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Design, Synthesis and Biological Activities of Thiazolyl-pyrazole-*bis*-coumarins

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In this study, we report the synthesis of thiazolyl-pyrazole-biscoumarins employing hydrochloric acid as an effective catalyst. The synthesized derivatives were subjected to biological evaluation for anti-inflammatory, antioxidant and antimicrobial activities. The results indicate that the oxygen containing heterocycles among the synthesized compounds exhibited notable anti-inflammatory and antioxidant properties while sulfur containing heterocycles demonstrated promising antimicrobial efficacy. This work highlights the potential of these thiazolyl-pyrazole-biscoumarin derivatives as multifunctional agents in therapeutic applications.

Keywords: Biscoumarins, Anti-inflammatory, Sulfur, Antimicrobial activity.

INTRODUCTION

The risks associated with inflammation present a significant challenge for medicinal chemists, driving the search for more effective anti-inflammatory agents. Many existing anti-inflammatory compounds, particularly those with established clinical efficacy are acidic, including well-known drugs such as aspirin, indomethacin, flufenamic acid and ibuprofen. Non-steroidal anti-inflammatory drugs (NSAIDs) are a major class of anti-inflammatory agents that alleviate inflammation by targeting affected tissues and inhibiting cyclooxygenase (COX) enzymes, which play a key role in the synthesis of prostaglandins [1-3].

The thiazole ring system has attracted considerable interest in drug development due to its potential for diverse therapeutic applications. Derivatives of thiazole exhibit a wide range of biological activities, including antimicrobial [4], antiviral [5], anti-inflammatory [6], anticancer [7], anticonvulsant [8] and antifungal [9] effects. Similarly, pyrazoles are also known for its aromaticity and electron-rich nature, and therefore are significant in medicinal chemistry owing to their broad spectrum of biological activity [10,11]. This heterocycle is a core component in various therapeutic agents, making it valuable for the development of drugs with applications such as analgesic [12], anti-inflammatory [13], antipyretic [14], antimicrobial [15], antidiabetic [16], antitubercular [17], antidepressant [18] and anticancer [19] properties.

Coumarins are heterocyclic compounds widely distributed in various plant species, with a notable presence in foods like apricots, cherries, cinnamon and strawberries. Both natural and synthetic coumarins exhibit a wide range of therapeutic properties, including antimicrobial [20], anti-HIV [21], antioxidant [22], anticoagulant [23], anti-inflammatory [24], anticonvulsant [25], anticancer [26] and antiviral [27] activities. Their broad biological efficacy and structural adaptability make them a significant focus of scientific research. Emerging research further suggests that coumarins act as lipid-lowering agents [28], with specific benefits in reducing triglyceride levels, underscoring their potential in metabolic health applications.

Considering these intriguing pharmacological characteristics, we aimed to develop a molecular framework by integrating above these three pharmacophores *viz*. coumarins, pyrazoles and thiazoles into a compact structure allows for an exploration of the biological activities of the resulting compounds.

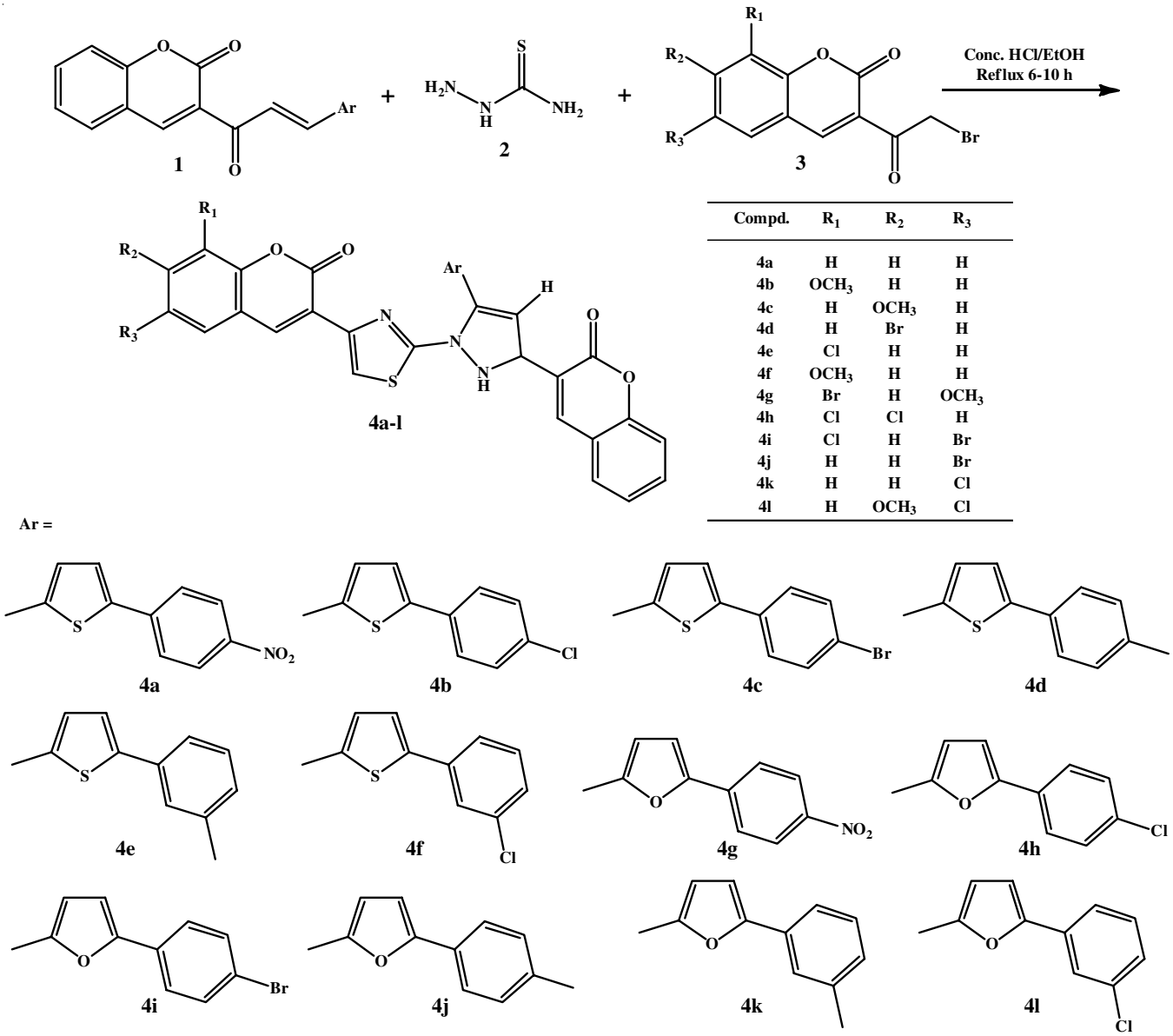
EXPERIMENTAL

The melting points were measured using an open capillary approach and are uncorrected. The IR spectra were measured with an FT-IR spectrometer (Shimadzu FTIR 440) using KBr

pellet. Using DMSO-d_6 as solvent and TMS as internal standard, the ^{1}H NMR spectra were captured using a JEOL 400 MHz NMR spectrometer. A Micro mass Q-Tof Micro LC mass spectrometer was used to record the mass spectra. Using silica gel plates (Merck) and petroleum ether:ethyl acetate (1:1) as the mobile phase, TLC was used to confirm the purity of the synthesized compounds.

Synthesis: An EtOH solution of thiosemicarbazide (**2**) (5 mmol) was added to a solution of coumarin chalcone (**1**) (5 mmol) in hot ethanol (10 mL) with constant stirring. The resulting solution was refluxed for 4-6 h in the presence of HCl. Then, an ethanolic solution (30 mL) of 3-(2-bromoacetyl)-coumarin (**3**) (1.34 g, 5 mmol) was added and the mixture was refluxed in a water bath for 2 h. After completion of the reaction [monitored by TLC] and neutralized with a saturated solution of $NaHCO_3$, the precipitated solid was filtered, washed with ethanol and recrystallized from a mixture of DMF-H_2O to afford the target compounds (**Scheme-I**).

3-(5-(5-(4-Nitrophenyl)thiophen-2-yl)-1-(4-(2-oxo-2*H*-chromen-3-yl)thiazol-2-yl)-2,3-dihydro-1*H*-pyrazol-3-yl)-2*H*-chromen-2-one (4a): Yield: 78%; m.p.: 285 ºC. IR (KBr, ν_{max}, cm^{-1}): 3500-3300 (-NH- *str.*), 1700-1500 (-C=C- *str.*), 1250-1020 (-CN- *str.*), 1210-1163 (-CO- *str.*); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 3.70 (2H, d, *J* = 16.6 Hz), 3.83 (3H, s), 4.77 (1H, d, *J* = 2.3 Hz), 5.99 (1H, d, *J* = 2.3 Hz), 6.60-6.89 (3H, 6.65 (d, *J* = 1.5 Hz), 6.74 (d, *J* = 8.8 Hz), 6.84 (s), 7.05-7.45 (9H), 7.11 (d, *J* = 8.7 Hz), 7.21 (d, *J* = 7.9 Hz), 7.23 (d, *J* = 8.7 Hz), 7.28 (d, *J* = 8.5 Hz), 7.28 (d, *J* = 7.9 Hz), 7.35 (d, *J* = 7.9 Hz), 7.39 (d, *J* = 8.5 Hz), 7.52 (1H, s), 7.73 (1H, d, *J* = 7.9 Hz), 7.86 (1H, s), 8.12 (1H, d, *J* = 8.8 Hz). ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 45.0 (1C, s), 56.0 (1C, s), 56.6 (1C, s), 98.7 (1C, s), 114.3 (1C, s), 116.9 (1C, s), 117.7 (2C, s), 118.2 (1C, s), 123.8 (1C, s), 124.0 (1C, s), 126.2 (1C, s), 127.0 (1C, s), 127.3-127.3 (2C, 127.3 (s), 127.3 (s), 127.6 (1C, s), 128.0 (1C, s), 128.3-128.5 (2C), 128.4 (s), 128.4 (s), 128.6 (2C, s), 132.1 (1C, s), 133.6-133.8 (2C), 133.7 (s), 133.7 (s), 134.3



Compd.	R_1	R_2	R_3
4a	H	H	H
4b	OCH_3	H	H
4c	H	OCH_3	H
4d	H	Br	H
4e	Cl	H	H
4f	OCH_3	H	H
4g	Br	H	OCH_3
4h	Cl	Cl	H
4i	Cl	H	Br
4j	H	H	Br
4k	H	H	Cl
4l	H	OCH_3	Cl

Scheme-I: General procedure for the synthesis of thiazolyl-pyrazole-biscoumarins

(1C, s), 134.9 (1C, s), 135.6 (1C, s), 136.8-137.0 (2C, 136.9 (s), 136.9 (s), 139.5 (1C, s), 151.1 (1C, s), 155.4 (1C, s), 159.2 (1C, s), 164.0 (1C, s), 165.7 (1C, s), 197.1 (1C, s). Mass: 645 [M+1].

3-(2-(5-(5-(4-Chlorophenyl)thiophen-2-yl)-3-(2-oxo-2*H*-chromen-3-yl)-2,3-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-8-methoxy-2*H*-chromen-2-one (4b): Yield: 76%; m.p.: 287 ºC. IR (KBr, ν_{max}, cm^{-1}): 3500-3300 (-NH- *str.*), 1700-1500 (-C=C- *str.*), 1250-1020 (-CN- *str.*), 1210-1163 (-CO- *str.*); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 3.73-3.92 (5H), 3.80 (d, *J* = 16.7 Hz), 3.87 (s), 4.75 (1H, d, *J* = 2.3 Hz), 6.01 (1H, d, *J* = 2.3 Hz), 6.63 (1H, d, *J* = 8.0 Hz), 7.03-7.67 (13H), 7.08 (s), 7.12 (d, *J* = 7.5 Hz), 7.19 (d, *J* = 8.6 Hz), 7.29 (d, *J* = 8.0 Hz), 7.33 (d, *J* = 8.1 Hz), 7.33 (s), 7.35 (d, *J* = 8.6 Hz), 7.44 (d, *J* = 7.9 Hz), 7.52 (d, *J* = 8.9 Hz), 7.55 (d, *J* = 8.1 Hz), 7.60 (d, *J* = 8.9 Hz), 7.79 (1H, d, *J* = 7.9 Hz), 7.98 (1H, s); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 45.0 (1C, s), 56.0 (1C, s), 56.6 (1C, s), 115.8-115.8 (2C), 115.8 (s), 115.8 (s), 116.9 (1C, s), 118.2-118.3 (2C), 118.2 (s), 118.2 (s), 123.8 (1C, s), 124.0 (1C, s), 126.9-127.0 (2C), 126.9 (s), 127.0 (s), 127.3-127.3 (2C), 127.3 (s), 127.6 (2C, s), 128.0 (1C, s), 128.4 (1C, s), 128.7 (2C, s), 129.4 (1C, s), 129.6 (1C, s), 132.1 (1C, s), 133.6-133.8 (3C, 133.7 (s), 133.7 (s), 133.7 (s), 134.3 (1C, s), 134.9 (1C, s), 136.8-137.0 (2C), 136.9 (s), 136.9 (s), 151.1 (1C, s), 154.1 (1C, s), 156.7 (1C, s), 159.2 (1C, s), 165.7 (1C, s), 197.1 (1C, s). Mass: 635 [M+], 637 [M+2].

3-(2-(5-(5-(4-Bromophenyl)thiophen-2-yl)-3-(2-oxo-2*H*-chromen-3-yl)-2,3-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-7-methoxy-2*H*-chromen-2-one (4c): Yield: 75%; m.p.: 289 ºC. IR (KBr, ν_{max}, cm^{-1}): 3500-3300 (-NH- *str.*), 1700-1500 (-C=C- *str.*), 1250-1020 (-CN- *str.*), 1210-1163 (-CO- *str.*); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 3.73-3.92 (5H), 3.80 (d, *J* = 16.7 Hz), 3.87 (s), 4.74 (1H, d, *J* = 2.3 Hz), 5.99 (1H, d, *J* = 2.3 Hz), 6.63 (1H, d, *J* = 8.0 Hz), 7.03-7.63 (13H), 7.08 (s), 7.12 (d, *J* = 7.5 Hz), 7.19 (d, *J* = 8.6 Hz), 7.29 (d, *J* = 8.0 Hz), 7.30 (d, *J* = 8.6 Hz), 7.33 (d, *J* = 8.1 Hz), 7.33 (s), 7.44 (d, *J* = 7.9 Hz), 7.47 (d, *J* = 8.9 Hz), 7.55 (d, *J* = 8.1 Hz), 7.57 (d, *J* = 8.9 Hz), 7.79 (1H, d, *J* = 7.9 Hz), 7.98 (1H, s); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 45.0 (1C, s), 56.0 (1C, s), 56.6 (1C, s), 115.8-115.8 (2C, 115.8 (s), 115.8 (s), 116.9 (1C, s), 118.2-118.3 (2C), 118.2 (s), 118.2 (s), 122.3 (1C, s), 123.8 (1C, s), 124.0 (1C, s), 126.9-127.0 (2C), 126.9 (s), 127.0 (s)), 127.3-127.3 (2C, 127.3 (s), 127.3 (s)), 127.9-128.1 (3C), 127.9 (s), 128.0 (s), 128.4 (1C, s), 129.4 (1C, s), 129.6 (1C, s), 131.7 (2C, s), 132.1 (1C, s), 133.6-133.8 (2C), 133.7 (s), 133.7 (s), 134.3 (1C, s), 134.9 (1C, s), 136.8-137.0 (2C, 136.9 (s), 136.9 (s), 151.1 (1C, s), 154.1 (1C, s), 156.7 (1C, s), 159.2 (1C, s), 165.7 (1C, s), 197.1 (1C, s). Mass: 708 [M+], 710 [M+2].

7-Bromo-3-(2-(3-(2-oxo-2*H*-chromen-3-yl)-5-(5-(*p*-tolyl)thiophen-2-yl)-2,3-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromen-2-one (4d): Yield: 74%; m.p.: 282 ºC. IR (KBr, ν_{max}, cm^{-1}): 3500-3300 (-NH- *str.*), 1700-1500 (-C=C- *str.*), 1250-1020 (-CN- *str.*), 1210-1163 (-CO- *str.*); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.30 (3H, s), 3.71 (2H, d, *J* = 16.6 Hz), 4.82 (1H, d, *J* = 2.3 Hz), 6.00 (1H, d, *J* = 2.3 Hz), 7.03-7.65 (12H, 7.08 (s), 7.16 (d, *J* = 8.7 Hz), 7.17 (d, *J* = 8.3 Hz), 7.19 (d, *J* = 8.2 Hz), 7.22 (d, *J* = 8.7 Hz), 7.33 (d, *J* = 8.1 Hz), 7.35 (d, *J* = 8.2 Hz), 7.44 (d, *J* = 7.9 Hz), 7.55 (d, *J* = 8.1 Hz), 7.54 (s), 7.60 (d, *J* = 1.9 Hz), 7.73-7.90 (3H), 7.79 (d, *J* = 7.9 Hz), 7.84 (d, *J* = 8.3 Hz), 7.98 (1H, s); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: δ 21.3 (1C, s), 45.0 (1C, s), 56.6 (1C, s), 115.8 (1C, s), 116.9 (1C, s), 118.2 (1C, s), 118.4 (1C, s), 123.8 (1C, s), 124.0 (1C, s), 126.9-127.0 (2C), 126.9 (s), 127.0 (s), 127.3 (2C), 127.8 (3C), 128.4 (1C, s), 129.1 (2C, s), 129.4 (1C, s), 131.0 (1C, s), 131.7 (1C, s), 132.1 (1C, s), 133.6-133.8 (2C), 133.7 (s), 134.3 (1C, s), 134.9 (1C, s), 135.6 (1C, s), 136.8-137.0 (2C, 136.9 (s), 141.5 (1C, s), 151.1 (1C, s), 154.1 (1C, s), 159.2 (1C, s), 165.7 (1C, s), 197.1 (1C, s). Mass: 693 [M+].

8-Chloro-3-(2-(3-(2-oxo-2*H*-chromen-3-yl)-5-(5-(*m*-tolyl)thiophen-2-yl)-2,3-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromen-2-one (4e): Yield: 73%; m.p.: 280 ºC. IR (KBr, ν_{max}, cm^{-1}): 3500-3300 (-NH- *str.*), 1700-1500 (-C=C- *str.*), 1250-1020 (-CN- *str.*), 1210-1163 (-CO- *str.*); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.29 (3H, s), 3.71 (2H, d, *J* = 16.6 Hz), 4.87 (1H, d, *J* = 2.3 Hz), 6.00 (1H, d, *J* = 2.3 Hz), 7.03-7.63 (13H), 7.08 (s), 7.17 (d, *J* = 7.6 Hz), 7.18 (d, *J* = 8.7 Hz), 7.23 (d, *J* = 8.7 Hz), 7.33 (d, *J* = 8.1 Hz), 7.34 (d, *J* = 7.8 Hz), 7.40 (d, *J* = 8.1 Hz), 7.44 (d, *J* = 7.9 Hz), 7.46 (d, *J* = 7.8 Hz), 7.50 (d, *J* = 8.1 Hz), 7.51 (s), 7.55 (d, *J* = 8.1 Hz), 7.55 (d, *J* = 1.9 Hz)), 7.67-7.86 (2H), 7.72 (t, *J* = 1.4 Hz), 7.79 (d, *J* = 7.9 Hz)), 7.98 (1H, s); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 21.3 (1C, s), 45.0 (1C, s), 56.6 (1C, s), 115.8 (1C, s), 116.9 (1C, s), 118.2 (1C, s), 123.8 (1C, s), 124.0 (1C, s), 126.9-127.0 (2C), 126.9 (s), 127.0 (s), 127.3-127.3 (2C), 127.3 (s), 127.3 (s), 127.4 (1C, s), 127.7-127.8 (2C, 127.7 (s), 127.8 (s), 128.0 (1C, s), 128.1 (1C, s), 128.4 (1C, s), 128.7 (1C, s), 128.9 (1C, s), 129.4 (1C, s), 130.4 (1C, s), 132.1 (1C, s), 133.6-133.8 (2C, 133.7 (s), 133.7 (s), 134.8-135.0 (2C, 134.8 (s), 134.9 (s), 135.1 (1C, s), 135.6 (1C, s), 136.8-137.0 (2C), 136.9 (s), 136.9 (s), 151.1 (1C, s), 154.1 (1C, s), 159.2 (1C, s), 165.7 (1C, s), 197.1 (1C, s). Mass: 649 [M+], 651 [M+2].

3-(2-(5-(5-(3-Chlorophenyl)thiophen-2-yl)-3-(2-oxo-2*H*-chromen-3-yl)-2,3-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-8-methoxy-2H-chromen-2-one (4f): Yield: 72%; m.p.: 279 ºC. IR (KBr, ν_{max}, cm^{-1}): 3500-3300 (-NH- *str.*), 1700-1500 (-C=C- *str.*), 1250-1020 (-CN- *str.*), 1210-1163 (-CO- *str.*); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 3.73-3.92 (5H, 3.80 (d, *J* = 16.7 Hz), 3.87 (s), 4.75 (1H, d, *J* = 2.3 Hz), 6.01 (1H, d, *J* = 2.3 Hz), 6.63 (1H, d, *J* = 8.0 Hz), 7.03-7.67 (13H, 7.08 (s), 7.12 (d, *J* = 7.5 Hz), 7.19 (d, *J* = 8.6 Hz), 7.29 (d, *J* = 8.0 Hz), 7.33 (d, *J* = 8.1 Hz), 7.33 (s), 7.35 (d, *J* = 8.6 Hz), 7.44 (d, *J* = 7.9 Hz), 7.52 (d, *J* = 8.9 Hz), 7.55 (d, *J* = 8.1 Hz), 7.60 (d, *J* = 8.9 Hz), 7.79 (1H, d, *J* = 7.9 Hz), 7.98 (1H, s); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 45.0 (1C, s), 56.0 (1C, s), 56.6 (1C, s), 115.8-115.8 (2C, 115.8 (s), 115.8 (s), 116.9 (1C, s), 118.2-118.3 (2C), 118.2 (s), 118.2 (s), 123.8 (1C, s), 124.0 (1C, s), 126.9-127.0 (2C, 126.9 (s), 127.0 (s), 127.3-127.3 (2C), 127.3 (s), 127.3 (s), 127.6 (2C, s), 128.0 (1C, s), 128.4 (1C, s), 128.7 (2C, s), 129.4 (1C, s), 129.6 (1C, s), 132.1 (1C, s), 133.6-133.8 (3C), 133.7 (s), 133.7 (s), 133.7 (s), 134.3 (1C, s), 134.9 (1C, s), 136.8-137.0 (2C), 136.9 (s), 136.9 (s), 151.1 (1C, s), 154.1 (1C, s), 156.7 (1C, s), 159.2 (1C, s), 165.7 (1C, s), 197.1 (1C, s). Mass: 635 [M+], 637 [M+2].

8-Bromo-6-methoxy-3-(2-(5-(5-(4-nitrophenyl)furan-2-yl)-3-(2-oxo-2*H*-chromen-3-yl)-2,3-dihydro-1*H*-pyrazol-

1-yl)thiazol-4-yl)-2*H*-chromen-2-one (4g): Yield: 70%; m.p.: 284 ºC. IR (KBr, ν_{max}, cm^{-1}): 3500-3300 (-NH- *str.*), 1700-1500 (-C=C- *str.*), 1250-1020 (-CN- *str.*), 1210-1163 (-CO- *str.*); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 3.71-3.85 (5H), 3.78 (d, *J* = 16.7 Hz), 3.80 (s), 4.51 (1H, d, *J* = 2.3 Hz), 5.93 (1H, d, *J* = 2.3 Hz), 6.79-6.98 (4H), 6.84 (d, *J* = 2.5 Hz), 6.86 (d, *J* = 2.5 Hz), 6.92 (d, *J* = 9.0 Hz), 7.03-7.63 (9H), 7.08 (s), 7.12 (d, *J* = 8.6 Hz), 7.20 (d, *J* = 8.6 Hz), 7.26 (s), 7.33 (d, *J* = 8.1 Hz), 7.38 (d, *J* = 9.0 Hz), 7.44 (d, *J* = 7.9 Hz), 7.55 (d, *J* = 8.1 Hz)), 7.79 (1H, d, *J* = 7.9 Hz), 7.98 (1H, s); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 45.0 (1C, s), 56.0 (1C, s), 56.6 (1C, s), 113.3 (1C, s), 114.3 (2C, s), 115.8 (1C, s), 116.0 (1C, s), 116.9 (1C, s), 118.2 (1C, s), 122.0 (1C, s), 123.8 (1C, s), 124.0 (1C, s), 126.9-127.0 (2C, 126.9 (s), 127.0 (s), 127.3-127.3 (2C), 127.3 (s), 127.3 (s), 128.0 (1C, s), 128.4 (1C, s), 128.6 (2C, s), 129.4 (1C, s), 132.1 (1C, s), 133.6-133.8 (2C, 133.7 (s), 133.7 (s), 134.3 (1C, s), 134.9 (1C, s), 136.8-137.0 (2C), 136.9 (s), 136.9 (s), 148.4 (1C, s), 151.1 (1C, s), 152.4 (1C, s), 154.1 (1C, s), 159.2 (1C, s), 165.7 (1C, s), 197.1 (1C, s). Mass: 754 [M+].

7,8-Dichloro-3-(2-(5-(5-(4-chlorophenyl)furan-2-yl)-3-(2-oxo-2*H*-chromen-3-yl)-2,3-dihydro-1*H*-pyrazol-1-yl)-thiazol-4-yl)-2*H*-chromen-2-one (4h): Yield: 74%; m.p.: 288 ºC. IR (KBr, ν_{max}, cm^{-1}): 3500-3300 (-NH- *str.*), 1700-1500 (-C=C- *str.*), 1250-1020 (-CN- *str.*), 1210-1163 (-CO- *str.*); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 3.84 (2H, d, *J* = 16.7 Hz), 4.83 (1H, d, *J* = 2.3 Hz), 6.04 (1H, d, *J* = 2.3 Hz), 7.03-7.67 (13H, 7.08 (s), 7.19 (d, *J* = 8.6 Hz), 7.25 (d, *J* = 7.9 Hz), 7.33 (d, *J* = 8.1 Hz), 7.35 (d, *J* = 8.6 Hz), 7.44 (d, *J* = 7.9 Hz), 7.50 (s), 7.52 (d, *J* = 8.9 Hz), 7.55 (d, *J* = 8.1 Hz), 7.57 (d, *J* = 7.9 Hz), 7.60 (d, *J* = 8.9Hz), 7.79 (1H, d, *J* = 7.9 Hz), 7.98 (1H, s); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 45.0 (1C, s), 56.6 (1C, s), 115.8 (1C, s), 116.9 (1C, s), 118.2 (1C, s), 123.8 (1C, s), 124.0 (1C, s), 126.9-127.0 (2C), 126.9 (s), 127.0 (s), 127.3-127.3 (2C), 127.3 (s), 127.3 (s), 127.6 (2C, s), 127.8 (1C, s), 128.0 (1C, s), 128.4 (1C, s), 128.7 (2C, s), 129.4 (1C, s), 130.3 (1C, s), 131.8 (1C, s), 132.0-132.2 (2C), 132.1 (s), 132.1 (s), 133.6-133.8 (3C), 133.7 (s), 133.7 (s), 133.7 (s), 134.3 (1C, s), 134.9 (1C, s), 136.8-137.0 (2C), 136.9 (s), 136.9 (s), 151.1 (1C, s), 154.1 (1C, s), 159.2 (1C, s), 165.7 (1C, s), 197.1 (1C, s). Mass: 704 [M+], 703 [M+2].

6-Bromo-3-(2-(5-(5-(4-bromophenyl)furan-2-yl)-3-(2-oxo-2*H*-chromen-3-yl)-2,3-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-8-chloro-2*H*-chromen-2-one (4i): Yield: 73%; m.p.: 290 ºC. IR (KBr, ν_{max}, cm^{-1}): 3500-3300 (-NH- *str.*), 1700-1500 (-C=C- *str.*), 1250-1020 (-CN- *str.*), 1210-1163 (-CO- *str.*); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 3.82 (2H, d, *J* = 16.6 Hz), 4.81 (1H, d, *J* = 2.3 Hz), 5.99 (1H, d, *J* = 2.3 Hz), 6.69 (1H, d, *J* = 1.6 Hz), 7.03-7.63 (12H, 7.08 (s), 7.19 (d, *J* = 8.6 Hz), 7.30 (d, *J* = 8.6 Hz), 7.33 (d, *J* = 8.1 Hz), 7.39 (s), 7.44 (d, *J* = 7.9 Hz), 7.47 (d, *J* = 8.9 Hz), 7.55 (d, *J* = 8.1 Hz), 7.57 (d, *J* = 8.9 Hz), 7.57 (d, *J* = 1.6 Hz), 7.79 (1H, d, *J* = 7.9 Hz), 7.98 (1H, s); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 45.0 (1C, s), 56.6 (1C, s), 115.8 (1C, s), 116.9 (1C, s), 118.2 (1C, s), 119.6 (1C, s), 122.3 (1C, s), 123.8 (1C, s), 124.0 (1C, s), 126.9-127.0 (2C, 126.9 (s), 127.0 (s), 127.3-127.3 (2C, 127.3 (s), 127.3 (s), 127.9-128.1 (3C), 127.9 (s), 128.0 (s), 128.4 (1C, s), 129.4 (1C, s), 130.4 (1C, s), 131.0 (1C, s), 131.7 (2C, s), 132.1 (1C, s), 132.7 (1C, s), 133.6-133.8 (2C), 133.7 (s), 133.7 (s), 134.3 (1C, s), 134.9 (1C, s), 136.8-137.0 (2C, 136.9 (s), 136.9 (s), 151.1 (1C, s), 154.1 (1C, s), 159.2 (1C, s), 165.7 (1C, s), 197.1 (1C, s). Mass: 791 [M+], 793 [M+1].

6-Bromo-3-(2-(3-(2-oxo-2*H*-chromen-3-yl)-5-(5-(*p*-tolyl)furan-2-yl)-2,3-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-2*H*-chromen-2-one (4j): Yield: 72%; m.p.: 289 ºC. IR (KBr, ν_{max}, cm^{-1}): 3500-3300 (-NH- str.), 1700-1500 (-C=C- *str.*). 1250-1020 (-CN- *str.*), 1210-1163 (-CO- *str.*); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.30 (3H, s), 3.71 (2H, d, *J* = 16.6 Hz), 4.86 (1H, d, *J* = 2.3 Hz), 6.00 (1H, d, *J* = 2.3 Hz), 7.03-7.63 (11H), 7.08 (s), 7.16 (d, *J* = 8.7 Hz), 7.17 (d, *J* = 8.3 Hz), 7.22 (d, *J* = 8.7 Hz), 7.25 (d, *J* = 1.5 Hz), 7.33 (d, *J* = 8.1 Hz), 7.44 (d, *J* = 7.9 Hz), 7.46 (d, *J* = 8.2 Hz), 7.50 (s), 7.55 (d, *J* = 8.1 Hz), 7.65-7.90 (4H), 7.71 (d, *J* = 8.2 Hz), 7.79 (d, *J* = 7.9 Hz), 7.84 (d, *J* = 8.3 Hz), 7.98 (1H, s); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 21.3 (1C, s), 45.0 (1C, s), 56.6 (1C, s), 115.8 (1C, s), 116.9 (1C, s), 118.2 (1C, s), 118.4 (1C, s), 123.8 (1C, s), 124.0 (1C, s), 126.9-127.0 (2C), 126.9 (s), 127.0 (s), 127.3-127.3 (2C, 127.3 (s), 127.3 (s), 127.8-127.8 (3C, 127.8 (s), 127.8 (s), 128.4 (1C, s), 129.1 (2C, s), 129.4 (1C, s), 131.0 (1C, s), 131.7 (1C, s), 132.1 (1C, s), 133.6-133.8 (2C, 133.7 (s), 133.7 (s), 134.3 (1C, s), 134.9 (1C, s), 135.6 (1C, s), 136.8-137.0 (2C, 136.9 (s), 136.9 (s), 141.5 (1C, s), 151.1 (1C, s), 154.1 (1C, s), 159.2 (1C, s), 165.7 (1C, s), 197.1 (1C, s). Mass: 693 [M+].

6-Chloro-3-(2-(3-(2-oxo-2*H*-chromen-3-yl)-5-(5-(*m*-tolyl)furan-2-yl)-2,3-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-2H-chromen-2-one (4k): Yield: 77%; m.p.: 284 ºC. IR (KBr, ν_{max}, cm^{-1}): 3500-3300 (-NH- *str.*), 1700-1500 (-C=C- *str.*), 1250-1020 (-CN- *str.*), 1210-1163 (-CO- *str.*); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 2.29 (3H, s), 3.71 (2H, d, *J* = 16.6 Hz), 4.86 (1H, d, *J* = 2.3 Hz), 6.00 (1H, d, *J* = 2.3 Hz), 7.03-7.63 (13H), 7.08 (s), 7.17 (d, *J* = 7.6 Hz), 7.18 (d, *J* = 8.7 Hz), 7.23 (d, *J* = 8.7 Hz), 7.31 (d, *J* = 1.5 Hz), 7.33 (d, *J* = 8.1 Hz), 7.34 (d, *J* = 7.8 Hz), 7.40 (d, *J* = 8.0 Hz), 7.44 (d, *J* = 7.9 Hz), 7.45 (d, *J* = 8.0 Hz), 7.46 (d, *J* = 7.8 Hz), 7.50 (s), 7.55 (d, *J* = 8.1 Hz)), 7.67-7.86 (2H), 7.72 (d, *J* = 1.4 Hz), 7.79 (d, *J* = 7.9 Hz), 7.98 (1H, s); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 21.3 (1C, s), 45.0 (1C, s), 56.6 (1C, s), 115.8 (1C, s), 116.9 (1C, s), 118.2 (1C, s), 123.8 (1C, s), 124.0 (1C, s), 126.9-127.0 (2C), 126.9 (s), 127.0 (s), 127.3-127.3 (2C, 127.3 (s), 127.3 (s), 127.4 (1C, s), 127.7-127.8 (2C), 127.7 (s), 127.8 (s), 128.0 (1C, s), 128.1 (1C, s), 128.4 (1C, s), 128.7 (1C, s), 128.9 (1C, s), 129.4 (1C, s), 130.4 (1C, s), 132.1 (1C, s), 133.6-133.8 (2C), 133.7 (s), 133.7 (s), 134.8-135.0 (2C, 134.8 (s), 134.9 (s), 135.1 (1C, s), 135.6 (1C, s), 136.8-137.0 (2C), 136.9 (s), 136.9 (s), 151.1 (1C, s), 154.1 (1C, s), 159.2 (1C, s), 165.7 (1C, s), 197.1 (1C, s). Mass: 648 [M+], 650 [M+2].

6-Chloro-3-(2-(5-(5-(3-chlorophenyl)furan-2-yl)-3-(2-oxo-2*H*-chromen-3-yl)-2,3-dihydro-1*H*-pyrazol-1-yl)thiazol-4-yl)-7-methoxy-2*H*-chromen-2-one (4l): Yield: 78%; m.p.: 280 ºC. IR (KBr, ν_{max}, cm^{-1}): 3504-3310 (-NH- *str.*), 1705-1510 (-C=C- *str.*), 1251-1025 (-CN- *str.*), 1211-1162 (-CO- *str.*). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 3.58-3.78 (5H, 3.65 (d, *J* = 16.7 Hz), 3.73 (s), 4.85 (1H, d, *J* = 2.3 Hz), 6.02 (1H, d, *J* = 2.3 Hz), 7.08 (1H, s), 7.14-7.65 (11H, 7.20 (d, *J* = 8.7

Hz), 7.24 (d, *J* = 0.5 Hz), 7.33 (d, *J* = 8.1 Hz), 7.39 (d, *J* = 8.1 Hz), 7.39 (d, *J* = 8.7 Hz), 7.44 (d, *J* = 7.9 Hz), 7.42 (d, *J* = 0.5 Hz), 7.47 (s), 7.54 (d, *J* = 8.1 Hz), 7.55 (d, *J* = 8.1 Hz), 7.59 (d, *J* = 7.7 Hz), 7.66-7.86 (2H), 7.71 (d, *J* = 1.5 Hz), 7.79 (d, *J* = 7.9 Hz), 7.98 (1H, s); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm: 45.0 (1C, s), 56.0 (1C, s), 56.6 (1C, s), 110.6 (1C, s), 115.8 (1C, s), 116.9 (1C, s), 118.2 (1C, s), 120.6 (1C, s), 123.8 (1C, s), 124.0 (1C, s), 126.9-127.0 (3C, 126.9 (s), 127.0 (s), 127.0 (s), 127.3-127.3 (2C), 127.3 (s), 127.3 (s), 127.7 (1C, s), 128.4 (1C, s), 128.7 (1C, s), 128.8-129.0 (2C, 128.9 (s), 128.9 (s), 129.4 (1C, s), 130.4 (1C, s), 132.1 (1C, s), 133.0 (1C, s), 133.6-133.8 (2C, 133.7 (s), 133.7 (s)), 134.9 (1C, s), 135.6 (1C, s), 136.8-137.0 (2C, 136.9 (s), 136.9 (s), 149.7 (1C, s), 151.1 (1C, s), 154.1 (1C, s), 159.2 (1C, s), 165.7 (1C, s), 197.1 (1C, s). Mass: 698 [M+], 700 [M+2].

Biological activity

***In vitro* anti-inflammatory activity:** The anti-inflammatory activity of the target compounds was measured using the denaturation of bovine serum albumin technique, adhering to the protocols outlined in the literature [29,30]. In the test sample, the pH of mixture, containing the test chemical and a 1% aqueous solution of bovine albumin fraction, was increased to 7.4. Furthermore, test samples were incubated for 20 min at 37 ºC before being heated to 51 ºC for 20 min. Using a UV-visible spectrophotometer, the turbidity of the sample was measured at 660 nm after it had cooled to room temperature. Diclofenac sodium, standard drug used in the investigation, was administered in triplicate.

Based on the percentage of inhibition of albumin denaturation, the anti-inflammatory activity was computed as follows:

$$\text{Inhibition (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Antioxidant activity: The Blois method was used to examine hydrazone analogs' ability to scavenge free radicals. At various doses (20-100 µg/mL), a recently prepared DPPH solution (0.004% w/v) was added to the sample solutions in methanol. The solution was allowed to sit at room temperature for 30 min in dark, the absorbance of mixture at 517 nm was measured. Ascorbic acid was used as a reference. Methanol served as the blank and the same volume of DPPH was used to create the control sample in the absence of any test samples. The lower absorbance value of the reaction mixture indicates that it has a higher free radical scavenging activity. Every test was administered three times in duplicate [31]. Using the following formula, the fraction of the DPPH free radical that was scavenged was calculated:

$$\text{Inhibition (\%)} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

where A_{test} is the absorbance of test sample and $A_{control}$ is the absorbance of control reaction.

Antibacterial activity: Fungal strain *C. albicans*, Gram-negative bacterial strain *i.e. E. coli* and Gram-positive bacterial strains *i.e. S. aureus B.* maintained at the microbial collection repository of Yenepoya Research Centre, Mangalore were used for the experiments. Muller Hinton broth media was used for growing the cultures.The compounds were tested to determine the MIC for each bacterial strain. A 100 µL of 10^6 cells/mL of each bacterial culture was inoculated in labelled 96 well microtiter plates in triplicates containing Muller Hinton broth. The test compounds at a concentration of 500 µg/mL were supplemented into the broth and incubated at 37 °C for 24 h. The MIC of the individual sample was determined by measuring the optical density at 600 nm [32].

RESULTS AND DISCUSSION

A series of thiazolyl-pyrazole-coumarin hybrids were successfully synthesized. Initially, an ethanolic solution of thiosemicarbazide (**2**) was combined with a coumarin chalcone (**1**) in ethanol of 3-(2-bromoacetyl)coumarin (**3**) was added to the reaction mixture. The mixture was refluxed in a water bath to facilitate nucleophilic substitution between the bromoacetyl group and the thiosemicarbazone, resulting in cyclization and the formation of the desired heterocyclic structure. Upon completion, the mixture was neutralized with a saturated NaHCO solution, leading to the precipitation of the desired product (**4a-l**). The precipitate was filtered, washed with ethanol to remove impurities and then recrystallized from a DMF/HO solvent system to improve purity and yield.

Anti-inflammatory activity: Diclofenac sodium was considered as a standard medication to assess the *in vitro* anti-inflammatory effectiveness of all the synthesized derivatives through the inhibition of protein denaturation (bovine albumin). Based on the IC_{50} values, it was evident that several synthetic compounds had superior activity (Table-1).

TABLE-1
ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITIES RESULTS OF THE SYNTHESIZED COMPOUNDS

Compound	IC_{50} values	
	Anti-inflammatory	Antioxidant
4a	78.9	67.4
4b	87.8	75.9
4c	69.5	56.3
4d	56.3	95.4
4e	47.4	54.6
4f	88.2	90.1
4g	32.8	17.58
4h	35.1	14.3
4i	36.4	22.1
4j	28.8	25.3
4k	29.1	18.5
4l	33.5	22.1
Standard	38.4	34.8

Antioxidant activity: Table-1 shows that compound **4h** demonstrated a higher efficacy in inhibiting radical damage, with an inhibition rate of 14.3%. This performance surpasses that of the standard antioxidant, ascorbic acid, which exhibited an inhibition rate of 34.8%. This suggests that compound **4h** is more effective than DPPH radicals compared to ascorbic acid in this assay.

Antibacterial activity: All the synthesized compounds (**4a-l**) exhibited effective antibacterial activity against *E. coli*.

All the compounds exhibited moderate to excellent antifungal activity against *C. albicans* and the results are presented in Table-2.

TABLE-2
ANTIMICROBIAL ACTIVITY RESULTS OF THE SYNTHESIZED COMPOUNDS

Compound	*S. aureus*	*E. coli*	*C. albicans*
4a	58.6	78.6	57.6
4b	78.9	85.3	96.4
4c	69.3	75.1	75.2
4d	84.7	75.1	81.0
4e	75.8	89.3	88.1
4f	92.4	75.3	83.7
4g	44.2	50.1	47.5
4h	56.8	32.4	22.8
4i	22.7	31.2	30.4
4j	19.2	22.8	34.7
4k	12.7	22.8	34.2
4l	12.8	10.7	12.5

Conclusion

The present study highlights the synthesis, characterization and biological evaluation of thiazolyl-pyrazole-biscoumarin derivatives. The findings indicate that oxygen-containing heterocycles exhibited enhanced anti-inflammatory and antioxidant properties, while sulfur-containing heterocycles demonstrated strong antimicrobial activity against both bacterial and fungal strains. These results suggest that thiazolyl-pyrazole-biscoumarin derivatives hold significant promise as candidates for further development in therapeutic applications, potentially addressing multiple health challenges associated with inflammation, oxidative stress and microbial infections.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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