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Synthesis and antimicrobial activity of Bis-Derivatives of 3a′, 6a′Dihydro-2'H-Spiro[Indole-3,1'-Pyrrolo[3,4-c]Pyrrole]-2,4',6'(1H, 3'H, 5'H)-Trione

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ABSTRACT

The interaction of isatins, α-amino acids and 1,6-bismaleimidohexane has been studied. It was found that for 1'-R^3-2'-R^2-3'-R^1-5'-(6-{1'-R^3-2'-R^2-3'-R^1-2,4',6'-trioxo-1,2,3',3'a,4',5',6',6'a-octahydro-2'H-spiro[indole-3,1'-pyrrolo[3,4-c]pyrrole]-5'-yl}hexyl)-1,2,3',3'a,4',5',6',6'a-octahydro-2'*H*-spiro[indole-3,1'-pyrrolo[3,4-c]pyrrole]-2,4',6'-triones were formed through three-component 1,3-dipolar cycloaddition of azomethine ylides generated *in situ* by the decarboxylative condensation of isatin and some *α*-amino acids with dipolarophile 1,6-bismaleimidohexane. This method has the advantages of mild reaction conditions, high atom economy and excellent yields. The most suitable conditions for this reaction are boiling alcohol-water medium. The obtained compounds showed a weak selective antimicrobial activity for the *Micrococcaceae* family.

INTRODUCTION

Isatin (**1**) and its derivatives are highly effective initial substrates for the synthesis of a wide range of heterocyclic compounds, particularly derivatives of 2-spiro-oxindole (Joaquim *et al*., 2001; Pandeya *et al*., 2005). The analysis of international experience shows the great attention of researchers to isatin as an initial synthon for the synthesis of spiroheterocyclic platform (Pavlovska *et al*., 2016; Singh and Desta, 2012), which has been named privileged structure for the synthesis and search for new biologically active compounds, in particular, novel antimicrobial agents (Ball-Jones *et al*., 2012). Thus, compounds with antibacterial action (Pandeya *et al*., 1999; Rane *et al*., 2016) and antiviral activity against smallpox virus (Pirrung *et al*., 2005) were discovered among derivatives of isatin; *bis*-Schiff base-based isatin exhibit a wide spectrum of antibacterial, antiviral and antifungal activity (Aliasghar *et al*., 2007). Some isatin β-phenylhydrazones approached to the level of antimicrobial activity against Mycobacterium tuberculosis H37Rv streptomycin and ciprofloxacin in vitro (Karki *et al*., 2011). Some novel isatin derivatives of sultams have been synthesized and these compounds possess significant antibacterial and moderate antifungal activities (Harikrishna and Ravindranath, 2015). In order to enhance the antibacterial action of fluoroquinolones, Mannich bases were synthesized on the basis of isatin, ciprofloxacin and lomefloxacin, respectively (Pandeya *et al*., 1998).

Derivatives of spiro[indole-3,2'-pyrrolo[3,4-c]pyrrole] (**3**) have bioisosteric properties with regard to their natural prototypes to the spiro[pyrrolidine-3,3'-oxindole] (**2**) core in several alkaloids (**5-6**) and their spiro structure is not flat, which provides for a much greater affinity to biotargets (Syumka *et al*., 2015).

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That is why the synthetic methods lead to these compounds, their chemical and pharmacological properties are of great interest in the last few decades (Yu *et al*., 2015). In addition, some 3,3'-pyrrolidinyl-dispirooxindole compounds have also been demonstrated to show promising bioactivities, including antimicrobial (**7**, **10**) (Babu *et al*., 2008) and anticancer (**8**) (Arun *et al*., 2013) properties. Due to their unique three-dimensional structural features, spirooxindoles have been identified as privileged chemotypes for antiviral drug development, e.g. (**9**) inhibitor activity against HIV (Ye *et al*., 2016) (Figure 1). At the same time, antimicrobial potential synthetic dispirooxindolic compounds have not been investigated.

In particular, the key idea for drug-design of new chemotherapeutic agents based on synthetic spirocyclic platform is the possibility of introducing further aliphatic substituents located in the areas of the ATP-binding pocket above or below the plane of the interaction with the hinge elements which are present in certain microbial cell kinases (Osolodkin *et al*., 2013). Because the vast majority of inhibitors of microbial enzymes known to date comprise planar or flattened structures, they cannot bind with specific biological target cavities above or below the plane of the molecule, which does not contribute to the selectivity of the inhibition.

Considering all the above-stated facts, the search for new antimicrobial agents among derivatives of spiro[2-oxindole-3,2'-pyrrole] (**3, 8-10**) is a promising direction for the development of new drugs having antimicrobial action.

The aim of this work is as follows: synthesis and search for drug-like molecules and estimation of their antimicrobial potential among hexamethylene-N,N'-bis-derivatives of spiro[indole-3,2'-pyrrolo[3,4-c]pyrrole] synthesized for the first time. The study compounds were obtained by multi-component one-pot condensation of isatins, α-amino acids and 1,6-bismaleimidohexane.

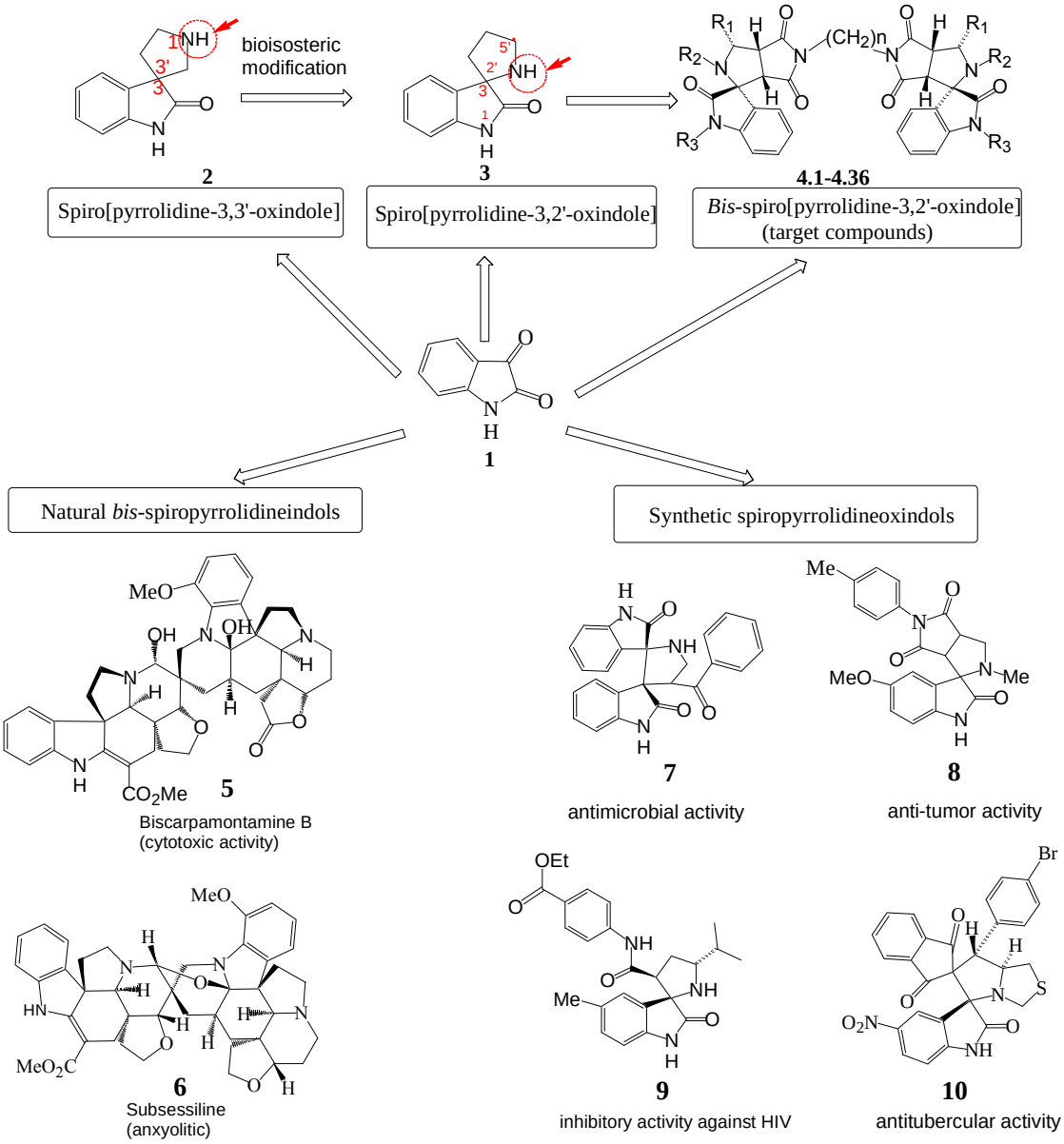


Fig. 1: Background for the synthesis of the target compounds

MATERIALS AND METHODS

Starting isatins and *bis*-hexamethylenemaleimide were obtained from commercial sources and used without further purification. Melting points were obtained on a Gallenkamp melting point apparatus, Model MFB-595 in open capillary tubes. ^{1}H NMR and ^{13}C NMR spectras were recorded on a Varian WXR (200 MHz) spectrometer in DMSO-d_6 using TMS as an internal standard (chemical shifts in parts per million); the references at the signal of the solvent 39.5 ppm for DMSO-d_6. Elemental analyses were carried out using Carlo Erba CHNS-O EA 1108 analyzer. Mass spectra were taken usang a Varian 1200L DIP (EI, 70 eV).

The progress of reactions and purity of the obtained compounds were monitored by TLC on Silufol UV-254 plates in the systems acetone-heptane, 4:1, H_2O–MeOH, 1:9. Commercially available reagents and solvents were used without additional purification.

Method for preparation of 1'-R_3-2'-R_2-3'-R_1- 5'-(6-{1'-R_3-2'-R_2-3'-R_1-2,4',6'-trioxo-1,2,3',3'a,4',5',6',6'a-octahydro -2'H-spiro [indole-3,1'-pyrrolo[3,4-c]pyrrole]-5'-yl}hexyl)- 1,2,3',3'a,4',5',6', 6'a-octahydro-2'H-spiro[indole-3,1'-pyrrolo[3,4-c]pyrrole] -2,4',6'-triones (4.1-4.36)

A mixture of the corresponding isatins **1** (2 mmol), α-amino acid **2** (2 mmol), and 1,6-bismaleimidohexane (1 mmol) in a mixture of *i*-PrOH–H_2O (3:1, 4 ml) was refluxed for 3-8 h, the reaction progress was checked by TLC and by changes in the reaction mixture color from red to yellow. The solution was cooled and placed into a freezer at -5^{o}C for 24 h for compounds **4.1-4.36**, or obtained precipitate was filtered off, washed with *i*-PrOH, and recrystallized, or the reaction mixture was then poured onto ice, and resulting precipitate was filtered and recrystallized from mixture of DMF–EtOH (1:1).

Antimicrobial activity study

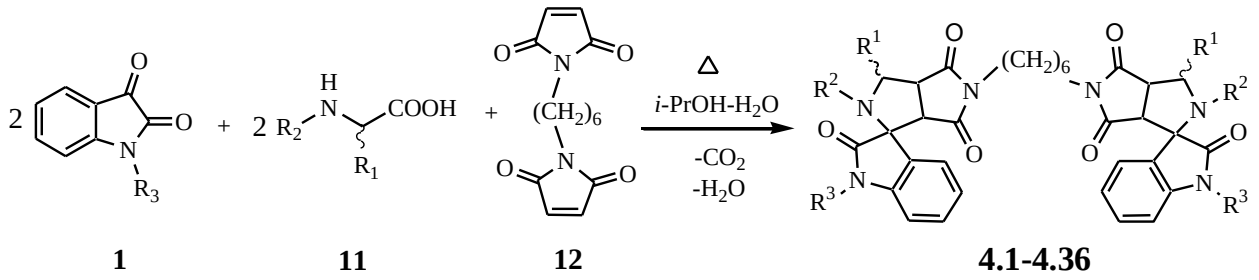
Microbiological experiment was performed by the Microbiology Department of Donetsk National Medical University (Ukraine). According to the WHO recommendations (Balouiri *et al*., 2016; Patel *et al*., 2015), the following strains of microorganisms were used to estimate the activity of the tested compounds: *Escherichia coli ATCC 25922, Enterobacter cloacae №487, Klebsiella pneumoniae No. 247, Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus ATCC 25923, Staphylococcus epidermidis №235, Candida albicans* ATCC653/885. Inoculum's density was 107 cells per 1 mL of media and was determined by comparing with McFarland standard. 18 to 24-hour old culture of the microorganism was used for the test. The studied compounds were introduced as 0.3 ml DMSO (100 µg/mL) solution aliquots. To prepare serial dilutions of substances, solution of basic solids synthesized in a solvent dimethylsulfoxide (DMSO) were prepared. Then we prepared a series of two-fold dilution working solution in sterile distilled water at concentrations 10 times higher than the maximum concentration required for this study. The pH value of the initial solution was about 6.5 units. The inhibiting effect (Minimum Inhibitory concentration (MIC) value) of the substances on microorganisms was determined using the method of serial dilutions in dense nutrient medium – meat-peptone agar (MPA). The MIC values were recorded after incubation at 28±2°C for 72hrs. Solutions were aseptically brought to bottles with autoclave-sterilized and cooled to 50˚C agar (1 part of the substance and 9 parts of the agar), and poured in Petri dishes with a layer thickness of 3-4 mm. The inoculum suspension was prepared using the Densi-La-Meter apparatus (made by PLIVA-Lachema, Czech Republic; 540-nm wavelength). The cultures were synchronized using the low temperature conditions (4 ^{o}C). When the agar hardened, a drop of prepared suspensions of different strains of microorganisms at a concentration 107 CFU/ml was placed onto the surface of agar using a bacteriological loop (with the loop diameter of 3 mm). The corresponding concentration was prepared by 10-times dilution of the initial suspension of microorganisms equalling to 0.5 units by the McFarland turbidity scale (108 CFU/mL). Mueller-Hinton agar was employed (HIMedia Laboratorles Pvt. Ltd India) for bacteria. The strain of *Candida albicans* was cultivated using Sabouraud agar (HIMedia Laboratorles Pvt. Ltd India).

All molecular descriptors were calculated using the Molinspiration Cheminformatics v2016.09 software system, 2016 (Molinspiration property engine v2016.10, Bratislava University, Slovakia) available online at http://www.molinspiration.com. The authors used a standard IBM PC-compatible personal workstation (PIV CPU clocked at 1.4 GHz, 512 MB RAM) running under Windows 2000 and Microsoft Office 2003.

RESULTS AND DISCUSSION

For this purpose, we synthesized bis-derivatives of 1,6-hexyliden 3a',6a'-2'*H*-dihydro-spiro[indole-3,1'-pyrrolo[3,4c] pyrrole]-2,4',6'(1*H*,3'*H*,5'*H*)-trione and studied antibacterial properties of 36 synthesized compounds vs. standard test strains and determined their antimicrobial potential.

Three-component condensations of isatin with amino acids and dipolarophiles containing an electron withdrawing substituents activated π-bond is the most promising method for forming the spiro heterocyclic system of [indole-3,2'-pyrrolo[3,4-c]pyrrole] (*Pavlovskaya et al. 2013*). Therefore, this approach has been used by us for the synthesis of target symmetric bis-spiro oxindole derivatives (**4.1-4.36**). The target **4.1-4.36** bis-derivatives were obtained using a *one-pot* procedure and three-component domino cyclocondensation of isatins **1** (R^3=H, CH_3, All, Bn) between α-amino acids **11** (sarcosine, glycine, *L*-proline, *L*-serine, *L*-valine, *L*-isoleucine, *L*-phenylglicine, *L*-phenylalanine, *L*-thyrosine, *L*-methyonine, *L*-glutaminic acid and *L*-glutamine) and 1,6-bismaleimidohexane **12**. The mechanism of interaction is the formation of unstable azomethine-ylides *in situ* as a result of decarboxylation of α-amino acids and isatin products, with subsequent 1,3-dipolar cycloaddition of the olefinic bond by symmetrical dipolarophile **12**.



1 **11** **12** **4.1-4.36**

Fig. 2: Synthesis of 1'-R_3-2'-R_2-3'-R_1-5'-(6-{1'-R_3-2'-R_2-3'-R_1-2,4',6'-trioxo-1,2,3',3'a,4',5',6',6'a-octahydro-2'H-spiro[indole-3,1'-pyrrolo[3,4-c]pyrrole]-5'-yl}hexyl)-1,2,3',3'a,4',5',6',6'a-octahydro-2'H-spiro[indole-3,1'-pyrrolo[3,4-c]pyrrole]-2,4',6'-triones **(4.1-4.36)**

Table 1: Optimization of solvent effect on the model reaction[a].

Solvent	Time	Yield [b] (%)
Isopropanol-water (3:1)	30 min	88
Ethanol-water (3:1)	1.5 h	80
Acetonitrile-water (3:1)	2 h	75
Tetrahydrofuran (THF)	8 h	26
1,4-Dioxane-water (3:1)	6 h	60

[a] Reaction conditions: isatin (0.5 mmol), *L*-valine (0.5 mmol) and 1,6-bismaleimidohexane (1 mmol) in solvent (10 mL) at reflux temperature; [b] Yields of isolated product **4.11**.

It was found that the regioselectivity of this reaction depends on the ratio of reagents. So, symmetrical bis-derivatives **4.1-4.36** can be obtained only at a molar double excess **1** and **11** in moderate to excellent yields (Figure 2, Table 1).

The reaction time largely depend on the reactivity of the employed α-amino acids. The longest reaction time (5-7 h) was found for sarcosine and glycine (5h), while the fastest reaction (30-45 min) was found for *L*-valine, *L*-isoleucine and *L*-proline as substrates. Selecting an appropriate solvent is of critical importance for effective synthesis. To optimize the reaction solvent, the reaction of isatin, *L*-valine, and 1,6-bismaleimidohexane was carried out in different organic solvents such as mixture (1:3) of isopropanol, ethanol, acetonitrile, 1,4-dioxane with water and THF at reflux condition. Water is desired to better dissolving of the *L*-amino acids in the reaction mixture. The results are summarized in Table 1. It was shown that the reactions in refluxing isopropanol-water (3:1) delivered the best results. This method has the advantages of mild reaction conditions, high atom economy and excellent yields.

In our case, spirooxindols **4.1-4.36** are exclusively formed. All new cycloadducts obtained by the above method were characterized by mass spectrometry, ^{1}H and ^{13}C NMR for compounds **4.1**, **4.3**, **4.19**, **4.34** (Table 2).

Signals for all proton-containing fragments are present in ^{1}H NMR spectras of compounds **4.1-4.36** (Table 2). Assignment of the signals of NH and OH groups were made with the aid of deuterium exchange with D_2O. The NH-proton of the oxindole moiety appeared as a singlet between 10.38–10.86 ppm. The resonance of the methine protons of the pyrrolo[3,4-c]pyrrole system was displayed as a doublet at 3.40-3.50 ppm for the H-6a' proton, a triplet at 3.50-3.60 ppm for the H-3a' proton, and a multiplet for the H-3' proton, which was located at 4.00-4.40 ppm in the spectra of compounds **4.1-4.36**. Values of the coupling constants of the H-3a' and H-6a' protons and of H-3' and H-3a' amounted to 7-8 *Hz*, which may point at their *cis* orientation. The benzylic (3'-C$\underline{H}_2$Ar) protons in compounds **4.5, 4.19, 4.20** were detected as multiplets with H-3' protons at δ 4.10-4.25 ppm and the benzylic (N-C$\underline{H}_2$Ar) **4.25-4.31** are exhibited a singlet at δ 4.65-5 ppm. The sharp singlet at δ 3.01-3.10 due to the N-methyl protons was seen for componds **4.32-4.36**. The signals of aliphatic protons of hexamethylene residue, which are observed as signal of CH_2CH_2 groups two multiplets at δ 0.83-1.29 and δ 1.11-1.75 ppm and the signal of 5'-NCH_2 two fragments is present in the region from δ 2.53 up to 3.52 ppm. The main feature of the ^{13}C spectra of compounds **4.1-4.36** is the presence of the signal of the 3C-spiro nucleus. For example, in the ^{13}C-NMR spectrums of compounds **4.1, 4.3, 4.19, 4.34**, the signal at δ 67-68 ppm due to the spirocarbons was observed.

Table 2: The properties of 1'-*R_3*-2'-*R_2*-3'-*R_1*-5'-(6-{1'-*R_3*-2'-*R_2*-3'-*R_1*-2,4',6'-trioxo-1,2,3',3'a,4',5',6',6'a-octahydro-2'*H*- spiro[indole-3,1'- pyrrolo[3,4-c]pyrrole] -5'-yl}hexyl)-1,2,3',3'a,4',5',6',6'a-octahydro-2'*H*-spiro[indole-3,1'-pyrrolo[3,4-c]pyrrole]-2,4',6'-triones (**4.1-4.36**).

Compound. №	Substituents	Reaction time, Spectral and Characterization data
4.1.	R^1=Ph, R^2=R^3=H	Compound **4.1.**: time 1 h; 75, % yield; 190 ^{o}C *; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.29 (m, 4H, CH_2CH_2), 1.43 (m, 4H, CH_2CH_2), 3.59-3.86 (m, 4H, 2×6'a-C$\underline{H}$, 2×5'-NC$\underline{H}_2$), 4.07-4.20 (2H, 2×3'a-CH, *J*=7.01 Hz), 4.34 (d, 2H, 2×2'-NH, *J*=4.27 Hz), 5.40-5.52 (m, 2H, 3'-CH), 6.98 (s, 5H, ArH), 7.21 (m, 9H, ArH), 7.34 (m, 4H, ArH), 10.38 (s, 2H, 1-NH) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6) δ 25.30, 25.60, 25.74, 27.12, 27.76, 37.67, 38.68, 38.89, 39.09, 39.51, 39.72, 39.93, 49.34, 50.59, 59.45, 61.84, 67.11, 109.08, 120.79, 126.42, 126.91, 127.14, 127.33, 127.40, 128.05, 128.83, 134.27, 139.29, 142.13, 174.46, 174.98, 180.67 ppm; EI-MS for $C_{44}H_{40}N_6O_6$ (M+1) 748.8.

4.2.	R^1-R^2=$(CH_2)_3$, R_3=H	Compound **4.2.**: time 40 min; 81, % yield; 150–152 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.29 (m, 4H, C$\underline{H_2}$C$\underline{H_2}$), 1.49 (m, 4H, C$\underline{H_2}$C$\underline{H_2}$), 2.27 (m, 4H, 2×5'-NCH_2), 3.26-3.41 (m, 14H, 2×6'a-CH, 2×C$\underline{H_2}$C$\underline{H_2}$C$\underline{H_2}$), 3.43-3.60 (m, 4H, 2×3'a-CH), 4.20 (q, 2H, 2×3'-CH, *J*=7.02 Hz), 6.73-6.96 (m, 6H, ArH), 7.11 - 7.31 (m, 2H, ArH), 10.52 (s, 2H, 2×1-NH) ppm; EI-MS for $C_{38}H_{40}N_6O_6$ (M+1) 676.7.
4.3.	R^1=CH_3, R^2=R^3=H	Compound **4.3.**: time 2 h; 62, % yield; 260–262 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.13 (d, 6H, 2×3'-C$\underline{H_3}$, *J*=6.41 Hz), 1.32 (m, 4H, CH_2CH_2), 1.52 (m, 4H, CH_2CH_2), 3.17-3.24 (d, 2H, 2×6'a-CH, *J*=7.9 Hz), 3.29-3.47 (m, 6H, 2×3'a-CH, 2×5'-NCH_2), 3.65 (d, 2H, 2×2'-NH, *J*=4.88 Hz), 4.19-4.38 (m, 2H, 2×3'-CH), 6.67-6.95 (m, 6H, ArH), 7.08-7.27 (m, 2H, ArH), 10.33 (s, 2H, 2×1-NH) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6) δ 16.90, 25.72, 25.77, 27.16, 37.95, 38.87, 39.07, 39.91, 40.12, 48.56, 51.74, 51.98, 67.79, 109.15, 120.92, 126.29, 127.51, 128.94, 142.12, 175.00, 176.33, 180.42 ppm; EI-MS for $C_{34}H_{36}N_6O_6$ (M+1) 624.7.
4.4.	R^1=H, R^2=CH_3, R^3=H	Compound **4.4.**: time 7 h; 69, % yield; 270 °C*; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.29 (m, 4H, CH_2CH_2), 1.48 (m, 4H, CH_2CH_2), 1.89 (s, 6H, 2×2'-NC$\underline{H_3}$), 2.57-2.74 (t, 4H, 2×5'-NCH_2), 2.91 (d, 2H, 2×6'a-CH), 3.14-3.26 (m, 2H, 2×3'a-CH), 3.51 (d, 4H, 2×3'-CH_2, *J*=7.02 Hz), 6.62-6.74 (m, 2H, ArH), 6.76-6.98 (m, 3H, ArH), 7.12-7.34 (m, 3H, ArH), 10.52 (s, 2H, 2×1-NH) ppm; EI-MS for $C_{34}H_{36}N_6O_6$ (M+1) 624.8.
4.5.	R^1=$CH_2C_6H_4$-*p*-OH, R^2=R^3=H	Compound **4.5.**: time 1 h; 55, % yield; 275–278 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.37 (m, 4H, CH_2CH_2), 1.57 (m, 4H, CH_2CH_2), 2.97-3.29 (m, 8H, 2×3'a-CH, 2×6'a-CH, 2×5'-NCH_2), 3.52 (d, 2H, 2'-NH, *J*=7.02 Hz), 4.27 (m, 2H, 2×3'-CH), 6.58 - 6.88 (m, 11H, ArH), 6.95-7.21 (m, 7H, ArH), 9.16 (m, 2H, ArOH), 10.30 (s, 2H, 2×1-NH) ppm; EI-MS for $C_{46}H_{44}N_6O_8$ (M+1) 808.9.
4.6.	R^1=R^2=R^3=H	Compound **4.6.**: time 5 h; 80, % yield; 182–185 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.16-1.34 (m, 4H, CH_2CH_2), 1.39-1.60 (m, 4H, CH_2CH_2), 3.16 (m, 4H, 2×5'-NCH_2), 3.42 (d, 2H, 2×6'a-CH, *J*=7.9 Hz), 3.54 (m, 4H, 2×3'a-CH), 3.75 (t, 2H, 2H, 2'-NH, *J*=6.10 Hz), 4.24 - 4.43 (t, 3'-CH_2 *J*=7.02 Hz), 6.71 - 6.93 (m, 6H, ArH), 7.18 (t, 2H, ArH, *J*=7.63 Hz), 10.37 (s, 2H, 2×1-NH) ppm; EI-MS for $C_{32}H_{32}N_6O_6$ (M+1) 596.7.
4.7.	R_1=$(CH_2)_2SCH_3$, R^2=R^3=H	Compound **4.7.**: time 1 h; 74, % yield; 180 °C*; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.23-1.39 (m, 6H, CH_2CH_2, 2×C$\underline{H}^aH^b$–CH_2S), 1.45-1.67 (6H, CH_2CH_2, 2×CH$^a\underline{H}^b$–CH_2S), 2.03 (s, 6H, 2×SC$\underline{H_3}$), 2.53 - 2.70 (m, 4H, 2×CH$^aH^b$–C$\underline{H_2}$S), 3.25 (d, 2H, 2×6'a-CH,, *J*=7.63 Hz), 3.33 (m, 4H, 2×5'-NCH_2), 3.38-3.51 (d, 2H, 2'-NH *J*=6.64 Hz), 3.67-3.84 (m, 2H, 2×3'a-CH), 4.14 - 4.27 (m, 2H, 2×3'-CH), 6.69-6.91 (m, 6H, ArH), 7.17 (t, 2H, ArH, *J*=8.09 Hz), 10.35 (s, 2H, 2×1-NH) ppm; EI-MS for $C_{38}H_{44}N_6O_6S_2$ (M+1) 744.9.
4.8.	R^1=$(CH_2)_2SC_2H_5$, R^2=R^3=H	Compound **4.8.**: time 1 h; 58, % yield; 170–172 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.16 (t, 6H, 2×C$\underline{H_3}$CH_2S, *J*=7.32 Hz), 1.26-1.42 (m, 6H, CH_2CH_2, 2×C$\underline{H}^aH^b$–CH_2S), 1.53 (6H, CH_2CH_2, 2×CH$^a\underline{H}^b$–CH_2S), 1.84-2.12 (m, 4H, 2×CH$^aH^b$–C$\underline{H_2}$S), 2.62 (m, 4H, 2×CH_3C$\underline{H_2}$S), 3.24 (d, 1H, 2'-NH, *J*=7.63 Hz), 3.40 - 3.50 (t, 2H, 2×3'a-CH,), 3.68-3.87 (m, 6H, 2×6'a-CH, 2×5'-NCH_2), 4.22 (m, 2H, 3'-C$\underline{H}$.), 4.34 (d, 2H, 2'-NH, *J*=3.97 Hz), 6.70-6.93 (m, 6H, ArH), 7.17 (t, 2H, ArH, *J*=7.32 Hz), 10.34 (s, 2H, 2×1-NH) ppm; EI-MS for $C_{40}H_{48}N_6O_6S_2$ (M+1) 772.9.
4.9.	R^1=$CH_2CH(CH_3)_2$, R^2=R^3=H	Compound **4.9.**: time 45 min; 63, % yield; 158–160 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.86 (d, 12H, 2×CH_2CH(C$\underline{H_3}$)$_2$, *J*=6.10 Hz), 1.15 - 1.31 (m, 6H, C$\underline{H_2}$C$\underline{H_2}$, 3'-CH-C$\underline{H_2}$), 1.34 - 1.50 (m, 6H, C$\underline{H_2}$C$\underline{H_2}$, 3'-CH-C$\underline{H_2}$), 1.74 (m, 2H, $CH_2C\underline{H}(CH_3)_2$), 3.16 - 3.27 (m, 6H, 2×6'a-CH, 2×5'-NCH_2), 3.38 (2H, 2×3'a-CH, *J*=7.69 Hz), 3.59 (d, 2H, 2×2'-NH, *J*=6.22 Hz), 4.22 (m, 2H, 2×3'-C$\underline{H}$), 6.68 - 6.91 (m, 6H, ArH), 7.06 - 7.25 (t, 2H, ArH, *J*=7.30 Hz), 10.31 (s, 2H, 2×1-NH) ppm; EI-MS for $C_{40}H_{48}N_6O_6$ (M+1) 708.9.
4.10.	R^1=$(CH_2)_4NH_2$, R^2=H, R^3=H	Compound **4.10.**: time 1.5 h; 70, % yield; 225–227 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.86 (d, 12H, 2×CH_2CH(C$\underline{H_3}$)$_2$, *J*=6.10 Hz), 1.15-1.31 (m, 6H, C$\underline{H_2}$C$\underline{H_2}$, 3'-CH-C$\underline{H_2}$), 1.34-1.50 (m, 6H, C$\underline{H_2}$C$\underline{H_2}$, 3'-CH-C$\underline{H_2}$), 1.74 (m, 2H, $CH_2C\underline{H}(CH_3)_2$), 3.16 - 3.27 (m, 6H, 2×6'a-CH, 2×5'-NCH_2), 3.38 (2H, 2×3'a-CH, *J*=7.69 Hz), 3.59 (d, 2H, 2×2'-NH, *J*=6.22 Hz), 4.22 (m, 2H, 2×3'-C$\underline{H}$), 6.68 - 6.91 (m, 6H, ArH), 7.06 - 7.25 (t, 2H, ArH, *J*=7.30 Hz), 10.31 (s, 2H, 2×1-NH) ppm; EI-MS for $C_{40}H_{50}N_8O_6$ (M+1) 738.9.
4.11.	R^1=$CH(CH_3)_2$, R^2=R^3=H	Compound **4.11.**: time 30 min; 88, % yield; 172–174 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 0.83 (d, 12H, 2×CH(C$\underline{H_3}$)$_2$, *J*=6.10 Hz), 1.11 - 1.20 (m, 4H, C$\underline{H_2}$C$\underline{H_2}$), 1.21 - 1.38 (m, 4H, CH_2CH_2), 1.38 - 1.59 (m, 4H, 2×5'-NCH_2), 3.23 (d, 2H, 2×6'a-CH, *J*=7.63 Hz), 3.48 (t, 2H, 2×3'a-CH *J*=7.17 Hz), 3.64 (d, 2H, 2×2'-NH, *J*=4.58 Hz), 3.68 - 3.84 (m, 2H, 2×3'-C$\underline{H}$), 6.65 - 6.90 (m, 6H, ArH), 7.08 - 7.22 (t, 2H, ArH, *J*=7.2 Hz), 10.29 (s, 2H, 2×1-NH) ppm; EI-MS for $C_{38}H_{44}N_6O_6$ (M+1) 680.8.
4.12.	R^1=CH_2OH, R^2=R^3=H	Compound **4.12.**: time 2 h; 79, % yield; 175–177 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.15 - 1.31 (m, 4H, C$\underline{H_2}$C$\underline{H_2}$), 1.35 - 1.50 (m, 4H, C$\underline{H_2}$C$\underline{H_2}$), 3.35 - 3.52 (m, 6H, 2×6'a-CH, 2×5'-NCH_2), 3.55-3.59 (t, 2H, 2×3'a-CH *J*=7.2 Hz), 3.76 (4H, 2×C$\underline{H_2}$O), 3.86 (d, 2H, 2×2'-NH, *J*=6.22 Hz), 4.13 - 4.33 (m, 2H, 2×3'-C$\underline{H}$), 4.50 (t, 2H, 2×$CH_2O\underline{H}$, *J*=5.19 Hz), 6.70 - 6.93 (m, 6H, ArH), 7.09 - 7.24 (t, 2H, ArH, *J*=7.2 Hz), 10.33 (s, 2H, 2×1-NH) ppm; EI-MS for $C_{34}H_{36}N_6O_8$ (M+1) 656.7.
4.13.	R^1=$(CH_2)_2COOH$, R^2=R^3=H	Compound **4.13.**: time 2 h; 45, % yield; 182 °C *; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.14 - 1.31 (m, 4H, C$\underline{H_2}$C$\underline{H_2}$), 1.36 - 1.51 (m, 4H, C$\underline{H_2}$C$\underline{H_2}$), 1.91 (t, 4H, 2×5'-NCH_2, *J*= 6.56 Hz), 2.36 (d, 2H, 6'a-CH, *J*=7.02 Hz), 3.24 (t, 2H, 2H, 2×3'a-CH, *J*=7.63 Hz), 3.31 - 3.50 (m, 8H, 2×C$\underline{H_2}$C$\underline{H_2}$COOH), 3.61 - 3.83 (t, 2H, 2×3'a-CH, *J*=7.6 Hz), 3.85 (d, 2H, 2×2'-NH, *J*=6.25 Hz), 4.01 - 4.20 (m, 2H, 2×3'-C$\underline{H}$), 6.69 - 6.93 (m, 6H, ArH), 7.16 (t, 2H, ArH, *J*=7.48 Hz), 10.34 (s, 2H, 2×1-NH), 11.74 (br s, 2H, COO$\underline{H}$) ppm; EI-MS for $C_{38}H_{40}N_6O_{10}$ (M+1) 740.7.
4.14.	R^1=CH_2COOH, R^2=R^3=H	Compound **4.14.**: time 3 h; 34, % yield; 210 °C *; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.15 - 1.31 (m, 4H, C$\underline{H_2}$C$\underline{H_2}$), 1.35 -1.50 (m, 4H, C$\underline{H_2}$C$\underline{H_2}$), 2.37 (d, 2H, 6'a-CH, *J*=4.88 Hz), 2.53 - 2.75 (t, 4H, 2×5'-NCH_2, *J*= 6.7 Hz), 3.30 (d, 4H, C$\underline{H_2}$COOH, *J*=7.17 Hz), 3.45 - 3.59 (m, 2H, 2×3'a-CH), 3.74 (d, 2H, 2×2'-NH, *J*=6.26 Hz), 4.36 - 4.53 (m, 2H, 2×3'-C$\underline{H}$), 6.67 - 6.92 (m, 6H, ArH), 7.07 - 7.25 (t, 2H, ArH, *J*=7.5 Hz), 10.37 (s, 2H, 2×1-NH), 11.63 (br s, 2H, COO$\underline{H}$) ppm; EI-MS for $C_{36}H_{36}N_6O_{10}$ (M+1) 712.8.
4.15.	R^1=H, R^2=CH_3, R^3=All	Compound **4.15.**: time 5 h; 78, % yield; 103–105 °C *; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.12 - 1.27 (m, 4H, C$\underline{H_2}$C$\underline{H_2}$), 1.38 - 1.47 (m, 8H, C$\underline{H_2}$C$\underline{H_2}$4H, 2×5'-NCH_2), 1.88 (s, 6H, 2×2'-NC$\underline{H_3}$), 3.23 (d, 2H, 2×6'a-CH, *J*=7.63 Hz), 3.48 (t, 2H, 2×3'a-CH *J*=7.15 Hz), 4.27 (m, 6H, 2×3'-C$\underline{H}$, 2×C$\underline{H_2}$CH=CH_2), 5.12 (m, 4H, 2×CH_2CH=C$\underline{H_2}$), 5.78 (m, 2H, 2×CH_2C$\underline{H}$=CH_2), 6.93 (m, 6H, ArH), 7.28 (t, 2H, ArH, *J*=7.5 Hz) ppm; EI-MS for $C_{40}H_{44}N_6O_6$ (M+1) 704.8.

4.16.	R^1=Ph, R^2=H, R^3=All	Compound **4.16.**: time 1.5 h; 80, % yield; 208–210 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.14-1.32 (m, 4H, CH_2CH_2), 1.46-1.75 (m, 8H, CH_2CH_2, 2×5'-NCH_2), 3.63 (d, 2H, 2×6'a-CH, *J*=7.63 Hz), 3.84 (t, 2H, 2×3'a-CH *J*=7.15 Hz), 4.15 - 4.43 (t, 2H, 2×3'-CH, *J*=7.69 Hz), 5.09 - 5.33 (4H, 2×CH_2CH=CH_2), 5.40-5.55 (m, 4H, 2×CH_2CH=CH_2), 5.72-5.98 (m, 2H, 2×CH_2CH=CH_2), 6.74-7.12 (м, 10 H, ArH), 7.15-7.32 (m, 4H, ArH), 7.32-7.48 (m, 4 H, ArH) ppm; EI-MS for $C_{50}H_{48}N_6O_6$ (M+1) 828.9
4.17.	R^1-R^2=$(CH_2)_3$, R^3=All	Compound **4.17.**: time 40 min; 47, % yield; 120 °C *; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.16-1.39 (m, 4H, CH_2CH_2), 1.43-1.62 (m, 4H, CH_2CH_2), 1.79 (8H, 2×5'-NCH_2, (CH_2)$_3$), 2.11-2.28 (m, 4H, (CH_2)$_3$), 2.28-2.41 (m, 4H, (CH_2)$_3$), 2.89 (d, 2H, 2×6'a-CH,, 7.17 Hz), 3.52 (t, 2H, 2×3'a-CH, *J*=7.71 Hz), 4.07-4.37 (m, 6H, 2×3'-CH, 2×CH_2CH=CH$_2$), 5.00 - 5.20 (m, 4H, 2×CH$_2$CH=CH_2), 5.70-5.93 (m, 2H, 2×CH$_2$CH=CH$_2$), 6.76 - 7.04 (m, 6H, ArH), 7.14-7.38 (m, 2 H, ArH) ppm; EI-MS for $C_{44}H_{48}N_6O_6$ (M+1) 756.9
4.18.	R^1=CH_3, R^2=H, R^3=All	Compound **4.18.**: time 3 h; 51, % yield; 150 °C *; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.16 (d, 6H, 2×CH_3, *J*=6.10 Hz), 1.14-1.33 (m, 4H, CH_2CH_2), 1.35-1.53 (m, 8H, CH_2CH_2, 2×5'-NCH_2), 3.17 - 3.28 (d, 2H, 2×6'a-CH, *J*=7.70 Hz), 3.60 - 3.83 (m, 4H, 2×3'a-CH, 2×2'-NH), 4.16-4.40 (m, 6H, 2×3'-CH, 2×CH_2CH=CH$_2$), 5.04-5.28 (m, 4H, 2×CH$_2$CH=CH_2), 5.67 -5.96 (m, 2H, 2×CH$_2$CH=CH$_2$), 6.73 - 7.02 (m, 6H, ArH), 7.10-7.33 (d, 2 H, ArH, *J*=7.0 Hz) ppm; EI-MS for $C_{40}H_{44}N_6O_6$ (M+1) 704.8
4.19.	R^1=$CH_2C_6H_4$-*p*-OH, R^2=H, R^3=All	Compound **4.19.**: time 1.5 h; 51, % yield; 230 °C *; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.21-1.43 (m, 4H, CH_2CH_2), 1.45-1.68 (m, 4H, CH_2CH_2, 2×5'-NCH_2), 3.20-3.48 (d, 2H, 2×6'a-CH, *J*=7.63 Hz), 3.57 (t, 2H, 2×3'a-CH, *J*=7.71 Hz), 3.67 - 3.85 (d, 2H, 2×2'-NH, *J*=6.02 Hz), 4.10-4.25 (m, 6H, 2×3'-CH, 2×C$H_2$$C_6H_4$-*4*-OH), 4.27 - 4.45 (4H, 2×CH_2CH=CH$_2$), 5.06 - 5.30 (m, 4H, 2×CH$_2$CH=CH_2), 5.68-5.94 (m, 2H, 2×CH$_2$CH=CH$_2$), 6.61-6.65 (d, 4H, ArH, *J*=7.94 Hz), 6.79 - 7.13 (m, 10H, ArH), 7.20 (d, 2H, ArH, *J*=7.02 Hz), 9.15 (br., s, 2H, 2×$CH_2C_6H_4$-*4*-OH) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6) δ 25.33, 25.61, 26.96, 27.05, 27.78, 36.08, 36.85, 38.01, 38.71, 38.92, 39.13, 39.55, 39.75, 39.96, 41.15, 47.88, 52.06, 59.27, 61.90, 67.20, 108.57, 114.84, 116.87, 121.45, 125.60, 126.86, 128.79, 129.67, 129.86, 131.90, 134.27, 142.58, 155.40, 170.93, 174.61, 176.29, 178.17 ppm; EI-MS for $C_{52}H_{52}N_6O_8$ (M+1) 889.0
4.20.	R^1=CH_2Ph, R^2=H, R^3=All	Compound **4.20.**: time 1 h; 79, % yield; 210 °C *; ^{1}H NMR (200 MHz, DMSO-d_6) 1.20 -1.37 (m, 4H, CH_2CH_2), 1.39 - 1.59 (m, 4H, CH_2CH_2), 2.43 - 2.52 (m, 4H, 2×5'-NCH_2), 3.33 - 3.55 (d, 2H, 2×6'a-CH, *J*=7.63 Hz), 3.60 - 3.70 (t, 2H, 2×3'a-CH, *J*=7.6 Hz), 3.70 - 3.85 (d, 2H, 2×2'-NH, *J*=6.5 Hz), 4.04 - 4.27 (m, 6H, 2×3'-CH, 2×CH_2Ph), 4.28 - 4.52 (4H, 2×CH_2CH=CH$_2$), 5.00 - 5.29 (m, 4H, 2×CH$_2$CH=CH_2), 5.66 - 5.93 (m, 2H, 2×CH$_2$CH=CH$_2$), 6.78 - 7.03 (m, 6H, ArH), 7.09 - 7.39 (m, 12H, ArH) ppm; EI-MS for $C_{52}H_{52}N_6O_6$ (M+1) 857.0
4.21.	R^1=$CH_2CH(CH_3)_2$, R^2=H, R^3=All	Compound **4.21.**: time 40 min; 43, % yield; 190 °C *; ^{1}H NMR (200 MHz, DMSO-d_6) 0.87 (d, 12H, 2×CH$_2$CH(CH_3)$_2$, *J*=5.80 Hz), 1.22 (t, 4H, 2×CH_2CH(CH$_3$)$_2$, *J*=6.41 Hz), 1.24 - 1.31 (m, 4H, CH_2CH_2), 1.40-1.63 (m, 8H, CH_2CH_2, 2×5'-NCH_2), 1.64 - 1.88 (m, 2H, CH$_2$CH(CH$_3$)$_2$), 3.24 (d, 2H, 2×6'a-CH, *J*=7.63 Hz), 3.36 - 3.50 (t, 2H, 2×3'a-CH, *J*=7.6 Hz), 3.61 - 3.73 (d, 2H, 2×2'-NH, *J*=6.6 Hz), 4.10 - 4.37 (m, 6H, 2×3'-CH, 2×CH$_2$CH=CH_2), 5.08 - 5.31 (m, 4H, 2×CH$_2$CH=CH_2), 5.72 - 5.96 (m, 2H, 2×CH$_2$CH=CH$_2$), 6.80 - 7.02 (m, 6H, ArH), 7.16 - 7.31 (t, 2H, ArH., *J*=7.6 Hz) ppm; EI-MS for $C_{46}H_{56}N_6O_6$ (M+1) 789.0
4.22.	R^1=CH(CH_3)$_2$, R^2= H, R^3=All	Compound **4.22.**: time 30 min; 49, % yield; 180 °C *; ^{1}H NMR (200 MHz, DMSO-d_6) 0.86 (d, 6 H, CH(CH_3)$_2$, *J*=6.10 Hz), 1.05 - 1.19 (d, 6 H, CH(CH_3)$_2$ *J*=6.10 Hz), 1.33 (m, 4H, CH_2CH_2.), 1.52 (m, 8H, CH_2CH_2, 2×5'-NCH_2), 1.79 - 1.85 (m, 2H, CH(CH$_3$)$_2$), 3.25 (d, 2H, 2×6'a-CH, *J*=7.63 Hz), 3.53 (t, 2H, 2×3'a-CH, *J*=7.02 Hz), 3.67 - 3.90 (d, 2H, 2×2'-NH, *J*=6.6 Hz), 4.09 - 4.41 (m, 6H, 2×3'-CH, 2×CH$_2$CH=CH_2), 5.09 - 5.35 (m, 4H, 2×CH$_2$CH=CH_2), 5.72 - 5.97 (m, 2H, 2×CH$_2$CH=CH$_2$), 6.77 - 7.05 (m, 6H, ArH), 7.18 - 7.33 (m, 2H, ArH) ppm; EI-MS for $C_{44}H_{52}N_6O_6$ (M+1) 760.9
4.23.	R^1=CH_2OH, R^2= H, R^3=All	Compound **4.23.**: time 2.5 h; 45, % yield; 122 – 124 °C; ^{1}H NMR (200 MHz, DMSO-d_6) 0.86 (d, 6 H, CH(CH_3)$_2$, *J*=6.10 Hz), 1.05 - 1.19 (d, 6 H, CH(CH_3)$_2$ *J*=6.10 Hz), 1.33 (m, 4H, CH_2CH_2.), 1.52 (m, 8H, CH_2CH_2, 2×5'-NCH_2), 1.79 - 1.85 (m, 2H, CH(CH$_3$)$_2$), 3.25 (d, 2H, 2×6'a-CH, *J*=7.63 Hz), 3.53 (t, 2H, 2×3'a-CH, *J*=7.02 Hz), 3.67 - 3.90 (d, 2H, 2×2'-NH, *J*=6.6 Hz), 4.09 - 4.41 (m, 6H, 2×3'-CH, 2×CH$_2$CH=CH_2), 5.09 - 5.35 (m, 4H, 2×CH$_2$CH=CH_2), 5.72 - 5.97 (m, 2H, 2×CH$_2$CH=CH$_2$), 6.77 - 7.05 (m, 6H, ArH), 7.18 - 7.33 (m, 2H, ArH) ppm; EI-MS for $C_{40}H_{44}N_6O_8$ (M+1) 736.8
4.24.	R^1=$(CH_2)_2$COOH, R^2= H, R^3=All	Compound **4.24.**: time 3 h; 45, % yield; 142 °C *; ^{1}H NMR (200 MHz, DMSO-d_6) 1.32 (m, 4H, CH_2CH_2), 1.43 - 1.73 (m, 4H, CH_2CH_2), 1.92 (4H, 2×5'-NCH_2,), 2.23 - 2.43 (m, 4H, (CH_2)$_2$COOH), 2.43 - 2.57 (m, 4H, m, 4H, (CH_2)$_2$COOH), 3.25 (d, 2H, 6'a-CH, *J*=7.63 Hz), 3.41 - 3.54 (t, 2H, 2×3'a-CH, *J*=7.63 Hz), 3.75 (d, 2H, 2×2'-NH, *J*=6.25 Hz), 4.02 - 4.37 (m, 6H, 2×3'-CH, 2×CH$_2$CH=CH_2), 5.05-5.31 (m, 4H, 2×CH$_2$CH=CH_2), 5.69 - 5.99 (m, 2H, 2×CH$_2$CH=CH$_2$), 6.79 - 7.04 (m, 6H, ArH.), 7.24 (t, 2H, ArH., *J*=7.63 Hz), 11.72 (br s, 2H, COOH) ppm; EI-MS for $C_{44}H_{48}N_6O_{10}$ (M+1) 820.9
4.25.	R^1=H, R^2=CH_3, R^3=Bn	Compound **4.25.**: time 5 h; 46, % yield; 140 °C *; ^{1}H NMR (200 MHz, DMSO-d_6) 1.19 - 1.33 (m, 4H, CH_2CH_2), 1.46 – 1.55 (m, 4H, CH_2CH_2), 1.89 (s, 6H, 2×2'-NCH_3), 2.41 - 2.53 (t, 4H, 2×5'-NCH_2, *J*=7.1 Hz), 3.21 - 3.48 (d, 2H, 2×6'a-CH, *J*=7.6 Hz), 3.58 (m, 2H, 2×3'a-CH,, *J*=6.10 Hz), 3.74 - 4.33 (d, 4H, 2×3'-CH_2), 4.79 - 4.99 (s, 4H, 2×CH_2Ph), 6.71 - 6.85 (m, 5H, ArH), 6.85 - 7.03 (m, 3H, ArH), 7.15 - 7.43 (m, 10H, ArH) ppm; EI-MS for $C_{48}H_{48}N_6O_6$ (M+1) 804.9
4.26.	R^1=Ph, R^2=H, R^3=Bn	Compound **4.26.**: time 1 h; 66, % yield; 110 °C*; ^{1}H NMR (200 MHz, DMSO-d_6) 1.17 - 1.33 (m, 4H, CH_2CH_2), 1.45 - 1.75 (m, 8H, CH_2CH_2, 2×5'-NCH_2), 3.55 (d, 2H, 2×6'a-CH, *J*=9.16 Hz), 3.65 (t, 2H, 2×3'a-CH, *J*=7.78 Hz), 3.76 (d, 2H, 2×2'-NH, *J*=6.6 Hz), 4.32 (t, 2H, 2×3'-CH, *J*=7.69 Hz), 4.87 (s, 4H, 2×CH_2Ph), 6.82 (d, 2H, ArH., *J*=7.94 Hz), 6.91 - 7.12 (m, 4H, ArH), 7.28 - 7.33 (m, 10 H, ArH), 7.45 - 7.50 (m, 2H, ArH., *J*=8.55 Hz) ppm; EI-MS for $C_{58}H_{52}N_6O_6$ (M+1) 929.1
4.27.	R^1-R^2=$(CH_2)_3$, R^3=Bn	Compound **4.27.**: time 35 min; 50, % yield; 220 °C*; ^{1}H NMR (200 MHz, DMSO-d_6) 1.17 - 1.33 (m, 4H, CH_2CH_2), 1.45 - 1.75 (m, 8H, CH_2CH_2, 2×5'-NCH_2), 3.55 (d, 2H, 2×6'a-CH, *J*=9.16 Hz), 3.65 (t, 2H, 2×3'a-CH, *J*=7.78 Hz), 3.76 (d, 2H, 2×2'-NH, *J*=6.6 Hz), 4.32 (t, 2H, 2×3'-CH, *J*=7.69 Hz), 4.87 (s, 4H, 2×CH_2Ph), 6.82 (d, 2H, ArH., *J*=7.94 Hz), 6.91 - 7.12 (m, 4H, ArH), 7.28 - 7.33 (m, 10 H, ArH), 7.45 - 7.50 (m, 2H, ArH., *J*=8.55 Hz) ppm; EI-MS for $C_{52}H_{52}N_6O_6$ (M+1) 857.0
4.28.	R^1=CH_3, R^2=H, R^3=Bn	Compound **4.28.**: time 1 h; 81, % yield; 190 °C*; ^{1}H NMR (200 MHz, DMSO-d_6) 1.18 (d, 6H, 2×CH_3, *J*=6.41 Hz), 1.28 - 1.32 (m, 4H, CH_2CH_2), 1.46 - 1.53 (m, 4H, CH_2CH_2), 3.35 - 3.49 (m, 6H, 2×3'a-CH, 2×5'-NCH_2), 3.69 - 3.89 (d, 2H, 2×2'-NH, *J*=4.88 Hz), 4.35 (m, 2H, 2×3'-CH), 4.83 (s, 4H, 2×CH_2Ph), 6.75 - 7.00 (m, 4H, ArH), 7.09 - 7.40 (m, 14H, ArH) ppm; EI-MS for $C_{48}H_{48}N_6O_6$ (M+1) 804.9

4.29.	R^1=$CH_2C_6H_4$-*p*-OH, R^2=H, R^3=Bn	Compound **4.29.**: time 45 min; 75, % yield; 198 – 200 °C; ^{1}H NMR (200 MHz, DMSO-d_6) 1.25 - 1.43 (m, 4H, CH_2CH_2), 1.45 - 1.68 (m, 4H, CH_2CH_2, 2×5'-NCH_2), 3.19 - 3.43 (d, 2H, 2×6'a-CH, *J*=7.63 Hz), 3.56 (t, 2H, 2×3'a-CH, *J*=7.71 Hz), 3.67 - 3.85 (d, 2H, 2×2'-NH, *J*=6.02 Hz), 4.69 (d, 2H, 2×3'-CH, *J*=16 Hz), 4.55 (s, 4H, 2×NCH_2Ph), 4.87 (d, 6H, 2×3'-CH, 2×$CH_2C_6H_4$-*4*-OH), 6.61 - 6.65 (d, 4H, ArH, *J*=7.94 Hz), 6.79 - 7.13 (m, 10H, ArH), 7.20 (d, 2H, ArH, *J*=7.02 Hz), 9.15 (br, s, 2H, 2×$CH_2C_6H_4$-*p*-OH) ppm; EI-MS for $C_{60}H_{56}N_6O_8$ (M+1) 989.1
4.30.	R^1=$CH(CH_3)_2$, R^2=H, R^3=Bn	Compound **4.30.**: time 30 min; 56, % yield; 153 – 155 °C; ^{1}H NMR (200 MHz, DMSO-d_6) 0.86 (d, 6H, $CH(CH_3)_2$, *J*=7 Hz), 0.98-1.08 (m, 2H, $CH(CH_3)_2$), 1.12 (d, 6 H, $CH(CH_3)_2$ *J*=6.0 Hz), 1.31 (m, 4H, CH_2CH_2.), 1.51 (m, 4H, CH_2CH_2, 2×5'-NCH_2), 1.70 - 1.97 (m, 2H, $CH(CH_3)_2$), 3.22 - 3.43 (14H, m, M07), 3.25 (m, 2H, 2×5'-NCH_2, 2×6'a-CH), 3.55 (t, 2H, 2×3'a-CH, *J*=7 Hz), 3.72 - 3.92 (d, 2H, 2×2'-NH, *J*=6.7 Hz), 4.65 - 5.00 (m, 4H, 2×CH_2Ph), 6.72 - 7.07 (m, 6H, ArH) 7.26 - 7.48 (m, 10H, ArH), 7.16 (t, 2H, ArH, *J*=7 Hz) ppm; EI-MS for $C_{52}H_{56}N_6O_6$ (M+1) 861.0
4.31.	R^1=CH_2OH, R^2=H, R^3=Bn	Compound **4.31.**: time 1 h; 79, % yield; 195 – 200 °C; ^{1}H NMR (200 MHz, DMSO-d_6) 0.86 (d, 6H, $CH(CH_3)_2$, *J*=7 Hz), 0.98-1.08 (m, 2H, $CH(CH_3)_2$), 1.12 (d, 6 H, $CH(CH_3)_2$ *J*=6.0 Hz), 1.31 (m, 4H, CH_2CH_2.), 1.51 (m, 4H, CH_2CH_2, 2×5'-NCH_2), 1.70 - 1.97 (m, 2H, $CH(CH_3)_2$), 3.22 - 3.43 (14H, m, M07), 3.25 (m, 2H, 2×5'-NCH_2, 2×6'a-CH), 3.55 (t, 2H, 2×3'a-CH, *J*=7 Hz), 3.72 - 3.92 (d, 2H, 2×2'-NH, *J*=6.7 Hz), 4.65 - 5.00 (m, 4H, 2×CH_2Ph), 6.72 - 7.07 (m, 6H, ArH) 7.26 - 7.48 (m, 10H, ArH), 7.16 (t, 2H, ArH, *J*=7 Hz) ppm; EI-MS for $C_{48}H_{48}N_6O_8$ (M+1) 836.9
4.32.	R^1=H, R^2=CH_3, R^3=CH_3	Compound **4.32.**: time 5 h; 78, % yield; 102 – 105 °C; ^{1}H NMR (200 MHz, DMSO-d_6) 1.01 (d, 6H, 2×2'-NCH_3 J=6.10 Hz,), 1.30-1.50 (m, 8H, 2×CH_2CH_2), 1.84 (s, 6H, 2×1'-NCH_3), 3.02 (d, 2H, 2×6'a-CH, J=3.05 Hz), 3.05 - 3.16 (m, 4H, 2×5'-NCH_2), 3.24 (d, 4H, 2×3'-CH_2, J=7.63 Hz), 3.61 - 3.85 (m, 2H, 2×3'a-CH) 6.74 (d, 2H ArH, J=7.32 Hz), 6.85 - 7.21 (m, 4H, ArH) 7.21 - 7.47 (m, 2H, ArH) ppm; EI-MS for $C_{36}H_{40}N_6O_6$ (M+1) 652.7
4.33.	R^1=Ph, R^2=H, R^3=CH_3	Compound **4.33.**: time 1 h; 82, % yield; 160 – 162 °C; ^{1}H NMR (200 MHz, DMSO-d_6) 1.30 (m, 4H, CH_2CH_2), 1.45 (m, 4H, CH_2CH_2), 3.10 (s, 6H, 2×1'-NCH_3), 3.04 - 3.17 (m, 4H, 2×5'-NCH_2), 3.59 - 3.92 (m, 4H, 2×3'a-CH,), 4.11 (m, 2H, 2×2'-NH) 4.34 (d, 2H, 2×6'a-CH, *J*=3.97 Hz,) 5.46 (d, 2H, 2×3'-CH, *J*=8.55 Hz), 6.89 - 7.45 (m, 16H, ArH), 7.54 (t, 2H, ArH, *J*=6.26 Hz) ppm; EI-MS for $C_{46}H_{44}N_6O_6$ (M+1) 776.9
4.34.	R^1-R^2=$(CH_2)_3$, R^3=CH_3	Compound **4.34.**: time 45 min; 55, % yield; 233 – 235 °C; ^{1}H NMR (200 MHz, DMSO-d_6) 1.30 (m, 4H, CH_2CH_2) 1.50 (m, 4H, CH_2CH_2) 1.80 (m, 8H, 2×5'-NCH_2, -$(CH_2)_3$), 2.11 - 2.40 (m, 8H, -$(CH_2)_3$-) 3.09 (s, 6H, 2×1'-NCH_3), 3.35 - 3.45 (m, 2H, 2×3'a-CH), 3.45 - 3.61 (m, 2H, 2×3'-CH) 4.23 (d, 2H, 2×6'a-CH, *J*=6.71 Hz,) 6.80 - 7.09 (m, 6H, ArH), 7.24 - 7.42 (m, 2H, ArH) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6) 23.24, 25.24, 26.75, 37.89, 38.73, 38.94, 39.77, 39.98, 44.60, 45.50, 54.83, 63.73, 68.00, 108.50, 121.53, 123.91, 126.43, 129.44, 143.87, 174.95, 175.52, 176.62 ppm; EI-MS for $C_{40}H_{44}N_6O_6$ (M+1) 704.9
4.35.	R^1=CH_3, R^2=H, R^3=CH_3	Compound **4.35.**: time 2 h; 47, % yield; 228 – 230 °C; ^{1}H NMR (200 MHz, DMSO-d_6) 1.02 (d, 6H, 2×3'-CH_3 J=6.10 Hz,), 1.30 (m, 4H, CH_2CH_2) 1.45 (m, 4H, CH_2CH_2), 3.01 (s, 6H, 2×1'-NCH_3), 3.04-3.17 (m, 4H, 2×5'-NCH_2) 3.59-3.92 (m, 2H, 2×2'-NH), 4.34 (d, 2H, 2×3'-CH, *J*=3.97 Hz) 5.46 (d, 2H, 2×6'a-CH, J=8.55 Hz), 6.91-7.45 (m, 4H, ArH) 7.54 (t, 4H, ArH, *J*=6.26 Hz) ppm; EI-MS for $C_{36}H_{40}N_6O_6$ (M+1) 652.7
4.36.	R^1=$CH_2C_6H_4$-*p*-OH, R^2=H R^3=CH_3	Compound **4.36.**: time 1.5 h; 72, % yield; 218 – 220 °C; ^{1}H NMR (200 MHz, DMSO-d_6) 1.02 (d, 6H, 2×3'-CH_3 J=6.10 Hz,), 1.30 (m, 4H, CH_2CH_2) 1.45 (m, 4H, CH_2CH_2), 3.01 (s, 6H, 2×1'-NCH_3), 3.04-3.17 (m, 4H, 2×5'-NCH_2) 3.59-3.92 (m, 2H, 2×2'-NH), 4.34 (d, 2H, 2×3'-CH, *J*=3.97 Hz) 5.46 (d, 2H, 2×6'a-CH, J=8.55 Hz), 6.91-7.45 (m, 4H, ArH) 7.54 (t, 4H, ArH, *J*=6.26 Hz) ppm; EI-MS for $C_{48}H_{48}N_6O_8$ (M+1) 836.9

In the course of the studies, we examined antibacterial properties of 36 synthesized substances including some novel spirooxindole derivatives **4.1-4.36** against different types of microorganisms (gram-positive and gram-negative). Most of the tested compounds showed a weak antimicrobial activity, and the most effective growth inhibitors are presented in Table 3.

Seven reference strains of microorganisms were used as control ones: *Escherichia coli ATCC 25922*, *Enterobacter cloacae №487, Klebsiella pneumoniae №247, Pseudomonas aeruginosa ATCC 27853*, *Staphylococcus aureus ATCC 25923*, *Staphylococcus epidermidis No. 235*, *Candida albicans* ATCC653/885. The microorganisms belong to different groups: gram-negative bacteria from the *Enterobacteriaceae* family *(E. coli, E. cloacae, K. pneumoniae) while P. aeruginosa* belong to non-fermenting gram-negative bacteria. Gram-positive cocci *S. aureus* and *S. epidermidis* belong to the *Micrococcaceae* family, and fungi *C. albicans* to the *Criptococcaceae* family. In order to detect antimicrobial properties of the compounds, the value of the minimal inhibitory concentration (MIC) of aqueous solutions of these **4.1-4.36** substances in five double dilutions 100 μg/mL, 50 μg/mL, 25 μg/mL, 12.5 μg/mL, 6.25 μg/mL, 3.125 μg/mL was determined.

Compounds **4.4, 4.5, 4.11, 4.15, 4.19, 4.21, 4.24, 4.25, 4.31** demonstrated the maximum activity against gram-positive microorganisms and are not active against gram-negative bacteria. Only compound **4.12** (R^1=CH_2OH, R^2=R^3=H) demonstrated a relatively weak growth inhibition of *C. albicans*. The N^1-benzyleted derevated substance **4.31** (R^1=CH_2OH, R^2=H, R^3=Bn) was found to be the most potent compound with antibacterial activity against *S. aureus* and *S. epidermidis* and *C. albicans* with MIC 6.25 μg/mL.

Compound **4.4** (R^1=H, R^2=CH_3, R^3=H) showed activity only with respect to staphylococci. Although none of the tested compounds was surpassed by its active reference-drugs comparison (Ciproflaxacin and Fluconazole), the obtained results make it possible to construct more active antimicrobial compounds based on bis-spirooxindoles structures in the future. It was found that test-strains of the *Micrococcaceae* family were more susceptible to the test compounds.

The important physicochemical properties (lipophilicity (LogP), topological molecular polar surface area (TPSA) and molar volume) for all most active compounds **4.4, 4.5, 4.11, 4.15, 4.19, 4.21, 4.24, 4.25, 4.31** were calculated by the Molinspiration server (Molinspiration property engine v2016.10) for the establishing structure activity relationship (SAR) and listed in Table 4.

Table 3: Antimicrobial screening data for the most active compounds and MIC (*μg/ml*).

Compound. №	***MIC*[1], μg/mL**						
	Gram "+ve" bacteria		**Gram "–ve" bacteria**				**Fungal strain**
	S. aureus	***S. epidermidis***	***E. coli***	***P. aeruginosa***	***K. pneumoniae***	***E. cloacae***	***C. albicans***
4.4.	6.25	6.25	growth[2]	growth	growth	growth	growth
4.31.	6.25	6.25	growth	growth	growth	growth	6.25
4.25.	50	50	growth	growth	growth	growth	growth
4.19.	50	25	growth	growth	growth	growth	100
4.5.	100	100	growth	growth	growth	growth	50
4.11.	100	50	growth	growth	growth	growth	growth
4.24.	100	100	growth	growth	growth	growth	growth
4.15.	100	100	growth	growth	growth	growth	100
4.21.	100	25	growth	growth	growth	growth	growth
4.12.	100	100	growth	growth	growth	growth	100
Control	growth	growth	growth	growth	growth	growth	growth
Ciproflox.[4]	0.78	2	0.39	1.0	0.78	2	N/T
Fluconazole[5]	N/T[3]	N/T	N/T	N/T	N/T	N/T	3.125

Notes:
1 – MIC (μg/mL) – minimal inhibitory concentration; it was accepted according inhibitory the action complete absence of growth on the Petri dishes with peptone meat agar and certain strains of microorganisms;
2 – "growth" – it is that investigated compound was not active in concentrations 100 *μg/ml*;
Control – DMSO solution in peptone meat agar (100 *μg/ml*);
3 – N/T – it was not tested.
4 – *Ciproflox.* – *Ciproflaxacin* DMSO solution;
5 – *Fluconazole* water solution

Table 4: Molinspiration predicted physicochemical parameters for most active compounds *in silico* and their comparision with results of antimicrobial activity investigation *in vitro.*

Compound.	LogP	TPSA, Å^2	Molecular volume (MV), Å^3	***MIC*, μg/mL (*in vitro*)**		
				S. aureus	***S. epidermidis***	***C. albicans***
4.4.	1.65	157.01	547.39	6.25	6.25	growth
4.31.	2.99	179.89	741.09	6.25	6.25	6.25
4.25.	4.94	139.43	724.57	50	50	growth
4.19.	5.40	179.89	796.55	50	25	100
4.5.	3.62	197.47	706.72	100	100	50
4.11.	3.21	157.01	614.16	100	50	growth
4.24.	2.32	214.03	725.30	100	100	growth
4.15.	3.26	121.85	637.93	100	100	100
4.21.	6.05	139.43	737.60	100	25	growth
4.12.	-0.30	197.47	563.90	100	100	100

From the obtained results, we tentatively assume that the activity may relate to contribution of the lipophilicity (LogP), topological molecular polar surface area (TPSA) and molecular volume (MV) descriptors. Molinspiration server has predicted that *N*-benzyleted derevated substance **4.31** is moderately lipophilic (LogP = 2.99) while **4.12** is hydrophylic (LogP= −0.30) and it has more polarity (TPSA=197.47 Å^2).

Obviously, more polar compound **4.12** exhibit less lipid solubility that limits cell membrane penetration (*Waterhouse*, 2003). Since all the compounds studied have the same bis-spirooxindolic system, the changes in the molar volume depend only on the structure of the substituents R^1, R^2, R^3.

This indicates that the antimicrobial activity of those compounds depends not only on the length of the longer substituents, but also on the presence of the second one that contributes to the hydrophobicity, polar surface area (TPSA) and on molecular volume of the whole molecule.

Therefore, a comparison of structures **4.12** (MV=563.90 Å^3) and **4.31** (MV=741.09 Å^3) shows that the introduction of a benzyl radical dramatically increases the molecular volume and lipophilicity. Nonetheless, that reduces the polarity of the molecule as a whole and it is possible to form chelate complexes with microelements (Mg, Fe, Cu) and DNA of microbes resulting in enzyme systems inhibition (Fig. 3). However, more analogues have to be compared to establish a proper structure-activity relationship.

The combination of new substituents should lead to an optimal balance of lipophilicity, polarity, and molecular volume. Further extensive studies are necessary to determine additional physicochemical and biological parameters, get deeper insight the structure-activity relationship and optimize the effectiveness and anti-staphylococci activity of hexamethylene-N,N'-*bis*-derivatives of 3a′,6a′-dihydro-2'*H*-spiro[indole-3,1'-pyrrolo[3,4-c]pyrrole]-2,4',6'(1*H*,3'*H*,5'*H*)-trione series of molecules.

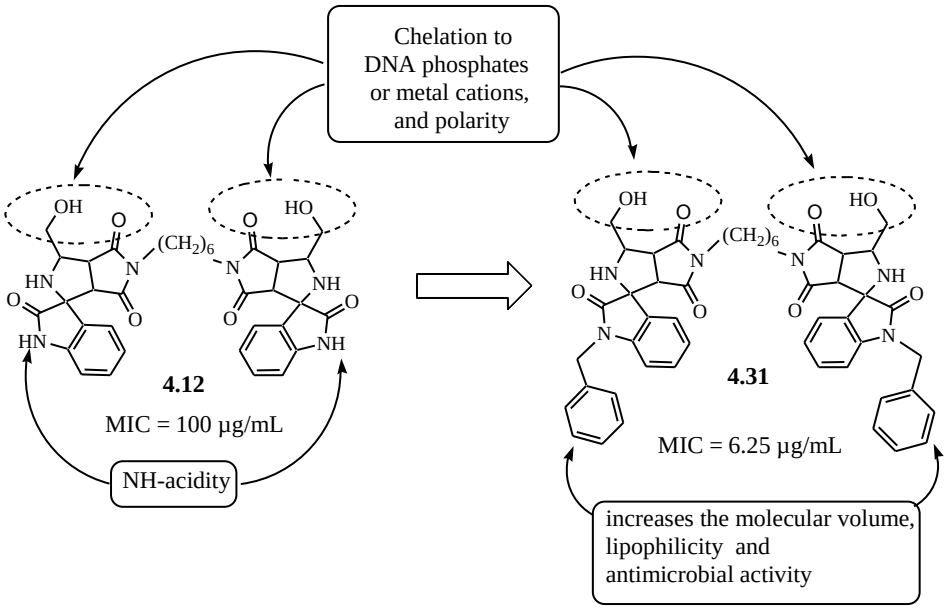


Fig. 3: Structure Activity Relationship illustration.

CONCLUSION

In summary, we have successfully developed a 1,3-dipolar cycloaddition of 1,6-bismaleimidohexane to azomethine ylides generated *in situ* from isatins and some α-amino acids, and 36 novel hexamethylene-N,N'-*bis*-derivatives of 3a′,6a′-dihydro-2'*H*-spiro[indole-3,1'-pyrrolo[3,4-c]pyrrole]-2,4',6'(1*H*,3'*H*,5'*H*)-trione **4.1-4.36** were synthesized and their structures were established by NMR, Mass and elemental analysis. The isolated yields in this one-pot protocol were moderate to high (43-88%) and provided the desired target compounds with a high purity after a simple filtration and recrystallization from an ethanol/DMF mixture (1:1). This method has the advantages of convenient operation, mild reaction conditions and high efficiency. It has the advantages of mild reaction conditions, high atom economy and excellent yields. The most suitable conditions for this reaction were boiling in the alcoholic-water media. It was interesting to notice that test compound **4.31** with N^1-benzyl and R^1=CH_2OH radicals led to a significant increase in antibacterial activity. Some obtained compounds showed a weak antimicrobial activity with regard to the *Micrococcaceae* family.

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