

Synthesis and *In Vitro* Evaluation of Novel Thiazole-Hydrazone Derivatives with Antioxidant and Cytotoxic Potential

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ABSTRACT


A new series of thiazole–hydrazone derivatives (2a–2l) was synthesized and evaluated for their *in vitro* antioxidant and cytotoxic properties. The structures of the synthesized compounds were confirmed by ¹H NMR, ¹³C NMR, and HRMS analyses. Antioxidant activity was assessed using the DPPH radical scavenging assay, while cytotoxicity was evaluated using the MTT assay in human colorectal cancer (HCT116) and non-small cell lung cancer (H460) cell lines. In the DPPH assay, compounds 2c and 2h exhibited the notable radical scavenging activity, with IC₅₀ values of 31.73 ± 0.004 μM and 31.13 ± 0.008 μM, respectively, comparable to the reference compound gallic acid (IC₅₀ = 29.48 ± 0.014 μM). Cytotoxicity screening performed at 100 μM for 24 h indicated that most derivatives did not significantly reduce cell viability in HCT116 cells. In contrast, a partial decrease in cell viability was observed in H460 cells for the selected compounds, with compound 2l showing a moderate effect (IC₅₀ = 82.00 ± 3.67 μM). The results indicate that the biological activity of these thiazole–hydrazone derivatives is strongly influenced by substitution patterns. While the compounds exhibited limited antiproliferative effects under the tested conditions, certain derivatives demonstrated notable antioxidant activity. These findings suggest that this scaffold may serve as a useful starting point for further structural optimization toward multifunctional bioactive molecules



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Keywords: Antioxidant activity, Cytotoxicity, DPPH assay, Hydrazone, MTT assay, Thiazole derivatives

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1. Introduction

Thiazole and hydrazone derivatives represent important structural motifs in medicinal chemistry due to their chemical versatility and broad pharmacological potential. Numerous studies have reported that these heterocyclic frameworks exhibit a wide range of biological activities, including antimicrobial, anti-inflammatory, antitubercular, antiviral, antiparasitic, and anticancer effects [1–7]. Because of their structural diversity and tunable electronic properties, thiazole-based compounds have attracted considerable attention as promising scaffolds in drug discovery [1,8]. The thiazole ring is also present in several biologically relevant molecules and clinically used drugs. For example, it constitutes an essential part of vitamin B1 (thiamine), which plays a key role in carbohydrate metabolism and neuronal function [9]. In addition, a variety of thiazole-containing pharmaceuticals have been reported in the literature, including antibiotics such as penicillin, antitumor agents such as bleomycin, antiviral drugs such as ritonavir, and antiulcer drugs such as nizatidine [10–12]. Furthermore, thiazole derivatives have been investigated as ligands for estrogen receptors and as antagonists of adenosine receptors, highlighting their importance as pharmacologically versatile heterocyclic frameworks

[13,14]. Hydrazone derivatives have likewise emerged as an important class of bioactive molecules. The presence of the azomethine (-NH–N=CH-) functional group provides structural flexibility and facilitates interactions with various biological targets. Consequently, hydrazone-containing compounds have been reported to exhibit diverse biological activities, including antimicrobial, antimalarial, anticonvulsant, anti-inflammatory, antiviral, and anticancer effects [15–19]. Because of these properties, hydrazone derivatives have been widely explored as pharmacophores in the design of novel therapeutic agents [20,21].

Recent studies have suggested that hybrid structures containing both thiazole and hydrazone moieties may exhibit enhanced biological activity. For example, several thiazole-based derivatives have been reported to demonstrate antioxidant, enzyme-inhibitory, and antiproliferative effects in different biological systems [22–24]. In addition, structure–activity relationship (SAR) analyses have shown that the introduction of electron-withdrawing substituents on aromatic rings can significantly influence the biological properties of thiazole-containing compounds, particularly their antioxidant and cytotoxic activities [22,25]. Despite these

advances, inden-based thiazole–hydrazone derivatives remain relatively underexplored, especially regarding their combined antioxidant and anticancer potential. Considering the important role of oxidative stress in cancer progression and the continuous need for new heterocyclic scaffolds with biological activity, further investigation of such hybrid molecules is warranted. Therefore, in the present study, a new series of thiazole hydrazone derivatives (2a–2l) was synthesized and structurally characterized by ^1H NMR, ^{13}C NMR, and HRMS analyses. The antioxidant potential of the synthesized compounds was evaluated using the DPPH radical scavenging assay, while their cytotoxic effects were investigated using the MTT assay in HCT116 and H460 cancer cell lines. The compounds exhibited limited antiproliferative effects under the tested conditions. The obtained results were further analyzed to explore preliminary structure–activity relationships (SAR) within the synthesized compound series.

2. Materials and Methods

2.1. Chemistry

All starting materials and chemicals used in the synthetic procedure were purchased from Sigma-Aldrich Chemicals (Sigma-Aldrich Corp., St. Louis, MO, USA) or Merck Chemicals (Merck KGaA, Darmstadt, Germany). Melting points of the obtained compounds were determined by MP90 digital melting point apparatus (Mettler Toledo, OH, USA) and were uncorrected. ^1H -NMR, and ^{13}C -NMR spectra of the synthesized compounds were registered by a Bruker 300 MHz and 75 MHz digital FT-NMR spectrometer (Bruker Bioscience, Billerica, MA, USA) in DMSO- d_6 , respectively. HRMS studies were performed on an LCMS-IT-TOF system (Shimadzu, Tokyo, Japan). All reactions were monitored by thin-layer chromatography (TLC) using Silica Gel 60 F254 TLC plates (Merck KGaA, Darmstadt, Germany).

2.2. Synthesis of 2-(5-methoxy-2,3-dihydro-1H-inden-1-ylidene)hydrazine-1-carbothioamide (1):

5-Methoxy-2,3-dihydro-1H-inden-1-one (3 gr, 0.012 mol) and thiosemicarbazide (2.02 gr, 0.022 mol) were dissolved in ethanol (25 mL). Then, it was stirred under reflux for 3 hours. At the end of the reaction, the mixture was cooled in an ice bath, and the precipitated product was filtered off [23] Yield: 87 %.

2.3. Synthesis of Target Compounds (2a-2l)

2-(5-Methoxy-2,3-dihydro-1H-inden-1-ylidene)hydrazine-1-carbothioamide (1) (0.2 gr, 0.001 mol) and the appropriate 2-bromoacetophenone derivative (0.001 mol) were dissolved in ethanol (15 mL). Then, it was stirred under reflux for 4 hours. At the end of the reaction, the mixture was cooled in an ice bath, and the precipitated product was filtered off [23].

The structures of the compounds were elucidated by ^1H NMR, ^{13}C NMR, and HRMS.

2-(2-(5-Methoxy-2,3-dihydro-1H-inden-1-ylidene)hydrazineyl)-4-(4-nitrophenyl)thiazole (2a):

Yield: 85% M.P.= 243.9 °C. ^1H -NMR (300 MHz, DMSO- d_6): δ : 2.90-2.92 (2H, m, CH₂), 2.99 (2H, s, CH₂), 3.80 (3H, s, OCH₃), 6.96 (1H, dd, $J_1=2.52$ Hz, $J_2=8.31$ Hz, Aromatic CH), 7.07 (1H, s, Aromatic CH), 7.28 (1H, d, $J=8.31$ Hz, Aromatic CH), 7.71 (1H, s, Aromatic CH), 8.12 (2H, d, $J=8.91$ Hz, Aromatic CH), 8.28 (2H, d, $J=8.94$ Hz, Aromatic CH), 11.25 (1H, s, NH). ^{13}C -NMR (75 MHz, DMSO- d_6): δ = 27.82, 28.73, 55.71, 104.21, 109.13, 118.26, 124.57, 126.74, 126.91, 139.34, 140.67, 141.34, 146.57, 149.06, 156.95, 159.23, 170.31. HRMS (m/z): [M+H]⁺ calcd for C₁₉H₁₆N₄O₃S: 381.1016; found: 381.1035.

2-(2-(5-Methoxy-2,3-dihydro-1H-inden-1-ylidene)hydrazineyl)-4-(4-methoxyphenyl) thiazole (2b):

Yield: 88 % M.P.= 229.4 °C. ^1H -NMR (300 MHz, DMSO- d_6): δ : 2.90 (2H, s, CH₂), 3.00 (2H, s, CH₂), 3.73 (3H, s, OCH₃), 3.79 (3H, s, OCH₃), 6.93-6.99 (3H, m, Aromatic CH), 7.08 (1H, s, Aromatic CH), 7.15 (1H, s, Aromatic CH), 7.26-7.27 (1H, m, Aromatic CH), 7.66 (1H, d, $J=8.13$ Hz, Aromatic CH), 7.80 (1H, d, $J=8.34$ Hz, Aromatic CH). ^{13}C -NMR (75 MHz, DMSO- d_6): δ = 27.82, 28.68, 55.65, 57.15, 114.25, 114.43, 117.68, 118.42, 121.87, 126.92, 127.36, 127.91, 129.18, 130.98, 141.14, 147.19, 159.31, 169.77. HRMS (m/z): [M+H]⁺ calcd for C₂₀H₁₉N₃O₂S: 366.1271; found: 366.1265.

2-(2-(5-Methoxy-2,3-dihydro-1H-inden-1-ylidene)hydrazineyl)-4-(4-cyanophenyl) thiazole (2c):

Yield: 77 % M.P.= 269.1 °C. ^1H -NMR (300 MHz, DMSO- d_6): δ : 2.88 (2H, s, CH₂), 2.98 (2H, s, CH₂), 3.80 (3H, s, OCH₃), 6.95-6.97 (1H, m, Aromatic CH), 7.07 (1H, s, Aromatic CH), 7.64-7.68 (1H, m, Aromatic CH), 7.90-7.93 (2H, m, Aromatic CH), 8.07-8.11 (2H, m, Aromatic CH). ^{13}C -NMR (75 MHz, DMSO- d_6): δ =27.82, 28.83, 55.76, 104.21, 118.57, 118.71, 119.04, 119.18, 126.57, 126.93, 128.86, 129.31, 132.62, 132.85, 133.15, 139.34, 159.25, 169.30. HRMS (m/z): [M+H]⁺ calcd for C₂₀H₁₆N₄OS: 361.1118; found: 361.1123.

4-([1,1'-biphenyl]-4-yl)-2-(2-(5-methoxy-2,3-dihydro-1H-inden-1-ylidene)hydrazineyl) thiazole (2d):

Yield: 81% M.P.= 219.3 °C. ^1H -NMR (300 MHz, DMSO- d_6): δ : 2.91 (2H, s, CH₂), 3.00 (2H, s, CH₂), 3.81 (3H, s, OCH₃), 6.97 (1H, dd, $J_1=2.49$ Hz, $J_2=8.31$ Hz, Aromatic CH), 7.09 (1H, s, Aromatic CH), 7.28-7.31 (1H, m, Aromatic CH), 7.38-7.39 (2H, m, Aromatic CH), 7.46-7.48 (2H, m, Aromatic CH), 7.71-7.74 (4H, m, Aromatic CH), 7.83-7.86 (1H, m, Aromatic CH), 7.97 (1H, d, $J=8.28$ Hz, Aromatic CH). ^{13}C -NMR (75 MHz, DMSO- d_6): δ = 27.83, 28.70, 55.74, 104.24, 104.48, 118.19, 126.58, 126.92, 127.04, 127.06, 127.29, 127.93, 128.34, 129.44, 134.24, 139.45, 140.63, 147.36, 156.78, 159.24, 170.00. HRMS (m/z): [M+H]⁺ calcd for C₂₅H₂₁N₃OS: 412.1478; found: 412.1496.

2-(2-(5-Methoxy-2,3-dihydro-1H-inden-1-ylidene)hydrazineyl)-4-(4-bromophenyl) thiazole (2e):

Yield: 79 % M.P.= 252.9 °C. ¹H-NMR (300 MHz, DMSO-d₆): δ: 2.89 (2H, s, CH₂), 2.99 (2H, s, CH₂), 3.80 (3H, s, OCH₃), 6.96 (1H, dd, *J*₁=2.49 Hz, *J*₂=8.28 Hz, Aromatic CH), 7.07 (1H, s, Aromatic CH), 7.28 (1H, d, *J*=8.28 Hz, Aromatic CH), 7.41 (1H, s, Aromatic CH), 7.54 (1H, s, Aromatic CH), 7.61 (1H, d, *J*=8.55 Hz, Aromatic CH), 7.83 (2H, d, *J*=8.52 Hz, Aromatic CH), 11.15 (1H, s, NH). ¹³C-NMR (75 MHz, DMSO-d₆): δ= 27.82, 28.69, 55.76, 104.21, 105.13, 118.68, 121.00, 124.13, 126.92, 128.02, 129.78, 130.80, 131.81, 132.00, 142.19, 159.03, 169.43.

2-(2-(5-Methoxy-2,3-dihydro-1H-inden-1-ylidene)hydrazineyl)-4-(4-methylphenyl) thiazole (2f):

Yield: 77 % M.P.= 234.6 °C. ¹H-NMR (300 MHz, DMSO-d₆): δ: 2.33 (3H, s, CH₃), 2.90 (2H, s, CH₂), 2.99 (2H, s, CH₂), 3.81 (3H, s, OCH₃), 6.96 (1H, dd, *J*₁=2.52 Hz, *J*₂=8.28 Hz, Aromatic CH), 7.07 (1H, s, Aromatic CH), 7.22 (3H, d, *J*=8.46 Hz, Aromatic CH), 7.28 (1H, d, *J*=8.25 Hz, Aromatic CH), 7.76 (2H, d, *J*=8.04 Hz, Aromatic CH). ¹³C-NMR (75 MHz, DMSO-d₆): δ= 21.28, 27.82, 28.70, 55.73, 103.36, 104.21, 118.23, 120.25, 125.99, 126.94, 127.64, 129.65, 129.96, 137.32, 139.40, 140.65, 159.22, 169.72. HRMS (m/z): [M+H]⁺ calcd for C₂₀H₁₉N₃OS: 350.1322; found: 350.1319.

2-(2-(5-Methoxy-2,3-dihydro-1H-inden-1-ylidene)hydrazineyl)-4-(3,4-dichlorophenyl) thiazole (2g):

Yield: 86 % M.P.= 260.8 °C. ¹H-NMR (300 MHz, DMSO-d₆): δ: 2.88 (2H, s, CH₂), 2.99 (2H, s, CH₂), 3.77 (3H, s, OCH₃), 6.95-6.99 (2H, m, Aromatic CH), 7.24-7.29 (3H, m, Aromatic CH), 7.70-7.74 (2H, m, Aromatic CH). ¹³C-NMR (75 MHz, DMSO-d₆): δ= 27.25, 28.35, 55.91, 101.68, 104.62, 110.60, 116.23, 118.55, 120.99, 121.60, 124.78, 129.55, 130.29, 131.16, 131.99, 140.79, 146.17, 159.98, 169.63.

2-(2-(5-Methoxy-2,3-dihydro-1H-inden-1-ylidene)hydrazineyl)-4-(2,4-difluorophenyl) thiazole (2h):

Yield: 80 % M.P.= 220.5 °C. ¹H-NMR (300 MHz, DMSO-d₆): δ: 2.90 (2H, s, CH₂), 2.99 (2H, s, CH₂), 3.80 (3H, s, OCH₃), 6.96 (1H, dd, *J*₁=2.49 Hz, *J*₂=8.31 Hz, Aromatic CH), 7.08 (1H, s, Aromatic CH), 7.19-7.22 (2H, m, Aromatic CH), 7.28 (1H, d, *J*=8.34 Hz, Aromatic CH), 7.33-7.39 (1H, m, Aromatic CH), 8.04-8.07 (1H, m, Aromatic CH), 11.22 (1H, s, NH). ¹³C-NMR (75 MHz, DMSO-d₆): δ= 27.82, 28.77, 55.72, 104.22, 104.67, 105.02, 105.37, 108.26, 108.44, 112.14, 112.45, 118.33, 126.93, 130.82, 139.33, 140.72, 157.17, 159.24, 169.28. HRMS (m/z): [M+H]⁺ calcd for C₁₉H₁₅N₃O₂F₂S: 372.0977; found: 372.1002.

2-(2-(5-Methoxy-2,3-dihydro-1H-inden-1-ylidene)hydrazineyl)-4-(4-chlorophenyl) thiazole (2i):

Yield: 82 % M.P.= 252.7 °C. ¹H-NMR (300 MHz, DMSO-d₆): δ: 2.93 (2H, s, CH₂), 3.07 (2H, s, CH₂), 3.79 (3H, s, OCH₃), 6.99 (1H, s, Aromatic CH), 7.16 (1H, s, Aromatic CH), 7.33 (2H, s, Aromatic CH), 7.56 (1H, s, Aromatic CH), 7.68 (2H, d, *J*=8.64 Hz, Aromatic CH), 7.96 (1H, d, *J*=8.64 Hz, Aromatic CH), ¹³C-NMR (75 MHz, DMSO-d₆): δ= 26.76,

28.51, 55.87, 104.40, 106.27, 113.90, 115.28, 120.28, 127.15, 128.99, 129.95, 131.86, 134.78, 144.16, 149.78, 154.54, 171.66. HRMS (m/z): [M+H]⁺ calcd for C₁₉H₁₆N₃O₂Cl: 370.0775; found: 370.0764.

2-(2-(5-Methoxy-2,3-dihydro-1H-inden-1-ylidene)hydrazineyl)-4-(2,4-dichlorophenyl) thiazole (2j):

Yield: 88 % M.P.= 190.8 °C. ¹H-NMR (300 MHz, DMSO-d₆): δ: 2.89 (2H, s, CH₂), 3.00 (2H, s, CH₂), 3.81 (3H, s, OCH₃), 6.97 (1H, dd, *J*₁=2.40 Hz, *J*₂=8.19 Hz, Aromatic CH), 7.08 (1H, s, Aromatic CH), 7.29 (1H, d, *J*=8.04 Hz, Aromatic CH), 7.41 (1H, s, Aromatic CH), 7.52 (1H, dd, *J*₁=2.13 Hz, *J*₂=6.36 Hz, Aromatic CH), 7.71 (1H, s, Aromatic CH), 7.95 (1H, d, *J*=8.49 Hz, Aromatic CH), 11.15 (1H, s, NH). ¹³C-NMR (75 MHz, DMSO-d₆): δ= 27.81, 28.72, 55.72, 104.17, 105.11, 109.75, 118.24, 119.00, 126.95, 127.95, 128.77, 130.23, 131.91, 132.70, 132.87, 139.38, 140.64, 159.23, 168.97.

2-(2-(5-Methoxy-2,3-dihydro-1H-inden-1-ylidene)hydrazineyl)-4-phenylthiazole (2k):

Yield: 87 % M.P.= 258.7 °C. ¹H-NMR (300 MHz, DMSO-d₆): δ: 2.90 (2H, s, CH₂), 2.99 (2H, s, CH₂), 3.81 (3H, s, OCH₃), 6.95-6.98 (1H, m, Aromatic CH), 7.08 (1H, s, Aromatic CH), 7.28-7.33 (3H, m, Aromatic CH), 7.40-7.45 (2H, m, Aromatic CH), 7.68 (1H, d, *J*=7.89 Hz, Aromatic CH), 7.86-7.89 (1H, m, Aromatic CH). ¹³C-NMR (75 MHz, DMSO-d₆): δ= 27.83, 28.79, 55.77, 104.25, 108.07, 118.48, 118.64, 126.03, 126.93, 127.83, 128.54, 128.79, 129.08, 130.84, 139.46, 159.24, 169.85. HRMS (m/z): [M+H]⁺ calcd for C₁₉H₁₇N₃OS: 336.1165; found: 336.1160.

2-(2-(5-Methoxy-2,3-dihydro-1H-inden-1-ylidene)hydrazineyl)-4-(3-nitrophenyl)thiazole (2l):

Yield: 86 % M.P.= 271.6 °C. ¹H-NMR (300 MHz, DMSO-d₆): δ: 2.90 (2H, s, CH₂), 3.00 (2H, s, CH₂), 3.81 (3H, s, OCH₃), 6.95-6.97 (1H, m, Aromatic CH), 7.05-7.07 (1H, m, Aromatic CH), 7.28 (1H, d, *J*=8.37 Hz, Aromatic CH), 7.51-7.55 (1H, m, Aromatic CH), 7.74-7.79 (2H, m, Aromatic CH), 8.20-8.23 (1H, m, Aromatic CH), 8.46-8.49 (1H, m, Aromatic CH). ¹³C-NMR (75 MHz, DMSO-d₆): δ= 27.84, 28.82, 55.78, 104.20, 114.10, 118.58, 118.78, 122.18, 122.92, 123.14, 126.94, 130.33, 130.58, 134.60, 136.42, 140.93, 148.15, 159.25, 169.33. HRMS (m/z): [M+H]⁺ calcd for C₁₉H₁₆N₄O₃S: 381.1016; found: 381.1039.

2.4. DPPH Radical Scavenging Activity

Blois's UV method was employed to screen the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. According to this method, 40 μM and 100 μM concentrations of the compounds and the reference molecule (gallic acid) were prepared in 20 μL methanol. 180 μL of 0.15 mM DPPH solution in methanol was added to each solution. After 20 min incubation at room temperature, remaining DPPH amount was measured at 520 nm (Varioskan Flash, Thermo Scientific, USA). The percent DPPH radical scavenging activity was calculated through the following formula;

$$\% = [(A \text{ control} - A \text{ sample}) / A \text{ control}] \times 100$$

where A sample is the absorbance of the compounds/reference, and A control is the absorbance of the control reaction. Each experiment was carried out in triplicate, and the standard error mean, or S.E.M., was used to reflect the average data [26].

2.5. Cell Culture Studies and Reagents

Colorectal cancer cell line HCT116 and lung carcinoma cell line H460 were cultured in Roswell Park Memorial Institute (RPMI)-1640 supplemented with 10% fetal bovine serum (FBS) and 100 U/mL penicillin-streptomycin (P/S) solution. The human colorectal cancer cell line HCT116 (ATCC CCL-247) and lung cancer cell line H460 (ATCC HTB-177) were obtained from ATCC and used according to standard cell culture procedures. The cells were grown in 25 cm² cell culture flasks at 37 °C in a humidified atmosphere containing 5% CO₂. All cells were tested for the absence of Mycoplasma. Test compounds were dissolved in dimethyl sulfoxide (DMSO) and diluted with culture medium [DMSO final concentration, 0.1% (v/v) served as the vehicle control].

2.5.1 Cytotoxicity assay

Cell viability was assessed using MTT, a tetrazolium salt. This salt is specific to the succinate dehydrogenase enzyme found in the mitochondria of living cells and is

based on the principle that this enzyme breaks down the tetrazolium ring of the MTT dye, forming a water-insoluble formazan salt. This formation is only observed in living cells with active mitochondria. The value obtained by dissolving water-insoluble formazan crystals with DMSO and measuring them in a spectrophotometer indicates the number of living cells. Cisplatin was used as a positive control under the same experimental conditions (100 μM, 24 h). After the cells were removed from the surface of the flask, the cell suspension formed was centrifuged, and the cell pellet was collected. The pellet was resuspended in the medium, seeded in a 96-well plate at 7x10³ cells/well, and incubated overnight at 37°C.

On the second day, the cells in the 96-well plates were exposed to the test substances at four different concentrations, in three repetitions, and incubated for 24 h. After the incubation period, 5 mg/ml of powdered MTT prepared in PBS was added to each well of the 96-well plates. Following 24 h of incubation, 100 μl of DMSO was added to dissolve the formazan crystals, and the absorbance was measured at 570 nm using a spectrophotometer. The data, repeated at least three times for each substance were compared. The relative % cell viability was then determined. At the end of the measurement, the percentage of cell proliferation was calculated using Equation 1 shown below.

$$Viability (\%) = \left[\frac{100 \times (\text{Mean absorbance of treated cells} - \text{Blank})}{\text{Mean absorbance of control cells} - \text{Blank}} \right] \quad (1)$$

Cell viability was calculated by normalizing the absorbance values of treated cells to the control group. Each experimental condition was performed with at least three independent biological replicates (n = 3), with three technical replicates per condition. Data are presented as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism (version 8.0). Differences between groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A p-value < 0.05 was considered statistically significant.

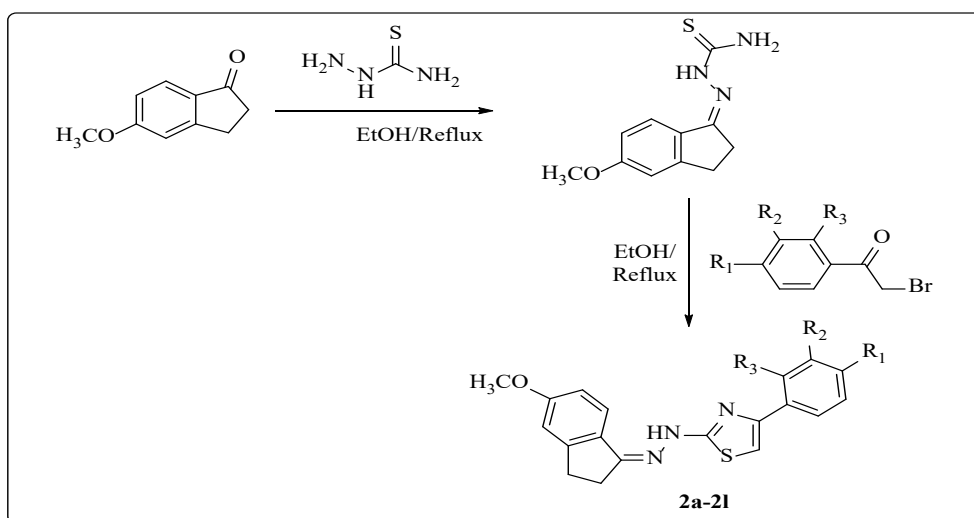
3. Results and Discussions

3.1. Chemistry

In this study, a new series of inden-thiazole hydrazone derivatives (2a-2l) was obtained via a classical Hantzsch-type thiazole synthesis starting from 5-methoxy-2,3-dihydro-1H-inden-1-one and thiosemicarbazide, followed by cyclization with appropriately substituted 2-bromoacetophenones. The use of thiosemicarbazone intermediates as versatile nucleophilic partners for α-haloketones is well established in heterocyclic chemistry and has been widely applied in the design of bioactive thiazole and benzothiazine systems [1,7,8,18]. The reaction proceeds through nucleophilic attack of the

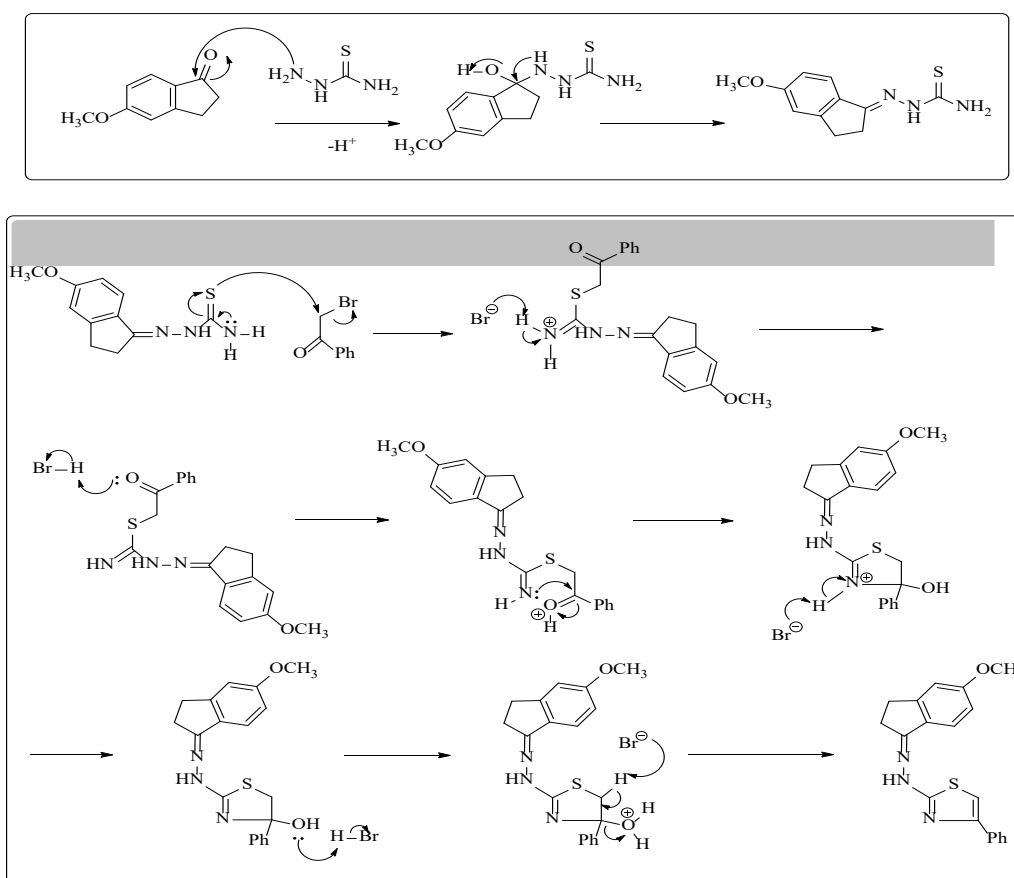
thioamide sulfur at the α-carbon of the α-bromoketone, facilitated by the electron-withdrawing amide moiety and the good leaving ability of the halogen, yielding the thiazole ring after cyclization and aromatization. This strategy is consistent with previous reports on the synthesis of thiazole-based scaffolds with promising pharmacological profiles, including antimicrobial, enzyme-inhibitory, and anticancer activities [1,5,22,24]. The structures of all final compounds were confirmed by ¹H NMR, ¹³C NMR, and HRMS analysis, in agreement with earlier studies on structurally related thiazole and hydrazone derivatives [1,5,6,16,17]. The presence of different electron-donating or electron-withdrawing substituents on the phenyl ring (-NO₂, -CN, -OCH₃, -CH₃, -F, -Cl, -Br, and biphenyl) enabled an initial structure-activity relationship (SAR) evaluation for both antioxidant and cytotoxic properties.

The general structures and synthetic procedure of title inden-thiazole derivatives 2a-2l synthesized in this study are shown in Scheme 1. 5-Methoxy-2,3-dihydro-1H-inden-1-one was treated with thiosemicarbazide in the first step to afford the thiosemicarbazone derivative, which then reacted with the appropriate 2-bromoacetophenones to synthesize title compounds (2a-2l). The structures of all products were elucidated through ¹H NMR, ¹³C NMR, and HRMS.



Comp.	R ₁	R ₂	R ₃
2a	-NO ₂	-H	-H
2b	-OCH ₃	-H	-H
2c	-CN	-H	-H
2d	-C ₆ H ₅	-H	-H
2e	-Br	-H	-H
2f	-CH ₃	-H	-H
2g	-Cl	-Cl	-H
2h	-F	-H	-F
2i	-Cl	-H	-H
2j	-Cl	-H	-Cl
2k	-H	-H	-H
2l	-H	-NO ₂	-H

Scheme 1. Chemical structure and general procedure for the synthesis of the final compounds 2a-2l



Scheme 2. Synthesis mechanism of compounds 2a-2l.

Hantzsch's thiazole synthesis was considered for the synthesis of the desired thiazole derivative compounds. According to this synthesis, the thiazole structure is formed as a result of the reaction of α -haloketones with thioamides. Sulfur (S) in the thioamide structure is the part that directs the reaction due to its strong nucleophilic character, which increases with the resonance structure coming from the amide group. While the carbonyl in the α -haloketone is expected to be the center of attack, the sulfur in the structure prefers the α -carbon because the halogens can be easily separated from the structure. As a result of this reaction mechanism, the cyclization of the thiazole ring occurred. The mechanism of the synthesized compounds is shown in Scheme 2.

3.2. DPPH Radical Scavenging Activity

The antioxidant activity of the target compounds was determined using DPPH assays. Gallic acid was used as the reference drug. IC_{50} values of the DPPH free radical-scavenging activity were calculated, and the IC_{50} values of the compounds are shown in Table 1. In the DPPH test, compounds 2c and 2h exhibited promising antioxidant activity with IC_{50} values of $31.73 \pm 0.004 \mu\text{M}$ and $31.13 \pm 0.008 \mu\text{M}$, respectively. In particular, compounds 2c and 2h, with para-cyano and 2,4-difluoro substituents on the phenyl ring, were found to have antioxidant activity close to the reference drug gallic acid. The presence of an electron-withdrawing group at positions 2 and 4 of the phenyl ring increases activity. However, this increase in activity was observed with the fluorine substituent. Substituting the fluorine group with the chlorine substituent decreases activity. While no activity was observed in compound 2i, which carries a chlorine substituent only at position 4, it was observed that the activity increased with the addition of a chlorine substituent to position 2 (compound 2j). This shows that the presence of electron-withdrawing groups at positions 2 and 4 is important for antioxidant activity.

Table 1. IC_{50} values (μM) of the synthesized compounds 2a-2l

Compound	IC_{50} (DPPH) μM
2a	> 60 μM
2b	46.19 ± 0.005
2c	31.73 ± 0.004
2d	43.96 ± 0.009
2e	51.18 ± 0.017
2f	57.02 ± 0.014
2g	> 60 μM
2h	31.13 ± 0.008
2i	> 60 μM
2j	43.91 ± 0.021
2k	59.26 ± 0.005
2l	> 200 μM
RUTIN 50 μM	-
RUTIN 100 μM	-
BHT 50 μM	-
BHT 50 μM	-
Gallic Acid	29.48 ± 0.014

These findings are consistent with previous reports indicating that electron-withdrawing substituents on the aromatic ring often favor radical-scavenging or redox-modulating properties in thiazole-based systems. Hussein et al. reported that newly synthesized thiazole derivatives bearing strong electron-withdrawing groups, such as halogens or nitro moieties, exerted notable antioxidant activity *in vitro* and showed a clear substituent-dependent trend [22]. Similarly, Taslimi et al. demonstrated that amide- and thiazolidinone-based structures on an acetophenone core displayed enhanced inhibition of oxidative-stress-related enzymes (carbonic anhydrase, acetylcholinesterase, butyrylcholinesterase), and that the introduction of electron-withdrawing groups increased their potency [25].

In the present series, the para-cyano group (2c) and the 2,4-difluoro pattern (2h) appear particularly favorable. The cyano group can stabilize the radical form through conjugation, while fluorine atoms at positions 2 and 4 may modulate electron density and hydrogen-bonding capacity, thereby increasing the efficiency of DPPH radical quenching. The SAR trend observed here parallels other thiazole and hydrazone studies, where substitution patterns significantly influenced antioxidant outcomes [7,21,23]. For instance, Rollas and Küçükgülmez summarized that the incorporation of electron-withdrawing groups onto hydrazone frameworks often correlates with improved radical-scavenging and metal-chelating properties [21].

On the other hand, compounds with less strongly electron-withdrawing groups, such as 4-methyl (2f) or unsubstituted phenyl (2k), exhibited weaker DPPH activity, suggesting that simple hydrophobicity is not sufficient to ensure potent antioxidant effects. Similar observations were made by Katariya et al., who found that quinoline-based hydrazone analogues required a balance of electronic effects and conjugation to achieve optimal antioxidant and anticancer activity [23]. Taken together, the current data support that the presence and position of electron-withdrawing substituents on the phenyl ring are critical determinants of antioxidant potency in this inden-thiazole hydrazone series.

3.3. Cytotoxicity Evaluation of the Compounds on HCT116 and H460 Cells

The cytotoxic effects of the synthesized compound series (2a–2l) were initially screened at a concentration of 100 μM for 24 h using the MTT assay in HCT116 and H460 cancer cell lines. Most compounds did not significantly reduce cell viability in HCT116 cells under these conditions. In contrast, several derivatives showed moderate growth inhibition in the H460 cell line. Based on the initial screening results, IC_{50} values were further estimated for the most active compound.

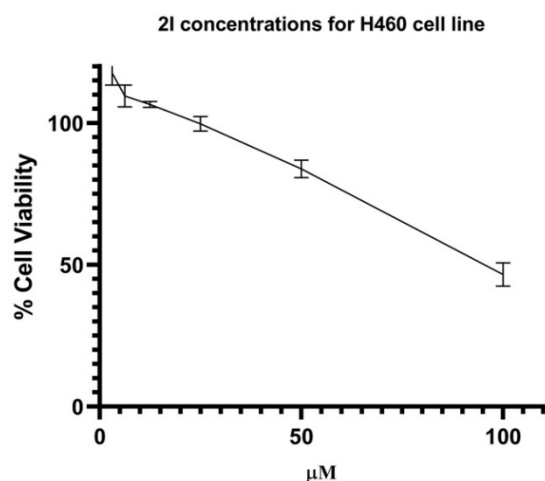


Figure 1. Dose–response curve of compound 2l in H460 cells after 24 h treatment. Data are presented as mean \pm SD of three independent experiments (* $p < 0.05$ vs. control) ($n=3$).

To address the dose–response relationship of the most active compound, a concentration-dependent cytotoxicity analysis was performed for compound 2l in H460 cells. As shown in Figure 1, compound 2l exhibited a clear concentration-dependent decrease in cell viability. The generated dose–response profile supports the calculated IC_{50} value ($82.00 \pm 3.67 \mu M$) and confirms the moderate cytotoxic activity of this compound. These results complement the initial single-dose screening and provide additional insight into the biological response pattern of the most active derivative. Among the tested derivatives, compound 2l exhibited the highest activity against H460 cells with an IC_{50} value of $82.00 \pm 3.67 \mu M$, whereas the remaining compounds showed IC_{50} values above $100 \mu M$ (Table 2). This study was designed as an initial single-dose screening, and all compounds dose–response curves were not generated. Therefore, the cytotoxicity findings should be interpreted as preliminary. In addition, a dose–response analysis was performed for the most active compound (2l) to further support its cytotoxic profile. All experiments were performed in triplicate, and the results are presented as mean \pm SD.

Table 2. Cell Viability (%) of HCT116 and H460 cell lines at $100 \mu M$ concentration for 24 h (** $p < 0.001$, * $p < 0.01$, * $p < 0.05$)

H460	Cell Viability (%)	HCT116	Cell Viability (%)
Control	100.00 \pm 13.56	Control	100.00 \pm 5.6
Cisplatin	23.97 \pm 12.84***	Cisplatin	45.75 \pm 14.48
2a	90.03 \pm 10.20	2a	87.10 \pm 8.4
2b	68.09 \pm 13.27*	2b	102.73 \pm 6.0
2c	70.25 \pm 12.00	2c	96.92 \pm 6.1
2d	72.78 \pm 6.01	2d	142.96 \pm 4.9
2e	82.79 \pm 3.68	2e	115.19 \pm 2.4
2f	132.90 \pm 11.42	2f	122.75 \pm 11.8
2g	61.78 \pm 7.85*	2g	122.82 \pm 6.7
2h	105.67 \pm 10.71	2h	111.56 \pm 9.1
2i	87.09 \pm 11.16	2i	102.59 \pm 3.9
2j	65.50 \pm 4.34*	2j	123.34 \pm 4.1
2k	106.97 \pm 1.16	2k	85.05 \pm 6.8
2l	41.48 \pm 4.97**	2l	119.62 \pm 12.6

The cytotoxic potential of the synthesized compound series (2a–2l) was assessed using the MTT assay on HCT116 and H460 cell lines at a concentration of $100 \mu M$ for 24 hours (Table 2). Data are presented as mean \pm SD ($n = 3$). Statistical significance was determined by one-way ANOVA followed by Tukey's post hoc test; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. control group. In the HCT116 cell line, most compounds did not significantly reduce cell viability, with several derivatives (e.g., 2d, 2e, 2f, 2g, 2h, 2j, 2l) even showing viability values above 100%, suggesting potential proliferative or non-toxic behavior. In contrast, compounds 2a and 2k exhibited slight cytotoxicity, while compound 2b showed viability comparable to that of the control. Some compounds—particularly 2l (41.48%), 2g (61.78%), 2j (65.50%), and 2b (68.09%) demonstrated notable cytotoxicity, while others (e.g., 2f, 2k, 2h) had no inhibitory effect on the H460 cell line. These findings indicate a differential sensitivity of the two cell lines to the tested compounds, with H460 cells being more susceptible to certain derivatives. However, most compounds did not exhibit strong cytotoxic activity at the tested concentration and exposure duration, suggesting limited anti-proliferative efficacy under these conditions. In addition, cisplatin was included as a positive control to validate the experimental system. Under the same conditions ($100 \mu M$, 24 h), cisplatin significantly reduced cell viability to $23.97 \pm 12.84\%$ in H460 cells and $45.75 \pm 14.48\%$ in HCT116 cells (Table 2). This confirms the sensitivity of the assay and provides a reference for interpreting the cytotoxic effects of the synthesized compounds. Statistical analysis revealed that the reduction in cell viability observed for compound 2l and cisplatin in H460 cells was statistically significant compared to the control group ($p < 0.05$).

The IC_{50} screening results obtained for the H460 and HCT116 cell lines indicate that the majority of the synthesized derivatives (2a–2k) exhibit low or negligible cytotoxicity at the tested concentration range, with IC_{50} values consistently above $100 \mu M$ in both cancer cell lines. This suggests that these compounds do not exert significant antiproliferative effects under the experimental conditions used. Notably, compound 2l demonstrated a measurable cytotoxic response in H460 cells, with an IC_{50} value of $82.00 \pm 3.67 \mu M$, indicating a moderate level of activity compared to the other derivatives. The data suggest a cell-line-dependent and structure-specific trend, where only compound 2l displayed selective activity toward H460 cells. At the same time, the remaining derivatives exhibited poor anticancer potential at both 24 h and $100 \mu M$ concentrations. When the structures and effects of the compounds are evaluated, it is seen that the presence of the NO_2 substituent, an electron-withdrawing group, at the 3rd position (compound 2l) increases the anticancer activity, and the activity decreases when this group is moved to the 4th position (compound 2a).

Table 3. IC₅₀ values of HCT116 and H460 cell lines at 100 μM concentration for 24 h

H460	IC ₅₀ μM	HCT116	IC ₅₀ μM
Control	>100	Control	>100
2a	>100	2a	>100
2b	>100	2b	>100
2c	>100	2c	>100
2d	>100	2d	>100
2e	>100	2e	>100
2f	>100	2f	>100
2g	>100	2g	>100
2h	>100	2h	>100
2i	>100	2i	>100
2j	>100	2j	>100
2k	>100	2k	>100
2l	82.00 ± 3.67	2l	>100

These results are in line with earlier observations that thiazole and hydrazone scaffolds can show highly variable anticancer profiles depending on substitution patterns and target cell lines. Dos Santos et al. reported that certain thiosemicarbazones and 1,3-thiazoles exhibited strong antiproliferative activity in Jurkat and HT-29 cells, while closely related analogues were considerably less active [4]. Likewise, Zaki et al. and Gomha et al. found that structural modifications on thiazole and pyrano[2,3-d]thiazole frameworks markedly affected cytotoxic potency and selectivity toward different tumor models [5,27].

Compared with highly potent thiazole-based anticancer agents reported in the literature—often exhibiting low micromolar IC₅₀ values in various human cancer cell lines [1,22,23]. The current series demonstrated limited antiproliferative effects under the tested conditions, with only compound 2l showing a measurable response (IC₅₀ < 100 μM) in H460 cells. These findings should be interpreted as preliminary, as the study was based on an initial single-dose screening design. Nevertheless, the moderate activity of 2l, together with its distinct substitution pattern, provides a starting point for further optimization. For example, combining the 3-nitrophenyl nitro group of 2l with the electron-withdrawing substituents that favor antioxidant activity (such as cyano or difluoro groups in 2c and 2h) might lead to hybrid structures with improved dual antioxidant and cytotoxic properties.

The dual evaluation of antioxidant and cytotoxic properties is particularly relevant for thiazole–hydrazone derivatives, as many bioactive small molecules act through combined redox modulation, enzyme inhibition, and direct cytotoxic mechanisms [7,22,25]. In this context, the present data suggest that the synthesized inden–thiazole series predominantly displays antioxidant rather than strong anticancer behavior under the tested conditions. Hussein et al. described thiazole derivatives with both antioxidant and anticancer activities, emphasizing that optimization of substitution patterns was necessary to shift the balance from primarily antioxidant to more potent cytotoxic effects [22].

Similarly, Pola highlighted that thiazole-based heterocycles constitute a versatile platform where fine-tuning of electronic and steric factors can modulate a wide spectrum of bioactivities, including enzyme inhibition, anticancer activity, and anti-inflammatory effects [7,21]. Furthermore, the modest cytotoxicity observed here may be linked to the hydrazone linker, which is known to contribute to diverse biological activities (antitubercular, antimicrobial, and anticancer) but whose impact depends strongly on the nature and orientation of aryl substituents [17,21,28]. Rollas and co-workers have repeatedly shown that hydrazone derivatives can be transformed from weakly active to highly potent molecules through targeted substitution on either side of the azomethine (–NH–N=CH–) linkage [16,17,21]. In our series, the significant difference between 2a and 2l illustrates how positional isomerism of a single nitro group can modulate cytotoxic response, especially in H460 cells.

In parallel, the relatively strong DPPH scavenging activity of 2c and 2h indicates that these molecules might be more suitable as antioxidant, enzyme-modulatory, or neuroprotective leads, similar to the carbonic anhydrase and cholinesterase inhibitory thiazoles reported by Karakaya et al. and Taslimi et al. [24,25]. Future work, including enzyme inhibition, redox biology assays, and detailed mechanistic studies (e.g., docking and ADME, as outlined in the Conclusion), will be essential to clarify whether these compounds act mainly as chemical antioxidants, enzyme inhibitors, or potential adjuvants that modulate oxidative stress pathways in cancer or other diseases. When integrated with the existing literature, the present findings support the notion that inden–thiazole hydrazone derivatives constitute a structurally promising but biologically still moderate platform. With judicious substitution and further derivatization—guided by SAR trends observed here and in previous thiazole/hydrazone studies—more potent and selective multifunctional agents may be developed.

The present findings suggest that the synthesized thiazole derivatives exhibit selective cytotoxic effects, particularly in H460 cells, which may be associated with their ability to interfere with intracellular signaling and redox balance. Although the exact molecular mechanism was not experimentally investigated in this study, previous reports have shown that thiazole-containing compounds can exert anticancer activity through mechanisms such as induction of apoptosis, disruption of mitochondrial function, and modulation of key signaling pathways including PI3K/Akt and MAPK pathways [29,30]. In comparison with earlier studies, the cytotoxic activity observed for compound 2l (IC₅₀ = 82.00 ± 3.67 μM) can be considered moderate but consistent with preliminary screening results reported for similar heterocyclic scaffolds in early-stage anticancer drug discovery [31]. Compared to cisplatin, the tested compounds demonstrated lower cytotoxic activity, indicating that these derivatives may require further structural optimization to achieve stronger antiproliferative effects. Importantly, the relatively lower toxicity toward non-

cancerous HUVEC cells suggests a degree of selectivity, which is a desirable characteristic for potential anticancer agents [30]. However, several limitations should be acknowledged. The biological evaluation was limited to in vitro cytotoxicity assays in a small number of cell lines, and detailed mechanistic studies such as apoptosis analysis, cell cycle evaluation, or molecular target validation were not performed. Although cisplatin was included as a positive control, a direct comparative dose–response analysis with standard anticancer agents was not performed. Therefore, further studies are required to elucidate the precise molecular mechanisms and to validate the therapeutic potential of these compounds using broader biological models and in vivo systems [29,31].

4. Conclusion

In this study, a series of inden–thiazole hydrazone derivatives was successfully synthesized and biologically evaluated in terms of antioxidant and antiproliferative properties. The findings indicate that the synthesized compounds predominantly exhibit antioxidant activity, while their cytotoxic effects remain limited under the tested conditions. The observed biological responses appear to be strongly influenced by substitution patterns, suggesting that structural modifications play a critical role in modulating activity. In particular, derivatives bearing electron-withdrawing groups demonstrated enhanced antioxidant potential, highlighting this scaffold as a promising starting point for further chemical optimization. The cytotoxicity data obtained in this study should be interpreted as preliminary, as the experimental design was based on an initial single-dose screening approach. A major limitation of this work is the absence of detailed dose–response analysis and mechanistic validation, which will be essential to fully elucidate the biological potential of these compounds. Taken together, the present results suggest that inden–thiazole hydrazone derivatives may serve as a versatile scaffold for the development of multifunctional bioactive molecules, particularly in the context of antioxidant-related applications. Future studies focusing on structural optimization, expanded biological evaluation, and mechanistic investigations are required to further advance this compound class.

Conflict of interest

No conflicts of interest have been declared by the authors.

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