum* with a rotary evaporator, the residue was taken up in chloroform and it was washed with 10% sodium carbonate solution and then with water. The chloroform solution was dried using anhydrous sodium sulphate and then the solvent was evaporated. The resulting substances were brown-yellow oils, and were purified by transformation in the corresponding hydrochlorides. Thus a solution of N-(2-dimethylaminoethyl)-N-((un)substituted phenyl-2-(4-methyl/methoxy-phenoxy-methyl) benzamide in 10 mL anhydrous ether was treated at 5°C with a saturated solution of hydrochloric acid in anhydrous ether which was dropwise added. The final hydrochlorides are white crystals from ethyl acetate.

Microbiological assays

Microbial strains. The antimicrobial activity of the investigated compounds was tested against the following bacterial strains: Gram-positive, i.e.: *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 6538, *Staphylococcus saprophyticus* ATCC 15305, vancomycin –resistant *Enterococcus faecalis* ATCC VA-R, *Bacillus subtilis* ATCC 6633 and Gram-negative, i.e.: *E. coli* ATCC 29252, *Klebsiella pneumoniae* ATCC 40, *Acinetobacter baumannii* ATCC 230 as well as yeasts, i.e.: *Cryptococcus neoformans* ATCC 204092. Bacterial suspensions of 0.5 McFarland density obtained from 18 hours bacterial cultures and 1 McFarland for yeasts developed on solid media were used in the experiments. The antimicrobial activity of the new compounds was tested on Mueller-Hinton agar medium.

Stock solutions. The tested compounds were solubilised in water and used for the antimicrobial activity screening at 10 mg/mL concentration.

Qualitative screening of the antimicrobial properties of the tested compounds. The qualitative screening was performed by an adapted disk diffusion method. For this purpose, Petri dishes with Mueller-Hinton medium were seeded with microbial standardized inoculum as for the classical antibiotic susceptibility testing (Kirby-Bauer method); then a volume of 5 µL were distributed on the seeded medium, at 30 mm distance. The plates were left at room temperature for 20 - 30 minutes and then incubated at 37°C for

24 hours. The positive results were read as the occurrence of an inhibition zone of microbial growth around the disk [14].

Quantitative assay of the antimicrobial activity. It was performed by the binary microdilution method, in Mueller Hinton broth (MHB) distributed in 96 multi-well plates, in order to establish the minimum inhibitory concentration (MIC). For this purpose, serial binary dilutions of the tested compounds were performed in a total volume of 200 µL volume of Mueller Hinton Broth (MHB) (from 1000 µg/mL to 7.8 µg/mL). Each well was seeded with 20 µL of microbial inoculum of 0.5/1 MacFarland density for bacterial/fungal strains. The plates were incubated for 24 hrs at 37°C, and MICs were read as the last concentration of the compound which inhibited the microbial growth, as compared to the positive control, represented by the MHB inoculated with the microbial suspensions.

Anti-biofilm activity assay. The anti-biofilm activity of the tested compounds was tested by the microtiter method. For this purpose, the microbial strains were grown in the presence of two-fold serial dilutions of the tested compounds performed in liquid nutrient broth/YPG, distributed in 96-well plates and incubated for 24 hours at 37°C for bacterial strains, respectively for 48 hours at 28°C for fungal strains. At the end of the incubation period, the plastic wells were emptied, washed three times with phosphate buffered saline (PBS), fixed with cold methanol and stained with 1% violet crystal solution for 30 minutes. The biofilm formed on the plastic wells was resuspended in 30% acetic acid. The intensity of the coloured suspensions was assessed by measuring the optical density at 490 nm. The last concentration of the tested compound that inhibited the development of microbial biofilm on the plastic wells was considered the minimum biofilm eradication concentration (MBEC), being also expressed in µg/mL [4, 5].

Results and Discussion

Chemistry

2-(4-Methyl-phenoxy-methyl)benzoic acid, 2-(4-methoxy-phenoxy-methyl)-benzoic acid and their acid chlorides were prepared according to procedures described before [6, 7, 9].

Synthesis of new N-(2-dimethylaminoethyl)-N-((un)-substituted phenyl-2-(4-methyl/methoxy-phenoxy-methyl)benzamide hydrochlorides (1a-d) (Figure 1) The new N-(2-dimethylaminoethyl)-N-((un)substituted phenyl-2-(4-methyl/methoxy-phenoxy-methyl)benz-amides (**2a-d**) were obtained in the reaction of 2-(4-methyl/methoxy-phenoxy-methyl)benzoyl chloride (**3a**, **3b**) with N-(2-dimethylaminoethyl)-(un)substituted anilines (**4a-c**), in the presence of triethylamine, using anhydrous toluene as reaction medium. The benzamide hydrochlorides (**1a-d**) resulted by treating the ethereal

solution of benzamides with ethereal hydrochloric acid at low temperature.

2-(4-Methyl/methoxy-phenoxy-methyl)benzoyl chloride (**3a, b**) was prepared by refluxing the corresponding acid with thionyl chloride in excess, the reaction medium being anhydrous 1,2-dichloroethane.

2-(4-Methyl/methoxy-phenoxy-methyl)benzoic acid (**5a, b**) was obtained from phthalide (**6**) which was refluxed with potassium *p*-methyl/methoxy-

phenoxide in xylene, resulting the potassium salt of 2-(4-methyl/methoxy-phenoxy-methyl)benzoic acid (**7a, 7b**) which was converted then in the corresponding acid using a mineral acid solution.

N-(2-Dimethylaminoethyl)-(un)substituted anilines (**4a-c**) resulted in the reaction of corresponding anilines (**8a-c**) with N-(2-chloroethyl)-N,N-dimethylamine hydrochloride, in anhydrous toluene, using anhydrous sodium carbonate as proton acceptor.

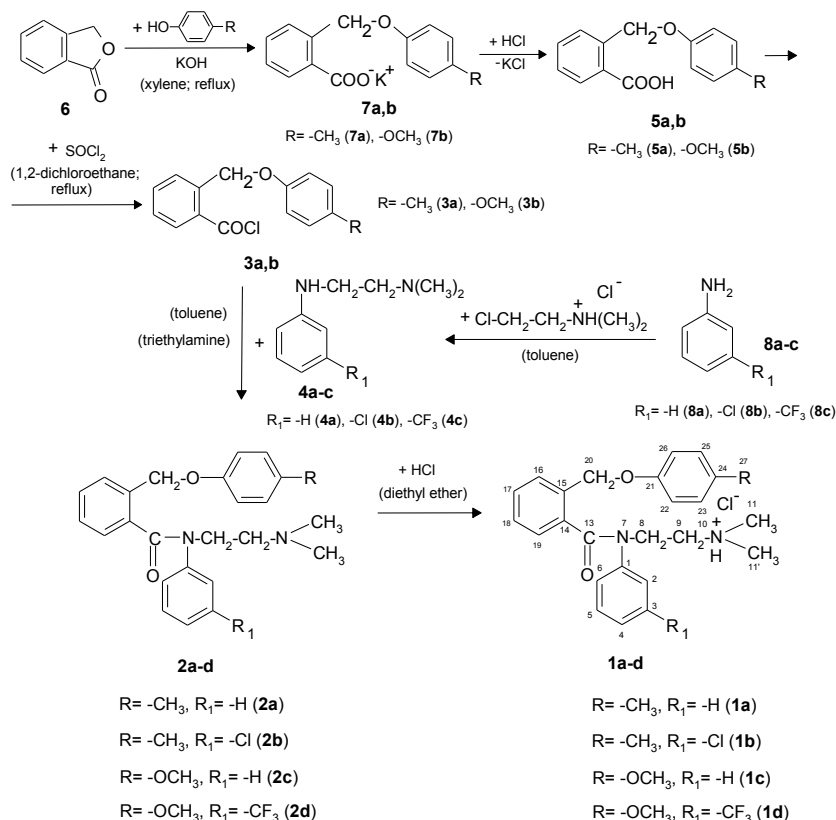


Figure 1.

The new N-(2-dimethylaminoethyl)-N-((un)substituted phenyl)-2-(4-methyl/methoxy-phenoxy-methyl)benzamide hydrochlorides (**1a-d**) synthesis

Physicochemical characterization of the new benzamides

We established the optimal reaction conditions in the synthesis process with high purity and good yields. The new compounds **1a-d** have been characterized by their physical and chemical constants (melting point, solubility). The structures of the original compounds and the intermediate compounds were established by elemental analysis, IR, ^1H -NMR and ^{13}C -NMR. The chemical shifts for hydrogen and carbon atoms were confirmed also by 2D-NMR experiments (COSY, HETCOR).

In the NMR spectra, chemical shifts were recorded as δ values in parts per million (ppm) downfield from tetramethylsilane as internal standard and the coupling constants (J) values in Hertz. Standard abbreviations indicating multiplicity are used as follows: s (singlet), d (doublet), t (triplet), m (multiplet),

dd (double doublet), td (triple doublet), tt (triple triplet) and br (broad) signal. The ^1H -NMR data are reported as follows: chemical shifts, multiplicity, the coupling constants, number of protons and signal/atom attribution. For the ^{13}C -NMR data the following order is: chemical shifts and signal/atom attribution (Cq- quaternary carbon).

N-(2-dimethylaminoethyl)-*N*-phenyl-2-(4-methyl-phenoxy-methyl)benzamide hydrochloride (**1a**): yield 61%; mp 167 - 168.7°C; ^1H -NMR (CDCl_3): 11.25 (br s, 1H, H-N $^+$); 7.28 (d, $J = 7.2$ Hz, 1H, H-19); 7.23 - 7.06 (m, 8H, H-2, H-3, H-4, H-5, H-6, H-16, H-17, H-18); 7.07 (d, $J = 8.6$ Hz, 2H, H-22, H-26); 6.86 (d, $J = 8.6$ Hz, 2H, H-23, H-25); 5.14 (s, 2H, H-20); 4.31 (t, $J = 7.3$ Hz, 2H, H-8); 2.88 (t, $J = 7.3$ Hz, 2H, H-9); 2.55 (s, 6H, H-11, H-11'); 2.24 (s, 3H, H-27); ^{13}C -NMR (CDCl_3): 170.13 (C-13); 156.14 (C-21); 141.51 (C-1); 134.97 (C-15); 134.43

(C-14); 130.0 (C-23; C-25); 129.45 (C-24); 129.39 (C-4); 129.24 (C-2; C-6); 128.21 (CH); 127.58 (C-3; C-5); 127.33 (CH); 127.15 (CH); 127.04 (CH); 114.29 (C-22; C-26); 68.15 (C-20); 54.05 (C-9); 44.29 (C-8); 42.91 (C-11; C-11'); 20.39 (C-27); IR (solid in ATR, cm^{-1}): 3356, 3041, 2974, 2931, 2536, 2335, 1645, 1595, 1510, 1477, 1453, 1389, 1326, 1313, 1293, 1243, 1178, 1147, 1113, 1082, 1021, 969, 887, 845, 788, 739, 655, 614, 564; Anal. calcd. for $\text{C}_{25}\text{H}_{29}\text{ClN}_2\text{O}_2$ (424.96): C, 70.65; H, 6.88; N, 6.59; Found: C, 70.43; H, 6.83; N, 6.51.

N-(2-dimethylaminoethyl)-*N*-(3-chlorophenyl)-2-(4-methylphenoxyethyl)benzamide hydrochloride (**1b**): yield 57%; mp 168.6 - 170.4°C; $^1\text{H-NMR}$ (CDCl_3): 12.49 (br s, 1H, H-N⁺); 7.42 - 7.18 (m, 9H, H-2, H-3, H-4, H-5, H-6, H-16, H-17, H-18, H-19); 7.06 (d, $J = 8.5$ Hz, 2H, H-22, H-26); 7.05 (d, $J = 8.5$ Hz, 2H, H-23, H-25); 5.33 (s, 2H, H-20); 4.48 (t, $J = 7.4$ Hz, 2H, H-8); 3.05 (m, 2H, H-9); 2.75 (d, $J = 4.6$ Hz, 6H, H-11, H-11'); 2.43 (s, 3H, H-27); $^{13}\text{C-NMR}$ (CDCl_3): 170.12 (C-13); 156.42 (C-21); 142.79 (C-1); 135.08 (C-15); 134.81 (C-3); 134.14 (C-14); 130.69 (C-5); 130.39 (CH); 130.13 (CH); 129.7 (C-24); 129.59 (C-23; C-25); 128.05 (CH); 127.83 (C-2); 127.63 (CH); 127.59 (C-6); 125.43 (C-4); 114.33 (C-22; C-26); 68.19 (C-20); 54.07 (C-9); 44.38 (C-8); 42.99 (C-11; C-11'); 20.40 (C-27); IR (solid in ATR, cm^{-1}): 3064, 3017, 2972, 2934, 2336, 1647, 1585, 1512, 1472, 1458, 1384, 1356, 1312, 1291, 1229, 1171, 1144, 1108, 1072, 1019, 963, 889, 834, 782, 749, 675, 622, 561; Anal. calcd. for $\text{C}_{25}\text{H}_{28}\text{Cl}_2\text{N}_2\text{O}_2$ (459.4): C, 65.36; H, 6.14; N, 6.10; Found: C, 65.51; H, 6.20; N, 6.03.

N-(2-dimethylaminoethyl)-*N*-phenyl-2-(4-methoxyphenoxyethyl)benzamide hydrochloride (**1c**): yield 63%; mp 157.9 - 159.3°C; $^1\text{H-NMR}$ (CDCl_3): 12.2 (br s, 1H, H-N⁺); 7.31 (d, $J = 6.7$ Hz, 1H, H-19); 7.21 (t, $J = 7.6$ Hz, 2H, H-3, H-5); 7.14 (tt, $J = 1.6$ Hz, $J = 7.6$ Hz, 1H, H-4); 7.09 (m, 3H, H-16, H-17, H-18); 7.02 (dd, $J = 1.6$ Hz, $J = 7.6$ Hz, 2H, H-2, H-6); 6.93 (d, $J = 9.2$ Hz, 2H, H-22, H-26); 6.87 (d, $J = 9.2$ Hz, 2H, H-23, H-25); 5.16 (s, 2H, H-20); 4.36 (t, $J = 7.5$ Hz, 2H, H-8); 3.75 (s, 3H, -OCH₃); 2.96 (t, $J = 7.5$ Hz, 2H, H-9); 2.63 (m, 6H, H-11, H-11'); $^{13}\text{C-NMR}$ (CDCl_3): 170.21 (C-13); 154.11 (C-24); 152.69 (C-21); 141.51 (C-1); 135.05 (C-15); 134.39 (C-14); 129.40 (C-4); 129.35 (CH); 129.26 (C-2; C-6); 128.25 (CH); 127.57 (C-3; C-5); 127.35 (CH); 127.16 (CH); 115.40 (C-23; C-25); 114.71 (C-22; C-26); 68.79 (C-20); 55.68 (-OCH₃); 54.03 (C-9); 44.28 (C-8); 42.98 (C-11; C-11'); IR (solid in ATR, cm^{-1}): 3122, 3069, 2952, 2839, 2414, 1643, 1581, 1502, 1482, 1448, 1389, 1370, 1332, 1297, 1265, 1219, 1175, 1149, 1109, 1077, 1024, 953, 887, 831, 772, 755, 732, 675, 622, 557; Anal. calcd. for $\text{C}_{25}\text{H}_{29}\text{ClN}_2\text{O}_3$ (440.96): C, 68.09; H, 6.63; N, 6.35; Found: C, 67.88; H, 6.58; N, 6.39.

N-(2-dimethylaminoethyl)-*N*-(3-trifluoromethylphenyl)-2-(4-methoxyphenoxyethyl)benzamide hydrochloride (**1d**): yield 69%; mp 147.4 - 149.6°C; $^1\text{H-NMR}$ (CDCl_3): 10.24 (br s, 1H, H-N⁺); 7.63 (br s, 1H, H-2); 7.53 - 7.25 (m, H-4, H-5, H-6, H-7, H-16, H-17, H-18, H-19); 7.06 (td, $J = 2.6$ Hz, $J = 6$ Hz, 2H, H-22, H-26); 6.96 (d, $J = 7.8$ Hz, 2H, H-23, H-25); 5.14 (s, 2H, H-20); 4.4 (m, 2H, H-8); 3.8 (s, 3H, -OCH₃); 3.23 (m, 2H, H-9); 2.77 (d, $J = 4.8$ Hz, 6H, H-11, H-11'); $^{13}\text{C-NMR}$ (CDCl_3): 173.55 (C-13); 159.40 (C-21); 142.57 (C-1); 134.93 (C-15); 132.68 (q; 33.2; C-3); 132.32 (C-14); 132.18 (C-24); 131.88 (C-5); 130.62 (C-6); 130.39 (C-23; C-25); 129.71 (CH); 128.24 (CH); 128.19 (CH); 128.00 (CH); 124.60 (d; 3.6; C-2); 124.41 (q; 3.2; C-4); 123.8 (q; 272.3; -CF₃); 115.50 (C-22; C-26); 69.09 (C-20); 56.03 (-OCH₃); 55.12 (C-9); 45.77 (C-8); 43.78 (C-11; C-11'); IR (solid in ATR, cm^{-1}): 3452, 3027, 2952, 2824, 2620, 2484, 2061, 1647, 1505, 1492, 1458, 1399, 1380, 1329, 1307, 1292, 1275, 1188, 1155, 1109, 1096, 1076, 1011, 983, 956, 903, 868, 812, 792, 785, 760, 731, 695, 652, 537; Anal. calcd. for $\text{C}_{26}\text{H}_{28}\text{F}_3\text{ClN}_2\text{O}_3$ (508.96): C, 61.35; H, 5.54; N, 5.51; Found: C, 61.51; H, 5.59; N, 5.46.

Antimicrobial activity

The qualitative screening of the antimicrobial activity was quantified by measuring the microbial growth inhibition zone induced by the compound solution distributed on the previously seeded Petri dish. The most susceptible strains to the tested compounds were *B. subtilis*, *C. neoformans*, *E. coli*, *S. saprophyticus* and *A. baumannii* (Table I). Out of the tested compounds, the compound (**1b**) proved to exhibit the widest antimicrobial spectrum, inducing the occurrence of growth inhibition zones ranging from 7 to 12.5 mm, against all tested microbial strains. The growth inhibition zone diameters indicated a better antimicrobial activity against the Gram-positive (9 - 12.5 mm) and fungal strains (11.5 mm), as compared to the Gram-negative strains (7 - 8 mm). The most intensive antimicrobial activity was proven by the compound (**1a**), active against seven of the tested microbial strains, inducing growth inhibition zones with diameters ranging from 6.5 to 9.5 mm. This compound proved to be more active on the fungal (9.5 mm) and Gram-negative strains (6.5 - 9.33 mm). The compound (**1d**) inhibited six of the nine microbial strains, the diameter values being of 7.5 - 11.5 mm. The highest activity was noticed against Gram-positive (9 - 11.5 mm) and fungal strain (10.5 mm). The highest activity of compounds (**1b**) and (**1d**) against the Gram-positive and fungal strains could be related with the presence of one chlorine atom for (**1b**) and of CF₃ for (**1d**) on the phenyl ring bonded to the amide nitrogen atom. Therefore, the presence of halogens seems to increase the antimicrobial activity against the Gram-positive bacterial

strains. The compound (**1c**) exhibited the narrowest antimicrobial activity spectrum, inducing the

occurrence of growth inhibition zones of 5.5 - 9 mm in diameter.

Table I

Qualitative assay of the antimicrobial activity (grown inhibition zones are expressed in mm)

Chemical compound	(1a)	(1b)	(1c)	(1d)
Microbial strains				
<i>Ent. faecalis</i> VA-R17	7.5	9	-	-
<i>S. aureus</i> 6538	-	9	-	10
<i>S. aureus</i> 25923	-	10	-	9
<i>B. subtilis</i> 6633	9.5	12.5	7	11.5
<i>S. saprophyticus</i> 15305	7	10	+/-	9.5
<i>K. pneumoniae</i> 40	7.5	8	-	-
<i>E. coli</i> 29252	6.5	7	6	7.5
<i>A. baumannii</i> 230	9.3	8	9	9
<i>C. neoformans</i> 20409	9.5	11.5	5.5	10.5

+/- = inconsistent inhibition of the microbial growth with the occurrence of microbial colonies inside the inhibition zone area

The quantitative assay of the antimicrobial activity sustained the results of the qualitative screening, the compound (**1b**) being the most effective, revealing the lowest MIC values from all tested benzamides, the most susceptible strains proving to be *S. aureus* 6538, *B. subtilis* 6633, *S. saprophyticus* 15305 and

C. neoformans 20409 (MIC of 250 µg/mL) (Table II). The compound (**1d**) exhibited the lowest MIC value against *A. baumannii* 230 (MIC of 250 µg/mL). The compounds (**1a**) and (**1c**) exhibited high MIC values against all tested microbial strains.

Table II

The MIC values (µg/mL) of the obtained compounds against the tested microbial strains

Chemical compound	(1a)	(1b)	(1c)	(1d)
Microbial strains				
<i>Ent. faecalis</i> VA-R17	> 1000	> 1000	-	-
<i>S. aureus</i> 6538	1000	250	1000	> 1000
<i>S. aureus</i> 25923	-	500	-	> 1000
<i>B. subtilis</i> 6633	500	250	> 1000	> 1000
<i>S. saprophyticus</i> 15305	1000	250	> 1000	> 1000
<i>K. pneumoniae</i> 40	> 1000	> 1000	> 1000	-
<i>E. coli</i> 29252	> 1000	500	> 1000	> 1000
<i>A. baumannii</i> 230	1000	500	1000	250
<i>C. neoformans</i> 20409	1000	250	> 1000	> 1000

All tested compounds, with few exceptions, exhibited very good anti-biofilm activity with very low MBEC values of 1.9 µg/mL against the majority of the tested

microbial strains (Table III). The most active anti-biofilm compound proved to be (**1d**) bearing a CF₃ group on the phenyl ring bonded to the amide nitrogen atom.

Table III

MBEC values (µg/mL) of the obtained compounds against the tested microbial strains

Chemical compound	(1a)	(1b)	(1c)	(1d)
Microbial strains				
<i>Ent. faecalis</i> VA-R17	1.9	3.9	1.9	1.9
<i>S. aureus</i> 6538	3.9	> 1000	1.9	3.9
<i>S. aureus</i> 25923	1.9	1.9	1.9	1.9
<i>B. subtilis</i> 6633	1.9	1.9	3.9	1.9
<i>S. saprophyticus</i> 15305	15.6	7.8	7.8	1.9
<i>K. pneumoniae</i> 40	125	1.9	31.2	15.6
<i>E. coli</i> 29252	1.9	1.9	1.9	1.9
<i>A. baumannii</i> 230	1.9	1.9	1.9	1.9
<i>C. neoformans</i> 20409	1000	1.9	> 1000	125

Conclusions

New benzamides were synthesized by optimized synthesis methods and characterized using IR, NMR and elemental analysis. The results of spectral analysis confirmed the synthesis pathway and the structure

of the resulted compounds. The obtained compounds proved to exhibit a specific spectrum of antimicrobial activity. The benzamides bearing one chlorine atom and respectively CF₃ on the phenyl ring bonded to the amide nitrogen atom proved to be more active against the Gram-positive and fungal strains. All

tested compounds, but especially that one bearing the CF₃ group exhibited a very good anti-biofilm activity, proving their potential for the further development of novel antimicrobial and anti-biofilm agents and surfaces. The concept of combining the antibacterial and analgesic - anti-inflammatory activities in a single therapeutic agent applies for the obtained compounds and is promising for the treatment of bacterial infections, particularly those accompanied by pain and inflammation.

References

- Hlasta D.J., Demers J.P., Foleno B.D., Fraga-Spano S.A., Guan J., Hilliard J.J., Macielag M.J., Ohemeng K.A., Sheppard C.M., Sui Z., Webb G.C., Weidner-Wells M.A., Barret J.F., Novel inhibitors of bacterial two-component systems with Gram positive antibacterial activity: Pharmacophore identification based on the screening hit closantel. *Bioorg. Med. Chem. Lett.*, 1998; 8: 1923-1928.
- Kobayashi I., Muraoka H., Hasegawa M., Saika T., Nishida M., Kawamura M., Ando R., *In vitro* anti-*Helicobacter pylori* activity of BAS-118, a new benzamide derivative. *J. Antimicrob. Chemother.*, 2002; 50: 129-132.
- Kumar A., Narasimhan B., Kumar D., Synthesis, antimicrobial, and QSAR studies of substituted benzamides. *Bioorg. Med. Chem.*, 2007; 15(12): 4113-4124.
- Limban C., Grumezescu A.M., Saviuc C., Voicu G., Predan G., Sakizlian R., Chifiriuc M.C., Optimized anti-pathogenic agents based on core/shell nano-structures and 2-((4-ethylphenoxy)methyl)-N-(substituted-phenylcarbamothioyl)-benzamides. *Int. J. Mol. Sci.*, 2012; 13: 12584-12597.
- Limban C., Măruțescu L., Chifiriuc M.C., Synthesis, spectroscopic properties and antipathogenic activity of new thiourea derivatives. *Molecules*, 2011; 16: 7593-7607.
- Limban C., Missir A.V., Chiriță I.C., Guță R., Nănuș-Andrescu D., Nițulescu G.M., Drăghici C., Căproiu M.T., Delcaru C., Chifiriuc M.C., Synthesis, structural characterization and microbiological assays of some new 2-methoxy-O-acyl-oximino-dibenz[b,e]oxepins. *Rev. Roum. Chim.*, 2010; 55(6): 313-319.
- Limban C., Missir A.V., Nuță D.C., Căproiu M.T., Papacoea M.T., Chiriță C., Synthesis of some new 2-((4-chlorophenoxy)methyl)-N-(arylcarbamothioyl) benzamides as potential antifungal agents. *Farmacia*, 2016; 64(5): 775-779.
- Limban C., Missir A.V., Grumezescu A.M., Oprea A.E., Grumezescu V., Vasile B.S., Socol G., Trușcă R., Caproiu M.T., Chifiriuc M.C., Gălățeanu B., Costache M., Morusciag L., Pircălăbioru G., Nuță D.C., Bioevaluation of Novel Anti-Biofilm Coatings Based on PVP/Fe₃O₄ Nanostructures and 2-((4-Ethylphenoxy)methyl)-N-(arylcarbamothioyl)benzamides. *Molecules*, 2014; 19(8): 12011-12030.
- Limban C., Missir A.V., Nuta D.C., Caproiu M.T., Morusciag L., Chirita C., Cupii A., Gurgu H., Advances in research of new 2-((4-ethylphenoxy)methyl)-N-(arylcarbamothioyl)benzamides. *Farmacia*, 2015; 63(3): 376-380.
- Mabkhot Y.N., Al-Majid A.M., Barakat A., Al-Showiman S.S., Al-Har M.S., Radi S., Naseer M.M., Hadda T.B., Synthesis and Biological Evaluation of 2-Aminobenzamide Derivatives as Antimicrobial Agents: Opening/Closing Pharmacophore Site. *Int. J. Mol. Sci.*, 2014; 15: 5115-5127.
- Macielag M.J., Demers J.P., Fraga-Spano S.A., Hlasta D.J., Johnson S.G., Kanojia R.M., Russel R.K., Sui Z.H., Weidner-Wells M.A., Werblow H., Foleno B.D., Goldschmidt R.M., Loeloff M.J., Webb G.C., Barrett J.F., Substituted salicylanilides as inhibitors of two-component regulatory systems in bacteria. *J. Med. Chem.*, 1998; 41: 2939-2945.
- McDonnell G., Russell A.D., Antiseptics and Disinfectants: Activity, Action, and Resistance. *Clin. Microbiol. Rev.*, 1999; 12(1): 147-179.
- Nathani B.R., Pandya K.S., Jeni M.M., Prasad A., Synthesis, characterisation and antimicrobial study of some new isatins derivatives. *J. Chem. Pharm. Res.*, 2011; 3(6): 687-692.
- Olar R., Badea M., Cristurean E., Lazar V., Cernat R., Balotescu M.C., Thermal behaviour, spectroscopic and biological characterization of Co(II), Zn(II), Pd(II) and Pt(II) complexes with N,N-dimethylbiguanide. *J. Therm. Anal. Calorim.*, 2005; 80: 451-455.
- Prajapati A.K., Modi V.P., Synthesis and biological activity of N-{5-(4-methylphenyl) diazenyl-4-phenyl-1,3-thiazol-2-yl}benzamide derivatives. *Quim. Nova*, 2011; 34(5): 771-774.
- Pratima G., Nikalje A., Patel M., Ranade Y., Deshpande R., Rajani D., Design and synthesis of novel N-substituted morpholino benzamide derivatives as antimicrobial agents. *Der Pharmacia Sinica*, 2012; 3(4): 462-469.
- Rotaru (Enache) I.D., Nuță D.C., Chiriță I.C., Căproiu M.T., Limban C., Missir A.V., Chiriță C., New synthesis in the N-[4 [(phenylcarbamoyl)-amino]-phenyl]benzenesulfonamide derivatives series. Note 1. *Farmacia*, 2016; 64(6): 828-833.
- Shinkai H., Ito T., Iida T., Kitao Y., Yamada H., Uchida I., 4-Aminoquinolines: novel nociceptin antagonists with analgesic activity. *J. Med. Chem.*, 2000; 43(24): 4667.
- Vinsova J., Imramovsky A., Buchta V., Ceckova M., Dolezal M., Staud F., Jampilek J., Kaustova J., Salicylanilide Acetates: Synthesis and Antibacterial Evaluation. *Molecules*, 2007; 12(1): 1-12.
- Yildiz Oren I., Aki-Sener E., Ertas C., Arpacı O.T., Yalcin I., Altanlar N., Synthesis and microbiological activity of some substituted N-(2-hydroxy-4-nitrophenyl)benzamides and phenylacetamides as possible metabolites of antimicrobial active benzoxazoles. *Turk. J. Chem.*, 2004; 28: 441-449.