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Antimicrobial Activity and Structural Study of Disubstituted Thiourea Derivatives

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Summary. The antimicrobial activity of six *N*-phenyl- and fourteen *N*-benzoylthiourea derivatives were evaluated from their Minimal Inhibitory Concentration (*MIC*) values using the microdilution procedure against ten microorganisms. Most of the compounds exhibited selective activity against fungi and *Gram*-positive bacteria, which were very effectively inhibited by some of the tested thioureas. Additionally, *SAR* considerations and four novel X-ray diffraction structures of *N*-benzoylthioureas are included.

Keywords. Benzoylthioureas; Phenylthioureas; X-ray structure.

Introduction

The search for compounds with antimicrobial activity is a theme of continuous interest because of the increased antimicrobial resistance developed by important pathogens [1]. The emergence of multidrug-resistant strains of *Gram*-positive bacterial pathogens is a problem of ever increasing significance.

Thiourea derivatives have attracted the attention of several research groups due to their potential in medicinal chemistry [2–8]. The broad spectra of biological activity of thiourea derivatives and their metal complexes have been investigated and diverse bioactivities, such as antifungal [7] and anti-malarial [8], have been reported. Thiourea-based non-nucleoside inhibitors of *HIV* reverse transcriptase have also been described [9–12].

Although the antimicrobial properties of thioureas have been related [7, 8, 15], they are limited both in terms of microorganisms' strains and structural diversity. To the best of our knowledge, no systematic screening has been reported for this class of compounds. Among the thioureas, the *N*-phenyl and *N*-benzoyl derivatives caught our attention because previously published results concerning the low toxicity of these thiourea derivatives [14, 15] suggested their potential as antibiotic drugs.

As part of our interest in the synthesis and screening of potentially bioactive compounds [13], we report herein our results concerning the antimicrobial evaluation of thioureas against a diversity of bacteria and fungi strains. Additionally, *SAR* considerations and the conformational bias from novel X-ray diffraction structures of *N*-benzoylthioureas are discussed.

Results and Discussion

Chemistry

To evaluate a representative set of thiourea derivatives with a diverse substitution pattern, two series of

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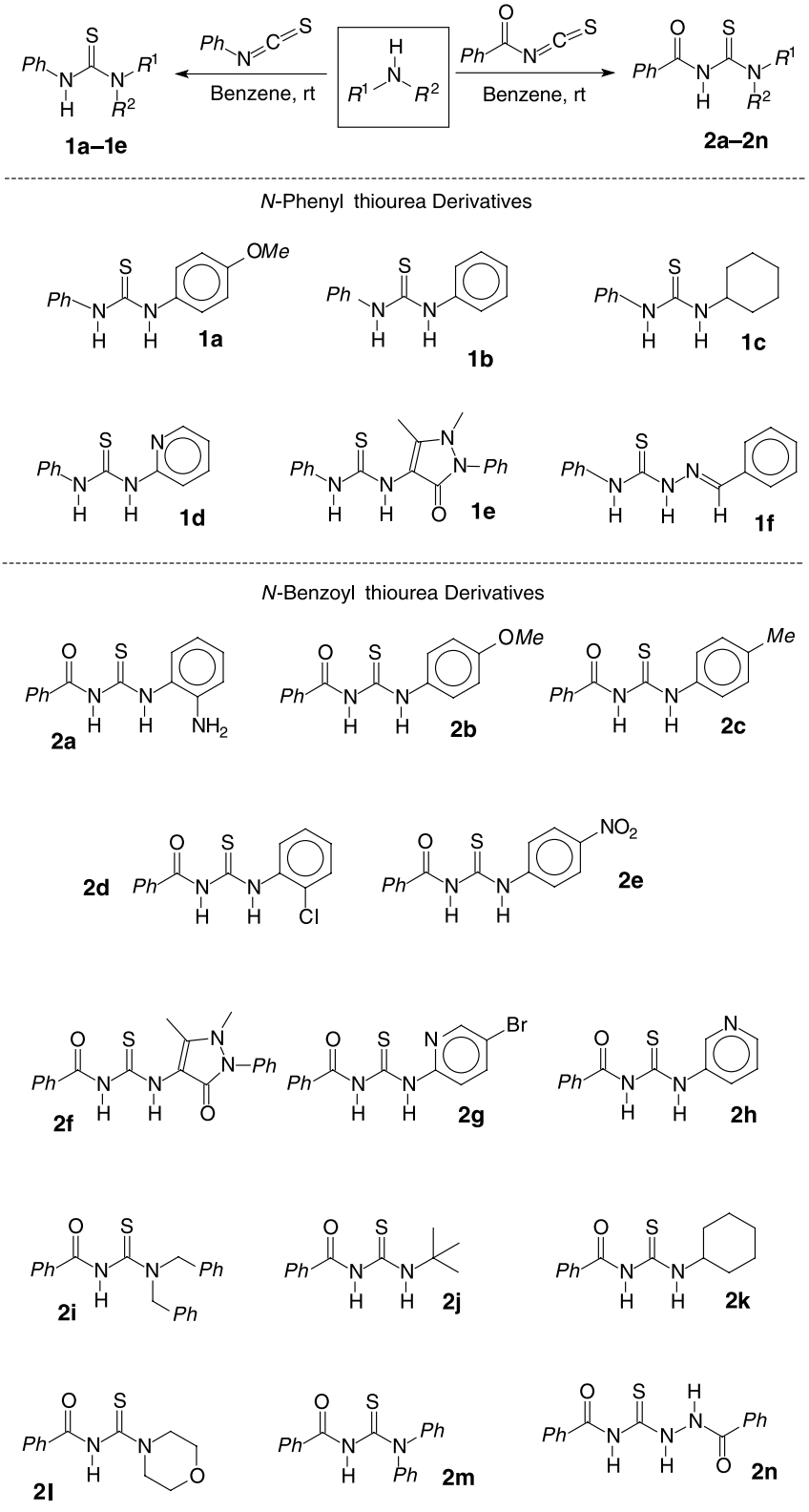
N^1,N^2-disubstituted thioureas were prepared by reaction of primary and secondary amines with both phenyl and benzoyl isothiocyanate, affording *N*-phenyl- **1a**–**1e** and *N*-benzoylthioureas **2a**–**2m**, Scheme 1 [16]. Similarly, the N^1-benzoyl-N^2-benzamidethiourea derivative **2n** was obtained by treat-



Scheme 1

Table 1. Minimal inhibitory concentration ($MIC/\mu g\,cm^{-3}$) of thiourea derivatives **1a–1e** and **2a–2n**

Entry	Thiourea	Microorganisms[#]						
		Bacteria tested						
		B. subtilis	*S. aureus*	*M. luteus*	*S. mutans*	*S. choleraesuís*	*E. coli*	*P. aeruginosa*
1	**1a**	>100	>100	>100	>100	>100	>100	>100
2	**1b**	>100	>100	>100	>100	>100	>100	>100
3	**1c**	>100	>100	100	>100	>100	>100	>100
4	**1d**	>100	>100	100	>100	>100	>100	>100
5	**1e**	>100	>100	50	>100	>100	>100	>100
6	**1f**	>100	>100	100	>100	>100	>100	>100
7	**2a**	25	50	50	>100	>100	>100	>100
8	**2b**	>100	>100	12.5	>100	>100	>100	>100
9	**2c**	>100	>100	25	>100	>100	>100	>100
10	**2d**	>100	100	>100	>100	>100	>100	>100
11	**2e**	>100	>100	12.5	>100	>100	>100	>100
12	**2f**	>100	>100	100	>100	>100	>100	>100
13	**2g**	>100	>100	12.5	>100	>100	>100	>100
14	**2h**	>100	>100	>100	>100	>100	>100	>100
15	**2i**	25	>100	100	>100	>100	>100	>100
16	**2j**	>100	>100	>100	>100	>100	>100	>100
17	**2k**	100	100	6.3	100	>100	>100	>100
18	**2l**	50	>100	100	>100	>100	>100	>100
19	**2m**	50	>100	>100	>100	>100	>100	>100
20	**2n**	6.3	3.1	3.1	25	50	25	>100
21	PC♣	3.1	6.3	0.78	6.3	6.3	3.1	125

Entry	Thiourea	Fungi tested		
		C. albicans	*A. niger*	*C. cladosporioides*
1	**1a**	100	>100	>100
2	**1b**	>100	100	100
3	**1c**	100	>100	50
4	**1d**	50	100	50
5	**1e**	>100	100	>100
6	**1f**	100	100	>100
7	**2a**	100	50	25
8	**2b**	100	>100	100
9	**2c**	>100	>100	>100
10	**2d**	100	>100	>100
11	**2e**	100	>100	100
12	**2f**	100	100	100
13	**2g**	100	>100	>100
14	**2h**	>100	100	>100
15	**2i**	>100	100	>100
16	**2j**	>100	100	100
17	**2k**	100	100	25
18	**2l**	>100	100	100
19	**2m**	>100	>100	>100
20	**2n**	25	3.1	3.1
21	PC♣	6.3	12.5	6.3

[#] *Bacillus subtilis ATCC* 6633, *Staphylococcus aureus ATCC* 6638, *Micrococcus luteus ATCC* 10240, *Streptococcus mutans ATCC* 24175, *Salmonella choleraesuis ATCC* 14028, *Escherichia coli ATCC* 94863, *Pseudomonas aeruginosa*, *Candida albicans ATCC* 18804, *Aspergillus niger ATCC* 16404, *Cladosporium cladosporioides IMI* 178517

♣ *PC* Positive control (chloramphenicol for bacteria, and ciclopirox olamine for fungi)

ment of ethyl benzoate with hydrazine followed by reaction of the formed hydrazide with benzoyl isothiocyanate, while benzaldehyde 4-phenylthiosemicarbazone **1f** was prepared by condensation of 4-phenyl thiosemicarbazide with benzaldehyde (see Experimental).

Biological Results

The minimal inhibitory concentrations of all obtained N^1,N^2-disubstituted thioureas were evaluated in bioassays involving *Gram*-positive and *Gram*-negative bacteria as well as fungi. As can be verified, all the compounds showed antimicrobial activity in some tested concentration (Table 1, shadowed entries).

Antibacterial Activities

The active thioureas showed a clear selectivity against the *Gram*-positive bacterias (*B. subtilis ATCC* 6633, *S. aureus ATCC* 6638, *M. luteus ATCC* 10240) even for the most potent tested compound **2n**, to which very low *MIC* values (3.1–6.3 $\mu g/cm^3$) were observed for these strains. Analysis of Table 1 also suggests that, in general, benzoylthioureas were more active than corresponding phenyl- ones as can be verified directly from comparison of the *MIC* values between **1c**/**2k** and **1a**/**2b**. *M. luteus* seems to be the most susceptible bacterial strain and was particularly inhibited by benzoylthioureas bearing a para-substituted aromatic ring at N^2 as observed for **2b**, **2c**, **2e**, **2g**. However, the substitution of the N^2-aromatic ring by a corresponding aliphatic group led to an even further decrease of the *MIC* (6.3 $\mu g/cm^3$ for **2l**) against the later microorganism. Conversely, the N^2 secondary benzoylthioureas were, in general, more effective against *B. subtilis* (see componds **2i**, **2l** and **2m**).

Antifungal Activities

The inhibitory activities of the thiourea derivatives were broad and more uniform for fungi than for bacterias. Similarly to *Gram*-positive bacterial strains, **2n** was the most bioactive compound against fungi cultures, followed by benzoylthioureas **2a** and **2k**, which showed low inhibition concentrations for *C. cladosporioides*.

X-Ray Analysis

To gain insight into the conformational bias, crystal structures of some of the obtained thioureas were analyzed by X-ray crystallography. The *ORTEP*-3 [17] representations of **2c**, **2j**, **2l**, and **1f** are shown in Fig. 1. The solid state structural features observed for the N^2 secondary derivative **2l** confirms its previously predicted structure [18] using *B3LYP*/6-31*G*. Despite four planar conformations are possible for N^1,N^2-disubstituted thioureas **2c**, **2j**, and **1f** [19], only the *cis-trans* conformation was observed. Regarding the crystal structure of **2c**, **2j**, and **2l**, there is one intramolecular hydrogen bond involving

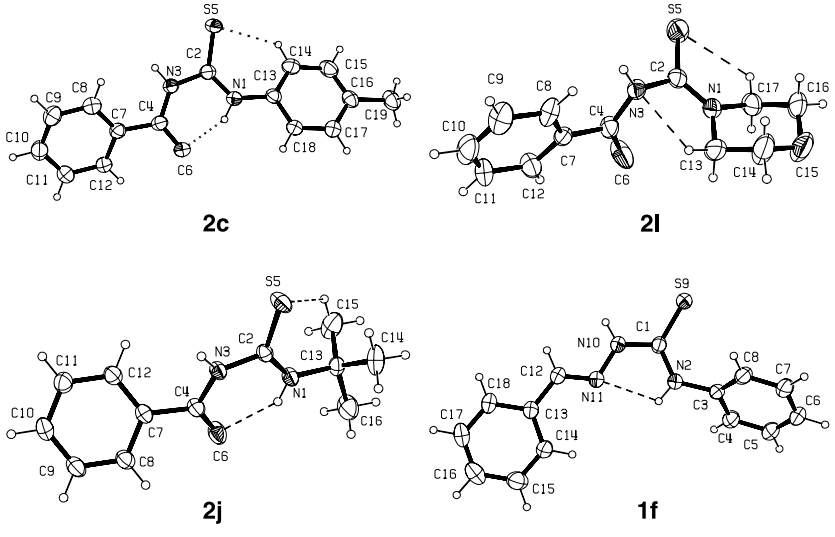


Fig. 1. *ORTEP* drawings for **2e**, **2j**, **2c**, and **1f**. Displacement ellipsoids are drawn at the 30% probability level and H atoms are shown as spheres of arbitrary radii. The intramolecular H-bonds are shown with dashed lines

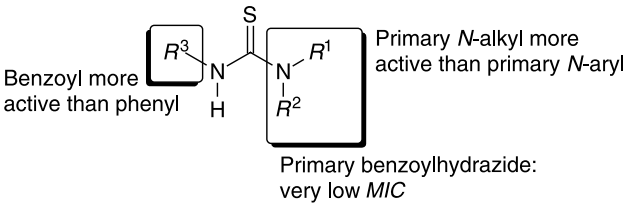


Fig. 2. *SAR* summary of the thiourea derivatives

atoms N1H1···O6 and a short contact interaction of the type CH···S5 in each structure, providing two *pseudo* six-membered rings (see Fig. 1). Curiously, this later weak intramolecular interaction is not observed for **1f**. Intermolecular hydrogen bonds involving N3H3···S5 are observed for **2c**, **2j**, and **1f**, while for **2l** this interaction involves N3H3···O6.

In conclusion, thiourea derivatives obtained by simple and inexpensive manner display selective antimicrobial activities against fungi and *Gram*-positive bacterial strains. Among the tested compounds, some benzoyl derivatives presented promising activity against *B. subtilis*, *M. luteus*, or *C. cladosporioides*. *MIC* values of the two series of thioureas against bacterial strains indicated the importance of the substitution pattern on N^2, Fig. 2. However, at this stage, the influence of the electronic nature of the N^2-group of the N^1-benzoyl-N^2-substituted thioureas and substitution at the phenyl ring are not clear. Thus, structure activities relationship studies on other series of thioureas inspired on structures **2a**, **2b**, **2k**, and particularly **2n** are currently underway.

Experimental

Melting points were determined on a Microquímica MQAPF 301 hot plate apparatus. Infrared spectra were recorded with KBr discs on a FT-IR BOMEM MB100 instrument. NMR spectra were obtained for ^1H at 300 MHz and for ^{13}C at 75 MHz using a Varian Gemini 300 spectrometer. All compounds had physical properties identical with reported values.

Typical Synthesis Procedures

*N-Phenylthioureas **1a–1e** and N-Benzoylthioureas **2a–2n***

The suitable isothiocyanate (5 mmol) was added dropwise to a solution of 5 mmol amine in 5 cm^3 benzene under stirring and ice bath cooling. The reaction mixture was left for 3 h at room temperature after which time the solvent was evaporated and the crude solid was triturated with petroleum ether. Recrystallization with CH_2Cl_2:petroleum ether (4:1) afforded the thiourea. Its structure was confirmed by mp and spectroscopic data compared to literature values (**1e**, **2f** [13]; **1b**, **1d**, **2d**, **2e**, and **2h** [16b]; **2b**, **2c** [16c]; **2l** [16e]; **2i**, **2m** [16i]; **2k** [16j]; **2j** [16k]; **2g** [16l]; **1a**, **1c** [16n]).

N-(Benzamido)-N′-phenylthiourea (**2n**)

A mixture of 105 mmol 50% aqu hydrazine in 10 cm^3 isopropanol and 7 mmol ethyl benzoate was refluxed for 3 h to afford the benzoylhydrazine as colorless hygroscopic solid after solvent evaporation. To a solution of this product in 30 cm^3 isopropanol, 7 mmol benzoyl isothiocyanate were added dropwise at room temperature until *TLC* showed the completion of the reaction. The mixture was filtered and the colorless solid was washed with isopropanol yielding product (91%) with mp 170–172°C (Ref. [16m] 172–174°C).

Benzaldehyde 4-phenylthiosemicarbazone (**1f**)

Phenyl isothiocyanate 18 mmol was added dropwise to a solution of 54 mmol 50% aqu hydrazine in 30 cm^3 isopropanol under stirring at room temperature for 1 h. The colorless solid was decanted and washed with cold isopropanol giving the 4-phenylthiosemicarbazide (70%). Then, 5 mmol benzaldehyde were added dropwise to a suspension of 5 mmol 4-phenylthiosemicarbazide in 25 cm^3 ethanol under stirring at room temperature for 4 h. The mixture was filtered and the colorless solid was washed with cold ethanol yielding **1f** (81%) with mp 189–191°C (lit. 189–191°; *B* **12** 413).

Determination of Minimal Inhibitory Concentrations [20]

Values are means of three experiments. The bacteria cultures used were grown for 24 h at 35°C on nutrient agar. The fungi and yeasts were cultivated for 72 h at 26°C on malt extract agar and yeast malt agar. The inocula for the assays were prepared by cells suspensions according to *McFarland* scale 0.5, except to filamentous fungi to which a modified method was used [11]. Broth microdilution method was carried out to determine the *MIC* of the compounds against the microrganisms in sterile 96-well microplates. The 20% *DMSO* aqueous stock solutions of the compounds were transferred into the first well from which serial dilutions were performed so that concentrations ranged from 100 to 0.78 $\mu g/cm^3$. Chloramphenicol and olamine ciclopirox were used as the reference drugs against bacteria and fungi. Aqueous *DMSO* (20%) was used as negative control. The inoculum was added to all wells and the plates were incubated in the appropriate conditions. After incubation, microorganisms growth were observed by the presence of turbidity on the well. *MIC* was defined as the lowest concentration of the substances that inhibited visible growth.

*Crystallographic Data Collection and Structure Determination of **2j**, **2l**, **2c**, and **1f***

Single crystals X-ray diffraction data were collected at room temperature using a Nonius CAD-4 diffratometer [12] with CuKα radiation ($\lambda = 1.54180$ Å). The structures were solved by direct methods and refined anisotropically with full-matrix least-squares on F^2 using *SHELXL97* [13]. The hydrogen atoms were placed at calculated positions except those involved in H-bonds and weak interactions, found on difference maps and refined with riding constraints. The crystallographic data were deposited at the Cambridge Crystallographic Data Center under the numbers *CCDC* 290675, *CCDC* 290676, *CCDC* 290677, and *CCDC* 606380 for **2j**, **2l**, **2c**, and **1f**. Copies of

the data can be obtained, free of charge *via* www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, *CCDC*, 12 Union Road, Cambridge, CB2 1EZ, UK; (Fax: +44 1223 336033; or E-mail: deposit@ccdc.cam.ac.uk).

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