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Studies on the antimicrobial activity of new compounds containing thiourea function

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ABSTRACT

New 2-((4-methylphenoxy)methyl)-N-(arylcarbamothioyl)benzamides, prepared from 2-(4-methyl-phenoxymethyl)benzoyl chloride *via* isothiocyanate formation followed by treatment with various substituted amines, were tested for their antimicrobial features. Despite their weak antimicrobial activity, some of the tested derivatives inhibited the microbial biofilm development on the inert substrata, encouraging further studies of their potential application as anti-biofilm agents.

Keywords: *acylthioureas, benzamides, antimicrobial, antibiofilm*

1. Introduction

Compounds containing thiourea function in their molecules seem to be suitable candidates for further chemical modifications and might be of interest as pharmacologically active compounds and ligands useful in coordination chemistry. Thiourea derivatives have been the subject of interest because it has been shown that they have antibacterial and antifungal activities. Some pyridazine derivatives carrying thiourea moiety are potent inhibitory activity against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *C. parapsilosis* [1]. 1-Aroyl-3-aryl-thioureas show moderat to potent activity against tested bacterial strains, especially against the *E. coli* resistant to standard drug [2]. Novel aroyl, thiophenoyl, morpholinoyl and butanoyl thiourea derivatives containing a thiazole moiety, such as N-[(1,3-thiazol-2-ylamino)carbonothioyl]thiophene-2-carboxamide and N-[(1,3-thiazol-2-ylamino)carbonothioyl]morpholine-4-carboxamide, possessed a broad spectrum of antifungal activity [3]. Metal complexes of the thiourea derivatives have been the subject of considerable study known for a long time. Acyl-thioureas containing carbonyl and thiocarbonyl groups have an important position as potential donor ligands for transition metal ions. These compounds can coordinate metal ions through oxygen and sulfur donor atoms by reaction with salts of transition metals. Furthermore, metal complexes of thioureas (i.e. Ag (I), Cu (I), Cu (II), Co (II), Ni (II), Pt (II), Pd (II), Te (II) complexes) can exhibit higher antibacterial activity [4, 5].

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2. Experimental section

2.1. Chemistry. All reagents used in this study were obtained from commercial suppliers (Merck, Fluka or Sigma-Aldrich) and used without further purification except *para*-methylphenol which was used freshly distilled, acetone was dried over K_2CO_3 and ammonium thiocyanate by heating at 100°C before use. The necessary liquid amines were dried with potassium hydroxide and afterwards distilled. Melting points were determined in open capillary tubes on Electrothermal 9100 apparatus and are uncorrected. The reaction was monitored by thin layer chromatography performed on silica gel plates $60F_{254}$ (Merck, 0.2 mm thick) using a mobile phase of 4: 6 chloroform/ethyl acetate, with visualization by ultraviolet light. Structural elucidation of these compounds was performed by IR, NMR spectroscopy and elemental analysis. Elemental analyses were done on a Perkin Elmer CHNS/O Analyzer Series II 2400 apparatus. The IR spectra were recorded with a Bruker Vertex 70 spectrophotometer using the ATR technique. ^{1}H-NMR and ^{13}C-NMR spectra were recorded in DMSO-d_6 on a Gemini 300BB instrument, at room temperature, operating at 300MHz for ^{1}H and 75MHz for ^{13}C and a Unity Inova 400 instrument, operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. The synthesis, physico-chemical characterization and spectral data of the new derivatives were presented in a previous paper [6].

2.2. Assessment of the antimicrobial and anti-pathogenic activity of the newly synthesized compounds. The *in vitro* antimicrobial tests were carried out by an adapted agar-disk diffusion technique using a bacterial suspension of 0.5 McFarland obtained from 24 hours cultures. The antimicrobial activities of the newly synthesized compounds were determined against the following microbial strains *S. aureus* 1694, *S. aureus* ATCC 25923, *Bacillus sp., S. epidermidis* 756, *E. faecalis* ATCC 29212, *Salmonella sp.* 9246, *Klebsiella pneumoniae* 1771, *E. coli* 1567, *E. coli* ATCC 25922, *P. aeruginosa* 1671, *C. albicans* 128 and *Aspergillus* sp. The compounds were solubilised in dimethylsulfoxide. A volume of 10 µL of each tested compounds solution was distributed directly on the solid medium previously seeded with the microbial inoculums. The inoculated plates were incubated for 24 hrs at 37°C. Antimicrobial activity was assessed by measuring the growth inhibition zones diameters [7, 8]. The quantitative assay of the minimal inhibitory concentration (MIC, µg/mL) was based on liquid medium two-fold microdilutions. After 24 hrs incubation at 37°C the bactericidal activity was quantified by measuring the absorbance of the liquid culture at 620 nm. At the end of the experiment the plastic wells were emptied, washed three times with phosphate buffered saline (PBS), fixed with cold methanol and stained with 1% violet crystal solution for 30 minutes. The biofilm formed on plastic wells was resuspended in 30% acetic acid. The intensity of the colored suspensions was assessed by measuring the absorbance at 490 nm [8, 9]. The tested substances were investigated for their influence on the expression of soluble enzymatic virulence factors (haemolysins and other pore-forming toxins, proteases activity, DNA-se and esculin hydrolysis with the production of esculethol as an iron-chelating). The expression of soluble enzymatic factors was investigated by spotting fresh cultures on specific culture media incubated at 37°C for 24 hrs. *Plate haemolysis*: the strains were streaked on blood agar plates containing 5% (vol/vol) sheep blood in order to obtain isolated colonies. After incubation 24 hours at 37°C, the clear areas (total lysis of red blood cells) around the colonies were registered as positive reactions [8, 9]. *Lipase production*: cultures were spotted on Tween 80 agar as a substrate at a final concentration of 1 % and were incubated at 37°C until 7 days. An opaque (precipitation) zone around zone around the spot was registered as positive reaction [8, 9]. *Lecithinase production*: cultures were spotted into 2,5% yolk agar and incubated at 37°C until 7 days. An opaque

(precipitation) zone around the spot indicated the lecithinase production [8, 9]. *DN-ase production* was studied on DNA medium. The strains were spotted and after incubation at 37°C for 24 hours, a drop of HCl 1N solution was added upon the spotted cultures, a clearing zone around the culture being interpreted as positive reaction. *Esculin hydrolysis*: the esculin hydrolysis was evidenced onto Esculin containing agar medium. In the presence of an iron salt, esculethol (an iron chelating agents) forms a brown-black complex that diffuses into the surrounding medium interpreted as positive reaction [8, 9].

3. Results section

The new thioureides synthesis (**1a-l**) involve the reaction of 2-(4-methyl-phenoxymethyl)benzoyl chloride (**2**) with ammonium thiocyanate in dry acetone, followed by condensation of the resulting 2-(4-methyl-phenoxymethyl)-benzoyl isothiocyanate (**3**) with an primary amine. The acide chloride (**2**) was prepared by refluxing the 2-(4-methyl-phenoxymethyl)benzoic acid (**4**) with thionyl chloride, using anhydrous 1,2-dichloroethane as reaction medium. The acid (**4**) was synthetized with the best yield using phtalide (**5**) which was treated with potassium *para*-cresolate in xylene under reflux. First, the potassium salt of 2-(4-ethyl-phenoxymethyl)benzoic acid (**6**) is obtained and, having a good solubility in a 10% potassium hydroxide aqueous solution, can be separated from xylene. The acid (**4**) then precipitated using a mineral acid solution. The necessary potassium *para*-cresolate was obtained from the corresponding phenol and potassium hydroxide in xylene. The resulting water was removed by azeotropic distillation. The general synthetic pathway and the structure of the new compounds are given in Scheme 1.

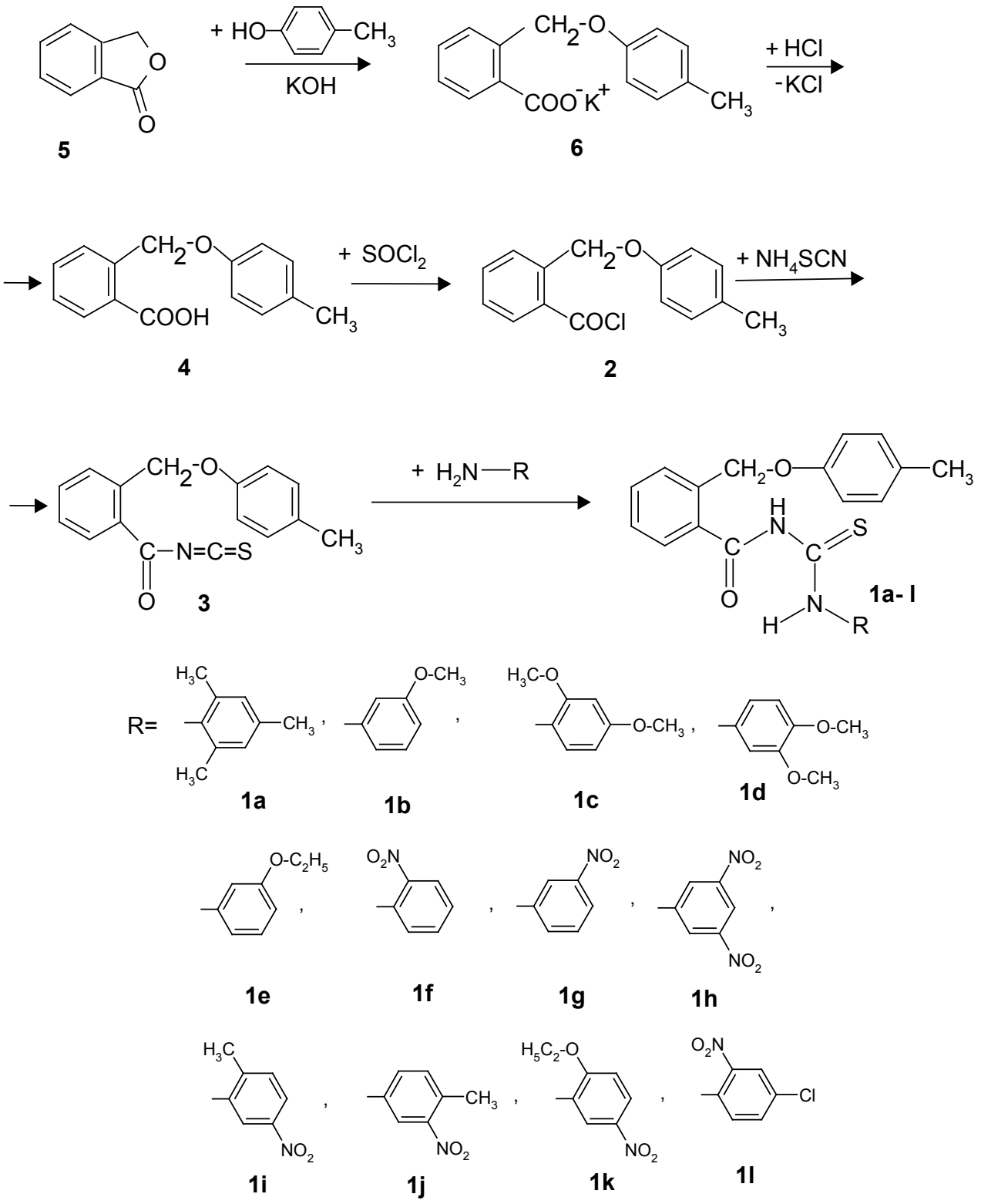
Table 1: Qualitative assay of the antimicrobial activity of the tested compounds

Microbial strain / **Chemical compound**	*Salmonella*	*Pseudomonas aeruginosa 1671*	*Staphyloccus aureus 1694*	*Staphyloccus aureus ATCC 25923*	*Aspergillus*	*Staphyloccus epidermidis 756*	*Klebsiella pneumoniae*	*Escherichia coli 1567*	*Escherichia coli ATCC 25922*	*Enterococcus faecalis ATCC*	*Candida 128*	*Bacillus sp.*
CTMP 11 (1a)	-	-	-	-	+/-	-	-		-	-	-	-
CTMP 25 (1b)	-	+/-	-	+	-	-	-	-	-	-	-	-
CTMP 27 (1c)	-	-	-	-	-	-	-	-	-	-	-	-
CTMP 28 (1d)	-	-	+	-	-	-	-	-	-	-	-	+/-
CTMP 31 (1e)	-	-	-	-	-	-	-	-	-	-	-	-
CTMP 33 (1f)	-	-	-	-	-	-	-	-	-	-	-	-
CTMP 34 (1g)	-	-	-	-	+	-	-	-	-	-	-	-
CTMP 37 (1h)	-	-	-	-	-	-	-	-	-	-	-	-
CTMP 40 (1i)	-	-	-	-	-	-	-	-	-	-	-	-
CTMP 41 (1j)	-	-	-	-	-	-	-	-	-	-	-	-
CTMP 42 (k)	-	-	-	-	-	-	-	-	-	-	-	-
CTMP 54 (l)	-	-	-	-	-	-	-	-	-	-	-	-
DMSO	-	-	-	-	-	+	-	+	-	-	-	+

Legend: "-" absence of any antimicrobial activity; "+" and "+/-" presence of the antimicrobial activity, quantified by the occurrence of a growth inhibition zone"

The qualitative screening of the susceptibility spectra of various microbial strains to the newly synthesized compounds showed that three compounds (encoded **1b, 1d** and **1l**) exhibited an antimicrobial effect at 10mg/mL, quantified by the occurrence of a growth inhibition zone (Table 1).

Scheme 1: The general scheme of synthesis



The quantitative assay was further carried out only for these compounds, against *S. aureus* 1694 and *Bacillus sp.*) for CTMP 28 **(1d)** and against *P. aeruginosa 1671* and *S. aureus ATCC 25923* for CTMP 25 (**1b**). The results of the quantitative assay revealed that the tested compounds did not exhibit an inhibitory activity on the bacterial growth at concentrations lower than that used for the qualitative assay (Fig. 1-2). In some cases, the absorbance values measured at 620 nm of the bacterial cultures grown in liquid medium in the presence of the tested compounds were higher than those obtained for the organic solvent (DMSO). Concerning the influence of these derivatives on biofilm development on inert substrata the results showed that some compounds exhibited an inhibitory effect on the microbial adherence compared to the positive control, while in some cases the absorbance values registered in case of the solvent (DMSO) were similar to those of the tested compounds. The compound **1b** produced an inhibitory effect on biofim development by *S. aureus ATCC* 25923, while it stimulated the adherence of *P. aeruginosa* 1671 cells (Fig. 3). The compound **1d** exhibited an inhibitory effect on microbial adherence, the effect being more pronounced in case of *Bacillus* strain (Fig. 4). Taken together, these results showed that the inhibitory effect exhibited by the newly synthesized compounds on bacterial adherence on inert substrata was preferentially expressed against the Gram positive tested strains. The tested compounds did not induce any change in the expression pattern of the soluble virulence factors.

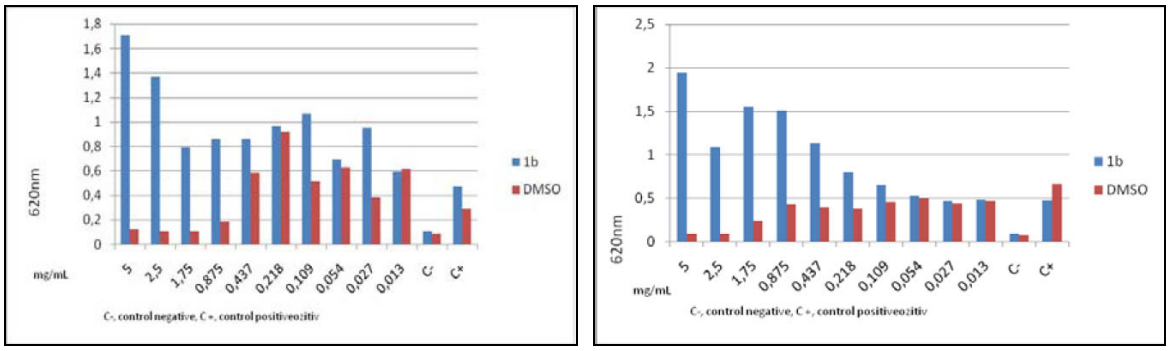


Figure 1: The influence of the compound 1b on bacterial growth (*S. aureus* ATCC 25923 (left) and *P. aeruginosa* 1671(right))

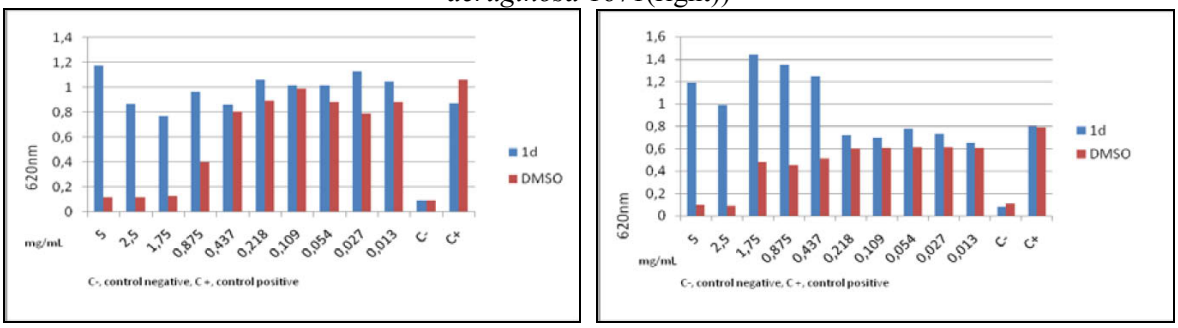


Figure 2: The influence of the compound 1d on bacterial growth (*Bacillus* sp.(left) and *S. aureus* 1694 (right))

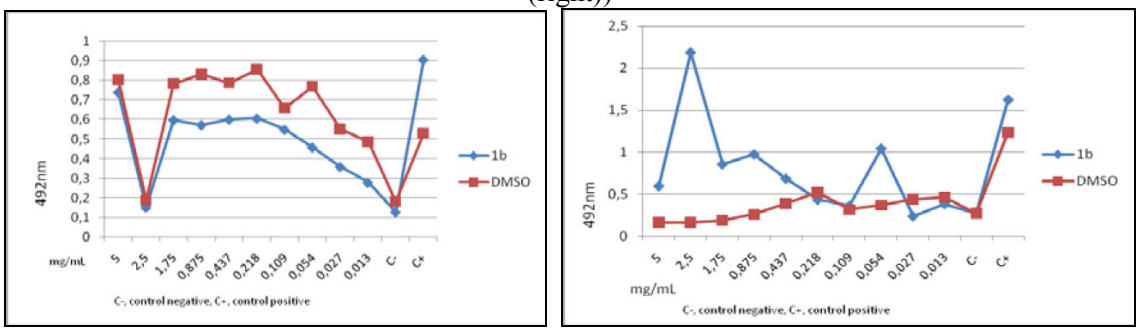


Figure 3: *S. aureus* ATCC 25923 (left) and *P. aeruginosa* 1671(right) biofilm development on inert substrata in the presence of the compound 1b.

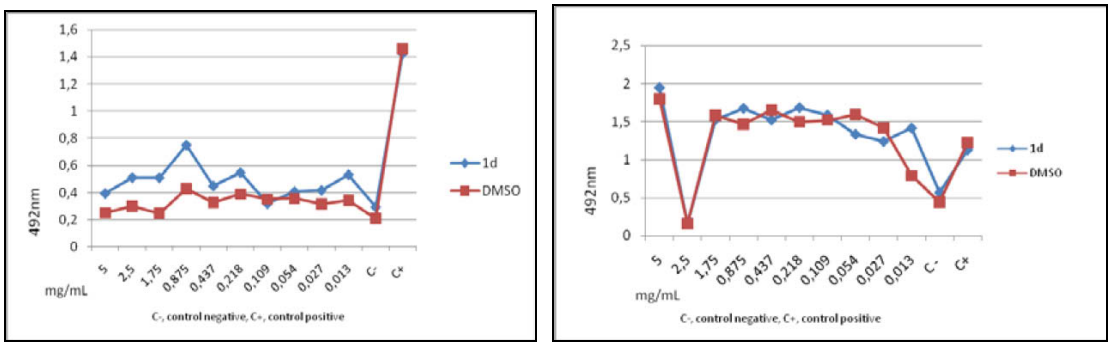


Figure 4: *Bacillus* sp.(left) and *S. aureus* 1694 (right) biofilm development on inert substrata in the presence of the compound 1d.

4. Conclusions

New 2-((4-methylphenoxy)methyl)-N-(arylcarbamothioyl)benzamides, prepared from 2-(4-methyl-phenoxymethyl)benzoyl chloride *via* isothiocyanate formation followed by treatment with various substituted amines, were tested for their antimicrobial features. Despite their weak antimicrobial activity, some of the tested derivatives inhibited the microbial biofilm development on the inert substrata, encouraging further studies of their potential application as anti-biofilm agents.

5. References

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