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ANTIOXIDANT ACTIVITY AND DFT STUDIES OF ETHYL 2-(2-((6-METHYL-4-OXO-4*H*-CHROMEN-3-YL) METHYLENE)HYDRAZINYL)THIAZOLE-4-CARBOXYLATE

Adriana GROZAV[a], Gabriel MARC[a*], Cristina AZAROV[a],
Violeta NECULA[b], Smaranda ONIGA[c],
Tamas LOVASZ[d], Ovidiu CRIȘAN[a]

ABSTRACT. The microwave-assisted improved synthesis of ethyl 2-(2-((6-methyl-4-oxo-4*H*-chromen-3-yl)methylene)hydrazinyl)thiazole-4-carboxylate is described. The antioxidant potential of the target compound was evaluated using spectrophotometric methods, including the DPPH• and ABTS•$^+$ radical scavenging assays, as well as three additional electron-transfer assays employing metal-based oxidizing agents. The title compound exhibited good antioxidant activity compared to reference antioxidants. For the title compound theoretical quantum chemical (DFT) calculations were performed.

Keywords: *microwave-assisted synthesis, antioxidant activity, DFT calculation, chromene, thiazole;*

1. INTRODUCTION

Free radicals are highly reactive molecular species that result from metabolic processes in the body and contain an unpaired electron. They can be neutralized by the body's natural defense systems, but when the regulatory capacity is exceeded, a condition known as oxidative stress occurs, in which free radicals attack biomolecules, causing their deterioration. Antioxidant

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[a] *"Iuliu Hațieganu" University of Medicine and Pharmacy, Faculty of Pharmacy, Department of Organic Chemistry, 8 Victor Babes, Cluj-Napoca, RO-400012, Romania.*
[b] *"Iuliu Hațieganu" University of Medicine and Pharmacy, ENT Department Cluj-Napoca, Romania.*
[c] *"Iuliu Hațieganu" University of Medicine and Pharmacy, Faculty of Pharmacy, Department of Therapeutical Chemistry, 12 Ion Creangă, Cluj-Napoca, RO-400010, Romania.*
[d] *Babeş-Bolyai University, Faculty of Chemistry and Chemical Engineering, 11 Arany Janos str., RO-400028, Cluj-Napoca, Romania.*
* *Corresponding author: marc.gabriel@umfcluj.ro*

compounds neutralize free radicals and are considered essential for maintaining health, reducing the risk of developing various diseases. The most well-known mechanism through which this effect is achieved consists in donating a hydrogen atom to the free radicals, eliminating the unpaired electron and transforming them into non-reactive species [1],[2],[3].

Compounds containing thiazole or hydrazinothiazole unit have been widely reported in the literature to exhibit a broad spectrum of biological activities, including antitumor [4,5], antimicrobial [6], and antioxidant activities [7–9].

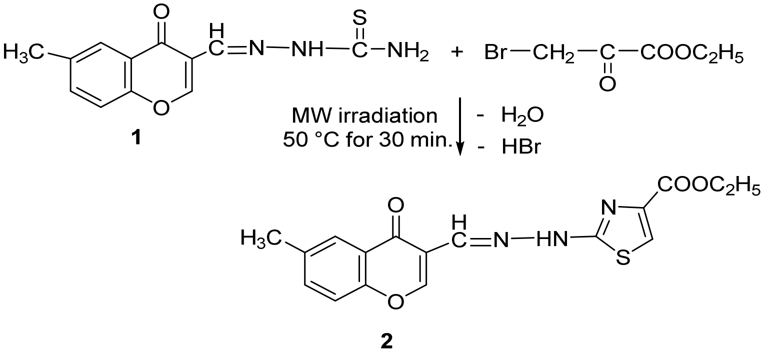
In addition, chromone (benzo-γ-pyrone) and its derivatives are versatile molecular scaffolds capable of interacting with various receptors, exhibiting diverse biological activities, including notable antioxidant properties [10–12].

This study aimed to integrate the two moieties into a single molecular entity using an improved approach by means of a microwave-assisted synthesis. Based on the aforementioned premises, the synthesized chromene–thiazole hybrid compound was subsequently evaluated for its antioxidant activity using multiple assays.

2. RESULTS AND DISCUSSION

2.1. Chemical synthesis

2-((6-Methyl-4-oxo-4H-chromen-3-yl)methylene) thiosemicarbazone (**1**) [13,14] was subjected to a Hantzsch-type cyclization with ethyl 3-bromo-2-oxopropanoate under microwave-assisted conditions. The reaction was performed in DMF at 50°C, 50W for 30 min. Under these experimental conditions, microwave-assisted synthesis lead to the obtention of ethyl 2-(2-((6-methyl-4-oxo-4H-chromen-3-yl)methylene) hydrazinyl)thiazole-4-carboxylate (**2**) in a substantially shorter reaction time than the conventional approach previously reported by our group [15].



Scheme 1. Synthesis of ethyl-2-(2-((6-methyl-4-oxo-4H-chromen-3-yl)methylene)hydrazineyl)thiazole-4-carboxylate (**2**)

2.2. Antioxidant activity evaluation

The determination of in vitro antioxidant activity of compound **2** was carried out using standard spectrophotometric methods based on the ability of compounds to scavenge free radicals or reduce metal-based oxidizing agents. The antioxidant potential of the newly synthesized chromone–thiazole derivative was evaluated using the following methods: DPPH (1,1-diphenyl-2-picrylhydrazyl), ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate)), FRAP (Ferric Reducing Antioxidant Power), RP (Reducing Power), and TAC (Total Antioxidant Capacity), which are based on hydrogen transfer mechanisms and electron transfer, respectively. The results of the antioxidants assays are presented in Tables 1 and 2.

Table 1. The antioxidant activity of compound 2 determined in the DPPH and ABTS radical scavenging assays expressed as IC50 (µM)

Compound	**DPPH$^{\bullet}$ scavenging**	**ABTS$^{\bullet+}$ scavenging**
2	64.38	18.92
Trolox	36.23	14.68

The results presented in Table 1 show a higher IC_{50} (64.38 µM) for compound 2 compared to the standard IC_{50} (36.23 µM), indicating an approximately twofold lower antioxidant potential in the DPPH radical scavenging method. On the other hand, the ABTS assay reveals a comparable antioxidant activity of compound 2 IC_{50} (18.92 µM) to that of the standard IC_{50} (14.68 µM).

Table 2. The antioxidant activity of compound 2 determined in the FRAP, RP and TAC assays, expressed as molar equivalents of reference agents – ascorbic acid, trolox and butylated hydroxytoluene, respectively

FRAP			**RP**			**TAC**		
AAE	**TE**	**BHTE**	**AAE**	**TE**	**BHTE**	**AAE**	**TE**	**BHTE**
1.78	0.57	0.86	0.49	0.62	0.47	0.36	0.57	0.78

AAE: Ascorbic Acid Equivalent Mol, **TE:** Trolox Equivalent Mol, **BHTE:** Butylated Hydroxytoluene Equivalent Mol

In the FRAP assay, compound 2 had the highest activity, particularly in ascorbic acid equivalents (1.78 AAE). This value surpasses the corresponding trolox (0.57 TE) and BHT equivalents (0.86 BHTE), suggesting that compound 2 exhibits a strong electron-donating capacity, more comparable to ascorbic acid than to the other reference antioxidants.

In the RP assay the reducing power values are lower and more uniform across standards, ranging from 0.47 to 0.62. Compound 2 displayed a moderate reducing ability, with slightly higher activity relative to trolox (0.62 TE). The title compound, under these conditions, had a stable but not exceptionally high reducing capacity, but approximately a half of the activity of the reference antioxidants.

The TAC assay yields the lowest values overall (0.36–0.78 molar equivalents), highlighting a reduced total antioxidant capacity compared to FRAP. However, compound 2 demonstrates relatively stronger activity when expressed in BHT equivalents (0.78 BHTE), suggesting that its efficiency in this assay may be influenced by radical scavenging mechanisms or by interactions specific to the assay’s reaction environment.

A comparison of the three methods shows that FRAP provides the strongest antioxidant potential for compound 2, whereas TAC reveals the weakest. This variability underscores that the antioxidant behavior of compound 2 is highly dependent on the redox system evaluated in each specific reaction environment.

2.3. DFT Calculations

DFT calculations were performed to evaluate some molecular properties and the frontier molecular orbital energies (HOMO and LUMO) in vacuum and in three solvents, to evaluate how the solvation environment may interact with compound **2**. The results are presented in Tables 3 and 4.

Table 3. Molecular properties of compound 2.

Area (Å^2)	**Polar Surface Area (Å^2)**	**Volume (Å^3)**	**LogP**	**Dipole moment (D)**
358.85	69.068	334.96	2.59	4.01

The calculated molecular properties of compound 2 indicate a balanced distribution between polar and nonpolar regions. The molecular volume (334.96 Å^3) is consistent with a compact structure, typical for molecules of this size. The calculated LogP value of 2.59 denotes moderate lipophilicity, implying that the compound can interact with both hydrophobic and hydrophilic environments, favoring membrane permeability. The dipole moment of 4.01 D reflects a moderate molecular polarity, which may contribute to favorable solvation in polar media while maintaining a hydrophobic character. The polar regions of the molecule electron-deficient were identified where electronegative atoms are found (the two chromone oxygen atoms, the oxygen atoms from the carboxylic ester, the azomethine nitrogen atom, the thiazole nitrogen atom), while the electron-rich regions are in the N-H group and in the position 5 of the thiazole. The graphical depiction of the aforementioned data is presented in Figure 1.

Table 4. The energy levels of HOMO and LUMO, across the four environments studied for compound 2 (eV).

Vacuum			**Nonpolar solvent (ε=7.43)**			**Polar solvent (ε=37.22)**			**Water**		
HOMO	**LUMO**	**gap**	**HOMO**	**LUMO**	**gap**	**HOMO**	**LUMO**	**gap**	**HOMO**	**LUMO**	**gap**
-6.03	-1.87	4.16	-6.01	-1.92	4.09	-6.01	-1.94	4.07	-6.01	-1.94	4.07

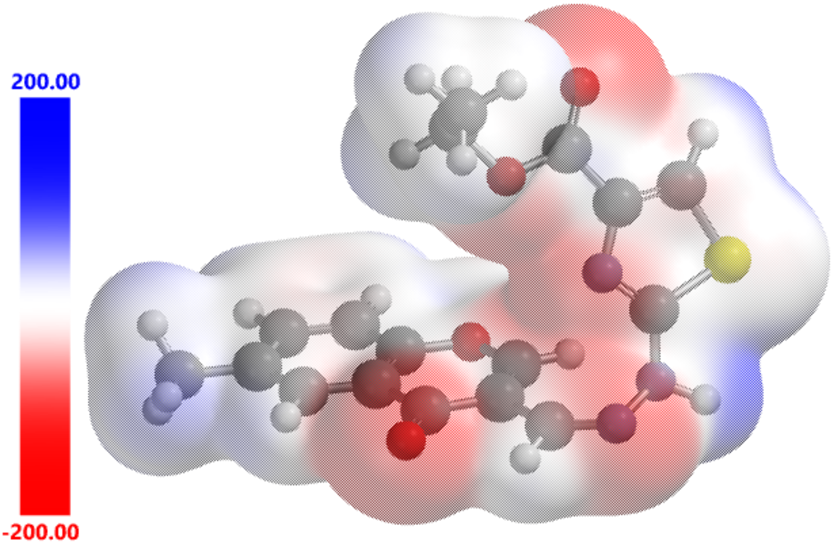


Figure 1. The electrostatic potential map for compound 2

In the studied compound, HOMO is found across the hydrazone and the thiazole ring and slight on the pyrone moiety from chromone, while LUMO is found across the whole chromone moiety (Figure 2). HOMO and LUMO energies show minimal variation across different environments. The HOMO level remains nearly constant at around −6.0 eV, while the LUMO decreases slightly (from −1.87 eV in vacuum to −1.94 eV in polar solvents). This indicates that solvent induced effect, even in nonpolar, polar or high polar media such as water, has some effect on the frontier orbital energies of the molecule, reducing the gap between the two frontier molecular orbitals. The respective effect is attributed mainly to the variation of LUMO energy levels and less to the change of the HOMO energy levels. The low change in HOMO levels, indicate a low variability of the antioxidant properties of the molecule 2 in different solvents with different electronic properties. The small stabilization of LUMO in solvents suggests a weak solvent–solute electronic interaction and limited charge delocalization upon solvation.

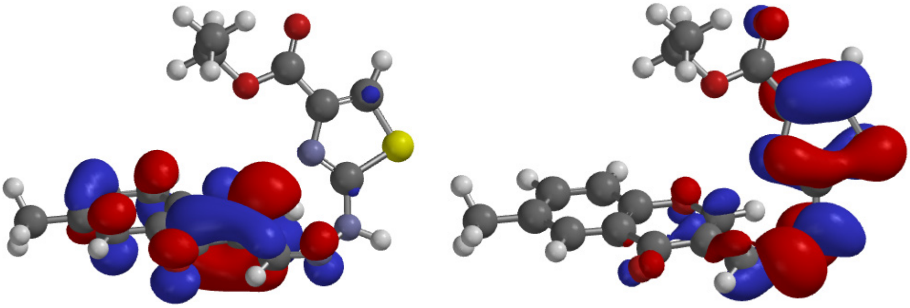


Figure 2. The graphical depiction of frontier molecular orbitals HOMO (left), LUMO (right) of compound 2.

The compound exhibited a good radical-scavenging activity against DPPH• and a very good against ABTS•+, together with a good reducing power in all three assays when compared to the reference compounds. These findings indicate that the conjugated π-system created through the hydrazone bridge effectively facilitates electron transfer—a key process involved in antioxidant reactions. The reducing character suggests that the molecule can readily donate electrons to stabilize oxidative species, which aligns with the behavior of other chromone-based antioxidants reported in the literature.

To better understand these results at a molecular level, DFT calculations were per-formed, which indicated the pyrone-hydrazone-thiazole being the moieties redox active in the present compound. These theoretical insights supported the experimental data by revealing that the electron-rich regions of the molecule—particularly those located across the extended aromatic system—play a central role in radical neutralization, consistent with the orbital distribution observed in previous studies on similar heterocyclic antioxidants. According to the distribution of electrons across the molecule, the substituent from the position 4 of the thiazole ring have no evident contribution to the antioxidant activity of the studied compound.

Overall, the combination of experimental assays and computational analysis highlights the potential of this chromone–thiazole hydrazone scaffold as a promising antioxidant candidate. Considering its favorable redox properties and structural versatility, future research should explore structural modifications aimed at further enhancing electronic delocalization, as well as biological evaluations in cellular oxidative stress models. These directions could provide deeper insight into structure–activity relationships and expand the applicability of this hybrid system in medicinal or materials chemistry.

3. CONCLUSIONS

The microwave-assisted synthesis of the ethyl-2-(2-((6-methyl-4-oxo-4H-chromen-3-yl)methylene)hydrazineyl)thiazole-4-carboxylate proved to be an efficient and advantageous approach, enabling the preparation of the chromene–thiazole derivative within a significantly reduced reaction time (30 minutes) compared to the conventional classical synthesis, which requires approximately 24 hours. The synthesized compound exhibited antioxidant activity comparable to that of the reference standards used (ascorbic acid, Trolox, and BHTE), highlighting its relevant biological potential. The experimental findings were further supported and validated by theoretical studies based on Density Functional Theory (DFT) calculations, which confirmed the correlation between structural properties and the observed antioxidant activity. Overall, these results emphasize the utility of microwave-assisted synthesis and the relevance of the obtained chromene–thiazole derivative as a potential candidate for future applications.

4. EXPERIMENTAL SECTION

4.1. Chemical synthesis

All reagents, solvents and chemicals were obtained from commercial sources (Sigma-Aldrich and Riedel-de Haënand used as received without further purification. The uncorrected melting point was measured with a

Buchi M-560 device (BÜCHI Labortechnik AG, Flawil, Switzerland). Colorimetric measurements were recorded with a UV-VIS Jasco V-530 spectrophotometer (Jasco International Co., Tokyo, Japan) at room temperature against specific blank samples. Thin-layer chromatography (TLC) was performed on 0.2 mm silica gel 60 plates (Fluka, Merck KGaA, Darmstadt, Germany). The microwave-assisted synthesis experiments were performed using the CEM Discover Microwave Synthesizer system (CEM Corporation, Matthews, NC, USA).

4.1.1. Microwave assisted synthesis of ethyl-2-(2-((6-methyl-4-oxo-4H-chromen-3-yl)methylene)hydrazineyl)thiazole-4-carboxylate (compound 2)

A mixture of 2-((6-methyl-4-oxo-4H-chromen-3-yl)methylene) thiosemicarbazone (87 mg, 0.33 mmol) and ethyl 3-bromo-2-oxopropanoate (60 mg, 0.33 mmol) in DMF (2 mL) was irradiated under microwave conditions for 30 min at 50 °C and 50 W. The progress of the reaction was monitored by thin-layer chromatography (TLC) using heptane/ ethyl acetate/ethanol (7:3:0.5, v/v/v) as the eluent on silica gel plates. After completion, the reaction mixture was neutralized to pH 7 with an aqueous $NaHCO_3$ solution (10%). The resulting precipitate was filtered, dried under reduced pressure, and recrystallized from DMF (5 mL) to afford a pale yellow powder (77 mg, 65% yield). Compound 2 was identified by melting point determination (m.p. = 248°C) in accordance with reported literature data [15] and mass spectrometry (m/z = 358.0874, APCI, $[M+H]^+$).

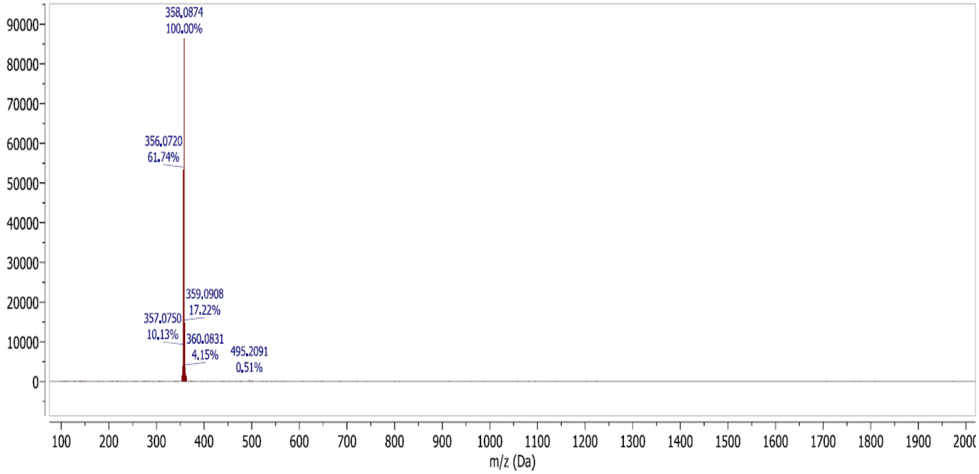


Figure 3. APCI mass spectra recorded for compound 2 (positive ionization mode).

4.2. Antioxidant activity evaluation

The protocols used for the antiradical assays and for the electron transfer assays were previously reported by our group in our previous papers [16]. Briefly, the respective assays will be presented in the following subsections [4].

4.2.1. Antiradical assays

For evaluation of the antiradical properties of the compound 2, two radical scavenging assays were employed - DPPH• and ABTS•+. The reducing of the absorbance of the two radicals was calculated using the following equation:

$$\text{radical scavenging (\%)} = \text{(control absorbance-sample absorbance)/}\\ \text{(control absorbance)}\times 100 \qquad (1)$$

The 1,1-diphenyl-2-picrylhydrazyl (DPPH•) scavenging test relies on the ability of the investigated compound to donate a hydrogen atom to the intensely colored DPPH• radical, which contains an unpaired electron and exhibits a characteristic absorption peak at 517 nm. A reduction in the absorbance of the reaction mixture indicates radical neutralization, and the scavenging activity was quantified using the presented equation.

The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) scavenging assay was carried out in a 0.1 M potassium phosphate buffer (pH=7.4) after generating the ABTS•+ radical with MnO_2. The reduction of absorbance at 734 nm was measured spectrophotometrically, and the percentage of radicals quenched was calculated according to the presented equation.

4.2.2. Electron transfer assays

All electron-transfer–based assays were carried out using equimolar amounts of the tested compound 2 and the corresponding reference antioxidants, under specific experimental conditions for each method [17].

The activity of compound 2, expressed relative to the activity of an equimolar quantity of the standard compound, was determined according to the following equation in the three following assays:

$$\text{\% of control activity} = \text{(sample absorbance)/}\\ \text{(reference absorbance)}\times 100$$

The Ferric Reducing Antioxidant Power (FRAP) assay followed the procedure originally described by Benzie and Strain, with minor modifications [18]. In this method, the analyte reduces Fe^{3+} from the reagent to Fe^{2+}, which subsequently forms an intense blue complex with a chromogenic ligand, exhibiting maximal absorbance at 593 nm.

Total Antioxidant Capacity (TAC) was assessed by monitoring the reduction of the phosphomolybdate reagent by the test compounds upon heating, resulting in the formation of a green-colored complex with an absorption peak at 695 nm.

For the Reducing Power (RP) assay, the principle involves the reduction of $[Fe(CN)_6]^{3-}$ to $[Fe(CN)_6]^{4-}$ by the electron-donating activity of the sample. The generated ferrocyanide reacts with ferric ions to produce a blue complex, which absorbs maximally at 700 nm.

4.3. DFT Calculations

To investigate the electronic and structural characteristics of the compound 2 in silico DFT calculations were performed with the B3LYP functional method and the 6-311G(2D,P) basis set using Spartan 24 1.3.1 (Wavefunction, Inc., Irvine, CA, USA) under Microsoft Windows 10, on a machine with Intel 12700KF CPU. The calculations were performed under vacuum, in a nonpolar solvent ($\varepsilon = 7.43$), a polar solvent ($\varepsilon = 37.22$), and in water.

Abbreviations

The following abbreviations are used in this manuscript:

ABTS	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate)
DFT	Density Functional Theory
DMF	Dimethylformamide
DPPH	1,1-diphenyl-2-picrylhydrazyl
FRAP	Ferric Reducing Antioxidant Power
HOMO	Highest Occupied Molecular Orbital
LUMO	Lowest Unoccupied Molecular Orbital
MP	Melting Point
RP	Reducing Power
TAC	Total Antioxidant Capacity

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