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Adamantane-1-carboxamides: synthesis and antimicrobial activity

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The rapid emergence of resistant bacteria highlights the urgent demand for new effective drugs. In view of the importance of adamantane skeleton in various antimicrobial drugs, herein the synthesis of *N*-adamantane-1-carboxamides of polyamine derivatives is described. The *in vitro* antibacterial activity of the new synthesized compounds against two Gram-positive (*Staphylococcus aureus, Bacillus subtilis*) and two Gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) bacteria, as well as the antifungal activity against *Candida albicans* was assessed. The results revealed that amongst the new synthesized bisamides, *N,N'*-bis-adamantane-1-carboxamide of 1,6-diaminohexane was the most effective one and inhibited both Gram-negative and Gram- positive strains with MIC of 125 μg.ml^{-1}. Moreover, the same amide showed the highest antifungal activity (MIC of 63 μg.ml^{-1}) against *Candida albicans.*

Keywords: Adamantane-1-carboxamides, Antibacterial activity, Antifungal activity

INTRODUCTION

The global emergence of infectious diseases caused by viruses, bacteria, fungi and protozoa represents a particularly worrying trend. The high rate of morbidity and mortality is a direct result of these infections. According to the World Health Organization (WHO) lethal outcomes from common infectious diseases (measles, tuberculosis, malaria, AIDS, respiratory and diarrheal diseases, etc.) account for more than 85 % [1].

In this regard, drug resistance has been recognized as a major health concern. A leading strategy to fight against resistant pathogens is the development of new effective antimicrobials.

Adamantane analogues have long been known for their manifold pharmacological activities. Since the discovery of 1-aminoadamantane (amantadine) and its methyl analogue (rimantadine) as effective M2 inhibitors against influenza virus type A [2], more attention has been paid to the adamantane scaffold. Despite the rapidly acquired resistance to M2-blockers and later to the second approved class of drugs (neuraminidase inhibitors-oseltamivir, zanamivir), the combination therapy of both class of inhibitors represents a good option for the control of resistant influenza viral infections [3]

In addition to the antiviral activity, several newly adamantyl analogues have been found to possess bactericidal or fungicidal activities [4-9]. Nowadays, the role of the adamantyl moiety as an essential pharmacophore in biologically active molecules is well known. The incorporation of adamantyl nucleus in molecules could substantially affect their lipophilicity, pharmacological properties and biological activity. Hence, adamantane could positively modulate the therapeutic index of the parent molecule and has been widely used in designing of agents with potential antimicrobial activity.

Other group of compounds that are considered in designing of invaluable chemotherapeutics are polyamines. Biogenic polyamines such as putrescine, spermidine, spermine and cadaverine are the most widely distributed and indispensable components of the living cells [10]. These linear aliphatic molecules possess: two primary (terminal) amino groups, in most cases – with one or more imino groups. Consequently, their basic groups are fully protonated under physiological pH 7 and could further interact with anions and negatively charged sites in cell components [11]. Moreover, these nitrogen containing organic constituents are also known to be involved in cell metabolism, division and differentiation [12].

On the other hand, being endogenous modulators of porin channel function, polyamines influence outer membrane permeability of bacteria [13]. Thus, they induce resistance to different antibiotics (e.g. cationic peptide, aminoglycoside, and quinolone antibiotics) [14]. Several studies

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have clearly indicated that some polyamines (cadaverine or spermine) can reduce bacterial susceptibility to antibiotic treatments [15-17] by decreasing outer membrane permeability.

Interestingly, Kwon *et al.* have shown that exogenous natural polyamines at millimolar levels can enhance the susceptibility of *P. aeruginosa* [14]. However, contradictory results have been obtained by Vaara *et al*., who concluded that polyamines had neither bactericidal nor sensitizing activity at sub-millimolar concentration [18].

It was recently reported that certain molecules including polyamine scaffold can serve as an efficient alternative mechanism to reduce the development of resistance by affecting membrane depolarization/or integrity membrane disruption [19]. In the same context, the importance of lipophilic functionalized polyamines for permeabilisation of an outer membrane of Gram-negative bacteria have been published by Katsu *et al.* [20]. Moreover, significant antimicrobial activities have been reported for polyamines conjugated to cholesterol, cholenic acid and bile acids [21-26].

Considering the potential impact of the hydrophobicity of the adamantane skeleton and polyamine chain as critical factors for the antimicrobial activity, herein we report the synthesis and *in vitro* antibacterial and antifungal activities of novel hybrid molecules consisting of these two fragments.

EXPERIMENTAL

General information

All chemicals used in this study were purchased from Sigma-Aldrich (FOT, Bulgaria). Synthesized compounds were purified by column chromatography using silica gel (Acros Organics, mesh 35-70) and identified by TLC, IR, NMR, and MS analysis. TLC was carried out on silica gel $60F_{254}$ (Merck) precoated aluminium plates. Melting points were determined using an apparatus „Stuart SMP10“ and are uncorrected. Attenuated total reflectance infrared spectroscopy (ATR-IR) measurements were performed using Thermo Scientific Nicolet iS10 FT-IR device with ID5 ATR accessory (diamond crystal). NMR spectra were recorded on a Bruker Avance III 400 spectrometer in dimethyl sulfoxide-d_6 (DMSO-d_6) solution and referenced to the solvent resonance peak at 2.49 ppm. One-dimensional (1D: ^{1}H, ^{13}C) and two-dimensional (2D: ^{1}H/^{1}H COSY, ^{1}H/^{13}C HSQC) NMR spectra were acquired using standard pulse sequences and experimental conditions. The spectra were recorded at a temperature of 25 °C and spectral width of 5000 Hz and 20000 Hz for ^{1}H and ^{13}C, respectively. The ESI mass spectra were recorded on an Esquire3000 plus instrument.

General procedure for synthesis of amides

The amide bond formation in the target carboxamides (**1–3**) was carried out under mild conditions by means of the coupling method EDC/HOBt, as described previously by us [27].

The physico-chemical parameters and the IR, NMR and MS spectral data of the compounds **1-3** and **1a** are as follows:

N^1-Adamantanoyl-N^6-(t-buthyloxycarbonyl)--1,6-diaminohexane

AdA-NH-$(CH_2)_6$-NH-Boc *(compound **1**);* Yield: 35 %; mp~127-129^{o}C; IR (ATR)u_{max}: 3320, 2965, 2902, 1681, 1628, 1533, 1450, 1388, 1363, 732 cm^{-1}; ^{1}H-NMR (DMSO-d_6, ppm): δ 1.23 (br. s, 4H, 2 x -CH_2-), 1.31 (s, 9H, 3 x –C$(CH_3)_3$), 1.38 (br. s, 4H, 2 x -CH_2-), 1.69 (12H, 6 x -CH_2-), 1.89 (br. s, 3H, 3 x >CH-), 3.11 (br. s, 4H, 2 x >NCH_2-), 7.24 (2H, 2 x >NH).

N^1,N^6–bis-Adamantanoyl-1,6-diaminohexane

AdA-NH-$(CH_2)_6$-NH-AdA *(compound **2**);* Yield: 22 %; mp ~ 206-208^{o}C; IR (ATR)u_{max}: 3318, 2900, 2848, 1710, 1630, 1547, 1447, 724 cm^{-1}; ^{1}H-NMR (DMSO-d_6, ppm): δ 1.20 (br. s, 4H, 2 x -CH_2-), 1.35 (br. s, 4H, 2 x -CH_2-), 1.64 (12H, 6 x -CH_2-), 1.73 (12H, 6 x -CH_2-), 1.94 (br. s, 6H, 6 x >CH-), 3.00 (br. s, 4H, 2 x >NCH_2-), 7.20 (2 x >NH); ^{13}C-NMR (DMSO-d_6, ppm): δ 25.8 (2 x -CH_2-), 27.6 (6 x >CH-), 29.02 (2 x -CH_2-), 36.1 (6 x -CH_2-), 38.2 (2 x >NCH_2-), 38.7 (6 x -CH_2-), 176.9 (-C(O)NH-); ESI-MS: 441.5 $[M+H]^+$, 463.4 $[M+Na]^+$, 479.4 $[M+K]^+$.

Boc-Oseltamivir Carboxamide of N^1-adamantoyl-1,6-diaminohexane

Boc-Os-NH-$(CH_2)_6$-NH-AdA *(compound **3**);* Yield: 24 %; m p ~ 144-146^{o}C; IR (ATR)u_{max}: 3294, 3079, 2906, 2851, 1686, 1626, 1525, 1453, 1390, 1366, cm^{-1}; ^{1}H-NMR (DMSO-d_6, ppm): δ 0.87 (t, J= 7.52 Hz, 3H, -CH_2CH_3), 0.93 (t, J= 7.34 Hz, 3H, -CH_2CH_3), 1.32 (m, 4H, 2 x -CH_2CH_3), 1.46 (br. s, 4H, 2 x -CH_2-), 1.49 (br. s, 4H, 2 x -CH_2-), 1.74 (s, 9H, -C$(CH_3)_3$), 1.84 (s, 3H, -C(O)CH_3), 1.87 (12H, 6 x -CH_2-), 2.04 (br. s, 3H, 3 x >CH-),), 2.31 (m, 1H, =CCH_{2a}-), 2.58 (dd, J=17.7, 5.0 Hz, 1H,=CCH_{2b}-), 3.11 (m, 1H, -NH-CH<), 3.16 (m, 1H, -NH-CH<), 3.42 (br. s, 4H, 2 x >NCH_2-), 3.46 (m, 1H, -OCHC=), 4.11 (m, 1H, -

OCH<), 6.37 (d, J=8.70 Hz, 1H, $H_3CC(O)NH$-), 6.54 (br.s, 1H, =CH-), 7.39 (d, J=9.17 Hz, 1H, -C(O)NH-), 7.86 (m, 1H,-C(O)NH-), 7.99 (m, 1H,-C(O)NH-); ESI-MS: 545.6 $[M+H-Boc+H]^+$, 645.6 $[M+H]^+$, 667.7 $[M+Na]^+$, 683.7 $[M+K]^+$.

Removal of the tert-butyloxycarbonyl (Boc) protecting group [28]

Deprotection of **AdA-NH-$(CH_2)_6$-NH-Boc** (compound **1**) to **AdA-NH-$(CH_2)_6$-NH_2.TFA** (compound **1a**) was readily achieved by 50% (v/v) TFA in CH_2Cl_2.

N^1-Adamantanoyl-1,6-diaminohexane.TFA salt

AdA-NH-$(CH_2)_6$-NH_2.TFA *(compound 1a);* Yield: 69%. ESI-MS: 279.5 $[M+H]^+$.

Microbiology

The antibacterial activity was tested against *Staphylococcus aureus 209* (G+), *Bacillus subtilis 1A95* (G+), *Pseudomonas aeruginosa 5749* (G-), *Escherichia coli WF+* (G-); the antifungal activity was tested against the pathogenic fungus *Candida albicans 5*62. All microorganisms were obtained from the Bulgarian National Collection for Microorganisms and Cell Cultures (NBIMCC).

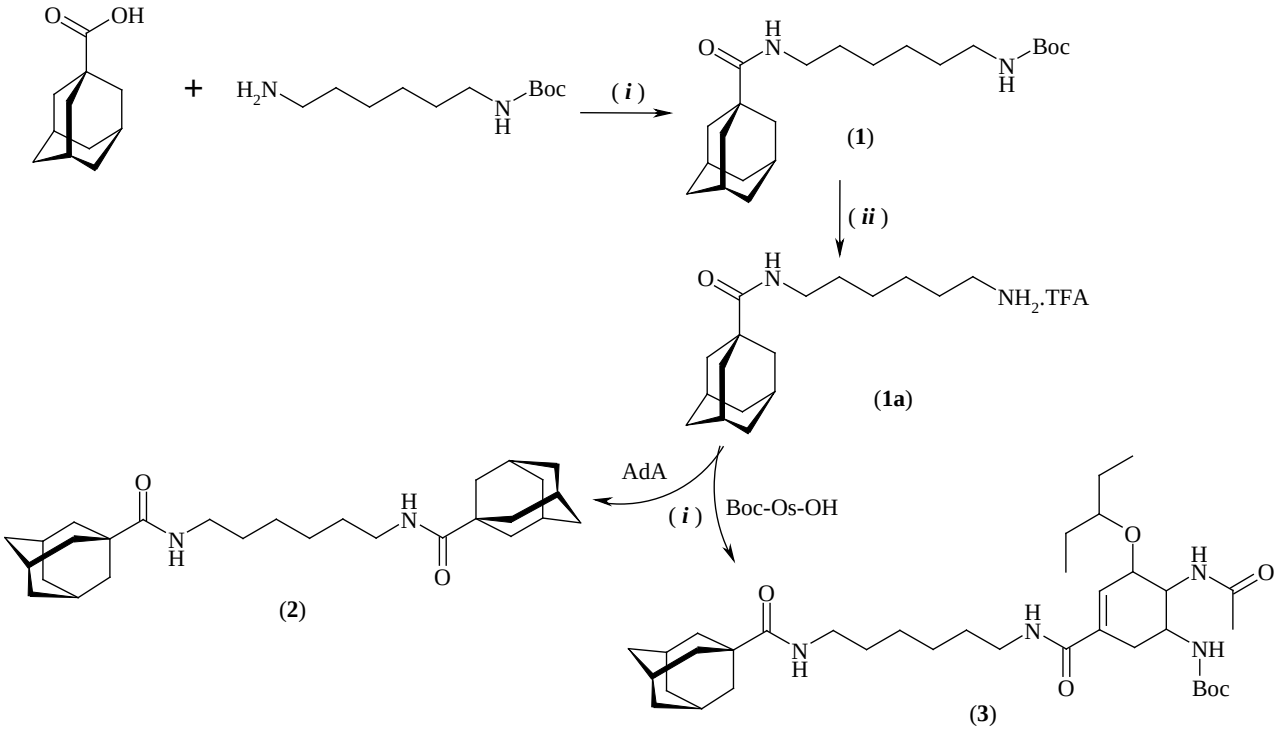
The minimal inhibitory concentration (MIC) of all samples was determined by the microdilution method described by Andrews [29]. Briefly, 50 µl of twofold diluted serial dilutions of the examined samples were added to 50 µl of microbial suspension adjusted to yield approximately 1.0×10^5 CFU ml^{-1}. MIC was determined as the lowest concentration of the examined sample that inhibits the visible microbial growth after 24 h incubation at 37°C. For positive controls commercially available antibiotics tobramycin and ketoconazole were used. The solvent DMSO was tested as negative control. Three replicates were done for each compound.

In the course of our program directed towards the design and development of novel adamantane derivatives with a potential antimicrobial and antiviral activity and better therapeutic index, we synthesized adamantane analogues, comprising hydrophobic (adamantane) and cationic (1,6-diaminohexane) components. 1,6-Diaminohexane was used considering the early reported properties of polyamines to inhibit the growth of various microorganisms [30] and their effect on antibiotic susceptibility in bacteria [19, 31].

Despite a wide range of different peptide methods are used for the amide bond formation, for the preparation of the new adamantane carboxamides we have chosen the method employing *N*-ethyl-*N*'-dimethylaminopropyl carbodiimide (EDC) as a coupling reagent [32]. The advantage of this coupling reagent in the peptide chemistry is well known and is due mainly to the formation of water-soluble urea, which facilitates the isolation of the reaction products. For acceleration of the reaction an efficient additive as HOBt was added.

However, the derivatization of polyamines often encounters problems with respect to the selective protection of amino functionalities. Therefore, herein we used commercial available mono-Boc (*tert*-butyloxycarbonyl) protected diaminohexane. It is well known that this urethane group can be easily removed under strong acid conditions.

The synthetic strategy towards preparing the target adamantane-1-carboxamides **2** and **3** is presented in Scheme 1. The three-stage synthetic procedure was based on a method recently reported by us for formation of amide bond under mild experimental conditions [27] and following coupling of the obtained amides with 1-adamantane caboxylic acid (AdA) or *N*-Boc-oseltamivir carboxylic acid (Boc-Os-OH). The reactions at all stages were monitored by TLC and the products were purified by column chromatography using silica gel using mobile phase composed of CH_2Cl_2:CH_3OH. The structures of the targeted compounds **1-3** were confirmed by melting points, NMR, IR and MS analysis.



Scheme 1. (***i***) EDC/HOBt, CH_2Cl_2; ***(ii)*** 50% TFA/CH_2Cl_2; **AdA**: 1-adamantane carboxylic acid; **Boc-Os-OH**: *N*-Boc-oseltamivir carboxylic acid

The first stage implies solution phase (EDC/HOBt) coupling of 1-adamantane carboxylic acid with *N-tert*-butyloxycarbonyl-1,6-hexanediamine.

As a result, the *N*-(Boc-aminohexyl) adamantane-1-carboxamide (**1**) after purification by column chromatography was obtained in a good yield.

The formation of the amide **1** was confirmed by the presence of characteristic resonance signals for the amide and urethane NH protons (7.24 ppm, 2H, >NH) and methyl-, methyne- and methylene protons (3.11 to 1.23 ppm) in the ^{1}H NMR spectra.

IR spectroscopy further confirmed the formation of amide bond by means of very strong absorption (C=O str.) at 1628 cm^{-1} (amide I) and amide II band caused by N-H bending at 1533 cm^{-1}.

The next stage involved deprotection of **1** (cleavage of the Boc group) by TFA in CH_2Cl_2, which allowed the isolation of the semi-product **1a** as TFA salt. Accordingly, the latter was further linked with 1-adamantane carboxylic acid (AdA) or *N*-Boc-oseltamivir carboxylic acid (Boc-Os-OH) through the use of the same coupling reagent EDC/HOBt, which afforded the corresponding symmetrical (**2**) and unsymmetrical (**3**) diamides.

Besides the common infrared characteristic amide group frequencies and characteristic absorption of hydrocarbons derived from adamantyl and diaminohexyl moieties (amides **2, 3**), the IR spectrum of compound **3** shows the specific frequency of the double-bond stretching vibration of a cyclohexenyl skeleton at 1686 cm^{-1}.

The structures of the targeted compounds **2** and **3** were confirmed by ^{1}H and ^{13}C NMR analysis. The complete assignment of the resonance signals in the spectra is presented in the Experimental part. The chemical shift and integral intensity of the observed resonance signals in the ^{1}H NMR spectra unambiguously demonstrated the formation of amide bond and incorporation of two adamantyl (AdA) moieties in **2** and adamantyl- and *N*-Boc-oseltamivir moieties (Boc-Os-OH) in **3**. The results from the analysis of the ^{13}C NMR spectra were consistent with those from ^{1}H NMR.

Additional information was gained from mass spectral data. The positive-ion ESI-MS of both compounds **2** (Mw=440.6) and **3** (Mw=644.8) displayed the corresponding intense $[M+Na]^+$ peaks at *m/z* 463.4 and *m/z* 667.7, accompanied by smaller signals for the $[M+H]^+$ and $[M+K]^+$ peaks. In the spectrum of compound **3** Boc-characteristic ion at *m/z* 545.6 $[M+H-Boc+H]^+$ was also observed.

Evaluation of antimicrobial activity in vitro

Inspired by 'Lipophilic bullet' of adamantane skeleton for providing novel antimicrobials, herein the synthesized adamantane-based diamino derivatives were estimated for their antimicrobial activity *in vitro*.

The antibiotic tobramycin and the antifungal drug ketoconazole were used as positive controls. The minimum inhibitory concentrations (MIC) of the tested compounds are summarized in Table 1. The results of antimicrobial screening indicate that compounds have demonstrated low to moderate antibacterial activity.

Surprisingly, amongst the tested amides, compound **2** has shown to be the most active (MIC = 125 μg/ml) against all tested Gram-positive and Gram-negative bacterial strains. The incorporation of two adamantyl residues in the molecule most probably affects its lipophilicity and enhances its penetration within the cellular membrane. The modification of 1,6-diaminohexane with two adamantyl residues (molecule **2**) suggests that an optimum hydrophobic interaction may be essential for the established activity. On the other hand, the results from the *in vitro* biological test (Table 1) show that the amides **1**, **1a** and **3** exhibit almost the same activity against different bacterial strains and fungus tested, which is significantly lower than that of compound **2**.

Moreover, the terminal free amino group in the amide **1a** seems to be not crucial for the antimicrobial activity (except for *S. aureus*) as compared with its *N*-Boc protected analogue (**1**).

Regarding the antifungal activity, compounds **1**, **1a** and **3** can be considered not to be so active against the pathogenic fungus *C. albicans* (with MIC of 313 μg/ml). Exception is bis-adamantyl carboxamide **2**, which shows MIC of 63 μg/ml. Considering the apparent antifungal activity of compound **2** amongst the other synthetic ones, it could be attributed to the presence of an additional lipophilic motif in its structure.

Finally, the activity of molecule **2** was 8-fold lower than that of the used antifungal drug ketoconazole.

CONCLUSIONS

In conclusion, two novel adamantane-1-carboxamides (**2**, **3**) were obtained, comprising a diamine linker. The antimicrobial activity of the covalently bonded hybrid structures was evaluated *in vitro*. The most active was the *N,N'*-bis-adamantane-1-carboxamide of 1,6-diaminohexane (compound **2**) with regard to the structure cell wall peculiarities of G+, G- bacteria and the fungus *Candida albicans*. All others compounds demonstrated low to moderate antibacterial activity with moderate antifungal activity.

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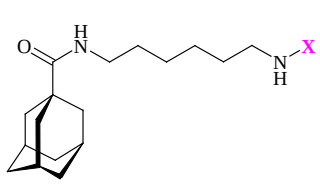
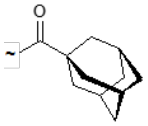
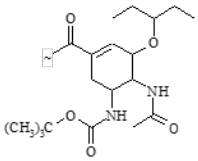
Table 1. *In vitro* antimicrobial activity of synthetic adamantane-1-carboxamides

Compound		X	MIC (μg/ml)				
			S. aureus	*E. coli*	*P. aerug.*	*B. subt.*	*C. albicans*
	1)	-Boc	625	625	313	625	313
	1a)	H	156	625	313	625	313
	**2)**		125	125	125	125	63
	3)		625	313	625	625	313
Tobramycin			15.6	19.5	NT	1.0	NT
Ketoconazole			NT	NT	NT	NT	7.8

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