Synthesis, Characterization and Antimicrobial Activity of Some New Hydrazine Metallic Complexes

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The present paper reports the results of the studies on the synthesis of new metallic complexes obtained by the condensation of the hydrazides of sulphonamidated aryloxyalkyl-carboxylic acids with various chlorides and acetates of transitional metals resulting in final products with potential anti-tumour, anti-oxidative, anti-tubercular and herbicidal and growing regulators properties. These compounds were properly characterized by both physico-chemical methods (elemental analysis) and IR, ^{1}NMR, ^{13}C-NMR, GC–MS and UV-Vis spectral measurements. The obtained data gave useful information about the coordination way of the metallic ion, the atoms involved in the bonds between the central ion and ligands and also about the purity of the final compounds. Since actually an increasing number of complex combinations are largely applied in anti-microbial therapy the above mentioned complexes were tested for their antimicrobial action on certain gram negative (Escherichia coli, Pseudomonas aeruginosa), and gram positive (Staphyloccocus aureus) germs and on yeasts (Candida albicans).

Keywords: hydrazide, metallic complexes, antimicrobial action, tested activity, spectral measurements

The studies concerning the synthesis and characterization of the metallic complexes with potential biological activity are particularly important due to their possible applications in pharmaceutical, medicinal, agricultural as well as nutritional fields [1-8]. There has been growing interest in studying hydrazides and their metal complexes due to their application as antifungal, [9,10] antibacterial, [9-11] anticonvulsant, [12] anti-inflammatory, [10] analgesic,[13] antituberculosis,[14] anticancer activities [15].

Hydrazides act as herbicides, insecticides, nematocides, rodenticides and plant growth regulators and antioxidants.

Generally, the metallic ions act within the living organisms as complex forms with biological activity involved in the electron transfers, oxygen transport, energy transfer, redox reactions in cells, blocking or replacement of certain functional groups, nitrogen fixation in photosynthesis, synthesis and decomposition of the basic biological molecules [16-18].

The introduction of the transitional metals of a great biological interest to the human organism can be improved by using some of their complexes. The transitional metals have a specific tendency of forming complexes due to the presence of unoccupied orbitals *d* and acceptation of an electron pair from the ligand [19, 20].

The studies performed with complexes of transitional metals containing molecules of biological interest as ligands made evident the increase in their biological activity compared to the pure ligands. The complexes of transitional metals with various molecules of a biological interest are strongly involved in catalytic, functional and structural processes in the living organisms. The biological activity of the complexes depends on the local structure around the metallic ions, on the type and strength of the newly formed chemical bonds [14-18, 20].

The complex combinations are largely applied in the anti-viral and antimicrobial therapies where the pharmacological action of the medicaments is explained based on their complex forming ability [2-5, 20].

According to the literature data the metals under study in the present paper (Cr (II), Co(II) and Mn(II)) have proved their efficiency in sustaining some vital functions in the organism and in fighting against certain diseases [16, 17, 20].

These considerations suggested us to synthesize new metallic complexes and to test their antimicrobial properties.

The comparative inhibiting action was estimated by the diffusimetric method in the overlay agar (for bacteria) and Sabouraud (for yeasts) media. 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 [21].

Experimental part

Materials and method

The starting chemicals were of analytical grade and provided by Merck.

Melting points were measured by means of an Kruss KSP1N & KSP1D electrical apparatus destined to melting point determination, in open capillaries. 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.

The IR spectra were recorded on a FT-IR Bruker Vertex 70 spectrometer, with horizontal device for attenuated

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reflectance and diamond crystal, on a spectral window ranging from 4000 to 400 cm^{-1}, at a spectral resolution of 2 cm^{-1}, using KBr (ν, cm^{-1}). The IR bands are denoted as: w-weak; m- medium; s- strong; vs- very strong.

The ^{1}NMR spectra were recorded on a Varian Gemini 300BB spectrometer (^{1}H-NMR at 300 MHz and ^{13}C-NMR at 75 MHz) using DMSO-d6 as a solvent. Tetramethylsilane (TMS) was taken as an internal standard both in proton and carbon spectra. The ^{1}H-NMR data are reported in the following order: chemical shift (ppm), multiplicity number of protons, assignment of the signal, coupling constant (J) in hertz. Spin multiplets are given as: s (singlet), d (doublet).

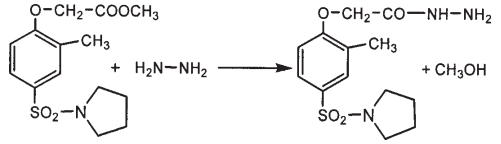
The ^{13}C-NMR data were reported in the following order: chemical shift (ppm), the signal / atom attribution, the coupling constant (J).

The UV spectra were recorded, in DMSO (~ 2.5–10.5 mol/L), on a Specord 40 Analytik Jena spectrophotometer.

MS spectra were recorded on a Shimadzu GCMS-QP 2010. The instrument running in chemical ionization (CI) mode. The system was equipped with a 25 m x 0.25 mm x 0.25μm DB-5ms capillary column. The ion source, quadrupole and interface temperatures were 330°C. Helium was used as carrier gas at constant flow (1.54 mL min^{-1}) with an initial pressure of 90.7 kPa, while methane was used as reagent gas in the mass spectrometer. The electron multiplier voltage was set at 1750 V. Two microliters of diluted solution was injected in cold pulsed splitless mode (initial injector temperature at 250°C). The temperature of the DB-5ms column was programmed from 50°C, let to stay for 2 min, then to 320°C at a rate of 15°C min^{-1} and finally let to stay for 5 min to 320°C.

Preparation of ligand [H_2L]

The ligand, 2-[2-(methyl-4-pyrolidinosulfonil)phenoxy] acetohydrazide (H_2L) was prepared (scheme 1) by adding amounts of [2-(methyl-4-pyrolidinosulfonil) phenoxy acetic acid methyl ester (3 g, 0.01 mol, in 20 mL of absolute ethanol) to hydrazine monohydrate (1 g, 0.02 mol). The mixture was refluxed under stirring for 40-45 h. The solid product which formed was filtered off, washed with ethanol, followed by recrystallized from ethanol and finally dried under vacuum over anhydrous $CaCl_2$. The yield was 92%.



Scheme 1. Preparation of the ligand

Preparation of the metal complexes

Preparation of complex of di-{2-[2-(methyl-4-pyrolidinosulfonil) phenoxy]acetohydrazide} with Mn **(1)**

0.783 g (0.002 mol) hydrazide were solved in 15 mL acetone and then 0.0013 mol manganese chloride solved in 5 mL water added. The reaction mixture was heated on a water bath under stirring for about 15 min. The solution was left at room temperature overnight, the resulting precipitate filtered and washed on the filter with water and with diethyl ether. The obtained product was purified by recrystallisation from ethanol or from acetone.

Preparation of complex of di-{2-[2-(methyl-4-pyrolidinosulfonil) phenoxy]acetohydrazide} with Cr **(2)**

0.783 g (0.002 mol) hydrazide were solved in 15 mL acetone and then 0.0013 mol chromium acetate solved in 5 mL water added. The reaction mixture was heated on a water bath under stirring for about 30 min. and the solution left at room temperature overnight. The formed precipitate was filtered and washed on the filter with water and diethyl ether. The obtained product was purified by recrystallisation from acetone.

Preparation of complex of di-{2-[2-(methyl-4-pyrolidinosulfonil) phenoxy] acetohydrazide} with Co **(3)**

0.783 g (0.002 mol) hydrazide were solved in 15 mL acetone and 0.0013 mol cobalt acetate solved in 5 mL water added. The reaction mixture was heated on a water bath under stirring for about 40 min. and the resulting precipitate filtered and washed on the filter with water and diethyl ether. The obtained product was purified by recrystallisation from ethanol or from acetone.

Compound **(1)**: Yield: 95.85%; m.p. 248-251°C; ochre pearl powder; Anal. Calcd. for $C_{26}H_{32}N_6O_8S_2Mn$: C, 46.22, H, 4.74, N, 12.44, Found: C, 46.16, H, 4.83, N, 12.49; FT-IR (KBr) cm^{-1}: 449.77 VW, 513.06 W, 543.92 W, 590.22 M, 613.36 M, 671.23 M, 725.23 W, 813.96 W, 964.41 W, 1006.70 M, 1060.85 M, 1103.28 M, 1141.86 VS, 1161.14 M, 1222.87 S, 1273.02 M, 1319.31 M, 1338.60 M, 1492.90 M, 1558.48 M, 1631.77 M, 1666.49 S, 1685.78 M, 1874.80 VW, 2248.99 VW, 2360.87 VW, 2850.78 W, 2985.80 M, 3024.38 M, 3174.82 M, 3386.99 W, 3846.05 VW; ^{1}H-NMR (300 MHz, DMSO-d6, δ/ppm): 1.62 (d, 4H, -(CH_2-)$_2$ pyrrolidine); 2.02 (s, 1H, -NH-); 2.31 (s,3H, Ar-CH_3); 2.77 (d, 4H, (-N-CH_2-)$_2$ pyrrolidine); 7.13 (s, 1H, H-7 aromatic); 7.67 (s, 1H, H-10, aromatic); 7.69 (s, 1H, H-6, aromatic); 8,2 (s, 1H, -NH- sec. amide); ^{13}C-NMR (75 MHz, DMSO-d6), δ/ppm: 14 (-CH_3, aliphatic, (2C)); 24.65 (-CH_2, pyrrolidine, (4C)), 57.55 (-N-CH_2, pyrrolidine, (4C)); Ar-C: [119.85 (2C); 125.57 (2C); 128.75 (2C); 133.55 (2C); 137.81(2C); 154.71 (2C)]; 157.75 (-N-C-O, amide (2C)); UV-vis (DMSO) λ_{max}/nm, (ε/ L . mol^{-1} . cm^{-1}): 276; 284; 363; GC–MS (m/z, (relative intensity, %)): 679 (1, M^+), 354(5), 313(2), 242(76), 70(100, base peak), 64(10), 60(10);

Compound **(2)**: Yield: 94.12%; m.p. 221-224°C; green pearl powder; Anal. Calcd. for $C_{26}H_{32}N_6O_8S_2Cr$: C, 46.42, H, 4.76, N, 12.50, Found: C, 46.35, H, 4.85, N, 12.57; FT-IR (KBr) cm^{-1}: 439.77 W, 540.07 W, 551.64 W, 590.22 S, 613.36 M, 671.23 M, 725.23 W, 813.96 W, 898.83 W, 964.41 W, 1006.84 M, 1060.85 M, 1103.28 M, 1141.86 VS, 1161.14 M, 1222.87 S, 1273.02 M, 1319.31M, 1338.60 S, 1381.03 M, 1450.47 S, 1496.76 M, 1558.48 S, 1593.20 S, 1631.77 S, 1666.49 VS, 1685.78 S, 1778.36 VW, 2877.79 W, 2958.80 M, 2997.37 M, 3174.82 M, 3385.99 W, 3664.44 W; ^{1}H-NMR (300 MHz, DMSO-d6, δ/ppm): 1.61(d, 4H, -(CH_2-)$_2$ pyrrolidine); 2.01 (s, 1H, -NH-); 2.31 (s, 3H, Ar-CH_3); 2.78 (d, 4H, (-N-CH_2-)$_2$ pyrrolidine); 7.2 (s, 1H, H-7 aromatic); 7.69 (s, 1H, H-10, aromatic); 7.7 (s, 1H, H-6, aromatic); 8,2 (s, 1H, -NH- sec. amide); ^{13}C-NMR (75 MHz, DMSO-d6), δ/ppm: 13.89 (-CH_3, aliphatic, (2C)); 24.71 (-CH_2, pyrrolidine, (4C)), 57.59 (-N-CH_2, pyrrolidine, (4C)); Ar-C: [120.15 (2C); 126.17 (2C); 129.12 (2C); 134.11 (2C); 138.10 (2C); 154.55 (2C)]; 157.76 (-N-C-O, amide (2C)); UV-vis (DMSO) λ_{max}/nm, (ε/L . mol^{-1} . cm^{-1}): 276; 284; 363. GC–MS (m/z, (relative intensity, %)): 672(2, M+), 313(1), 355(5), 242(78) 70(100, base peak), 64(12), 61(95);

Compound **(3)**: Yield: 96%; m.p. 198-200°C; pink purple powder; Anal. Calcd. for $C_{26}H_{32}N_6O_8S_2Co$: C, 46.15, H, 4.73, N, 12.42, Found: C, 46.03, H, 4.81, N, 12.48; FT-IR (KBr) cm^{-1}: 439.77 W, 513.06 W, 551.64 W, 590.22 S, 613.36 S, 671.23 M, 721.38 W, 756.09 W, 813.96 W, 894.97 W, 964.41 W, 1006.84 M, 1060.85 M, 1103.28 M, 1141.86 VS, 1161.14

S, 1222.87 S, 1273.02 M, 1296.16 M, 1319.31 S, 1338.60 S, 1400.32 M, 1427.32 S, 1446.61 S, 1496.76 M, 1558.48 VS, 1589.34 S, 1631.77 M, 1666.49 VS, 1685.78 S, 1782.22 VW, 2777.49 VW, 2850.78 W, 2958.80 M, 3024.38 M, 3174.82 M, 3340.70 W, 3386.99 VW, 3664.74 VW, 3745.75 VW; ^{1}H-NMR (300 MHz, DMSO-d6, δ/ppm): 1.6 (d, 4H, -(CH_2-)$_2$, pyrrolidine); 2.02 (s, 1H, -NH-); 2.30 (s,3H, Ar-CH_3); 2.78 (d, 4H, (-N-CH_2-)$_2$ pyrrolidine); 7.19 (s, 1H, H-7 aromatic); 7.7 (s, 1H, H-10, aromatic); 7.72(s, 1H, H-6, aromatic); 8,0(s, 1H, -NH- sec. amide); ^{13}C-NMR (75 MHz, DMSO-d6), δ/ppm: 14 (-CH_3, aliphatic, (2C)); 24.45 (-CH_2, pyrrolidine, (4C)), 56.95 (-N-CH_2, pyrrolidine, (4C)); Ar-C: [119.96 (2C); 125.17 (2C); 128.45 (2C); 133.25 (2C); 137.55 (2C); 154.34 (2C)]; 157.74 (-N-C-O, amide (2C)); UV-vis (DMSO) λ_{max}/ nm, (ε/L. mol^{-1}. cm^{-1}): 276; 284; 363; GC–MS (m/z, (relative intensity, %)): 676(1, M+), 313(1), 356(12), 242 (34), 70 (50), 61(100, base peak);

Testing of antimicrobial activity

The Mueller-Hinton agar (for bacteria) and Sabouraud (for yeasts) media were taken as culture media and placed in Petri dishes as uniform layers of 4 mm thickness, the *p*H values of 7.2–7.4 (for bacteria) and 6.5 (for yeasts) being previously measured. These media have nutritive value allowing the optimum growing of a great variety of micro-organisms and do not contain inhibitors of some anti-microbial substances.

From the young cultures of micro-organisms (18 h – bacteria, 72 h – yeasts) microbial suspensions of 1/100 for the micro-organisms to be tested, namely *Staphylococcus aureus* and *Candida albicans,* and of 1/1000 for *Escherichia coli* and *Pseudomonas aeruginosa* have been prepared. The inoculum of the micro-organism under study must be a representative one including all the microbial populations although sometimes heterogeneous regarding their resistance. Every plate was inoculated with 3 mL of the obtained suspensions and let to stay for 3-5 min for the inoculum absorption. After removing the inoculum the plates were maintained for 30 min at the room temperature. Then stainless steel cylinders were applied on the medium surface by means of sterile nippers and 200 μL of every tested sample placed into them. The plates were incubated with the cover down, at 37°C for 24 h with bacteria and at 28°C for 72 h with yeasts. The microorganism cultures were used for the impregnation of both samples and standard samples (represented by DMSO) since in every experimental model the three compounds were tested with the samples under study and also in comparison with impregnated standard samples under identical cultivation conditions.

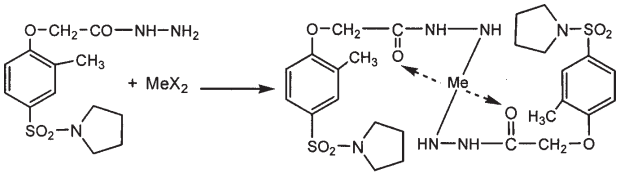
Reading and expressing of results

The readings were made only on the plates showing cultures corresponding to the purity and density requirements. The readings were accomplished with the naked eye by measuring the diameter of the inhibition area (mm) 2-3 times on different directions by means of a rule.

The expressing of the results was performed by the direct transcription of the inhibition area diameter into the categories of sensitive and resistant strains, respectively [22-24].

Results and discussions

New metallic complexes were synthesized by the condensation of the hydrazides of sulphonamidated aryloxyalkyl-carboxylic acids with various chlorides (manganese chloride) and acetates of transitional metals (acetate of chromium, acetate of cobalt) [25-28] (scheme 2).



MeX_2= $MnCl_2$·$4H_2O$, Cr $(CH_3COO)_2$·$4H_2O$, Co $(CH_3COO)_2$·$4H_2O$

Scheme 2. Synthesis of new compounds

The syntheses were carried out by treating the hydrazides of the sulphonamidated aryloxyalkylcarboxylic acids solved in acetone or other solvent (methanol or ethanol) with the metallic chlorides and acetates taken in a small excess (with respect to the 2:1 mole ratio) and dissolved in either water or methanol as the case was. The mixture was heated under stirring for 30-40 min. The resulting precipitate was filtered out and washed with ethylic ether on the filter. The final compound was purified from hot ethanol or toluene.

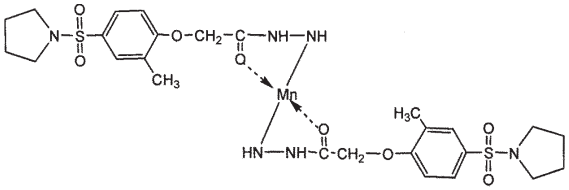
The newly synthesized compounds, their denominations, some physico-chemical characteristics are given in scheme 3.

Spectral measurements

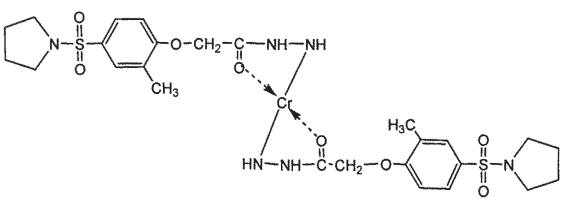
The structures of the newly obtained derivatives were elucidated by IR, ^{1}H-NMR, ^{13}C-NMR, GC-MS and UV-Vis spectral measurements.

The IR spectral characteristics

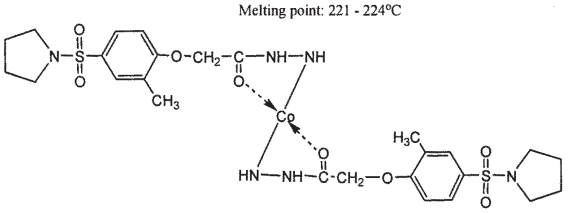
The C=O vibration band between 1631.77 -1666.49 cm^{-1} is very strong and the C=C one between 1558.48-1631.77 cm^{-1} is strong or very strong. The peaks corresponding to the -SO_2-NH- group are to be found at 1155.36 - 1159.22 cm^{-1} and those characteristic of Ar-O- can be seen at 1220.94 - 1253.73 cm^{-1}. The C=S band is placed within the 1099.42 - 1105.21 cm^{-1} range while the C=O and S-N



Complex with Mn of di-{2-[2-(methyl-4-pyrolidinosulfonil)phenoxy] acetohydrazide} (1)
Chemical formula: $C_{26}H_{32}N_6O_8S_2Mn$
Molecular weight: 675
Melting point: 248 - 251°C



Complex with Cr of di-{2-[2-(methyl-4-pyrolidinosulfonil)phenoxy] acetohydrazide} (2)
Chemical formula: $C_{26}H_{32}N_6O_8S_2Cr$
Molecular weight: 672
Melting point: 221 - 224°C



Complex with Co of di-{2-[2-(methyl-4-pyrolidinosulfonil)phenoxy] acetohydrazide} (3)
Chemical formula:$C_{26}H_{32}N_6O_8S_2Co$
Molecular weight: 676
Melting point: 198 - 200°C

Scheme 3. Structures of new compounds

bands are noticed between 1060.85 and 1101.35 cm^{-1}. The bands corresponding to the ring vibration are placed within the 418.55-601.79 cm^{-1} and 914 - 1002.35 cm^{-1} ranges. The C-N vibration band can be seen at 1105.21 - 1138.00 cm^{-1} and the deformation vibration bands of δCH_3 sym. and δCH_3 asym. of medium and strong absorptions at 1375.24 cm^{-1} and 1431.18 cm^{-1}, respectively [29-33]. The spectra correspond entirely to the structures of the metallic complexes.

The ^{1}H-NMR spectra confirm the presence of the structure elements characteristic of every compound. In the aliphatic region of the spectra the $-SO_2-NH_2$ and methyl groups ($Ar-CH_3$) are identified at the corresponding δ values. The aromatic protons in the phenyl residue could be differentiated in function of vicinities and couplings. The chemical shifting values and intensities of the peaks in the H-NMR spectra are in full agreement with the proton types and number in every complex. The NMR spectra confirm for a certainty the structures of the obtained compounds [34, 35].

UV-Vis spectra

The results and discussion on the structure-reactivity-applicability correlation were directed according to the chromophore types by making evident the spectral shifting function of the type, number and position of the substitutes in the constituent aromatic radicals.

The spectral characteristics of the synthesized compounds draw the attention by the presence of three spectral regions.

The spectral regions are influenced by the solvent type and are represented by: the band I – λ = 265 - 276 the band II – λ= 275 - 284, the band III – λ = 283 - 363. '

The spectral shifting is explained as dependent on the substitute type, number and position, sensitiveness of forming intra-molecular and inter-molecular hydrogen bonds as well as on the solvent polarity [36, 37].

Mass spectra:

The proposed molecular formula of compounds was confirmed by comparing their molecular formula weight with the m/z values. In the mass spectra of the compounds, peaks were attributed to the molecular ions, m/z: 313 for the ligand, 679 [M]+ for complex 1; 672 [M]+ for complex 2, and 676 [M]+ for complex 3. These data are in good agreement with the proposed molecular formula of the ligand and its metal complexes 1–3. In addition to the peaks due to the molecular ion, the spectra exhibited peaks assignable to various fragments arising from the cleavage of the compounds in interaction with the accelerated electrons.

Antimicrobial activity

The sensitivity of micro-organisms towards the compounds **1, 2** and **3** was tested "*in vitro*" by applying the optimum and standardized cultivation conditions (culture medium, inoculum, incubation time etc.) [38]. For this purpose the *Kirby-Bauer* diffusion method has been applied as a reference method according to the CLSI standards [14] of USA. This common method is largely applied in laboratories where a rather small number of microbial strains with rapid growth and with no significant differences in their growing rates from one another are tested. By placing the cylinders containing amounts of 200 µL of the samples to be tested, denoted as **1**, **2**, **3** compounds, on the surface of a solid medium impregnated with a microbial culture, the active antimicrobial compound will diffuse through the medium showing a constant decrease in the concentration gradient from the cylinder edge to the periphery.

After a certain incubation time two distinct areas can clearly be noticed: one where the microbial growth is inhibited by the antimicrobial compound while the other is a growth area where the compound concentration is too low for inhibiting the growth.

The larger the diameter of the inhibition area the more sensitive the micro-organism meaning that the amount of the substance necessary to the micro-organism inhibition is lower and inverse. The Kirby-Bauer technique allowed the estimation of the critical diameters making possible the classification of the micro-organisms under study as "sensitive" and "resistant".

The antimicrobial activities of the compounds **1**, **2** and **3** were tested on the micro-organism strains of *Staphylococcus aureus* ATCC–25923, *Escherichia coli* ATCC–25922, *Pseudomonas aeruginosa* ATCC–27853 and *Candida albicans* ATCC–10231. The obtained results are shown in table 1.

Table 1

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

Microorganism test	Sample test			
	1	2	3	Standard sample (DMSO)
Staphylococcus aureus ATCC–25923	10	12	0	0
Escherichia coli ATCC–25922	35	37	17	0
Pseudomonas aeruginosa ATCC–27853	0	18	12	0
Candida albicans ATCC–10231	18	11	16	0

The anti-bacterial activity was made evident for the compounds **1** (fig. 1a) and **2** (fig. 1b), with the *Staphylococcus aureus* species where the inhibition area diameters were of rather close values (10 mm for **1** and 12 mm for **2**) while **3** did not show anti-bacterial effect (fig. 1c) since the inhibition area diameter was 0 as with the standard sample (fig. 1d).

As revealed by the data obtained with the *Escherichia coli* species all the tested compounds showed anti-bacterial action decreasing in the following order: **2 < 1 < 3** (fig. 2a-c). The results are close to those of the standard sample (fig. 2d).

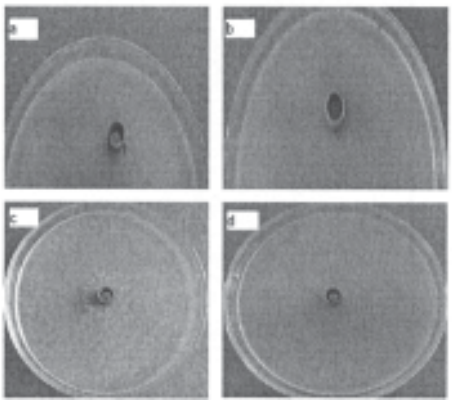


Fig. 1. Testing of the antimicrobial action of the compounds against *Staphylococcus aureus*

a) Testing of the antimicrobial action of the 1 compound
b) Testing of the antimicrobial action of the 2 compound
c) Testing of the antimicrobial action of the 3 compound
d) Testing of the antimicrobial action of the standard sample (DMSO)

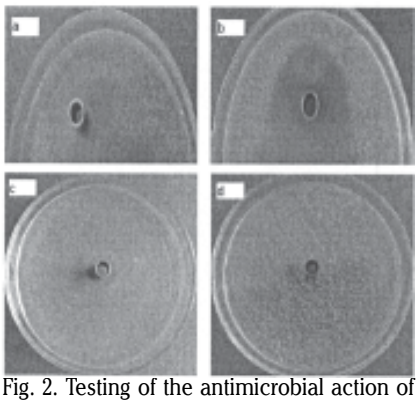


Fig. 2. Testing of the antimicrobial action of the compounds against *Escherichia coli*
a) Testing of the antimicrobial action of the 1 compound
b) Testing of the antimicrobial action of the 2 compound
c) Testing of the antimicrobial action of the 3 compound
d) Testing of the antimicrobial action of the standard sample (DMSO)

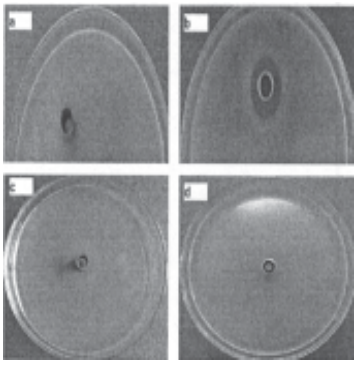


Fig. 3. Testing of the antimicrobial action of the compounds against *Pseudomonas aeruginosa*
a) Testing of the antimicrobial action of the 1 compound
b) Testing of the antimicrobial action of the 2 compound
c) Testing of the antimicrobial action of the 3 compound
d) Testing of the antimicrobial action of the standard sample (DMSO)

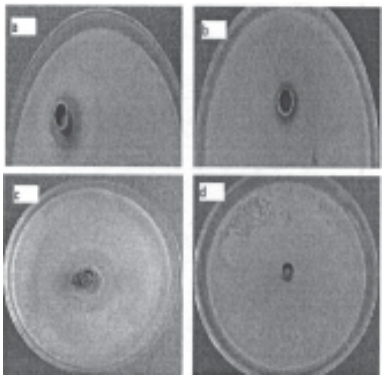


Fig. 4. Testing of the antimicrobial action of the compounds against *Candida albicans*
a) Testing of the antimicrobial action of the 1 compound
b) Testing of the antimicrobial action of the 2 compound
c) Testing of the antimicrobial action of the 3 compound
d) Testing of the antimicrobial action of the standard sample (DMSO)

The species *Pseudomonas aeruginosa* was not inhibited by the compound **1** (fig. 3a) unlike the compounds **2** and **3** giving inhibition areas of diameters of 18 mm (fig. 3b) and 12 mm (fig. 3c), respectively, as compared to the diameter of 0 mm noticed with the standard sample (fig. 3d).

With *Candida albicans* the inhibition area diameters were different, especially for compound **1** -18 mm (fig. 4a) and **3** - 16 mm (fig. 4c), while a slighter inhibition was noticed with compound **2** with an inhibition area diameter of only 11 mm (fig. 4b) compared to the standard sample (fig. 4d).

As made evident by the data of the anti-microbial tests the sensitivity/resistance of the micro-organisms is different toward the tested compounds due to both the different chemical structures of the compounds and the different types of the micro-organisms under study differing in their cell ultra-structures and response manner to the chemical compounds: Gramm-positive (*Staphylococcus aureus*), Gramm-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*) and yeasts (*Candida albicans*).

Conclusions

Three new compounds derivatives of sulphonamidated phenoxyalkylcarboxylic acids (metallic complexes) were thus synthesized and the pure products purified finally by recrystallization from ethanol or toluene. They were characterized by means of elemental analysis data and spectral measurements (IR, ^{1}H-NMR, ^{13}C-NMR, UV-Vis and GC–MS) that undoubtedly confirmed the advanced structures.

The antimicrobial activity was estimated by measuring the growth inhibition area against four types of microorganism strains.

By analysing the antimicrobial activity of the tested compounds (**1**, **2** and **3**) a clear inhibition of the microorganism growth and multiplication was noticed in the presence of the compounds under study especially in case of *Escherichia coli* species and somehow lower for the levura *Candida albicans*.

The tested compounds were active toward the microorganisms taken in the study excepting for the compounds **1** and **3** which showed no activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, respectively.

Among the tested compounds the **2** compound had a clear inhibiting effect on the growth and multiplication of the most microorganisms taken in the study which showed an increased sensitivity.

A slight anti-microbial effect of the compound **3** against the tested microorganisms was noticed.

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