Synthesis, Characterization and Evaluation Antimicrobial Activity of Some New Derivatives Theophylline Sulfonyl Phenoxyacetic Acids

ANCA MIHAELA MOCANU[1]*, CONSTANTIN LUCA[1], ION SANDU[2], SIMONA ISABELA DUNCA[3]

[1] Gheorghe Asachi Technical University of Iasi, Faculty of Chemical Engineering and Environmental Protection, Department of Organic, Biochemical and Food Engineering, 71A D.Mangeron Blvd., 700050, Iasi, Romania.

[2] Alexandru Ioan Cuza University of Iasi, ARHEOINVEST Interdisciplinary Platform, 22 Carol I Blvd., 700506, Iasi, Romania

[3] Alexandru Ioan Cuza University of Iasi, Department of Biology, Faculty of Biology, Microbiology Laboratory, 20A Carol I Blvd.,700506, Iasi, Romania

The purpose of this study is to synthesis and assess antibacterial activity of new derivates of theophylline-sulphonyl phenoxyacetic acids. The compounds were obtained by hydrazides condensation of the acids with a series of alheides. Due to the biologically activity found for previously known theophylline-sulphonyl phenoxyacetic acids we considered opportune to synthesize and characterize new compounds of the same class. The synthesis stages of the new products (azomethine) are presented as well as the elemental analysis data and IR, ^{1}H-RMN spectral measurements made for elucidating the chemical structures, and microbial properties were confirmed by the microbiological tests. The synthetized compounds were evaluated taking into account their microbial activity, in vitro, by measuring zone diameters of bacterial growth inhibition of two different types of strains microorganisms: Pseudomonas aeruginosa 'and Escherichia coli.

Keywords: hydrazide, azomethine, spectral measurements, microbiological tests, antimicrobial activity.

The therapeutic efficacy of theophylline and its derivates in various affection has imposed continuous research studies on the impovement on its biological quality [1-3]. The main focus being on the selectivity, lack of toxicity and reduction of side effects such as: anorexia, tachycardia, cephalalgia, nervosity [4-8]. Xanthine derivatives belong to alkaloids which are described as natural bases having nitrogen atoms in molecular structure and have strong physiological effect on human organism.

Literature studies frequently point out, new xantinic derivatives with hypotensive or antihypertensive actions, coronarodilator, vasodilators, antiarrhythmic, antiedema [9-12]. Moreover, among bronchodilator, diuretics and those excised on cardiocirculatory the focus is on the theophylline potential to stimulate the central nervous system [13-19]. An important characteristic of theophylline is the ability to interfere with the citocinese and the activity of these compous whose electronic affinity make it useful in cancer treatment [20-27].

Taking into account the importance of these compounds we have conducted research in the field on synthesis of new theophylline derivatives (azomethines), starting with of hydrazides theophylline-sulphonyl phenoxyacetic acids.

The antimicrobial activities of the newly obtained compounds were estimated against a Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli)* bacteria. The comparative inhibiting action was estimated by the diffusimetric method in the overlay agar Mueller Hinton. The measured critical diameters afford the germs under study to be classified as "sensitive" and "resistant". The obtained results were expressed by the direct transcription of the inhibition area diameter [28].

Experimental part

Materials, methods and equipment

Theophylline anhydrous 99%, hydrazine monohydrate 98%, 4-hydroxy-benzaldehyde 97%, 4-metoxybenzaldehyde 97%, 4-nitrobenzaldehyde 97%, and 4-methylbenzaldehyde 97% were purchased from Sigma–Aldrich. All reagents and solvents had purity grade and were obtained from commercial suppliers.

The ^{1}H-NMR spectra were recorded in DMSO-d6 with a Bruker Avance 300 MHz instrument. The chemical shifts were expressed in ppm using tetramethylsilane (TMS) as internal standard. Spin multiplets are given as: s (singlet), d (doublet). FT-IR spectra were performed on a Biorad FT-IR- FTS 570^{o}C spectrometer. All melting points were determined on Bruker Vertex 70 Melting Point apparatus. Elemental analyses were carried out using a Perkin Elmer CHNS/O Analyzer Series II 2400 apparatus, and the results were within ±0.4% of theoretical values.

General procedure of the hidrazide synthesis

The hydrazides of theophylline-sulphonyl phenoxyacetic acids were obtained by hydrazine monohydrate on methyl esters of theophylline-sulphonyl phenoxyacetic acids. The methyl ester of acid 4-(theophylline-7-sulphonyl) phenoxyacetic was dilluted in 25 mL ethylic alcohol, on the solution obtained is added hydrazine monohydrate. The solution is refluxed for 60 minuted then the alchool is removed by distillation. On the remaining waste is added 25 mL of water, when it preccipitates 4-(theophylline-7-sulphonyl) phenoxyacetic hydrazides is filted and recrystallized in a mixture of ethanol-water (1:1).

Esters: FT-IR (KBr, cm^{-1}): 1754 (-C=O ester), 1656 (-C=N-), 1464 (-N-CH_3), 1432 (-CH_2-ester), 1366 (-CH_3 ester), 1291 (C-O-C), 1212 (-C-N-), 1152 (-N-SO_2-) cm^{-1}; ^{1}H-NMR d/ppm (400 MHz, DMSO): 2.35 (s, 1H, CH_3), 2.64 (d, 6H, $2CH_3$), 3.59 (s, 1H, -$COOCH_3$), 3.75(s, 3H, -OCH_3), 4.58 (s, 2H, -CH_2-), 7.03 (s, 1H, Ar-H), 7.76 (s, 1H, Ar-H), 7.87 (s, 1H, Ar-H), 8.55 (s, 1H, -N=CH-N-).

Hydrazides*:* FT-IR (KBr, cm^{-1}): 3436, 3308, 3296 (-NH-NH_2), 1658 (-C=O), 1549 (-N-C-) cm^{-1}; ^{1}H-NMR d/ppm (400 MHz, DMSO): 1.95 (s, 2H, NH_2), 2.35 (s, 3H, Ar-CH_3), 2.64

* email: ancamocanu2004@yahoo.com

(d, 6H, $2CH_3$), 4.58 (s, 2H, $-CH_2-$), 7.03 (s, 1H, Ar-H), 7.76 (s, 1H, Ar-H), 7.87 (s, 1H, Ar-H), 8.14 (s, 1H, NH-), 8.55 (s, 1H, -N=CH-N-).

Preparation of 4-hydroxybenzaldehyde {2-[4-(theophylline-7-yl sulfonyl)-2-chloro-phenoxy]ethyl} hydrazone) (**1**)

0.5 g (1.2 mmol) hydrazide, dissolved in 15 mL acetone, was treated with 0.15 g (1.2 mmol) of *p*-hydroxy-benzaldehyde in 10 mL ethanol and 3 drops of acetic acid and heated for 45 min. under stirring. The reaction mixture was finally cooled and the solid product filtered off and washed with ethanol on the filter. The obtained reaction yields of 87.85% were much dependent on the product solubility in ethanol. An white powder was obtained after purification from toluene or from ethanol as well as from the DMF-ethylic ether two-solvent mixture.

Yield: 87.85%; m.p. 225-227 °C; white powder; Anal. Calcd. for $C_{22}H_{19}N_6O_7SCl$: C, 48.30, H, 3.47, N, 15.37, Found: C, 48.26, H, 3.54, N, 15.42; FT-IR (KBr, cm^{-1}): 1643 (-C=N-), 1602 (C=C), 1578 (-C-C-), 3042 (C-H), 1234 (C-S), 1145 ($-SO_2$-N-), 1595 (-C=O), 3248 (-NH-), 1229 (-C-N), 3112 (-OH); 6856 (-C-Cl) cm^{-1}; ^{1}H-NMR d/ppm (400 MHz, DMSO): 2.76 (d, 6H, $-CH_3$ theophylline), 4.8 (s, 2H, $-CH_2-$), 5.49 (s, 1H, Ar-OH), 6.86, 7.51 (d, 4H, Ar-H), 7.0, 7.86 (d, 4H, Ar-H), 8.0 (s, 1H, -NH-), 8.18 (s, 1H, -CH=N-), 8.36 (s, 1H, -N=CH-N-).

Preparation of 4-methoxybenzaldehyde{2-[4-(theophylline-7-yl sulfonyl)-2-methyl-phenoxy]ethyl} hydrazone) (**2**)

0.5 g (1.23 mmol) hydrazide, dissolved in 15 mL acetone, was treated with 0.168 g (1.23 mmol) of *p*-methoxy-benzaldehyde in 10 mL ethanol and 3 drops of acetic acid and heated for 45 min. under stirring. The reaction mixture was finally cooled and the solid product filtered off and washed with ethanol on the filter. The obtained reaction yields of 86.35% were much dependent on the product solubility in ethanol. An white powder was obtained after purification from toluene or from ethanol as well as from the DMF-ethylic ether two-solvent mixture.

Yield: 86.35%; m.p. 232-234 °C; white powder; Anal. Calcd. for $C_{24}H_{24}N_6O_7S$: C, 53.33, H, 4.44, N, 15.55, Found: C, 53.29; H, 4.52, N, 15.60; FT-IR (KBr, cm^{-1}): 1645 (-C=N-), 1600 (C=C), 1576 (-C-C-), 3045 (C-H), 1235 (C-S), 1146 ($-SO_2$-N-), 1593 (-C=O), 3250 (-NH-), 1228 (-C-N), 2814.35 ($-OCH_3$) cm^{-1}; ^{1}H-NMR d/ppm (400 MHz, DMSO): 2.32(s, 3H, Ar-CH_3) 2.78 (d, 6H, $-CH_3$ theophylline), 3.73 (s, 3H, -OCH_3), 4.81 (s, 2H, $-CH_2-$), 6.84, 7.48 (d, 4H, Ar-H), 6.91 (s, 1H, Ar-H), 7.59 (d, 2H, Ar-H), 8.1 (s, 1H, -NH-), 8.2 (s, 1H, -CH=N-), 8.36 (s, 1H, -N=CH-N-).

Preparation of 4-nitrobenzaldehyde{2-[4-(theophylline-7-yl sulfonyl)-2-methoxy-phenoxy]ethyl} hydrazone) (**3**)

0.5 g (1.23 mmol) hydrazide, dissolved in 15 mL acetone, was treated with 0.185 g (1.23 mmol) of p-nitro-benzaldehyde in 10 mL ethanol and 3 drops of acetic acid and heated for 45 min. under stirring. The reaction mixture was finally cooled and the solid product filtered off and washed with ethanol on the filter. The obtained reaction yields of 88.78 % were much dependent on the product solubility in ethanol. An white powder was obtained after purification from toluene or from ethanol as well as from the DMF-ethylic ether two-solvent mixture.

Yield: 88.78%; m.p. 206-208 °C; white powder; Anal. Calcd. for $C_{23}H_{21}N_7O_9S$: C, 48.33, H, 3.67, N, 17.16, Found: C, 48.28, H, 3.74, N, 17.21; FT-IR (KBr, cm^{-1}): 1648 (-C=N-), 1604 (C=C), 1576 (-C-C-), 3039 (C-H), 1236 (C-S), 1144 ($-SO_2$-N-), 1593 (-C=O), 3245 (-NH-), 1228 (-C-N), 1330.88 ($-NO_2$) cm^{-1}; ^{1}H-NMR d/ppm (400 MHz, DMSO): 2.72 (d, 6H, $-CH_3$ theophylline), 3.69 (s, 3H, $-OCH_3$), 4.81 (s, 2H, $-CH_2-$), 6.87 (s,1H, Ar-H), 7.35 (d, 2H, Ar-H), 7.76, 8.14 (d, 4H, Ar-H), 8.1 (s, 1H, -NH-), 8.2 (s, 1H, -CH=N-), 8.36 (s, 1H, -N=CH-N-).

Preparation of 4-methylbenzaldehyde{2-[4-(theophylline-7-yl sulfonyl)phenoxy]ethyl}hydrazone) (**4**)

0.5 g (1.32 mmol) hydrazide, dissolved in 15 mL acetone, was treated with 0.158 g (1.32 mmol) of p-methyl-benzaldehyde in 10 mL ethanol and 3 drops of acetic acid and heated for 45 min. under stirring. The reaction mixture was finally cooled and the solid product filtered off and washed with ethanol on the filter. The obtained reaction yields of 86.00% were much dependent on the product solubility in ethanol. An white powder was obtained after purification from toluene or from ethanol as well as from the DMF-ethylic ether two-solvent mixture.

Yield: 86.00%; m.p. 211-213°C; white powder; Anal. Calcd. for $C_{23}H_{22}N_6O_6S$: C, 51.11, H, 4.07, N, 15.55, Found: C, 51.07, H, 4.13, N, 15.60; FT-IR (KBr) 1644 (-C=N-), 1604 (C=C), 1576 (-C-C-), 3040 (C-H), 1236 (C-S), 1147 ($-SO_2$-N-), 1595 (-C=O), 3248 (-NH-), 1227 (-C-N), 2942 (Ar-CH_3) cm^{-1}; ^{1}H-NMR d/ppm (400 MHz, DMSO): 2.30 (s, 3H, Ar-CH_3), 2.76 (d, 6H, $-CH_3$ theophylline), 4.81 (s, 2H, $-CH_2-$), 6.98, 7.78 (d,4H, Ar-H), 7.0, 7.64 (d, 4H, Ar-H), 8.1 (s, 1H, -NH-), 8.2 (s, 1H, -CH=N-), 8.36 (s, 1H, -N=CH-N-).

Testing of antimicrobial activity

The microbial investigation consisted in testing antimicrobial actity of new obtained compounds (azomethines), conventionally noted with 1-4, against the two tested microorganisms (*Staphylococcus aureus* ATCC 25923 si *Escherichia coli* ATCC 25922) by using the difuzimetric method Kirby-Bauer [28]**.**

The bacterial inoculum is the result of bacterial cultures for 18 h, which was standardized according to McFarland scale, obtaining 10^7-10^8 CFU/mL. The medium used (Muller Hinton agariza) was then inoculated. On the medium surface was applied stainless steel cylinders using a sterile tweezer splitting (about 200 μL) of the tested compund (1/10, 1/100, 1/1000 and 1/10000 dillutions). The plates hatch was performed at 37°C for 24 h. The microorganisms cultures were used both for samples inoculation and standard sample also (DMSO), therefore maintaining identical growth conditions.

After incubation can define two distinct zones: firstly, in which the microbial growth is inhibited by concentrations of antimicrobial substances and secondly a growth medium where the substance concentration is too small to inhibit the growth. As the diameter of the inhibition medium gets larger the germ reaches high sensitivity levels, meaning that the substance quantity required for tested microorganism inhibition is reduced and reverse [29-32].

Reading and expressing of results consisted of measuring the diameter of inhibition zone (in mm) using a line scale and classification in categories of sensitive strains [33-35].

Results and discussions

The new derivatives of theophylline-sulfonyl phenoxy-acetic acids were synthesized according to the method described in our previous paper [36, 37]. In first stage, the esters of theophylline-sulfonyl phenoxyacetic acids reacted with hydrazine monohydrate and the corresponding hydrazides of theophylline-sulfonyl phenoxyacetic acids was obtained. Finally, this hydrazides was condensed with various aldehyde (4-hydroxy-benzaldehyde, 4-methoxy-benzaldehyde p-nitrobenz-aldehyde and 4-methyl-

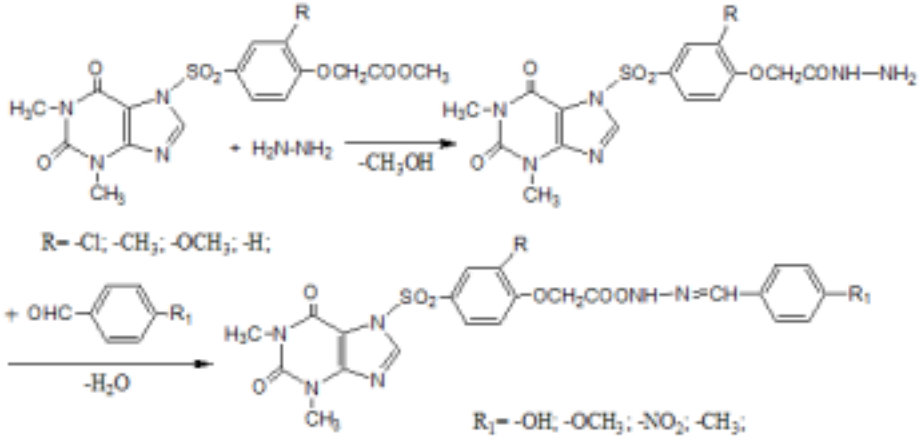

Fig.1. Synthesis of new compounds

benzaldehyde) the corresponding new derivatives (azomethines) was obtained (fig. 1) [36-38].

The syntheses were carried out with a reagent equimolecular ratio by refluxing in ethanol medium with low amounts of acetic acid as a catalyst. The reaction time was between 0.5-1 h with the less reactive aldehydes (*p*-hydroxy-, *p*-methoxy-, *p*-nitro-, and *p*-methyl-substituted) as well as in cases where the separation did not happen during heating. The obtained reaction yields between 85-90% were much dependent on the product solubility in ethanol. The products were purified from organic solvents such as ethanol, toluene, *o*-xylene as well as from two-solvent mixture: DMF-ethylic ether, ethyl acetate-ethylic ether, toluene-petroleum ether.

The newly synthesized compounds, their denominations, some physical-chemical characteristics and elemental analysis data are given in figure 2.

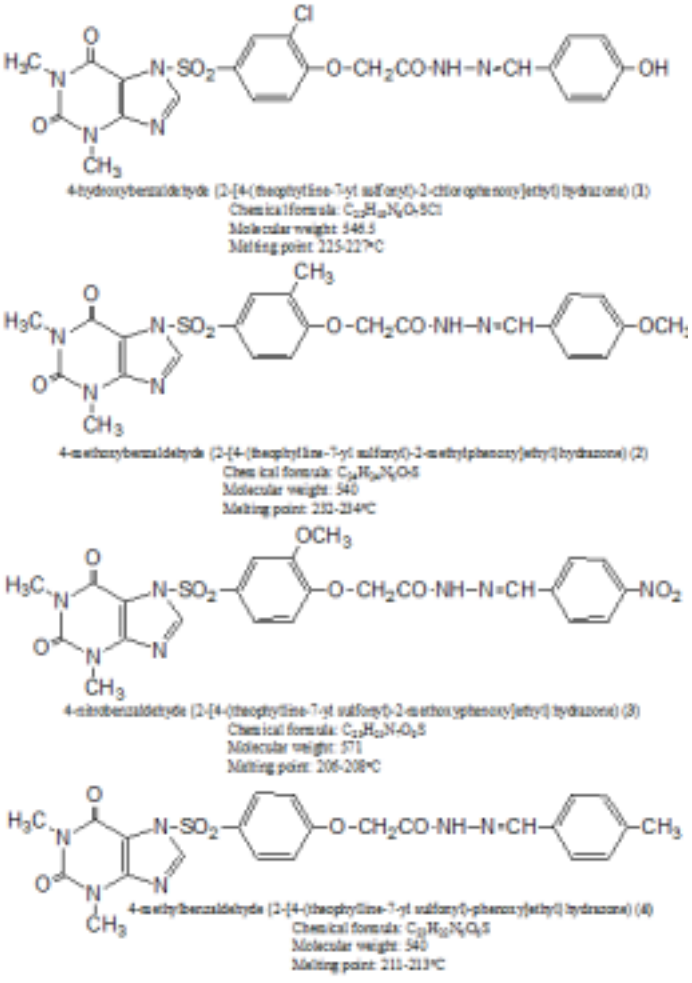

Fig.2. Structures of new compounds

The structures of the newly obtained derivatives were also confirmed by IR, ^{1}H-NMR spectral measurements and elemental analysis. The spectral analyses were in accordance with the assigned structures.

The IR spectrum of methyl esters of theophylline-sulfonyl phenoxyacetic acids confirm the presence of the functional methyl group through stretching vibration bands, narrow of medium intensity at 1432 cm^{-1} respectively 1436 cm^{-1}. The carbonyl (-C=O) from ester group is proved by a narrow absorption band of medium intensity at 1753-1754 cm^{-1}. The imidic group was included in six terminals is attributed the band length of 1710 cm^{-1}, corresponding to vibration of symmetric valence of group (-C=O) and band length from 1654-1656 domain, corresponding to the same groups (both being in the theophylline IR spectrum). For valence vibration of chain (-C=N-) the absorbtion was at 1654-1656 cm^{-1}. The presence of sulphonyl group (-SO_2-) was highlighted by the two valence vibration bands, first at 1330 cm^{-1} (symmetrical) while the second one appears at 1150-1152 cm^{-1} (asymmetrical). The characteric absorbtions of valence vibration ($C_{arqmathic}$-S) were observed at 1080 cm^{-1} and at 600 cm^{-1}. The valence vibrations of chains at medium intensity (C-H) from the aromatic chaing are found around 3050 cm^{-1} and those of deformation at 800 cm^{-1}. The medium intensity bands given by valence vibrations for chain (-C=C-) are found at about 1460-1464 cm^{-1}. In the ^{1}H-NMR spectrum of methyl esters of theophylline-sulfonyl phenoxyacetic acids, the protons of methyl group (-CH_3) of segment theophylline or aromatic ring of the rest phenoxyacetic appear as a singlet at 2.15-2.35 ppm. The protons of the ethyl group from the ester appear as multiplet (-CH_2-) at 4.58 ppm. In region 6.8-7.87 are presented the signals of aromatic protons and in the domain 8.45-8.55 ppm the protone from the rest of theophylline.

The structure of hydrazides is confirmed by the appearance in the IR spectrum of the characteristic bands for -NH-NH_2- group at 3436 cm^{-1}, 3308 cm^{-1} and 3296 cm^{-1}. The structure was strongly supported by the disappearance of the proton signals characteristic of the ester group that was replaced by the hydrazide group. In the ^{1}H-NMR spectrum of hydrazides protones of group –NH_2 from hydrazine fraction appear as a singlet at 1.95 ppm and protons adjacent to carbonyl string (-CO-NH-) could be identified under the shape of a singlet at 8.14 ppm.

In the IR spectra of the azomethines a quite strong absorption band attributable to the νC=N vibrations is to be found between 1643-1648 cm^{-1}. The benzene rings are responsible for the band between 1576-1578 cm^{-1} corresponding to the νC-C vibrations as well as for one or two absorptions between 3039-3045 cm^{-1} generated by

Sample test	Dilutions tested	Microorganism test	
		Staphylococcus aureus ATCC 25923	*Escherichia coli* ATCC 25922
1	1/10	12	11
	1/100	11	11
	1/1000	11	10
	1/10000	11	10
2	1/10	12	10
	1/100	12	10
	1/1000	11	9
	1/10000	10	8
3	1/10	17	20
	1/100	14	16
	1/1000	12	14
	1/10000	10	12
4	1/10	15	16
	1/100	14	13
	1/1000	11	12
	1/10000	10	11
Standard sample (DMSO)	-	0	0

Table 1
DIAMETERS OF THE INHIBITION AREAS/MM RESULTING FROM THE TESTS ON THE ANTIMICROBIAL ACTION OF NEW COMPOUNDS

the aromatic íC-H vibrations. Vibration bands for νNO_2 were noticed within the 1330.88 cm^{-1}, as a very strong band. Other bands show middle absorptions at values between 1346-1357 cm^{-1} and about 1427-1492 cm^{-1} for the deformation vibrations $äCH_3$ sym. and $äCH_3$ asym., respectively. The absorption band given by the valence vibration of the C-S bond is less intense and can be found within the 1234-1236 cm^{-1} range and that of the valence vibration of the C-O bond is noticed at about 1230-1234.44 cm^{-1}. The very weak valence vibration of the -N-H bond is to be found within the 3245-3250 cm^{-1} range. The S-N bands are noticed between 1072 and 1080 cm^{-1} being of a middle intensity, while the peaks of the $–SO_2$-NH- group placed between 1145-1147 cm^{-1} are intense and very intense [39, 40]. The structure of these compounds was strongly confirmed by ^{1}H-NMR spectral data. In the azometine spectrum the heterocyclic -N- group of adequate ä values is to be found. Within the domain of the aromatic protons the presence of the ethylene =C- proton can be noticed. The proton of the -N=CH- group is the most unscreened one and it occurs after the aromatic protons. The values of the chemical shifts and the peak intensities in the ^{1}H-NMR spectra are in good agreement with the proton types and number in azomethine.

As the research show there are different levels of sensitivity to the tested compounds against of the two microorganisms (table 1).

The research results prove the antibacterial action of the four compounds tested against microorganisms. In the case of testing the antibacterial action of compounds 1 and 2 there was highlighted a greater sensitivity of positive Gram bacteria (*Staphylococcus aureus* ATCC 25923) compared with negative Gram bacteria (*Escherichia coli* ATCC 25922), phenomenon given by the presence of substitutes of chlorine and hydroxyl type (-OH) of compound 1 and metoxi group ($-CH_3$) from compound 2, which can obviously influence the growth and spreading of this microorganism (fig. 3a, b, e and f).

Compunds 3 and 4 influence, also, differentially the development of the two microorganisms, antibacterial activity being obvious in case of negative Gram bacteria (*Escherichia coli* ATCC 25922) which presents a higher level of sensitivity to nitro groups ($-NO_2$) from compound 3 and methyl ($-CH_3$) from compound 4 from aldehyde (fig. 3c and d).

The inhibition diameter zones vary between 12-22 mm (compound 3) and 11-16 mm (compound 4) in case of *Escherichia coli* species and 10-17 mm (compound 3), respectively 10-15 mm (compound 4) in case testing the *Staphylococcus aureus* species (fig. 3c, d, g and h).

Generally, it was noticed a decrease of the level of sensitivity of the tested microorganism, assessed by the diameter of inhibition zone, as the dilution rate increased the inhibition values decreased from 1/10 dillution to 1/10000, compared to standard samples where the microorganism resistance to DMSO solvent was noticed (fig. 3i and j).

The comparative analysis of the antimicrobial action of the four tested compounds against two microorganisms we particularly notice the compound 3 which contains besides the basis compounds of these 3 compounds

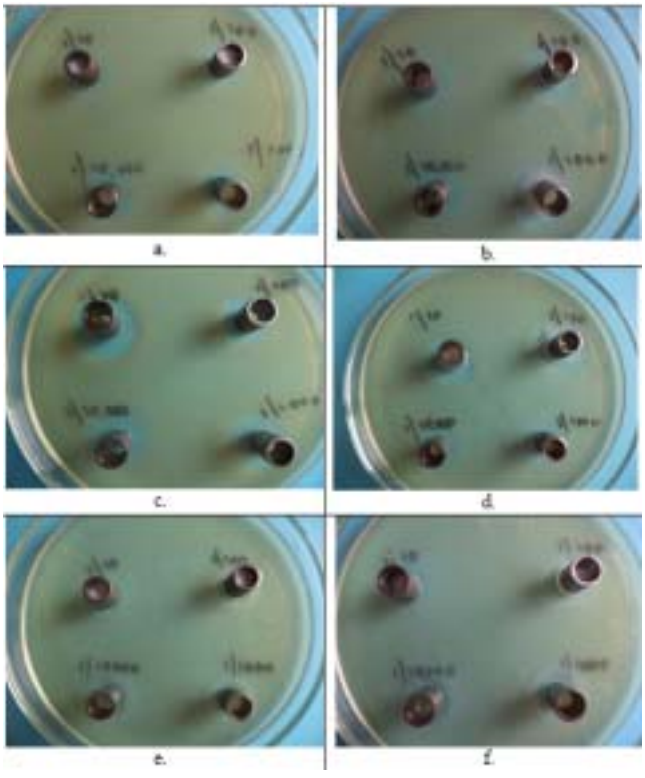

Fig. 3. Testing of the antimicrobial action: a - Compound 1 against *Staphylococcus aureus* ATCC 25923; b - Compound 2 against *Staphylococcus aureus* ATCC 25923; c - Compound 3 against *Staphylococcus aureus* ATCC 25923; d - Compound 4 against *Staphylococcus aureus* ATCC 25923: e - Compound 1 against *Escherichia coli* ATCC 25922; f - Compound 2 against *Escherichia coli* ATCC 25922

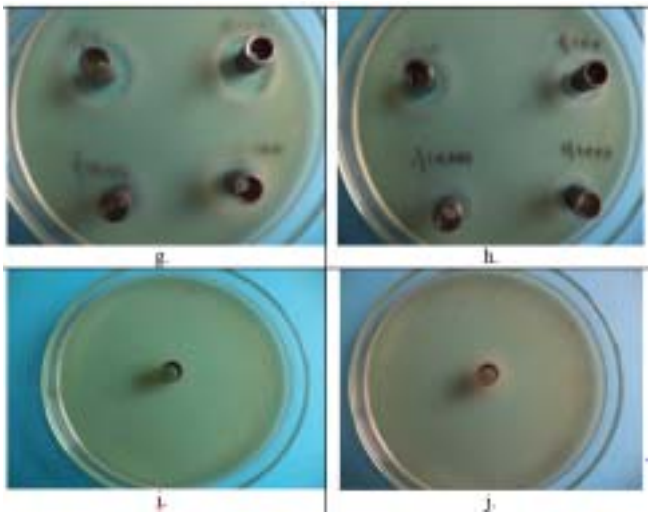

Fig. 3. Testing of the antimicrobial action: g - Compound 3 against *Escherichia coli* ATCC 25922; h - Compound 4 against *Escherichia coli* ATCC 25922; i - Standard sample (DMSO) against *Staphylococcus aureus* ATCC 25923; j - Standard sample (DMSO) against *Escherichia coli* ATCC 25922

(theophylline, phenoxy and group from aldehydes), nitro substitute (NO_2), that determine the antibacterial effect in the case of this compound.

Conclusions

New derivatives of theophylline-sulfonyl phenoxyacetic acids have been synthesized and their structure was proved through IR, ^{1}H-NMR spectral measurements.

The newly obtained final products were tested and show antimicrobial properties. The antimicrobial activity was estimated by measuring the growth inhibition area against two types of microorganism strains.

The antibacterial activity was detected in the case of the four compounds tested, demonstrating that substituents of the type chloro, hydroxy, methyl, methoxy or nitro, can influence this activity.

It was noticed a higher level of sensitivity of bacteria (*Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922) to compound 3, which contains as substitute nitro group.

References

1. ABBATE, F., CASINI, A., OWA, T., SCOZZAFAVA, A., SUPURAN, C.T., Bioorg. & Med. Chem. Lett., **14**, no 1, 2004, p. 217.
2. AMR, A.G., SABRRY, N.M., ABDALLA, M.M., ABDEL-WAHAB, B.F., Eur. J. Med. Chem., **44**, 2009, p. 725.
3. SUPURAN, C.T., SCOZZAFAVA, A., CASINI, A., Med. Res. Rev., **23**, no. 2, 2003, p. 146.
4. SLAWINSKI, J., BRZOZOWSKI, Z., Eur. J. of Med. Chem., **41**, no.10, 2006, p. 1180.
5. BEEDKAR, S.D., KHOBRAGADE, C.N., CHOBE, S.S., DAWANE, B.S., YEMUL, O.S., Int. J. Biol. Macromol., **50**, 2012, p. 947.
6. LINDAMOOD, C., LAMB, J.C., BRISTOL, D., COLLINS, J.J., HEATH, J.E., PREJEAN, D., Toxicol. Sci., **10**, 1987, p. 477.
7. LUPASCU, F.G., DRAGOSTIN, O.M., FOIA, L., LUPASCU, D., PROFIRE, L., Molecules, **18**, 2013, p. 9684.
8. MANZINI, S., PERRETTI, F., ABELLI, L., EVANGELISTA, S., SEEDS, E.A.M., PAGE, C.P., Eur. J. Pharmacol., **249**, 1993, p. 251
9. HANSEN, L.B., CHRISTRUP, L.L., BUNDGAARD, H., Int. J. Pharm., **88**, 1992, p. 237.
10. MOTEGI, T., KATAYAMA, M., UZUKA, Y., OKAMURA, Y., Res. Vet. Sci., **95**, 2013, p. 600.
11. OSORIO, M., ARAVENA, J., VERGARA, A., TABORGA, L., BAEZA, E., CATALÁN, K., GONZÁLEZ, C.,CARVAJAL, M., CARRASCO, H., ESPINOZA, L., Molecules, **17**, 2012, p. 556.
12. GUO, J.H., Drug Dev. Ind. Pharm., **20**, 1994, p. 2809.
13. ZUMLA, A., NAHID, P., COLE, S.T., Nat. Rev. Drug Disc., **12**, 2013, p. 388.
14. GOUDA, M.A., ABU-HASHEM, A.A., Arch. Pharm., **344**, 2011, p. 170.
15. GAREA, S.A., GHEBAUR, A., Mat. Plast., **49**, no. 1, 2012, p. 1.
16. CACIC, M., TRKOVNIK, M., CACIC, F., HAS-SCHON, E., Molecules, **11**, 2006, p. 134.
17. CARIOU, B., CHARBONNEL, B., STAELS, B., Trends Endocrin. Met., **23**, 2012, p. 205.
18. GHOSH, A.K., PRETZER, E., CHO, H., HUSSAIN, K.A., DUZGUINESS, N., Antivir. Res., **54**, 2002, p. 29.
19. EL-GABY, M.S., EL-HAG, A.G.A., EL-MAGHRABY, A.A., ABD EL-RAHMAN, M.T., HELAL, M.H., Eur. J. Med. Chem., **44**, 2009, p. 4148.
20. MOTEGI, T., KATAYAMA, M., UZUKA, Y., OKAMURA, Y., Res. Vet. Sci., **95**, 2013, p. 600.
21. DALY, J.W., Cell. Mol. Life Sci., **64**, 2007, p. 2153.
22. RAHAMAN, S.H., FUN, H.K., GHOSH, B.K., Polyhedron, **24**, no. 18, 2005, p. 3091.
23. LUPASCU, F.G., DASH, M., SAMAL, S.K, DUBRUEL, P., LUPUSORU C.E., LUPUSORU, R.V, DRAGOSTIN, O., PROFIRE, O., Eur. J. Pharm. Sci., **77**, 2015, p. 122
24. POBUDKOWSKA, A., DOMAN´SKA, U., KRYSKA, J.A., J. Chem. Thermodyn., **79**, 2014, p. 41
25. VOYNIKOV, Y., VALCHEVA, V., MOMEKOV, G., PEIKOV, P., STAVRAKOV, G., Bioorg. & Med. Chem. Lett., **24**, 2014, p. 3043
26. TEODORESCU, D., HODOSAN, C., NISTOR, L., HUBCA, G., Mat. Plast., **51**, no. 2, 2014, p. 296.
27. SLAWINSKI, J., BRZOZOWSKI, Z., Eur. J. of Med. Chem., 41, no.10, 2006, p. 1180.
28. JORGENSEN, J.H., TURNIDGE, J.D., Susceptibility test methods: dilution and disk diffusion methods. In: Manual of clinical microbiology, 9th ed. ASM Press, Washington, D.C., 2007, p. 1152.
29. MOCANU, A.M., LUCA, C., CIOBANU, G., DUNCA, S.I., SANDU, I.G., LUCA, A.C., Rev. Chim. (Bucharest), **66**, no. 8, 2015, p. 1137.
30. MOCANU, A.M., LUCA, C., DUNCA, S.I., CIOBANU, G., Rev. Chim. (Bucharest), **65**, no. 11, 2014, p. 1363
31. MOCANU, A.M., Rev. Chim. (Bucharest), **62**, no. 11, 2011, p. 1055.
32. TANTARU, G., MARIN, L., VIERIU, M., PANAINTE, A.D., POIATA, A., APOSTU, M., BIBIRE, N., Rev. Chim. (Bucharest), **66**, no. 12, 2015, p. 1965.
33. BORHAN, O., MURESAN, A., RADU, C.D., MURESAN, E., RIMBU, C., SANDU, I.G., Rev. Chim. (Bucharest), **66**, no. 11, 2015, p. 1796.
34. CHIRILA, L., SANDU, I., CEREMPEI, A., ECSNER, A.I., POPESCU, A., POPESCU, C., RIMBU, C., Rev. Chim. (Bucharest), **65**, no. 8, 2014, p. 911.
35. GRIGORIU, A.M., LUCA, C., HOROBA, E., DUNCA, S., Rev. Chim. (Bucharest), **64**, no. 6, 2013, p. 606.
36. MOCANU, A.M., Chem. Ind. Chem. Eng. Q., **16**, no.1, 2010, p. 89.
37. MOCANU, A.M., LUCA, C., Rev. Chim. (Bucharest), **65**, no. 2, 2014, p. 185.
38. MOCANU, A.M., ODOCHIAN, L., CARJA, G., ONISCU, C., Roum. Biotechnol. Lett., **13**, no.6, 2008, p. 3990.
39. OZKAN-DAGLIYAN, I., SAHIN, F., KOKSAL, M., Rev. Chim. (Bucharest), **64**, no. 5, 2013, p. 534.
40. NIKNAFS, B. N., AHMADI, A., Rev. Chim. (Bucharest), **63**, no.2, 2012, p. 136

Manuscript received: 12.10.2015