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SYNTHESIS, STRUCTURE DETERMINATION AND ANTIOXIDANT ACTIVITY OF CHALCONE–THEIAZOLE–COUMARINE HYBRID MOLECULES

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ABSTRACT

Compounds with free radical scavenging properties have garnered significant interest among researchers seeking to develop molecules with such capabilities. Chalcones, thiazoles, and coumarins are pharmacophores known for their potent antioxidant activities and have been the subject of extensive investigation. In this study, ten hybrid molecules incorporating three pharmacophores—chalcone, coumarin, and thiazole—were successfully synthesized. The synthesis was achieved through the Hantzsch cyclization reaction between 3-(bromoacetyl) coumarin and chalcone-thiourea as starting materials, employing various substituents. Chalcone-thiourea derivatives with electron-donating substituents yielded higher product yields (80-99%), whereas those with electron-withdrawing substituents resulted in lower yields (70-80%). The molecular structures of the synthesized compounds were elucidated using spectroscopic techniques, including UV-Vis, FTIR, HR-ESIMS, ^{1}H-NMR, and ^{13}C-NMR. The antioxidant activity was evaluated using DPPH and ABTS assays, revealing that the synthesized compounds exhibited moderate antioxidant activity.

Keywords: Hybrid Molecule, Chalcone, Thiazole, Coumarin, Antioxidant.

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INTRODUCTION

Under physiological conditions, Reactive Oxygen Species (ROS) play a role in cellular processes such as defense against infectious agents. At low concentrations, ROS are involved in inducing mitogenic responses.[1] Conversely, an excess of ROS, along with Reactive Nitrogen Species (RNS), disrupts the body's redox balance, leading to oxidative stress.[2]

Oxidative stress is a key factor that triggers cellular and structural damage[3] and is believed to contribute to the development of various diseases, including cardiovascular diseases[4], diabetes[5], neurodegenerative disorders[6], cancer[7], and aging.[8] In response to these concerns, many researchers are focused on developing drugs or compounds with antioxidant properties to mitigate the harmful effects of excess ROS and RNS. Compounds with antioxidant activity have been extensively published. Antioxidant activity describes the kinetics of each reaction formed between antioxidants and target radicals.

The role of antioxidant compounds is to inhibit reactions during the initiation and propagation phases of free radicals by interacting with the radicals themselves or with radical mediators.[9] With the advancement of technology and the increasing need for antioxidants, many researchers are performing molecular hybridization of compounds with antioxidant activity as a step to enhance their antioxidant properties. Molecular hybridization is a relatively new concept in drug design and development, based on the combination of pharmacophoric groups from various bioactive compounds.

The aim is to produce new hybrid molecules with better affinity compared to the parent drugs.[10] Some examples of hybrid molecules that have been developed include coumarin-thiazole[11], coumarin-chalcone[12], triazole-chalcone derivatives[13], and thiadiazole-chalcone derivatives.[14]

This article reports and discuss the synthesis, structure elucidation, and antioxidant evaluation some hybrid molecules consist of three pharmacophores - coumarin, thiazole, chalcone- simultaneously.

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EXPERIMENTAL

Materials and Methods

Chemicals and Instruments

All chemicals used in this research were obtained from commercial suppliers, including Sigma Aldrich (St. Louis, USA), E. Merck (Darmstadt, Germany), and TCI Tokyo Chemical Industry Co. Ltd (Tokyo, Japan), in synthesis or pro-analysis grade. UV-Vis spectra were recorded using a Shimadzu UV-1800 UV-Vis spectrophotometer, while IR spectra were obtained using a Shimadzu IRTracer-100 FTIR spectrophotometer. The molecular weights of the synthesized compounds were determined with a Waters Q-Tof MS Xevo HR-ESI mass spectrometer. NMR spectra were recorded on a JEOL JNM-ECS 400 NMR spectrometer, operating at 400 Hz for ^{1}H-NMR and 101 Hz for ^{13}C-NMR. Antioxidant assays were conducted using a Biochrom EZ Read 800 microplate reader.

General Procedure

Synthesis of compound 1 (3-acetyl coumarine)

The reaction mixture, consisting of 8 mmol (852 µL) of salicylaldehyde, 8 mmol (1020 µL) of ethyl acetoacetate, and 5 mol% (34.5 µL) of morpholine as a catalyst, was placed in a round-bottom flask and stirred for 3 hours at 40°C. Upon completion of the reaction, the mixture was washed with cold ethanol, filtered, and then dried at room temperature. The purity of the product was assessed using Thin Layer Chromatography (TLC).[15] *3-acetyl-coumarine **1***: white crystal (1370 mg; 91%); R_f = 0.51 (n-hexane: dichloromethane = 1:3); HRESI-MS $[M+Na]^+$ calculated for $C_{11}H_8O_3Na^+$ 211.03711 observed 211.03715; UV-Vis (EtOH) λmax (log ε): 298 (4.02), 337 (3.80); FTIR (DRIFT, KBr, ῡ, cm^{-1}): 3030 (C–H sp2), 2930 (C–H sp3), 1742 (C=O lactone), 1678 (C=O ketone), 1614 (C=C alkene), 1557 (C=C aromatic), 1211 (C–O lactone); ^{1}H-NMR (400 MHz, $CDCl_3$) δH (ppm): 8.50 (m, 1H); 7.65 (m, 2H); 7.34 (m, 2H); 2.71 (m, 3H); ^{13}C-NMR (101 MHz, $CDCl_3$, APT) δC (ppm): 195.6 (C); 159.4 (C); 155.4 (C); 147.6 (CH); 134.5 (CH); 130.4 (CH); 125.1 (CH); 124.6 (C); 118.3 (C); 116.8 (CH); 30.7 (CH_3).

Synthesis of Compound 2 (3-bromo acetyl coumarin*)*

The bromination of 3-acetylcoumarin was carried out using a slightly modified method based on the procedure from a previous study.[16] In a reaction vessel, 2 mmol (376.4 mg) of 3-acetylcoumarin was dissolved in 4 mL of ethyl acetate, followed by the addition of 2.4 mmol (187.2 mg) of DMSO and 2.4 mmol (187.2 mg) of HBr. The reaction mixture was stirred at room temperature until completion, as monitored by TLC. After the reaction, the solvent was fully evaporated, and the crude product was recrystallized from glacial acetic acid. The purity of the final product was confirmed by TLC analysis.

3-(2-bromoacetyl)-2H-chromen-2-one 2

yellow crystal (531 mg; 99%); R_f = 0.50 (n-hexane: dichloromethane=1:3); EI-MS $[M^+]$ m/z (%): 18 (6), 39 (<1), 63 (10), 89 (19), 115 (7), 145 (6), 173 (100), 187 (80), 266 (<1); UV-Vis ($CHCl_3$) λmax (log ε): 292 (4.32), 348 (4.04); FTIR (DRIFT, KBr, ῡ, cm^{-1}): 3034 (C–H sp2), 2959 (C–H sp3), 1746 (C=O lactone), 1728 (C=O ketone), 1603 (C=C alkene), 1557 (C=C aromatic), 1180 (C–O lactone), 671 (C–Br); ^{1}H-NMR (400 MHz, $CDCl_3$) δH (ppm): 8.64 (s, 1H); 7.70 (m, 2H); 7.39 (m, 2H); 4.75 (s, 2H); ^{13}C-NMR (101 MHz, $CDCl_3$, APT) δC (ppm): 189.0 (C); 159.0 (C); 155.5 (C); 149.7 (CH); 135.3 (CH); 130.6 (CH); 125.4 (CH); 122.2 (C); 118.2 (C); 117.0 (CH); 359 (CH_2).

Synthesis of Compound 3 (4-acetylphenylthiourea)

In a reaction flask, 4 mmol (540 mg) of 4-aminoacetophenone, 6 mL of 1N HCl, and 8 mmol (688 mg) of ammonium thiocyanate dissolved in 3 mL of water were added. The reaction mixture was stirred at a temperature of 80-90°C until the reaction was complete, as monitored by TLC. The mixture was then filtered, washed with 1N HCl, and dried. The purity of the final product was confirmed by TLC analysis[17].

1-(4-acetylphenyl)thiourea 3

yellow crystal (579.5 mg; 75%); R_f = 0.51, ($CHCl_3$: ethylacetate = 2:3); UV-Vis (EtOH) λmax (log ε): 307 (4.26), 256 (4.06); FTIR (DRIFT, KBr, ῡ, cm^{-1}): 3346 (N-H primary), 3163 (N-H secondary), 3120 (C-H sp2), 2992 (C-H sp3), 1676 (C=O), 1528 (C=C alkene), 1254 (C=S); ^{1}H-NMR (400 MHz, DMSO-d_6) δH (ppm): 10.01 (s, 1H), 7.90 (d, *J* = 8.9 Hz, 2H), 6.98-8.33 (br, 2H), 7.67 (d, *J* = 8.9 Hz, 2H), 2.53 (s, 3H);

^{13}C NMR (101 MHz, DMSO-d_6) δc (ppm): 197.1 (C); 181.7 (C); 144.4 (C); 132.5 (C); 129.0 (CH); 120.9 (CH); 27.0 (CH_3).

Synthesis of Compounds 4 (chalcone-thiourea derivatives)

A solution of 2 mmol (383.6 mg) of 4-acetylphenylthiourea, 2 mmol of a benzaldehyde derivative, and 10 mL of ethanol were prepared and maintained at a temperature of ≤10°C. Subsequently, 2 mL of a 40% w/w NaOH solution was added dropwise while keeping the temperature below 10°C. The temperature was then allowed to rise to room temperature, and the mixture was stirred until the reaction was complete. The reaction mixture was poured into crushed ice in a beaker, followed by the dropwise addition of 5 mL of 4 M HCl. The resulting precipitate was filtered, washed with cold distilled water, and dried. The purity of the product was confirmed by TLC analysis.[18]

1-(4-cinnamoylphenyl)thiourea 4.1

orange powder (546.7 mg; 97%); R_f = 0.57 (ethyl acetate: n-hexane = 3:1); HR-ESI-MS $[M+H]^+$ calculated for $C_{16}H_{15}N_2OS$ 283.0905, observed 283.0897; FTIR (DRIFT, KBr, ῡ (cm^{-1}) 3388;3278;3176 (N-H), 3176 (C-H sp2), 1654 (C=C alkene), 1608 (C=O), 1589; 1573 (C=C aromatic), 1222 (C=S); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.14 (s, 1H); 7.24-8.22 (m, br, 2H); 8.14 (d, J = 8.7 Hz, 2H); 7.94 (d, J = 15.7, 1H); 7.88 (dd, J_1 = 2.9 Hz, J_2 = 6.5, 2H); 7.76 (d, J= 8.7 Hz, 2H); 7.71 (d, J =15.7, 1H); 7.46 (dd, J_1 = 1.8 Hz, J_2 = 5.1 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δc (ppm) 187.6; 181.1; 144.1; 143.4; 134.8; 132.5; 130.5; 129.5; 128.9; 128.8; 122.0; 120.9.

(E)-1-(4-(3-(4-chlorophenyl)acryloyl)phenyl)thiourea 4.2

pale yellow powder (617.8 mg; 98%); R_f = 0.60 (ethyl acetate: n-hexane: 3:1); HR-ESI-MS $[M+H]^+$ calculated for $C_{16}H_{14}N_2OSCl^+$ 317.0515, observed 317.0514; FTIR (DRIFT, KBr, ῡ (cm^{-1}) 3469; 3277; 3184 (N-H), 3051;3034 (C-H sp2), 1658 (C=C alkene), 1610 (C=O), 1490; 1404 (C=C aromatic), 1222 (C=S), 813 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.07 (s, 1H); 7.12-8.35 (m, br, 2H); 8.14 (d, J = 8.8 Hz, 2H); 7.97 (d, J = 15.6 Hz, 1H), 7.92 (d, J = 8.5 Hz, 2H), 7.74 (d, J = 8.8 Hz, 2H), 7.71 (d, J = 15.6 Hz, 1H), 7.52 (d, J = 8.5 Hz, 2H); ^{13}C NMR (400 MHz, DMSO-d_6) δc (ppm) 187.5; 181.1; 144.1; 141.9; 134.9; 133.7; 132.4; 130.5; 129.5; 128.9; 122.7; 121.0.

(E)-1-(4-(3-(4-fluorophenyl)acryloyl)phenyl)thiourea 4.3

pale yellow powder (594.3 mg; 98%); R_f = 0.63 (ethyl acetate: n-hexane: 3:1); HR-ESI-MS $[M+H]^+$ calculated for $C_{16}H_{14}N_2O_2SF$ 301,0811, observed 301.0827; FTIR (DRIFT, KBr, ῡ (cm^{-1}) 3385; 3275; 3172 (N-H), 3053 (C-H sp2), 1658 (C=C alkene), 1600 (C=O), 1508; 1413 (C=C aromatic), 1222 (C=S), 1159 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.09 (s, 1H); 6.94-8.32 (m, br, 2H); 8.14 (d, J = 8.8 Hz, 2H); 7.97 (dd, J_1 = 5.7 Hz; J_2 = 8.7, 2H), 7.92 (d, J = 15.6 Hz, 1H), 7,73 (d, J = 8.8 Hz, 2H), 7.72 (d, J = 15.6 Hz, 1H), 7.30 (t, J = 8.7 Hz, 2H); ^{13}C NMR (400 MHz, DMSO-d_6) δc (ppm) 187.5; 181.1; 164.6; 162.1; 144.1; 142.2; 132.5; 131.5 (d, J = 8 Hz); 131.2 (d, J = 32 Hz); 129.5; 121.9; 121.0; 115.9.

(E)-1-(4-(3-(4-methoxyphenyl)acryloyl)phenyl)thiourea 4.4

yellow powder (398.0 mg; 64%); R_f = 0.60 (ethyl acetate: n-hexane = 3=1); HR-ESI-MS $[M+H]^+$ calculated for $C_{17}H_{17}N_2O_2S^+$ 313.1011, observed 313.1010; FTIR (DRIFT, KBr, ῡ (cm^{-1}) 3406; 3267; 3178 (N-H), 3091 (C-H sp2), 2993 (C-H sp3), 1658; 1625 (C=C alkene), 1591 (C=O), 1421; 1411 (C=C aromatic), 1215 (C=S), 1170 (C_{aryl}-O-C_{alkyl}); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.05 (s, 1H); 6.88-8.12 (m, br, 2H); 8.11 (d, J = 8.6 Hz, 2H); 7.83 (d, J = 8.7 Hz, 2H), 7.79 (d, J = 15.5 Hz, 1H), 7.72 (d, J = 8.7 Hz, 2H), 7.67 (d, J = 15.5 Hz, 1H), 7.06 (d, J = 8.6 Hz, 2H); 3.81 (s, 3H); ^{13}C NMR (400 MHz, DMSO-d_6) δc (ppm) 187.5; 181.1; 161.3; 143.9; 143.4; 132.9; 130.7; 129.3; 127.4; 121.1; 119.5; 114.4; 55.4.

(E)-1-(4-(3-(4-bromophenyl)acryloyl)phenyl)thiourea 4.5

pale yellow powder (702.5 mg; 97%); R_f = 0,60 (ethyl acetate: $CHCl_3$ = 3=2); HR-ESI-MS $[M+H]^+$ calculated for $C_{16}H_{14}N_2OSBr^+$ 361.0010, observed 361.0009; FTIR (DRIFT, KBr, ῡ (cm^{-1}) 3466; 3275; 3180 (N-H), 3053 (C-H sp2), 1660 (C=C alkene), 1608 (C=O), 1562; 1487 (C=C aromatic), 1222 (C=S), 810 (C-Br); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.17 (s, 1H); 7.00-8.35 (m, br, 2H); 8.14 (d, J = 8.8 Hz, 2H); 7.99 (d, J = 15.6 Hz, 1H), 7.86 (d, J = 8.5 Hz, 2H), 7.75 (d, J = 8.8 Hz, 2H), 7.71 (d, J = 15.6 Hz, 1H),

7.66 (d, J = 8.5 Hz, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δc (ppm): 188.0 (C); 181.6 (C); 144.7 (C); 142.6 (CH); 134.6 (C); 132.9 (C); 132.4 (CH); 131.3 (CH); 130.0 (CH); 124.4 (C); 123.2 (CH); 121.5 (CH).

(E)-1-(4-(3-(2,5-dimethoxyphenyl)acryloyl)phenyl)thiourea 4.6
yellow powder (360.1 mg; 52%); R_f = 0.51 (ethyl acetate: n-hexane = 3:1); HR-ESI-MS $[M+H]^+$ calculated for $C_{18}H_{19}N_2O_3S^+$ 343.1116, observed 343.1114; FTIR (DRIFT, KBr, ῡ (cm^{-1}) 3348; 3280; 3151 (N-H), 3003 (C-H sp2), 2835 (C-H sp3), 1660 (C=C alkene), 1602 (C=O), 1494; 1429; 1409 (C=C aromatic), 1222 (C=S), 1286, 1259 (C_{aryl}-O-C_{alkyl}); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.09 (s, 1H); 6.94-8.32 (m, br, 2H); 8.14 (d, J = 8.8 Hz, 2H); 8.02 (d, J = 15.7 Hz, 1H), 7.91 (d, J = 15.7 Hz, 1H), 7.74 (d, J = 8.7 Hz, 2H), 7.55 (m, 2H), 7.04 (m, 1H); 3.814 (s, 3H); 3.80 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δc (ppm): 187.7 (C); 181.1 (C); 153.3 (C); 152.7 (C); 144.0 (C); 137.7 (CH); 132.7 (C); 129.5 (CH); 123.6 (C); 121.9 (CH); 121.0 (CH); 118.1 (CH); 113.1 (CH); 112.5 (CH); 56.2 (CH3); 55.7 (CH_3).

(E)-1-(4-(3-(2,4-dimethoxyphenyl)acryloyl)phenyl)thiourea 4.7
yellow powder (589.0 mg; 86%); R_f = 0.51 (ethyl acetate: n-hexane = 3:1); HR-ESI-MS $[M+H]^+$ calculated for $C_{18}H_{19}N_2O_3S^+$ 343.1116, observed 343.1114; FTIR(DRIFT, KBr, ῡ (cm^{-1}) 3441; 3321; 3201 (N-H), 3074 (C-H sp2), 2995; 2941 (C-H sp3), 1643 (C=C alkene), 1604 (C=O), 1517; 1502; 1463 (C=C aromatic), 1220 (C=S), 1157; 1120 (C_{aryl}-O-C_{alkyl}); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.10 (s, 1H); 6.88-8.31 (m, br, 2H); 8.07 (d, J = 8.8 Hz, 2H); 7.98 (d, J = 15.6 Hz, 1H), 7.91 (d, J = 8.4 Hz, 1H), 7.76 (d, J = 15.6 Hz, 1H), 7.72 (d, J = 8.8 Hz, 2H), 6.63 (m, 2H); 3.90 (s, 3H); 3.84 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δc (ppm): 188.2 (C); 181.6 (C); 163.6 (C); 160.4 (C); 144.2 (C); 138.8 (CH); 133.6 (C); 130.6 (CH); 129.8 (CH); 121.6 (CH); 119.5 (CH); 116.5 (C); 107.3 (CH); 106.8 (CH); 98.8 (CH); 56.4 (CH_3); 56.1 (CH_3).

(E)-1-(4-(3-(2,3-dimethoxyphenyl)acryloyl)phenyl)thiourea 4.8
yellow powder (475.6 mg; 69%); R_f = 0.60 (ethyl acetate: n-hexane = 3:1); HR-ESI-MS $[M+H]^+$ calculated for $C_{18}H_{19}N_2O_3S^+$ 343.1116, observed 343.1114; FTIR (DRIFT, KBr, ῡ (cm^{-1}) 3392;3273;3183 (N-H), 3088 (C-H sp2), 2964;2935 (C-H sp3), 1649 (C=C alkene), 1602 (C=O), 1577; 1477 (C=C aromatic), 1269 (C=S), 1178;1070 (C_{aryl}-O-C_{alkyl}); ^{1}H NMR (400 MHz, DMSO-d_6) δH (ppm) δH (ppm): 10.07 (s, 1H); 7.18-8.31 (m, br, 2H); 8.12 (d, J = 8.8 Hz, 2H); 7.98 (d, J = 15.8 Hz, 1H); 7.89 (d, J = 15.8 Hz, 1H); 7.74 (d, J = 8.8 Hz, 2H); 7.62 (t, J = 4.7 Hz, 1H); 7.16 (d, J = 4.7 Hz, 2H); 3.84 (s, 3H); 3.80 (s, 3H); ^{13}C NMR (400 MHz, DMSO-d_6) δc (ppm) 187.70, 181.12, 152.77, 148.23, 144.02, 137.43, 132.56, 129.45, 128.28, 124.31, 122.82, 121.01, 119.15, 114.93, 60.98, 55.83.

(E)-1-(4-(3-(2,4,6-trimethoxyphenyl)acryloyl)phenyl)thiourea 4.9
pale yellow powder (702.5 mg; 97%); R_f = 0.60 (ethyl acetate: $CHCl_3$ = 3:2); HR-ESI-MS $[M+H]^+$ calculated for $C_{16}H_{14}N_2OSBr^+$ 361.0010, observed 361.0009; FTIR (DRIFT, KBr, ῡ (cm^{-1}) 3387; 3290; 3180 (N-H), 3001 (C-H sp2), 2976; 2943; 2837 (C-H sp3), 1651 (C=C alkene), 1606 (C=O), 1469; 1456; 1415 (C=C aromatic), 1213 (C=S), 1155; 1114; 1022 (C_{aryl}-O-C_{alkyl}); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.08 (s, 1H); 7.27-8.26 (m, br, 2H); 8.09 (d, J = 15.7 Hz, 1H); 795 (d, J = 8.6 Hz, 2H), 7.88 (d, J = 15.7 Hz, 1H), 7.70 (d, J = 8.6 Hz, 2H), 6.32 (s, 2H), 3.92 (s, 6H); 3.85 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δc (ppm): 188.5 (C); 185.7 (C); 181.1 (C); 163.3 (C); 161.4 (C); 143.4 (C); 134.7 (CH); 133.5 (C); 128.9 (CH); 121.2 (CH); 120.2 (CH); 105.2 (C); 91.0 (CH); 90.8 (CH); 56.2 (CH_3); 55.6 (CH_3).

(E)-1-(4-(3-(4-morpholinophenyl)acryloyl)phenyl)thiourea 4.10
brown powder (575.0 mg; 78%); R_f = 0.63 ($CHCl_3$: acetonitrile = 4:1); HR-ESI-MS $[M+H]^+$ calculated for $C_{20}H_{22}N_3O_2S^+$ 368.1433, observed 368.1424; FTIR(DRIFT, KBr, ῡ (cm^{-1}) 3414; 3265; 3174 (N-H), 2974; 2956; 2852; 2825 (C-H sp3), 1653; 1627 (C=C alkene), 1600 (C=O), 1514; 1446; 1409 (C=C aromatic), 1219 (C=S), 1176; 1114 (C_{aryl}-O-C_{alkyl}); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.11 (s, 1H); 6.61-8.60 (m, br, 2H); 8.11 (d, J = 8.6 Hz, 2H); 7.74 (d, J = 8.9 Hz, 2H), 7.72 (d, J = 8.6 Hz, 2H), 7.70 (d, J = 15.4 Hz, 1H), 7.66 (d, J = 15.4 Hz, 1H), 6.99 (d, J = 8.9 Hz, 2H); 3.73 (m, 4H); 3.24 (m, 4H); ^{13}C NMR (101 MHz, DMSO-d_6) δc (ppm): 187.3 (C); 181.1 (C); 152.6 (C); 144.0 (CH); 143.7 (C); 133.1 (C); 130.5 (CH); 129.3 (CH); 124.9 (C); 121.0 (CH); 117.7 (CH); 114.2 (CH); 65.9 (CH_2); 47.2 (CH_2).

Synthesis of compounds 5 (chalcone-thiazole-coumarine hybrid molecules)
A mixture of compound **4** (0.5 mmol), compound **2** (0.5 mmol), and sodium carbonate (0.5 mmol) was dissolved in 4 mL of acetone in a round-bottom flask and stirred for 30-60 minutes at 50°C. The progress of the reaction was monitored by TLC using a 3:2 n-hexane mixture as the eluent. The reaction mixture was then washed with a 30% ethanol-water solution (v/v), and the resulting precipitate was filtered and washed with distilled water. The crude product was subsequently recrystallized from dimethylformamide.

3-(2-((4-cynnamoilphenyl)amino)thiazole-4-yl)-2H-cromen-2-on
yellow-brown powder (167 mg; 74%); R_f = 0.53 (n-hexane/ethyl acetate = 2:1); HRESI-MS $[M+H]^+$ calculated for $C_{27}H_{18}N_2O_3SH^+$ 451.1116 observed 451.1112; UV-Vis (MeCN) λmax (log ε): 297 (4.56), 354 (4.60); FTIR (DRIFT, KBr, ύ, cm^{-1}) 3312 (N–H secondary); 3065 (C–H aromatic); 1717 (C=O lactone); 1609 (C=N), 1553 (C=C aromatic); 1179 (C–N); 831 (C–S); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.88 (s,1H); 8.75 (s, 1H); 8.25 (J = 8.9 Hz, 2H); 8.05-7.85 (m, 6H); 7.89 (s, 1H); 7.73 (d, J = 15.6 Hz, 1H); 7.67-7.40 (m, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 187.1; (C); 161.7 (C); 158.8 (C); 152.4 (C); 145.1 (C); 143.8 (C); 143.0 (CH); 138.9 (CH); 134.9 (C); 131.8 (CH); 130.6 (CH); 130.5 (C); 130.4 (CH); 129.1 (CH); 129.0 (CH); 128.7 (CH); 124.7 (CH); 122.1 (CH); 120.2 (C); 119.3 (C); 116.4 (CH); 116.3 (CH); 115.9 (CH).

***(*E)-3-(2-((4-(3-(4-chlorophenyl)acryloil)phenyl)amino)thiazole-4-yl)-2H-cromen-2-one**
yellow crystal (172 mg; 71%); R_f = 0.53 (n-hexane: ethyl acetate = 2:1); HRESI-MS $[M+H]^+$ calculated for $C_{27}H_{17}ClN_2O_3SH^+$ 485.0648 observed 485.0723; UV-Vis (MeCN) λmax (log ε): 297 (4.65), 354 (4.65); FTIR (DRIFT, KBr, ῡ, cm^{-1}) 3298 (N–H secondary); 3065 (C–H aromatic); 1721 (C=O lactone); 1607 (C=N), 1547 (C=C aromatic); 1177 (C–N); 1094 (C_{aryl}–Cl); 814 (C–S); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.87 (s, 1H); 8.75 (s, 1H); 8.25 (d, J = 8.8 Hz, 2H); 8.05-7.85 (m, 6H); 7,89 (s, 1H); 7.71 (d, J = 15.6 Hz, 1H); 7.65-7.35 (m, 5H); ^{13}C- NMR (101 MHz, DMSO-d_6) δ 187.0 (C); 161.7 (C); 158.8 (C); 152.4 (C); 145.2 (C); 143.8 (C); 141.5 (CH); 138.9 (CH); 134.8 (C); 133.9 (C); 131.8 (CH); 130.6 (CH); 130.4 (CH); 130.4 (C); 129.0 (CH); 128.9 (CH); 124.7 (CH); 122.9 (CH); 120.2 (C); 119.3 (C); 116.4 (CH); 116.2 (CH); 115.9 (CH).

(E)-3-(2-((4-(3-(4-fluorophenyl)acryloil)phenyl)amino)thiazole-4-yl)-2H-cromen-2-one
Brown powder (200 mg; 85%); R_f = 0.58 (n-hexane: ethyl acetate = 2:1); HRESI-MS $[M+H]^+$ calculated for $C_{27}H_{17}FN_2O_3SH^+$ 469.0944 observed 469.1022; UV-Vis (MeCN) λmax (log ε): 298 (4.54), 348 (4.63); FTIR (DRIFT, KBr, ῡ, cm^{-1}) 3308 (N–H secondary); 3073 (C–H aromatic); 1713 (C=O lactone); 1601 (C=N), 1537 (C=C aromatic); 1179 (C–N); 1157 (C_{aryl}–F); 820 (C–S); ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.86 (s, 1H); 8.74 (s, 1H); 8.24 (d, J = 8.5 Hz, 2H); 8.05-7.85 (m, 6H); 7.89 (s, 1H); 7.73 (d, J = 15.6 Hz, 1H); 7.68-7.27 (m, 5H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 187.0 (C); 163.3 (d, J = 250.38 Hz, C); 161.6 (C); 158.8 (C); 152.4 (C); 145.1 (C); 143.8 (C); 141.7 (CH); 138.8 (CH); 131.8 (CH); 131.6 (C); 131.1, CH); 130.5 (C); 130.5 (CH); 129.0 (CH); 124.7 (CH); 122.0 (CH); 120.2 (C); 119.3 (C); 116.4 (CH); 116.2 (CH); 116.0 (d, J = 21.21 Hz, CH); 115.9 (CH).

(E)-3-(2-((4-(3-(4-methoxyphenyl)acryloil)phenyl)amino)thiazole-4-yl)-2H-cromene-2-one
Yellow crystal (230 mg; 96%); R_f = 0.42 (n-hexane: ethyl acetate = 2:1); HRESI-MS $[M+H]^+$ calculated for $C_{28}H_{20}N_2O_4SH^+$ 481.1144 observed 481.1222; UV-Vis (MeCN) λmax (log ε): 302 (4.40), 355 (4.69); FTIR (DRIFT, KBr, ῡ, cm^{-1}) 3310 (N–H secondary); 3065 (C–H aromatic); 2959 (C–H aliphatic); 1719 (C=O lactone); 1603 (C=N), 1510 (C=C aromatic); 1258 (C_{aryl}–O– C_{alkyl}); 1173 (C–N); 814 (C–S); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.86 (s, 1H); 8.77 (s, 1H); 8.23 (d, J = 8.92 Hz, 2H); 8.02 (d, J = 7.76 Hz, 2H); 7.90 (s, 1H); 7.84 (d, J = 15.72 Hz, 1H); 7.70 (d, J = 15.52 Hz, 1H); 7.66 (m, 1H); 7.48 (d, J = 8.72 Hz, 1H); 7.43 (m, 1H); 7.03 (d, J = 8.72 Hz, 2H); 3.83 (s, 3H); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 190.1 (C); 163.3 (C); 158.4 (C); 151.3 (C); 148.7 (CH); 147.3 (CH); 141.5 (C); 139.2 (C); 135.2 (CH); 134.7 (CH); 134.6 (C); 133.8 (C); 131.2 (CH); 131.0 (CH); 130.9 (CH); 125.2 (CH); 125.0 (CH); 116.3 (CH); 116.2 (CH); 116.1 (C); 116.0 (C); 114.5 (CH); 114.3 (C); 55.4 (CH_3).

(E)-3-(2-((4-(3-(4-bromophenyl)acryloyl)phenyl)amino)thiazol-4-yl)-2H-chromen-2-one
brown powder (192 mg; 78%); R_f = 0.43 (n-hexane: ethyl acetate = 2:1); HRESI-MS $[M+H]^+$ calculated for $C_{27}H_{17}BrN_2O_3SH^+$ 529.0143 observed 529.0222; UV-Vis (DMSO) λmax (log ε): 303 (4.79), 368 (4.77);

FTIR (DRIFT, KBr, ῡ, cm^{-1}) 3278 (N–H secondary); 3182 (C–H aromatic); 1735 (C=O chalcone); 1716 (C=O lactone); 1607 (C=N), 1541 (C=C aromatic); 1182 (C–N); 820 (C–S); 756 (C_{aryl}–Br). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.88 (s, 1H); 8.76 (s, 1H); 8,25 (d, J = 8.92 Hz, 2H); 7.0 (s, 1H); 7.85-8.02 (m, 5H); 7.66 (m, 2H); 7.64 (m, 1H); 7.47 (d, J = 8.2 Hz, 1H); 7.42 (m, 1H).

(E)-3-(2-((4-(3-(2,5-dimethoxyphenyl)acryloyl)phenyl)amino)thiazol-4-yl)-2H-chromen-2-one
green powder (254 mg; 99%); R_f = 0.42 (n-hexane: ethyl acetate = 2:1); HRESI-MS $[M+H]^+$ calculated for $C_{29}H_{22}N_2O_5SH^+$ 511.1328 observed 511.1328; UV-Vis (MeCN) λmax (log ε): 295 (4.31), 367 (4.45); FTIR (DRIFT, KBr, ῡ, cm^{-1}) 3446 (N–H secondary); 3076 (C-H aromatic); 2945 (C-H aliphatic); 1734 (C=O chalcone); 1718 (C=O lactone); 1606 (C=N), 1543 (C=C aromatic); 1254 (C_{aryl}–O– C_{alkyl}); 1174 (C–N); 827 (C–S). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.82 (s, 1H); 8.71 (s, 1H); 8.21 (d, J = 8.96 Hz, 2H); 7.96 (d, J = 8.96 Hz, 2H); 7.98 (d, J = 15.72 Hz, 1H); 7.90 (d, J = 15.76 Hz, 1H); 7.85 (s, 1H); 7.85-8.00 (m, 3H); 7.60 (m, 1H); 7.51 (d, J = 2.4 Hz, 1H); 7.42 (d, J = 8.24 Hz, 1H); 7.38 (m, 1H); 7.00 (d, J = 2.4 Hz, 1H); 3.82 (s, 3H); 3.78 (s, 3H); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 187.2 (C); 161.7 (C); 158.8 (C); 153.3 (C); 152.6 (C); 152.4 (C); 145.0 (C); 143.8 (C); 141.5 (C); 138.9 (CH); 137.3 (CH); 131.8 (CH); 130.6 (C); 130.5 (CH); 129.1 (CH); 124.7 (CH); 123.7 (C); 122.1 (CH); 120.2 (C), 119.3 (C); 117.8 (CH); 116.4 (CH); 115.9 (CH); 113.0 (CH); 112.6 (CH); 111.2 (CH); 56.2 (CH_3); 55.7 (CH_3).

(E)-3-(2-((4-(3-(2,4-dimethoxyphenyl)acryloyl)phenyl)amino)thiazol-4-yl)-2H-chromen-2-one
orange crystal (212 mg; 83%); R_f = 0.48 (n-hexane: ethyl acetate = 4:3); HRESI-MS $[M+H]^+$ calculated for $C_{29}H_{22}N_2O_5SH^+$ 511.1311 observed 511.1328; UV-Vis (DMSO) λmax (log ε): 292 (4.10), 379 (4.44); FTIR (DRIFT, KBr, ῡ, cm^{-1}) 3290 (N–H secondary); 3068 (C-H aromatic); 2930 (C-H aliphatic); 1737 (C=O chalcone); 1707 (C=O lactone); 1602 (C=N), 1543 (C=C aromatic); 1255 (C_{aryl}–O– C_{alkyl}); 1180 (C–N); 825 (C–S); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.83 (s, 1H); 8.76 (s, 1H); 8.19 (d, J = 8.92 Hz, 2H); 8.01 (d, J = 8.08 Hz, 2H); 7.96 (d, J = 8.96 Hz, 2H); 7.94 (d, J = 15.16 Hz, 1H); 7.89 (s, 1H); 7.79 (d, J = 15.64 Hz, 1H); 7.65 (m, 1H); 7.42 (d, J = 8.28 Hz, 1H); 7.41 (m, 1H); 6.64 (d, J = 8.4 Hz, 2H); 3.92 (s, 3H); 3.85 (s, 3H). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 187.2 (C); 163.0 (C); 161.8 (C); 159.9 (C); 158.9 (C); 152.4 (C); 144.9 (C); 143.8 (C); 139.0 (CH); 137.8 (CH); 131.9 (CH); 131.0 (C); 130.4 (CH); 130.0 (CH); 129.2 (CH); 124.8 (CH); 120.3 (C), 119.4 (C); 119.1 (CH); 116.4 (CH); 116.1 (C); 116.0 (CH); 106.4 (CH); 98.4 (CH); 55.9 (CH_3); 55.6 (CH_3).

(E)-3-(2-((4-(3-(2,3-dimethoxyphenyl)acryloyl)phenyl)amino)thiazol-4-yl)-2H-chromen-2-one
yellow powder (206 mg; 81%); R_f = 0.46 (n-hexane: ethyl acetate = 3:2); HRESI-MS $[M+H]^+$ calculated for $C_{29}H_{22}N_2O_5SH^+$ 511.1326 observed 511.1328; UV-Vis (MeCN) λmax (log ε): 296 (4.39), 354 (4.49); FTIR (DRIFT, KBr, ῡ, cm-1) 3300 (N–H secondary); 3072 (C-H aromatic); 2937 (C-H aliphatic); 1734 (C=O chalcone); 1718 (C=O lactone); 1604 (C=N), 1541 (C=C aromatic); 1271 (C_{aryl}–O– C_{alkyl}); 1174 (C–N); 781 (C–S); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.84 (s, 1H); 8.71 (s, 1H); 8.20 (d, J = 8.96 Hz, 2H); 7.96 (d, J = 8.96 Hz, 2H); 7.95 (d, J = 15.84 Hz, 1H); 7,88 (d, J = 15.72 Hz, 1H); 7.86 (s, 1H); 7,61 (m, 1H); 7.57 (m, 1H); 7.42 (d, J = 8.24 Hz, 1H); 7.38 (m, 1H); 7.13 (d, J = 2.6 Hz, 1H); 3,82 (s, 3H); 3,78 (s, 3H); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 187,2 (C); 161,6 (C); 158,8 (C); 152.8 (C); 152,4 (C); 148.2 (C); 145.1 (C); 143.8 (C); 141.5 (C); 138.9 (CH); 136.9 (CH); 131.8 (CH); 130.5 (CH); 130.5 (C); 129.1 (CH); 128.4 (C); 124.7 (CH); 124.3 (CH); 122.9 (CH); 120.2 (C), 119.3 (C); 119.1 (CH); 116.4 (CH); 115.9 (CH); 114.8 (CH); 112.6 (CH); 111.2 (CH); 60.9 (CH_3); 55.8 (CH_3).

(E)-3-(2-((4-(3-(2,4,6-trimethoxyphenyl)acryloyl)phenyl)amino)thiazol-4-yl)-2H-chromen-2-one
Yellow brownish powder (242 mg; 90%); R_f = 0.40 (n-hexane: ethyl acetate = 4:3); HRESI-MS $[M+H]^+$ calculated for $C_{30}H_{24}N_2O_6SH^+$ 541.1437 observed 541.1433; UV-Vis (MeCN) λmax (log ε): 252 (4.30), 297 (4.19), 365 (4.60); FTIR (DRIFT, KBr, ῡ, cm-1) 3267 (N–H secondary); 3071 (C-H aromatic); 2943 (C-H aliphatic); 1739 (C=O chalcone); 1720 (C=O lactone); 1606 (C=N), 1543 (C=C aromatic); 1273 (C_{aryl}–O– C_{alkyl}); 1172 (C–N); 823 (C–S). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.81 (s, 1H); 8.76 (s, 1H); 8.10 (d, J = 15.76 Hz, 1H); 8.08 (d, J = 8.88 Hz, 2H); 8.01 (dd; J = 1.52 Hz; 7.52 Hz; 1H); 7.93 (d, J = 15.72 Hz, 1H); 7.92 (d, J = 8.88 Hz, 2H); 7.87 (s, 1H); 7.63 (m, 1H); 7.57 (m, 1H); 7.45 (d, J = 8.32 Hz, 1H); 7.40 (m, 1H); 7.13 (s, 2H); 3.94 (s, 6H); 3.86 (s, 3H). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 188.2 (C); 163.2 (C); 161.8 (C); 161,4 (C); 158,9 (C); 152,4 (C); 144,7 (C); 143,9 (C); 139,0 (CH); 134,3 (CH); 131,8

(CH); 131,9 (CH); 131,4 (C); 129,2 (CH); 124,8 (CH); 124,3 (CH); 120,3 (CH), 120,2 (C); 119,4 (C); 116,5 (CH); 115,9 (CH); 105,3 (C); 91,1 (CH); 56,2 (CH_3); 55,6 (CH_3).

(E)-3-(2-((4-(3-(4-morpholinophenyl)acryloyl)phenyl)amino)thiazol-4-yl)-2H-chromen-2-one
Green crystal (214 mg; 80%); R_f = 0.45 (n-hexane: ethyl acetate = 3:2); HRESI-MS $[M+H]^+$ calculated for $C_{31}H_{25}N_3O_4SH^+$ 536.1642 observed 536.1644; UV-Vis ($CHCl_3$) λmax (log ε): 255 (4.34), 282 (4.30), 380 (4.60); FTIR (DRIFT, KBr, ῡ, cm^{-1}) 3267 (N–H secondary); 3059 (C-H aromatic); 2849 (C-H aliphatic); 1730 (C=O chalcone); 1720 (C=O lactone); 1603 (C=N), 1537 (C=C aromatic); 1174 (C–N); 817 (C–S). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.83 (s, 1H); 8.75 (s, 1H); 8.21 (d, *J* = 8.9 Hz, 2H); 8.01 (dd; *J* = 1.5 Hz; 7.8 Hz; 1H); 7.95 (d, *J* = 15.8 Hz, 1H); 7.75 (m, 3H); 7.69 (s, 1H); 7.65 (m, 1H); 7.47 (d, *J* = 8.3 Hz, 1H); 7.42 (td, 1H); 6.99 (d, *J* = 9.0 Hz, 2H); 3.74 (t, 4H); 3.25 (t, 4H). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 187.0 (C); 161.8 (C); 158.9 (C); 152.4 (C); 144.8 (C); 143.8 (C); 143.5 (CH); 131.9 (CH); 131.5 (CH); 131.1 (C); 130.3 (CH); 129.1 (CH); 125.1 (C); 124.8 (CH); 120.3 (C); 119.3 (C); 117.9 (CH); 116.4 (CH); 116.0 (CH); 114.2 (CH); 66.0 (CH_2); 47.3 (CH_2).

Determination of Antioxidant Activity

DPPH Scavenging Activity Assay

The scavenging activity against the free radical DPPH was assessed following the protocol outlined by Ramadhan *et al.*[19] with minor modifications. A 0.10 mM DPPH solution was prepared by dissolving DPPH powder in 100 mL of methanol. The antioxidant activity was evaluated using a 96-well plate format, wherein 20 µL of the sample at varying concentrations was mixed with 80 µL of the DPPH solution. The mixture was incubated for 30 minutes in a dark environment to prevent light interference. The absorbance was then measured at 515-517 nm using a microplate reader. A blank was prepared by following the same procedure without the addition of the sample. Each concentration was tested in seven independent replicates. The scavenging activity was calculated using the following equation:

$$\text{Percentage Scavenging Activity} = (A_{blank} - A_{sample})/A_{blank} \times 100\%$$

ABTS Assay

The antioxidant activity of the synthesized compounds was assessed using the ABTS assay, following the protocols established by Xiao *et al.*[20] and Ramadhan *et al.*[19], with minor modifications. The working solution was prepared by mixing a 7 mM ABTS•+ solution with a 2 mM potassium persulfate solution, followed by incubation in a dark environment for 12-16 hours at room temperature. The antioxidant activity was evaluated by mixing 20 µL of the sample with 80 µL of the ABTS•+ solution and incubating the mixture for 60 minutes under dark conditions. Absorbance was measured at wavelengths between 734 and 750 nm using a microplate reader. Each concentration was tested in seven independent replicates. The percentage of inhibition and IC_{50} values were calculated using the same formula as employed in the DPPH assay.

RESULTS AND DISCUSSION

Synthesis and Structure Elucidation of Target Molecules

The target compounds are hybrid molecules comprising a chalcone moiety, a thiazole ring, and a coumarin structure.These compounds were synthesized through a five-step reaction sequence,as illustrated in Fig.-1.

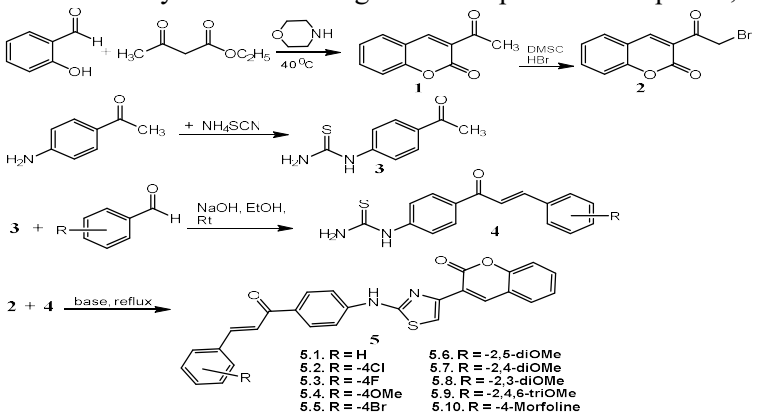

Fig.-1: Reaction Sequence for the Synthesis of the Target Molecules

3-Acetyl coumarin (**1**), serving as the starting material, was synthesized through a one-step, solvent-free reaction involving transesterification followed by a Knoevenagel condensation, using salicylaldehyde and ethyl acetoacetate as reagents and morpholine as a base catalyst.[21,22] The infrared (IR) spectra of this compound confirmed the presence of the lactone moiety, indicated by the vibration bands at 1211 cm^{-1} for the C-O lactone and 1742 cm^{-1} for the carbonyl lactone, respectively.[23,24] The mass spectra showed a close match between the calculated molecular mass (m/z 211.03711) and the observed value (m/z 211.03715) for the $[C_{11}H_8O_3Na]^+$ ion. Proton NMR spectra displayed two multiple aromatic signals at 6.65 and 7.34 ppm, along with an alkene proton signal at 8.50 ppm. Meanwhile, the carbon NMR spectra revealed deshielded signals at 195.6 and 159.4 ppm, corresponding to the carbonyl ketone and carbonyl lactone, respectively. The bromination of compound **1** to yield 3-(2-bromoacetate) coumarin (**2**) was carried out using DMSO and HBr. The transformation of the acetyl group to a bromoacetyl moiety was confirmed by the presence of a mass spectrometric peak at m/z 266, consistent with the expected mass, and by the C-Br infra-red vibration band at 671 cm^{-1}.[25] A nucleophilic addition reaction was employed to synthesize 1-(4-acetylphenyl) thiourea (**3**) by reacting 4-aminoacetophenone with ammonium thiocyanate. The formation of compound **3** was confirmed by infrared (IR) spectroscopy, which indicated the presence of a urea moiety through characteristic vibration bands at 3346 cm^{-1} for the primary amine, 3163 cm^{-1} for the secondary amine, and 1254 cm^{-1} for the thiocarbonyl group.[26] The assignment of the ^{1}H-NMR spectrum revealed that the -NH_2 group appeared as a broad signal at a chemical shift of 6.98-8.33 ppm, while the -NH- group was observed as a singlet at 10.01 ppm. The ^{13}C-NMR spectrum provided evidence of the C=S group at a chemical shift of 181.7 ppm and the C=O group at 197.1 ppm. The observed molecular masses of the synthesized chalcone-thiourea derivatives (4.1–4.10) were consistent with the calculated values. The structures of these chalcone-thiourea derivatives were characterized using infrared (IR) spectroscopy, as well as ^{1}H- and ^{13}C-NMR spectroscopy, with particular attention to the carbonyl-α, β-unsaturated moiety of the chalcones. In the IR spectra, the carbonyl group exhibited vibration bands at 1591–1610 cm^{-1}, while the C=C alkene group showed vibration bands at 1625–1660 cm^{-1}. Additionally, the carbonyl group of the chalcones was identified in the ^{13}C-NMR spectra by signals at 187.3–188.2 ppm. The ^{1}H-NMR spectra revealed the Hα of the enone moiety as signals at 7.66–7.91 ppm, and the Hβ as signals at 7.70–8.02 ppm, with a coupling constant of 15.4–15.8 Hz, indicating a trans conformation. The structure of the targeted hybrid molecules (5.1–5.10), which consist of coumarin, a thiazole ring, and chalcones, was synthesized by reacting compounds **2** and **4** using sodium carbonate as a base and acetone as a solvent in a Hantzsch reaction. The structural determination of the coumarin and chalcone moieties has been previously discussed; therefore, the focus here is on the identification of the thiazole ring based on spectroscopic data. The UV-Vis spectra of the target compounds exhibited two maximum absorption wavelengths in the ranges of 282–303 nm (with ε values of 4.10–479) and 354–380 nm (with ε values of 4.44–4.77). The first absorption maximum is attributed to the benzene ring of the coumarin and the benzoyl group of the chalcone moiety, while the second corresponds to the chromophore of the coumarin-thiazole and the cinnamoyl group of the chalcone moieties.[27,28] Both absorptions result from $\pi \rightarrow \pi^*$ electronic transitions in the target molecules.[28-31] The IR spectra revealed the presence of characteristic vibration bands, including C=N stretching at 1602–1607 cm^{-1}, C-N stretching at 1172–1182 cm^{-1}, and C-S stretching in the fingerprint region at 781–827 cm^{-1}. The ^{1}H-NMR spectrum displayed a singlet for a methine proton at a chemical shift of 7.88–7.89 ppm, along with a singlet for an -NH- proton at 10.81–10.87 ppm. Additionally, the ^{13}C-NMR spectrum provided further confirmation with a signal for the methine carbon at a chemical shift of 160–162 ppm.[11,32,33]

Antioxidant Activity Assay

The antioxidant activity of the target compounds was evaluated using the DPPH[34] and ABTS[35] assays. The maximum wavelength used for the DPPH assay was 517 nm, while for the ABTS assay, it was 750 nm.

Table-1: Radical Scavenging Activity of the Prepared Compound

Compd (100 ppm)	DPPH (% inhibition ± SD)		ABTS (% inhibition ± SD)	
	30 min	20 h	1 h	20 h
5.1	30.12 ± 0.735	68.25 ± 0.000	36.37 ± 0.100	37.59 ± 1.54
5.2	44.41 ± 1.016	61.11 ± 1.375	31.16 ± 0.265	19.74 ± 0.109

5.3	50.95 ± 0.804	73.28 ± 0.458	19.54 ± 0.100	28.10 ± 0.762
5.4	51.63 ± 0.584	56.08 ± 1.212	35.70 ± 0.452	24.20 ± 0.377
5.5	54.39 ± 1.027	86.24 ± 1.833	33.17 ± 0.501	16.40 ± 0.109
5.6	59.73 ± 1.993	57.94 ± 3.637	38.18 ± 0.609	29.04 ± 0.576
5.7	45.78 ± 0.326	70.90 ± 5.741	39.31 ± 0.153	20.80 ± 0.653
5.8	48.20 ± 1.018	60.85 ± 0.458	35.87 ± 1.041	14.08 ± 0.218
5.9	38.90 ± 0.530	59.52 ± 1.375	36.37 ± 0.100	26.08 ± 1.728
5.10	45.61 ± 0.516	57.41 ± 0.916	36.44 ± 0.834	50.09 ±0.762
Gallic acid	72.63 ± 0.329	not determined	94.66 ± 0.153	not determined

The assay results were quantified as percentage inhibition, with higher values indicating increased antioxidant activity. The antioxidant potential of the target compounds was evaluated in comparison to gallic acid, which served as the positive control. To investigate whether the target molecules maintained their antioxidant efficacy over prolonged periods, the incubation time (excluding gallic acid) was extended up to 20 hours. This approach aligns with previous studies that also assess the influence of varying incubation times on antioxidant[33,34]. The findings are summarized in Table-1. In the DPPH assay, the inhibition activity ranged from 30% to 60% after 30 minutes of incubation, and from 56% to 86% after 20 hours of incubation. Conversely, the ABTS assay showed inhibition activity between 19% and 39% after 30 minutes, and between 14% and 50% after 20 hours. The comparatively lower inhibition activity observed for the tested compounds in the ABTS assay is likely due to the decreasing activity of the ABTS radical over time, attributable to its metastable nature[36]. Furthermore, the target compounds demonstrated relatively lower activity when compared to gallic acid. The sulfur atom within the aromatic thiazole ring system of the target molecules may undergo tautomerization to form a –SH group, which could react with free radicals. This reaction may reduce the capacity of the target molecules to neutralize free radicals, particularly in assays with incubation periods shorter than one hour. Additionally, no linear correlation was found between antioxidant activity and the position or type of substituents. This absence of correlation could be explained by the spatial separation of the substituents from the functional groups, such as the -NH group and sulfur atom, which are most likely to display antioxidant activity. The findings demonstrated that the synthesized hybrid molecules exhibited antioxidant activity through multiple mechanisms. This aligns with the research conducted by Swathi and Sundararajan[37], which suggested that hybrid molecules may possess more than one biological activity, warranting further studies to gain a deeper understanding of their mechanisms of action.

CONCLUSION

In conclusion, we have successfully synthesized a series of molecular-hybrid compounds integrating three pharmacophores: coumarin, thiazole, and chalcone. The synthesis followed a five-step reaction sequence, including bimolecular nucleophilic substitution, 5-exo-trig intramolecular cyclization via nucleophilic addition, and the Hantzsch thiazole reaction. The antioxidant properties of the synthesized compounds were evaluated using DPPH and ABTS assays, with the latter showing superior antioxidant activity. However, the results indicated no significant correlation between antioxidant activity and the type or position of substituents on the molecules.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID IDs, given below:

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