ORIGINAL ARTICLE

SYNTHESIS, STRUCTURAL, PHISICO-CHEMICAL CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY SCREENING OF NEW THIOUREA DERIVATIVES

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Abstract

A number of seven new thiourea derivatives having thiophene skeleton, obtained from 2-thiopheneacetic acid, have been synthesized, characterized by their physical properties (melting point, solubility), FT-IR, NMR spectroscopy and tested by qualitative and quantitative microbiological methods on various bacterial and fungal strains in order to identify their antimicrobial and antibiofilm activities, planktonic and biofilm growth state. The new compounds were prepared by the reaction of 2-thienyl-isothiocyanate with various primary aromatic amines. The antimicrobial activity of the obtained thiourea derivatives was evaluated by using both qualitative and quantitative assays, allowing to establish the Minimal Inhibitory Concentration (MIC) as well as the spectrum of antimicrobial and anti-biofilm activities. The compounds N-(2-trifluoromethylphenyl)-N'-(2-thienyl)-thiourea (**4a**) and N-(4-trifluoromethylphenyl)-N'-(2-thienyl)-thiourea (**4c**), proved to be the most efficient, showing antimicrobial activity on the majority of the tested strains in planktonic growth state. The compounds N-(3,5-dimethoxyphenyl)-N'-(2-thienyl)-thiourea (**4d**) and N-(3-trifluoromethylphenyl)-N-(2-thienyl)-thiourea (**4b**) had the broadest spectrum of anti-biofilm activity, which included the most clinically relevant biofilm forming microorganisms, i.e. *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*.

Rezumat

Au fost sintetizați șapte noi derivați ai tioureei obținuți de la acidul 2-tiofenacetic, care au fost caracterizați cu ajutorul proprietăților lor fizice (temperatură de topire, solubilitate), spectroscopiei FT-IR, RMN și au fost testați prin metode microbiologice calitative și cantitative pe diferite tulpini bacteriene și fungice, pentru determinarea activității lor antimicrobiene și anti-biofilm. Noii compuși au fost obținuți în urma reacției izotiocianatului de 2-tienil cu diverse amine primare aromatice. Activitatea antimicrobiană a acestor derivați de tioureea a fost evaluată față de nouă tulpini microbiene pentru stabilirea Concentrației Minime Inhibitorii (CMI) utilizând metoda microdiluțiilor în mediu lichid. Compușii N-(2-trifluorometilfenil)-N'-(2-tienil)-tiourea (**4a**) și N-(4-trifluorometilfenil)-N'-(2-tienil)-tiourea (**4c**) au fost cei mai activi, prezentând activitate antimicrobiană față de majoritatea tulpinilor testate în stare planctonică. Compușii N-(3,5-dimetoxifenil)-N'-(2-tienil)-tiourea (**4d**) și N-(3-trifluorometilfenil)-N'-(2-tienil)-tiourea (**4b**) au prezentat cel mai larg spectru de activitate anti-biofilm, incluzând microorganismele cele mai relevante clinic pentru producerea infecțiilor asociate biofilmelor, respectiv *Staphylococcus aureus*, *Pseudomonas aeruginosa* și *Candida albicans*.

Keywords: thioureas, 2-thiopheneacetic acid, antimicrobial, anti-biofilm

Introduction

In the last few years, antimicrobial resistance has led to an increase in the number of deaths or exacerbation of infectious diseases severity. Moreover, micro-organisms do not exist in nature as pure cultures of dispersed single cells but instead, they accumulate at interfaces to form polymicrobial aggregates such as films, mats, flocs, sludge, or "biofilms". Biofilms are microbial communities of surface-attached cells embedded in a self-produced extracellular polymeric matrix. In the biofilm structure, the microbial cells are well protected and despite of high doses of antibiotics, the biofilms cannot be eliminated, rendering them difficult to eradicate and requiring complex multi-drug strategies especially when biofilms are polymicrobial [18]. The mechanisms of the biofilms

tolerance to antimicrobial agents are represented but not limited to: failure of or limited penetration of antimicrobial agents into biofilms, entry into states of slow growth or starvation, modified gene expression, accumulation of antibiotic inactivating enzymes inside biofilms [19].

Biofilms are involved in much chronic infectious pathology, such as: cystic fibrosis, osteomyelitis, prostatitis and pyelonephritis. The presence of biofilm on synthetic surfaces explains the occurrence of persistent infections related to implantable medical devices (catheters, vascular prostheses etc.) [19].

The increasing bacterial resistance, both genetic and phenotypic, makes necessary an improvement of drugs quality and development of new antimicrobial active substances [7].

The thiourea derivatives, according to the literature data, are of great importance because of their diversity of biological effects: antibacterial [3, 17], antitubercular [8, 11], antiparasitic agents [16, 13], anticonvulsant [4, 12], analgesic [15, 23], antiarrythmic, antihyperlipidemic, local anaesthetic [20], antiaggregating, antiproliferative [10], fungicidal [6], insecticides [21, 22], plant-growth regulators [14, 24]. There are also some reports showing the anti-biofilm activity of thiourea derivatives that has been attributed to its *quorum*-quenching activity, due to its structural analogy with the Gram-negative bacterial quorum sensing signalling molecules, represented by N-acyl homoserine lactones [5]. The quorum sensing phenomenon is considered today the mechanism that allows pathogenic bacteria to coordinate their virulence factors expression for escaping the host immune response and establishing an infection [5, 9].

The present paper represents a follow up study of our previous research [1, 2] and aimed to synthesize new thiourea derivatives, to characterize them by their physical properties (melting point, solubility) and to identify their structures by spectral data. The antimicrobial activity of the synthesized compounds was performed by an *in vitro* assay, using reference strains, cultivated in planktonic and adherent state.

Materials and Methods

The chemicals used in this synthesis were all purchased from commercial suppliers (Merck, Sigma-Aldrich or Fluka) and used as received, without any further purification. Acetone and ammonium thiocyanate were dried before use.

The melting points were recorded using an Electrothermal 9100 apparatus in open capillary tubes and are uncorrected.

Infrared spectra were recorded on a Fourier transform infrared spectroscopy attenuated total reflectance (FT-IR-solid in ATR) spectrometer and the signal intensities (height) were denoted by the following abbreviations: m = medium, s = strong, i = intense.

The NMR spectra were recorded in DMSO-d_6 using a Gemini 300BB instrument, operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C. Tetramethylsilane (TMS) was used as internal standard.

The chemical shifts are expressed in δ units (ppm) values and the coupling constants are in Hertz. The spectra were recorded at room temperature. The splitting patterns are abbreviated as following: s = singlet; d = doublet; t = triplet; dd = double doublet; m = multiplet; b = broad.

The elemental analyses were performed on a Perkin Elmer CHNS/O Analyser Series II 2400 apparatus and the results were in agreement with the calculated values.

General synthesis procedure of the new thioureas

The necessary 2-thiopheneacetic acid chloride (2) was obtained from 2-thiopheneacetic acid (1) and thionyl chloride according to the reaction scheme shown in Figure 1. The new thioureas, **4a** - **4g**, were prepared by heating the 2-thienyl-isothiocyanate (3) (obtained from the corresponding acid chloride (2) with ammonium thiocyanate in dry acetone under reflux for one hour), with primary aromatic amines in dry acetone under reflux for 1 h. The general synthesis pathway of the new compounds is presented in Figure 1.

The new seven acylthioureas (**4a** - **4g**) were synthesized according to the general method presented in our previous papers [1, 2], as follows: a solution of 2-thiopheneacetic acid chloride (0.01 mol) (2) in dry acetone (15 mL) was added to a solution of ammonium thiocyanate (0.01 mol; 0.76 g) in dry acetone (5 mL) and the reaction mixture was kept under reflux for 1 h. The intermediate 2-thienyl-isothiocyanate (3) was not isolated.

After cooling, the solution of primary aromatic amine (0.01 mol) in dry acetone (5 mL) was added to the reaction mixture upon stirring and refluxed for 1 h. After completion of the reaction, the mixture was poured into cold water (500 mL). The precipitated thioureas were separated by filtration and recrystallized from isopropanol.

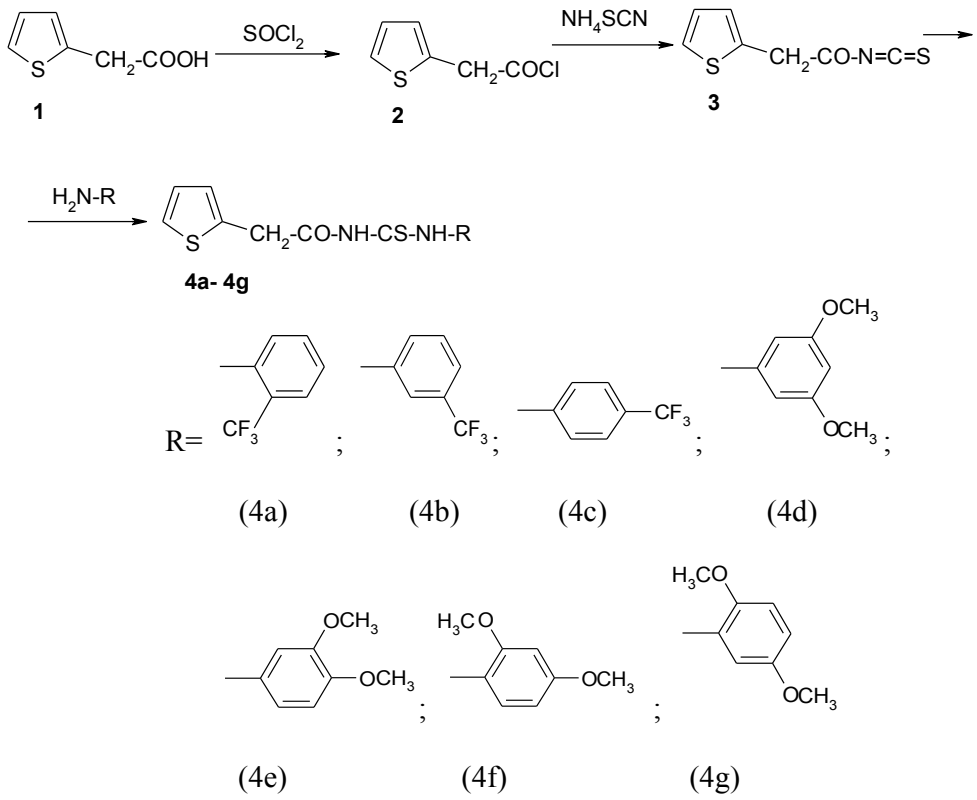


Figure 1.
The synthesis pathway of the new thiourea derivatives

Antimicrobial activity assay

The antimicrobial properties were tested against nine microbial strains, including Gram-positive (*S. aureus* ATCC 25923, *Staphylococcus aureus* ATCC BA 1026, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* 53(100), *Bacillus licheniformis* 12195, *Bacillus subtilis* IC 6633) and Gram-negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) bacteria, as well as a fungal strain (*Candida albicans* ATCC 26790); all tested strains are included in the microbial collection of the Microbiology Department of the Faculty of Biology, University of Bucharest.

The qualitative screening was accomplished using the disk diffusion method, while the quantitative assay was performed by the binary micro-dilution method in 96-well culture plates, in order to establish the minimal inhibitory concentration (MIC). The anti-biofilm activity assay was performed by the violet crystal microtiter method, as previously described [3, 5].

The qualitative assay of the antimicrobial activity of the obtained compounds using the spot technique

Bacterial suspensions of 0.5 McFarland IU density were obtained from 18 h bacterial cultures developed on solid media. The antimicrobial activity of the new compounds was tested on solid Mueller-Hinton agar. In this purpose, paper filter disks were impregnated with 10 µL of the tested compounds and were equally distributed on Petri dishes previously seeded "in layer" with the corresponding bacterial strain inoculums. The plates have been left to stay at room temperature for adsorption of the solution droplet into the medium, after that the plates have been incubated at 37ºC with the cover down, for 24 hours. The occurrence of growth inhibition areas around the disk indicated the presence of an antimicrobial activity. DMSO solvent was also tested for the anti-microbial activity. The diameters of the growth inhibition zones obtained for the compounds were compared to those induced by DMSO.

Quantitative assay of the antimicrobial activity by the serial microdilution method in liquid medium

The quantitative assay of the antimicrobial activity of the new compounds was performed by using the two fold microdilution method in liquid broth distributed in 96 plates, in order to establish the minimum inhibitory concentration (MIC), corresponding to the minimum amount of the chemical compound able to inhibit the visible growth of microbial cells. Over 270 µL liquid medium distributed in each well, 30 µL of each chemical compound stock solution were added and serial two fold dilutions, ranging from 1 mg/mL up to 0.015 mg/mL were obtained. Finally, in each well there were added 15 µL microbial suspension of 0.5 MacFarland density. The plates were incubated for 24 hours at 37ºC and MICs were determined as the last concentration of the respective

tested compound which inhibited the microbial growth. In the following wells, including the growth control one, the culture medium was opalescent as a result of microbial growth. The content of the negative sterility control remained clear.

Anti-biofilm activity assay

The evaluation of development of the microbial biofilm was performed by the microtiter method, using sterile 96 plates. After cultivating the bacteria for 24 hours at 37ºC in liquid medium in the presence of the binary dilutions of the tested compounds the plates have been emptied and washed twice with sterile saline solution. Subsequently, adhered cells have been fixed for 5 minutes with 150 µL methanol 80%. After mounting, the plates have been coloured with violet crystal alkaline solution 1% (150 µL/well) for 15 minutes. The staining solution was removed and then the plates have been washed under cool tap water. The microbial biofilms developed on the plastic plates have been resuspended in acetic acid 33% (by splashing), and the intensity of the coloured suspension has appreciated by visual examination and quantified, by comparison with the positive and negative controls.

Results and Discussion

Using the mentioned general method of synthesis, we obtained seven new acylthioureas and their structures were confirmed by elemental analysis, IR and ^{1}H-NMR and ^{13}C-NMR spectra.

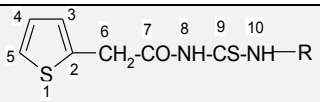
The elemental analysis results were in good agreement with those calculated using the suggested formula and the accuracy of experimental values in respect to the theoretical values.

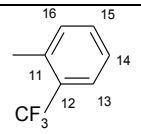
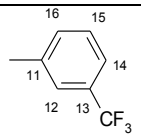
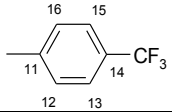
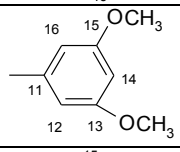
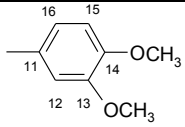
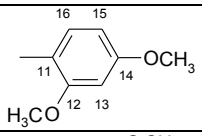
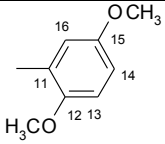
The chemical structures of all compounds were characterized by spectroscopic methods, and the spectral data were in full agreement with the proposed structures.

All new acylthioureas (Table I) are white or light-yellow crystalline solids, insoluble in water and soluble in acetone and chloroform at normal temperature and in aliphatic alcohols, benzene, toluene and xylene at higher temperatures.

Table I

The characterization data for the new compounds



Compound	R	Molecular formula	Molecular weight
4a		$C_{14}H_{11}S_2ON_2F_3$	344.378
4b		$C_{14}H_{11}S_2ON_2F_3$	344.378
4c		$C_{14}H_{11}S_2ON_2F_3$	344.378
4d		$C_{15}H_{16}S_2O_3N_2$	336.433
4e		$C_{15}H_{16}S_2O_3N_2$	336.433
4f		$C_{15}H_{16}S_2O_3N_2$	336.433
4g		$C_{15}H_{16}S_2O_3N_2$	336.433

The ^{1}H-NMR data are reported in the following order: chemical shifts, multiplicity, the coupling constants, number of protons and signal/atom attribution.

For the ^{13}C-NMR data the order was: chemical shifts and signal/atom attribution. The chemical structure, molecular formula and molecular weight of the new thioureas are presented in Table I.

Following, there are presented the melting points (m.p.), reaction yield and the spectral data for the new compounds (**4a** - **4g**), which showed all the expected signals.

*Compound **4a**: N-(2-trifluoromethylphenyl)-N'-(2-thienyl)-thiourea*: m.p. 181 - 183ºC; yield 70%. ^{1}H-NMR (DMSO-d6, δ ppm, *J* Hz): 12.25 (s, 1H, NH-10); 11.99 (s, 1H, NH-8); 7.68-7.82 (m, 3H, H-13, H-15, H-16); 7.52 (bt, 1H, H-14, 7.5); 7.42 (dd, 1H, H-5, 4.9, 1.2); 7.02 (m, 2H, H-3, H-4); 4.09 (s, 2H, H-6). ^{13}C-NMR (DMSO-d6, δ ppm, *J* Hz): 181.29 (C-9); 172.41 (C-7); 135.69 (C-11); 134.95 (C-2); 132.89 (C-16); 130.86 (C-15); 127.86 (C-14); 127.43 (C-3); 126.98 (q, J^{3}_{F} = 4.8, C-13); 126.94 (C-4); 125.84 (C-3); 125.24 (q, J^{2}_{F} = 29.8, C-12); 124.16 (q, J^{1}_{F} = 273.1, CF_3); 36.68 (C-6). FT-IR (solid in ATR, υ cm^{-1}): 3301m; 3179m; 3017s; 2964s; 2831s; 1663i; 1507i; 1314m; 1169i; 1107i; 759m; 709i.

*Compound **4b**: N-(3-trifluoromethylphenyl)-N'-(2-thienyl)-thiourea*: m.p. 145 - 147ºC; yield 71%. ^{1}H-NMR (DMSO-d6, δ ppm, *J* Hz): 12.36 (s, 1H, NH-10); 11.84 (s, 1H, NH-8); 8.12 (bs, 1H, H-12); 7.40 - 7.95 (m, 3H, H-14, H-15, H-5); 7.45 (dd, 1H, H-5, 1.5, 4.8); 7.00 (m, 2H, H-3, H-4); 4.06(s, 2H, H-6). ^{13}C-NMR (DMSO-d6, δ ppm, *J* Hz): 179.45 (C-9); 171.46 (C-7); 138.65 (C-11); 134.76 (C-2); 129.88 (C-15); 129.16 (q, J^{2}_{F} = 31.9, C-13); 128.81 (C-3); 126.93 (C-4); 125.83 (C-5); 123.98(q, J^{1}_{F} = 272.5, CF_3); 122.94 (C-14); 121.35 (C-12); 30.67 (C-6). FT-IR (solid in ATR, υ cm^{-1}): 3300m; 3176m; 3014s; 2958m; 2829s; 1669i; 1508i; 1321m; 1170i; 1104i; 761m; 754m; 698m.

*Compound **4c**: N-(4-trifluoromethylphenyl)-N'-(2-thienyl)-thiourea*: m.p. 157 - 160ºC; yield 69%. ^{1}H-NMR (DMSO-d6, δ ppm, *J* Hz): 12.46 (s, 1H, NH-10); 11.85 (s, 1H, NH-8); 7.88 (d, 2H, H-13, H-15, 8.2); 7.75 (d, 2H, H-12, H-16, 8.2); 7.61 (dd, 1H, H-5, 4.9, 1.4); 7.05 (m, 2H, H-3, H-4); 4.06 (s, 2H, H-6). ^{13}C-NMR (DMSO-d6, δ ppm, *J* Hz): 179.16 (C-9); 171.92 (C-7); 141.45 (C-11); 134.94 (C-2); 128.34 (q, J^{2}_{F} = 32.4, C-14); 127.86 (q, J^{3}_{F} = 4.0, C-13,15); 127.45 (C-3); 126.53 (C-4); 125.81 (C-5); 124.79 (C-12, C-16); 124.12 (q, J^{1}_{F} = 271.9, CF_3); 36.73 (C-6). FT-IR (solid in ATR, υ cm^{-1}): 3298m; 3173m; 3011s; 2959m; 2824s; 1650m; 1509i; 1322i; 1265i; 1147i; 847i; 673i.

*Compound **4d**: N-(3,5-dimethoxiphenyl)-N'-(2-thienyl)-thiourea*: m.p. 165 - 168ºC; yield 56%. ^{1}H-NMR (DMSO-d6, δ ppm, *J* Hz): 12.35 (s, 1H, H-10); 11.71 (s, 1H, H-8); 7.43 (dd, 1H, H-5, 4.8, 1.6); 7.03 (m, 2H, H-3, H-4); 6,91 (d, 2H, H-12, H-16, 2.2); 6.39 (t,1H, H-14, 2.2); 4.04 (s, 2H, H-6); 4.04 (s, 3H, 15-OCH_3); 3.72 (s, 3H, 13-OCH_3). ^{13}C-NMR (DMSO-d6, δ ppm): 178.35 (C-9); 171.99 (C-7); 160.29 (C-13, C-15); 139.25 (C-11); 134.99 (C-2); 127.41 (C-3); 126.92 (C-4); 125.75 (C-5); 102.61 (C-12, C-16); 98.32 (C-14); 55.36 (OCH_3); 36.68 (C-6). FT-IR (solid in ATR, υ cm^{-1}): 3298m; 3182m; 2968m; 2831m; 1658i; 1338m; 1364i; 1243i; 1133m; 1059m; 778m.

*Compound **4e**: N-(3,4-dimethoxiphenyl)-N'-(2-thienyl)-thiourea*: m.p. 141 - 144ºC; yield 62%. ^{1}H-NMR (DMSO-d6, δ ppm, *J* Hz): 12.22 (s, 1H, H-10); 11.67 (s, 1H, H-8); 7.44 (dd, 1H, H-5, 4.8, 1.8); 7.28 (d, 1H, H-12, 2.4); 7.13 (dd, 1H, H-16, 8.2, 2.4); 7.04 (m, 2H, H-3, H-4); 6.94 (d, 1H, H-15, 8.2); 4.04 (s, 2H, H-6); 3.75 (s, 3H, 13-OCH_3); 3.73 (s, 3H, 14-OCH_3). ^{13}C-NMR (DMSO-d6, δ ppm): 178.52 (C-9); 171.91 (C-7); 161.31 (C-13); 148.83 (C-14); 147.06 (C-15); 135.10 (C-2); 130.69 (C-11); 127.36 (C-3); 126.92 (C-4); 125.73 (C-5); 116.44 (C-16); 108.78 (C-12), 55.63 (13-OCH_3); 55.87 (14-OCH_3); 36.69 (C-6). FT-IR (solid in ATR, υ cm^{-1}): 3296m; 3171m; 3080m; 1654i; 1510m; 1410m; 1355m; 1278i; 1145i; 775i; 710i.

*Compound **4f**: N-(2,4-dimethoxiphenyl)-N'-(2-thienyl)-thiourea*: m.p. 168 - 170ºC; yield 59%. ^{1}H-NMR (DMSO-d6, δ ppm, *J* Hz): 12.38 (s, 1H, H-10); 11.61 (s, 1H, H-8); 8.26 (d, 1H, H-16, 9.0); 7.44 (dd, 1H, H-5, 4.4, 1.8); 7.01 (m, 2H, H-3, H-4); 6.66 (d, 1H, H-13, 2.6); 6.54 (dd, 1H, H-15, 9.0, 2.6); 4.03 (s, 2H, H-6); 3.81 (s, 3H, 12-OCH_3); 3.73 (s, 3H, 14-OCH_3). ^{13}C-NMR (DMSO-d6, δ ppm): 177.42 (C-9); 171.82 (C-7); 158.22 (C-14); 152.02 (C-12); 135.18 (C-2); 127.37 (C-3); 126.95 (C-4); 125.72 (C-5); 124.55 (C-16); 119.79 (C-11); 103.87 (C-15); 98.66 (C-13); 55.05 (12-OCH_3); 55.43 (14-OCH_3); 36.65 (C-6). FT-IR (solid in ATR, υ cm^{-1}): 3285m; 3183m; 3019s, 2975s; 2931m; 2845m; 1655m; 1532i; 1323m; 1160m; 829m; 719i.

*Compound **4g**: N-(2,5-dimethoxiphenyl)-N'-(2-thienyl)-thiourea*: m.p. 153 - 156ºC; yield 64%. ^{1}H-NMR (DMSO-d6, δ ppm, *J* Hz): 12.75 (s, 1H, H-10); 11.70 (s, 1H, H-8); 8.42 (d, 1H, H-16, 3.0); 7.44 (dd, 1H, H-5, 4.4, 1.8); 7.02 (d, 1H, H-13, 9.0); 7.01 (m, 2H, H-3, H-4); 6.77 (dd, 1H, H-14, 9.0, 3.0); 4.04 (s, 2H, H-6); 3.78 (s, 3H, 12-OCH_3); 3.70(s, 3H, 15-OCH_3). ^{13}C-NMR (DMSO-d6, δ ppm): 177.19 (C-9); 171.85 (C-7); 152.27 (C-15); 144.34 (C-12); 135.12 (C-11); 135.11 (C-2); 127.40 (C-3); 126.97 (C-4); 125.75 (C-5); 111.94 (C-13); 110.32 (C-14); 108.99 (C-16); 56.47 (15-OCH_3); 55.45 (12-OCH_3); 36.67 (C-6). FT-IR (solid in ATR, υ cm^{-1}): 3340s; 3181s; 2962m; 2854m; 1661m; 1532i; 1352m; 1226i; 1147i; 1043m; 725i.

The results of the qualitative screening of the anti-microbial activity of the obtained compounds are presented in Table II.

Table II

Results of the qualitative assay of the antimicrobial activity of the obtained compounds

Microbial strain / Compound	*Staphylococcus aureus* ATCC 25923	*Staphylococcus aureus* ATCC BA 1026	*Enterococcus faecalis* ATCC 29212	*Bacillus cereus* 53(100)	*Bacillus Licheniformis* 12195	*Bacillus subtilis IC 6633*	*Pseudomonas aeruginosa* ATCC 27853	*Escherichia coli* ATCC 25922	*Candida albicans* ATCC 26790
4a	-	-	-	-	-	-	-	-	-
4b	+/-	+/-	+/--	+	-	+	+	+	+
4c	-	-	-	+	-	-	-	-	-
4d	-		-	-	+	+	-	-	-
4e	-	-	-	-	+	+	-	-	-
4f	-	-	-	-	+	+	-	-	-
4g	-	-	-	-	-	-	-	-	-
DMSO	-	+/--	-	-	-	+/--	-	-	-

DMSO = dimethyl sulfoxide standard; (-) no growth inhibition zone; (+) clear area of growth inhibition; (+/-) a weak inhibition of the microbial growth; (+/--) a very weak inhibition of the microbial growth.

The qualitative assay indicated the compound **4b** as the most active, exhibiting the largest spectrum of antimicrobial activity including Gram-positive bacteria (*B. cereus, B. subtilis*), Gram-negative fermentative (*E. coli*) and non-fermentative rods (*P. aeruginosa*), as well as fungal strains (*C. albicans*), followed by the compounds **4d**, **4e** and **4f**, revealing a similar and narrower spectrum of antimicrobial activity, represented by two strains of *Bacillus* sp. (Table II). However, it must be noticed that the results of the qualitative assay are not sufficient for evaluating the antimicrobial potential of a new drug, because the poor water solubility could limit the diffusion of the active substance from the paper disk into the culture medium. Therefore, some active compounds could occur as lacking any activity in this assay, while others could be active, but in very high concentrations, which are impossible to achieve *in vivo* due to side effects.

In the quantitative assay, based on literature data, we considered a strong antimicrobial effect for MICs ranging from 0.125 mg/mL to 0.015 mg/mL, while a MIC of 0.250 mg/mL represented a moderate antimicrobial effect.

The results of the quantitative assay of the antimicrobial activity of the tested compounds are presented in Table III.

Table III

The MIC (mg/mL) values of the tested compounds

Microbial strain / Compound	*Staphylococcus aureus* ATCC 25923	*Staphylococcus aureus* ATCC BA 1026	*Enterococcus faecalis* ATCC 29212	*Bacillus cereus* 53(100)	*Bacillus Licheniformis* 12195	*Bacillus subtilis IC 6633*	*Escherichia coli* ATCC 25922	*Pseudomonas aeruginosa* ATCC 27853	*Candida albicans* ATCC 26790
4a	0.250	0.250	0.062	0.125	0.062	0.062	0.125	1	1
4b	> 1	> 1	> 1	> 1	> 1	> 1	> 1	1	> 1
4c	0.031	0.500	0.015	0.031	> 1	0.500	0.031	0.500	> 1
4d	1	> 1	1	> 1	0.500	0.500	> 1	1	> 1
4e	1	> 1	1	1	0.500	0.500	> 1	1	> 1
4f	1	> 1	1	> 1	0.500	0.500	> 1	1	> 1
4g	1	> 1	1	1	1	> 1	> 1	1	> 1
DMSO	> 1	> 1	> 1	> 1	> 1	> 1	> 1	> 1	> 1

DMSO = dimethyl sulfoxide control

The quantitative assay results showed that the tested compounds exhibited different intensities and ranges of the antimicrobial activity, the most active being the compound **4a**, exhibiting a high or moderate antimicrobial activity against seven of the nine tested microbial strains, i.e. the two *S. aureus* strains, *E. faecalis*, *B. licheniformis*, *B. cereus*, *B. subtilis* and *E. coli*, followed by **4c**, with very low MIC values against four microbial strains, i.e. *S. aureus*, *E. faecalis*, *B. cereus* and *E.coli*. Thus, the compounds **4a** and **4c** can be considered broad-spectrum antimicrobial agents, active against both Gram-positive and Gram-negative bacterial strains, confirming their promising potential for the development of novel antimicrobial strategies. These new compounds were also tested for any possible anti-biofilm activity. The obtained thiourea derivatives have exhibited varying

degrees of anti-biofilm activity against all tested pathogens.

All tested thiourea derivatives exhibited anti-biofilm activities on different microbial strains. The biofilm development ability of the two *Bacillus* sp. strains proved to be inhibited by all tested derivatives. Compound **4d** exhibited the largest spectrum of the anti-biofilm activity including, besides, two *Bacillus* sp. strains, the three most clinically relevant biofilm forming microbial species, i.e. *S. aureus*, *P. aeruginosa* and *C. albicans*. Compounds **4b, 4e** and **4f** exhibited anti-biofilm activity on the three *Bacillus* sp. strains, while **4b** was also active against *P. aeruginosa* biofilms. Considering the anti-biofilm activity, the efficiency of the obtained compounds was **4d > 4b > 4a = 4e = 4f > 4c = 4g** (Table IV).

Table IV

Results of the qualitative assay of the biofilm formation of the obtained compounds

Microbial strain / Compound	*Staphylococcus aureus* ATCC 25923	*Staphylococcus aureus* ATCC BA 1026	*Enterococcus faecalis* ATCC 29212	*Bacillus cereus* 53(100)	*Bacillus Licheniformis* 12195	*Bacillus subtilis IC 6633*	*Pseudomonas aeruginosa* ATCC 27853	*Escherichia coli* ATCC 25922	*Candida albicans* ATCC 26790
4a	-	-	+/-	++	++	+	-	+/-	-
4b	-	-	-	++	++	+	+	-	-
4c	-	-	-	++	++	+/-	-	-	-
4d	+	-	-	++	++	+/-	++	-	++
4e	-	-	+/-	++	++	+	-	+/-	-
4f	-	-	-	++	+	++	-	-	-
4g	-	-	-	++	++	+/-		-	-

(-) no anti-biofilm activity; (+) good biofilm inhibition; (++) very good biofilm inhibition; (+/-) weak biofilm inhibition

Conclusions

We have synthesized seven new thioureas derived from 2-thiopheneacetic acid. The new compounds were obtained by the reaction of 2-thienyl-isothiocyanate with various primary aromatic amines.

Their structure was confirmed by spectral analysis (IR, ^{1}H-NMR, ^{13}C-NMR) and by elemental analysis.

The obtained compounds have been characterized by some physico-chemical properties.

It was noted a trend toward precipitation of compounds after adding them in the culture medium used for the MIC value determination, which could suggest the extremely hydrophobic character of these compounds which must be addressed in further studies for the design of potential antimicrobial agents.

The antimicrobial activity tested against Gram-positive and Gram-negative bacteria and fungal strains showed that the tested compounds exhibited specific antimicrobial and anti-biofilm activity.

The compound **4a** proved to be the most active against planktonic bacterial strains as revealed by the low MIC values, whereas **4d** and **4b** exhibited a large spectrum of anti-biofilm activity including the most potent biofilm forming agents, i.e. *S. aureus*, *P. aeruginosa* and *C. albicans*. The new thiourea derivatives could therefore present a promising potential for the development of novel antimicrobial and anti-biofilm agents. An ideal approach will include a combination of anti-biofilm molecules, with an anti-pathogenic effect, active at sub-inhibitory concentrations to reduce the risk of developing resistance and with low toxicity for the host cells.

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