Synthesis and Antimicrobial Evaluation of Some New 2-(4-fluoro-phenoxymethyl) Benzoic Acid Thioureides

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2-(4-Fluoro-phenoxymethyl)benzoic acid thioureides were prepared from the reaction of 2-(4-fluoro-phenoxymethyl)benzoyl isothiocyanate with various primary aromatic amines. These new compounds were characterized by their physical properties (melting point, solubility), the structures were confirmed by elemental analysis, ^{1}H-NMR, ^{13}C-NMR, IR and UV-Vis spectral methods, and then were tested by qualitative and quantitative methods on various microbial and fungal strains and proved to be active at low concentrations on gram positive, gram negative bacteria and fungi.

Keywords: acylthiourea, 4-fluoro-phenoxymethylbenzoic acid, ^{1}H-NMR, ^{13}C-NMR, antimicrobial activity

Acylthiourea compounds are attractive structures due to their biological significance. Some derivatives exhibit antibacterial and antifungal activity [1-8].

Based on this, we have synthesized new thioureides of the 2-(4-fluoro-phenoxymethyl)benzoic acid by addition of some primary aromatic amines to 2-(4-fluoro-phenoxymethyl)benzoyl isothiocyanate. The spectral and elemental analysis confirmed the new compound structures and also the synthesis that we had done. As part of our interest in the screening of potentially bioactive compounds [9-19], we evaluated the *in vitro* antimicrobial activity, using reference and clinical multidrug resistant strains.

Experimental part

All the starting materials were purchased and used as received, except acetone which was dried over potassium carbonate and distilled, and ammonium thiocyanate, by heating at 100°C. All melting points were recorded with an Electrothermal 9100 apparatus and are uncorrected. Elemental analysis was realized using a Perkin Elmer CHNS/O Analyser Series II 2400 apparatus.

The NMR spectra were recorded on a Gemini 300BB instrument, at room temperature, operating at 300MHz for ^{1}H and 75MHz 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 and coupling constants (J) values are reported in Hertz. Spin multiplets are given as s (singlet), d (doublet), t (triplet), m (multiplet), bs (broad singlet), bt (broad triplet), bd (broad doublet), and dd (double doublet). The ^{13}C-NMR data are reported in the following order: chemical shifts and signal/ atom attribution.

The IR spectra were performed using potassium bromide tablet, with a Biorad FTS 135 apparatus.

The UV-Vis spectra of the new compounds have been recorded in the spectral range from 200 to 400 nm using a Perkin-Elmer Lamda 2 UV-VIS spectrophotometer, using a cuvettes of 1 cm. For these molecules that possess π bonding as chromophore groups (C=O, C=S) and aromatic ring, energy that is available can promote electrons from a π bonding molecular orbital to a π antibonding molecular orbital ($\pi \rightarrow \pi^*$ transition).

The new compounds were prepared following the next procedures.

The synthesis of 2-(4-fluoro-phenoxymethyl)benzoic acid (1)

A solution containing 11.21 g (0.1 mol) of p-fluorophenol (mol. wt. 112.10) in 60 mL xylene was placed in a round-bottom flask, equipped with a Dean- Stark trap device. Subsequently, 6.17 g (0,11 mol) potassium hydroxide (mol. wt. 56,11) were added. The reaction mixture was refluxed until the resulting water was removed by azeotropic distillation while potassium p-fluorophenoxid precipitated at the bottom. 13.41 g (0.1 Mol) phtalide (mol. wt. 134.14) was 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 water. The aqueous phase was separated and acidulated with 1M hydrochloric acid solution until the mixture became acidic (pH= 3), when 2-(4-fluoro-phenoxymethyl)benzoic acid (mol. wt. 246.23) precipited. The resulting precipitate (17.9g) (72.7% yield), crystallized from water: isopropanol (1: 1) mixture shows a m.p. 113- 115^{0}C.

The synthesis of 2-(4-fluoro-phenoxymethyl)-benzoyl chloride (4)

4.92 g (0.02 Mol) of 2-(4-fluoro-phenoxymethyl)benzoic acid, 80 mL dry 1,2-dichlorethane and 5 g (3 mL) (0.042 mol) (mol. wt. 119; d_4^{20} 1.638) thionyl chloride were placed in a round-bottom flask equipped with condenser and drying tube. The mixture was refluxed for 3 h. The thionyl chloride in excess and the solvent were removed by reduced pressure. For the next step, the acid chloride, was used in the crude status.

The synthesis of the new thioureides (5a-g) (general procedure)

To a solution of ammonium thiocyanate (0.76 g, 0.01 mol) (mol. wt. 76,13) in 5 mL dry acetone was added a solution of 2-(4-fluoro-phenoxymethyl)benzoyl chloride

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(0.01 mol)(mol. wt. 264.677) in 15 mL dry acetone. The acetone was dried over potassium carbonate and the ammonium thiocyanate by heating at 100^{0}C. The reaction mixture was refluxed one hour in a one round-bottom flask with condenser and a drying tube. After cooling, 0.01 mol of 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 cooled reaction mixture was poured into 500 mL water. The crude thioureides obtained were crystallised from isopropanol with active carbon.

Spectral data

N-[2-(4-Fluoro-phenoxymethyl)-benzoyl]-N'phenyl-thiourea (5a)

^{1}H-NMR(δ ppm, *J* Hz): 7.40- 7.68 (m, 4H, H-4- H-7); 5.28 (s, 2H, H-8); 6.99 (dd, 2H, H-10, H-14, 4.5, 8.9); 7.10 (t, 2H, H-11, H-13, 8.9); 7.59 (bd, 2H, H-17, H-21, 8.1); 7.26 (bt, 1H, H-19, 8.1); 7.41 (t, 2H, H-18, H-20, 8.1); 11.85 (s, 1H, NH) and 12.41 (s, 1H, NH)

^{13}C-NMR(δ ppm): 169.54 (C-1); 133.13 (C-2); 137.69 (C-3); 127.59 (C-4); 130.83 (C-5); 127.60 (C-6); 128.14 (C-7); 68.08 (C-8); 154.35 (2.0)(C-9); 116.09 (8.2)(C-10, C-14); 115.42 d (23.0)(C-11, C-13); 157.56 d (236.4) (C-12); 178.40 (C-15); 135.26 (C-16); 128.25 (C-17, C-21); 128.37 (C-18, C-20); 126.0 (C-19)

FT-IR (KBr, ν cm^{-1}): -CH_2-O- (νCH_2 sym: 2856.0; íCH$_2$ asym: 2929.4; íC-O-C sym: 1019.5; íC-O-C asym: 1269.7); -CO-NH- (νC=O: 1671.7; νC-N: 1534.3); -NH-CS-NH- (νC=S: 1166.7); aromatic ring (νC=C: 1506.3); νC-F: 1095

UV-Vis (CH_3OH, λ, nm, lgε); 259± 2nm (3.86); UV-Vis (CH_3CN, λ, nm, lgε); 257± 2nm (3.61); UV-Vis ($CHCl_3$, **λ**, nm, lg**ε**); 264±2nm (3.80)

N-[2-(4-Fluoro-phenoxymethyl)-benzoyl]-N'-(*para*-tolyl)-thiourea (5b)

^{1}H-NMR(δ ppm, *J* Hz): 2.31 (s, 3H, -CH_3); 7.56- 7.61 (m, 4H, H-4- H-7); 5.27 (s, 2H, H-8); 7.08 (d, 2H, H-10, H-14, 8.5); 7.01 (dd, 2H, H-11, H-13, 4.5, 9.3); 7.47 (m, 2H, H-17, H-21); 7.20 (d, 2H, H-18, H-20, 8.2); 11.80 (bs, NH); 12.35 (bs, NH)

^{13}C-NMR(δ ppm): 20.57 (-CH_3); 170.09 (C-1); 133.33 (C-2); 135.63 (C-3); 124.17 (C-4); 131.02 (C-5); 128.54 (C-6); 124.17 (C-7); 68.06 (C-8); 158.25 (C-9); 115.96 (C-10, C-14); 116.06 (C-11, C-13); 155.12 (C-12); 178.83 (C-15); 135.26 (C-16); 128.54 (C-17, C-21); 129.02 (C-18, C-20); 135.41 (C-19)

FT-IR (KBr, ν cm^{-1}): CH_2-O- (νCH_2 sym: 2852.2; νCH_2 asym: 2923.2; νC-O-C sym: 1036.8; νC-O-C asym: 1220.2); -CO-NH- (νC=O: 1669.8; νC-N: 1534.3 and 1245.1); -NH-CS-NH- (νC=S: 1156.4); aromatic ring (νC=C: 1506.0); νNH: 3044.2; νC-F: 1095

UV-Vis (CH_3OH, λ, nm, lgε); 264± 2nm(3.89); UV-Vis (CH_3CN, l, nm, lge); 262± 2nm (3.77); UV-Vis($CHCl_3$, λ, nm, lgε); 268± 2nm (3.97)

N-[2-(4-Fluoro-phenoxymethyl)-benzoyl]-N'-(*para*-methoxyphenyl)-thiourea (5c)

^{1}H-NMR(δ ppm, *J* Hz): 3.76 (s, 3H, -OCH_3), 7.45- 7.64 (m, 4H, H-4- H-7); 5.27 (s, 2H, H-8); 7.00 (dd, 2H, H-10, H-14, 8.9, 4.4); 7.10 (t, 2H, H-11, H-13, 8.9); 7.48 (d, 2H, H-17, H-21, 8.9); 6.96 (d, 2H, H-18, H-20, 8.9); 11.78 (s, 1H, NH) and 12.25 (s, 1H, NH)

^{13}C-NMR(δ ppm): 55.04 (-OCH_3); 169.65 (C-1); 133.10 (C-2); 135.17 (C-3); 127.62 (C-4); 130.79 (C-5); 127.71 (C-6); 128.19 (C-7); 67.86 (C-8); 154.26 (C-9); 116.51d (7.0)(C-10, C-14); 116.30 d (21.9)(C-11, C-13); 157.18 d (235.8) (C-12); 178.51 (C-15); 133.16 (C-16); 128.30 (C-17, C-21); 113.53 (C-18, C-20); 158.04 (C-19)

FT-IR (KBr, ν cm^{-1}): -CH_2-O- (νCH_2 sym: 2842.5; νCH_2 asym: 2921.7; νC-O-C sym: 1036.7; νC-O-C asym: 1213.5); -CO-NH- (νC=O: 1673.5; νC-N: 1535.5 and 1250.4); -NH-CS-NH- (νC=S: 1156.5); aromatic ring (νC=C: 1508.6); íNH: 3060.6; νC-F: 1098.6

UV-Vis (CH_3OH, λ, nm, lgε); 270± 2nm (3.87); UV-Vis (CH_3CN, λ, nm, lgε); 270± 2nm (3.93); UV-Vis ($CHCl_3$, λ, nm, lgε); 275± 2nm (3.85)

N-[2-(4-Fluoro-phenoxymethyl)-benzoyl]-N'-(*para*-chlorophenyl)-thiourea (5d)

^{1}H-NMR(δ ppm, *J* Hz): 7.40- 7.68 (m, 4H, H-4- H-7); 5.28 (s, 2H, H-8); 6.99 (dd, 2H, H-10, H-14, 8.7, 4.6); 7.10 (t, 2H, H-11, H-13, 8.7); 7.46 (d, 1H, H-17, H-21, 8.8); 7.59 (d, 1H, H-18, H-20, 8.8); 11.92 (s, 1H, NH) and 12.41 (s, 1H, NH)

^{13}C-NMR(δ ppm): 169.73 (C-1); 133.02 (C-2); 136.58 (C-3); 127.62 (C-4); 130.89 (C-5); 127.94 (C-6); 127.97 (C-7); 67.84 (C-8); 154.27 (C-9); 116.46 (7.9)(C-10, C-14); 116.32 d (22.9)(C-11, C-13); 157.45 d (236.5) (C-12); 178.83 (C-15); 135.28 (C-16); 128.19 (C-17, C-21); 128.31 (C-18, C-20); 132.96 (C-19)

FT-IR (KBr, νcm^{-1}): -CH_2-O- (νC-O-C sym: 1036.3; νC-O-C asym: 1215.0); -CO-NH- (νC=O: 1674.7; νC-N: 1533.9 and 1249.5); -NH-CS-NH- (νC=S: 1159.0); aromatic ring (νC=C: 1505.1); νNH: 3055.1; νC-F: 1095.3

UV-Vis (CH_3OH, λ, nm, lgε); 262± 2nm (3.85); UV-Vis (CH_3CN, λ, nm, lgε); 259± 2nm (3.76); UV-Vis ($CHCl_3$, λ, nm, lgε); 268± 2nm (3.55)

N-[2-(4-Fluoro-phenoxymethyl)-benzoyl]-N'-(2,4-dichlorophenyl)-thiourea (5e)

^{1}H-NMR(δ ppm, *J* Hz): 7.42- 7.64 (m, 4H, H-4- H-7); 5.27 (s, 2H, H-8); 6.98 (dd, 2H, H-10, H-14, 9.1, 4.4); 7.09 (t, 2H, H-11, H-13, 9.1); 7.74 (d, 1H, H-18, 2.3); 7.47 (dd, 1H, H-20, 8.0, 2.3); 7.97 (d, 1H, H-21, 8.0); 12.12 (s, 1H, NH) and 12.41 (s, 1H, NH)

^{13}C-NMR(δ ppm): 169.97 (C-1); 132.9 (C-2); 135.1 (C-3); 127.12 (C-4); 130.91 (C-5); 127.73 (C-6); 128.70 (C-7); 67.91 (C-8); 154.23 (C-9); 115.73 d (9.0)(C-10, C-14); 115.58 d (20.2)(C-11, C-13); 156.47 d (234,8) (C-12); 179.64 (C-15); 131.13 (C-16); 132.97 (C-17); 128.75 (C-18); 135.20 (C-19); 128.80 (C-20); 128.42 (C-21)

FT-IR (KBr, ν cm^{-1}): -CH_2-O- (νCH_2 sym: 2856.0; νCH_2 asym: 2921.7; νC-O-C sym: 1036.9;νC-O-C asym: 1219.1); -CO-NH- (νC=O: 1674.3;νC-N: 1529.1 and 1250); -NH-CS-NH- (νC=S: 1164.0); aromatic ring (νC=C: 1507.1); νNH: 3280.7; νC-F: 1100.8

UV-Vis (CH_3OH, λ, nm, lgε); 254± 2nm (3.84); UV-Vis ($CHCl_3$, λ, nm, lgε); 270± 2nm (3.55) and 311± 2nm (3.42);

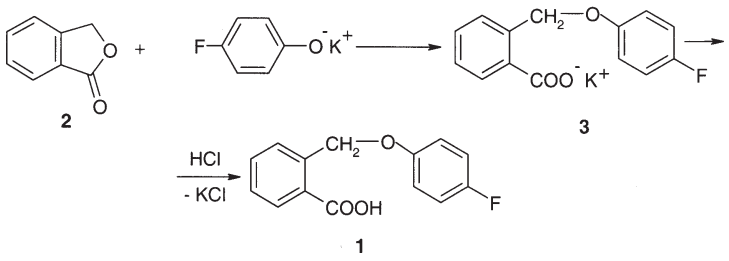
N-[2-(4-Fluoro-phenoxymethyl)-benzoyl]-N'-(2,6-dichlorophenyl)-thiourea (5f)

^{1}H-NMR(δ ppm, *J* Hz): 7.43- 7.64 (m, 4H, H-4- H-7); 5.26 (s, 2H, H-8); 6.99 (dd, 2H, H-10, H-14, 9.2, 4.4); 7.10 (t, 2H, H-11, H-13, 9.2); 7.53 (d, 2H, H-18, H-20, 7.9); 7.36 (t, 1H, H-19, 7.9); 11.87 (s, 1H, NH) and 12.14 (s, 1H, NH)

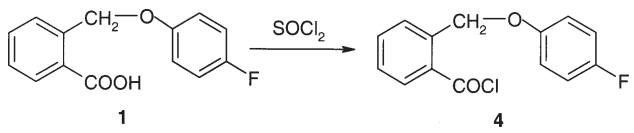
^{13}C-NMR(δ ppm): 169.65 (C-1); 133.12 (C-2); 135.08(C-3); 127.84 (C-4); 130.89 (C-5); 128.2 (C-6); 128.4 (C-7); 67.88 (C-8); 154.23 (C-9); 115.84 d (8.0)(C-10, C-14); 115.54 d (23.0)(C-11, C-13); 156.46 d (235.0) (C-12); 180.56 (C-15); 134.01 (C-16); 133.87 (C-17); 128.20 (C-18, C-20); 128.5 (C-19); 133.87 (C-21)

FT-IR (KBr, ν cm^{-1}): -CH_2-O- (νCH_2 sym: 2856.0; νCH_2 asym: 2929.4; νC-O-C sym: 1033.8; νC-O-C asym: 1200.1); -CO-NH- (νC=O: 1692.7; νC-N: 1568.7 and 1261.0); -NH-CS-NH- (νC=S: 1173.7); aromatic ring (νC=C: 1506.1); νNH: 3072.2; νC-F: 1098.2

UV-Vis (CH_3OH, λ, nm, lgε); 279± 2nm (3.52); UV-Vis (CH_3CN, λ, nm, lgε); 279± 2nm (3.91); UV-Vis ($CHCl_3$, λ, nm, lgε);281± 2nm (3.67)



Scheme 1. The synthesis of 2-(4-fluoro-phenoxymethyl)benzoic acid



Scheme 2. The synthesis of 2-(4-fluoro-phenoxymethyl)benzoyl chloride

N-[2-(4-Fluoro-phenoxymethyl)-benzoyl]-N'-(*para*-bromophenyl)-thiourea (5g)

^{1}H-NMR(δ ppm, *J* Hz): 7.45- 7.63 (m, 4H, H-4- H-7); 5.28 (s, 2H, H-8); 6.99 (dd, 2H, H-10, H-14, 8.7, 4.5); 7.10 (t, 2H, H-11, H-13, 8.7); 7.59 (s, 2H, H-17, H-21); 7.59 (s, 2H, H-18, H-20); 11.91 (s, 1H, NH) and 12.40 (s, 1H, NH)

^{13}C-NMR(δ ppm): 169.70 (C-1); 132.98 (C-2); 136.94 (C-3); 128.06 (C-4); 130.87 (C-5); 127.61 (C-6); 128.34 (C-7); 67.83 (C-8); 154.25 (C-9); 116.42 d (6.1)(C-10, C-14); 116.30 (22.9) (C-11, C-13); 156.47 d (236.1) (C-12); 178.64 (C-15); 135.26 (C-16); 128.06 (C-17, C-21); 128.18 (C-18, C-20); 118.26 (C-19)

FT-IR (KBr, ν cm^{-1}): -CH_2-O- (νCH_2 sym: 2852.2; νCH_2 asym: 2929.4; νC-O-C sym: 1032.6; νC-O-C asym: 1218.3); -CO-NH- (νC=O: 1676.4; νC-N: 1553.5 and 1256.5); -NH-CS-NH- (νC=S: 1151.7); aromatic ring (νC=C: 1507.1); νNH: 3043.1; νC-F: 1070.3

UV-Vis (CH_3OH, λ nm, lgν); 262± 2nm (3.94); 306± 2nm (3.60); UV-Vis (CH_3CN, λ, nm, lgε); 260± 2nm (3.94); 303± 2nm (3.62); UV-Vis ($CHCl_3$, λ, nm, lgε); 267± 2nm (3.90); 310± 2nm (3.56)

Antimicrobial activity assay

The qualitative screening of the susceptibility spectra of different microbial strains versus these substances was performed by adapted diffusion techniques, while the quantitative assay of minimal inhibitory concentration (MIC, μg/ mL) value was based on liquid medium serial microdilutions. The compounds were solubilised in DMSO (dimethylsulfoxide) to a final concentration of 2048μg/mL. The *in vitro* biological screening effects were tested against a microbial inoculum of ~1.5x10^8 UFC/cm^3, corresponding to 0.5 McFarland density, represented by Gram-positive (*Listeria monocytogenes, Staphylococcus aureus-methicilin resistant - MRSA, Bacillus subtilis*), Gram-negative (*Pseudomonas aeruginosa- microorganism known for high natural resistance to antibiotics, Escherichia coli- producing extended spectrum beta-lactamases- ESBL, Salmonella enteritidis), as well as*, *Candida albicans*, using both reference and clinical multidrug resistant strains.

The qualitative screening was performed by an adapted disk diffusion method. Petri dishes with Mueller Hinton (for bacterial strains) /YPG (for yeasts) medium were seeded with bacterial inoculums as for the classical antibiotic susceptibility testing disk diffusion method (Kirby- Bauer); 5 mm diameter paper filter disks were placed on the seeded medium, at 30 mm distance. Subsequently, the disks were impregnated with 5 mL tested compound solution (2048 μg/ mL concentration). The plates were left at room temperature for 20- 30 min and then incubated at 37°C for 24 h. The positive results were read as the occurrence of an inhibition zone of microbial growth around the disk.

The quantitative assay of the antimicrobial activity was performed by binary micro dilution method, in 96 multi-well plates, in order to establish the minimal inhibitory concentration. In this purpose, serial binary dilutions of the tested compounds (ranging between 1024 and 64 μg/ mL) were performed in a 200 mL volume of nutrient broth and each well was seeded with 50 mL microbial inoculum. The plates were incubated for 24 h at 37°C, and MICs were read as the lowest concentration of compound which inhibited the microbial growth.

Flow cytometry and annexin V assay

HeLa cells were grown in DMEM supplemented with 10% foetal calf serum (FCS) and 24 h later 100 μg/mL of the tested compound were added. Cells from the supernatant and monolayer were harvested and 1×10^5 cells were stained with annexin V and propidium iodide using the Immunotech Annexin V-FITC Kit (Beckman Coulter Company, France) following the manufacturer's instructions. Cells were analyzed by flow cytometry using a Coulter EPICS XL flow cytometer (Beckman Coulter). Green fluorescence (525 nm; FITC annexin V) and red fluorescence (613 nm; propidium iodide) were measured. The experiment was repeated three times.

Results and discussion

The title compounds were preparated in three step synthesis.

1. *The synthesis of 2-(4-fluoro-phenoxymethyl)benzoic acid* (scheme 1)

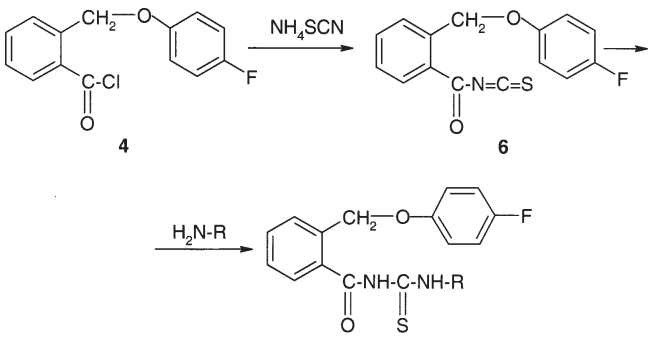
In the first stage, the 2-(4-fluoro-phenoxymethyl)benzoic acid (**1**) was prepared by refluxing the phtalide (**2**) with potassium *para*-fluorophenoxide in xylene. The resulted potassium salt of 2-(4-fluoro-phenoxymethyl)benzoic acid (**3**), which has good solubility in 10% aqueous potassium hydroxide solution, allows its facile separation from xylene. The acid **1** is then precipitated using a mineral acid solution. The potassium *para*-fluorophenoxide was obtained using the *para*-fluorophenol and potassium hydroxide in xylene, the resulting water being removed by azeotropic distillation. These reactions are presented in scheme 1.

2. *The synthesis of 2-(4-fluoro-phenoxymethyl)benzoyl chloride* (scheme 2)

The 2-(4-fluoro-phenoxymethyl)benzoyl chloride (**4**) results by refluxing acid **1** with thionyl chloride, using as reaction medium anhydrous 1,2-dichlorethane. Scheme 2 presents the mentioned reaction.

3. *The synthesis of the new thioureides* (scheme 3)

The new compounds (**5a–g**) were prepared by refluxing the 2-(4-fluoro-phenoxymethyl)benzoyl isothiocyanate (**6**) with primary aromatic amines in dry acetone. The isothiocyanate **6** was obtained through the reaction



R= -C_6H_5(**5a**); -$C_6H_4(CH_3)$ (4)(**5b**); -$C_6H_4(OCH_3)$ (4)(**5c**); -$C_6H_4(Cl)$ (4)(**5d**); -$C_6H_3(Cl)_2$ (2,4)(**5e**); -$C_6H_3(Cl)_2$ (2,6)(**5f**); -$C_6H_4(Br)$(4) (**5g**)

Scheme 3. The synthesis of the new thioureides 5a- g

between 2-(4-fluoro-phenoxymethyl)benzoyl chloride (**4**) and ammonium thiocyanate in dry acetone, as shown in scheme 3. The isothiocyanate was not isolated and the necessary amines were directly added to the reaction mixture to give the thioureides.

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

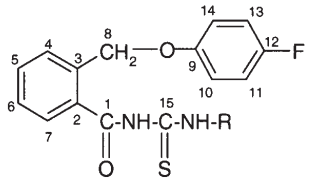
The structure, elemental analysis, melting point and yield of the new thioureides are presented in table 1.

The calculated formula provided by the elemental analysis results (table 1) is in good agreement with the expected structures.

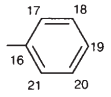
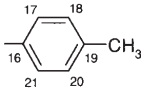
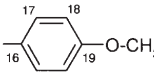
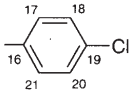
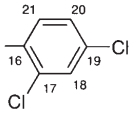
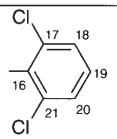
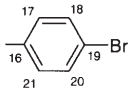
In the ^{1}H-NMR the methylene group situated near the oxygen produces a singlet at 5.26-5.28 ppm. The aromatic protons produce signals in the range 6.96-7.74 ppm, excepting the compound *5e* where H-21 has shift value of 7.97 ppm. The –NH protons produce singlets in the 11.78-12.12 ppm and 12.14- 12.41 range. In the ^{13}C-NMR spectra are characteristic the thiocarbonyl carbon signal (178.40-

Table 1

DATA ON THE NEW THIOUREIDES 5a-g



5a-g

No.	R	C%		H%		N%		S%		Melting point (^{0}C)	Yield (%)
		c.	e.	c.	e.	c.	e.	c.	e.		
5a		66.24	65.94	4.47	4.42	7.36	7.43	8.41	8.37	128.1- 130.3	83
5b		66.92	66.64	4.82	4.74	7.10	7.31	8.11	8.21	122.4- 124.2	79.5
5c		64.32	63.99	4.63	4.58	6.82	7.13	7.80	7.80	111.3- 114	78.5
5d		60.74	60.59	3.86	3.92	6.75	6.85	7.71	7.63	127.7- 130	85
5e		56.08	55.87	3.34	3.50	6.23	6.37	7.12	6.91	158.5- 160.2	84.5
5f		56.08	55.84	3.34	3.47	6.23	6.31	7.12	7.03	179- 180.9	87
5g		54.86	54.69	3.48	3.44	6.10	6.13	6.97	6.97	147.9- 150	89

where: c = calculated, e = experimental

Table 2
THE RESULTS OF THE ANTIMICROBIAL ACTIVITY (MIC VALUES) OF THE NEW THIOUREIDES (μg/ mL)

Strains / No.	*Escherichia coli*	*Salmonella enteritidis*	*Pseudomonas aeruginosa*	*Listeria monocytogenes*	*Staphylococcus aureus*	*Bacillus subtilis*	*Candida albicans*
5a	> 1024	> 1024	> 1024	> 1024	**256**	> 1024	> 1024
5b	> 1024	**256**	> 1024	> 1024	> 1024	> 1024	> 1024
5c	> 1024	> 1024	> 1024	> 1024	> 1024	> 1024	**256**
5d	**256**	**256**	**256**	> 1024	> 1024	**256**	**256**
5e	> 1024	> 1024	> 1024	> 1024	> 1024	> 1024	**256**
5f	> 1024	**256**	> 1024	> 1024	**128**	> 1024	> 1024
5g	**256**	> 1024	> 1024	> 1024	> 1024	**256**	**64**

180.56 ppm), the carbonyl carbon signal (169.54- 170.09) ppm, the methylene carbon near the oxygen signal (67.84-68.08 ppm), the methyl carbons signal (20.57 ppm) and the methoxy carbons signal (55.04 ppm).

In the IR spectra the νC=O vibrations produce a medium sharp stretching band in the region 1670-1693 cm^{-1}. The νC=O band of all the compounds appeared at 1670-1693 cm^{-1}, which is lower than that of the ordinary carbonyl absorption (1730 cm^{-1}). The formation of an intramolecular H-bond between the carbonyl oxygen and the hydrogen atom on the nitrogen atom, leads an increase of their polarity, so the strength of their double bond decreased, and the absorption moved to lower wavenumber. These compounds also show a typical alkyl-aryl ether signal at 1200-1270 cm^{-1}, for the antisymmetric vibration, and at 1020-1037 cm^{-1} for the symmetric one. The νC=S stretching vibrations were discovered in the range of 1152-1174 cm^{-1}, which is in agreement with literature data. The fluoro presence is proved by the stretching band situated at 1070-1159 cm^{-1} (for νC_{ar}-F).

In the UV-Vis, for these molecules that possess π bonding as chromophore groups (C=O, C=S) and aromatic ring, energy that is available can promote electrons from a π bonding molecular orbital to a π antibonding molecular orbital ($\pi \rightarrow \pi^*$ transition).

Antimicrobial activity

The obtained thioureides were investigated to determine their antimicrobian activity.

Our results showed that the most efficient qualitative method proved to be the direct spotting of the tested solutions on the seeded medium, the reading of the results was performed by measuring the microbial growth inhibition zones around the filter disks impregnated with the testing compounds and around the wells, respectively.

It was noticed a good correlation of these results with the results of the MIC quantitative assay. The used solvent exhibited no antimicrobial activity, this being a practical advantage for the antimicrobial activity testing of the water insoluble compounds. The MIC was read by wells observation: in the first wells containing high concentrations of the tested compounds the culture growth was not visible, the microbial cells being killed or inhibited by the tested compound. At lower concentrations of the tested compounds, the microbial culture becomes visible. The lowest concentration which inhibited the visible microbial growth was considered the MIC (μg/ mL) value for the tested compound. In the next wells, including the standard culture growth control wells, the medium become muddy as a result of the microbial growth. In the sterility control wells series the medium had to remain clear. From the last well without any visible microbial growth and from the first one with a microbial growth, Gram stained smears were performed for the results confirmation.

In table 2 we present the results of the quantitative assay of the antimicrobial and antifungal activities of the new compounds. By reporting at the active substance charge impregnated in standard antibiotic disks (i.e. up to 30 μg/ disk), we considered that a low concentration of 64 μg/ mL represents a strong effect and a 256 μg/ mL concentration represents a moderate effect. The tested compounds presented an antimicrobial activity at concentrations from 1024 to 64 μg/ mL. Our results showed that the tested compounds exhibited specific antimicrobial activity, the highest activity being noticed against suspended fungal cells (MIC ranging from >1024 to 65 μg/ mL), followed by *S. aureus* (MICs from >1024 to 128 μg/ mL). The most active compound prove to be N-[2-(4-Fluoro-phenoxymethyl)-benzoyl]-N'-(*para*-chlorophenyl)-thiourea.

Flow cytometry and annexin V assay

The cytotoxicity of the antimicrobial compounds was tested with their possible use in much more in depth studies for the design of new antimicrobial chemical compounds in mind.

The cytotoxicity assay has demonstrated that the compounds exhibited low cytotoxicity levels, comparable to those obtained for the cell control, demonstrating the possibility to be configured for the in vivo antimicrobial use.

Conclusions

In order to obtain new compounds with antimicrobian activity, we continue our research concerning synthesis in the thioureides series and we have synthesized new 2-(4-fluoro-phenoxymethyl)benzoic acid thioureides. Their structure was confirmed by spectrophotometric (IR, NMR, UV) methods and by elemental analysis.

The tested compounds exhibited specific antimicrobial activity against different bacterial and fungal strains, recently isolated from clinical samples and exhibiting

resistance features to conventional antibiotics, with MIC values ranging from >1024 to 64 μg/ mL.

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