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Thiazolyl-pyrazole-biscoumarin synthesis and evaluation of their antibacterial and antioxidant activities

Nosrat O. Mahmoodi[1] · **Shahryar Ghodsi**[2]

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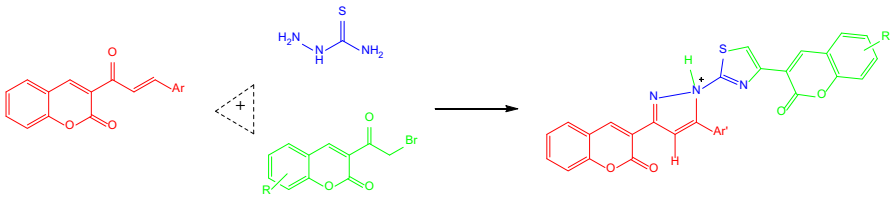
Abstract A series of novel 3-(2-oxo-2*H*-chromen-3-yl)-1-(4-(2-oxo-2*H*-chromen-3-yl)thiazol-2-yl)-5-aryl-1*H*-pyrazol-1-ium bromides have been prepared through a one-pot three-component cyclocondensation of various coumarin chalcones, thiosemicarbazide and 2-bromocoumarin. The key features of this reaction are the incorporation of four heterocyclic rings in the structure of target products, using commonly available and inexpensive catalysts, high yields, and simple reaction conditions. Final salt products were obtained by self-capturing of a proton by the nitrogen of the pyrazole moiety. The easy work-up and mild reaction conditions are notable features of this protocol. The antioxidant, antibacterial, and anti-fungal activities of the synthetic products were examined. Most of the compounds showed good biological capacity in comparison to references.

Graphical Abstract



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✉ Nosrat O. Mahmoodi
mahmoodi@guilan.ac.ir; nosmahmoodi@gmail.com

[1] Department of Organic Chemistry, Faculty of Sciences, University of Guilan, PO Box 41335-1914, Rasht, Iran

[2] Faculty of Science, School of Chemistry, University of Tehran, Tehran, Iran

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Introduction

In recent years, many studies, either by our research group or others, were carried out on heterocyclic systems bearing thiazole and pyrazoline groups as pharmacophores. Compounds possessing pyrazoles and pyrazolines are an interesting class of heterocycles because of their synthetic flexibility and useful biological activities [1], serving as antimicrobials [2, 3], antivirals [4], anti-inflammatories [5, 6], antidepressants [7], antituberculars [8], antiamoebics [9], and analgesics [10].

Similarly, the thiazole moiety is a prevalent scaffold in a number of naturally occurring and synthetic molecules with remarkable medicinal value due to their potential chemotherapeutic [11], fungicidal [12], antiviral [13], antibacterial [14], anti-HIV [15], hypnotic [16], pesticidal [17], anticonvulsant, anti-inflammatory [18] enzyme inhibition [19] and antitumour activities [20]. They have also found broad application in the treatment of allergies [21], schizophrenia [22], hypertension [23], and, more recently, pain [24], as fibrinogen receptor antagonists with antithrombotic activity [25]. The presence of a toxophoric moiety (S–C=N) is responsible for such properties, especially the antimicrobial activity, of these molecules [26].

In addition, coumarin and its derivatives are representative of one the most important classes of heterocyclic compounds, which encompass a wide variety of biological activities [1] that include antibacterial [27], antifungal [28, 29], antitumor [30, 31], herbicidal, anti-inflammatory [32], anticancer [33], anti-HIV [33], and monoamino oxidase (MAO) enzymatic inhibition [33] properties.

On the other hand, coumarin chalcones exhibit versatile pharmacological and biological activities like antimicrobial, anti-inflammatory, anti-HIV, anticoagulant, anticancer, antihypertensive, antimalarial, hypoglycemic, and antileshmanial activities [34]. An important feature of chalcones is their ability to act as an intermediate for the synthesis of various valuable heterocyclic compounds such as cyclohexenone, isoxazoline, pyrazoline, pyridine and pyrimidine derivatives, and so on with considerable biological and medicinal properties [35, 36]. Keeping in mind these interesting pharmacological features, we planned to design of molecular skeleton by combination of these three valuable pharmacophores: coumarins, pyrazoles and thiazoles together in a compact structure, and study the biological activities of the resulting compounds (Fig. 1).

Experimental

Materials and methods

Melting points (uncorrected) were determined by using a Mettler Fp5 apparatus. The infrared (IR) spectra were recorded on a Shimadzu IR-470 spectrophotometer using KBr disc method. Proton (^{1}H) and carbon-13 (^{13}C) spectra were recorded on a

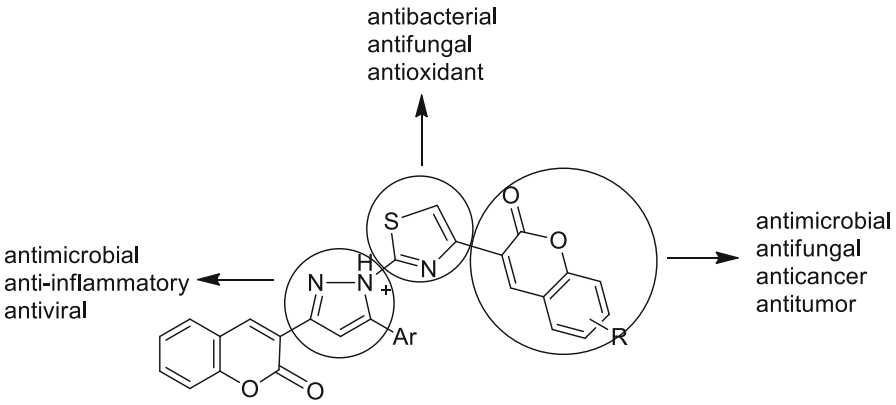


Fig. 1 Structure of products **4a–q** and defining biological activities of each moiety

Avance III Bruker 400 MHz spectrometer at 25 °C using tetramethylsilane as an internal standard and deuterated dimethyl sulfoxide (DMSO-d_6) as the solvent. Chemical shifts were reported in ppm (δ). Splitting patterns were assigned as: s for singlet, br s for broad singlet, d for doublet, t for triplet, td for triplet of doublets, dd for doublet of doublets, ddd for doublet of doublet of doublets, and m for multiplet. Elemental analyses were performed on a Perkin Elmer series II, 2400 CHN analyzer and experimental data were within ±0.4 % of the theoretical values. A microwave oven (ETHOS 1600, Milestone) with a power of 600 W specially designed for organic synthesis was used. Sonication was performed by a Fisher sonicator (with a frequency of 25 kHz and a nominal power 600 W). The X-ray data were obtained with a Bruker SMART diffractometer (Fig. 2).

General procedure for the synthesis of compounds 4a–q

An EtOH solution of thiosemicarbazide **3** (5.00 mmol) was added to a solution of the appropriate prepared coumarin chalcone **1a–d** (5.00 mmol) in hot EtOH (10 mL) with stirring. The resulting solution was refluxed for 4–6 h in the presence of glacial AcOH. Then, an ethanolic solution (30 mL) of 3-(2-bromoacetyl)-coumarin **2a–d** (1.34 g, 5 mmol) was added to the resulting 4,5-dihydropyrazol-1-thiocarboxamide **3** and the mixture was refluxed on a water bath for 2 h. After completion of the reaction [monitored by thin layer chromatography (TLC)] and neutralization with a saturated solution of $NaHCO_3$, the precipitated solid was filtered, washed with EtOH, and recrystallized from a mixture of dimethylformamide (DMF)/H_2O to afford the target compounds.

3-(2-Oxo-2H-chromen-3-yl)-1-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)-5-phenyl-1H-pyrazol-1-ium ***(4a)*** Dark cream-colored solid (2.296 g, 77 %); Mp 287–290 °C; FT-IR (KBr; ν max/cm^{-1}): 3419 (stretch N–H), 3118 (stretch C–H Ar), 1722, 1676 (stretch O–C=O), 1612 (stretch C=N), 1562, 1455 (stretch C=C), 1419 (bending C–H), 1278, 1204, 1089, 1111 (stretch C–O, bending C–N, C–S),

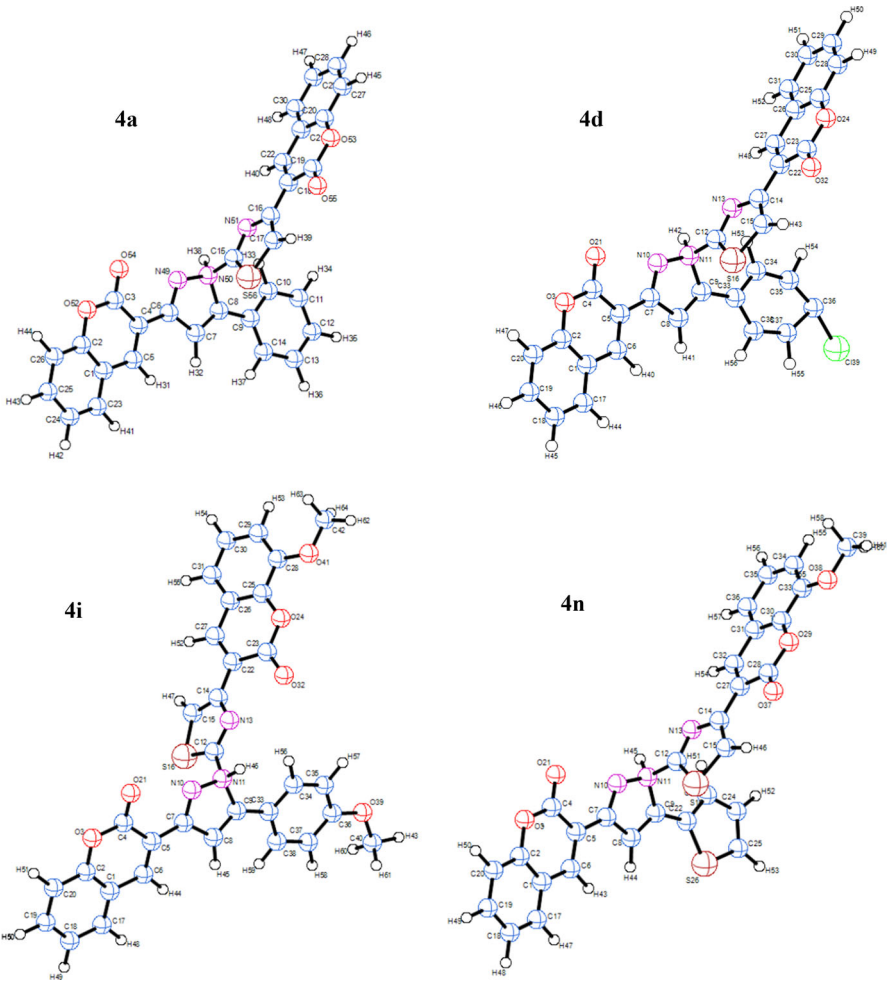


Fig. 2 X-ray single crystal structure of compounds **4a**, **4d**, **4i** and **4n**

934, 861, 756, 700 (OOP C–H); ^{1}H nuclear magnetic resonance (NMR; 400 MHz, DMSO-d_6): δ = 11.23 (br s, 1H, NH, H_r), 8.62 (s, 1H, H_e), 8.52 (d, J = 5.2 Hz, 1H, H_d), 8.42 (dd, J_1 = 7.6, J_2 = 1.6 Hz, 1H, H_b), 8.24–8.25 (m, 2H, H_c, H_a), 8.1 (s, 1H, H_m), 7.917 (s, 1H, H_l), 7.61–7.88 (m, 10H, H_f, H_g, H_h, H_i, H_j, H_k, H_n, H_o, H_p, H_q) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ; 165.2 (C=O), 162.4, 159.8, 153.9, 150.8, 143.6, 141.9, 135.3, 132.6, 130.9, 128.5, 126.7, 125.3, 124.9, 123.7, 119.2, 118.2, 117.8, 116.7, 116, 112.8, 106; MS (EI) *m/z*: 516.10 $(M)^+$; Anal. calcd. for $C_{30}H_{18}N_3O_4S^+$: C, 69.76; H, 3.51; N, 8.13. Found: C, 69.89; H, 3.61; N, 8.19 %.

1-(4-(8-Methoxy-2-oxo-2H-chromen-3-yl)thiazol-2-yl)-3-(2-oxo-2H-chromen-3-yl)-5-phenyl-1H-pyrazol-1-ium ***(4b)*** Lemon-yellow solid (2.505 g, 80 %); Mp 275–278 °C; FT-IR (KBr; ν max/cm^{-1}): 3428 (stretch N–H), 3136 (stretch C–H

Ar), 1721, 1676 (stretch O–C=O), 1611(stretch C=N), 1565, 1458 (stretch C=C), 1366 (bending C–H), 1276, 1203, 1108, 1033 (stretch C–O, bending C–N, C–S), 933, 860, 759, 699 (OOP C–H); ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.23$ (br s, 1H, NH, H_r), 8.61 (s, 1H, H_e), 8.42 (d, $J = 8$ Hz, 1H, H_d), 8.24 (s, 1H, H_m), 7.87 (d, $J = 7.6$ Hz, 1H, H_n), 7.71 (t, $J = 7.8$ Hz, 2H, H_c and H_o), 7.63 (t, $J = 7.2$ Hz, 2H, H_h and H_j), 7.43–7.53 (m, 8H, H_a, H_b, H_f, H_g, H_i, H_k, H_l, H_p), 3.93 (s, 3H, OCH_3); MS (EI) *m/z*: 546.11 $(M)^+$; Anal. calcd. for $C_{31}H_{20}N_3O_5S^+$: C, 68.12; H, 3.69; N, 7.69. Found: C, 68.20; H, 3.80; N, 6.76 %.

1-(4-(7-Methoxy-2-oxo-2H-chromen-3-yl)thiazol-2-yl)-3-(2-oxo-2H-chromen-3-yl)-5-phenyl-1H-pyrazol-1-ium ***(4c)*** Brown solid (2.505 g, 80 %); Mp 233–237 °C; FT-IR (KBr; *v* max/cm^{-1}): 3433 (stretch N–H), 3136 (stretch C–H Ar), 1720, 1681 (stretch O–C=O), 1611 (stretch C=N), 1557, 1456 (stretch C=C), 1368 (bending C–H), 1279, 1220, 1152, 1024 (stretch C–O, bending C–N, C–S), 933, 845, 761, 702 (OOP C–H); ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.23$ (br s, 1H, NH, H_r), 8.61 (s, 1H, H_e), 8.41 (d, $J = 8$ Hz, 1H, H_d), 8.24 (s, 1H, H_m), 7.87 (d, $J = 7.8$ Hz, 1H, H_b), 7.85 (d, $J = 7.8$ Hz, 1H, H_a), 7.71 (t, $J = 7.8$ Hz, 1H, H_c), 7.63 (t, $J = 7.8$ Hz, 2H, H_h, H_j), 7.43–7.53 (m, 8H, H_f, H_g, H_j, H_k, H_l, H_n, H_o, H_q); MS (EI) *m/z*: 546.11 $(M)^+$; Anal. calcd. for $C_{31}H_{20}N_3O_5S$: C, 68.12; H, 3.69; N, 7.69. Found: C, 68.24; H, 3.75; N, 6.71 %.

5-(4-Chlorophenyl)-3-(2-oxo-2H-chromen-3-yl)-1-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)-1H-pyrazol-1-ium ***(4d)*** Brick-red solid (2.428 g, 77 %); Mp 283–287 °C; FT-IR (KBr; *v* max/cm^{-1}): 3413 (stretch N–H), 3116 (stretch C–H Ar), 1722, 1674 (stretch O–C=O), 1611 (stretch C=N), 1561, 1454 (stretch C=C), 1366, 1322 (bending C–H), 1276, 1203,1088, 1024 (stretch C–O, bending C–N, C–S), 930, 860, 753, 698 (OOP C–H); ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.24$ (br s, 1H, NH, H_r), 8.63 (s, 1H, H_e), 8.43 (d, $J = 7.2$ Hz, 1H, H_d), 8.26 (s, 1H, H_m), 7.87 (d, $J = 7.6$ Hz, 2H, H_a, H_q), 7.72 (t, $J = 7.8$ Hz, 2H, H_h, H_j), 7.65 (t, $J = 8.3$ Hz, 2H, H_g, H_k), 7.40–7.55 (m, 7H, H_b, H_c, H_f, H_l, H_n, H_o, H_p); ^{13}C NMR (100 MHz, DMSO-d_6): δ; 169 (C=O), 161.3, 153.8, 150.8, 144, 143.5, 139.8, 138.9, 135.4, 133.16, 131.67, 129.66, 128.84, 125.36, 124.7, 123.56, 122.3, 120.8, 119.68, 118.37, 117.92, 116.63, 116.55, 113.52. MS (EI) *m/z*: 550.06 $(M)^+$. Anal. calcd. for $C_{30}H_{17}ClN_3O_4S^+$: C, 65.40; H, 3.11; N, 7.63. Found: C, 65.31; H, 3.06; N, 7.58 %.

5-(4-Chlorophenyl)-1-(4-(8-methoxy-2-oxo-4a,8a-dihydro-2H-chromen-3-yl)thiazol-2-yl)-3-(2-oxo-2H-chromen-3-yl)-1H-pyrazol-1-ium ***(4e)*** Golden solid (2.643 g, 80 %); Mp 296–299 °C; FT-IR (KBr; *v* max/cm^{-1}): 3427 (stretch N–H), 3132 (stretch C–H Ar), 1721, 1676 (stretch O–C=O), 1612 (stretch C=N), 1564, 1456 (stretch C=C), 1367, 1321 (bending C–H), 1277, 1204, 1091, 1033 (stretch C–O, bending C–N, C–S), 936, 861, 758, 700 (OOP C–H); ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.23$ (br s, 1H, NH, H_r), 8.61 (s, 1H, H_e), 8.41 (d, $J = 7.2$ Hz, 1H, H_d), 8.24 (s, 1H, H_m), 7.85 (d, $J = 7.6$ Hz, 1H, H_a), 7.71 (t, $J = 7.8$ Hz, 2H, H_h,H_j), 7.63 (t, $J = 7.8$ Hz, 2H, H_g,H_k), 7.43–7.53 (m, 7H, H_b, H_c, H_f, H_l, H_n, H_o, H_p), 3.93 (s, 3H, OCH_3); MS (EI) *m/z*: 580.07 $(M)^+$; Anal. calcd. for $C_{31}H_{19}ClN_3O_5S^+$: C, 64.08; H, 3.30; N, 7.23. Found: C, 64.20; H, 3.44; N, 7.41 %.

5-(2-Chlorophenyl)-3-(2-oxo-2H-chromen-3-yl)-1-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)-1H-pyrazol-1-ium ***(4f)*** Lemon-yellow solid (2.523 g, 80 %); Mp 292–296 °C; FT-IR (KBr; *v* max/cm^{-1}): 3418 (stretch N–H), 3118 (stretch C–H Ar), 1722, 1675 (stretch O–C=O), 1611 (stretch C=N), 1561, 1454 (stretch C=C), 1372 (bending C–H), 1277, 1204, 1103, 1028 (stretch C–O, bending C–N, C–S), 935, 862, 756, 699 (OOP C–H); ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.22$ (br s, 1H, NH, H_r), 8.61 (s, 1H, H_e), 8.41 (d, $J = 8$ Hz, 1H, H_d), 8.23 (s, 1H, H_m), 7.85 (d, $J = 7.6$ Hz, 2H, H_a, H_q), 7.71 (t, $J = 7.8$ Hz, 2H, H_b, H_p), 7.63 (t, $J = 7.4$ Hz, 2H, H_c, H_o), 7.43–7.53 (m, 7H, H_f, H_g, H_h, H_i, H_j, H_k, H_l); MS (EI) *m/z*: 550.06 $(M)^+$; Anal. calcd. for $C_{30}H_{17}ClN_3O_4S^+$: C, 65.40; H, 3.11; N, 7.63. Found: C, 65.51; H, 3.16; N, 7.75 %.

5-(2-Chlorophenyl)-1-(4-(8-methoxy-2-oxo-2H-chromen-3-yl)thiazol-2-yl)-3-(2-oxo-2H-chromen-3-yl)-1H-pyrazol-1-ium ***(4g)*** Light cream-colored solid (2.610 g, 79 %); Mp 298–300 °C; FT-IR (KBr; *v* max/cm^{-1}): 3420 (stretch N–H), 3122,2931 (stretch C–H Ar), 1722, 1675 (stretch O–C=O), 1611 (stretch C=N), 1563, 1455 (stretch C=C), 1380 (bending C–H), 1277, 1203, 1110, 1089 (stretch C–O, bending C–N, C–S), 934, 861, 756, 699 (OOP C–H); ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.23$ (br s, 1H, NH, H_r), 8.62 (s, 1H, H_e), 8.5 (dd, $J_1 = 15.4$, $J_2 = 1.2$ Hz, 1H, H_d), 8.4 (s, 1H, H_m), 8.22 (dd, $J_1 = 17.4$, $J_2 = 1.2$ Hz, 1H, H_b), 7.89–7.95 (m, 1H, H_c), 7.86 (s, 1H, H_l), 7.25–7.69 (m, 9H, H_a, H_f, H_h, H_i, H_j, H_n, H_o, H_p, H_k), 3.94 (s, 3H, OCH_3) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ; 168.5, 161.3, 153.8, 150.8, 146.8, 146.6, 144, 143.5, 135.4, 133.1, 131.7, 129.6, 128.8, 128.4, 126.8, 125.9, 125.4, 124.7, 121.2, 119.7, 119.3, 119, 118.7, 117.8, 116.5, 113.5, 111.3, 100.8, 89.65, 56.6; MS (EI) *m/z*: 580.07 $(M)^+$; Anal. calcd. for $C_{31}H_{19}ClN_3O_5S^+$: C, 64.08; H, 3.30; N, 7.23. Found: C, 64.16; H, 3.37; N, 7.24 %.

5-(4-Methoxyphenyl)-3-(2-oxo-2H-chromen-3-yl)-1-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)-1H-pyrazol-1-ium ***(4h)*** Cream-colored solid (2.505 g, 80 %); Mp 295–298 °C; FT-IR (KBr; *v* max/cm^{-1}): 3417 (stretch N–H), 3115 (stretch C–H Ar), 1719, 1672 (stretch O–C=O), 1610 (stretch C=N), 1560, 1457 (stretch C=C), 1369 (bending C–H), 1272, 1202, 1088 (stretch C–O, bending C–N), 931, 858, 755, 699 (OOP C–H); ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.23$ (br s, 1H, NH, H_r), 8.62 (s, 1H, H_e), 8.43 (dd, $J_1 = 7.4$, $J_2 = 1.2$ Hz, 1H, H_d), 8.26 (d, $J = 1.2$, 1H, H_m), 7.87 (dd, $J_1 = 7.8$, $J_2 = 1.2$ Hz, 2H, H_a, H_q), 7.72 (dt, $J_1 = 7.9$, $J_2 = 1.6$ Hz, 2H, H_b, H_p), 7.65 (dt, $J_1 = 7.6$, $J_2 = 1.2$ Hz, 2H, H_c, H_o), 7.47–7.56 (m, 7H, H_f, H_g, H_h, H_j, H_k, H_l, H_n) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ; 161.32, 159.7, 153.8, 150.8, 144, 143.5, 135.4, 135, 133.2, 132.5, 131.7, 129.6, 129.1, 125.8, 125.3, 124.7, 121.9, 119.7, 118.4, 117.9, 116.6, 116.5, 113.5, 106.18, 80.65; MS (EI) *m/z*: 546.11 $(M)^+$; Anal. calcd. for $C_{31}H_{20}N_3O_5S^+$: C, 68.12; H, 3.69; N, 7.69. Found: C, 68.15; H, 3.79; N, 7.78 %.

1-(4-(8-Methoxy-2-oxo-2H-chromen-3-yl)thiazol-2-yl)-5-(4-methoxyphenyl)-3-(2-oxo-2H-chromen-3-yl)-1H-pyrazol-1-ium ***(4i)*** Cream-colored solid (2.626 g, 80 %); Mp 292–296 °C; FT-IR (KBr; *v* max/cm^{-1}) : 3422 (stretch N–H), 3117 (stretch C–H Ar), 1722, 1675 (stretch O–C=O), 1611 (stretch C=N), 1562, 1455 (stretch C=C), 1380, 1323 (bending C–H), 1276, 1203, 1089, 1028 (stretch C–O, bending C–N, C–

S), 931, 860, 753, 698 (OOP C–H); ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.22$ (br s, 1H, NH, H_r), 8.60 (s, 1H, H_e), 8.40 (d, $J = 8$ Hz, 1H, H_d), 8.22 (s, 1H, H_m), 7.85 (d, $J = 7.6$ Hz, 2H, H_g, H_k),7.70 (t, $J = 7.8$ Hz, 2H, H_b, H_p), 7.63 (t, $J = 7.8$ Hz, 2H, H_c, H_o), 7.43–7.53 (m, 6H, H_a, H_g, H_h, H_j, H_k, H_l), 3.932 (s, 6H, $2OCH_3$) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ; 179.59, 164.6, 161.37, 159.69, 153.8, 150.8, 143.98, 143.48, 142.87, 139.56, 136.3, 135.34, 134.35, 133.16, 131.66, 129.66, 128.46, 127.72, 127.19, 125.83, 125.35, 124.66, 122.23, 120.45, 118.36, 117.91, 116.61, 116.54, 113.49, 56.6, 56.9; MS (EI) *m/z*: 576.12 $(M)^+$; Anal. calcd. for $C_{32}H_{22}N_3O_6S^+$: C, 66.66; H, 3.85; N, 7.29. Found: C, 66.54; H, 3.73; N, 7.18 %.

5-(4-Nitrophenyl)-3-(2-oxo-2H-chromen-3-yl)-1-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)-1H-pyrazol-1-ium ***(4j)*** Orange solid (2.405 g, 75 %); Mp 235–238 °C; FT-IR (KBr; *v* max/cm^{-1}): 3404 (stretch N–H), 3161 (stretch C–H Ar), 1724, 1679 (stretch O–C=O), 1607 (stretch C=N), 1504, 1462 (stretch C=C), 1334 (bending C–H), 1239, 1107 (stretch C–O, bending C–N), 1000, 954, 845, 752, 698, 588 (OOP C–H); ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.23$ (br s, 1H, NH, H_r), 8.62 (s, 1H, H_e), 8.51 (d, $J = 19.6$, 1H, H_d), 8.44 (s, 1H, H_m), 8.32 (d, $J = 8.8$, 1H, H_n), 7.99 (t, $J = 10.2$ Hz, 2H, H_b, H_p), 7.72 (dt, $J_1 = 7.8$, $J_2 = 1.6$ Hz, 2H, H_c, H_o), 7.49–7.56 (m, 8H, H_a, H_f, H_l, H_g, H_h, H_j, H_k, H_q); MS (EI) *m/z*: 561.09 $(M)^+$; Anal. calcd. for $C_{30}H_{17}N_4O_6S^+$: C, 64.17; H, 3.05; N, 9.98. Found: C, 64.04; H, 3.02; N, 9.90 %.

3-(2-Oxo-2H-chromen-3-yl)-1-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)-5-(thiophen-2-yl)-1H-pyrazol-1-ium ***(4k)*** Cream-colored solid (2.409 g, 80 %); Mp 290–292 °C; FT-IR (KBr; *v* max/cm^{-1}): 3414 (stretch N–H), 3129 (stretch C–H Ar), 1722, 1675 (stretch O–C=O), 1609 (stretch C=N), 1560, 1456 (stretch C=C), 1372 (bending C–H), 1276, 1204, 1099, 1028 (stretch C–O, bending C–N, C–S), 935, 860, 756, 699 (OOP C–H); ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.22$ (br s, 1H, NH, H_r), 8.61 (s, 1H, H_e), 8.41 (d, $J = 8$ Hz, 1H, H_i), 8.23 (s, 1H, H_m), 7.85 (d, $J = 7.6$ Hz, 2H, H_d, H_n), 7.70 (t, $J = 7.6$ Hz, 2H, H_b, H_p), 7.63 (t, $J = 7.6$ Hz, 2H, H_c, H_o), 7.43–7.53 (m, 6H, H_a, H_h, H_i, H_f, H_l, H_q); MS (EI) *m/z*: 522.06 $(M)^+$; Anal. calcd. for $C_{28}H_{16}N_3O_4S_2^+$: C, 64.35; H, 3.09; N, 8.04. Found: C, 64.43; H, 3.16; N, 8.15 %.

1-(4-(7-Methoxy-2-oxo-2H-chromen-3-yl)thiazol-2-yl)-3-(2-oxo-2H-chromen-3-yl)-5-(thiophen-2-yl)-1H-pyrazol-1-ium ***(4l)*** Beige solid (2.530 g, 80 %); Mp 272–277 °C; FT-IR (KBr; *v* max/cm^{-1}): 3360 (stretch N–H), 3131 (stretch C–H Ar), 1721, 1677 (stretch O–C=O), 1611 (stretch C=N), 1561, 1455 (stretch C=C), 1367 (bending C–H), 1277, 1206, 1088, 1024 (stretch C–O, bending C–N, C–S), 968, 931, 853, 754, 699 (OOP C–H); ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.23$ (br s, 1H, NH, H_r), 8.61 (s, 1H, H_e), 8.41 (d, $J = 8$ Hz, 1H, H_i), 8.23 (s, 1H, H_m), 7.84–7.86 (d, $J = 7.8$ Hz, 2H, H_d, H_n), 7.71 (t, $J = 7.9$ Hz, 1H, H_b), 7.63 (t, $J = 7.7$ Hz, 2H, H_c, H_o), 7.43–7.53 (m, 6H, H_a, H_h, H_i, H_f, H_l, H_q), 3.89 (s, 3H, OCH_3); MS (EI) *m/z*: 552.07 $(M)^+$; Anal. calcd. for $C_{29}H_{18}N_3O_5S_2^+$: C, 63.03; H, 3.28; N, 7.60. Found: C, 63.09; H, 3.33; N, 7.66 %.

1-(4-(6-Bromo-2-oxo-2H-chromen-3-yl)thiazol-2-yl)-3-(2-oxo-2H-chromen-3-yl)-5-(thiophen-2-yl)-1H-pyrazol-1-ium ***(4m)*** Golden solid (2.793 g, 82 %); Mp

283–287 °C; FT-IR (KBr; *v* max/cm^{-1}): 3421 (stretch N–H), 3156 (stretch C–H Ar), 1720, 1675 (stretch O–C=O), 1610 (stretch C=N), 1562, 1458, 1417 (stretch C=C and N–H bending), 1364 (bending C–H), 1276, 1203, 1086, 1032 (stretch C–O, bending C–N, C–S), 989, 931, 860, 813, 754, 698 (OOP C–H); ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.23$ (br s, 1H, NH, H_r), 8.61 (s, 1H, He), 8.41 (d, $J = 7.6$ Hz, 1H, Hd), 8.23 (s, 1H, Hm), 7.85 (d, $J = 8$ Hz, 2H, Hi, Ha), 7.71 (t, $J = 7.2$ Hz, 2H, H_b, H_p), 7.63 (t, $J = 7$ Hz, 2H, H_c, H_i), 7.43–7.53 (m, 5H, H_h, H_f, H_l, H_n, H_q); MS (EI) *m/z*: 599.97 $(M)^+$; Anal. calcd. for $C_{28}H_{15}BrN_3O_4S_2^+$: C, 55.91; H, 2.51; N, 6.99. Found: C, 55.80; H, 2.46; N, 6.94 %.

1-(4-(8-Methoxy-2-oxo-2H-chromen-3-yl)thiazol-2-yl)-3-(2-oxo-2H-chromen-3-yl)-5-(thiophen-2-yl)-1H-pyrazol-1-ium ***(4n)*** Light cream-colored solid (2.403 g, 76 %); Mp 295–297 °C; FT-IR (KBr; *v* max/cm^{-1}) 3422 (stretch N–H), 3119 (stretch C–H Ar), 1723, 1675 (stretch O–C=O), 1611 (stretch C=N), 1592, 1455 (stretch C=C), 1418 (bending C–H), 1277, 1204, 1110, 1088 (stretch C–O, bending C–N, C–S), 934, 861, 755, 699 (OOP C–H); ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.23$ (br s, 1H, NH, H_r), 8.63 (s, 1H, H_e), 8.44 (d, $J = 6.8$ Hz, 1H, H_d), 8.26 (d, $J = 1.2$, 1H, H_a), 7.88 (dd, $J_1 = 7.6$, $J_2 = 1.6$ Hz, 2H, H_i, H_n), 7.73 (td, $J_1 = 7.8$, $J_2 = 1.6$ Hz, 1H, H_b), 7.65 (td, $J_1 = 7.4$, $J_2 = 1.6$ Hz, 1H, H_c), 7.31–7.56 (m, 7H, H_h, H_g, H_f, H_l, H_m, H_o, H_p), 3.98 (s, 3H, OCH_3) ppm ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ; 171.2, 161.3, 159.7, 153.8, 146.9, 145.7, 143.5, 137.7, 136.3, 135.7, 135.2, 134.1, 133.7, 132.6, 131.6, 130.5, 129.7, 125.8, 125.4, 124.7, 123.8, 121, 120.54, 119.7, 117.9, 116.64, 116.55, 114.09, 113.54, 56.6; MS (EI) *m/z*: 552.07 $(M)^+$; Anal. calcd. for $C_{29}H_{18}N_3O_5S_2^+$: C, 63.03; H, 3.28; N, 7.60. Found: C, 62.84; H, 3.10; N, 7.41 %.

5-(1H-indol-3-yl)-1-(4-(8-methoxy-2-oxo-2H-chromen-3-yl)thiazol-2-yl)-3-(2-oxo-2H chromen-3-yl)-1H-pyrazol-1-ium ***(4o)*** Cream chocolate-colored solid (2.662 g, 80 %); Mp 217–220 °C; FT-IR (KBr; *v* max/cm^{-1}): 3427 (stretch N–H), 3171 (stretch C–H Ar), 1719, 1682 (stretch O–C=O), 1610 (stretch C=N), 1568, 1467 (stretch C=C), 1369 (C–H bending), 1272, 1202, 1098 (stretch C–O, bending C–N), 934, 858, 820, 765, 719 (OOP C–H); ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.76$ (br s, 1H, NH indole), 11.23 (br s, 1H, NH, H_r), 8.62 (s, 1H, H_e), 8.42 (d, $J = 8$ Hz, 1H, H_d), 8.24 (s, 1H, H_m), 7.95 (s, 1H, H_g), 7.87 (t, $J = 7.4$ Hz, 1H, H_b), 7.71 (t, $J = 7.6$ Hz, 1H, H_c), 7.64 (t, $J = 7.4$ Hz, 1H, H_j), 7.28–7.54 (m, 9H, H_a, H_f, H_h, H_i, H_k, H_l, H_n, H_o, H_p), 3.94 (s, 3H, OCH_3) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ; 177.7, 163.8, 160.6, 158.9, 157.7, 153.1, 150, 146.2, 144.6, 143.2, 142.7, 142.1, 134.6, 132.4, 130.9, 128.9, 125.1, 124.7, 124.6, 123.9, 123.7, 120, 119.7, 118.9, 118.8, 117.6, 117.2, 115.8, 115.6, 114, 112.7, 105.4, 55.9; MS (EI) *m/z*: 585.12 $(M)^+$; Anal. calcd. for $C_{33}H_{21}N_4O_5S^+$: C, 67.68; H, 3.61; N, 9.57. Found: C, 67.61; H, 3.49; N, 9.51 %.

5-(1H-indol-3-yl)-1-(4-(7-methoxy-2-oxo-2H-chromen-3-yl)thiazol-2-yl)-3-(2-oxo-2H-chromen-3-yl)-1H-pyrazol-1-ium ***(4p)*** Brick-red solid (2.662 g, 80 %); Mp 292–295 °C; FT-IR (KBr; *v* max/cm^{-1}): 3415 (stretch N–H), 3117 (stretch C–H Ar), 1722, 1675 (stretch O–C=O), 1611 (stretch C=N), 1560, 1454, 1419 (stretch C=C and N–H bending), 1366 (bending C–H), 1277, 1203, 1111, 1088, 1025

(stretch C–O, bending C–N, C–S), 931, 859, 754, 699 (OOP C–H); ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.74$ (br s, 1H, NH indole), 11.22 (br s, 1H, NH, H_r), 8.61 (s, 1H, H_e), 8.41 (d, $J = 7.2$ Hz, 1H, H_d), 8.23 (s, 1H, H_m), 7.87 (s, 1H, H_g), 7.84 (d, $J = 7.8$ Hz,1H, H_a), 7.71 (t, $J = 7.8$ Hz, 1H, H_b), 7.63 (t, $J = 7.7$ Hz, 2H, H_c, H_j), 7.43–7.53 (m, 7H, H_a, H_h, H_i, H_l, H_n, H_o, H_q); MS (EI) *m/z*: 585.12 $(M)^+$; Anal. calcd. for $C_{33}H_{21}N_4O_5S^+$: C, 67.68; H, 3.61; N, 9.57. Found: C, 67.73; H, 3.69; N, 9.70 %.

5-(1H-indol-3-yl)-3-(2-oxo-2H-chromen-3-yl)-1-(4-(2-oxo-2H-chromen-3-yl)thiazol-2-yl)-1H-pyrazol-1-ium ***(4q)*** Brick-red solid (2.446 g, 77 %); Mp 223–225 °C; FT-IR (KBr; *v* max/cm^{-1}): 3421 (stretch N–H), 3120 (stretch C–H Ar), 1723, 1676 (stretch O–C=O), 1612 (C=N), 1562, 1455, 1417 (stretch C=C and N–H bending), 1380 (bending C–H), 1273, 1204, 1111, 1088 (stretch C–O, bending C–N, C–S), 935, 861, 756, 699 (OOP C–H); ^{1}H NMR (400 MHz, DMSO-d_6): $\delta = 11.76$ (br s, 1H, NH indole), 11.24 (br s, 1H, NH, H_r), 8.63 (s, 1H, H_e), 8.43 (dd, $J_1 = 8$ Hz, $J_2 = 1.2$ Hz 1H, H_d), 8.26 (s, 1H, H_m), 7.89 (s, 1H, H_g), 7.87 (dd, $J_1 = 7.8$ Hz, $J_2 = 1.2$–1.6 Hz, 1H, H_n), 7.72 (td, $J_1 = 7.8$ Hz, $J_2 = 1.6$ Hz, 2H, H_b,H_p), 7.65 (t, $J_1 = 7.7$ Hz, $J_2 = 1.6$ Hz, 2H, H_c,H_o), 7.46–7.55 (m, 7H, H_a, H_f, H_h, H_i, H_l, H_k, H_q); MS (EI) *m/z*: 555.11 $(M)^+$; Anal. calcd. for $C_{32}H_{19}N_4O_4S^+$: C, 69.18; H, 3.45; N, 10.08. Found: C, 69.31; H, 3.52; N, 10.17 %.

Biological methods

Antibacterial assay

The antibacterial activities of the synthesized compounds were evaluated by an agar well diffusion method. First, nutrient agar and nutrient broth cultures were prepared according to the manufactures' instructions and incubated at 37 °C. After incubation for the appropriate time, nutrient agar medium (25 mL) was poured into each petri plate and the agar plates were swabbed with 40 μL inocula of each test bacterium and kept for 15 min for adsorption. Using a sterile cork borer of a 5-mm diameter wells were bored into seeded agar plates and these were loaded with a 30-μL volume. Solutions of the test compounds and standard were prepared in DMSO at concentrations of 2.0 mg/mL. Dilutions of the compounds (2, 4, 8, 0.5 mg/mL) were inoculated to the corresponding wells. All the plates were incubated at 37 °C for 18–24 h. Antibacterial activity of the compounds was evaluated by measuring the zone of growth inhibition compared with gentamycin as a reference drug. DMSO was used as a negative control.

DPPH radical-scavenging assay

The antioxidant properties of the subject compounds were evaluated quickly and efficiently using a 2,2-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging model[51] in which the decrease in the strong absorption band at *v* 517 nm due to the unpaired electron in DPPH decreased stoichiometrically upon scavenging an

electron or hydrogen atom. The DPPH solution was prepared by dissolving an appropriate amount of DPPH in MeOH to give a concentration of 6.25×10^{-5} M. Then, 3.9 mL of this solution was added to 0.1 mL of sample solutions of **4a–q** in different concentration (2000, 1000, 500, 250, 125, 62.5 μg/mL in MeOH). The samples were shaken vigorously and kept in the dark for 30 min The decrease in the absorbance of the resulting solution was measured at 517 nm and the radical scavenging activity was expressed as IC_{50} (half maximal inhibitory concentration) values, which is the concentration of tested compound required to scavenge 50 % of the DPPH radical concentration (6.25×10^{-6} M in this case on dilution). All of the DPPH assays were conducted in triplicate and a synthetic antioxidant, ascorbic acid, was used as reference standard. MeOH was used as blank and a sample of 3.9 mL DPPH containing 0.1 mL of MeOH instead of sample was used as control. The percentage inhibition was calculated for the standard ascorbic acid and other test compounds and comparison was made. The percentage of inhibition of free radical DPPH was calculated according to the formula:

$$\text{Radical scavenging activity } \% = \left(\frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}}\right) \times 100.$$

where A_{blank} is the absorbance of negative control (containing all reagents except test compounds) and A_{sample} is the absorbance of the test compounds and all the reagents. IC_{50} of the samples was calculated by plotting the radical scavenging percentage against sample concentration.

Anti-fungal assay

For anti-fungal activity assays, Sabouraud dextrose agar (Merck) media were used for the cultivation of fungi. Normal saline was used to make a suspension of spores of the fungal strain. A loopful of a particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. A solution of agar media (20 mL) was poured into each petri dish. The excess of suspension was decanted and the plates were dried. After drying, wells were made using an agar punch and test samples, reference standard and negative control (DMSO) were placed in labeled wells in each petri plate. The petri plates were incubated at 37 °C for 48 h.

Results and discussion

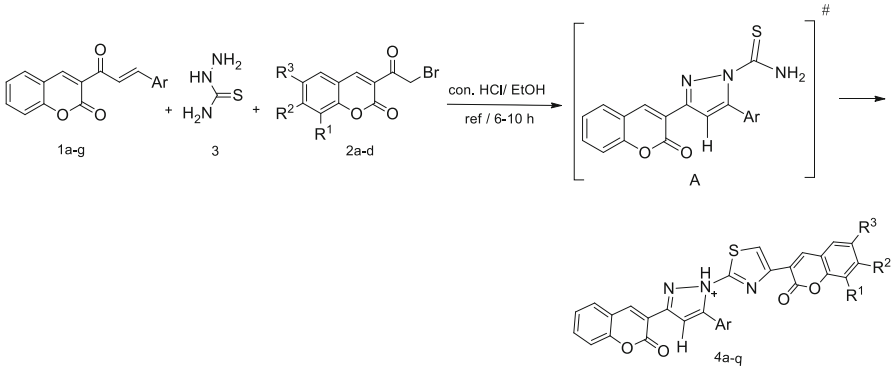
In a previous study, a series of thiazolyl-coumarin hybrids were designed, synthesized, and evaluated for their antioxidant properties [37]. It was well known that the coumarin moiety plays an important role in promoting the antioxidant properties of molecules. Therefore, inspiring study on the interesting pharmacological features of coumarin derivatives has encouraged us to synthesize new thiazolyl-coumarin compounds **4a–q**, through a reaction of coumarin chalcones **1a–g**, thiosemicarbazide **3**, and 2-bromocoumarin derivatives **2a–d**. The general route followed for the synthesis of coumarin derivatives is illustrated in Scheme 1.

The synthesis of new compounds **4a–q** is started from preparation of (1-phenylprop-1-en-2-yl)-2*H*-chromen-2-one (coumarin chalcone **1a–g**) through aldolic condensation of 3-acetylcoumarin with various aryl or heteroaryl aldehydes in the presence of a catalytic amount of piperidine in EtOH by either refluxing or microwave irradiation in accordance with what is reported in literatures [35, 39]. It is noteworthy that the reaction which required 5–7 h using conventional methods was completed efficiently, in 20–30 min with 78–92 % yields, under microwave conditions (Scheme 1). In the other pot, 3-bromoacetylcoumarins **2a–d** were readily synthesized from 3-acetylcoumarin as reported in literature [40, 41].

In the beginning, coumarin chalcones **1a–g** were treated with thiosemicarbazide **3** and allowed to reflux in EtOH in the presence of catalytic amount of HCl for 4–5 h. After in situ formation of pyrazolethioamide (monitored by TLC), 3-bromoacetylcoumarin **2a–d** in EtOH was added to the reaction mixture, and refluxing was continued for an additional 1–2 h. Neutralization with a saturated solution of $NaHCO_3$, followed by filtration and purification by recrystallization, gave bromide salts of **4a–q** in satisfactory yields (70–80 %; see Table 1; Scheme1).

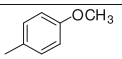
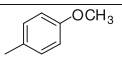
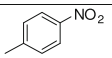
Addition of thiosemicarbazide to coumarin chalcones is a regioselective reaction proceeding via addition of the NH_2 of thiosemicarbazide to the chalcone carbonyl, and subsequent intramolecular cycloaddition of N–H to the olefinic bond of the propenone **6**, according to our previously reported work [38].

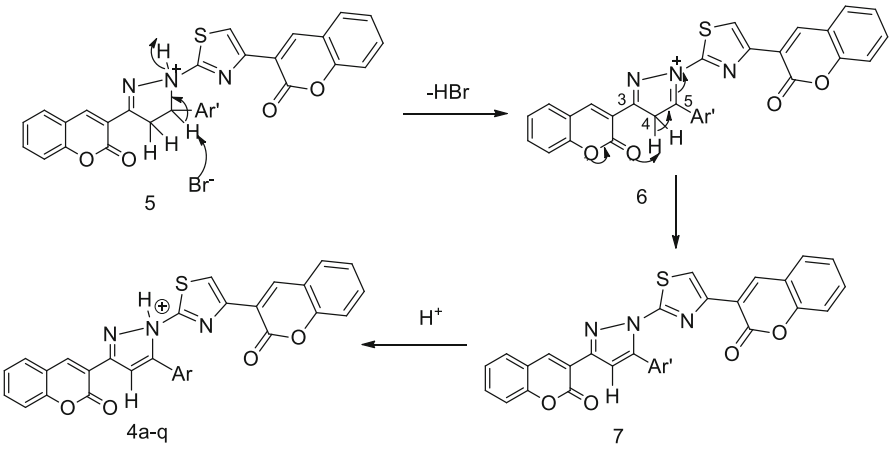
Formation of in situ-obtained pyrazolethioamide **A** is followed by the Hantzsch thiazole synthesis. Initially, nucleophilic substitution of the Br of 3-bromoacetyl-coumarin **2a–d** by the S-atom of thioamide generates the isothiourea, which subsequently undergoes cyclocondensation and H_2O elimination to give the thiazole ring of **4a–q** (Scheme 2). Unexpectedly, in this work the presence of the carbonyl group of coumarin **5** followed by dehydrogenation of C-5 led to aromatic pyrazole **7** by deprotonation of C-4 of the pyrazoline ring via anchimeric assistance. Proton capturing by the nitrogen of the pyrazole ring under the reaction conditions gave target compounds **4a–q** (Scheme 2).



Scheme 1 Synthetic protocol of the title compounds (**4a–q**)

Table 1 Reaction products obtained from coumarin chalcones **1**, 3-bromoacetylcoumarins **2**, and thiosemicarbazide **3**

Entry	1	Ar	2	R^1	R^2	R^3	4
1	**1a**		**2a**	H	H	H	**4a**
2	**1a**		**2b**	OCH_3	H	H	**4b**
3	**1a**		**2c**	H	OCH_3	H	**4c**
4	**1b**		**2a**	H	H	H	**4d**
5	**1b**		**2b**	OCH_3	H	H	**4e**
6	**1c**		**2a**	H	H	H	**4f**
7	**1c**		**2b**	OCH_3	H	H	**4g**
8	**1d**		**2a**	H	H	H	**4h**
9	**1d**		**2b**	OCH_3	H	H	**4i**
10	**1e**		**2a**	H	H	H	**4j**
11	**1f**		**2a**	H	H	H	**4k**
12	**1f**		**2c**	H	OCH_3	H	**4l**
13	**1f**		**2d**	H	H	Br	**4m**
14	**1f**		**2b**	OCH_3	H	H	**4n**
15	**1g**		**2b**	OCH_3	H	H	**4o**
16	**1g**		**2c**	H	OCH_3	H	**4p**
17	**1g**		**2a**	H	H	H	**4q**



Scheme 2 Proposed mechanism for production of final thiazolyl-pyrazolium salt

To identify the optimal process, various reaction conditions were studied in order to synthesize the target coumarin derivatives. For example, a model reaction was carried out with 3-(2-bromoacetyl)-2*H*chromen-2-one **2a–d**, thiosemicarbazide **3**, and coumarin chalcone **1a–g** in both EtOH without acidic catalyst and in EtOH containing a catalytic amount of HCl, both at reflux. In EtOH, after even 12 h reflux, only a 75 % yield of **4a–q** was obtained, while in EtOH containing a catalytic amount of HCl, within 4–6 h, a 90 % yield of products was obtained. When these reactions are performed under ultrasonic irradiation similar yield ratios of the products as in previous experiments were obtained within 6 and 3 h, respectively.

All the synthesized compounds have been characterized on the basis of their physical data and spectral analysis. Structures of all of the newly synthesized compounds were established by IR, NMR (^{1}H, ^{13}C) and elemental analyses.

The IR spectra of the compounds **4a–q** reveals the presence of a broad band at 3400 cm^{-1} for the N–H stretching vibration, 1675–1722 cm^{-1} for the C=O of ester, which is corroborated by the presence of a lactone C–O at 1276 cm^{-1}. Peaks at 1609–1612 cm^{-1} are for the C=N group, and peaks in the regions 1355–1375 cm^{-1} and 1055–1098 cm^{-1} indicate the presence of C–S and C–N groups. In the ^{1}H NMR spectra, the C–H peak of the OCH_3 group at 3.93 ppm confirms the formation of the thiazole moiety. Aromatic protons appear in the expected region (7.30–8.63 ppm) and 11.23 ppm for NH. ^{13}C NMR spectra also confirm structural identities, with resonances observed at δ_C of 55.9–56.6 ppm for OCH_3, 169.1–170.3 ppm (thiazole 2-C), 159.6–162.6 ppm (C=O), and 153.2–156.3 ppm (C=N). Through single crystal X-ray diffraction, the adducts of reactions of coumarin chalcones **1a–g** with thiosemicarbazide **3** and 3-bromoacetylcoumarins **2a–d** were clearly determined (Fig. 1).

Pharmacology

Antibacterial activity

The new compounds **4a–q** were evaluated for their in vitro antibacterial activity against *Escherichia coli* (*E. coli*), *Micrococcus luteus* (*M. luteus*), *Pseudomonas aeruginosa* (*Ps. aeruginosa*), and *Staphylococcus aureus* (*S. aureus*) by the zone inhibition method [40]. The results are presented in Table 2. All the tested compounds possessed moderate to good antibacterial activity against Gram-positive bacteria (*S. aureus* and *M.luteus*) as well as Gram-negative bacteria (*E. coli* and *P. aeruginosa*). On the basis of zone of inhibition against the test bacterium, compounds **4b**, **4c**, **4e**, **4f**, **4k**, **4l**, **4m**, and **4o** were found to be most effective against *S. aureus* (Gram-positive bacteria), compounds **4n** and **4f** were found to be most active against *E. coli,* and compounds **4j** and **4p** were effective-against *M. luteus* (Gram-positive bacteria), as compared with the standard drug gentamycin. This may be due to the incorporation of the thiophene ring and the presence of electron-withdrawing groups like –Cl in comparison to the other compounds. All the synthesized compounds showed weak antibacterial activity against *Ps. aeruginosa* (a Gram-negative bacteria). However, in terms of MIC (minimal inhibitory concentration), none of the compounds has shown activity.

Table 2 The antibacterial and antifungal activities of the synthesized compounds

Compound	Mean zone of inhibition (mean ± SD; mm)					
	M. luteus	*S. aureus*	*E. coli*	*Ps. aeruginosa*	*A. niger*	*A. flavus*
4a	–	–	8 ± 1.52	–	–	–
4b	–	16 ± 0.70	–	–	12 ± 0.85	12 ± 0.85
4c	9 ± 0.57	14 ± 0.57	–	–	10 ± 0.70	12 ± 0.70
4d	10 ± 0.57	10 ± 0.70	8 ± 1.52	11 ± 1.15	11 ± 0.89	14 ± 0.90
4e	12 ± 0.57	16 ± 0.70	8 ± 1.52	11 ± 1.15	11 ± 0.94	14 ± 0.90
4f	–	17 ± 0.70	12 ± 1.0	–	11 ± 0.83	13 ± 0.80
4g	10 ± 0.57	13 ± 0.57	–	12 ± 0.57	13 ± 0.70	13 ± 0.70
4h	9 ± 0.57	9 ± 1.15	–	–	12 ± 0.89	11 ± 0.90
4i	9 ± 0.57	9 ± 0.57	–	–	12 ± 0.70	11 ± 0.70
4j	15 ± 1.41	10 ± 0.57	–	–	9 ± 0.89	12 ± 0.85
4k	9 ± 0.70	15 ± 1.52	–	11 ± 0.57	14 ± 0.70	13 ± 0.70
4l	9 ± 0.70	15 ± 1.52	11 ± 1.0	–	13 ± 0.70	11 ± 0.70
4m	11 ± 0.57	15 ± 1.52	8 ± 1.0	–	16 ± 0.94	17 ± 0.90
4n	–	13 ± 1.52	13 ± 1.0	–	13 ± 0.83	12 ± 0.90
4o	–	15 ± 1.52	11 ± 0.57	–	13 ± 0.70	14 ± 0.70
4p	13 ± 1.41	9 ± 1.15	11 ± 1.0	–	–	–
Gentamycin[a]	18	21	20	19	–	–
Floconazol[a]	–	–	–	–	25	25
DMSO[b]	–	–	–	–	–	–

[a] Positive control, [b] negative control

Antioxidant activity

Most of the synthesized compounds showed antioxidant activity. According to Fig. 3, radical scavenging activity was dose-dependent and increased with rise in concentration of the compounds **4a–q**. Compound **4i** exhibited the highest DPPH radical scavenging, while compound **4a** had the lowest. The IC_{50} values were calculated according to line equations and were reported in Table 3. The IC_{50} values were between 0.25 and 6.16 mg/mL. Compound **4i** in comparison to ascorbic acid (0.07 mg/mL) had the lowest IC_{50} value (0.25 mg/mL). It was worth noting that compounds **4b**, **4c**, **4k**, **4e**, **4m**, **4o,** and **4p** exhibited very high activities against the DPPH radical (at a concentration of 6.25 × 10^{-}6 M) with IC_{50} values on the order of 4.33 × 10^{2} to 1.2 × 10^{4} μM. The antioxidant activity of compounds **4a–q** can be ascribed to the N–H groups of the pyrazole component which can donate a hydrogen

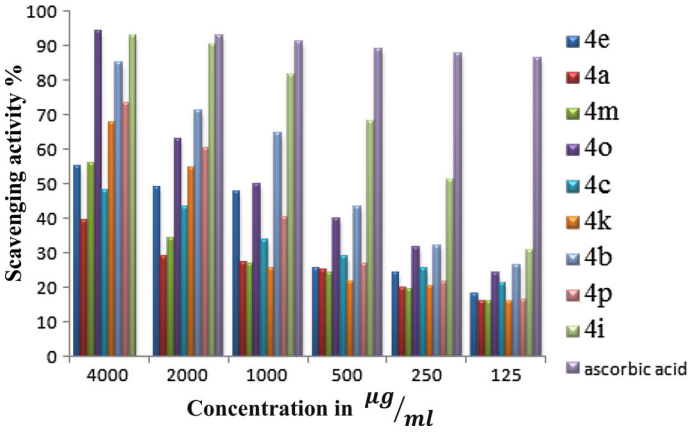


Fig. 3 Evaluation of antioxidant properties by DPPH radical scavenging assay of compounds **4a–q**

Table 3 IC_{50} for DPPH assay of compounds **1a–q** and comparison to ascorbic acid

Compound	DPPH assay IC_{50} (mg/L)
4a	6.16
4b	1.06
4k	2.43
4m	3.69
4e	3.13
4o	1.35
4p	2.1
4c	4.21
4i	0.25
Ascorbic acid	0.07

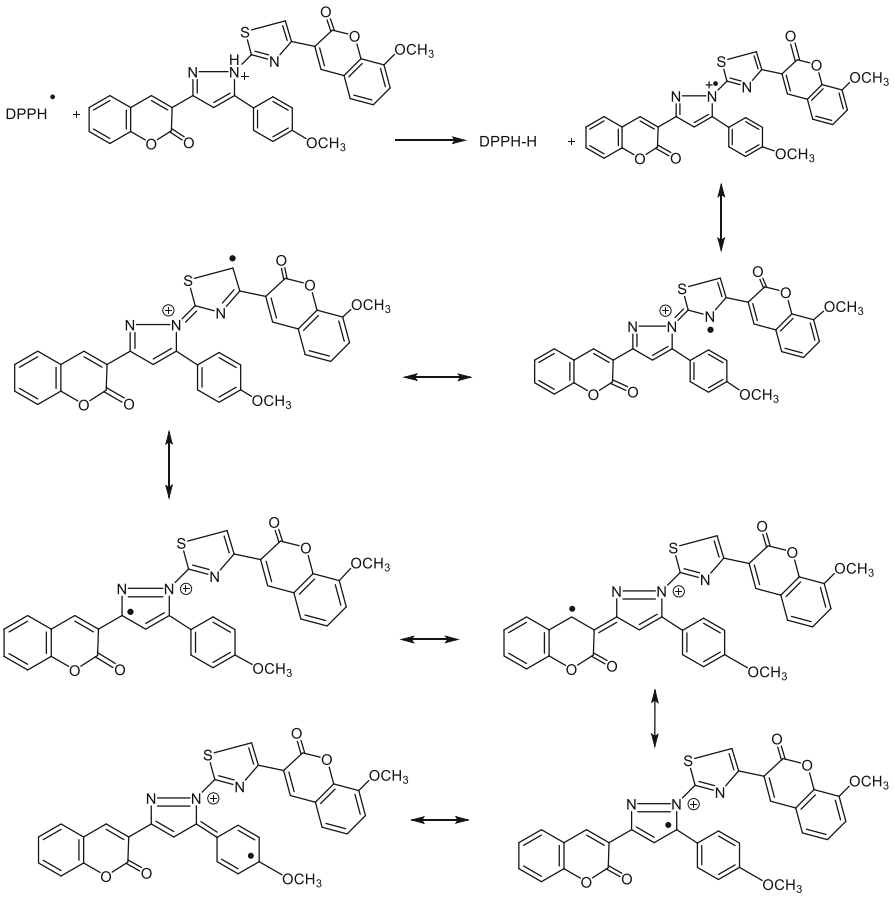
IC_{50} half maximal inhibitory concentration

atom to DPPH and then delocalize the resulting unpaired electron through the conjugated system.

The highest antioxidant capacity of compound **4i** in comparison to the other analogs may be due to the two electron-donating methoxy groups on the aromatic rings which can better stabilize the radical cation. The proposed radical scavenging mechanism for compound **4i** is depicted in Scheme 3.

Anti-fungal activity

The in vitro anti-fungal activity of compounds **4a–q** was also evaluated against *Aspergillus niger* and *Aspergillus flavus* using the drug fluconazole as a reference standard. Compound **4m** indicated promising activity, whereas the antifungal activity of the other compounds was reasonable or minor. On the basis of obtained results, the presence of bromine improves the anti-fungal activity.



Scheme 3 Proposed radical scavenging mechanism for **4i**

Conclusion

In conclusion, we reported here a three-component cyclo-condensation protocol synthesis with advantages such as easy an work-up, a fast and regioselective method for the simultaneous preparation of the target library, together with the use of inexpensive material and environmentally friendly procedure and providing antimicrobial and antioxidant properties and so on for the title products. DPPH assays indicate that most of the synthesized pyrazolium thiazolyl coumarin derivatives such as **4a**, **4b**, **4c**, **4e**, **4i**, **4k**, **4m**, **4o**, and **4p** had considerable radical scavenging activity that was comparable to the ascorbic acid. The pyrazole and thiazole moieties contribute to the free radical scavenging activity. However, incorporation of the coumarin motif further optimized the radical scavenging activity and improved the antioxidant activity. Also, some of the compounds exhibited prominent antimicrobial and anti-fungal properties that may be due to the incorporation of a thiophene ring and electron-withdrawing groups like –Cl.

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