ORIGINAL ARTICLE

PHYSICOCHEMICAL INVESTIGATIONS ON SOME 2-PHENETHYLBENZOYL THIOUREA DERIVATIVES

TAMARA COTELEA[1], GEORGE MIHAI NIŢULESCU[2]*, PETUHOV OLEG[3], LAURENŢIU MORUŞCIAG[2]

[1] *"Nicolae Testemiţanu" State University of Medicine and Pharmacy, Faculty of Pharmacy, 165 Stefan cel Mare si Sfint, Chisinau, Republic of Moldova*
[2] *"Carol Davila" University of Medicine and Pharmacy, Faculty of Pharmacy, 6 Traian Vuia, 020956, Bucharest, Romania*
[3] *Institute of Chemistry of Academy of Sciences of Moldova, 3 Academiei, Chisinau, Republic of Moldova*

**corresponding author: nitulescu_mihai@yahoo.com*

Manuscript received: March 2014

Abstract

Several physicochemical methods are proposed for the analysis of some potential antituberculosis derivatives of phenetylbenzoyl thiourea. Spectrophotometric parameters were selected in order to identify the compounds using high performance liquid chromatography (HPLC) methods. The thermogravimetric method (TGA) and differential scanning calorimetry (DSC) were used to determine the thermal stability of 2-(2-phenethyl)-benzoic acid thioureides and the method's utility was demonstrated.

Rezumat

Studiul propune o serie de metode fizico-chimice de analiză a unor derivaţi de 2-fenetilbenzoil-tiouree cu potenţial antituberculos. Au fost selectaţi parametrii spectrofotometrici care au permis identificarea compuşilor utilizând o metodă cromatografică de înaltă performanţă (HPLC). S-a aplicat şi s-a demonstrat utilitatea metodei termogravimetrice (TGA) şi calorimetriei diferenţiale prin scanare (DSC) pentru determinarea stabilităţii termice a tioureidelor acidului 2-(2-fenetil)-benzoic.

Keywords: differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), antituberculosis

Introduction

Thiourea derivatives exhibit a large spectrum of biological activities and have important potential applications. Several studies were performed and reported the valuable antiparasitic effects on *Trypanosoma* [2], on intestinal nematodes [3] and on *Plasmodium* species [8]. Some acylthiourea derivatives [4], as well as their metal complexes [13], have good antifungal properties. The most important application of the thiourea acyl derivatives is their antimicrobial activity against pathogenic bacteria. A large range of structural parameters were modulated and several series of compounds were synthesized and tested using aromatic [3, 4, 11] or heterocyclic acids [12]. The best results were observed for the compounds bearing halogens in their molecule [7].

Isoxyl is a thiourea derivative used in the treatment of tuberculosis, with a considerable *in vitro* activity being effective against multi-drug resistant strains of *Mycobacterium tuberculosis* inhibiting the synthesis of oleic, tuberculostearic and mycolic acids [14]. Using the structural pattern of this molecule and using our previous results with phenoxymethylbenzoic acid derivatives [3], we synthesized several phenethylbenzoyl thiourea derivatives as potential antituberculosis drugs [9, 10].

The objective of this research is the study of the newly synthesized compounds in order to develop identification, qualitative and quantitative analysis methods in order to obtain new more efficient remedies for the treatment of multidrug-resistant tuberculosis.

Materials and Methods

This research was focused on the complex physicochemical investigations of N-(2-phenethylbenzoyl)-N-(3,5-dichlorophenyl)-thiourea (a), N-(2-phenethylbenzoyl)-N-(3,4,5-trifluorophenyl)-thiourea (b) and N-(2-phenethylbenzoyl)-N-(2,4,6-trifluorophenyl)-thiourea (c). The compounds were obtained starting from phenethylbenzoic acid that was transformed in the acid chloride, which was condensed with ammonium thiocyanate to form 2-phenethylbenzoyl isothiocyanate and then the suitable substituted anilines were added. The details of the synthesis are mentioned in previous papers [9, 10]. All starting materials and solvents were purchased from common commercial suppliers and used without purification, unless otherwise noted.

Drugs characterization and analysis require various physical and chemical methods. Spectrophotometry allows an analytical study of medicinal substances in solution, being used both for identification, structures' validation and for equilibrium studies. UV spectra of the studied substances were recorded on a Perkin Elmer Lambda 25 UV/Vis Spectrometer, in the range 220 - 400 nm, on compounds acetonitrile solutions having a concentration of 16.67 mg/L.

A high performance liquid chromatography (HPLC) method was developed for the identification and separation of the new thiourea compounds. The chromatographic conditions were optimized to provide the maximum selectivity. The chromatographic analysis was carried out on a Jasco apparatus equipped with an UV detector. Chromatographic analysis conditions: UPTISPHERE C18-ODB 3UM 150X4.6mm column, temperature 30°C, the mobile phase consisted of acetonitrile-water (87:13, v/v) and the flow-rate was 1.0 mL/min, using UV detection at 272 nm. The column was injected with 10 µL of compounds acetonitrile solution using concentrations of 10 mg/L for the determination of the substance, and of 500 mg/L for the determination of impurities. The basic HPLC method was optimized for the identification of 2-(2-phenethyl)-benzoic acid as expected impurity.

Thermal analysis is applied extensively in the research of the pharmaceutical products to establish purity, to identify polymorphic structures [16], to measure residual solvents and moisture [3], to assess thermodynamic constants and predict stability [15]. Kinetic degradation of the compounds was measured as the temperature dependence of weight loss on a Mettler-Toledo TGA/SDTA 851e thermo-gravimetric analyzer. Samples of 5 mg were placed in standard 40 µL aluminum crucibles and heated from 25°C to 700°C with a temperature raising speed of 10°C/min and a nitrogen flow rate of 70 mL/min.

Results and Discussion

Following the aforementioned synthesis procedure, we obtained the desired N-(2-phenethylbenzoyl)-thiourea derivatives (a-c), that were characterized by NMR and IR spectra in our previous papers [9, 10]. The compounds were analysed using UV spectrophotometry, in order to develop the optimal HPLC method. In the following figure there are presented the compounds spectra (Figure 1).

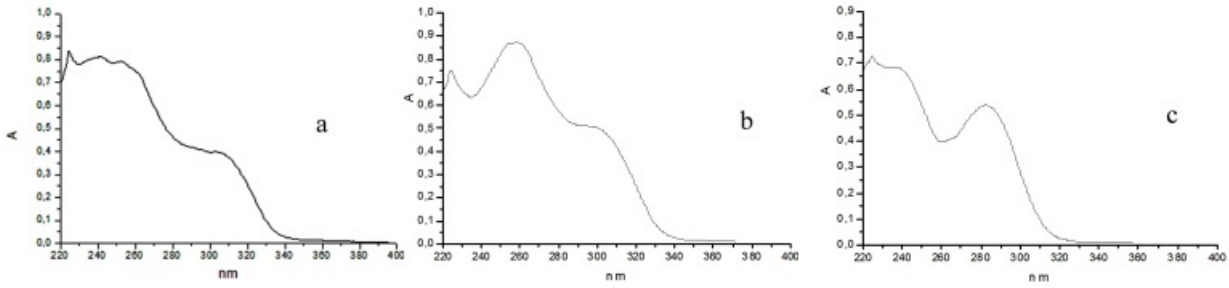

Figure 1.
Compounds UV spectra. N-(2-phenethylbenzoyl)-N-(3,5-dichlorophenyl)-thiourea (a), N-(2-phenethylbenzoyl)-N-(3,4,5-trifluorophenyl)-thiourea (b) and N-(2-phenethylbenzoyl)-N-(2,4,6-trifluorophenyl)-thiourea (c)

A HPLC method for the identification of the compounds was established and the purity of the compounds was therefore validated. In the Figure 2 are presented compounds chromatograms.

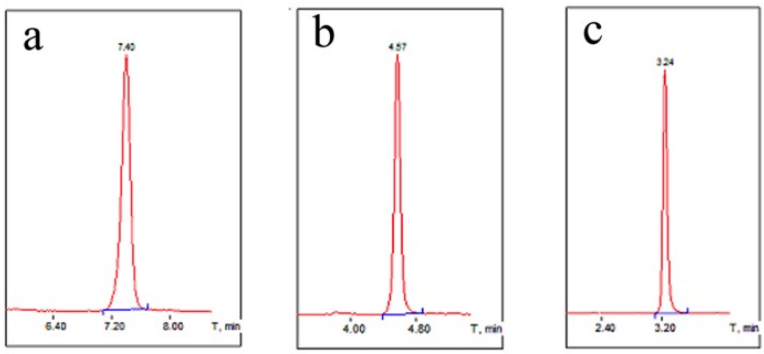

Figure 2.
Compounds (a-c) chromatograms

The N-(2-phenethylbenzoyl)-N-(3,5-dichlorophenyl)-thiourea (a) degradates totally near 600°C and is more stable than its fluoro congeners, b and c, for which the decomposition is complete at 350°C.

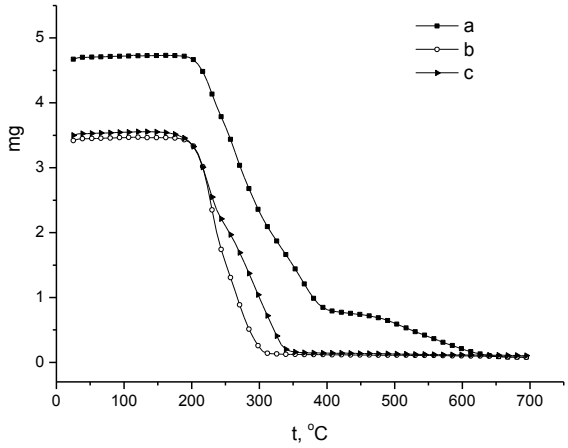

Figure 3.
Weight loss variation over temperature for compounds (a-c)

The 1st derivative curve of the TGA is displayed in Figure 4 and shows the inflection points that indicate the greatest rate of change on the weight loss curve and the number of distinct thermal events.

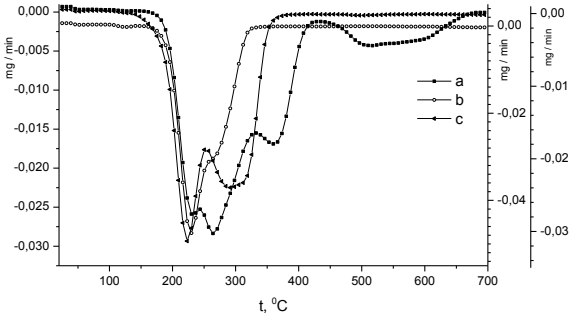

Figure 4.
Compounds (a-c) TGA curves

The analysis of the TGA and DSC curves shows different patterns of degradation for the new thiourea derivatives. The compounds b and c have two inflection points corresponding to a two stage degradation process, whereas compound a is degradated in four phases. In the Table I are presented the mass losses for each compound depending on temperature.

Table I
Compounds weight loss for each temperature interval

Compound	Temperature range/weight loss			
a	206-291°C 49.64%	291-389°C 35.67%	389-481°C -	481-632°C 14.21%
b	196-235°C 52.16%	235-297°C 45.69%	-	-
c	194-232°C 38.06%	259-330°C 59.48%	-	-

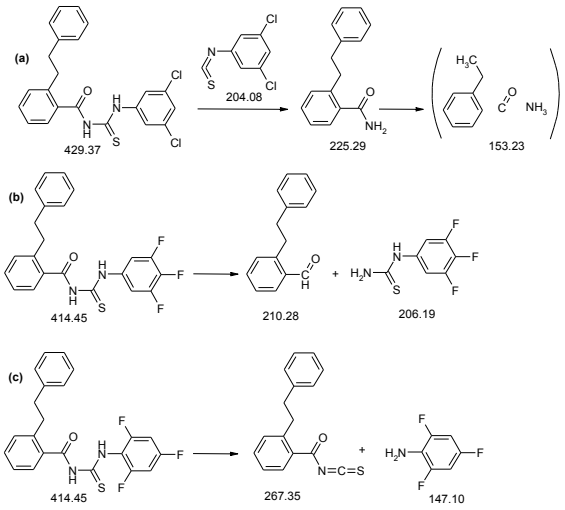

Figure 5.
Thermic degradation mechanism for the new compounds (a-c)

The structure analysis of the data presented in the above table can provide the decomposition reaction mechanism for each thiourea derivative. Compound a suffers a first reaction of degradation in the range of 206-291°C resulting in 2-phenethylbenzamide that afterwards is decomposed in ammonia, carbon monoxide and ethylbenzene. The compounds b and c are degradated approximately at the same temperature, but in different compounds. In the case of the compound b the amide bound is broken resulting in 3,4,5-trifluorophenylthiourea, whereas the compound **c** suffers the reverse process of the synthesis reaction. This difference can be explained by the steric effect of the two ortho fluor atoms that hinders the formation of the internal hydrogen bond in the compound c. The following figure presents the thermic degradation pattern for each compound.

Conclusions

We obtained a series of potential antituberculosis substances, derivatives of N-(2-phenethylbenzoyl)-thiourea, and complex physicochemical investigations were performed to assess their purity and stability. A HPLC method was developed for the identification and separation of the new

compounds. The stability of the compounds and their degradation profile were studied using thermal analyses.

Acknowledgements

The authors acknowledge the financial support offered by the National Authority for Scientific Research of Romania, through Romania - Republic of Moldova joint collaboration 419/03.06.2010 and by the Academy of Sciences of Moldova, project 10.820.09.12 RoA.

References.

1. Dranca I., Vyazovkin S., Thermal Stability of Gels: Effect of Preparation conditions on activation energy barrier to melting. *Polymer*, 2009; 50: 4859-4867.
2. Du X., Hansell E., Engel J.C., Caffrey C.R., Cohen F.E., McKerrow J.H., Aryl ureas represent a new class of anti-trypanosomal agents. *Chem. Biol.*, 2000; 7: 733-742.
3. Duan L.P., Xue J., Xu L.L., Zhang H.B., Synthesis 1-acyl-3-(2-aminophenyl) thioureas as anti-intestinal nematode prodrugs. *Molecules*, 2010; 15: 6941-6947.
4. Kulakov I.V., Nurkenov O.A., Akhmetova S.B., Seidakhmetova R.B., Zhambekov Z.M., Synthesis and antibacterial and antifungal activities of thiourea derivatives of the alkaloid anabasine. *Pharm. Chem. J.*, 2011; 45: 15-18.
5. Limban C., Missir A.V., Chiriţă I.C., Nițulescu G.M., Ilie C., Căproiu M.T., The synthesis and characterization of some new thioureides of 2-(4-methyl-phenoxymethyl)benzoic acid with antimicrobial activity. *Revista de Chimie*, 2008; 59(11): 1245-1248.
6. Limban C., Missir A.V., Chiriţă I.C., Nițulescu G.M., Căproiu M.T., Chifiriuc M.C., Israil A.M., Synthesis and antimicrobial properties of new 2-((4-ethylphenoxy)methyl)benzoylthioureas. *Chem. Pap.*, 2011; 65(1): 60-69.
7. Limban C., Missir A.V., Chiriţă I.C., Neagu A.F., Drăghici C., Chifiriuc M.C., Synthesis and antimicrobial evaluation of some new 2-(4-fluoro-phenoxymethyl) benzoic acid thioureides. *Revista de Chimie*, 2011; 62: 168-173.
8. Mishra A., Srivastava K., Tripathi R., Puri S.K., Batra S., Search for new pharmacophores for antimalarial activity. Part III: Synthesis and bioevaluation of new 6-thioureido-4-anilino-quinazolines. *Eur. J. Med. Chem.*, 2009; 44: 4404-4412.
9. Missir A.V., Moruşciag L., Nițulescu G.M., Chiriţă I.C., Căproiu M.T., Drăghici C., New thioureides of the 2-phenethylbenzoic acid with potential antimicrobial activity. VI. *Revista de Chimie*, 2011; 62(4): 365-370.
10. Missir A.V., Moruşciag L., Nițulescu G.M., Chiriţă I.C., Căproiu M.T., New thioureides of the 2-phenetylbenzoic acid with potential antimicrobial activity. IV. *Revista de Chimie*, 2010; 61(11): 1024-1027.
11. Moruşciag L., Missir A.V., Ilie C., Guţă R., Nănău-Andreescu D., New thioureides of the 2-phenetylbenzoic acid with potential antimicrobial activity. *Revista de Chimie*, 2009; 60(8): 805-809.
12. Ciolan D.F., Lupuleasa D., Synthesis of new thioureas derived from 2-(3,4-dimethyl-phenoxy-methyl)- and 2-(2,3-dimethyl-phenoxymethyl)-benzoic acid with potential antimicrobial activity. *Farmacia*, 2013; 61(5): 1018-1026.
13. Nural Y., Kilincarslan R., Dondas H.A., Cetinkaya B., Serin M.S., Grigg R., Ince T., Kilner C., Synthesis of Ni(II), Pd(II) and Cu(II) metal complexes of novel highly functionalized aroylaminocarbo-N-thioyl pyrrolidines and their activity against fungi and yeast. *Polyhedron*, 2009; 28: 2847-2854.
14. Phetsuksiri B., Jackson M., Scherman H., McNeil M., Besra G.S., Baulard A.R., Slayden R.A., DeBarber A.E., Barry C.E.3rd, Baird M.S., Crick D.C., Brennan P.J., Unique mechanism of action of the thiourea drug isoxyl on *Mycobacterium tuberculosis*. *J. Biol. Chem.*, 2003; 278(52): 53123-53130.
15. Bădiceanu C.D., Dimcevici Poesina N., Missir A.V., Stecoza C.E., Dinu M., The phytobiological testing of some new thiourea derivatives. *Farmacia*, 2014; 62(1): 23-33.
16. Urakami K., Characterization of pharmaceutical polymorphs by isothermal calorimetry. *Curr. Pharm. Biotechnol.*, 2005; 6(3): 193-203.