Synthesis, Characterization and Antimicrobial Evaluation of Some New Thioureas derived from 3-thiophenecarboxylic Acid

CARMELLINA DANIELA BADICEANU[1]*, CONSTANTIN DRAGHICI[2], ALEXANDRU-VASILE MISSIR[1], MARIANA CARMEN CHIFIRIUC[3], CAMELIA ELENA STECOZA[1]

[1]University of Medicine and Pharmacy, „Carol Davila" Bucharest, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 6 Traian Vuia, 020956, Romania

[2]Romanian Academy, Organic Chemistry Center „C.D. Nenitescu", 202B Splaiul Independentei, 060023, Bucharest, Romania

[3] University of Bucharest, Faculty of Biology, Department of Microbiology and Immunology, 1-3 Portocalelor Alley, Bucharest, Romania

New thioureas derived from 3-thiophenecarboxylic acid have been synthesized, characterized by their physical properties (melting point, solubility) and structurally elucidated by spectral analysis (^{1}H-NMR, ^{13}C-NMR) and elemental analysis. The new compounds were prepared by the reaction of 3-thenoyl-isothiocyanate with various primary aromatic amines. All the compounds were tested by qualitative and quantitative methods on various bacterial and fungal strains. The assay revealed for all compounds the presence of antifungal activity.

Keywords: thioureas, 3-thiophenecarboxylic acid, antimicrobial activity

According to the literature data, a diversity of biological effects is associated with thiourea derivatives: anticonvulsant [1], analgesic [2, 3], antiviral [4-6], anti-aggregating, antiproliferative, antiarrythmic, anti-hyperlipidemic, local anaesthetic [7], antitubercular [8, 9], antibacterial [fungicidal [10-13]. The thioureas are also used as antitumor agents [14, 15], insecticides [16], plant-growth regulators [17], antiparasitic agents [18].

The remarkable pharmacological importance of the thioureas and the fact that last years have been dominated by an incessant need for the development of new antimicrobial agents, in response to the multiple drug resistance, prompted us to synthesize new derivatives having this structure and to evaluate their antimicrobial activity.

The present paper is a continuation of our researches [19-24] and presents the synthesis, structure confirmation and antimicrobial activity evaluation of some new thiourea derivatives.

Experimental part

All reagents used in this study were purchased from commercial suppliers (Merck, Sigma-Aldrich or Fluka) and used without purification. The necessary liquid amines were dried with potassium hydroxide and afterwards distilled. Acetone and ammonium thiocyanate were dried before use.

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

The elemental analysis was performed on a Perkin Elmer CHNS/O Analyser Series II 2400.

The ^{1}H-NMR spectra were obtained at 300 MHz and the ^{13}C-NMR spectra were recorded at 75.075 MHz with a Varian Gemini 300BB apparatus, using solutions in DMSO-d6 as solvent and tetramethylsilane (TMS) as internal standard. The chemical shifts are expressed in δ ppm values and the coupling constants are in Hertz. The spectra were recorded at room temperature in usual conditions and sometimes Apt and Cosy sequences were used.

In ^{1}H-NMR spectra the splitting patterns are abbreviated as following: s, singlet; bs, broad singlet; d, doublet, bd, broad doublet, dd, double doublet; t, triplet; td, triple doublet; tt, triple triplet.

General synthesis procedure of the new thioureas

A solution of 3-thiophenecarboxylic acid chloride (0.01 mol) (**2**) in dry acetone (15 mL) was added to a solution of ammonium thiocyanate (0.01 mol) in dry acetone (5 mL). The reaction mixture was heated under reflux for one hour, and then cooled to room temperature. The intermediate, 3-thenoyl-isothiocyanate (**3**) was not isolated. A solution of primary aromatic amine (0.01 mol) in dry acetone (5 mL) was added, the mixture was heated under reflux for one hour and afterwards poured into 500 mL cold water. The crude thioureas were separated by filtration and recrystallized from isopropanol.

The necessary 3-thiophenecarboxylic acid chloride (**2**) was obtained from 3-thiophenecarboxylic acid (**1**) and thionyl chloride, as previously described [23].

Analytical and spectral data of the new thioureas are presented in tables 1-3.

Antimicrobial activity assay

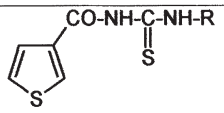
The antimicrobial properties were tested against reference microbial strains belonging to *Staphylococcus aureus, Bacillus subtilis*, *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Candida albicans* species, from Cantacuzino Institute Culture Collection Laboratory.

The qualitative screening was accomplished using the disc 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), as previously described [25].

Results and discussions

The title compounds were prepared by addition of various primary aromatic amines to 3-thenoyl-isothiocyanate (**3**). The intermediate (**3**) was obtained from

* *email: bcarmellina@yahoo.com; Tel 0740149597*



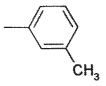
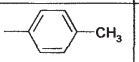
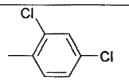
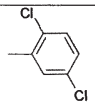
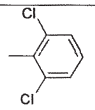
Comp. no.	R	C%		H%		N%		S%	
		t.	e.	t.	e.	t.	e.	t.	e.
4.a		56.50	55.87	4.38	4.33	10.14	10.02	23.20	22.94
4.b		56.50	56.10	4.38	4.34	10.14	10.06	23.20	23.03
4.c		56.50	55.93	4.38	4.33	10.14	10.03	23.20	22.96
4.d		43.51	42.98	2.43	2.40	8.46	8.35	19.36	19.12
4.e		43.51	43.11	2.43	2.40	8.46	8.36	19.36	19.18
4.f		43.51	42.90	2.43	2.39	8.46	8.34	19.36	19.08

Table 1
ELEMENTAL ANALYSIS RESULTS FOR THE NEW COMPOUNDS (t-TEORETICAL; e-EXPERIMENTAL)

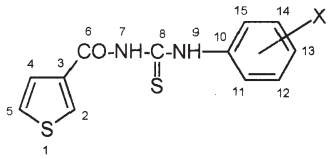


Table 2
^{1}H-NMR DATA FOR THE NEW COMPOUNDS (DMSO-d6, J ppm, J Hz)

No.	X	H-2	H-4	H-5	H-7	H-9	H-11	H-12	H-13	H-14	H-15	X
4.a	11-CH_3	8.76 dd (1.4; 2.7)	7.70 dd (2.6; 5.2)	7.76 dd (1.4; 5.2)	11.82 bs		–	7.18.....7.32 m			7.59 bd (7.8)	2.2 s
4.b	12- CH_3	8.75 dd (1.7; 2.6)	7.70 dd (2.6; 5.2)	7.70 dd (1.7; 5.2)	11.50 bs		7.50 bs	–	7.05 bd (8.0)	7.30 t (8.0)	7.51 bd (8.0)	2.32 s
4.c	13- CH_3	8.76 dd (1.6; 2.5)	7.71 dd (2.5; 5.2)	7.75 dd (5.2; 1.6)	12.10 bs		7.53 d (8.2)	7.20 d (8.2)	–	7.20 d (8.2)	7.53 d (8.2)	2.31 s
4.d	11,13 Cl_2	8.80 dd (1.4; 2.7)	7.71 dd (2.7; 5.2)	7.78 dd (1.4; 5.2)	12.10 bs		–	7.90 d (2.5)	–	7.50 dd (2.5; 8.6)	8.02 d (8.6)	–
4.e	11,14 Cl_2	8.78 dd (2.6; 1.5)	7.70 dd (2.6; 5.2)	7.78 dd (1.5; 5.2)	11.80 bs		–	7.62 d (8.5)	7.40 dd (8.5; 2.5)	–	8.25 d (2.5)	–
4.f	11,15 Cl_2	8.79 dd (2.6; 1.4)	7.71 dd (2.6; 5.2)	7.77 dd (1.4; 5.2)	11.75 s	12.0 s	–	7.55 d (8.0)	7.39 tt (8.0)	7.55 d (8.0)	–	–

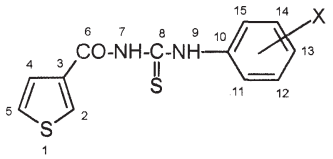
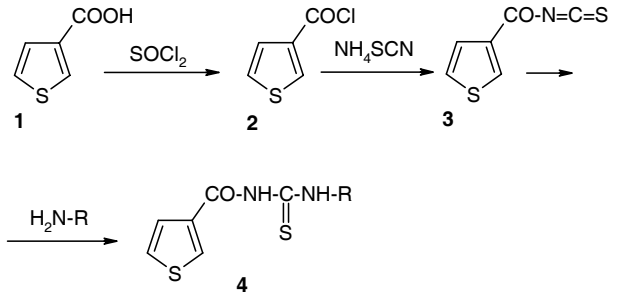


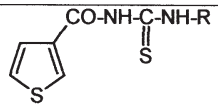
Table 3
^{13}C-NMR DATA FOR THE NEW COMPOUNDS (DMSO-d6, δ ppm)

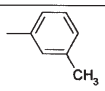
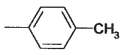
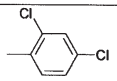
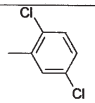
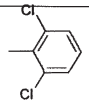
No.	X	C-2	C-3	C-4	C-5	C-6	C-8	C-10	C-11	C-12	C-13	C-14	C-15	X
4.a	11-CH_3	133.56	134.53	127.66	127.42	162.95	179.89	136.87	133.40	130.43	127.08	126.64	126.15	17.66
4.b	12- CH_3	133.56	134.55	127.66	127.46	162.94	178.78	138.16	124.61	137.84	127.00	128.54	121.29	20.97
4.c	13- CH_3	134.55	133.37	127.63	127.41	162.89	178.79	135.66	124.17	129.11	135.37	129.11	124.17	20.62
4.d	11,13 Cl_2	133.97	134.28	127.69	127.52	163.11	180.30	134.70	129.59	129.33	131.47	127.45	129.33	–
4.e	11,14 Cl_2	134.06	134.23	127.71	127.55	163.13	180.09	136.65	131.18	130.84	127.78	134.23	127.24	–
4.f	11,15 Cl_2	133.85	134.31	127.66	127.55	162.87	181.18	133.74	133.85	128.53	129.81	128.53	133.74	–



Scheme 1. The synthesis pathway of the new thioureas

Table 4
CHARACTERIZATION DATA OF THE NEW COMPOUNDS



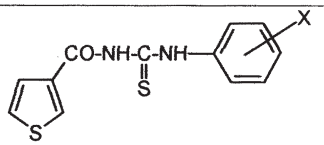
Compound no.	R	Molecular formula	Molecular weight	Melting point (^{0}C) (isopropanol)	Yield (%)
4.a		$C_{13}H_{12}S_2ON_2$	276.38	168.2-169.9	66
4.b		$C_{13}H_{12}S_2ON_2$	276.38	144.6-145.7	65
4.c		$C_{13}H_{12}S_2ON_2$	276.38	161.1-162.7	32
4.d		$C_{12}H_8S_2ON_2Cl_2$	331.24	181.2-18.6	70
4.e		$C_{12}H_8S_2ON_2Cl_2$	331.24	197.2-198.7	71
4.f		$C_{12}H_8S_2ON_2Cl_2$	331.24	191.4-192.8	69

3-thiophenecarboxylic acid (**1**) via 3-thiophenecarboxylic acid chloride (**2**). The general synthesis pathway of the new compounds is depicted in scheme 1.

The new compounds (**4a-f**) are solid, crystallized, white or light yellow, soluble at room temperature in chloroform and acetone and by heating in inferior alcohols, benzene, toluene and xylene, insoluble in water.

The structure, molecular formula, molecular weight, melting point and yield of the new thioureas are presented in table 4.

Structural elucidation of the new compounds was performed by spectral analysis (^{1}H-NMR, ^{13}C-NMR) and elemental analysis. All elemental analyses results were within ±0.4% of the theoretical values, and the ^{1}H - NMR and ^{13}C-NMR spectra show all the expected signals.



Compound no.	X	*K. pneumoniae IC 13420*	*E.coli IC 13529*	*S.aureus IC 13204*	*P.aeruginosa IC 13202*	*B. subtilis IC 6633*	*C.albicans IC 249*
4.a	-2CH_3	125	125	125	62.5	125	31.2
4.b	-3CH_3	125	125	125	62.5	125	62.5
4.c	-4CH_3	125	125	125	62.5	125	62.5
4.d	-2,4Cl_2	125	125	125	62.5	125	125
4.e	-2,5Cl_2	125	125	125	62.5	125	62.5
4.f	-2,6 Cl_2	125	125	125	62.5	125	15.6

Table 5
THE MINIMAL INHIBITORY CONCENTRATION (MIC VALUES) (μg/mL)

In the quantitative assay the minimal inhibitory concentration was read by wells observations. In the first wells containing high concentrations of compounds the culture growth was not visible, the microbial cells being killed or inhibited by the tested compound.

The lowest concentration which inhibited the visible microbial growth represents the MIC (μg/mL) value for the tested compound.

The quantitative assay results of the new thioureas are presented in table 5.

Based on the literature data, we considered a strong antimicrobial effect for MICs ranging between 15.6 μg/mL and 62.6 μg/mL, while a MIC of 250 μg/mL concentration represented a moderate effect.

Our results showed that the tested compounds exhibited specific antimicrobial activity, both on Gram-positive, Gram-negative bacteria and fungi, the highest activity being noticed against fungal strains *(Candida albicans)*. The tested compounds presented an antifungal activity at concentrations from 125 to 15.6 μg/ mL. The most active compound proved to be N-(2,6-dichlorophenyl)-N'-(3-thenoyl)-thiourea (**4.f**).

Conclusions

We have synthesized new thioureas derived from 3-thiophenecarboxilic acid. The target compounds were obtained by reaction of 3-thenoyl-isothiocyanate with various primary aromatic amines. All the new derivatives were characterized by their physical properties (melting point, solubility) and their structure was confirmed by spectral analysis (^{1}H-NMR, ^{13}C-NMR) and elemental analysis.

The new thioureas were screened for their *in vitro* antibacterial activity against Gram-positive, Gram-negative bacteria as well as fungi, the highest activity being noticed against fungal strains *(Candida albicans)*.

References

1. CELEN A.Ö., KOÇYI IT-KAYMAKÇIO LU B., GÜMRÜ S., TOKLU H.Z., ARICIO LU F.,
Marmara Pharmaceutical Journal, 15, 2011, p. 43
2. PARK, H.-G., PARK, M.-K., CHOI, J.-Y., CHOI, S.- H, LEE, J., PARK, B.-S., KIM, M. G., SUH, Y. -G., CHO, H., OH, U., LEE, J., KIM, H.-D., PARK, Y. –H., KOH, H. –J., LIM, K.M., MOH, J. –H., JEW, S.-S, Bioorg. Med. Chem. Lett., 13, nr. 4, 2003, p. 601
3. YAHYAZADEH A., GHASEMI Z., Eur. Chem. Bull., 2, nr. 8, 2013, p. 573
4. LIU J, YANG S, LI X, FAN H, BHADURY P, XU W, WU J, WANG Z., Molecules, 15, nr. 8, 2010, p. 5112
5. PATEL, R., B., CHIKHALIA, K., H., PANNECOUQUE, C., DE CLERCQ, E., J. Chem. Soc., 18, nr. 2, 2007, p. 312
6. SUN, C., HUANG, H., FENG, M., SHI, X., ZHANG, X., ZHOU, P., Bioorg. Med. Chem. Lett., 16, nr. 1, 2006, p. 162
7. RANISE A., SPALLAROSSA A., BRUNO O., SCHENONE S., FOSSA P., MENOZZI G., BONDAVALLI F., MOSTI L., CAPUANO A., MAZZEO F., FALCONE G., FILIPPELLI W., Farmaco., 58, nr. 9, 2003, 765
8. ÇIKLA, P.,Ţ. KÜÇÜKGÜZEL,G., KÜÇÜKGÜZEL, Ý., ROLLAS, S., CLERCQ, E., PANNECOUQUE, C., ANDREI, G., SNOECK, R., ŢAHIN, F., BAYRAK, ÖF., Marmara Pharmaceutical Journal, 14, nr. 1, 2010, p. 13
9. DHARMARAJAN S., PERUMAL, Y., MURUGESAN, D., RATHINASABABATHY, J., Antimicrob. Chemoter., 59, nr. 6, 2007, p. 1194
10. STRUGA M., ROSOLOWSKI S., KOSSAKOWSKI J., STEFANSKA J., Arch Pharm Res., 33, nr. 1, 2010, p. 47
11. LIMBAN C., MISSIR AL. V., CHIRIŢĂ I.C., NEAGU A.F., DRĂGHICI C., CHIFIRIUC M.C., Rev. Chim. (Bucharest), **62**, no. 2, 2011, p. 168
12. SAEED S., RASHID N., ALI M., HUSSAIN R., JONES P., Eur. J. Chem., 1, nr. 3, 2010, p. 221
13. SAEED, S., RASHID, N., JONES, P., G., HUSSAIN, R., BHATTI, M., H., Cent. Eur. J. Chem., 8, nr. 3, 2010, p. 50
14. HERNANDEZ, W., SPODINE, E., BEYER, L., SCHRODER, U., RICHTER, R., FERREIRA, J., PAVANI, M., Bioinorg. Chem. Appl., 3, nr. 3-4, 2005, p. 299
15. KOCA Ý. , ÖZGÜR A., COŞKUN K.A., TUTAR Y., Bioorganic & Medicinal Chemistry, 21, nr. 13, 2013, p. 3859
16. PAUL, A., HARRINGTON, L., C., SCOTT, J.,G., J. Med. Entomol., 43, nr. 1, 2006, p. 55
17. KUMAR, S., AWASTHI, V., KANAWAR, J., K., Hort. Sci. (Prague), 34, nr. 2, 2007, p. 77
18. MISHRA, A., SRIVASTAVA, K., TRIPATHI, R., PURI, S., K., BATRA, S., Eur. J. Med. Chem., 44, nr. 11, 2009, p. 4404
19. BĂDICEANU C. D., MISSIR AL., Rev. Roum. Chim., 54, nr. 1, 2009, p. 27
20. BĂDICEANU C. D, MISSIR AL., Farmacia, 57, nr. 3, 2009, p. 339
21. BĂDICEANU C. D, MISSIR AL., DRĂGHICI C., LARION C., Farmacia, 57, nr. 6, 2009, p. 771
22. BĂDICEANU C. D., LARION C., Farmacia, 57, nr. 4, 2009, p. 473
23. BĂDICEANU C. D, MISSIR AL., Farmacia, 55, nr. 6, 2007, p. 710
24. BĂDICEANU C. D, MISSIR AL, Farmacia, 55, nr. 4, 2007, p. 416
25. STECOZA C. E., CĂPROIU M. T., DRĂGHICI C., CHIFIRIUC M. C., DRĂCEA N. O., Rev. Chim. (Bucharest), **60**, no. 2, 2009, p. 137

Manuscript received: 29.07.2013