ANTIOXIDANT ACTIVITY AND DRUG PROFILE OF SEVERAL THIOUREA DERIVATIVES OF 2-THIOPHENE CARBOXYLIC ACID

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Abstract
Oxidative stress plays an important role in the development of several diseases (cancer, stroke, diabetes etc.). Therefore, the discovery of new compounds with antioxidant activity and a safe pharmacological profile has become an area of great interest. The aim of our study was the evaluation of antioxidant capacity and drug profile of several 2-thiophene carboxylic acid thiourea derivatives. The antioxidant activity was determined by means of scavenger activity towards 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS\(^+\)) free radicals and ferric reducing power assay. The pharmacological profile of the analysed compounds was evaluated by in silico protocols (bioavailability, pharmacokinetics etc.). The electronic and steric molecular descriptors of the thiourea derivatives have been also determined. According to our results, all compounds showed antioxidant activity. For the most part, in silico studies revealed a safe pharmacological profile for the analysed compounds.

Rezumat
Stresul oxidativ reprezintă un factor cheie în patologia multor afecțiuni (cancer, boli cardiovasculare, metabolice etc.). În ultimii ani, s-a observat un interes crescut în descoperirea de noi compuși cu proprietăți antioxidante și cu un profil farmacologic sigur. Scopul lucrării a constat în evaluarea potențialului antioxidant și a profilului medicamentos ale unor tipuri de substanțe chimice. Oxiuriură, potențialul antioxidant și a profilului farmacologic al unor compuși au fost prezentate și cu un profil farmacologic sigur. Studiile in silico au evidențiat, în general, un profil farmacologic sigur.

Keywords: thiourea derivatives, 2-thiophene carboxylic acid, antioxidant activity, bioavailability

Introduction
The role of oxidative stress in aging and development of human diseases (cancer, cardiovascular, metabolic conditions, diabetes, autoimmune and neurodegenerative disorders) is well known [5]. Reactive oxygen species (ROS) including free radicals (hydroxyl - OH•; superoxide – O₂•–; peroxyl – RO₂•; nitric oxide – NO•) and non-radicals (hypochlorous acid – HOCl; hydrogen peroxide – H₂O₂; singlet oxygen – ¹O₂; peroxynitrite – ONOO•) are constantly formed in the human body [5]. Indigenous sources of ROS result from mitochondria functions, cytochrome P450 metabolism or inflammatory cell response [5]. Among exogenous factors, exposure to UV light, X-ray irradiation, pesticides, pollution, or smoking are of great importance for ROS production [5]. Oxidative stress is considered as an imbalance between the production of ROS and the ability of the natural antioxidant defence system to fight against free radicals and prevent oxidation of lipids, proteins, or DNA [5]. It is well known that the human antioxidant defence system is represented by enzymatic (superoxide dismutase – SOD, catalase, glutathione peroxidase – GPx) and non-enzymatic antioxidants (GSH – glutathione, vitamin E, vitamin C etc.). Antioxidants are molecules that neutralize free radicals and prevent oxidation of a
substrate by means of various mechanisms: (i) keeping ROS production at a minimum level; (ii) scavenger activity; or (iii) repair of affected cells and tissues [5].

Taking into consideration the negative effect of oxidative stress upon human health, considerable research has been directed towards synthesis of new organic compounds with potential antioxidant properties. Over the years, thiourea derivatives have become an important class of compounds due to their various biological properties: antibacterial [21], antitubercular [23], antiparasitic [24], anticonvulsant [12], antiarrhythmic [6], local anaesthetic, antiproliferative, antiplatelet [19], antifungal [26], insecticidal [13], or plant-growth regulators [18].

The aim of our paper was the evaluation of antioxidant activity and drug profile of several 2-thiophene carboxylic acid thiourea derivatives.

Materials and Methods

Tested compounds (Table I) were 2-thiophene carboxylic acid thiourea derivatives encoded as follows: N-(o-iodophenyl)-N’-(2-thienyl)-thiourea (C1), N-(m-iodo-phenyl)-N’-(2-thienyl)-thiourea (C2), N-(p-iodophenyl)-N’-(2-thienyl)-thiourea (C3), N-(2,4-dichlorophenyl)-N’-(2-thienyl)-thiourea (C4), N-(2,5-dichlorophenyl)-N’-(2-thienyl)-thiourea (C5), N-(2,6-dichlorophenyl)-N’-(2-thienyl)-thiourea (C6) and N-(3,4-dichlorophenyl)-N’-(2-thienyl)-thiourea (C7). All compounds were synthesized and characterized, as previously described [3]. All reagents were commercially obtained (Fluka or Sigma-Aldrich, Germany), unless otherwise stated.

Preparation of samples for antioxidant activity evaluation: thiourea derivatives were dissolved in a mixture of dimethylosulfoxide (DMSO):96% ethanol = 1/99 (v/v). The concentration of each stock solution was 2 mM. Subsequent analysis, were performed using the same concentration range (0.025 - 2 mM).

The antioxidant activity was determined based on several well-known methods – the scavenger activity towards DPPH (2,2-diphenyl-1-picyridylhydrazyl), ABTS⁺⁺ (2,2’-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) free radicals and ferric reducing power assay [2, 4, 10].

| Compound | R | Molecular formula | Molecular weight | Melting point (°C) (isopropanol) | Yield (%) |
|----------|---|------------------|------------------|-----------------------------|-----------|
| C1 | | C₁₂H₁₈SnON₁₂I | 388.25 | 168 - 172 | 53 |
| C2 | | C₁₂H₁₈SnON₁₂I | 388.25 | 148 - 150 | 61 |
| C3 | | C₁₂H₁₈SnON₁₂I | 388.25 | 171 - 173 | 62 |
| C4 | | C₁₂H₁₈SnON₁₂Cl₂ | 331.24 | 181 - 183 | 70 |
| C5 | | C₁₂H₁₈SnON₁₂Cl₂ | 331.24 | 197 - 199 | 71 |
| C6 | | C₁₂H₁₈SnON₁₂Cl₂ | 331.24 | 191 - 193 | 69 |
| C7 | | C₁₂H₁₈SnON₁₂Cl₂ | 331.24 | 159 - 162 | 57 |

DPPH radical scavenging assay was determined according to Ohnishi M et al., with some modifications [7, 15]. Briefly, 0.5 mL of 0.025 - 2 mM solutions were mixed with 3 mL DPPH ethanolic solution (0.1 mM). The mixture was kept in the dark, at room temperature for 30 min. The absorbance was measured at λ = 515 nm (Jasco V-530 spectrophotometer, Jasco, Japan) before (A_control) and 30 min. after adding the sample (A_sample). The absorbance was measured against a blank (A_blank) that contained 0.5 mL of each solution, except for DPPH reagent.

DPPH free radical scavenger activity (%) = [A_control - (A_sample - A_blank)]/A_control x 100
ABTS™ radical cation scavenging assay was determined according to Re R et al. [20] with slight modifications [7]. Briefly, the ABTS™ free radical was generated by incubation of ABTS diammonium salt (7 mM) with potassium persulphate (2.45 mM) in the dark, at room temperature, for 16 h. The absorbance of the ABTS™ solution was equilibrated to a value of 0.700 ± 0.02, at λ = 734 nm, after dilution with 96% ethanol. 0.5 mL ABTS™ radical scavenger activity (%) = [(A_{control} - A_{sample} - A_{blank})]/A_{control} x 100 filters using SwissADME website [28]. These filters imposed: (i) molecular weight no more than 500, log P no more than 5, hydrogen bond acceptors no more than 10, and hydrogen bond donors no more than 5 (Lipinski’s filter); (ii) molecular weight between 160 and 480, log P between -0.4 and 5.6, molar refractivity between 40 and 130, number of atoms between 20 and 70 (Ghose filter); (iii) number of rotatable bonds no more than 10 and the total polar surface area no more than 140 (Veber filter) and (iv) log P no more than 5.88 and total polar surface area no more than 131.6 (Egan filter).

Predicted molecular features of thiourea derivatives.

The molecular features of a certain compound (induced by its specific chemical structure) represent a crucial selection criterion during the evaluation of a drug profile. The molecular properties of compounds C1-C7 were estimated using the descriptors computation facility of Discovery Studio. Due to the discrete structural differences among thiourea derivatives, we have determined the most important molecular features that may describe their therapeutic effects. Few of them were taken into account for subsequent analysis: (i) electronic features represented by orbital energies (e_HUMO, e_LUMO), dipole moment (dipole); (ii) steric features represented by accessible solvent surface area (ASA) and its derivative ASA-negative and (iii) charge distribution.

Computational ADME-Tox profile of thiourea derivatives.

Predicted ADME (Absorption Distribution Metabolism Excretion) profile of tested compounds was performed using pkCSM database [29] and SwissADME website [28], based on their chemical SMILES files. By using SwissADME website the following parameters were quantified: (i) gastrointestinal (GI) absorption; (ii) blood-brain barrier permeability (BBB); (iii) P-gp (P-glycoprotein 1) substrate and (iv) inhibitory activity upon CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4 isozymes. If using pkCSM database, we estimated: (i) AMES toxicity; (ii) hepatotoxicity; (iii) hERG (potassium channels encoded by hERG genes) affinity; (iv) LD50 (median lethal dose); (v) skin sensitisation and (vi) the maximum tolerated dose (humans).

Results and Discussion

Antioxidant activity

Antioxidants methods are mainly based on single electron transfer (SET) or hydrogen atom transfer...
(HAT). These mechanisms usually occur together, but the balance is determined by the antioxidant chemical structure and pH values [17]. A single method is not sufficient for the evaluation of a certain compound antioxidant activity, since antioxidants act by various mechanisms [1, 2, 17].

DPPH is a stable violet coloured free radical, with a high absorption value at \( \lambda = 518 \) nm [8]. As its odd electron becomes paired off in the presence of a free radical scavenger, the absorption diminishes and the observed degree of decolourization can be taken as a measure of the antioxidant activity [2, 8]. 

DPPH assay is mainly a single electron transfer based method [8], nevertheless some authors consider that it has a mixed mechanism [17]. For all tested compounds, the free radical inhibition (%) increased with concentration (Figure 1A). The inhibition varied among iodine thiourea derivatives from 13.12% (for \( C1 \) – 0.025 mM) to 40.40% (for \( C3 \) – 2 mM). Among dichloro derivatives, at the highest concentration (2 mM) compound \( C4 \) had the best scavenger activity (38%) (Figure 1A). As shown in Table II, higher values for ascorbic acid equivalents were seen for \( C1, C3 \) and \( C4 \) compounds, similar to ferric reducing power assay.

Figure 1.
Radical scavenging activity and ferric reducing power of the analysed compounds
(A – DPPH assay; B – ABTS\(^*\) assay; C – Ferric reducing power assay)

Regarding the \( ABTS^* \) assay, the bluish – green radical cation is reduced in the presence of antioxidants (both lipophilic and hydrophilic compounds) [17]. The method is based on both HAT and SET mechanisms [1, 2, 8, 17].

Among the analysed compounds, \( ABTS^* \) scavenger activity varied between 9.94% (for \( C5 \) – 0.025 mM) to 96.61% (for \( C7 \) – 2 mM) (Figure 1B). According to our results, the dichloro-substituted derivatives (compounds \( C5, C6 \) and \( C7 \)) showed a higher antioxidant capacity compared to iodine derivatives (Table II). The highest antioxidant capacity was found for compound \( C6 \).
reducing power properties (Table II). Therefore, we assume that the structural differences between thiourea derivatives have a major impact upon their antioxidant potential [1, 2, 8]. Among thiourea derivatives, compound C5 showed the weakest ferric reducing power effect, with absorbance values ranging between 0.05 (for 0.025 mM) to 0.1901 (for 0.5 mM) (Figure 1C).

Table II
Antioxidant activity of the analysed compounds

| Compound | METHOD – mM ascorbic acid/g | DPPH | ABTS* | Ferric reducing power |
|----------|-----------------------------|------|-------|-----------------------|
| C1       |                             | 1.0880 ± 0.9479* | 3.4864 ± 2.7751** | 1.6200 ± 0.2470*** |
| C2       |                             | 0.3338 ± 0.3117*$| 4.6364 ± 2.6112***| 1.5466 ± 0.4505*** |
| C3       |                             | 1.5545 ± 0.6829**| 4.1647 ± 3.8540** | 2.5085 ± 0.1743*** |
| C4       |                             | 1.5965 ± 1.5927**| 2.9954 ± 2.9156** | 1.7065 ± 0.2982** |
| C5       |                             | 0.6019 ± 0.5187* | 7.2755 ± 2.9389** | nd                    |
| C6       |                             | 0.2017 ± 0.1385| 16.5125 ± 1.3805***| 0.4191 ± 0.0682** |
| C7       |                             | 0.4726 ± 0.3176| 10.8840 ± 0.8533***| 0.9830 ± 0.3430** |

Results are mean ± SD (n = 3); nd – not determined. Two different letters for a compound (in the same column) means significant difference between antioxidant activity; *(p < 0.05); **(p < 0.001); *** (p < 0.0001)

We have found a positive correlation between the antioxidant assays (Table III). However, a negative correlation (although not significant) was observed between ABTS* and the ferric reducing power assays. This is probably the consequence of a different mechanism of action that is strongly influenced by thiourea derivatives chemical structure [1, 2].

Table III
Pearson correlation coefficients

| DPPH | ABTS* | Ferric reducing power |
|------|-------|-----------------------|
| DPPH| -     | 0.2857***              |
| ABTS*| 0.2857*** | -                     |
| Ferric reducing power | 0.0375* | -1.1501** |

ns – not significant (p > 0.05); * significant (p < 0.05)

In silico studies
Drug-likeness, lead-likeness and ADME-Tox features of thiourea derivatives

Our results generated by the medicinal chemistry filters (Lipinski, Ghose, Veber and Egan) (Table IV) and ADME-Tox analyses (Table V) revealed that all thiourea derivatives comply with drug-likeness rules, suggesting that these compounds may have therapeutic effects and an appropriate bioavailability (Table IV). Since medicinal chemistry filtering was respected, we had the opportunity to evaluate ADME-Tox features for all analysed thiourea derivatives.

Table IV
The bioavailability score and drug-likeness filtering of THE analysed thiourea derivatives

| Compound | Lipinski | Ghose | Veber | Egan | Bioavailability score |
|----------|----------|-------|-------|------|-----------------------|
| C1       | yes      | yes   | yes   | yes  | 0.55                  |
| C2       | yes      | yes   | yes   | yes  | 0.55                  |
| C3       | yes      | yes   | yes   | yes  | 0.55                  |
| C4       | yes      | yes   | yes   | yes  | 0.55                  |
| C5       | yes      | yes   | yes   | yes  | 0.55                  |
| C6       | yes      | yes   | yes   | yes  | 0.55                  |
| C7       | yes      | yes   | yes   | yes  | 0.55                  |

Table V
Predicted ADME features of the analysed thiourea derivatives

| ADME | C1 | C2 | C3 | C4 | C5 | C6 | C7 |
|------|----|----|----|----|----|----|----|
| Gl absorption | high | high | high | high | high | high | high |
| BBB permeability | no | no | no | no | no | no | no |
| P-gp substrate | no | no | no | no | no | no | no |
| CYP1A2 inhibitor | yes | yes | yes | yes | yes | yes | yes |
| CYP2C19 inhibitor | yes | yes | yes | yes | yes | yes | yes |
| CYP2D6 inhibitor | no | no | no | no | no | no | no |
| CYP3A4 inhibitor | yes | yes | yes | yes | yes | yes | yes |

Gl – gastro-intestinal; BBB – blood-brain barrier; P-gp – P-glycoprotein1
According to our ADME analyses (Table V), all thio-urea derivatives have an appropriate gastro-intestinal absorption. The high GI absorption together with a proper bioavailability score promotes thio-urea derivatives as future candidates for oral administration. None of the analysed compounds had blood brain barrier permeability. Moreover, analysed compounds are not a substrate for P-glycoprotein 1. On addition, all thio-urea derivatives inhibit CYP1A2, CYP3A4, CYP2C9 and CYP1A2 isoforms.

Interesting results were obtained regarding the toxicological profiles of the tested compounds (Table VI).

### Table VI

| Toxicity profile                              | C1 | C2 | C3 | C4 | C5 | C6 | C7 |
|-----------------------------------------------|----|----|----|----|----|----|----|
| AMES toxicity                                 | no | no | no | no | no | no | no |
| Max. tolerated dose (human) (log mg/kg/day)   | 0.22 | 0.429 | 0.41 | 0.32 | 0.61 | 0.61 | 0.48 |
| hERG I/II inhibitors                          | no/no | no/no | no/no | no/no | no/yes | no/yes | no/no |
| Oral Rat Acute Toxicity (LD₅₀) (mol/kg)      | 2.198 | 2.328 | 2.26 | 2.38 | 2.51 | 2.53 | 2.35 |
| Hepatotoxicity                                | no | no | no | no | no | no | no |
| Skin sensitisation                            | no | no | no | no | no | no | no |

Table VII

| Compound | $E_{LUMO}$ (eV) | $E_{HUMO}$ (eV) | Dipole (D) | ASA (Å²) | ASA-negative (Å²) |
|----------|----------------|----------------|------------|----------|-----------------|
| C1       | -0.87          | -8.66          | 4.06       | 515.08   | 246.25          |
| C2       | -0.86          | -8.68          | 4.45       | 522.14   | 250             |
| C3       | -1.12          | -8.81          | 5.22       | 518.75   | 248.70          |
| C4       | -0.99          | -8.74          | 5.20       | 526.62   | 273.40          |
| C5       | -0.96          | -8.72          | 5.42       | 521.24   | 266.88          |
| C6       | -0.84          | -8.62          | 6.35       | 514.65   | 258.20          |
| C7       | -0.95          | -8.80          | 4.53       | 521.34   | 261.60          |

Our results have shown that analysed thiourea derivatives don’t have a mutagenic effect; they don’t induce skin sensitisation or hepatotoxicity (Table VI). Regarding cardiovascular toxicity, our results pointed out those dichloro-derivatives (mainly C4 and C5 compounds) are hERG I/II inhibitors (responsible for large QT syndrome or torsade de pointes arrhythmia) [27]. Regarding oral rat acute toxicity, our results marked out that all compounds have similar LD₅₀ values (2.19 - 2.53 mol/kg).

Predicted molecular features of thiourea derivatives

In silico tool Discovery Studio was used for evaluation of electronic and steric molecular descriptors of analysed compounds (Table VII).

### Table VII

| Compound | $E_{LUMO}$ (eV) | $E_{HUMO}$ (eV) | Dipole (D) | ASA (Å²) | ASA-negative (Å²) |
|----------|----------------|----------------|------------|----------|-----------------|
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| C3       | -1.12          | -8.81          | 5.22       | 518.75   | 248.70          |
| C4       | -0.99          | -8.74          | 5.20       | 526.62   | 273.40          |
| C5       | -0.96          | -8.72          | 5.42       | 521.24   | 266.88          |
| C6       | -0.84          | -8.62          | 6.35       | 514.65   | 258.20          |
| C7       | -0.95          | -8.80          | 4.53       | 521.34   | 261.60          |

Figure 2.

 Charge distribution on C6 compound

We have noticed a significant variation of LUMO orbital energies, with values ranging from -1.12 eV (for C3) to -0.84 eV (for C6). Still, the HUMO orbital energies were similar among analysed compounds. Regarding the dipole moment, a higher value (6.35) was determined for compound C6 (Table VII). A discrete variation was observed for the steric descriptors ASA, yet analysing the ASA negative forms a significant variation (273.40 Å² for C4 and only 246.25 Å² for C1). According to our results, dichloro derivatives have higher values for ASA-negative and dipole moment compared to iodine derivatives. Among dichloro derivatives, C4 is more active than C6 regarding orbital energy and charge distribution (Figure 2 and Figure 3).
Conclusions

All thiourea derivatives showed in vitro antioxidant capacity. Overall, iodine thiourea derivatives (compounds C1, C2, C3) act as DPPH free radical scavenger agents, whereas dichloro derivatives (compounds C5, C6, C7) showed better results regarding the ABTS™ assay. In general, all tested compounds had ferric reducing power properties. In silico studies have demonstrated the drug profile of each tested compound. On the whole, thiourea derivatives showed a safe pharmacological profile, except for dichloro derivatives (compounds C4 and C5) that act as hERG I/II inhibitors. Future research is needed in order to establish the exact mechanism of action, regarding the antioxidant activity and the in vivo pharmacological profile.

Conflict of interest

The authors declare no conflict of interest.

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