

NEW THIOUREIDES OF 2-THIOPHENEACETIC ACID WITH POTENTIAL PHARMACOLOGICAL ACTIVITY. NOTE 2.

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Abstract

In this paper we present the synthesis of new thioureides from 2-thiopheneacetic acid performed by the addition of various primary aromatic amines to the 2-thienyl isothiocyanate. The best reaction conditions in order to obtain with good yields high pure compounds were established. The chemical structure and the purity of the new compounds were confirmed by ¹H-NMR and ¹³C-NMR spectra. The obtained substances were tested by qualitative and quantitative methods on various microbial and fungal strains.

Rezumat

În această lucrare prezentăm sinteza unor noi tioureide ale acidului 2-tiofenacetic obținute în urma adității diferitelor amine aromatice primare la isotiocianatul de 2-tienil. Au fost stabilite condițiile optime de reacție cu scopul de a obține compuși puri și cu randamente cât mai mari. Structura chimică și puritatea noilor compuși a fost confirmată prin spectrometrie ¹H-RMN și ¹³C-RMN. Compușii obținuți au fost testați prin metode calitative și cantitative pe diferite tulpini microbiene și fungice.

Keywords thiourea derivatives; 2-thiopheneacetic acid

Introduction

The present application is a continuation-in-part of our research concerning the synthesis and characterization of new thioureides from 2-thiopheneacetic acid [8, 9]. The remarkable pharmacological properties of some thiourea derivatives are well known such as: anthelmintic (nematodes), antidepressant, anticonvulsant, antihistaminic, anesthetic (local), antitussive, analgesic action [1, 2]. Their low side effects and the positive results of our previous synthesized thioureides, led us to obtain new thiourea derivatives from 2-thiopheneacetic acid.

In our previous papers we presented the synthesis, the structural proving and we did some research on the antimicrobial action of some

thioureides from 2-thiophenecarboxylic acid, 3-thiophenecarboxylic acid and 2-thiopheneacetic acid [4-9]. The satisfying results determined us to continue this research resulting in the obtain of 2-thiopheneacetic acid thioureides. The general synthesis method used was the addition of some primary aromatic amines to the 2-thienyl-isothiocyanate, putting into practice the method used by G. J. Durant and col.[3]. The chemical structures were confirmed by ^1H -NMR and ^{13}C -NMR spectral analysis.

A qualitative screening assay was made on this new 2-thiopheneacetic 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.

Materials and methods

According to the literature data [1, 2], the thiourea derivatives can be highly active and the preliminary positive results determined us to continue our research and to obtain new 2-thiopheneacetic acid thioureides.

The synthesis of the new compounds was carried out in two steps.

The first stage was the synthesis of 2-thiopheneacetic acid chloride (**2**) by treating the 2-thiopheneacetic acid (**1**) with thionyl chloride. The acid chloride was used for the next step of the synthesis in crude status, after thionyl excess was removed under reduced pressure.

In the second stage of our synthesis the 2-thiopheneacetic acid chloride was treated with ammonium thiocyanate which was priory dried at 100°C . The 2-thienyl isothiocyanate (**3**) resulted after refluxing the reaction mixture for one hour in dry acetone.

The isothiocyanate was not isolated, the new thioureides (**4**) were obtained by adding the necessary amines. The aromatic amines were Merck or Fulka products, dried on potassium hydroxide and freshly distilled. The reaction mixture was refluxed, under continuous stirring, for one hour.

The reaction scheme is presented in the Fig. 1.

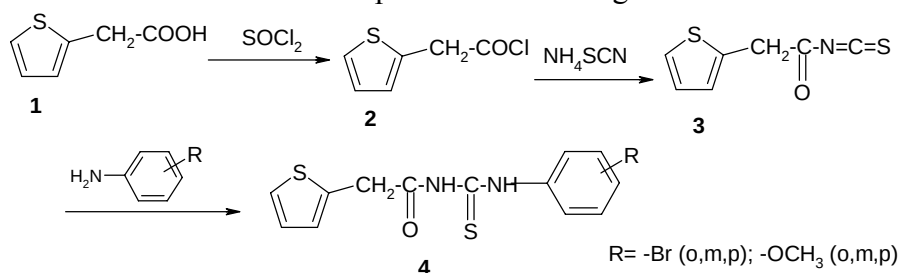


Figure 1

The synthesis of the new thioureides (**4**)

The 2-thiopheneacetic acid chloride synthesis

0.0070 mol (1 g) 2-thiopheneacetic acid and 0.2064 mol (15 mL, 24.57 g) thionyl chloride were placed in a round-bottom flask equipped with condenser and drying tube. The mixture was refluxed for 3 hours. The thionyl chloride excess was removed at reduced pressure. For the next step, the 2-thiopheneacetic acid chloride was used in the crude status.

General procedure for the synthesis of the new thioureaides

A solution of 2-thiopheneacetic acid chloride (0.01 mol) in 15 mL dry acetone was added into a solution of ammonium thiocyanate (0.01 mol) in 5 mL dry acetone. Previously, the acetone was dried over potassium carbonate and the ammonium thiocyanate by heating at 100°C. 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 primary aromatic amine dissolved in 2 mL dry acetone were added to the reaction mixture while stirring. The mixture was afterwards refluxed for one hour. The product precipitated after the cooled reaction mixture was poured into 500 mL water. The raw obtained thioureaides were recrystallised from isopropanol with charcoal.

Antimicrobial and antifungal activity assay

The testing of the antimicrobial and antifungal activity of the new thioureaides 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:

- method 1: the technique of paper filter disks impregnated with the testing compounds in DMF solution,
- method 2: the testing of the compounds antimicrobial activity by agar well-plates method,
- method 3: qualitative antimicrobial effect assay of the compound spot placed in bacterial inoculums seeded medium.

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).[10, 11]

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°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 performed (there were obtained concentrations from 250 µg/mL to 7.8 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 37°C.

For the technique 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 than 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 strain.

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 inhibited by the compound. The smallest concentration of the well that produces a visible microbial culture growth

inhibition represents the MIC (minimal inhibitory concentration) ($\mu\text{g/mL}$) 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.

Results and discussion

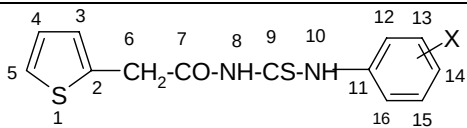
These new compounds are well crystallized, having white or light-yellow colour; they are soluble at normal temperature in acetone and chloroform and by heating in inferior alcohols, benzene, toluene and xylene, insoluble in water.

The melting points were established in glass capillary tubes on Electrothermal 9100 apparatus, verified with a Boetius apparatus and are uncorrected.

The structure, molecular formula, molecular weight, melting point and yield of the new thioureides are given in Table I:

Table I

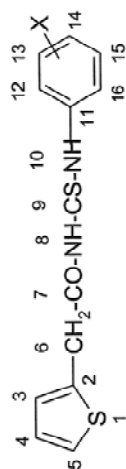
The new compounds characteristics

					
Comp. no.	X	Molecular formula	Molecular weight (g/mol)	Melting point ($^{\circ}\text{C}$) (isopropanol)	Yield (%)
4a	12-Br	$\text{C}_{13}\text{H}_{11}\text{S}_2\text{ON}_2\text{Br}$	355.2	$113^{\circ}\text{-}115^{\circ}\text{C}$	41
4b	13-Br	$\text{C}_{13}\text{H}_{11}\text{S}_2\text{ON}_2\text{Br}$	355.2	$124^{\circ}\text{-}127^{\circ}\text{C}$	46
4c	14-Br	$\text{C}_{13}\text{H}_{11}\text{S}_2\text{ON}_2\text{Br}$	355.2	$148^{\circ}\text{-}151^{\circ}\text{C}$	37
4d	12- OCH_3	$\text{C}_{14}\text{H}_{14}\text{S}_2\text{O}_2\text{N}_2$	306.4	$175^{\circ}\text{-}177^{\circ}\text{C}$	36
4e	13- OCH_3	$\text{C}_{14}\text{H}_{14}\text{S}_2\text{O}_2\text{N}_2$	306.4	$114^{\circ}\text{-}116^{\circ}\text{C}$	44
4f	14- OCH_3	$\text{C}_{14}\text{H}_{14}\text{S}_2\text{O}_2\text{N}_2$	306.4	$154^{\circ}\text{-}157^{\circ}\text{C}$	48

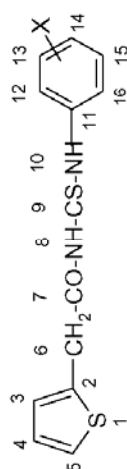
Spectral data

The NMR spectra were recorded on a Gemini 300BB instrument, at room temperature, operating at 300MHz for ^1H 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.

The most important ^1H -NMR and ^{13}C -NMR spectral values (DMSO-d_6 , δ ppm, JHz) for the new compounds are presented in the Tables II and III.

Table II. ¹H-NMR (DMSO-d₆, δppm, JHz)

Comp no.	X	H-3	H-4	H-5	H-6	H-8	H-10	H-12	H-13	H-14	H-15	H-16
4a	12-Br	7.03 m	7.03 m	7.41 dd (1.8;5.2)	4.05 s	11.88 s	12.21 s	—	7.70 dd (1.7; 8.2)	7.21 td (1.7;8.0)	7.40 td (1.4; 8.0)	7.84 dd (1.4;8.0)
4b	13-Br	7.02 m	7.02 m	7.42 dd (1.8;5.3)	4.05 s	11.78 s	12.31 s	7.96 sl	—	7.40 dl (8.0)	7.36 t (8.0)	7.53 dl (8.0)
4c	14-Br	7.02 m	7.02 m	7.42 dd (1.7;5.2)	4.05 s	11.75 s	12.27 s	7.58 s	7.58 s	—	7.58 s	7.58 s
4d	12-OMe 3.83s	7.02 m	7.02 m	7.45 dd (1.7;5.0)	4.05 s	11.67 s	12.40 s	—	7.11 dd (1.7;8.2)	7.21 td (1.7; 8.2)	6.98 td (1.7; 8.2)	7.50 dd (1.7; 8.2)
4e	13-OMe 3.75s	7.02 m	7.02 m	7.43 dd (1.7;5.3)	4.05 s	11.70 s	12.35 s	7.35 t (2.5)	—	6.81 ddd (1.3; 2.5; 8.3)	7.28 t (8.3)	7.16 dd (1.3;8.0)
4f	14-OMe 3.75s	7.03 m	7.03 m	7.41 dd (1.8;5.2)	4.04 s	11.64 s	12.15 s	7.48 d (9.1)	6.92 d (9.1)	—	6.92 d (9.1)	7.48 d (9.1)

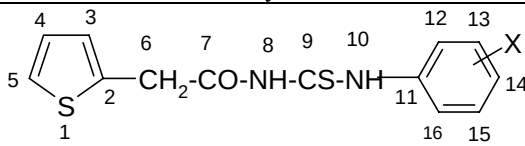
Table III. ^{13}C -NMR (DMSO- d_6 , δ ppm)

Comp. no.	X	C-2	C-3	C-4	C-5	C-6	C-7	C-9	C-11	C-12	C-13	C-14	C-15	C-16
4a	12-Br	135.30	127.73	127.25	126.09	36.94	172.33	180.34	136.97	119.61	133.00	129.01	128.19	129.08
4b	13-Br	135.28	127.71	127.22	126.07	37.04	172.16	179.48	139.66	127.35	121.31	129.45	130.87	124.00
4c	14-Br	134.91	127.29	126.82	125.06	36.64	171.76	178.93	137.12	126.52	131.44	118.59	131.44	126.52
4d	12-OMe 56.33	135.45	127.71	127.03	126.04	37.00	172.17	177.96	127.02	150.87	111.64	127.27	120.10	123.43
4e	13-OMe 55.18	134.96	127.30	126.83	125.65	36.64	171.90	178.50	138.74	109.62	159.27	111.81	129.41	116.18
4f	14-OMe 55.24	135.04	127.25	126.82	125.62	36.64	171.77	178.84	130.54	125.96	113.74	157.43	113.74	125.90

The most efficient qualitative method was the antimicrobial effect assay of the compound spot placed in bacterial inoculums seeded medium, the results being very good correlated with the quantitative assay results.

In the Table IV are presented the results obtained from the quantitative assay of the antimicrobial and antifungal activity of the new compounds. The tested compounds presented an antimicrobial activity at concentrations from 250 to 7.8 µg/mL.

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

							
Comp. No.	R	<i>K. pneumoniae</i> IC 13420	<i>E. coli</i> IC 13529	<i>S. aureus</i> IC 13204	<i>P. aeruginosa</i> ATCC 27853	<i>B. subtilis</i> ATCC 6633	<i>C. albicans</i> IC 249
4a	12-Br	125	125	250	125	125	15.6
4b	13-Br	125	125	250	125	125	7.8
4c	14-Br	125	125	250	125	125	7.8
4d	12-OCH ₃	125	125	250	125	125	62.5
4e	13-OCH ₃	125	125	250	125	125	31
4f	14-OCH ₃	125	125	250	125	125	62.5
Blank	DMSO	125	125	250	62.5	125	62.5

It's worth to notice the antimicrobial activity of the tested compounds against *Candida albicans* which can represent new therapeutical options in the treatment of this infection.

Conclusions

Continuing our research in the antimicrobial substances field we synthesized six new compounds, thioureides of the 2-thiopheneacetic acid. The synthesis of the new thioureides was completed through the condensation of 2-thienyl isothiocyanates with different primary aromatic amines in anhydrous acetone. The obtained compounds have been characterized by some physical properties. The chemical structure of the synthesized compounds has been confirmed by NMR spectroscopy. The

obtained thioureides were investigated to determinate their antimicrobial activity and proved to be active on *Candida albicans*.

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