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NEW 2-(4-ETHYL-PHENOXYMETHYL)BENZOIC ACID THIOUREIDES. SYNTHESIS, SPECTRAL ANALYSES AND MICROBIOLOGICAL ASSAYS

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We present in this paper the synthesis of new thioureides of the 2-(4-ethyl-phenoxymethyl)benzoic acid performed by the addition of various primary aromatic amines to the 2-(4-ethyl-phenoxymethyl)benzoyl isothiocyanate. We established the best reaction conditions in order to obtain with good yields high pure compounds. The chemical structure and the purity of the new compounds were confirmed by ^{1}H-NMR, ^{13}C-NMR, FT-IR spectra and using elemental analysis. The obtained substances were tested by qualitative and quantitative methods on various microbial and fungal strains and proved to be active at low concentrations both on gram positive, gram negative bacteria and fungi being a possible future therapeutical solution.

INTRODUCTION

The specialized literature mentions a series of thioureides tested for the antibacterial, antifungal and anthelmintic activity.[1-5] In previous papers we presented the synthesis, the structural characterization and we did some research on the antimicrobial action of some thioureides of namely the 2-phenoxymethylbenzoic acid, 2-(4-methyl-phenoxymethyl) benzoic acid, 2-(4-methoxy-phenoxymethyl) benzoic acid and 2-(4-chloro-phenoxymethyl) benzoic acid.[6-13]

The satisfying results determined us to continue this research in order to obtain 2-(4-ethyl-phenoxymethyl)benzoic acid thioureides. The general synthesis method used was the addition of some primary aromatic amines to the 2-(4-ethyl-phenoxymethyl)benzoyl isothiocyanate. The chemical structures were confirmed by ^{1}H-NMR, ^{13}C-NMR and IR, spectral analysis and by elemental analysis.

A qualitative screening assay was made on this new 2-(4-ethyl-phenoxymethyl) benzoic acid thioureides to determine the sensibility spectrum on different microbial strains, by adapted variants of the diffusion method. The quantitative assay of the antimicrobial activity was performed with a microdilution method and the minimal inhibitory concentrations (MIC) were measured.

RESULTS AND DISCUSSION

The new 2-(4-ethyl-phenoxymethyl)benzoic acid thioureides synthesis was completed in three stages. In the first step 2-(4-ethyl-phenoxymethyl)benzoic acid (I) was obtained by treating phtalide (II) with potassium *para*-ethyl-phenoxide in xylene. First, the potassium salt of 2-(4-ethyl-phenoxymethyl)benzoic acid (III) is obtained and, having a good solubility in a 10% potassium hydroxide aqueous solution, can be separated from xylene. The acid is removed from salt by treatment with a hydrochloric acid solution. The potassium *p*-ethyl phenoxide was synthesized from *p*-ethylphenol and solid potassium hydroxide

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in a xylene reaction medium, the resulting water being removed by azeotropic distillation.

In the synthesis second stage the 2-(4-ethyl-phenoxymethyl)benzoyl chloride was obtained by reacting the acid (I) with thionyl chloride in 1,2-dichlorethane. After the removal of the excess reactants, the raw acid chloride being used in the next stage.

In the third stage 2-(4-ethyl-phenoxymethyl)benzoyl chloride was reacted with ammonium thiocyanate to obtain 2-(4-ethyl-phenoxymethyl)benzoyl isothiocyanate (V). The reaction time was one hour and the reaction medium was acetone dried on potassium carbonate. The obtained isothiocyanate was not separated and the new thioureides (VIa-f) resulted after adding various primary aromatic amines in the reaction medium, continuing the reflux for another hour.[14]

The mentioned reactions are presented in Fig. 1.

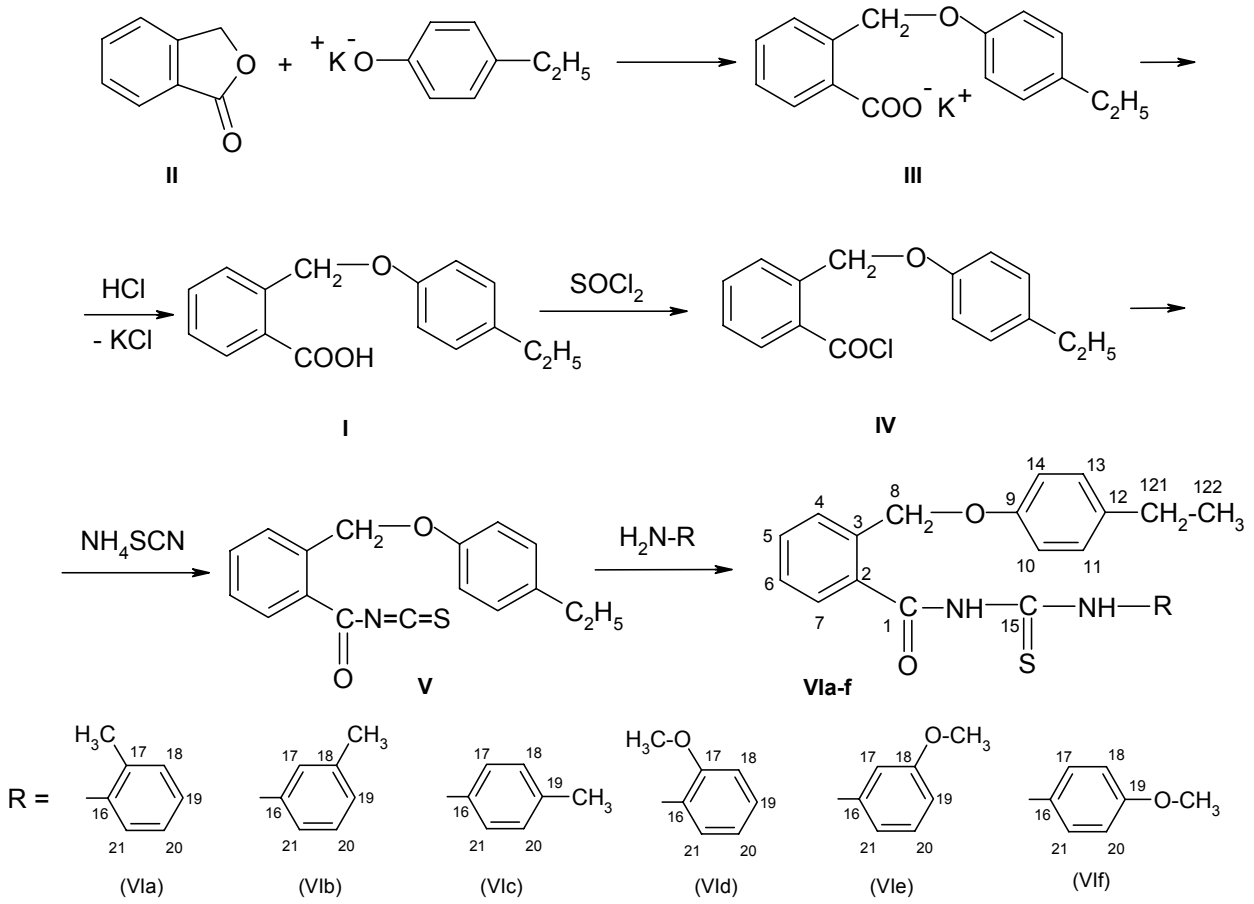

Fig. 1 – The synthesis pathway for the new thioureides.

The structure, molecular formula, molecular weight, melting point and synthesis yield for the new thioureides are presented in Table 1. All melting points were recorded with an Electrothermal 9100 apparatus and are uncorrected.

Table 1

The new compounds characteristics

No.	R	Molecular formula	Molecular weight	Melting point (^{0}C)	Yield (%)
VIa	-$C_6H_4CH_3$ (2)	$C_{24}H_{24}N_2O_2S$	404.43	98-99	72
VIb	-$C_6H_4CH_3$ (3)	$C_{24}H_{24}N_2O_2S$	404.43	129-130	81
VIc	-$C_6H_4CH_3$ (4)	$C_{24}H_{24}N_2O_2S$	404.43	99.3- 102.5	89
VId	-$C_6H_4OCH_3$ (2)	$C_{24}H_{24}N_2O_3S$	404.43	118-119	75
VIe	-$C_6H_4OCH_3$ (3)	$C_{24}H_{24}N_2O_3S$	404.43	109-110	79
VIf	-$C_6H_4OCH_3$ (4)	$C_{24}H_{24}N_2O_3S$	404.43	98.6- 101.3	63

The elemental analysis was realized using a Perkin Elmer CHNS/O Analyser Series II 2400 apparatus and the results are presented in Table 2, where t values represent the theoretical percentage and the e value, the experimentaly obtained percentage.

Table 2

The elemental analysis of the new thioureides

No	C%		H%		N%		S%	
	t.	e.	t.	e.	t.	e.	t.	e.
VIa	71.27	71.09	5.96	5.83	6.93	6.94	7.93	7.96
VIb	71.27	71.51	5.96	5.79	6.93	6.93	7.93	7.88
VIc	71.27	71.39	5.96	5.88	6.93	6.99	7.93	7.92
VId	68.56	68.72	5.73	5.61	6.66	6.59	7.63	7.68
VIe	68.56	68.40	5.73	5.81	6.66	6.62	7.63	7.57
VIf	68.56	68.67	5.73	5.73	6.66	6.57	7.63	7.61

Spectral data

The NMR spectra were recorded on a Gemini 300BB instrument, at room temperature, operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C, and Unity Inova 400 instrument, operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. The new thioureides were dissolved in DMSO-d_6 and the chemical shifts were recorded as δ values in parts per million (ppm) relative with tetramethylsilane used as internal standard. The IR spectra were performed using a Brucker Vertex 70 apparatus with diamond optics using the ATR technique.

The NMR chemical shifts for hydrogen and carbon atoms were established on the basis of multipliticity, the coupling constants and two dimensional experiments.In the ^{1}H-NMR spectra the ethyl group produces a characteristic quartet and triplet, the methylene group situated near oxygen produces a singlet at 5.24-5.32 ppm. The aromatic protons produce signals in the range 6.88-7.68 ppm, excepting the compound VId where H-21 has shift value of 8.57 ppm. The –NH protons produce broad singlets in the 11.76-12.80 ppm range. In the ^{13}C-NMR spectra characteristics are the thiocarbonyl carbon signal: 178.44-179.88 ppm, the carbonyl carbon signal: 170.12-171.35 ppm, the methylene carbon near the oxygen signal: 67.58-68.86 ppm and the ethly carbons signals: 27.31-28.35 pmm and 15.72-16.88 ppm.

In the IR spectra the νC=O vibrations produce a very intense sharp stretching band in the region 1662-1691 cm^{-1}. The region of 2965-2955 cm^{-1} and the region of 2912-2929 cm^{-1} are typical for methylene group and methyl group bound to an aromatic nucleus. These compounds also show a typical alkyl-aryl ether signal at 1225-1243 cm^{-1}, for the antisymmetric vibration, and at 1024-1039 cm^{-1} for the symmetric one.

Antimicrobial and antifungal activity results

The most efficient qualitative method was the assay of the compound antimicrobial effect spotted in bacterial inoculums seeded medium. The results present a very good correlation with the quantitative assay results.

In Table 3 are presented the results obtained from the quantitative assay of the antimicrobial and antifungal activity of the new compounds, being known that a concentration of 31.25 μg/mL represents a very strong effect and a 250 μg/mL concentration represents a moderate effect. The tested compounds presented an antimicrobial activity at concentrations from 1000 to 31.25 μg/mL.

Some of the tested compounds presented broad spectrum antimicrobial activity (*e.g.* the compounds VIc and VIf) and they are active at low concentrations both on gram positive, gram negative bacteria and on fungus.

It's worth to notice that the antimicrobial activity of the tested compounds against *E. coli* IC 13529, *S. aureus* IC 13204, *P. aeruginosa* 1246, *P. aeruginosa* IC 13202, *C. albicans* IC 249 can represent new therapeutical options in the treatment of this infections, infections difficult to treat and remove, because of the very high levels of natural and gained resistance of this microorganisms.

EXPERIMENTAL

The 2-(4-ethyl-phenoxymethyl)benzoic acid synthesis

A solution containing 0.10 mol of freshly distilled *para*-ethylphenol (mol. wt. 122.17) in 60 mL xylene was placed in a round-bottom flask equipped with a water removing device. Subsequently, 0.11 mol of potassium hydroxide (mol. wt. 56.11) were added. The reaction mixture was refluxed until the resulting water was removed by azeotropic distillation, while potassium *para*-ethylphenoxide precipitated at the bottom.

Table 3

The antimicrobial activity results of the new thioureides (MIC, μg/mL)

Microbial strain	**VIa**	**VIb**	**VIc**	**VId**	**VIe**	**VIf**
K. pneumoniae 1204	1000	125	62.5	250	250	62.5
K. pneumoniae IC 13420	1000	500	62.5	500	125	62.5
E. coli 13147	500	15.6	125	500	500	125
E. coli IC 13529	125	62.5	62.5	62.5	62.5	62.5
S. aureus 1263	1000	500	125	500	1000	125
S. aureus IC 13204	250	250	125	250	125	62.5
P. aeruginosa 1246	250	62.5	125	31.25	62.5	125
P. aeruginosa IC 13202	125	62.5	62.5	31.25	31.25	62.5
B. subtilis IC 12488	3.9	3.9	250	62.5	500	250
C. albicans IC 249	62.5	31.25	62.5	62.5	62.5	62.5

0.10 Mol of phtalide (mol. wt. 134.14) were added and the mixture was refluxed until it solidified. 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 2-(4-ethyl-phenoxymethyl)benzoic acid precipited. The resulting precipitate is crystallized from a water: isopropanol (1: 1) mixture and shows a m.p. 109- 111ºC. It was obtained 19.6 g of 2-(4-Ethyl-phenoxymethyl)benzoic acid (mol. wt. 256.29) with a 76.5% yield.

The 2-(4-ethyl-phenoxymethyl)-benzoyl chloride synthesis

0.02 Mol of 2-(4-ethyl-phenoxymethyl)benzoic acid, 80 mL of dry 1,2-dichloroethane and 0.042 mol of thionyl chloride (mol. wt. 119; d_4^{20} 1.638) were placed in a round-bottom flask equipped with condenser and drying tube. The mixture was refluxed for 3 hours. The excess thionyl chloride and the solvent were removed by reduced pressure. For the next step, the 2-(4-ethyl-phenoxymethyl)benzoyl chloride was used in the crude status.

General procedure for the synthesis of the new thioureides

A solution of (0.01 mol) 2-(4-ethyl-phenoxymethyl) benzoyl chloride (mol. wt. 286.75) in 15 mL dry acetone was added into a (0.01 mol) solution of ammonium thiocyanate (mol. wt. 76.13) in 5 mL dry acetone. Previously, the acetone was dried over potassium carbonate and the ammonium thiocyanate by heating at 100^0C. The reaction mixture was refluxed one hour in a round-bottom flask coupled with a condenser and a drying tube.

After cooling, 0.01 mol of dry and freshly distilled primary aromatic amine dissolved in 2 mL dry acetone were added to the reaction mixture while stirring. The mixture was afterwords refluxed for one hour. The product precipitated after the cooled reaction mixture was poured into 500 mL water. The raw obtained thioureides were crystallised a few times from isopropanol with active carbon.

Compounds spectra data

Compound VIa: N-[2-(4-Ethyl-phenoxymethyl)-benzoyl]-N'-(2-methylphenyl)-thiourea

^{1}H-NMR (dmso, δ ppm, J Hz): 12.21 (s, 1H, NH); 11.95 (bs, 1H, NH); 7.68 (dd, H-7, 1.7, 7.9); 7.64 (dd, H-4, 1.7, 7.9); 7.59 (td, H-5, 1.7, 7.9); 7.53 (td, H-6, 1.7, 7.9); 7.25-7.34 (m, 4H); 7.11 (d, H-11, H-13, 8.2); 6.96 (d, H-10, H-14, 8.2); 5.32 (s, H-8); 2.5 (q, H-121, 7.5); 2.13 (s, CH_3); 1.19 (t, H-122, 7.5)

^{13}C-NMR (dmso-d6, δ ppm): 179.88 (C-15); 170.34 (C-1); 156.32 (C-9); 136.80 (Cq); 136.21 (Cq); 135.71 (Cq); 133.54 (Cq); 133.30 (Cq); 130.92 (CH); 130.37 (CH); 128.64 (C-11, C-13); 128.55 (CH); 128.50 (CH); 127.81 (CH); 126.98 (CH); 126.47 (CH); 126.03 (CH); 114.53 (C-10, C-14); 67.58 (C-8); 27.31 (C-121); 17.49 (-CH_3); 15.90 (C-122).

FT-IR (ATR in solid, νcm^{-1}): 3142; 3020; 2965; 2929; 2869; 1879; 1675; 1607; 1508; 1458; 1382; 1332; 1297; 1240; 1157; 1072; 1020; 947; 869; 827; 776; 729; 657; 614; 579; 544; 511; 467; 436.

Compound VIb: N-[2-(4-Ethyl-phenoxymethyl)-benzoyl]-N'-(3-methylphenyl)-thiourea

^{1}H-NMR (dmso, δ ppm, J Hz): 12.4 (bs, 1H, NH); 11.9 (bs, 1H, NH); 7.64 (dd, H-7, 1.7, 7.9); 7.60 (dd, H-4, 1.7, 7.9); 7.59 (td, H-5, 1.7, 7.9); 7.50 (td, H-6, 1.7, 7.9); 7.47 (m, H-21); 7.38 (m, H-17); 7.32 (t, H-20, 7.8); 7.12 (d, H-11, H-13, 8.4); 6.93 (d, H-10, H-14, 8.4); 5.29 (s, H-8); 2.49 (q, H-121, 7.5); 2.35 (s, CH_3); 1.15 (t, H-122, 7.5)

^{13}C-NMR (dmso-d6, δ ppm): 179.93 (C-15); 171.35 (C-1); 157.37 (C-9); 139.16 (Cq); 138.76 (Cq); 137.29 (Cq); 136.82 (Cq); 134.43 (Cq); 132.07 (CH); 129.72 (CH); 129.70 (CH); 129.69 (CH); 129.52 (C-11, C-13); 128.87 (CH); 128.05 (CH); 125.70 (CH); 122.42 (CH); 115.62 (C-10, C-14); 68.64 (C-8); 28.35 (C-121); 21.95 (-CH_3); 16.88 (C-122).

FT-IR (ATR in solid, νcm^{-1}): 3370; 3238; 3128; 3032; 2964; 2922; 2866; 1669; 1597; 1551; 1505; 1444; 1376; 1335; 1300; 1270; 1225; 1170; 1139; 1052; 1025; 958; 891; 834; 809; 785; 737; 718; 682; 656; 598; 541; 512; 480; 440.

Compound VIc: N-[2-(4-Ethyl-phenoxymethyl)-benzoyl]-N'-(4-methylphenyl)-thiourea

^{1}H-NMR (dmso, δ ppm, J Hz): 12.33 (s, 1H, NH); 11.78 (bs, 1H, NH); 7.62-7.41 (m, H-4, H-5, H-6, H-7, H-17, H-21); 7.19 (d, H-18, H-20, 8.3); 7.08 (d, H-11, H-13, 8.5); 6.88 (d, H-10, H-14, 8.5); 5.24 (s, H-8); 2.49 (q, H-121, 7.6); 2.29 (s, CH_3); 1.11 (t, H-122, 7.6)

^{13}C-NMR (dmso-d6, δ ppm): 178.98 (C-15); 170.20 (C-1); 156.40 (C-9); 136.41 (C-3); 135.83 (C-12); 135.37 (C-16); 133.46 (C-19); 131.10 (C-5); 131.08 (C-2); 129.13 (C-18, C-20); 128.66 (C-11, C-13); 128.54 (C-6); 128.44 (C-7); 127.87 (C-4); 124.10 (C-17, C-21); 114.78 (C-10,C-14); 67.77 (C-8); 27.32 (C-121); 20.61 (-CH_3); 15.73 (C-122).

FT-IR (ATR in solid, νcm^{-1}): 3323; 3158; 3030; 2964; 2912; 1873; 1667; 1597; 1531; 1505; 1450; 1379; 1335; 1297; 1228;

1148; 1122; 1024; 950; 856; 821; 763; 726; 668; 639; 614; 548; 504; 432.

Compound VId: N-[2-(4-Ethyl-phenoxymethyl)-benzoyl]-N'-(2-methoxyphenyl)-thiourea

^{1}H-NMR (dmso, δ ppm, J Hz): 12.8 (bs, 1H, NH); 11.8 (bs, 1H, NH); 8.57 (d, H-21, 7.2); 7.66 (dd, H-7, 1.7, 7.9); 7.63 (dd, H-4, 1.7, 7.9); 7.61 (td, H-5, 1.7, 7.9); 7.52 (td, H-6, 1.7, 7.9); 7.28 (td, H-19, 1.6, 8.2); 7.16 (dd, H-18, 1.6, 8.2); 7.11 (d, H-11, H-13, 8.6); 7.04 (td, H-20, 1.6, 8.2); 6.93 (d, H-10, H-14, 8.6); 5.32 (s, H-8); 3.88 (s, OCH_3); 2.49 (q, H-121, 7.5); 1.15 (t, H-122, 7.5)

^{13}C-NMR (dmso-d6, δ ppm): 178.44 (C-15); 170.86 (C-1); 156.91 (C-17); 156.90 (C-9); 151.23 (Cq); 136.89 (Cq); 136.34 (Cq); 134.12 (Cq); 131.66 (CH); 129.24 (C-11, C-13); 128.52 (CH); 127.50 (CH); 127.29 (CH); 123.82 (CH); 120.40 (CH); 120.39 (CH); 115.30 (C-10, C-14); 111.95 (CH); 68.34 (C-8); 56.63 (-OCH_3); 27.95 (C-121); 16.47 (C-122).

FT-IR (ATR in solid, νcm^{-1}): 3269; 3122; 3030; 2961; 2929; 2867; 2837; 1890; 1662; 1602; 1553; 1532; 1512; 1462; 1364; 1326; 1292; 1242; 1230; 1181; 1149; 1120; 1028; 954; 928; 829; 786; 741; 681; 645; 613; 554; 489; 433.

Compound VIe: N-[2-(4-Ethyl-phenoxymethyl)-benzoyl]-N'-(3-methoxyphenyl)-thiourea

^{1}H-NMR (dmso, δ ppm, J Hz): 12.49 (bs, 1H, NH); 11.86 (bs, 1H, NH); 7.67 (dd, H-7, 1.7, 7.9); 7.62 (dd, H-4, 1.7, 7.9); 7.60 (td, H-5, 1.7, 7.9); 7.52 (td, H-6, 1.7, 7.9); 7.39 (t, H-17, 2.2); 7.34 (t, H-20, 8.2); 7.16 (dd, H-19, 1.6, 8.2); 7.14 (m, H-21); 7.12 (d, H-11, H-13, 8.4); 6.93 (d, H-10, H-14, 8.4); 5.3 (s, H-8); 3.78 (s, OCH_3); 2.49 (q, H-121, 7.5); 1.14 (t, H-122, 7.5)

^{13}C-NMR (dmso-d6, δ ppm): 179.75 (C-15); 170.97 (C-1); 160.52 (C-18); 157.41 (C-9); 140.05 (Cq); 137.43 (Cq); 136.86 (Cq); 134.39 (Cq); 132.01 (CH); 130.37 (CH); 129.52 (C-11, C-13); 129.33 (CH); 128.73 (CH); 117.17 (CH); 117.06 (CH); 115.88 (C-10, C-14); 113.00 (CH); 110.84 (C-17); 68.86 (C-8); 56.34 (-OCH_3); 28.21 (C-121); 16.39 (C-122).

FT-IR (ATR in solid, νcm^{-1}): 3265; 3028; 2995; 2955; 2928; 2865; 2830; 1674; 1613; 1596; 1568; 1529; 1510; 1490; 1465; 1445; 1430; 1386; 1350; 1330; 1312; 1278; 1254; 1243; 1232; 1181; 1153; 1120; 1068; 1039; 987; 962; 899; 852; 835; 821; 799; 780; 745; 680; 644; 614; 567; 545; 490; 443.

Compound VIf: N-[2-(4-Ethyl-phenoxymethyl)-benzoyl]-N'-(4-methoxyphenyl)-thiourea

^{1}H-NMR (dmso, δ ppm, J Hz): 12.25 (bs, 1H, NH); 11.76 (bs, 1H, NH); 7.61-7.42 (m, H-4, H-5, H-6, H-7); 7.43 (d, H-17, H-21, 8.5); 7.08 (d, H-11, H-13, 8.6); 6.95 (d, H-18, H-20); 6.89 (d, H-10, H-14, 8.6); 5.25 (s, H-8); 3.76 (s, OCH_3); 2.48 (q, H-121, 7.5); 1.11 (t, H-122, 7.5)

^{13}C-NMR (dmso-d6, δ ppm): 179.15 (C-15); 170.12 (C-1); 157.61 (C-9); 156.40 (C-19); 136.43 (C-3); 135.83 (C-12); 133.50 (C-2); 131.07 (C-5); 128.87 (C-6); 128.67 (C-16); 128.55 (C-11); 128.55 (C-13); 128.43 (C-7); 127.87 (C-4); 125.92 (C-17, C-21); 114.80 (C-10, C-14); 113.93 (C-18, C-20); 67.77 (C-8); 55.43 (-OCH_3); 27.32 (C-121); 15.72 (C-122)

FT-IR (ATR in solid, νcm^{-1}): 3160; 3029; 2960; 2881; 2835; 1883; 1691; 1609; 1584; 1510; 1460; 1382; 1333; 1298; 1243; 1161; 1116; 1079; 1026; 883; 830; 739; 661; 604; 555; 512.

Antimicrobial and antifungal activity assay

The testing of the antimicrobial and antifungal activity of the new thioureides was investigated by the qualitative screening of the sensibility spectrum of various microbial strains to the tested compounds using adapted variants of the diffusion method: the technique of paper filter disks impregnated with the testing compounds DMF solution (method 1), the testing of the compounds antimicrobial activity in agar well-plates (method 2) and the qualitative antimicrobial effect assay of the compound spot placed in bacterial inoculums seeded medium (method 3).

The quantitative assay of the antimicrobial and antifungal activity of the new compounds was performed by the microdilution method in liquid medium (Mueller Hinton broth and YPG broth).

The materials used were: sterile Petri dishes (10 cm diameter) incorporated with Mueller-Hinton agar medium 1.5% (pH= 7.2- 7.4) or YPG (Yeast Peptone Glucose) medium (for yeast strains) of 4 mm thickness; bacterial and fungal strains recently isolated from clinical specimens belonging to the following generes and species: *Klebsiella pneumoniae* 1204, *Klebsiella pneumoniae* IC 13420, *Escherichia coli* 13147, *Escherichia coli* IC 13529, *Staphylococcus aureus* 1263, *Staphylococcus aureus* IC 13204, *Pseudomonas aeruginosa* 1246, *Pseudomonas aeruginosa* IC 13202, *Bacillus subtilis* IC 12488, *Candida albicans* IC 249; inoculum prepared from these strains, represented by a suspension in Mueller-Hinton or SDW (sterile distilled water) of a young culture (4-5 isolated colonies) developed on solid medium for 15-18 hours, with a 1-3x10^8 UFC/ mL density, nephelometric (using a McFarland standard 0.5 = 1.5x10^8 UFC/ mL).

In the first qualitative screening method, 5 µL of the compound solution were equally distributed on paper filter disks placed on Petri dishes previously seeded "in layer" with the tested bacterial strain inoculums. In the 2nd variant, 5 µL of the tested compounds solutions were placed in the agar wells cut in the solidified culture medium seeded with the microbial inoculum. In the 3rd qualitative method, 5 µL of the compounds solutions were spotted on Petri dishes seeded with bacterial or yeast inoculum. In all three variants, the Petri dishes were left at room temperature to ensure the equal diffusion of the compound in the medium or to allow the drop of the solution to be adsorbed in the medium and afterwards the dishes were incubated at 37^{O}C for 24 hours. The solvent used was also tested by all three methods to evaluate a potential antimicrobial activity.

For the quantitative assay of the antimicrobial activity of the new compounds by the microdilution method in liquid medium distributed in 96-well plates, binary serial dilutions of the tested compounds solutions were perfomed (there were obtained concentrations from 1000 µg/mL to 31.25 µg/mL) in a 200 µL culture medium final volume, afterwards each well was seeded with a 50 µL microbial suspension of 0.5 MacFarland density. In each test a microbial culture control (a series of wells containing exclusively culture medium with microbial suspension) and a sterility control (a series of wells containing exclusively culture medium) were performed. The plates were incubated for 24 hours at 37ºC.

For the technic of paper filter disks impregnated with the testing compounds solution and the antimicrobial activity testing by agar well-plates method the reading of the results is done by measuring the microbial inhibition growth zones around the filter disks impregnated with the testing compounds and around the wells, respectively.

First, there are examined the standard culture plates to read and to analyze the qualitative method's results, where the culture stripes has to be observed. If in a plate containing a compound the inoculated strain didn't grow, then that compound has a bactericide effect. If in the plate a bacterial growth can be observed, the culture density is compared with that of the culture standard plate. In the case of a bacterial

growth less abundant then that of the culture standard plate, we can say that the substance has a bacteriostatic effect.

A superior bacterial growth in the presence of the tested compound compared to the culture standard may be observed, in this case the compound possesses a stimulator effect on the bacterial growth. If the growth intensity is comparable for the tested plate and for the standard, then the substance doesn't influence notably the growth and the development of the tested bacterial stain.

The used solvent testing revealed that it hasn't antimicrobial activity, this being a practic advantage for antimicrobial activity tests of the water insoluble compounds.

In the case of the quantitative assay of the antimicrobial activity of the tested compounds by the microdilution method in liquid medium the minimum inhibitory concentration is read by wells observation: in the first wells containing big concentrations of compound the culture growth is not visible, the microbials being killed or inhibated by the compound. The smallest concentration of the well that produces a visible microbial culture growth inhibition represents the MIC (μg/mL) value for the tested compound. In the next wells, including the growth standard wells, the medium becomes muddy as a result of the microbial growth. In the sterility wells series the medium has to remain clear. From the last well with antimicrobial activity and from the first one with a microbial growth Gram stain smears are performed for the results confirmation.

CONCLUSIONS

Continuing our research in the antimicrobial substances field we synthesized six new compounds, thioureides of the 2-(4-ethyl-phenoxymethyl)benzoic. The chemical structure of the synthesized compounds have been confirmed by elemental analysis, IR and NMR spectroscopy. The obtained thioureides were investigated to determinate their antimicrobial activity and proved to be active at low concentrations both on gram positive and gram negative bacteria and on fungi.

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REFERENCES

1. A.W. Chou, USA Pat. 4438135, Mar., 20, **1984**.
2. K.R. Desai and P.H. Desai, *J. Inst. Chem*, **1989**, *61,* 19–20.
3. K.K. Kapoor, P. Somal, R. Gupta and P.L. Kachroo, *J. Indian Chem. Soc.,* **1991**, *68*, 104–106.
4. J. Lueshun, W. Yulu, L. Yongfang, H. Fangshu and S. Yuyfang,, *Yougi Huaxue*, **1993,** *13*, 250-252.
5. V. Zikan, J. Sluka, J. Danek, CS Pat. CS262597, July, 14, **1989**, cf. Chem. Abstr., 1990, 112, 198384
6. C. Limban, Al.V. Missir and I.C. Chiriță, *Farmacia*, **2000**, *48*, 73-78.
7. C. Limban, Al.V. Missir and I.C. Chiriță, *Farmacia*, **2004**, *52*, 7-12.
8. C. Limban, Al.V. Missir and I.C. Chiriță, *T. M. J.*, **2005**, *55, supl.5,* 42-44.
9. M.C. Balotescu, C. Limban, Al.V. Missir, I.C. Chiriță and G.M. Nițulescu, *Rev. Chim. (Bucureşti)*, **2007**, *58,* 1064-1068.
10. C. Limban, M.C. Balotescu Chifiriuc, Al.V. Missir, I.C. Chiriță and C. Bleotu, *Molecules*, **2008**, *13,* 567-580.
11. C. Limban, Al.V. Missir, I.C. Chiriță, C.D. Bădiceanu, C. Drăghici, M.C. Balotescu and O. Stamatoiu, *Rev. Roum. Chim.*, **2008**, *53,* 595- 602.
12. C. Limban, Al.V. Missir, I.C. Chiriță, C. Ilie and M.T. Căproiu, *Rev. Chim. (Bucureşti)*, **2008**, *59*, 1136-1139.
13. C. Limban, Al.V. Missir, I.C. Chiriță, G.M. Nițulescu, M.T. Căproiu and C. Ilie, *Rev. Chim. (Bucureşti)*, **2008**, *59*, 1245- 1248.
14. G. J. Durant, G. M. Smith, R. G. W. Spickett, S. H. B. Wright, *J. Med. Chem.*, **1966**, *9*, 22-27.