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SYNTHESIS, ABSORPTION, DISTRIBUTION, METABOLISM, EXCRETION AND ANTIOXIDANT ASSAY OF SOME N4 – SUBSTITUTED THIOSEMICARBAZONES OF CINNAMALDEHYDE

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ABSTRACT

Introduction The increasing number of cancer diagnoses is a pressing issue in modern medicine. One contributing factor to cancer development is oxidative stress caused by free radicals, which can damage proteins, lipids, and DNA. Antioxidants are commonly used to mitigate these effects by neutralizing free radicals and reducing their harmful impact. Recent studies have shown that thiosemicarbazones may have antioxidant properties. These compounds are also known for their potential as anticancer, antibiotic, and antifungal agents. In this research, a series of thiosemicarbazones were synthesized and their antioxidant capacity was evaluated. Additionally, the ADME (Absorption, Distribution, Metabolism, Excretion) parameters of the synthesized compounds were evaluated to determine their potential as pharmaceutical candidates.

Material and methods All starting reagents were purchased from Sigma Aldrich or Alfa Aesar. The synthesis of the compounds was carried out using classical methods, adopted for this study. ADME analysis was performed using ADMETLabs 3.0 platform. Antioxidative properties were investigated using the standard assay method.

Results Five thiosemicarbazones were synthesized. The presence of characteristic functional groups was confirmed by FTIR spectroscopy. Synthesized compounds have a good ADME profile, characteristic of biologically active compounds. It was found that compounds 1 and 5 exhibit antioxidative activity in 6 times and 3 times higher than Trolox.

Conclusions Obtaining new thiosemicarbazones based on cinnamaldehyde with the study of antioxidant properties and ADME screening would allow in the future that these products can be tested in the clinical and preclinical stages with rigorous evaluations. The synthesized products represent an increased antioxidant potential for the biopharmaceutical field.

Keywords N4-substituted thiosemicarbazones; cinnamaldehyde; antioxidant activity; ADME analysis.

SINTEZA, STUDIUL ADME ȘI ACTIVITATEA ANTIOXIDANTĂ A UNOR TIOSEMICARBAZONE N4 SUBSTITUITE ALE ALDEHIDEI CINAMICE

Introducere Una dintre problemele actuale ale medicinei moderne o constituie creșterea permanentă a cazurilor de cancer. Deseori, cancerul reprezintă consecința stresului oxidativ, cauzat de radicalii liberi. Acesta conduce la deteriorarea ADN-ului și a proteinelor. Pentru combaterea stresului oxidativ sunt folosite substanțe cu efect antioxidant. Ele captează radicalii liberi, reducând efectele nocive ale acestora. Mulți cercetători raportează că tiosemicarbazonele pot manifesta efecte antioxidante. Pe lângă aceasta, compușii respectivi sunt cunoscuți și pentru efectele lor anticancerigene, antibiotice și antifungice. În cadrul cercetării a fost sintetizată o serie de tiosemicarbazone și a fost examinată proprietatea lor antioxidantă. De asemenea au fost evaluați parametrii ADME, în scopul determinării potențialului farmaceutic al compușilor sintetizați.

Material și metode Toți reagenții au fost achiziționați de la Sigma Aldrich sau Alfa Aesar. Sinteza compușilor 1-5 a fost realizată conform procedurilor de sinteză clasică, cu unele adaptări de rigoare, în cazul nostru. Analiza ADME a fost efectuată utilizând ADMET Labs 3.0. Analiza antioxidantă a fost realizată prin intermediul metodei standard.

Rezultate Au fost sintetizate cinci tiosemicarbazone noi. Prezența grupelor funcționale caracteristice a fost confirmată prin intermediul spectroscopiei FTIR. Compușii sintetizați au un profil ADME bun, caracteristic substanțelor biologic active. S-a observat, în cazul compușilor 1 și 5, o activitate antioxidantă de 6 și de 3 ori mai mare decât în cazul Trolox, substanță de referință.

Concluzii Obținerea de noi tiosemicarbazone pe bază de cinamaldehidă, prin studiul proprietăților antioxidante și screening ADME, ar permite în viitor testarea acestor produse în stadiile clinice și preclinice cu evaluări riguroase. Produsele sintetizate reprezintă un potențial antioxidant sporit pentru domeniul biofarmaceutic.

Cuvinte-cheie Tiosemicarbazone N4-substituite, cinamaldehidă, activitate antioxidantă, analiza ADME.

INTRODUCTION

Medicinal chemistry, like many scientific disciplines, is continually evolving to meet emerging health challenges. The recent SARS-CoV-2 pandemic has underscored humanity's vulnerability to global health crises and emphasized the urgent need for the development of novel therapeutic agents. The rising incidence of cancer remains a major concern. As shown in Figure 1, the annual incidence is expected to nearly double by 2050 (1). One contributing factor to cancer development is the presence of free radicals – highly reactive species capable of inducing oxidative stress (2). These species can damage cellular proteins, impairing their function, and disrupt lipid membranes, thereby compromising membrane integrity (3). Additionally, free radicals can induce DNA damage and genetic mutations (4). For example, mutations in the gene encoding phosphoinositide 3-kinase (PI3K), a key enzyme in the PI3K/AKT/mTOR signaling pathway, can result in permanent activation of PI3K, leading to uncontrolled cell growth (5). Antioxidants are commonly used to combat free radicals. These compounds have the ability to trap and bind free radicals, neutralizing their harmful effects. This process can reduce or even prevent oxidative damage and mutations.

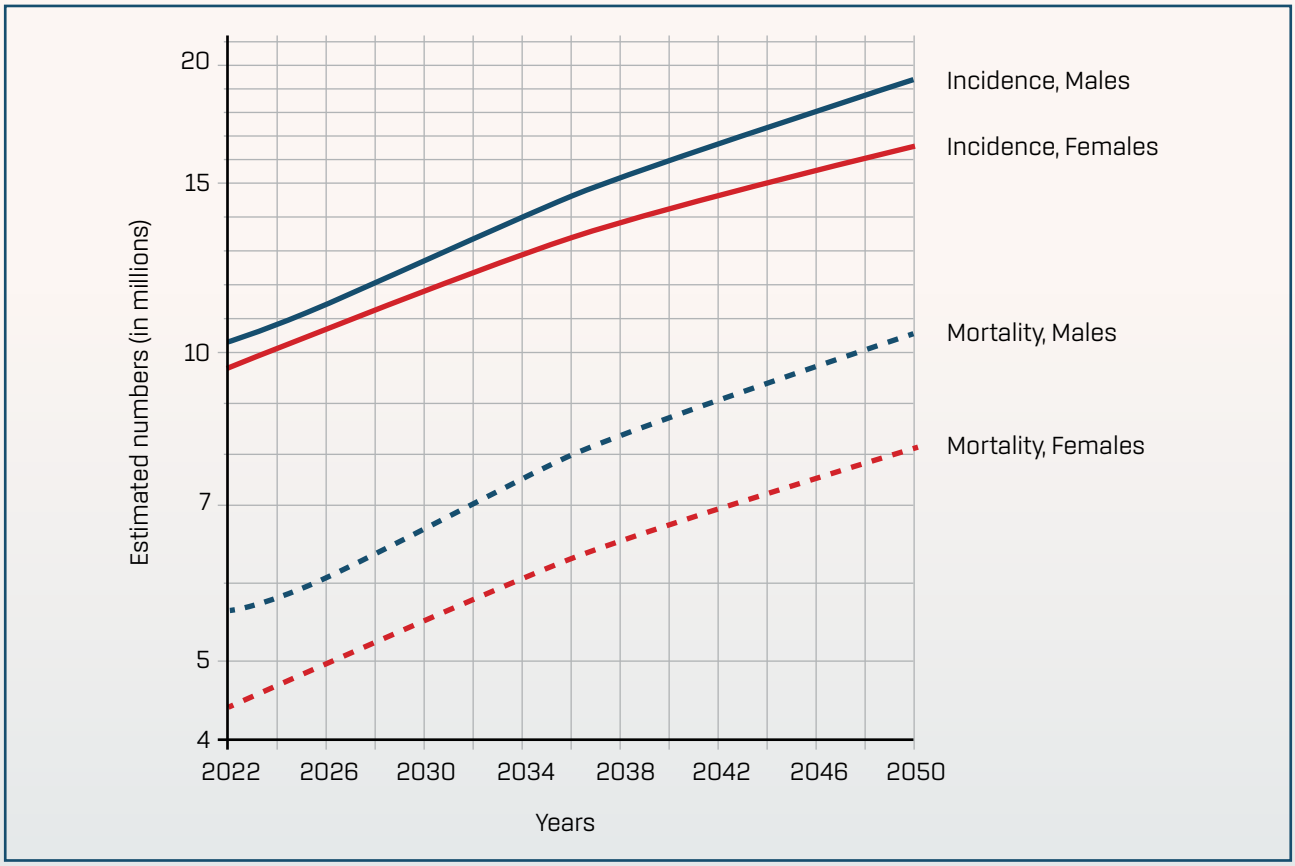

Figure 1. Estimated numbers of cancer incidence and mortality for 2022-2050 period, for males and females (1).

In this context, the development of novel compounds with antioxidant properties represents a highly promising area of research. Thiosemicarbazones, which can be considered derivatives of Schiff bases, have attracted considerable attention due to their diverse biological activities. Several studies (6, 7) have reported the antioxidant properties of specific thiosemicarbazones and their metal complexes, some of which have demonstrated greater activity than reference antioxidants such as Trolox and Rutin. Additionally, other

studies (8, 9) have described the antiproliferative activity of thiosemicarbazones against HL-60 leukemia cells. These compounds have also exhibited antimicrobial (10–12), antifungal (12, 13), and antitumor (14, 15) properties. Despite their promising biological effects, it is crucial for thiosemicarbazones to exhibit low toxicity levels for practical use. One potential solution to this issue is the introduction of natural compounds in their synthesis. In this study, thiosemicarbazones were synthesized using cinnamaldehyde, a compound naturally found in the bark of cinnamon trees (16). Previous research has shown that cinnamaldehyde itself possesses antimicrobial activity (17) and it is safe for human consumption, as it is commonly used in the food industry. This article reports the synthesis of five cinnamaldehyde-based thiosemicarbazones and evaluates their antioxidant activity and ADME (Absorption, Distribution, Metabolism, and Excretion) profiles. The aim of this study was to obtain thiosemicarbazone derivatives with antioxidant properties and favorable pharmacokinetic parameters, supporting their potential for future pharmaceutical development.

MATERIAL AND METHODS

All starting reagents were purchased from Sigma Aldrich or Alfa Aesar and used without any further purification. FTIR spectroscopy was performed using a Brucker Alpha spectrometer at room temperature. ADME analysis was performed using ADMETLab3.0 (18). Antioxidant activity assay was performed using the procedure described in source (19). General procedures for compounds synthesis have been taken and adapted from sources (20, 21, 22).

GENERAL PROCEDURE FOR ISOTHIOCYANATES SYNTHESIS

The primary amine (1.0 equiv.) was dissolved in a biphasic mixture of hexane and water, followed by the addition of sodium bicarbonate ($NaHCO_3$, 2.0 equiv.). The reaction mixture was cooled to approximately 0 °C, and a solution of thiophosgene (1.0 equiv.) in hexane was added dropwise under stirring. The mixture was then stirred at room temperature until the red-orange color disappeared. The organic (hexane) layer was separated, washed three times with saturated $NaHCO_3$ solution, and dried over anhydrous Na_2SO_4. After removal of the solvent under reduced pressure, the resulting pale yellow oils were obtained, affording hexyl isothiocyanate in 76% yield and octyl isothiocyanate in 83% yield.

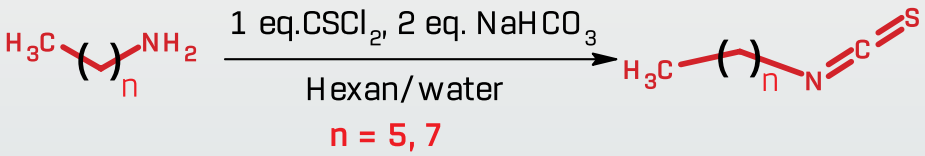

Figure 2. Scheme of isothiocyanates synthesis.

GENERAL PROCEDURE FOR THIOSEMICARBAZIDES SYNTHESIS

The reaction for the formation of thiosemicarbazides was carried out according to the following synthesis protocol: the appropiate isothiocyanate dissolved in tetrahydrofuran is added dropwise to the solution of hydrazine monohydrate in THF while the reaction mixture was cooled in an ice and salt bath during the mixing of the initial reagents. After the addition was complete, the mixture was stirred at room temperature for 30 minutes. The progress of the reaction was monitored by thin-layer chromatography (TLC).

The resulting precipitate was filtered, washed with cold ethanol, and then washed three times with diethyl ether. The reaction afforded white solids: N^4-hexylthiosemicarbazide (85% yield), N^4-octylthiosemicarbazide (92% yield), and N^4-norbornylthiosemicarbazide (91% yield). The melting points and IR spectra of the products were consistent with those reported in the literature.

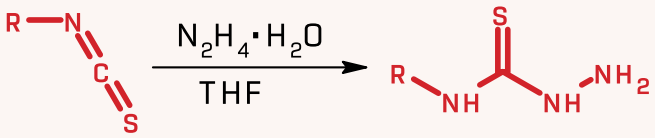

Figure 3. Scheme of thiosemicarbazides synthesis, R = *exo*-norborn-2-yl, hex-1-yl and oct-1-yl.

MORPHOLINE-THIOSEMICARBAZIDE SYNTHESIS

Thiophosgene (1.0 equiv.) was dissolved in chloroform and, with constant stirring, added to a chloroform solution of morpholine (1.0 equiv.) and triethylamine (1.0 equiv.), which had been cooled in an ice–salt bath. The reaction mixture was then stirred at room temperature for 3 hours. Diethyl ether was subsequently added until triethylamine hydrochloride fully precipitated. The precipitate was removed by filtration, and the resulting solution was concentrated using a rotary evaporator. Cold hexane was added to the concentrated solution to induce crystallization. The crystalline solid was collected by filtration and air-dried to afford morpholine-4-carbothioyl chloride as a pale yellow solid in 55% yield.

To a solution of hydrazine monohydrate (1.0 equiv.) in absolute 1,4-dioxane, a solution of morpholine-4-carbothioyl chloride (1.0 equiv.) was added dropwise. The reaction mixture was stirred for 30 minutes at room temperature, leading to the formation of a light pink precipitate. The product was filtered, washed with diethyl ether, and dried to yield morpholine-thiosemicarbazide (IUPAC name: morpholine-4-carbothiohydrazide) as a white solid in 67% yield.

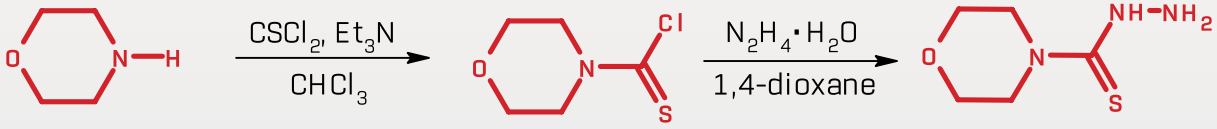

Figure 4. Scheme of morpholine-4-carbothiohydrazide synthesis.

GENERAL PROCEDURE FOR THIOSEMICARBAZONES SYNTHESIS

In a round-bottomed flask, 1 equivalent of the corresponding thiosemicarbazide and 1 equivalent of cinnamaldehyde were added, using ethanol as a solvent. A small amount of glacial acetic acid (3-5 drops) was then added as a catalyst. The mixture was refluxed for 2-4 hours, with the consumption of the initial compounds monitored using chromatography. After cooling, the reaction mixture was filtered and the solid product was washed with ethanol and dried.

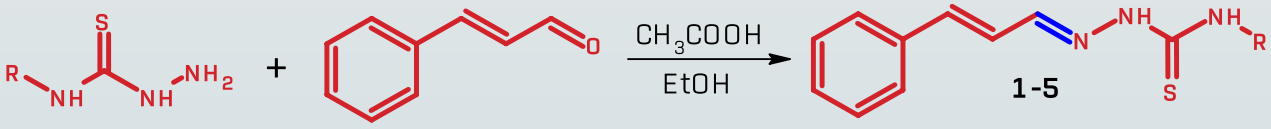

Figure 5. Scheme of thiosemicarbazones synthesis R= H, norborn-2-yl, hex-1-yl and oct-1-yl, *morpholin-1*-yl.

RESULTS

COMPOUNDS SYNTHESIS

The thiosemicarbazones were synthesized using a general synthetic procedure, resulting in good yields of 79-93% with the exception of compound 5, which was obtained in 66% yield. The presence of functional groups was confirmed using FTIR spectroscopy, indirectly confirming the structure of the synthesized compounds. The structures of the synthesized compounds are shown in Figure 6.

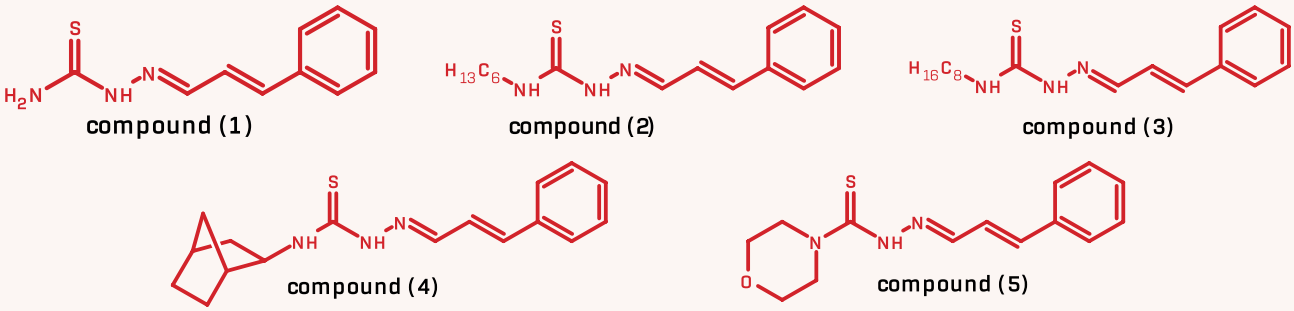

Figure 6. Structure of synthesised compounds **1-5**.

Compound (**1**) *(2E)-2-[(2E)-3-phenylprop-2-en-1-ylidene]hydrazine-1-carbothioamide*

It was synthesized according to the general procedure. Yellow solid, reflux time – 2.5 hours. Yield: 93%. FTIR (cm^{-1}): 3340 ν (N-H, secondary amine); 3058 ν (C-H, Alkene); 1545 ν (C=N, imine); 765, 696 ν (C-H, monosubstituted benzene ring), 1245 ν (C=S).

Compound (**2**) *(2E)-N-hexyl-2-[(2E)-3-phenylprop-2-en-1-ylidene]hydrazine-1-carbothioamide*

It was synthesized according to the general procedure. Yellow solid, reflux time – 3.5 hours. Yield: 85%. FTIR (cm^{-1}): 3344 ν (N-H, secondary amine); 3132, 3004, 2924, 2853 ν (C-H, Alkane); 3056 ν (C-H, Alkene); 1540 ν (C=N, imine); 750, 691 ν (C-H, monosubstituted benzene ring), 1258 ν (C=S).

Compound (**3**) *(2E)-N-octyl-2-[(2E)-3-phenylprop-2-en-1-ylidene]hydrazine-1-carbothioamide*

It was synthesized according to the general procedure. Yellow solid, reflux time – 4.5 hours. Yield: 88%. FTIR (cm^{-1}): 3347 ν (N-H, secondary amine); 3139, 3008, 2921, 2861 ν (C-H, Alkane); 3060 ν (C-H, Alkene); 1553 ν (C=N, imine); 751, 689 ν (C-H, monosubstituted benzene ring), 1246 ν (C=S).

Compound (**4**) *(E)-N-((1R,2S,4S)-bicyclo[2.2.1]heptan-2-yl)-2-((E)-3-phenylallylidene) hydrazinecarbo-thioamide*

It was synthesized according to the general procedure. Yellow solid, reflux time – 5 hours. Yield: 79%. FTIR (cm^{-1}): 3313 ν (N-H, secondary amine); 3123, 2983, 2945, 2866 ν (C-H, Alkane); 3037 ν (C-H, Alkene); 1533 ν (C=N, imine); 745, 689 ν (C-H, monosubstituted benzene ring), 1231 ν (C=S).

Compound (**5**) *N'-[(1E,2E)-3-phenylprop-2-en-1-ylidene]morpholine-4-carbothiohydrazide (**5**)*

It was synthesized according to the general procedure. Yellow solid, reflux time – 6 hours. Yield: 66%. FTIR (cm^{-1}): 2965, 2859 ν (C-H, Alkane); 3026 ν (C-H, Alkene); 1450 ν (C=N, imine); 754, 693 ν (C-H, monosubstituted benzene ring), 1240 ν (C=S).

ADME analysis

ADME analysis is an essential initial screening tool for assessing the potential biological activity of compounds. Additionally, analyzing the data obtained from this assay provides some valuable insights into key parameters for the compound's future use. Table 1 presents the calculated physicochemical properties, including the logarithm of the octanol-water partition coefficient (Log $P_{o/w}$), which indicates the ratio of a compounds solubility in lipophilic (octanol) and hydrophilic (water) phases. Ideally, Log P value should not exceed 5, as it indicates that the compound will primarily remain in the cell membrane. Conversely, values below 1 suggests that the compound will not penetrate the membrane at all. It is worth noting that all synthesized compounds, except for 3, have acceptable partition coefficient values. Furthermore, the pK_a values for the synthesized compounds fall within the range of 8.3-9.9, indicating that they are more likely to be absorbed in the intestine rather than the stomach.

Table 1. Some physicochemical properties calculated for compounds 1-5.

Physicochemical properties					
Compound	Number of H-bond acceptors	Number of H-bond donors	Molecular weight	Log $P_{o/w}$	pK_a
1	1	2	205.28	2.81	8.36
2	1	2	289.44	4.82	9.48
3	1	2	317.49	5.55	9.87
4	1	2	299.43	4.56	9.26
5	2	1	275.37	3.01	8.83

Log$P_{o/w}$ – logarithm of the octanol-water partition index value, pK_a – acidity constant

In general, the descriptors in Table 1-namely, the number of H-bond acceptors, number of H-bond donors, molecular weight, and Log $P_{o/w}$) can be evaluated using drug likeness rules to identify potential drug-like compounds with promising biological activity. As shown in Table 2, all compounds, with the exception of compound 3, meet all commonly used rules, indicating a high likelihood of biological activity. However, compound 3 does not meet the Veber and Muegge rule due to its high Log $P_{o/w}$ value.

Table 2. Correspondence to drug-likeness rules of synthesized compounds.

Drug-likeness rules correspondence					
Compound	Lipinski	Ghose	Veber	Egan	Muegge
1	+*	+	+	+	+
2	+	+	+	+	+
3	+	+	-**	+	-
4	+	+	+	+	+
5	+	+	+	+	+

**+ correspond; **- not correspond*

Table 3 presents calculated ADME data, which shows that all compounds, except for 5, are not inhibitors or substrates for permeability glycoprotein (Pgp), also known as MDR1 – multidrug resistance protein 1.

These findings suggest that compounds 1–4 are unlikely to exhibit drug resistance effects mediated by this protein. The calculated plasma clearance val-

ues indicate a relatively slow clearance rate, which may result in prolonged retention in the body and potentially lower dosing requirements. However, this could also increase the risk of accumulation and associated toxicity. Additionally, all compounds – except for compound 5 – are predicted to have oral bioavailability (F% > 20%), indicating favorable systemic exposure following oral administration.

None of the synthesized compounds are predicted to cross the blood–brain barrier, suggesting a low likelihood of central nervous system–related side effects. Furthermore, all compounds are expected to exhibit good intestinal absorption, with predicted absorption rates of at least 30%.

Table 3. Calculated ADME parameters for compounds 1-5.

ADME parameters						
Compound	Plasma clearance, mL/min/kg	Pgp inhibitor	Pgp substrate	HIA	BBB	F20%
1	3.36	-	-	>30%	-**	+*
2	5.46	-	-	>30%	-	+
3	5.23	-	-	>30%	-	+
4	3.10	-	-	>30%	-	+
5	6.79	-	+	>30%	-	-

HIA – human intestinal absorption, BBB – crossing of blood-brain barrier, F20% – bioavailability >20%; Pgp – permeability glycoprotein
**+Yes; **-No*

ANTIOXIDANT ACTIVITY ASSAY

The antioxidant activity of the synthesized compounds was also assessed, following the procedure described in (19). The results are shown in Figure 7. The data reveals that compounds 2, 3, and 4 do not possess significant antioxidant activity. However, compound 5 exhibits three times the antioxidant activity of Trolox, and compound 1 has six times the activity.

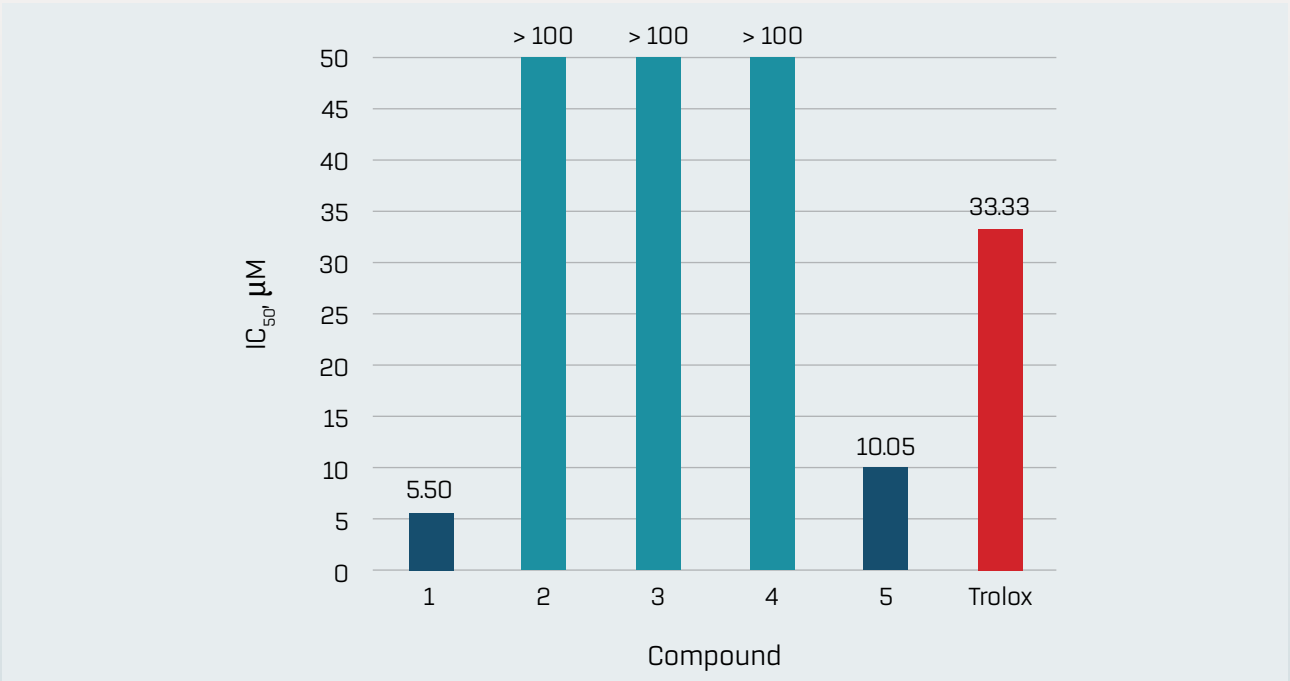

Figure 7. Antioxidant activity IC_{50} of tested compounds compared with Trolox.

DISSCUSIONS

The compounds were synthesized using to the procedures outlined in our previous work. The final yields of the compounds 1-5 can be considered good. All compounds, with the exception of compound 3, correspond to all the most widespread used drug likeness rules. The activity of the synthesized compounds, with the exception of compound 5, is not expected to be affected by MDR1- mediated drug resistance. Furthermore, the ADME profile obtained from the results indicates that the synthesized compounds possess drug likeness indices characteristic of medicinal compounds. Antioxidant analysis revealed that compound 1 and 5 exhibit stronger antioxidant properties than Trolox. Based on these findings and the ADME analysis, it can be assumed that compound 1 has the highest potential for reducing oxidative stress in biochemical systems.

CONCLUSIONS

1. Five thiosemicarbazone derivatives based on cinnamaldehyde were successfully synthesized, and their structures were confirmed through infrared spectroscopy.
2. The synthesized compounds exhibited favorable pharmacokinetic properties, including absorption, distribution, metabolism, and excretion, with the exception of compound 5, which deviates from commonly accepted drug-likeness criteria.
3. These compounds are not expected to cross the blood-brain barrier, thereby reducing the risk of adverse effects related to central nervous system interactions.
4. With the exception of compound 5, the compounds are unlikely to act as substrates or inhibitors of permeability glycoprotein, suggesting their bioavailability and activity are not significantly influenced by this efflux transporter.
5. Antioxidant assays revealed that compounds 1 and 5 exhibit significantly enhanced activity compared to Trolox, being approximately 6-fold and 3-fold more potent, respectively.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization, A.G.; methodology and compounds synthesis, R.R. and A.C.; validation, R.R., A.C., and A.G.; Antioxidant activity, A.C.; investigation, A.C., R.R.; writing – original draft preparation, A.C.; writing – review and editing, R.R., A.C., and A.G.; visualization, A.G, R.R., A.C; supervision, A.C., R.R., A.G.; project administration, A.G.; All authors have read and agreed to the published version of the manuscript.

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