Synthesis and Characterization of New Heterocyclic Compounds from 2-thioxo-4,5-imidazolidinedione Class and Their Evaluation for Antimicrobial Activity

FLORIN MIHALCEA¹, STEFANIA-FELICIA BARBUCEANU², CAMELIA CRISTEA², CONSTANTIN DRAGHICI³, CRISTIAN ENACHE-PREOTEASA⁴, GABRIELA LAURA ALMAJAN⁵, GABRIEL SARAME⁶

¹University of Medicine and Pharmacy “Carol Davila”, Faculty of Pharmacy, Organic Chemistry Department, 6 Traian Vuia Str., 020956, Bucharest, Romania
²Centre of Applied Biochemistry and Biotechnology “Biotehnol” USAMV, 59 Marasti Blvd., 011464, Bucharest, Romania
³ Romanian Academy, Organic Chemistry Centre “Costin D. Nenitescu”, 2028 Splaiul Independenței, 060023, Bucharest, Romania
⁴Central Phytosanitary Laboratory, 11 Voluntari Blvd., 077190, Voluntari, Romania
⁵University of Medicine and Pharmacy “Carol Davila”, Faculty of Pharmacy, Pharmaceutical Techniques Department, 6 Traian Vuia Str., 020956, Bucharest, Romania

This paper presents the synthesis of new heterocyclic compounds from 2-thioxo-4,5-imidazolidinedione class known as thioparabanic acids and their evaluation for antimicrobial activity. The new N¹-[4-(4-X-phenylsulfonyl)benzamido]-N³-[2-methoxyphenyl]/(3-methoxyphenyl)-2-thioxo-4,5-imidazolidinediones were synthesized by the reaction of N¹-[4-(4-X-phenylsulfonyl)benzoyl]-N³-[2-methoxyphenyl]/(3-methoxyphenyl)-thiosemicarbazides with oxalyl chloride. Acylthiosemicarbazides were obtained from 4-(4-X-phenylsulfonyl)-benzoic acid hydrazides with 2-methoxyphenyl/3-methoxyphenyl-isothiocyanate. The structures were confirmed by elemental analysis and spectral methods: IR, UV-Vis, ¹H-NMR, ¹³C-NMR, MS.

Keywords: 2-thioxo-4,5-imidazolidinedione, thioparabanic acid, acylthiosemicarbazide, oxalyl chloride

Compounds containing a 2-thioxo-4,5-imidazolidinedione heterocycle (thioparabanic acids) possess a broad range of biological properties such as: antiviral [1], anticancer [1-5], aldose reductase inhibitors [6,7], potassium channel openers [8,9], antibacterial [10,11], serum HDL-cholesterol elevating properties [12] and usefulness in treating atherosclerosis [13,14].

On the other hand diphenylsulfone derivatives possess antibacterial and antiinflammatory activity [15-18]. Therefore 2-thioxo-4,5-imidazolidinedione heterocycle substitution with diphenylsulfone moiety could increase the biological activity of a such molecular system.

Keeping this observation in view and in continuation of our research on the synthesis of heterocyclic compounds with expected biological activity, in this paper we describe the synthesis of some new N¹-[4-(4-X-phenylsulfonyl)-benzoic acid hydrazides with 2-methoxyphenyl/3-methoxyphenyl-isothiocyanate. The content of C, H, and N were done with ECS-40-10-Costeh micro-dosimeter.

Experimental part
All reagents used in synthesis were purchased from Merck, Sigma-Aldrich and Fluka Companies. Melting points were determined on a Böetius apparatus and are uncorrected. The UV spectra were determined on a SPECORD 40 Analytik Jena spectrophotometer, using methanolic solutions (2.5 × 10⁻⁵ M). The IR spectra were recorded on a Vertex 70 Bruker spectrophotometer recorded in KBr disc. The ¹H and ¹³C-NMR spectra were recorded on a Varian Gemini 300BB spectrometer (at 300 MHz for ¹H-NMR and 75 MHz for ¹³C-NMR), in DMSO-d₆ as a solvent and tetramethylsilane (TMS) as internal standard.

The mass spectra were obtained with a triple quadrupole mass spectrometer Varian 1200 L/MS/MS with electrospray interface (ESI) at 20 eV collision energy and 1.5 mTorr argon, coupled with a high performance liquid chromatograph with Varian ProStar 240 SDM ternary pump. The sample solution (2 μg/mL in CHCl₃/CH₃OH 1/1, v/v) was introduced in the ESI interface by direct infusion, after a tenth dilution with methanol, at a flow rate of 20 μL/min.

Synthesis of new compounds
The most common thioparabanic acids synthesis was found to be the reaction of oxalyl chloride with thioureas [1,2,6,7,11,13,14,19-24] and there are only few references in the literature where N¹-acylthiosemicarbazides are used instead of thioureas in reaction with oxalyl chloride [25-27].

This paper presents our contributions to the reaction of oxalyl chloride with N¹-[4-(4-X-phenylsulfonyl)benzoyl]-N³-[2-methoxyphenyl]/(3-methoxyphenyl)-thiosemicarbazides (2a-3c).

Key intermediates (2a-3c) used in the synthesis of new 2-thioxo-4,5-imidazolidinediones (4a-5c) were obtained by nucleophilic addition of 4-(4-X-phenylsulfonyl)-benzoic acid hydrazides (1a-c) [28] to 2-methoxyphenyl/3-methoxyphenyl-isothiocyanate [29,30] (scheme 1).

The new thioparabanic acids derivatives were obtained by treating the acylthiosemicarbazides (2a-3c) with oxalyl chloride in dichloromethane at room temperature. In the yellow solution formed, petroleum ether was added and, after filtration, the solid was heated in ethanol at reflux yielding 4a-5c (scheme 1).

*email: stefaniemelia_barbuceanu@yahoo.com
Synthesis of $N^1-N^1'$-[4-(4-X-phenylsulfonyl)benzoyl]-
$N^4-N^4'$-[2-methoxyphenyl]/[3-methoxyphenyl]-
thiosemicarbazides (2a-c) and (3a-c)
A mixture of acid hydrazide 1a-c (4 mmol) and 2-
methoxyphenyl/3-methoxyphenyl-isothiocyanate (4
mmol) was refluxed in anhidrous ethanol for 8 h, cooled to
room temperature and the formed precipitate was filtered
off and recrystallized from ethanol.

$N^1-N^1'$-[4-(phenylsulfonyl)benzoyl]-$N^4-N^4'$-[2-
methoxyphenyl]-thiosemicarbazide (2a)
m.p. = 147-8°C, (lit. 148-9°C); yield = 91.2%

$N^1-N^1'$-[4-(4-chlorophenylsulfonyl)benzoyl]-$N^4-N^4'$-[2-
methoxyphenyl]-thiosemicarbazide (2b)
m.p. = 173-4°C, (lit. 173-5°C); yield = 90.5%

$N^1-N^1'$-[4-(4-bromophenylsulfonyl)benzoyl]-$N^4-N^4'$-[2-
methoxyphenyl]-thiosemicarbazide (2c)
m.p. = 176-7°C, (lit. 176-7°C); yield = 94.5%

$N^1-N^1'$-[4-(phenylsulfonyl)benzoyl]-$N^3-N^3'$-[2-
methoxyphenyl]-2-thioxo-4,5-imidazolidinediones (4a-c)
A mixture of 2a-3c (2 mmol) and oxalyl chloride (2
mmol) was stirred in dichloromethane (5 mL) at room
temperature for 3 h. In the reaction mixture petroleum ether
was added and the bright yellow solid formed was filtered
off. The solid was dissolved in ethanol (5 mL) and heated
at reflux for 2 h. The pale yellow alcoholic solution was
evaporated to dryness and the residue was recrystallized
from chloroform-petroleum ether (v:v=1:1).

$N^1-N^1'$-[4-(4-X-phenylsulfonyl)benzamide]-
$N^4-N^4'$-[2-methoxyphenyl]-2-thioxo-4,5-imidazolidinedione (4a)
m.p. = 138-9°C; yield = 51.3%;
IR (KBr; cm$^{-1}$): 3277m, 3093w, 3067w, 3005w, 2946w,
2841w, 1793vs, 1702s, 1601m, 1578s, 1505s, 1467m,
1414m, 1380s, 1341s, 1284s, 1256s, 1160s;
$^1$H-NMR (DMSO-d$_6$, $\delta$, ppm, $J$, Hz): 12.21 (s, 1H, H-19);
8.18 (s, 4H, H-7,H-8,H-10,H-11); 8.02 (dd, 7.5, 1.7, 2H, H-
13, H-17); 7.75 (tt, 7.5,1.7, 1H, H-15); 7.66 (bt, 7.5, 2H, H-
14, H-16); 7.55 (m, 1H); 7.40 (bd, 8.2, 1H); 7.26 (dd, 1.1,
8.4, 1H, H-25); 7.13 (td, 7.7, 1.1, 1H, H-24); 3.79 (s, 3H,
OCH$_3$);
$^1$C-NMR (DMSO-d$_6$, $\delta$, ppm): 179.07 (C-2); 164.07 (C-
18); 155.07 (C-5); 153.72 (C-4); 152.60 (C-21); 144.63 (C-
9); 140.30 (C-12); 135.00 (C-6); 134.09 (C-15); 131.73 (C-
23); 129.87 (C-8, C-10); 129.37 (C-25); 127.89 (C-7, C-11);
127.33 (C-13, C-17); 120.70 (C-24); 120.23 (C-20); 112.76
(C-22); 56.02 (OCH$_3$);
UV (CH$_3$OH, $\lambda_{max}$(nm), lg $\varepsilon$): 205.3 (4.45); 2467.4 (4.17);
Elemental analysis: found: C:55.84; H:3.34; N:8.57 %;
calcd. for C$_{23}$H$_{17}$N$_3$O$_6$S$_2$ (495.53 g/mol): C:55.75; H:3.46;
N:8.48 %;
ESI-MS, m/z (abundance %): 494 (74) [M-H$^-$].

$N^1-N^1'$-[4-(4-chlorophenylsulfonyl)benzamide]-
$N^4-N^4'$-[2-methoxyphenyl]-2-thioxo-4,5-imidazolidinedione (4b)
m.p. = 143-5°C; yield = 49.4%;
IR (KBr; cm$^{-1}$): 3292m, 3091w, 3038w, 3009w, 2946w,
2841w, 1793vs, 1702s, 1601m, 1578s, 1505s, 1447m,
1414m, 1380s, 1341s, 1283s, 1256s, 1160s, 766s;
\textsuperscript{1}H-NMR (DMSO-\textit{d}_6, \delta, ppm, J, Hz): 12.23 (s, 1H, H-19); 8.20 (d, 9.0, 2H, H-7, H-11); 8.19 (d, 9.0, 2H, H-8, H-10); 8.03 (d, 8.6, 2H, H-13, H-17); 7.74 (d, 8.6, 2H, H-14, H-16); 7.54 (bt, 7.5, 1H, H-24); 7.05 (bd, 7.5, 1H, H-25); 7.26 (dd, 1.0, 7.5, 1H, H-22); 7.07 (d, 1.0, 7.5, 1H, H-24); 3.79 (s, 3H, OCH\textsubscript{3}).

\textsuperscript{13}C-NMR (DMSO-\textit{d}_6, \delta, ppm): 179.10 (C-2); 164.05 (C-18); 155.07 (C-5); 153.77 (C-4); 152.64 (C-21); 144.17 (C-9); 139.30 (C-12); 139.12 (C-6); 131.77 (C-15); 131.23 (C-13); 126.06 (C-14); 126.93 (C-22); 125.97 (C-13, C-17); 129.48 (C-7, C-11); 128.02 (C-8, C-10); 120.74 (C-24); 120.25 (C-20) - 112.79 (C-22); 56.06 (OCH\textsubscript{3}).

UV (CH\textsubscript{3}OH, \lambda_{\text{max}} (nm), \varepsilon): 205.3 (4.48); 251.1 (4.24); Elementar analysis: found: C:52.12; H:3.04; N:7.93 %; calc. for C\textsubscript{23}H\textsubscript{16}BrN\textsubscript{3}O\textsubscript{6}S\textsubscript{2} (574.42 g/mol): C:48.09; H:2.81; N:8.48 %; ESI-MS, m/z (abundance %): 574 (94.8) [M-H]; 317 (100, BP) [C\textsubscript{14}H\textsubscript{10}O\textsubscript{3}N\textsubscript{4}SO\textsubscript{5}CONHNHCO\textsubscript{2}] –

\textbf{N}-\textsuperscript{13}(4-(4-chlorophenylsulfonyl)benzamide]-N\textsuperscript{13}-\textsuperscript{13}(3-methoxyphenyl)-2-thioxo-4,5-imidazolidinedione (5b))

m.p. = 225-6°C; yield = 50.4%; IR (KBr; cm\textsuperscript{-1}): 3255m, 3089w, 3010w, 2928w, 2836w, 1798s, 1705s, 1610m, 1606s, 1519s, 1493m, 1410m, 1377s, 1341s, 1285s, 1246s, 1165s, 766s; \textsuperscript{1}H-NMR (DMSO-\textit{d}_6, \delta, ppm, J, Hz): 12.14 (s, 1H, H-19); 8.17 (s, 4H, H-7, H-8, H-10, H-11); 7.03 (d, 8.8, 2H, H-13, H-17); 7.80 (d, 8.8, 2H, H-14, H-16); 7.54 (bt, 1H, H-23); 7.39 (bd, 7.9, 1H, H-25); 7.12 (bt, 7.5, 1H, H-24); 3.79 (s, 3H, OCH\textsubscript{3}).

\textsuperscript{13}C-NMR (DMSO-\textit{d}_6, \delta, ppm): 179.08 (C-2); 164.12 (C-18); 156.06 (C-6); 153.75 (C-4); 152.63 (C-22); 144.11 (C-9); 139.28 (C-12); 139.13; 135.37 (C-15); 130.16 (C-14, C-16); 129.55 (C-13, C-17); 129.47; 128.42 (C-15); 128.01 (C-7, C-11); 120.74 (C-24); 120.26 (C-20) - 112.80 (C-22); 56.06 (OCH\textsubscript{3}).

UV (CH\textsubscript{3}OH, \lambda_{\text{max}} (nm), \varepsilon): 205.3 (4.61); 252.4 (4.41); Elementar analysis: found: C:52.16; H:2.98; N:7.96 %; calc. for C\textsubscript{23}H\textsubscript{16}BrN\textsubscript{3}O\textsubscript{6}S\textsubscript{2} (574.97 g/mol): C:52.12; H:3.04; N:7.93 %; ESI-MS, m/z (abundance %): 528 (100, BP) [C\textsubscript{14}H\textsubscript{10}O\textsubscript{3}N\textsubscript{4}SO\textsubscript{5}CONHNHCO\textsubscript{2}] –

\textbf{N}-\textsuperscript{13}(4-(4-bromophenylsulfonyl)benzamide]-N\textsuperscript{13}-\textsuperscript{13}(3-methoxyphenyl)-2-thioxo-4,5-imidazolidinedione (5c))

m.p. = 223-4°C; yield = 52.3%; IR (KBr; cm\textsuperscript{-1}): 3350m, 3089w, 3005w, 2940w, 2837w, 1782s, 1724s, 1607m, 1592s, 1574s, 1495m, 1424m, 1379s, 1373s, 1288s, 1258s, 1155s, 572m; \textsuperscript{1}H-NMR (DMSO-\textit{d}_6, \delta, ppm, J, Hz): 12.14 (s, 1H, H-19); 8.17 (s, 4H, H-7, H-8, H-10, H-11); 7.94 (d, 8.8, 2H, H-13, H-17); 7.87 (d, 8.8, 2H, H-14, H-16); 7.54 (bt, 1H, H-23); 7.39 (bd, 7.9, 1H, H-25); 7.12 (bt, 7.5, 1H, H-24); 3.79 (s, 3H, OCH\textsubscript{3}).

\textsuperscript{13}C-NMR (DMSO-\textit{d}_6, \delta, ppm): 179.07 (C-2); 164.12 (C-18); 156.04 (C-6); 153.75 (C-4); 152.63 (C-22); 144.12 (C-9); 139.35 (C-12); 135.19 (C-13); 130.37 (C-14, C-16); 131.77; 129.83 (C-25); 129.58 (C-13, C-17); 129.47; 128.42 (C-15); 128.01 (C-7, C-11); 120.74 (C-24); 120.26 (C-20) - 112.80 (C-22); 56.06 (OCH\textsubscript{3}).

UV (CH\textsubscript{3}OH, \lambda_{\text{max}} (nm), \varepsilon): 206.2 (4.58); 257.3 (4.36); Elementar analysis: found: C:48.15; H:2.78; N:7.36 %; calc. for C\textsubscript{23}H\textsubscript{16}BrN\textsubscript{3}O\textsubscript{6}S\textsubscript{2} (574.42 g/mol): C:48.09; H:2.81; N:7.32 %; ESI-MS, m/z (abundance %): 572 (100, BP) [C\textsubscript{14}H\textsubscript{10}O\textsubscript{3}N\textsubscript{4}SO\textsubscript{5}CONHNHCO\textsubscript{2}] –

\textbf{Antimicrobial activity}

\textit{In vitro} antimicrobial study was carried out on Muller Hinton agar (Hi-media) plates (37°C, 24 h) by agar diffusion cup plate method [51]. All the compounds were screened for antimicrobial activity at 2048 mg/ml concentration against the following bacterial strains: Escherichia coli ATCC 25922, Pseudomonas aeroginosa ATCC 27853, Bacillus subtilis ATCC 6663. Antifungal activity was tested on Sabouraud

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dextrose agar (Himedia) plates (26°C, 48-72 h) by cup plate method [31] against Candida scotti at the concentration 2048 μg/mL. Tobramycin, Ciprofloxacin, Eritromicin were used as a standards for comparison of antibacterial activity and Nistatin for antifungal activity. DMSO was used as a solvent control for both antibacterial and antifungal activities.

Determination of MIC was made by serial dilutions in liquid broth method [32]. The materials used were well plates, suspensions of microorganism (0.5 McFarland), Muller–Hinton broth (for bacteria), and Sabouraud dextrose agar (for yeasts), solutions of the substances to be tested (2048 mg/mL in DMSO). After incubation at 37°C for 18–20 h for bacterial strains and for 48 h for C. scotti, the MIC for each tested substance was determined by macroscopic observation of microbial growth. It corresponds to the well with the lowest concentration of the tested substance where microbial growth was clearly inhibited. Tobramycin, Ciprofloxacin and Eritromicin for bacteria and Nistatin for the yeasts were used as standard drugs.

Results and discussions
Chemistry

Acylthiosemicarbazide moiety provides an opportunity to perform cyclocondensations as well as addition-cyclization reactions based on molecular existence of a NH exchangeable protons. They provide existence of tautomeric forms (scheme 2) that could participate on nucleophilic displacement with oxalyl chloride both nitrogen atoms and sulfur atom.

Nitrogen atom N1 from I has an extremely low probability to participate on nucleophilic substitution because of its low nucleophilicity produced by R1CO group through conjugation effect.

In the reaction with oxalyl chloride, the three tautomers could give three heterocycle structures: a thioparabanic acid (IV) and two imino-thiazolidinediones (V and VI, V major comparative to VI) (scheme 3).

Imino-thiazolidinediones forming proceeds via nucleophilic attack by the thiosemicarbazide sulfur (after tautomeric shift to the thiol form with the hydrogen emanating from N2 or N4 to carbonylic carbon of oxalyl chloride. This way may afford two possible isomeric intermediates (A) and (B), which then are able to undergo different reaction routes to form one of the cyclic products (V) or (VI) (scheme 4).

Thioparabanic acid (IV) probably appears in dichloromethane at room temperature as a direct nucleophilic attack of the N2 and N4 to the oxalyl chloride carbon atoms or by partial isomerization of the imino-thiazolidinediones. This isomerisation is quantitative in ethanol at heating (scheme 5) [19].

The presence of imino-thiazolidinediones together with thioparabanic acid in the reaction mixture is proven by spectral techniques (IR and NMR).
In IR spectra a proof for imino-thiazolidinediones is an absorption band at 1665-1680 cm\(^{-1}\) characteristic to \(\nu\)C=N [19], the other absorption bands can not be considered proofs because of the closer to those of thioparabolic acids.

In \(^1\)H-NMR (CDCl\(_3\)) spectra the existence of the two forms (IV) and (V) is evidenced by two NH singlet signals which appear at \(\delta=9.19-10.00\), also in \(^{13}\)C-NMR (CDCl\(_3\)) spectra are present two closer signals around 56 ppm which can be attributed to the carbon atoms from methoxy groups present in both thioparabolic acid and imino-thiazolidinedione.

The mixture formed from two compounds which we obtained, after heating in ethanol, NMR and IR analysis indicated the presence of only one compound thioparabolic acid form (IV).

In IR spectra of new compounds 4a-5c two absorption bands characteristic of C=O groups vibration are present, which appear at \(\sim1700\) cm\(^{-1}\) (RCONH) and \(\sim1790\) cm\(^{-1}\) respectively (corresponding of two C=O from imidazolidine ring which overlaps). Also the \(\nu\)C=S vibration band is present \(\sim1250\) cm\(^{-1}\). In the region 3255-3350 cm\(^{-1}\) only one absorption band characteristic to NH group was observed. In the region 1665-1680 cm\(^{-1}\) no absorption band was found unlike the reaction mixture in which an absorption band is present in the region and is attributed to the C=N group from imino-thiazolidinedione form.

\(^1\)H-NMR (DMSO-d\(_6\)) spectra of new compounds 4a-5c present only one singlet signal of one NH proton at 12.12-12.23 ppm. The signal observed at \(-3.79\) ppm corresponds to three protons and was assigned to OCH\(_3\) group.

The new compounds 4a-5c present in the \(^{13}\)C-NMR (DMSO-d\(_6\)) spectra three characteristic signals of imidazolidine nucleus: the signals of two carbons from C=O group were observed around 153.19-153.82 ppm (for C-4) and 154.20-159.60 ppm (for C-5) respectively, while C=S signal was recorded at \(\sim179\) ppm. Also the carbon signal of OCH\(_3\) group was observed at \(\delta=55-56\) ppm. The signals of phenylsulfonylphenyl and phenylene fragments were recorded in \(^1\)H-NMR and \(^{13}\)C-NMR spectra at the expected chemical shifts in accordance with literature [29,30].

### Antimicrobial activity

The preliminary results of antimicrobial activities indicated that the tested compounds exhibited a low activity against Gram-negative bacteria: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, one Gram-positive bacteria: *Bacillus subtilis* ATCC 6663 and one yeast: *Candida scotti* (table 1).
Minimal inhibitory concentration (MIC) for 4a-5c was determined to be 1024 μg/mL for all tested strains.

The obtained results indicated that the nature of the diphenylsulfonyl moiety (X=H, Cl, Br) and of the substituent on the N1 from thioparabanic acid does not influence the antimicrobial activity of these compounds which is lower than reference drugs.

Conclusions

In this paper we report the synthesis and characterisation of six new compounds from 2-thioxo-4,5-imidazolidinedione class. These new compounds were obtained by the reaction of N1-[4-(4-X-phenylsulfonyl)benzoyl]-N4-(2-methoxyphenyl)/(3-methoxyphenyl)-thiosemicarbazides with oxalyl chloride.

The structure of new compounds was confirmed by elemental analysis and spectral methods.

All tested compounds had a low activity on growing of some gram-negative bacteria: Escherichia coli, Pseudomonas aeroginosa, gram-positive bacteria: Bacillus subtilis and fungi: Candida scotti.

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