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NEW THIOUREIDES OF 2-(4-METHYL-PHENOXYMETHYL)-BENZOIC AND 2-(4-METHOXY-PHENOXYMETHYL)-BENZOIC ACIDS WITH BIOLOGICAL ACTIVITY

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The present application is a continuation of our research concerning the synthesis and characterization of thioureides of 2-(4-methyl-phenoxymethyl)-benzoic acid and 2-(4-methoxy-phenoxymethyl)-benzoic acid with biological activities. The new compounds, are prepared in three stages by addition of some primary aromatic amines at 2-(4-methyl- or 4-methoxy-phenoxymethyl)-benzoyl isothiocyanate. Chemical structure of the synthesized compounds has been elucidated by their ^{1}H-NMR, ^{13}C-NMR and IR spectra and by elemental analysis. The *in vitro* qualitative and quantitative antimicrobial activity assay showed that the new thioureides exhibited antimicrobial activity.

INTRODUCTION

It is well known that many compounds bearing thioureide structure have been reported to have antimicrobial activity. Some of these compounds with thioureide structure are found to be associated with other therapeutical activities such as antitumoral, antiviral, antimycobacterial, antifungal, anthelmintic, diuretic, platelet aggregation inhibitor, anticonvulsant, H_2-antagonist, antidiabetic, insecticidal or pesticidal.

Keeping these biological activities, in the previous papers[1-8] we have presented the synthesis and characterization of some thioureides of the 2-phenoxymethyl-benzoic acid substituted with a methyl or methoxy group and with chloro, some complex combinations of transitional metals with some of these thioureides and also their antimicrobial activity.

In this study, some thioureides of 2-(4-methyl-phenoxymethyl)-benzoic acid and 2-(4-methoxy-phenoxymethyl)-benzoic acid with biological activities were synthesized and the chemical structures of the compounds have been confirmed by ^{1}H-NMR, ^{13}C-NMR and IR spectra and by elemental analysis.

RESULTS AND DISCUSSION

The synthesis of the new thioureides were performed in three stages.

The 2-(4-methyl-phenoxymethyl)-benzoic acid and the 2-(4-methoxy-phenoxymethyl)-benzoic acid synthesis

In the first step, 2-(4-methyl-phenoxymethyl)-benzoic acid (**1**) and 2-(4-methoxy-phenoxymethyl)-benzoic acid (**2**) were obtained by treating phtalide (**3**) with potassium p-cresolate and potassium *para*-methoxyphenoxide in xylene, under reflux. First the potassium salts of 2-(4-methyl-phenoxymethyl)-benzoic acid (**4**) or 2-(4

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methoxy-phenoxymethyl)benzoic acid (**5**) were obtained and, by having a good solubility in a 10% sodium hydroxide aqueous solution, can be separated from xylene. The acids **1** and **2** were removed from the salts by treatment with a hydrochloric acid solution.

The potassium p-cresolate and the potassium *para*-methoxyphenoxide were obtained through the reaction of *para*-cresol, respectively *para*-methoxyphenol with potassium hydroxide in xylene reaction medium. The resulting water was removed by azeotropic distillation.

The reactions are presented in Fig. 1.

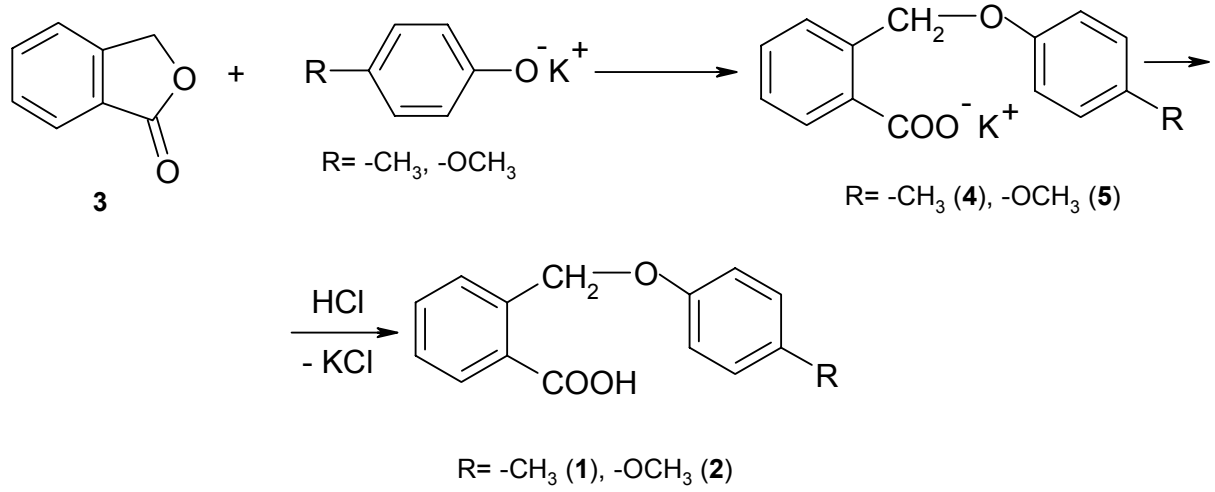


Fig. 1 – The synthesis of 2-(4-methyl-phenoxymethyl)-benzoic acid and 2-(4-methoxy-phenoxymethyl)-benzoic acid.

The 2-(4-methyl-phenoxymethyl)-benzoyl chloride and the 2-(4-methoxy-phenoxymethyl)-benzoyl chloride synthesis

In the second stage of the synthesis, the 2-(4-methyl-phenoxymethyl)-benzoyl chloride (**6**) and the 2-(4-methoxy-phenoxymethyl)-benzoyl chloride (**7**) were obtained by reacting, for three hours, the acid (**1**), respectively (**2**) with thionyl chloride, in anhydrous 1,2-dichlorethane. After the removal of the excess of the reactant and the reaction solvent, the raw acid chloride was used in the next stage.

Fig. 2 presents the mentioned reaction.

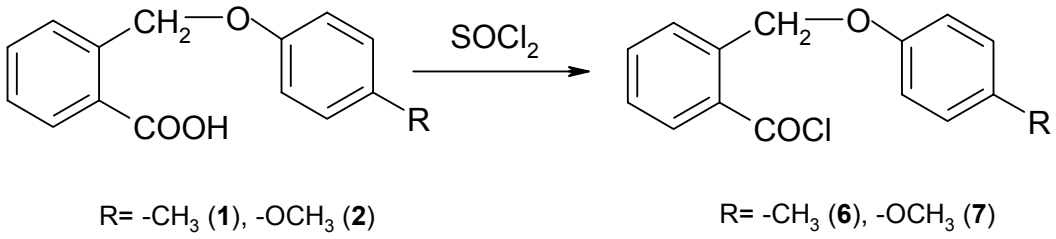


Fig. 2 – The synthesis of 2-(4-methyl-phenoxymethyl)-benzoyl chloride and 2-(4-methoxy-phenoxymethyl)-benzoyl chloride.

The new thioureides synthesis

In the third stage, the 2-(4-methyl-phenoxymethyl)-benzoyl chloride and the 2-(4-methoxy-phenoxymethyl)benzoyl chloride were reacted with ammonium thiocyanate, dried at 100^{O}C, and 2-(4-methyl-phenoxymethyl)-benzoyl isothiocyanate (**8**), respectively 2-(4-methoxy-phenoxymethyl)benzoyl isothiocyanate (**9**) was obtained. The reaction time was one hour and the reaction medium was acetone dried on potassium carbonate. The isothiocyanates were not separated and the new thioureides (**10 a-c and 11 a-c**), resulted after adding of some primary aromatic amines in the reaction medium, while the reflux continued for another hour, were obtained (Fig. 3).

The structure, molecular formula, molecular weight, melting point and the yield of the new thioureides are presented in Table 1.

The melting points were determined at Electrothermal 9100 apparatus and are uncorrected.

The new thioureides as solid, crystallized, white or light yellow are solubles, at normal temperature in acetone, chloroform and by heating in inferior alcohols, benzene, toluene, xylene and insolubles in water.

The elemental analysis of the newly obtained compounds, presented in Table 2, was performed with a Perkin Elmer CHNS/ O Analyser Series II 2400 apparatus.

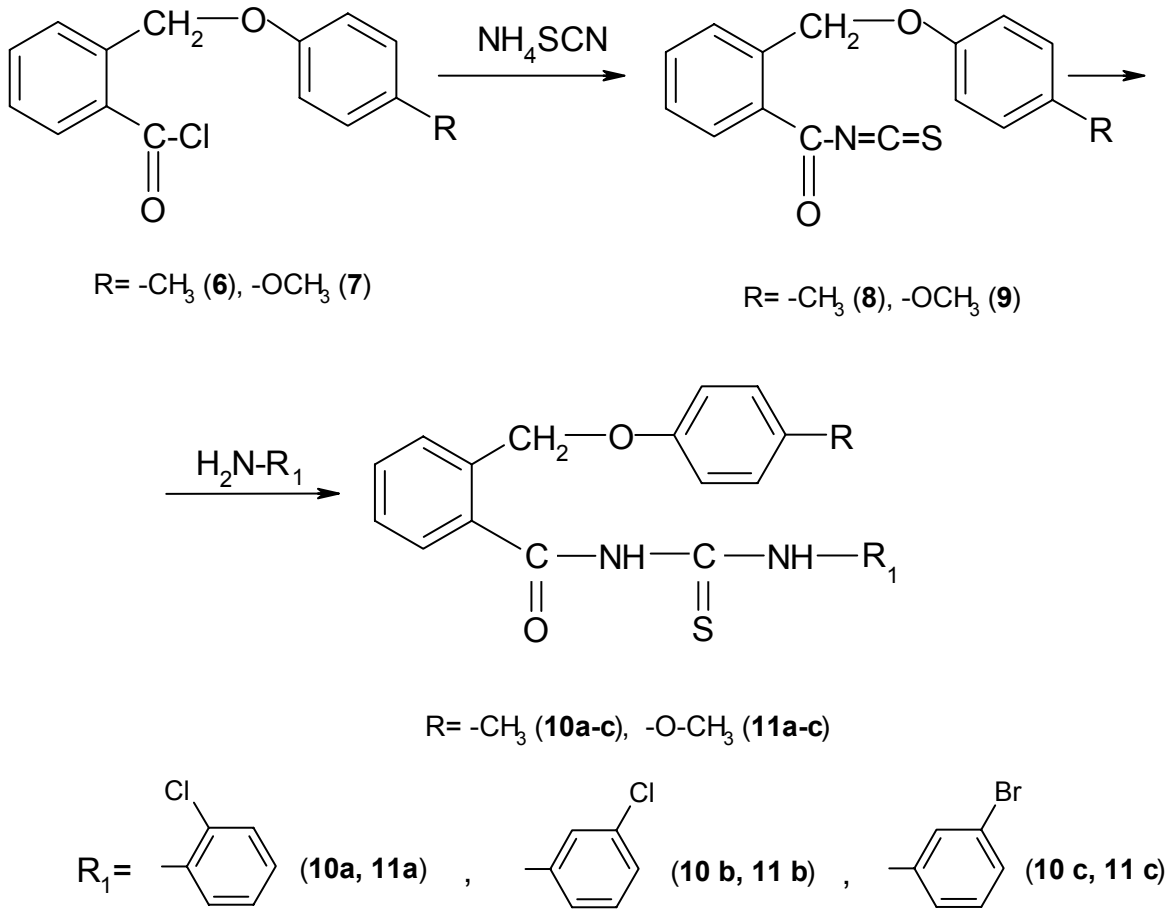
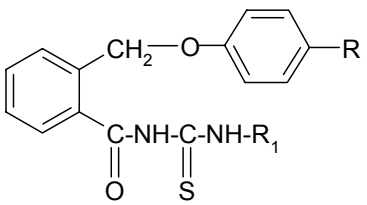


Fig. 3 – The synthesis of the new thioureides.

Table1

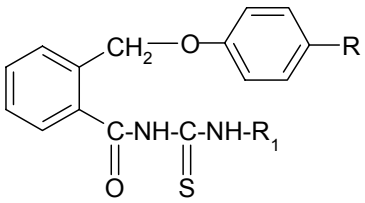
Some characteristics of the new compounds



Compound	R	R_1	Molecular formula	Molecular weight	Melting point (^{0}C)	Yield (%)
10a	$-CH_3$	$-C_6H_4Cl$ (2)	$C_{22}H_{19}ClN_2O_2S$	410.91	98.2- 101.4	74
10b	$-CH_3$	$-C_6H_4Cl$ (3)	$C_{22}H_{19}ClN_2O_2S$	410.91	144.5- 146.8	77
10c	$-CH_3$	$-C_6H_4Br$ (3)	$C_{22}H_{19}Br\ N_2O_2S$	455.37	155.6- 159.1	65
11a	$-OCH_3$	$-C_6H_4Cl$ (2)	$C_{22}H_{19}ClN_2O_3S$	426.91	95.3-97.5	87
11b	$-OCH_3$	$-C_6H_4Cl$ (3)	$C_{22}H_{19}Cl\ N_2O_3S$	426.91	118- 121.4	67
11c	$-OCH_3$	$-C_6H_4Br$ (3)	$C_{22}H_{19}BrN_2O_3S$	471.37	129.9- 133.2	62

Table 2

Elemental analysis of compounds **10a-c** and **11a-c**



Compound	R	R_1	C%		H%		N%		S%	
			t.	e.	t.	e.	t.	e.	t.	e.
10a	$-CH_3$	$-C_6H_4Cl$ (2)	64.30	64.07	4.66	4.54	6.82	6.89	7.80	7.66
10b	$-CH_3$	$-C_6H_4Cl$ (3)	64.30	64.13	4.66	4.75	6.82	6.71	7.80	7.91
10c	$-CH_3$	$-C_6H_4Br$ (3)	58.02	57.89	4.21	4,33	6.15	6.09	7.04	6.89
11a	$-OCH_3$	$-C_6H_4Cl$ (2)	61.89	61.55	4.48	4.39	6.56	6.47	7.51	7.58
11b	$-OCH_3$	$-C_6H_4Cl$ (3)	61.89	61.67	4.48	4.32	6.56	6.43	7.51	7.33
11c	$-OCH_3$	$-C_6H_4Br$ (3)	56.05	56.31	4.06	4.17	5.94	5.79	6.80	6.67

where t .- calculated, e.- experimental (obtained)

Spectral data

The molecular structure of the new compounds were confirmed by IR spectra, collected with a Buck M500 spectrometer. All measurements were made in KBr pressed disks.

The stretching bands due to νN-H of the amide group can be found to the highest values of the wave numbers.These are sharp peaks with a medium intensity occured in the region 3231-3378 cm^{-1}. The tioamide group shows a less intense stretching band at 3117-3136 cm^{-1} and, with a high probability, the band situated at 1389 cm^{-1} can be attributed to the tioamide group. For the antisymmetric stretching vibrations, methyl and methylene groups give a saturated (sp^3) νC-H stretch at about 2955 cm^{-1} and respectively, 2924 cm^{-1}; these bands are typical for aromatic compounds containing some saturated carbon. A very intense sharp stretching band, shown in the IR spectrum of these compounds in the region 1666-1687cm^{-1}, is due to the νC=O vibrations. Near this peak lies the intense band of νN-H, with a maximum at 1513 cm^{-1}, which overlaps the aromatic core vibrations. These compounds also show a typical alkyl-aryl ether at 1231 cm^{-1}, for the antisymmetric vibration, and 1030 cm^{-1} for the symmetric one. Halogens presence, in the molecules of new compounds, is proved by stretching bands situated at 1030-1044 cm^{-1} (for νC_{ar}-Cl) and at 823-868 cm^{-1} (for νC_{ar}-Br). The bands due by νC_{ar}-Cl overlaps the νC-O-C symmetric stretching band.

NMR spectra were performed at 300 MHz, (^{1}H) and at 75 MHz (^{13}C-) using an Varian Gemini 300BB equipment in hexadeuterodimethylsulfoxid $(CD_3)_2SO$ as solvent. Chemical shifts were recorded as δ values in parts per milion (ppm) with tetramethylsilane, $Si(CH_3)_4$, as internal standard. Multiplicities are given together with coupling constants in Hz. The multiplicity and the chemical shifts are influenced by the nature and the substituent position. For unambiguous assignment, ^{1}H-decoupling COSY ^{1}H-^{1}H and COSY ^{1}H-^{13}C were used.

^{1}H-NMR and ^{13}C-NMR spectra of the synthesised thioureides are presented in Table 3, respectively 4.

The chemical structure of the synthesized compounds has been confirmed by elemental analysis, IR and NMR spectroscopy.

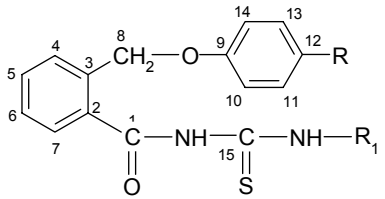
The antimicrobial activity was tested against Gram-positive (*Staphylococcus aureus*), Gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) bacteria and fungal (*Candida albicans*) strains.

Our results showed that the tested compound exhibited specific antimicrobial activity, the highest activity being noticed against suspended and adhered fungal cells. Only one compound was exhibited antimicrobial activity against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.*

In Table 5 are presented the results of the quantitative assay of the antimicrobial and antifungal activities of the new compounds, being known that a concentration of 4 μg/mL represents a very strong effect and at 512 μg/mL the compounds are inactivs.

Table 3

^{1}H-NMR data for the new compounds (δ ppm, J Hz)



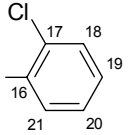
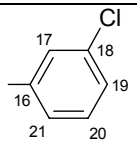
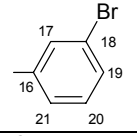
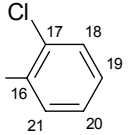
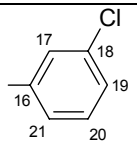
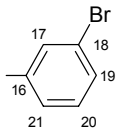
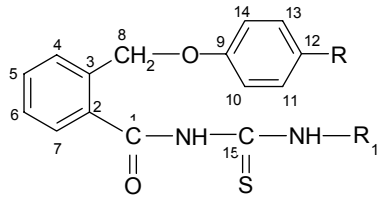
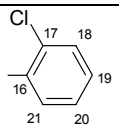
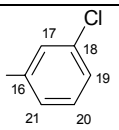
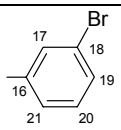
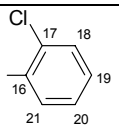
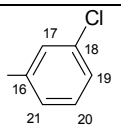
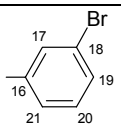
Compound	R	R_1	H_4- H_7	^{8}-CH_2-	H_{10}	H_{11}	H_{13}	H_{14}	H_{17}	H_{18}	H_{19}	H_{20}	H_{21}	-NH-
10a	-CH_3 2.19 s		7.30- 7.65 m	5.25 s	6.86 d (8.7)	7.04 d (8.7)	7.04 d (8.7)	6.86 d (8.7)	-	7.94 dd (8.2; 1.8)	7.30- 7.65 m			12.04 s 12.48 s
10b	-CH_3 2.19 s		7.42- 7.61m	5.24 s	6.86 d (8.5)	7.08 d (8.5)	7.08 d (8.5)	6.86 d (8.5)	7.74 t (0.9)	-	7.42- 7.61 m			11.90 s 12.40 s
10c	-CH_3 2.20 s		7.41- 7.65 m	5.24 s	6.86 d (7.7)	7.03 d (7.7)	7.03 d (7.7)	6.86 d (7.7)	7.86 t (1.9)	-	7.41- 7.65 m	7.35 t (7.9)	7.41- 7.65 m	11.91 s 12.39 s
11a	-OCH_3 3.70 s		7.40- 7.62 m	5.23 s	6.80 d (9.1)	6.89 d (9.1)	6.89 d (9.1)	6.80 d (9.1)	-	7.89 dd (7.9; 1.4)	7.40- 7.62 m			12.08 s 12.52 s
11b	-OCH_3 3.65 s		7.32- 7.65m	5.22 s	6.81 d (9.1)	6.91 d (9.1)	6.91 d (9.1)	6.81 d (9.1)	7.78 sl	-	7.32- 7.65 m			11.90 s 12.42 s
11c	-OCH_3 3.71 s		7.42- 7.51 m	5.27 s	6.87 d (9.1)	7.08 d (9.1)	7.08 d (9.1)	6.87 d (9.1)	7.87 tl (1.3)	-	7.42- 7.51 m	7.39 t (7.8)	7.42- 7.51 m	12.15 s 12.66 s

Table 4

^{13}C-NMR data for the new compounds (δ ppm)

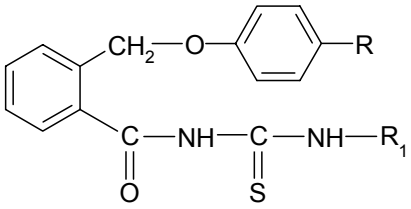


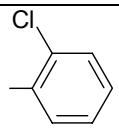
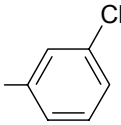
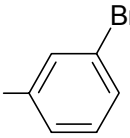
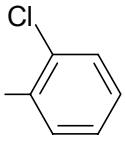
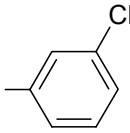
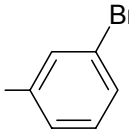
C \ R_1						
C_1	170.68	170.31	170.18	170.47	170.31	170.32
C_2	133.50	131.24	133.49	133.47	132.83	133.48
C_3	135.95	135.93	139.57	135.94	135.98	135.97
C_4	128.33	126.34	127.93	128.04	126.30	127.14
C_5	131.34	130.46	131.23	131.27	131.25	131.24
C_6	128.40	128.02	127.99	128.24	128.02	128.39
C_7	128.78	128.53	128.41	128.64	126.35	128.02
C_8	67.86	67.70	67.83	67.95	68.27	68.28
C_9	156.28	156.26	156.32	156.30	153.82	153.82
C_{10}	114.81	114.66	114.79	114.91	114.69	114.69
C_{11}	129.97	129.98	131.14	115.90	115.81	115.83
C_{12}	130.02	129.90	130.69	152.35	152.36	152.36
C_{13}	129.97	129.98	131.14	115.90	115.81	115.83
C_{14}	114.81	114.66	114.79	114.91	114.69	114.69
C_{15}	180.25	179.50	179.52	180.10	179.48	179.42
C_{16}	135.42	132.80	135.97	135.39	139.42	139.55
C_{17}	128.07	123.39	123.58	127.83	123.37	123.82
C_{18}	129.71	130.42	121.08	129.83	133.45	121.09
C_{19}	128.00	124.17	127.06	127.31	124.15	127.01
C_{20}	128.15	128.61	129.96	127.77	128.65	129.23
C_{21}	127.44	123.27	123.54	121.31	123.24	123.60
R - CH_3	20.31	20.24	20.22	-	-	-
-OCH_3	-	-	-	55.46	55.46	55.47

The antimicrobial research will be expended in order to obtain new compounds with a similar structure and to be analysed to obtain a structure-action relationship, the number of compounds in these study being to small too lead to a pertinent conclusion about the substitutions influence on the biological action.

Table 5

MIC values (expressed in μg/mL)



Compound	R	R_1	*S. aureus*	*E. coli*	*Ps. aeruginosa*	*Candida albicans*
10a	**-CH_3**		**> 512**	**>512**	**>512**	**>512**
10b	**-CH_3**		**> 512**	**>512**	**>512**	**4**
10c	**-CH_3**		**> 512**	**>512**	**>512**	**4**
11a	**-OCH_3**		**> 512**	**>512**	**>512**	**>512**
11b	**-OCH_3**		**> 512**	**>512**	**>512**	**>512**
11c	**-OCH_3**		**4**	**64**	**4**	**4**

EXPERIMENTAL

The 2-(4-methyl-phenoxymethyl)-benzoic acid and the 2-(4-methoxy-phenoxymethyl)-benzoic acid synthesis

A solution containing 0.05 mol of freshly distilled *para*-cresol or *para*-metoxyphenol in 30 mL xylene was placed in a round-bottom flask, equipped with a water removing device. Subsequently, 0,055 mol of potassium hydroxide were added.

The reaction mixture was refluxed until resulting water was removed by azeotropic distillation, while potassium *para*-cresolate, respectively potassium *para*-methoxyphenoxide precipitated at the bottom.

0.05 Mol of phtalide were added and the mixture was refluxed until it solidifies.

The precipitate was heated for solubilisation with 10% potassium hydroxide solution and then was diluted with 50 mL of water.

The aqueous phase was separated and acidulated with 1M hydrochloric acid solution until the mixture became acidic (pH 3), when the acid **1**, and the acid **2** precipited. The resulting precipitates, which crystallized from water: ethanol (1: 1) mixture (acid **1**), or a water: isopropanol (1: 3) mixture (acid **2**), shows a m.p. 122.5- 125.5^{0}C (acid **1**) and 178- 180^{0}C (acid **2**). 7.2 g Acid **1** (Wt 242.26) and 6.3 g acid **2** (Wt 258.26) were obtained (59.5% respectively 48.8% yield).

The 2-(4-methyl-phenoxymethyl)-benzoyl chloride and the 2-(4-methoxy-phenoxymethyl)-benzoyl chloride synthesis

0.02 Mol of acid **1** or acid **2**, 30 mL of dry 1,2-dichloroethane and 0.042 mol of thionyl chloride were placed in a round-bottom flask equipped with condenser and drying tube. The mixture was refluxed for 3 hours. The thionyl chloride in excess and the solvent were removed by reduced pressure. For the next step the acid chloride **6** and respectively **7** were used in the crude status.

The new thioureides synthesis (general procedure)

To a solution of ammonium thiocyanate (0.01 mol) in 5 mL dry acetone was added a solution of 2-(4-methyl-phenoxymethyl)-benzoyl chloride (0.01 mol) or 2-(4-methoxy-phenoxymethyl)-benzoyl chloride(0.01 mol) in 10 mL dry acetone.

The reaction mixture was refluxed one hour in a one round-bottom flask with a condenser and drying tube. After cooling, 0.01 mol of dry and freshly distilled primary aromatic amine in 2 mL dry acetone were added, by stirring, to the reaction mixture. The mixture was then refluxed for one hour. The product was precipitated after the cool reaction mixture was poured into 500 mL water.

The crude thioureides obtained, were crystallised from isopropanol with active carbon.

The *in vitro* **antimicrobial activity** was evaluated by qualitive and quantitative methods using compounds stock solutions in DMF of 1 mg/mL concentration.

The *in vitro* antimicrobial activity was evaluated by qualitive screening of the susceptibility spectra of different microbial strains to these compounds using adaptated diffusion methods: paper filter disk impregnation with the tested substances solutions, the disposal of tested solutions in agar wells and the spotting of tested solutions on microbial inoculums seeded medium.

The quantitative assay of the antimicrobial activity was performed by two comparative methods: the nutrient broth microdilution method in order to establish the minimal inhibitory concentration and the measurement of the absorbance of the microbial cells adhered to the plate wells and resuspended after staining with violet crystal.

CONCLUSIONS

New thioureides of 2-(4-methyl-phenoxymethyl)-benzoic acid and 2-(4-methoxy-phenoxymethyl)-benzoic acid were obtained based on the reaction of some primary aromatic amines with 2-(4-methyl- or 4-methoxy-phenoxymethyl)-benzoyl isothiocyanate.

This compounds were characterized by ^{1}H-NMR, ^{13}C-NMR and IR spectra and by elemental analysis.

The tested compound exhibited specific antimicrobial activity, the highest activity being noticed against suspended and adhered fungal cells.

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