Antibacterial Activity of Some New Hydrazide Derivatives with Potential Biological Action

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In the present paper the results of some studies on the synthesis of some new derivatives of sulfonamidated aryloxyalkylcarboxylic acids obtained by the condensation of their hydrazides with several substrata affording final compounds with potential biological activities such as anti-tumour, anti-oxidative, anti-tubercular as well as fungide, acaricide and plant growth regulating actions are reported. The synthesis stages of the new products (azomethines and diazoaminoderivatives) are presented as well as the elemental analysis data and IR, ^{1}H-NMR spectral measurements aimed to elucidate the chemical structures while the anti-microbial properties were confirmed by microbiological tests. The anti-microbial activity was estimated by measuring the growth inhibition area for four different strains of microorganisms: Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Candida albicans. The tests performed have confirmed the potential biological properties of the newly obtained compounds.

Keywords: azomethines, diazoaminoderivatives, IR, ^{1}H-NMR spectra, antimicrobial activity

The researches performed by now have elucidated the main characteristics of the sulphonamidated aryloxyalkylcarboxylic acid derivatives with various applications in both medicinal and agricultural fields. By extending these studies new potentially biological active compounds could be synthesized starting from the corresponding derivatives [1-7].

We have directed our studies to the obtaining of new sulphonamidated aryloxyalkyl-carboxylic derivatives (azomethines and diazoaminoderivatives) since the sulphonamidated aryloxyalkylcarboxylic derivatives show a low toxicity, are biodegradable, do not show cumulative properties within the organism and do not cause harmful effects.

The azomethines and diazoaminoderivatives have been found to show antibacterial, anti-convulsive and antitubercular activities [8-11]. Apart from this, they could also be used as herbicides, acaricides, fungicides and plant growing stimulators [12-16].

Following these considerations, in the present paper the synthesis of new azomethines and diazoaminoderivatives as well as the results of their antimicrobial activity testing are reported.

The antimicrobial activities of the newly obtained compounds were estimated against a Gram-positive (*Staphylococcus aureus*) and two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa)* bacteria and against a levura (*Candida albicans*).

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 [17].

Experimental part

General procedure of the azomethine synthesis

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 0.5-1.0h with the less reactive aldehydes (*o*-nitro-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.

General procedure of the diazoaminoderivative synthesis

The diazotization was carried out by treating the hydrazide with aqueous hydrogen chloride, in a 1:4.5 or 1:5 HCl/amine ratio. The suspension was cooled at 0-5°C, and the required amount of 10% $NaNO_2$ solution then added stepwise, under stirring. The mixture was stirred for another 15 minutes at the same temperature.

The diazotizations are usually carried out at low temperatures (0-5°C), sometimes below 0°C, in order to avoid the degradation of the diazonium salts although the reaction rate much increases with increasing temperature.

Coupling: The salicylic acid and the acid H were treated with aqueous NaOH solution.

Then the coupling component was poured stepwise on the diazonium salt. To maintain the alkaline *p*H (8-8.5) solid Na_2CO_3 solid was added.

The diazoaminoderivatives were purified from organic solvents such as ethanol, toluene, *o*-xylene or two combined solvents (DMF-ethylic ether, ethylacetate-ethylic ether, toluene-petroleum ether).

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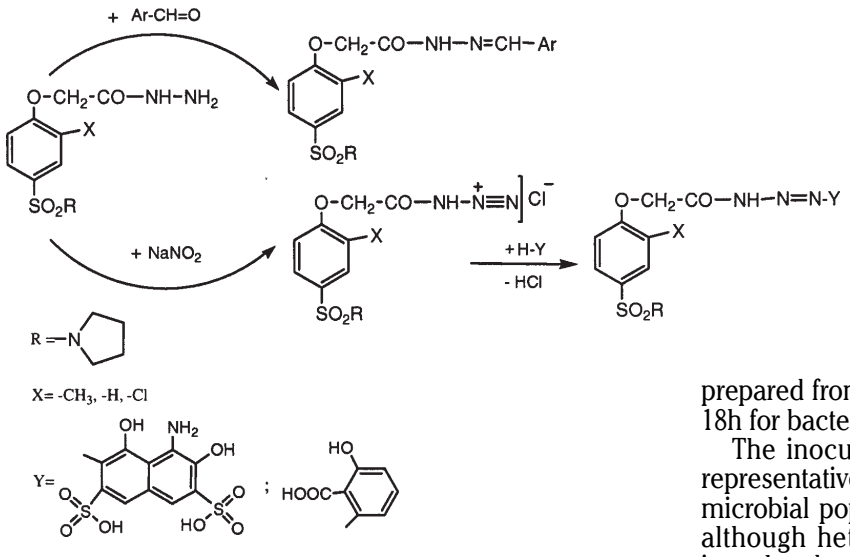


Fig.1. Synthesis of new compounds (azomethine and diazoaminoderivatives)

Testing of antimicrobial activity

The sensitivity of microorganisms against the four compounds was tested "*in vitro*" by applying optimum and standardized cultivation conditions (culture medium, inoculum, incubation time etc.) [18]. On this purpose the *Kirby-Bauer*, diffusimetric method was applied as a reference method according to the CLSI standards of USA [17]. This common method is largely applied in the laboratories testing rather few microbial strains of quick growth with no significant differences in the growing rate from one strain to another When the cylinders containing 200μL of the samples to be tested and denoted by P1, P2, P3 and P4 are set down on the surface of a solid medium impregnated with a microbial culture the active antimicrobial substance will diffuse through the medium showing a constant decrease in the concentration gradient from the cylinder edge to the periphery.

After an incubation time two distinct areas could be noticed: one where the microbial growth is inhibated by the concentrations of antimicrobial substance and the other as a growth area where the substance concentration is too low for inhibiring the growth.

The larger diameter of the inhibition area is the more sensitive the germ is, meaning that the substance amount necessary to the tested microorganism inhibition is lower and inversely. By the Kirby-Bauer technique the critical diameters could be measured making possible the germs under study to be classified as "sensitive" and "resistant".

Standardization of working technical conditions

Every component involved into the test performing can affect the diameter of the inhibition area and thus the interpretation criteria. Thus, a too abundant inoculum would result in a rather diminished inhibition diameter and inversely.

The technical conditions referring to the culture medium (composition, *p*H), inoculum, type of the cylinders, test performing, inoculation require an exact standardization.

Working technique

The overlay agar (for bacteria) and Sabouraud (for yeasts) media taken as culture media were placed in Petry plates as uniform layer of 4 mm thickness, *p*H = 7.2–7.4 (for bacteria) and *p*H = 6.5 (for yeasts) as measured prior to being poured into the plates. The nutrient values of these media promote the optimum development of a large variety of germs and apart from this they do not contain inhibitors of bacterial substances.

Microbial suspensions of 1/100 for the microorganisms *Staphylococcus aureus* and *Candida albicans* and of 1/1000 for *Escherichia coli* and *Pseudomonas aeruginosa* were prepared from the young cultures of microorganisms (of. 18h for bacteria and 72h for yeasts).

The inoculum from the germ under study must be representative, that is it must include all the categories of microbial population from the resistance point of view, although heterogeneous sometime. The plates were inoculated with 3mL of the obtained suspensions each, then let to stay for 3-5 min. for the inoculum absorption. After removing the inoculum the plates were maintained for 30min. at the room temperature. Then stainless steel cylinders were applied on the medium surface by means of sterile nippers and 200μL of every tested sample placed into them. The plates were incubated with the cover down, at 37°C for 24h with bacteria and at 28°C for 72h with yeasts; placing of more than two plates one over the other is not recommended. The micro-organism cultures were used for the impregnation of both samples and standard samples (represented by DMSO) since in every experimental model the four compounds were tested with the samples under study and also with impregnated standard samples by applying identical cultivation conditions.

Reading and expressing of results

Only the plates with cultures corresponding in purity and density were read. The reading was made to the naked eye by measuring 2-3 times the diameter of the inhibition area /mm in 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 [19-21].

Results and discussions

Synthesis of new azomethine and diazo-aminoderivatives

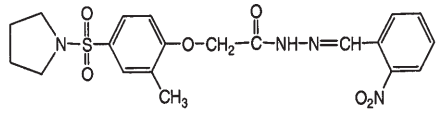
According to our purpose new compounds (azomethines and diazoaminoderivatives) were synthesized by the condensation of the sulphonamidated aryloxyalkylcarboxylic hydrazides with *o*-nitrobenzaldehyde and some coupling compounds such as salicylic acid and acid H [22-24] (fig. 1).

The performing of the diazotization-coupling reactions made possible the preparation of diazoaminiderivatives of structures similar to the azomethines with the only difference of replacing the imino group (-N=CH-) by the azo group.

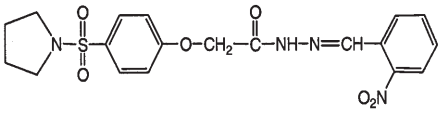
The newly synthesized compounds, their denominations, some physical-chemical characteristics and elemental analysis data are given in figure 2.

Compound (P1): Yield: 87.85%; m.p. 214-216°C; white powder; Anal. Calcd. for $C_{20}H_{22}N_4O_6S$: C, 53.81, H, 4.93, N, 12.55, Found: C, 53.70, H, 5.08, N, 12.64;

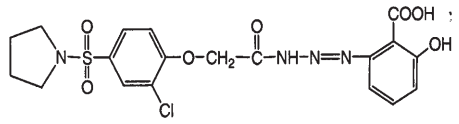
Compound (P2): Yield: 89.35%; m.p. 195-197°C; yellow powder; Anal. Calcd. for $C_{19}H_{20}N_4O_6S$: C, 52.77, H, 4.62, N, 12.96, Found: C, 52.68; H, 4.73, N, 13.05;



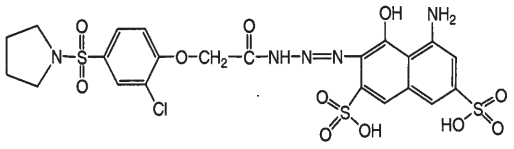
2-nitrobenzaldehyde {2-[4-(pyrolidinosulfonil)-2-methylphenoxy]ethyl}hydrazone) (P1)
Chemical formula: $C_{20}H_{22}N_4O_6S$
Molecular weight: 446
Melting point: 214-216°C



2-nitrobenzaldehyde {2-[4-(pyrolidinosulfonil)-phenoxy]ethyl}hydrazone) (P3)
Chemical formula: $C_{19}H_{20}N_4O_6S$
Molecular weight: 432
Melting point: 195-197°C



1-(3-{2-[4-(pyperazinilosulfonil)2-chlorphenoxy]ethyl}triaz-1-enyl)-salycilyc acids (P4)
Chemical formula:$C_{19}H_{19}N_4O_7SCl$
Molecular weight: 482.5
Melting point: 221-223°C.



1-(3-{2-[4-(pyperazinilosulfonil)2-chlorphenoxy]ethyl}triaz-1-enyl)-H acid (P5)
Chemical formula:$C_{22}H_{22}N_5O_{11}S_3Cl$
Molecular weight: 663.5
Melting point: 206-208°C.

Fig. 2. Structures of new compounds

Compound (P3): Yield: 85.78%; m.p. 221-223°C; brown powder; Anal. Calcd. for $C_{19}H_{19}N_4O_7SCl$: C, 47.24, H, 3.93, N, 11.60, Found: C, 47.12, H, 4.02, N, 11.69;

Compound (P4): Yield: 90.00%; m.p. 206-208°C; yellow powder; Anal. Calcd. for $C_{22}H_{22}N_5O_{11}S_3Cl$: C, 39.78, H, 3.31, N, 10.55, Found: C, 39.67, H, 3.40, N, 10.62;

Structure elucidation by spectral measurement

The structures of the newly obtained derivatives were also confirmed by IR and ^{1}H-NMR spectral measurements.

The IR spectral characteristics of the four newly synthesized compounds are given in table 1.

In the IR spectra of the azomethines a quite strong absorption band attributable to the νC=N vibrations is to be found between 1638-1674cm^{-1}. The benzene rings are responsible for the band between 1577.77-1581.63cm^{-1} corresponding to the νC-C vibrations as well as for one or two absorptions between 3041.65-3088.10cm^{-1} generated by the aromatic νC-H vibrations. Vibration bands for νNO_2 were noticed within the 1330.88 - 1346.31cm^{-1} range, as a very strong band. Other bands show middle absorptions at values between 1346-1357cm^{-1} and about 1427-1492cm^{-1} for the deformation vibrations δCH_3 sym. and δCH_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 600-700cm^{-1} range and that of the valence vibration of the C-O bond is noticed at about 1230-1234.44cm^{-1}. The very weak valence vibration of the -N-H bond is to be found within the 3300-3500cm^{-1} range. The S-N bands are noticed between 1072.42 and 1080cm^{-1} being of a middle intensity, while the peaks of the $-SO_2$-NH- group placed between 1141.86-1157.29cm^{-1} are intense and very intense.

Since the diazoaminoderivatives are of an aromatic structure, their vibrations are to be found especially within the νC-H (2941.01-2948cm^{-1}) and νC-H (1469.11-1585.48cm^{-1}) valence vibration or C-H (663-875cm^{-1}) deformation vibration ranges [25-27].

The azo group gives weak infrared absorptions, for even unsymmetrical molecules. With the aromatic azo-derivatives absorption bands at 1553±8cm^{-1} and 1070±14cm^{-1} are to be found but their position in spectra is rather difficult to be exactly identified [25-29].

The ^{1}H-NMR spectra show the signals given in table 2.

In the azomethine spectrum the heterocyclic -N- group of adequate δ values have 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.

The fact can be noticed that by carrying out the diazotization-coupling pair operations the possibility of preparing diazoaminoderivatives of a structure similar to the azomethines has occurred. Their only structural difference is the imino group (-N=CH-) replacement by the azo group. This structure modification causes significant physico-chemical features for every compound.

Compounds	Characteristic bands, (KBr , cm^{-1})
1	443.63 VW, 513.06 W, 551.64 W, 590.22 M, 613.36 M, 671.23 M, 736.81 W, 810.10 W, 894.97 W, 983.69 W, 1010.70 M, 1072.42 M, 1103.28 M, 1141.86 VS, 1157.29 M, 1234.44 S, 1276.87 M, 1323.16 M, 1346.31 VS, 1492.90 M, 1535.33 S, 1577.77 M, 1638.28 M, 1681.92 S, 1697.35 S, 1870.95 VW, 2252.85 VW, 2360.87 VW, 2877.79 W, 2985.80 M, 3008.95 W, 3041.65 W, 3186.40 W
2	435.91 VW, 482.20 VW, 543.92 W, 578.64 M, 613.36 M, 659.65 M, 678.94 W, 833.25 W, 871.82 W, 910.40 W, 991.41 W, 1006.84 M, 1037.70 W, 1080.13 M, 1157.29 S, 1199.72 M, 1230.58 M, 1300.02 M, 1330.88 S, 1357.88 S, 1427.32 M, 1539.19 M, 1581.63 M, 1616.34 W, 1674.21 VS, 1701.21 M, 2877.79 W, 3088.10 W, 3379.28 W.
3	418.55 VW, 466.77 W, 538.14 M, 543.92 M, 615.29 M, 663.44 M, 700.16 M, 742.59 S, 839.03 W, 860.25 M, 875.68 W, 991.41 M, 1014.55 M, 1039.63 W, 1082.06 M, 1143.79 M, 1157.29 S, 1195.86 M, 1257.59 S, 1284.59 S, 1284.59 S, 1301.95 S, 1334.74 S, 1377.17 S, 1429.23 M, 1469.11 VS, 1489.75 S, 1553.55 VS, 1675.07 VS, 2872.72 M, 2941.01M, 2996.17 M, 3446.79 S, 3646.50 M, 3665.18 M, 3861.34 M, 3917.42 M, 3930.28 M.
4	420.48 W, 522.71 W, 590.22 W, 615.29 M, 663.51 W, 719.45 W, 748.38 W, 837.10 VW, 910.40 VW, 1014.55 W, 1070.49 W, 1155.36 M, 1222.87 W, 1301.95 VW, 1334.74 W, 1361.74 W, 1388.74 W, 1471.86 W, 1585.48 W,. 1678.07 M, 2586.54 W, 2948.51 M, 3111.17 M, 3446.79 VS, 3454.50 VS, 3734.18 M, 3801.69 M, 3901.99 M, 3946.28 M.

Table 1
IR SPECTRA OF THE OBTAINED COMPOUNDS, CHARACTERISTIC BANDS AND THEIR INTENSITY, (VS = very intense, S = intense, M =medium intensity, W = weak, VW = very weak)

Compounds	Characteristic bands (200 MHz , DMSO-d_6), δ/ppm
1	1,61(d, 4H, -(CH_2-)$_2$ pyrrolidine); 2.33 (s, 3H, -CH_3); 2.76(d, 4H, H-15, H-18 pyrrolidine); 4.78 (s, 2H,-CH_2-); 6.86 (s, 1H, H-10 aromatic); 7.58 (s, 1H, H-1aromatic); 7.60 (d, 2H, H-11, H-13aromatic); 7.67 (d, 1H, H-2 aromatic); 7.78(s, 1H, H-14 aromatic); 7.95 (s, 1H, H-3); 8.1 (s, 1H, -NH- sec. amide); 8.2(s, 1H, -CH-); 8.3 (s, 1H, H-6 aromatic);
2	1,61(d, 4H, -(CH_2-)$_2$ pyrrolidine); 2.78(d, 4H, H-15, H-18 pyrrolidine); 4.79 (s, 2H,-CH_2-); 6.9 (d, 2H, H-10, H-14 aromatic); 7.58 (s, 1H, H-1 aromatic); 7.67 (d, 1H, H-2 aromatic); 7.78(d, 2H, H-11, H-13 aromatic); 7.92 (s, 1H, H-3 aromatic); 8.1 (s, 1H, -NH-); 8.2 (s, 1H, -CH-); 8.3 (s, 1H, H-6 aromatic);
3	1,61(d, 4H, -(CH_2-)$_2$ pyrrolidine); 2.78(d, 4H, H-15, H-18 pyrrolidine); 4.79 (s, 2H,-CH_2-); 5(s, 1H, aromatic C-OH); 6.86 (s, 1H, H-3 aromatic); 7.01 (s, 1H, H-10 aromatic); 7.10 (s, 1H, H-5 aromatic); 7.45(s, 1H, H-4 aromatic);7.68 (s, 1H, H-11 aromatic); 7.79 (s, 1H, H-13 aromatic); 8.1 (s, 1H, -NH-); 11.2 (s, 1H, -OH carboxylic acid);
4	1,61(d, 4H, -(CH_2-)$_2$ pyrrolidine); 2 (d,2H,-S-OH alcohol); 2.78 (d, 4H, H-15, H-1 pyrrolidine 8); 3.98 (s, 1H, -NH-); 4.79 (s, 2H,-CH_2-); 5(s, 1H, aromatic C-OH); 6.86 (s, 1H, H-13 aromatic); 7.3 (s, 1H, H-2 aromatic);7.68 (s, 1H, H-14 aromatic); 7.78 (s, 1H, H-4 aromatic); 7.8 (s, 1H, H-16 aromatic); 7.92 (s, 1H, H-6 aromatic); 8.0 (s, 1H, -NH-);

Table 2
^{1}H-NMR SPECTRA OF THE SAMPLES

Microorganism tested	Tested sample				
	P1	P2	P3	P4	Standard (DMSO)
Staphylococcus aureus	0	13	10	15	0
Escherichia coli	30	20	27	40	0
Pseudomonas aeruginosa	0	0	0	0	0
Candida albicans	6	7	8	19	0

Table 3
DIAMETERS OF THE INHIBITION AREAS/MM RESULTING BY THE ACTION OF THE COMPOUNDS UNDER STUDY ON THE TESTED MICROORGANISMS

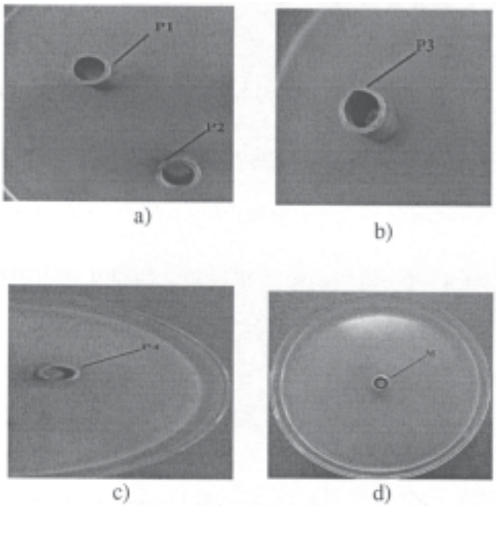


Fig. 3. Testing of the antimicrobial action of the compounds against *Staphylococcus aureus* a) Testing of the antimicrobial action of the P1 and P2 compounds
b) Testing of the antimicrobial action of the P3 compound
c) Testing of the antimicrobial action of the P4 compounds
d) Testing of the antimicrobial action of the standard sample (DMSO)

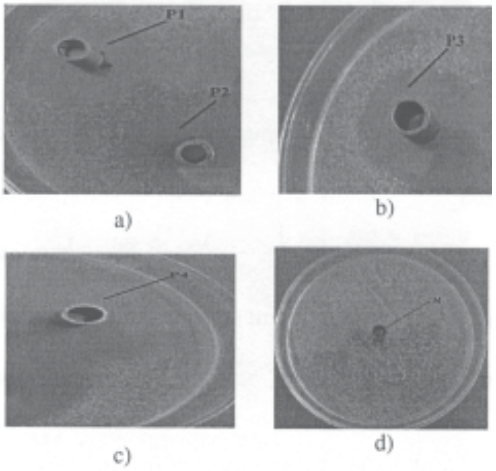


Fig. 4. Testing of the antimicrobial action of the compounds against *Escherichia coli*
a) Testing of the antimicrobial action of the P1 and P2 compounds
b) Testing of the antimicrobial action of the P3 compound
c) Testing of the antimicrobial action of the P4 compounds
d) Testing of the antimicrobial action of the standard sample (DMSO)

The ^{1}H-NMR spectra confirm the presence of the characteristic structural elements in every compound. The spectrum aliphatic region shows all types of the methyl groups (-CH_3, Ar-CH_3) at the corresponding δ values. The aromatic protons in the phenyl residue could be differentiated according to their vicinities and couplings.

With the compounds containing non-symmetrically substituted benzene ring, two singlets of very close values correspond to them [29]. The values of the chemical shifts and the peak intensities in the ^{1}H-NMR spectra are in full agreement with the proton types and number in every diazoaminoderivative.

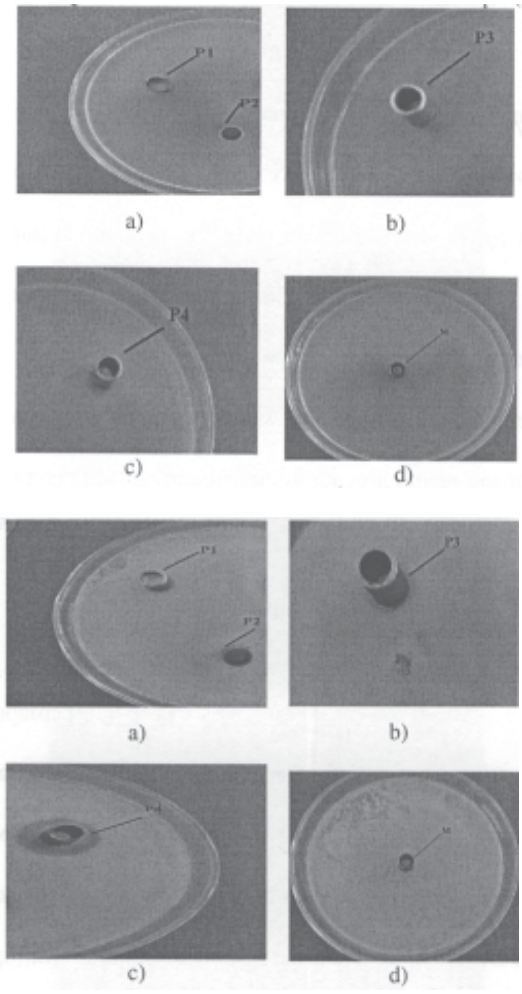


Fig. 5. Testing of the antimicrobial action of the compounds against *Pseudomonas aeruginosa*
a) Testing of the antimicrobial action of the P1 and P2 compounds
b) Testing of the antimicrobial action of the P3 compound
c) Testing of the antimicrobial action of the P4 compounds
d) Testing of the antimicrobial action of the standard sample (DMSO)

Fig. 6. Testing of the antimicrobial action of the compounds against *Candida albicans*
a) Testing of the antimicrobial action of the P1 and P2 compounds
b) Testing of the antimicrobial action of the P3 compound
c) Testing of the antimicrobial action of the P4 compounds
d) Testing of the antimicrobial action of the standard sample (DMSO)

The ^{1}H-NMR spectra confirm undoubtedly the structures of the newly obtained diazoaminoderivatives.

Testing of antimicrobial activity

Testing of the antimicrobial activity of the four types of compounds was made against a Gram positive (*Staphylococcus aureus*) and two Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria and a levura (*Candida albicans*) supplied by the Laboratory of Microbiology, Faculty of Biology of „Alexandru Ioan Cuza" University in Iasi.

As made evident by the data in table 3 no antibacterial action was shown by the P1 sample against *Staphylococcus aureus* (fig. 3a) where the inhibition area diameter is 0. The samples P2 (fig.3a), P3 (fig.3b) and P4 (fig.3c) have shown an antibacterial action against this microorganism similar to that of the standard sample (M) (fig. 3d).

By testing the antibacterial action with *Escherichia coli* a clear antibacterial effect shown by every sample under study was made evident. This effect is significant with the P4 compound (diameter of the inhibition area of 40 mm) (fig. 4c) decreasing then from P1 (fig. 4a) to P3 (fig. 4b) and P2 (fig. 4a) with the diameters of the inhibition areas of 30 mm, 27 mm and 20 mm, respectively, while no inhibition area was noticed with the standard (fig. 4d).

The results obtained with the Gram negative bacillus *Pseudomonas aeruginosa* are indicative of the resistance phenomenon for every sample P1, P2 (fig. 5a), P3 (fig. 5b) and P4 (fig. 5c) where the inhibition area diameter is 0 as with the standard sample (fig. 5d).

All the tested compounds have shown fungistatic activity against *Candida albicans* since the inhibition area diameters were of 6mm (sample P1), 7mm (sample P2) (fig. 6a), 8mm (sample P3) (fig. 6b) and 19 mm (sample P4) (fig. 6c) compared to the standard sample (0 mm) (fig. 6d).

The obtained results are indicative of an increased sensitivity of the *Escherichia coli* bacterium as well as the resistance of the *Pseudomonas aeruginosa* species to the tested compounds.

Conclusions

Due to the significant biological properties of the sulphonamidated phenoxyalkyl-carboxylic acid derivatives our studies were further directed to the obtaining of new active compounds with potential applications as pharmaceuticals and selective herbicides.

Four new compounds, azomethines and diazo-aminoderivatives as derivatives of sulphonamidated phenoxyalkylcarboxylic acids were thus synthesized and the pure products purified finally by recrystallization from ethanol or two combined solvent, DMF - diethyl ether. They were characterized by means of elemental analysis data and spectral measurements (IR, ^{1}H-NMR) 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 (P1, P2, P3 and P4) 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 microbial resistance was made evident especially with the *Pseudomonas aeruginosa* species towards the

compounds P1, P2, P3 and P4 and with *Staphylococcus aureus* towards the compound P1.

The general observation could be made that the type of the compounds under study affects the antimicrobial sensitivity/resistance.

Excepting the species *Pseudomonas aeruginosa*, with the other microorganisms the growth and multiplication inhibition was noticed especially with the compound P4.

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Manuscript received: 19.12.2013