N-(1-ADAMANTYLCARBAMOTHIOYL)BENZAMIDES: SYNTHESIS, BIOLOGICAL EVALUATION AND ADME PREDICTIONS

HOREA GURGU^{1#}, EMIL ȘTEFĂNESCU^{2#}, DIANA CAMELIA NUȚĂ^{1*}, MIRON TEODOR CĂPROIU⁴, FLOREA DUMITRAȘCU⁴, MARIANA CARMEN CHIFIRIUC^{5,6}, CORALIA BLEOTU⁷, GEORGE MIHAI NIȚULESCU¹, FLAVIAN ȘTEFAN RĂDULESCU³, CARMEN LIMBAN¹

¹“Carol Davila” University of Medicine and Pharmacy, Faculty of Pharmacy, Pharmaceutical Chemistry Department, 6 Traian Vuia Street, 020956, Bucharest, Romania

²“Carol Davila” University of Medicine and Pharmacy, Faculty of Pharmacy, Pharmacology and Clinical Pharmacy Department, 6 Traian Vuia Street, 020956, Bucharest, Romania

³“Carol Davila” University of Medicine and Pharmacy, Faculty of Pharmacy, Drug Industry and Pharmaceutical Biotechnologies Department, 6 Traian Vuia Street, 020956, Bucharest, Romania

⁴“Costin D. Nenițescu” The Organic Chemistry Centre of Romanian Academy, 202B Splaiul Independenței, 060023, Bucharest, Romania

⁵University of Bucharest, Faculty of Biology, Department of Microbiology, 1-3 Portocalelor Alley, 060101, Bucharest, Romania

⁶University of Bucharest, Research Institute of the University of Bucharest - ICUB, 91-95 Splaiul Independenței, 050095, Bucharest, Romania

⁷“Prof. Șt. Nicolau” Institute of Virology, 36 Mihai Bravu Road, 021326, Bucharest, Romania

*corresponding author: diananuta@yahoo.com

#Authors with equal contribution.

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Abstract

This paper presents the synthesis of five novel benzamide derivatives containing adamantane moiety. The compounds structure was proven through elemental analysis, FT-IR, ¹H-NMR and ¹³C-NMR spectroscopy. Their therapeutic potential was evaluated by the *in vitro* testing of the antimicrobial activity. Two different parameters of the antimicrobial activity were determined: minimum inhibitory concentration (MIC) and minimal biofilm eradication concentration (MBEC). Also, an *in vitro* cytotoxicity study was carried out on Hep-2 cell line. An *in silico* study of the compounds determined their ADME profile. The study reports the preliminary biological screening of five novel benzamides with adamantane scaffold, that evidenced a good toxicological and ADME profile, thus representing favourable candidates for further studies.

Rezumat

În această lucrare este prezentată sinteza a cinci noi derivați ai benzamidei care conțin fragmentul adamantan. Structura compușilor a fost dovedită prin analiză elementală, spectroscopie FT-IR, ¹H-RMN și ¹³C-RMN. Potențialul lor terapeutic a fost evaluat prin testarea *in vitro* a activității antimicrobiene. S-au determinat doi parametri diferiți ai activității antimicrobiene, respectiv: concentrația minimă inhibitoare (MIC) și concentrația minimă de eradicare a biofilmului (MBEC). De asemenea, a fost efectuat un studiu de citotoxicitate *in vitro*, pe linia celulară Hep-2. Un studiu *in silico* al compușilor a determinat profilul lor ADME. Studiul raportează *screening*-ul biologic preliminar a cinci noi benzamide cu fragment de adamantan, care prezintă profil toxicologic și ADME favorabil, prezentând astfel un potențial promițător pentru studii ulterioare.

Keywords: benzamides, adamantane, microbiological assay, cytotoxicity

Introduction

Fragments of adamantane and thiourea are important components of bioactive molecules, representing veritable building blocks for the pharmaceutical chemistry.

Some of the adamantane derivatives have been already introduced in clinical use: amantadine, rimantadine, adapromine and tromantadine as anti-virals, memantine, a N-methyl-D-aspartate receptor antagonist used in Alzheimer's disease, dopamantine and carmantadine as antiparkinsonian drugs, saxagliptin and vildagliptin,

inhibitors of dipeptidyl peptidase 4 as type II anti-diabetes, adapalene, an inhibitor of keratinocyte differentiation and proliferation, in the treatment of acne. All these compounds have the adamantane scaffold substituted at 1 position [14].

Compounds with thiourea skeleton are already used as drugs: noxytiolin as anti-infective agents, sulfathiourea as a bacteriostatic sulfamide, loflucarban for its anti-fungal properties, thiocarlide as anti-tuberculosis drug, burmamide and metiamide for the treatment of peptic ulcer, phenethyl-thiazolyl-thiourea and trovirdin as non-nucleoside reverse transcriptase inhibitors.

The wide range of pharmacological properties associated with thiourea derivatives makes them important templates for future drugs design. It was reported compounds with thiourea scaffold which have demonstrated the varied biological activities, such as antimicrobial [28], antiparasitic [29], antiviral [13], antitumour [32], antidiabetic [10], anti-inflammatory [23], insecticidal [31], herbicidal [33] and fungicidal properties [27].

Novel 1-(adamantane-1-carbonyl)-3-halophenyl thioureas and 1-(adamantane-1-carbonyl)-3-(2,4,6-trimethylphenyl)thiourea were synthesized and their structural and vibrational properties were determined [25, 26], and Al-Omary and co-workers reported the synthesis and crystal structure of the potentially bio-active 1-(adamantan-1-yl)-3-(4-chlorophenyl)thiourea [1].

A series of novel 1-(2-(adamantan-1-yl)-1*H*-indol-5-yl)-3-substituted thiourea derivatives were obtained and evaluated for their anti-proliferative activity, being found active against H460 lung cancer, HepG2 liver cancer and MCF-7 breast cancer cells. The molecular docking studies have shown that some of these derivatives show promising binding affinity and can modulate Nur77 receptor activity. These findings provide a new direction for the development of anticancer agents having as potential therapeutic target this receptor [12].

4-(3-Adamantan-1-yl-thioureido)-N-(2,6-dimethoxypyrimidin-4-yl)benzene-sulfonamide was designed and synthesized by the addition of N-(2,6-dimethoxypyrimidin-4-yl)-4-isothiocyanatobenzenesulfonamide to the 1-aminoadamantane, as potential antitubercular agent. The structure of the synthesized compound was inspired from the second line of antituberculosis pro-drugs thioacetazone, thiocarbide, ethionamide and prothionamide [11].

1-(Adamantane-2-yl)-3-phenyl/benzyl/phenethyl-thiourea and 1-(1-(adamantan-1-yl)ethyl)-3-phenyl/benzyl/phenethyl-thiourea have been synthesized and their inhibitory activity against mammalian and human soluble epoxide hydrolase have been examined [5]. Our research group has been involved in the synthesis and biological assay of different thiourea derivatives [16, 17, 19-21, 24].

In the present study, we report the synthesis of five novel N-(1-adamantylcarbamoithiyl)benzamides, through a simple and efficient method, employing good atom economy, readily available starting materials, low amounts of solvents and less hazardous synthesis [6]. By variation of different building blocks (phenols, phthalides, adamantanamines) the synthesis can be adapted for obtaining large numbers of compounds with adamantane scaffolds useful in biological screening. The novel compounds were assessed *in vitro* for their antimicrobial properties against several bacterial and fungal strains, as well as for their cytotoxic activity, and their ADME profile was established by *in silico*

studies, in order to further synthesize and develop potential leading compounds with therapeutic applications.

Materials and Methods

Chemistry

Reagents and solvents used for the synthesis were purchased from Merck (Darmstadt, Germany).

Melting points were recorded on an Electrothermal 9100 capillary melting point apparatus (Bibby Sci. Ltd, Stone, UK) and are uncorrected.

The IR spectra were measured using a Bruker Vertex 70 FT-IR spectrometer (Bruker Corporation, Billerica, MA, USA), at room temperature, with an attenuated total reflection (ATR) technique.

The ¹H-NMR and ¹³C-NMR spectra were recorded in DMSO-d₆ with a Varian Gemini 300BB instrument (Varian Medical Systems, Palo Alto, CA, USA) operating at 300 MHz for ¹H and 75 MHz for ¹³C, using tetramethylsilane as internal standard.

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

For the efficient synthesis of the novel N-(1-adamantylcarbamoithiyl) benzamides (**6a-e**) we used a four steps approach.

The general synthesis procedure is described in Figure 1 and was performed as described in previous studies [2-4, 15, 18, 21].

For the synthesis of 2-(4-R-phenoxyethyl)benzoic acids (**3**), phthalide (**1**) was refluxed with substituted phenols (**2**), in alkaline medium and the resulting potassium salts of 2-(4-R-phenoxyethyl)benzoic acids gave the corresponding acids (**3**) by acidification.

The 2-(4-R-phenoxyethyl)benzoic acid chlorides (**4**), were obtained by treating the corresponding benzoic acids (**3**) with thionyl chloride using anhydrous 1,2-dichloroethane as reaction medium. The acid chlorides (**4**) were used in the next step of the synthesis in crude state, after the removal of 1,2-dichloroethane and excess thionyl chloride by distillation under reduced pressure.

The synthesis of N-(1-adamantylcarbamoithiyl)-benzamides (6a-e)

To a stirred solution of 2-(4-R-phenoxyethyl)benzoic acid chloride (**4**) (0.01 mol) in acetone (15 mL) was added a solution of ammonium thiocyanate (0.01 mol) in acetone (5 mL). The reaction mixture was heated for 1 h, to obtain the benzoyl isothiocyanates (**5**). The mixture was cooled at room temperature and a solution of 1-adamantylamine (0.01 mol) in acetone (2 mL) was added and heated under reflux for 8 h. The reaction mixture was poured into cold water, the solid was isolated by filtration. The final compounds **6a-e** were purified by recrystallization from isopropanol with active charcoal.

Figure 1.
Synthesis of N-(1-adamantylcarbamothioyl)benzamides **6a-e**

Microbiological assays

The antimicrobial activity of the newly synthesized compounds was investigated on five different micro-organisms, respectively Gram-positive: *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212 and Gram-negative: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 bacterial strains, as well as the fungal strain *Candida albicans* ATCC 10231.

Bacterial suspensions of 0.5 McFarland density obtained from 24 hours microbial cultures developed on solid media were used in the experiments.

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

The antimicrobial activity of the novel compounds was tested on Muller-Hinton agar medium.

Qualitative screening by disk diffusion method

The qualitative screening, of the antimicrobial properties of the tested compounds, was performed by an adapted disk diffusion method. Petri dishes with Muller-Hinton medium were seeded with microbial standardized inoculum as for the classical antibiotic susceptibility testing; then a volume of 10 μL of the tested compounds was distributed on the seeded medium, at 30 mm distance. The plates were left at room temperature for 30 minutes and then incubated at 37°C for 24 h. The positive results were read as the occurrence of an inhibition zone of microbial growth around the disk. DMSO was used as negative control for the tested compounds.

Quantitative screening by determination of MIC values

Quantitative assay of the antimicrobial activity was performed by binary microdilution method, in Muller-Hinton broth (MHB) distributed in 96-well plates, in order to establish the MIC. The protocol was performed

in accordance with the CLSI guidelines [7, 8]. Serial binary dilutions were prepared starting from stock solution of the tested compounds. In the first well, a volume of 90 μL MHB was added with 90 μL of tested compound. 90 μL were transferred from the first well to the second one and so on until the final well with a compound concentration corresponding to 9.78 $\mu\text{g/mL}$. Each well was seeded with 10 μL of microbial inoculum of 0.5 McFarland density. The plates were incubated for 24 h at 37°C, and MICs were read as the last concentration of the compound which inhibited the visual microbial growth.

Anti-biofilm activity assay by determination of MBEC values

The anti-biofilm activity was tested by the microtiter method. For this purpose, the microbial strains have been grown in the presence of two-fold serial dilutions of the tested compounds performed in liquid nutrient, distributed in 96-well plates, and incubated for 24 h at 37°C. At the end of the incubation period, the plastic wells were emptied, washed three times with sterile distilled water, fixed with cold methanol and stained with 1% violet crystal solution for 15 minutes. The biofilm formed on plastic wells was resuspended in 33% acetic acid. The intensity of the coloured suspensions was assessed by measuring the absorbance at 492 nm. The last concentration of the tested compound that inhibited the development of microbial biofilm on the plastic wells was considered the MBEC and was also expressed in $\mu\text{g/mL}$ [30].

In vitro cytotoxicity assay

The human epidermoid laryngeal carcinoma (HEp-2) cells were maintained in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM: F12) culture medium, supplemented with 10% heat-inactivated foetal bovine serum (FBS), penicillin 100 U/mL and

streptomycin 100 µg/mL. The cytotoxicity treatments were done in triplicate using HEp-2 cells plated in 96-well plates at a density of 10,000 cells *per* well. After overnight incubation at 37°C in a 5% CO₂ incubator, with the tested compounds, the number of remaining live cells was determined using The CellTiter 96[®] AQueous One Solution Cell Proliferation Assay.

ADME and molecular property predictions

The chemical structures were written with ACD/Chemsketch[™] 2016.2, ACD/Labs and their molecular descriptors were generated by ADMET Predictor[™] software, version 8.0.4.6., Simulation Plus Inc.

Drug-like properties of the compounds were determined, in order to apply the Lipinski Rule of five (RO5) criterion: molecular weight (MW, g/mol), n-octanol/water partition coefficient (Moriguchi, MLogP), number of hydrogen bond donors (HBD), number of hydrogen bond acceptors (HBA).

The computed molecular descriptors were extended to parameters essential for the pharmacokinetic assessment of a drug. Solubility profile was predicted as Sw – solubility in water (mg/mL). The estimated penetration through various types of membranes was characterized by: Peff – human jejunal effective permeability (cm/s x 10⁴) and MDCK – apparent permeability in Madin-Darby Canine Kidney Cells (cm/s x 10⁷). Two blood-brain barrier permeation models were applied: BBB – qualitative permeability model and LogBB – blood-brain partition coefficient. Other pharmacokinetic predictions include: Vd – volume of distribution (L/kg), RBP – blood-to-plasma concentration ratio, Fup – percent of drug unbound to plasma proteins (%) and Pgp_Substr – Likelihood of intestinal efflux by permeability-glycoprotein (P-gp) transporter in human (%) [9].

Results and Discussion

Chemistry

The compounds were obtained through an efficient method, from readily available reagents, with low generation of waste material (no column chromatography is necessary for the purification stages), thus being an environmentally friendly procedure.

The novel benzamides with adamantane scaffold are white or light yellow, crystallized solids, soluble at room temperature in acetone, chloroform, DMSO, after heating in inferior alcohols (methanol, ethanol, isopropanol) and insoluble in water.

Elemental analysis data were in accordance with the calculated values.

In the IR spectra the compounds exhibited absorption bands for νC=O in the region 1662 - 1680 cm⁻¹. νN-H of the thioamide group appears at 3168 - 3188 cm⁻¹. Methyl and methylene groups νC-H are in the 2904 - 2961 cm⁻¹ respectively 2856 - 2893 cm⁻¹ regions. The vibration for δN-H amide group is found as a very

strong signal at 1502 - 1536 cm⁻¹. The alkyl-aryl ether bands appear at 1220 - 1247 cm⁻¹ for the anti-symmetric vibration, and at 1019 - 1032 cm⁻¹ for the symmetric vibration, while the νC=S stretching band is in the 1148 - 1155 cm⁻¹ range. The IR signals are given as w – weak, m – medium, s – strong, vs – very strong.

In the ¹H-NMR spectra, chemical shifts were given as δ values in parts per million (ppm) relative to tetramethylsilane as internal standard. Coupling constants *J* were given in Hz. Spin multiplets are given as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (double doublet), td (triple doublet) and br (broad). The chemical shifts for hydrogen and carbon atoms were established also by homonuclear correlation spectroscopy (COSY), heteronuclear multiple-bond correlation spectroscopy (HMBC) and heteronuclear multiple quantum correlation (HMQC) experiments. The ¹H-NMR data are reported in the following order: chemical shifts, multiplicity, and the coupling constants, number of protons and signal/atom attribution.

In the ¹H-NMR spectra the NH protons resonated as singlet in the range of 11.19 - 11.25 ppm and 10.75 - 10.79 ppm. The -CH₂-O- protons signal was observed in the region 5.16 - 5.23 as singlet. The ethyl group protons appear as a triplet at 1.13 ppm for -CH₃ group, and as quartet at 2.52 ppm for -CH₂-group. The methyl group protons have a signal at 2.22 ppm, as singlet. Protons of methoxy group signals are at 3.69 ppm, as singlet. In case of the adamantane moiety there are three different signals: 2.17 or 2.18 ppm bs or s for 6 protons, 2.05 or 2.06 ppm bs or s for 3 protons and 1.63 or 1.64 ppm bs or s for 6 protons.

The ¹³C-NMR data are reported in the following order: chemical shifts and signal/atom attribution. The C=O and C=S carbons gave signals in the regions 170.18 - 170.31 ppm, and 177.19 - 179.75 ppm respectively. The methylene carbon of -CH₂-O- group appears at 67.33 - 68.20 ppm, the ethyl group carbons appear at 15.97 ppm (-CH₃) and 27.35 ppm (-CH₂-). Carbon of methyl group signal is at 19.95 ppm and carbon of methoxy group is at 55.36 ppm. There are four different kinds of signals for the adamantane carbons: 54.02 - 54.14 ppm (C-17), 39.68 - 39.71 ppm (C-18; C-19; C-20), 35.79 - 36.65 ppm (C-24; C-25; C-26) and 28.64 - 28.80 (C-21; C-22; C-23).

N-(1-Adamantylcarbamothioyl)-2-(phenoxyethyl)-benzamide (**6a**)

Yield 67%; mp 144 - 145°C. ¹H-NMR: 11.20 (s, 1H, NH, deuterable); 10.77 (s, 1H, NH, deuterable); 7.56 - 7.49 (m, 3H, H-4, H-6, H-7); 7.43 (td, *J* = 7.8 Hz, *J* = 2.4 Hz, 1H, H-5); 7.29 (t, *J* = 7.8 Hz, 2H, H-11, H-13); 6.97 (t, *J* = 7.8 Hz, 1H, H-12); 6.95 (d, *J* = 7.8 Hz, 2H, H-10, H-14); 5.23 (s, 2H, H-8); 2.18 (s, 6H, H-18, H-19, H-20); 2.06 (s, 3H, H-21, H-22, H-23); 1.63 (s, 6H, H-24, H-25, H-26). ¹³C-NMR: 177.31 (C-16); 170.18 (C-1); 158.25 (C-9); 135.38 (C-3); 133.61 (C-2); 130.81 (C-4 or C-6 or C-7); 129.51 (C-11,

C-13); 128.46 (C-4 or C-6 or C-7); 128.37 (C-4 or C-6 or C-7); 127.84 (C-5); 121.00 (C-12); 114.25 (C-10, C-14); 67.33 (C-8); 54.05 (C-17); 39.69 (C-18, C-19, C-20); 35.80 (C-24, C-25, C-26); 28.79 (C-21, C-22, C-23). FT-IR (ν cm^{-1}): 3184m, 3056w, 2904s, 2889m, 2847m, 1679s, 1597w, 1536vs, 1492s, 1458m, 1354m, 1302m, 1256m, 1243s, 1229s, 1165m, 1149s, 1125m, 1069w, 1032m, 889w, 755s, 727m, 688m, 663m. Anal. calcd. for $\text{C}_{25}\text{H}_{28}\text{N}_2\text{O}_2\text{S}$ (420.57): C, 71.40; H, 6.71; N, 6.66; S, 7.62%; Found: C, 70.91; H, 6.81; N, 6.65; S, 7.67%.

N-(1-Adamantylcarbamothioyl)-2-((4-fluorophenoxy)-methyl)benzamide (**6b**)

Yield 70%; mp 157 - 158°C. $^1\text{H-NMR}$: 11.20 (s, 1H, NH, deuterable); 10.76 (s, 1H, NH, deuterable); 7.56 - 7.49 (m, 3H, H-4, H-6, H-7); 7.43 (td, $J = 7.8$ Hz, $J = 2.4$ Hz, 1H, H-5); 7.13 (t, $J = 9.2$ Hz, 2H, H-11, H-13); 6.96 (dd, $J(\text{F-H}10,14) = 4.5$ Hz, $J(\text{H}10,14-\text{H}11,13) = 9.2$ Hz, 2H, H-10, H-14); 5.21 (s, 2H, H-8); 2.17 (s, 6H, H-18, H-19, H-20); 2.06 (s, 3H, H-21, H-22, H-23); 1.64 (s, 6H, H-24, H-25, H-26). $^{13}\text{C-NMR}$: 177.19 (C-16); 170.02 (C-1); 156.64 (d, C-12, $J(\text{F-C}12) = 234.4$ Hz); 154.45 (C-9); 135.11 (C-3); 133.55 (C-2); 130.74 (C-4 or C-6 or C-7); 128.43 (C-4 or C-6 or C-7); 128.31 (C-4 or C-6 or C-7); 27.83 (C-5); 116.00 (d, C-10, C-14, $J(\text{F-C}10,14) = 6.9$ Hz); 115.68 (d, C-11, C-13, $J(\text{F-C}11,13) = 21.7$ Hz); 68.18 (C-8); 54.03 (C-17); 39.71 (C-18, C-19, C-20); 35.79 (C-24, C-25, C-26); 28.80 (C-21, C-22, C-23). FT-IR (ν cm^{-1}): 3182m, 3058w, 2904s, 2856m, 1677s, 1537vs, 1502vs, 1458m, 1352m, 1300m, 1247m, 1198m, 1149s, 1123m, 1027m, 831m, 754m, 731m, 663w. Anal. calcd. for $\text{C}_{25}\text{H}_{27}\text{FN}_2\text{O}_2\text{S}$ (438.56): C, 68.47; H, 6.21; F, 4.33; N, 6.39; S, 7.31%; Found: C, 68.03; H, 6.02; N, 6.29; S, 7.23%.

N-(1-Adamantylcarbamothioyl)-2-((4-methylphenoxy)-methyl)benzamide (**6c**)

Yield 63%; mp 140 - 141°C. $^1\text{H-NMR}$: 11.20 (s, 1H, NH, deuterable); 10.75 (s, 1H, NH, deuterable); 7.54 - 7.50 (m, 3H, H-4, H-6, H-7); 7.42 (td, $J = 7.8$ Hz, $J = 2.4$ Hz, 1H, H-5); 7.08 (d, $J = 8.3$ Hz, 2H, H-11, H-13); 6.83 (d, $J = 8.3$ Hz, 2H, H-10, H-14); 5.18 (s, 2H, H-8); 2.22 (s, 3H, H-12'); 2.17 (bs, 6H, H-18, H-19, H-20); 2.06 (bs, 3H, H-21, H-22, H-23); 1.64 (bs, 6H, H-24, H-25, H-26). $^{13}\text{C-NMR}$: 177.31 (C-16); 170.19 (C-1); 56.20 (C-9); 129.68 (C-12); 135.56 (C-3); 133.64 (C-2); 130.79 (C-4 or C-6 or C-7); 129.84 (C-11, C-13); 128.47 (C-4 or C-6 or C-7); 128.35 (C-4 or C-6 or C-7); 127.82 (C-5); 114.62 (C-10, C-14); 67.63 (C-8); 54.14 (C-17); 39.68 (C-18, C-19, C-20); 36.65 (C-24, C-25, C-26); 28.64 (C-21, C-22, C-23); 19.95 (C-12'). FT-IR (ν cm^{-1}): 3168m, 3029w, 2910m, 2886s, 2849m, 1671s, 1544vs, 1508vs, 1456m, 1355m, 1302w, 1227s, 1155s, 1121m, 1019m, 808w, 738m, 665w. Anal. calcd. for $\text{C}_{26}\text{H}_{30}\text{N}_2\text{O}_2\text{S}$ (434.60): C, 71.86; H, 6.96; N, 6.45; S, 7.38%; Found: C, 71.07; H, 6.76; N, 6.49; S, 7.31%.

N-(1-Adamantylcarbamothioyl)-2-((4-ethylphenoxy)-methyl) benzamide (**6d**)

Yield 65%; mp 117 - 118°C. $^1\text{H-NMR}$: 11.25 (s, 1H, NH, deuterable); 10.77 (s, 1H, NH, deuterable); 7.60 - 7.38 (m, 4H, H-4- H-7); 7.11 (d, $J = 8.7$ Hz, 2H, H-11, H-13); 6.86 (d, $J = 8.6$ Hz, 2H, H-10, H-14); 5.18 (s, 2H, H-8); 2.52 (q, $J = 7.6$ Hz, 2H, H-12'); 2.17 (bs, 6H, H-18, H-19, H-20); 2.05 (bs, 3H, H-21, H-22, H-23); 1.63 (bs, 6H, H-24, H-25, H-26); 1.13 (t, $J = 7.6$ Hz, 3H, H-12'). $^{13}\text{C-NMR}$: 179.75 (C-16); 170.31 (C-1); 156.25 (C-9); 20.70 (C-12); 136.27 (C-3); 129.67 (C-2); 128.54 (CH); 128.45 (CH); 127.78 (CH); 127.54 (CH); 128.65 (C-11, C-13); 114.72 (C-10, C-14); 67.57 (C-8); 54.10 (C-17); 39.70 (C-18, C-19, C-20); 35.79 (C-24, C-25, C-26); 28.80 (C-21, C-22, C-23); 27.35 (C-12'); 15.97 (C-12'). FT-IR (ν cm^{-1}): 3324w, 3188w, 3058w, 2961m, 2895vs, 2850m, 1662s, 1606w, 1546s, 1510vs, 1451m, 1356m, 1300m, 1220s, 1148m, 1113m, 1023m, 832m, 812w, 707m, 673w. Anal. calcd. for $\text{C}_{27}\text{H}_{32}\text{N}_2\text{O}_2\text{S}$ (448.63): C, 72.29; H, 7.19; N, 6.24; S, 7.15%; Found: C, 71.76; H, 7.23; N, 6.32; S, 7.19%.

N-(1-Adamantylcarbamothioyl)-2-((4-methoxyphenoxy)methyl)benzamide (**6e**)

Yield 71%; mp 122 - 123°C. $^1\text{H-NMR}$: 11.19 (s, 1H, NH, deuterable); 10.79 (s, 1H, NH, deuterable); 7.58 - 7.48 (m, 3H, H-4, H-5, H-7); 7.42 (td, $J = 7.7$ Hz, $J = 1.4$ Hz, 1H, H-6); 6.89 (d, $J = 9.4$ Hz, 2H, H-11, H-13); 6.84 (d, $J = 9.4$ Hz, 2H, H-10, H-14); 5.16 (s, 2H, H-8); 3.69 (s, 3H, H-12'); 2.18 (bs, 6H, H-18, H-20); 2.06 (bs, 3H, H-21, H-23); 1.64 (bs, 6H, H-24, H-26). $^{13}\text{C-NMR}$: 177.32 (C-16); 170.20 (C-1); 153.69 (C-12); 152.29 (C-9); 135.60 (C-3); 133.67 (C-2); 130.78 (C-4, C-5, C-7); 128.50 (C-4, C-5, C-7); 128.34 (C-4, C-5, C-7); 127.81 (C-6); 115.74 (C-11, C-13); 114.58 (C-10, C-14); 68.20 (C-8); 55.36 (C-12'); 54.05 (C-17); 39.71 (C-18, C-20); 35.80 (C-24, C-26); 28.80 (C-21, C-23). FT-IR (ν cm^{-1}): 3182m, 3057w, 2910m, 2879m, 2853w, 2830w, 1680s, 1539vs, 1506vs, 1457m, 1354m, 1304m, 1256m, 1226s, 1151m, 1124m, 1029m, 1026m, 830w, 751m, 730m, 663w. Anal. calcd. for $\text{C}_{26}\text{H}_{30}\text{N}_2\text{O}_3\text{S}$ (450.60): C, 69.30; H, 6.71; N, 6.22; S, 7.12%; Found: C, 69.92; H, 6.86; N, 6.29; S, 7.22%.

Antimicrobial activity

Following the screening of the antimicrobial activity by the disk diffusion method the synthesized compounds (**6a-e**) exhibited no growth inhibition zones. This result could be explained by the fact that the tested compounds were water-insoluble, and therefore, the active compounds did not diffuse in the culture medium, from the deposited spot.

This limitation is overcome in the quantitative assay performed in liquid medium, which allows a better contact between the tested compounds and the microbial cells. The analysis of the MIC values obtained for the tested compounds showed that they exhibited a slightly increased activity on the fungal strain *C. albicans*, as compared with the bacterial strains

(Table I). The most resistant strain was *Enterococcus faecalis*, followed by *Staphylococcus aureus*. The two Gram-negative strains exhibited similar susceptibility

to the tested compounds, as revealed by the same MIC value of 1250 µg/mL.

Table I

The MIC (µg/mL) values of the tested compounds

Microbial strains		Compound – MIC (µg/mL)				
		6a	6b	6c	6d	6e
Gram-positive bacterial strains	<i>Staphylococcus aureus</i> ATCC 25923	2500	2500	1250	1250	1250
	<i>Enterococcus faecalis</i> ATCC 29212	> 5000	> 5000	> 5000	5000	> 5000
Gram-negative bacterial strains	<i>Escherichia coli</i> ATCC 25922	1250	1250	1250	1250	1250
	<i>Pseudomonas aeruginosa</i> ATCC 27853	1250	1250	1250	1250	1250
Fungal strain	<i>Candida albicans</i> ATCC 10231	1250	1250	625	625	625

Study of the influence of tested compounds on the development of bacterial biofilms to the inert substratum revealed that, similarly to the MIC assay, the biofilms formed by the Gram-negative strains proved to be more sensitive to the tested compounds, as compared with the Gram-positive strains they have no anti-biofilm activity (Table II). A slight difference could be noticed in the anti-biofilm efficiency of

the tested compounds in case of the two Gram-negative strains, the compounds **6a**, **6b** and **6e** being more active on *Escherichia coli* biofilm, while **6a**, **6b**, **6c** and **6e** on *Pseudomonas aeruginosa* strain.

The biofilm quantification could not be performed for the fungal strain which did not show biofilm-forming capacity in the experimental conditions.

Table II

The MBEC (µg/mL) values of the tested compounds

Microbial strains		Compound – MBEC (µg/mL)				
		6a	6b	6c	6d	6e
Gram-positive bacterial strains	<i>Staphylococcus aureus</i> ATCC 25923	> 5000	> 5000	> 5000	> 5000	> 5000
	<i>Enterococcus faecalis</i> ATCC 29212	2500	2500	2500	> 5000	2500
Gram-negative bacterial strains	<i>Escherichia coli</i> ATCC 25922	625	625	2500	> 5000	625
	<i>Pseudomonas aeruginosa</i> ATCC 27853	625	625	625	> 5000	625

Figure 2.

Hep-2 cells viability (%) in the presence of different concentrations of the tested compounds

The cytotoxicity activity

The results of the cytotoxicity activity have shown that all tested compounds showed no cytotoxicity at the lowest tested concentration, of 12.5 µg/mL. The cytotoxicity was very low, with HEp-2 cells preserving their viability over 80% at 25 µg/mL concentration. In exchange, at higher concentrations, the cytotoxicity of the tested compounds was variable. At 50 µg/mL

concentration, only compounds **6d** and **6e** proved a low cytotoxicity, as revealed by HEp-2 cells viability over 80%, while in case of the highest tested concentration of 100 µg/mL, only **6d** exhibited an acceptable cytotoxicity.

Thus, the results of the *in vitro* cytotoxicity assay indicate that the compounds **6d** and **6e** proved to be the least cytotoxic (Figure 2).

ADME study

The results of the ADME *in silico* screening are presented in Tables III and IV.

Lipinski's RO5 is a general criterion to assess the drug-like properties of a molecule, with special regard to absorption and permeation. A candidate molecule should have at least 3 of these characteristics: a molecular weight (MW) < 500 g/mol, logarithm of the compound's partition coefficient between n-octanol and water (logP) < 5 (or MlogP < 4.15), less than five hydrogen bond donors (HBD) < 5 and less than ten hydrogen bond acceptors (HBA) < 10 [22].

Four out of five compounds violated only one of Lipinski's rules and one had none violations (Table III). In case of compound **6e**, the presence of a methoxy group proved beneficial for hydrophilic-lipophilic balance of the molecule, fact that also correlates with a lower cytotoxicity in the *in vitro* test.

Table III

Molecular descriptors in relation to Lipinski RO5

Identifier	MW (g/mol)	HBD	HBA	MlogP	Rule of 5 violation
6a	420.577	2	2	4.19	1
6b	438.567	2	3	4.561	1
6c	434.604	2	2	4.395	1
6d	448.631	2	2	4.596	1
6e	450.603	2	3	3.875	0

Bolded values indicate parameters outside the RO5; MW = molecular weight; HBD = hydrogen bond donors; HBA = hydrogen bond acceptors; Moriguchi octanol-water partition coefficient (MlogP).

In order to better understand the pharmacokinetic profile various physicochemical and biopharmaceutical properties were taken in consideration, from water solubility, to permeability through different membranes or percentage of protein binding.

The predicted water solubility values show a poor solubility profile for **6a-e**, all being below the 0.01 threshold. This problem should be addressed in future structural modification by introducing more polar moieties. Thus, being the case of compound **6e** which has a slightly higher water solubility.

Regarding membrane permeability, both parameters effective (Peff) and apparent (MDCK) have values over the recommended range. Usually good human jejunal permeability (Peff) is correlated with passive transcellular diffusion [9].

Blood-brain barrier permeation was determined by using two methods. The quantitative BBB model classifies molecules in two categories: low and high

penetration. Compounds that are predicted to have high penetration are evaluated with the regression model (logBB), which should be over the -0.1 threshold. The good blood-brain barrier permeation profile of **6a-e** recommend them for further studies as central nervous system agents.

In case of more specific pharmacokinetic variables, the synthesized compounds exhibited a volume of distribution and blood-to-plasma concentration ratio within limits. Values of RBD over 1.0 suggests partitioning to erythrocytes. On the other hand, all of the compounds are estimated to have high binding affinity to plasma proteins.

P-gp is an efflux membrane transporter, responsible for regulating cellular uptake and distribution of xenobiotics. It is also implicated in many drug-drug interactions. Thus, not being a substrate for P-gp, reduces the chance for **6a-e** of unwanted drug interactions and possible side effects.

Table IV

Important computed ADME properties

Identifier	Sw	Peff	MDCK	LogBB	BBB	Vd	RBP	F _{up}	Pgp Substr
6a	0.002	3.956	165.627	0.035	High	1.199	0.843	1.864	No (79%)
6b	0.001	3.421	176.017	0.208	High	1.134	0.836	1.647	No (68%)
6c	0.001	3.93	114.948	-0.031	High	1.238	0.801	1.53	No (75%)
6d	0.001	4.19	145.947	0.014	High	1.275	0.806	1.275	No (71%)
6e	0.003	2.907	147.022	-0.003	High	1.028	0.724	1.822	No (71%)

Bolded values exceed the recommended ranges: Sw (mg/mL) recommended range: ≥ 0.010 ; Peff (cm/s $\times 10^4$) recommended range: ≥ 0.5 ; MDCK (cm/s $\times 10^7$) recommended range: ≥ 30 ; LogBB recommended range: > -0.1 ; Vd (L/kg) recommended range: ≤ 3.7 ; RBP recommended range: < 1.0 ; Fup(%) recommended range: $> 10\%$ [9].

Overall, the *in silico* study revealed an ADME profile characterized by low solubility, high permeability and reduced drug interactions. The high permeability is probably induced by the strong lipophilic character of the adamantane moiety.

Conclusions

We synthesized, characterized and bio-evaluated a novel series of N-(1-adamantylcarbamothioyl)benz-amides as potential pharmacological agents.

By variation of the 2-phenoxyethylbenzoic acid and/or of the amine, in this case 1-aminoadamantane, a large series of biologically active products can be obtained.

The method of synthesis is relatively simple. The compounds were easily purified and produced in high yields.

Preliminary biological evaluation revealed that these compounds have no antimicrobial properties.

The reduced cytotoxicity and good ADME profile, especially high permeability through blood-brain barrier recommends these novel benzamides for further studies as potential neurotherapeutic agents.

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