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Synthesis and biological evaluation of some new coumarin derivatives as potential antimicrobial, analgesic and anti-inflammatory agents

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ABSTRACT

*A new series of 4-methyl-7-methoxycoumarin derivatives linked triazoles and oxadiazole were prepared starting from 2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy) acetohydrazide, compound **4**. The purity of all new compounds was checked by TLC and elucidation of their structures was confirmed by **IR**, **^{1}H** and **^{13}C NMR** along with High Resolution Mass Spectrometry (**HRMS**). All the target compounds were evaluated for their possible anti-inflammatory activities. The result showed that most of the tested compounds exhibited significant anti-inflammatory activity in comparison to indomethacin as a reference drug. The compound **8l** was the most active one. Compounds **6a**, **8g**, **8i**, **8k** and **8l** were tested for their analgesic effects in comparison to indomethacin as a reference drug. Compounds **6a** and **8i** showed activity comparable to the reference drug. Compounds **6a**, **8d** and **8l** were examined for their effect on gastric mucosa and showed no gastric ulcerogenic effect at dose 60 mg/Kg. Moreover, LD_{50} of compounds **6a** and **8l** were determined; they exhibited no-toxic effect up to 240 and 300 mg/kg (i.p.) respectively. Finally, compounds **7** and **8a-8j** were evaluated for their possible antimicrobial activity. Most of the tested compounds showed moderate to good antimicrobial activity against most of the strains used in comparison with ciprofloxacin and fluconazole as reference drugs.*

Keywords: Coumarin, triazole, oxadiazole, anti-inflammatory, analgesic, antibacterial

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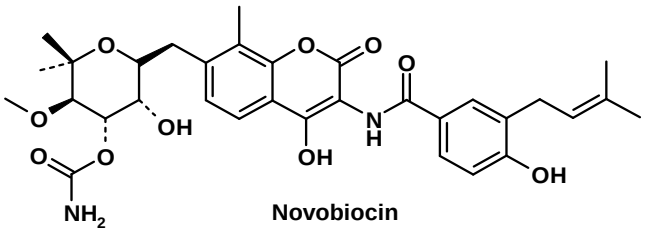
INTRODUCTION

Coumarins (2*H*-1-benzopyran-2-ones) are important oxygen containing fused heterocycles used in preparation of pharmaceutical compounds & dyes [1]. They are the family of lactones containing benzopyrone skeletal framework that was isolated from plant as well as total synthesis in the laboratory [2]. The incorporation of other heterocyclic moiety either as substituent group or as a fused component into the parent coumarin alters the property of parent coumarin and converts it into more useful products[3]. Natural coumarins are known to have antidiabetic activity [4], anabolic, antioxidant and hepato protective activities [5,6]. Substituted coumarin derivatives have been reported

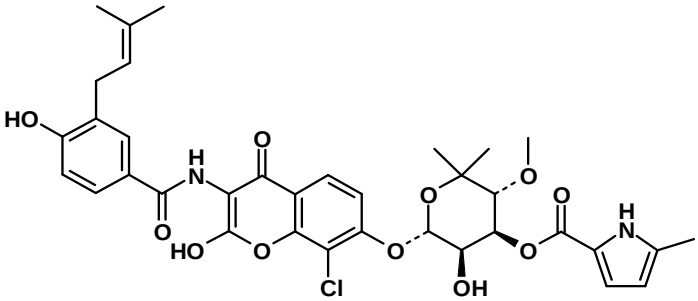
to have variety of biological activities including anticoagulant [7], HIV protease inhibition [8], CNS depressant [9], analgesic [10] and anti-inflammatory activities [11]. The potent antibiotics like novobiocin [12, 13] and chlorobiocin [14] (chart 1) are coumarin derivatives. Recently, the interest on these compounds has been reviewed owing to their use as fluorescent markers in the biochemical determination of enzymes [15].

On other hand 1,3,4 oxadiazole and 1,2,4 triazole derivatives has attracted widespread attention due to their diverse biological activities, including antimicrobial [16,17], anti- inflammatory [18,19], analgesic [20] and antitumor activity [21].

Enlightened by the aforementioned studies, the present work aims at the synthesis of new coumarin derivatives incorporating oxadiazole and triazole moieties to be subjected for preliminary *invitro* and *invivo* screening of their antibacterial, antifungal, analgesic and anti-inflammatory activities.



Novobiocin



Chlorobiocin

Chart 1: Novobiocin and chlorobiocin structures

MATERIALS AND METHODS

2.1. Chemistry

Melting points were determined on an electro thermal melting point apparatus [Stuart Scientific, model SMP3, England, UK], and were uncorrected. A pre-coated silica gel plate (kieselgel 0.25 mm, 60G F254, Merck, Germany) was used for TLC monitoring of reactions. IR spectra (KBr discs) were recorded on a shimadzu IR-470 spectrometer (Shimadzu, Kyoto, Japan) at Faculty of Pharmacy, Assiut University, Assiut, Egypt. NMR spectra were taken using Varian Unity INOVA 400 MHz spectrometers for proton and carbon at university of Aberdeen, United Kingdom. Chemical shifts are expressed in δ-value (ppm) relative to DMSO-d6 as internal standard, and deuterium oxide was used for the detection of exchangeable protons. High resolution mass spectrometric data were obtained using the EPSRC mass spectrometry Centre in Swansea and Thermo Instruments MS system (LTQ XL/LTQ Orbitrap Discovery) coupled to a Thermo Instruments HPLC system (Accela PDA detector, Accela PDA autosampler and Pump) at university of Aberdeen, United kingdom. Reagents used for synthesis were purchased from Sigma-Aldrich and Merck. All solvents were obtained from commercial suppliers and used without further purification.

The starting materials 7-hydroxy-4-methyl coumarin **2**[22], ethyl 2-(4-methyl-2-oxo-2*H*-chromen-7-yloxy) acetate **3**[23], 2-(4-methyl-2-oxo-2*H*-chromen-7-yloxy) acetohydrazide **4**[24] and 7-((5-mercapto-1,3,4-oxadiazol-2-yl)methoxy)-4-methyl-2*H*-chromen-2-one **5**[25] were synthesized according to reported procedures.

2.1.1. Synthesis of 7-((5-(alkyl/aralkylthio)-1,3,4-oxadiazol-2-yl)methoxy)-4-methyl-2*H* chromen-2-one (6a-e)

To a suspension of 7-((5-mercapto-1,3,4-oxadiazol-2-yl)methoxy)-4-methyl-2*H*-chromen-2-one **5** (0.1 mmol) and anhydrous potassium bicarbonate (0.1 mmol) in acetone (15 ml), an appropriate alkyl/aralkyl halide (0.1 mmol) was added. The reaction mixture was stirred overnight at the ambient temperature and then poured onto water. The precipitated solid was filtered, washed with water, dried, and crystallized from ethanol.

2.1.1.1. 7-((5-(ethylthio)-1,3,4-oxadiazol-2-yl)methoxy)-4-methyl-2H-chromen-2-one 6a.

IR (KBr, cm^{-1}) 1703 (C=O), 1602 (C=N), 1457 (C=C Ar), 1146 (C-O-C), 1253, 1195 (C-S-C). **^{1}H-NMR** (400 MHz, DMSO-d_6) δ1.37 (t, J = 7.3 Hz, 3H, $CH_2C\underline{H}_3$), 2.38 (s, 3H, CH_3), 3.25 (q, J = 7.2, 2H, $C\underline{H}_2CH_3$), 5.53 (s, 2H, OCH_2), 6.26 (s, 1H, coumarin-H), 7 (d, J = 8.8 Hz, 1H, coumarin-H), 7.14 (d, J = 2.5 Hz, 1H, coumarin-H), 7.7 (d, J = 8.8 Hz, 1H, coumarin-H). **^{13}C-NMR** (100 MHz, DMSO-*d*6) 14.7, 18.1, 26.6, 59.8, 101.9, 111.7, 112.3, 114.0, 126.6, 153.2, 154.4, 159.9, 160.0, 163.0, 165.0. HRESI-MS **(m/z)**: calcd. $[M+H]^+$ for $C_{15}H_{14}N_2O_4S$: 319.0747, found: 319.0739. Yield: (80%); m.p. 165-167 ^oC.

2.1.1.2. 7-((5-(allylthio)-1,3,4-oxadiazol-2-yl)methoxy)-4-methyl-2H-chromen-2-one 6b.

IR (KBr, cm^{-1}) 1723 (C=O), 1600 (C=N), 1500 (C=C Allyl), 1450 (C=C Ar), 1139 (C-O-C), 1251, 1196 (C-S-C). **^{1}H-NMR** (400 MHz, DMSO-d_6) δ 2.39 (s, 3H, CH_3), 3.91 (d, J = 7 Hz, 2H, SCH_2), 5.1-5.3 (dd, J = 16.9, 10.1, 2H, $CH=C\underline{H}_2$), 5.54 (s, 2H, OCH_2), 5.95 (ddt, J = 16.9, 10.0, 6.9, 1H, $C\underline{H}=CH_2$), 6.24 (s, 1H, coumarin-H),7.03 (d, J = 8.6 Hz, 1H, coumarin-H), 7.14 (d, J = 2.5 Hz, 1H, coumarin-H), 7.7 (d, J = 8.8 Hz, 1H, coumarin-H). **^{13}C-NMR** (100 MHz, DMSO-*d*6) 18.1, 34.5, 59.8, 101.9, 111.7, 112.4, 114.0, 119.4, 126.6, 132.4, 153.2, 154.4, 159.9, 159.9, 163.2, 164.3. HRESI-MS **(m/z)**: calcd. $[M+H]^+$ for $C_{16}H_{14}N_2O_4S$: 331.0747, found: 331.0738. Yield: (85%); m.p. 179-181^oC.

2.1.1.3. 7-((5-(benzylthio)-1,3,4-oxadiazol-2-yl)methoxy)-4-methyl-2H-chromen-2-one 6c.

IR (KBr, cm^{-1}) 1711 (C=O), 1601 (C=N), 1456 (C=C Ar), 1147 (C-O-C), 1253, 1190 (C-S-C), 743, 695. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ 2.4 (s, 3H, CH_3), 4.52 (s, 2H, SCH_2), 5.54 (s, 2H, OCH_2), 6.25 (s, 1H, coumarin-H), 7 (d, J = 8.8 Hz, 1H, coumarin-H), 7.14 (d, J = 2.6 Hz, 1H, coumarin-H), 7.22-7.36 (m, 3H, Ar-H), 7.37-7.46 (m, 2H, Ar-H), 7.7 (d, J = 8.9 Hz, 1H, coumarin-H). **^{13}C-NMR** (100 MHz, DMSO-*d*6) 18.1, 35.8, 59.8, 101.9, 111.7, 112.4, 114.1, 126.6, 127.7, 128.5, 128.9, 136.3, 153.2, 154.4, 159.9, 159.9, 163.2, 164.4. HRESI-MS **(m/z)**: calcd. $[M+H]^+$ for $C_{20}H_{16}N_2O_4S$: 381.0904, found: 381.0905. Yield: (85%); m.p. 163-165^oC.

2.1.1.4. 4-methyl-7-((5-(methylthio)-1,3,4-oxadiazol-2-yl)methoxy)-2H-chromen-2-one 6d.

IR (KBr, cm^{-1}) 1714 (C=O), 1599 (C=N), 1459 (C=C Ar), 1140 (C-O-C), 1251, 1196 (C-S-C)). **^{1}H-NMR** (400 MHz, DMSO-d_6) δ 2.39 (s, 3H, CH_3), 2.7 (s, 3H, SCH_3), 5.53 (s, 2H, OCH_2), 6.24 (s, 1H, coumarin-H), 7.07 (d, J = 8.8 Hz, 1H, coumarin-H), 7.14 (d, J = 2.4 Hz, 1H, coumarin-H), 7.7 (d, J = 8.9, 1H, coumarin-H). **^{13}C-NMR** (100 MHz, DMSO-*d*6) 14.2, 18.1, 59.8, 101.9, 111.7, 112.4, 114.0, 126.6, 153.2, 154.4, 159.9, 160.0, 163.0, 165.9. HRESI-MS **(m/z)**: calcd. $[M+H]^+$ for $C_{14}H_{12}N_2O_4S$: 305.0591, found: 305.0584. Yield: (83%); m.p. 160-162 ^oC.

2.1.1.5. 4-methyl-7-((5-((4-methylbenzyl)thio)-1,3,4-oxadiazol-2-yl)methoxy)-2H-chromen-2-one 6e.

IR (KBr, cm^{-1}) 1712 (C=O), 1601 (C=N), 1457 (C=C Ar), 1149 (C-O-C), 1256, 1199 (C-S-C), 833. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ 2.24 (s, 3H, p-C_6H_4-$C\underline{H}_3$), 2.42 (s, 3H, CH_3), 4.46 (s, 2H, SCH_2), 5.54 (s, 2H, OCH_2), 6.25 (s, 1H, coumarin-H), 7.03-7.16 (m, 4H, Ar-H), 7.28 (d, J = 7.8 Hz, 2H, coumarin-H), 7.72 (d, J = 8.8 Hz, 1H, coumarin-H). **^{13}C-NMR** (100 MHz, DMSO-*d*6) 18.1, 20.6, 30.6, 35.6, 59.7, 101.9, 111.7, 112.4, 114.1, 126.6, 128.8, 129.0, 133.2, 137.0, 153.2, 154.4, 159.9, 159.9, 163.2, 164.5. HRESI-MS **(m/z)**: calcd. $[M+H]^+$ for $C_{21}H_{18}N_2O_4S$: 395.1060, found: 395.1049. Yield: (80%); m.p. 175-177 ^oC.

2.1.2. a. Synthesis of 7-((4-amino-5-mercapto-4*H*-1, 2, 4-triazol-3-yl)methoxy)-4-methyl-2*H*-chromen-2-one (7)

Procedure (A): To a suspension of (2-(4-methyl-2-oxo-2*H*-chromen-7-yloxy)acetohydrazide **4** (0.1 mmol) in absolute ethanol (30 mL), potassium hydroxide (0.15 mmol) was added and the reaction mixture was refluxed for 30 mins, the reaction mixture was cooled to room temp. then carbon disulphide (0.2 mmol) was add dropwise. The mixture was stirred at room temperature for another 20 h. The solvent was evaporated and the obtained salt was air dried and used in the next step without further purification.

To a suspension of the obtained potassium salt (0.1 mmol) in ethanol (30 ml), 99% hydrazine hydrate (0.2 mmol) and water (10 mL) were added. The reaction mixture was heated under reflux for 3 h. After cooling, the reaction mixture was acidified with 10% hydrochloric acid until pH 3. The yellowish precipitate was filtered, washed with water, dried and crystallized from ethanol to afford the desired compound **7**.

2.1.2.b. Procedure (B): To a solution of 7-((5-mercapto-1,3,4-oxadiazol-2-yl)methoxy)-4-methyl-2*H*-chromen-2-one **5** (0.1 mmol) in absolute ethanol (20 mL), 99% hydrazine hydrate (0.2 mmol) was added. The reaction mixture was refluxed for 4 h. After cooling, the formed precipitate was filtered and dried. The crude product was purified by dissolving in a minimum amount of 1% aqueous KOH and acidified with 10% hydrochloric acid until pH 3. The resulted product was recrystallized from ethanol.

IR (KBr, cm^{-1}) 3400, 3370 (NH_2), 3220 (NH), 1680 (C=O), 1603 (C=N), 1150 (C-O-C), 1494 (C=C Ar). **^{1}H-NMR** (400 MHz, DMSO-d_6) δ 2.40 (s, 3H, CH_3), 4.5 (s, 2H, NH_2, D_2O exchangeable), 5.41 (s, 2H, OCH_2), 6.27 (s, 1H, coumarin-H), 6.94 (d, *J* = 8.8 Hz, 1H, coumarin-H), 7.09 (d, *J* = 2.5 Hz, 1H, coumarin-H), 7.71 (d, *J* = 6.1 Hz, 1H, coumarin-H), 13.78 (s, 1H, SH, D_2O exchangeable). **^{13}C-NMR** (100 MHz, DMSO-*d*6) 18.3, 65.2, 101.6, 111.2, 112.3, 113.3, 126.3, 153.3, 154.5, 154.5, 161.3, 163.6, 168.4. HRESI-MS **(m/z)**: calcd. $[M+H]^+$ for $C_{13}H_{12}N_4O_3S$: 305.0703, found: 305.0709. Yield: (40%); m.p. 221-222 ^oC.

2.1.3. General procedure for preparation of derivatives (8a-l)

To a suspension of 7-((4-amino-5-mercapto-4*H*-1, 2, 4-triazol-3-yl)methoxy)-4-methyl-2*H*-chromen-2-one **7** (2.0 mmol) in ethanol (10 mL) and an appropriate aryl aldehyde or acetophenone derivatives (2.0 mmol) and 2 drops of glacial acetic acid were added. The reaction mixture was heated under reflux for 4-6 h. After cooling, the formed precipitate was filtered, washed with diethyl ether and crystallized from ethanol.

2.1.3.1. (E)-7-((4-((4-chlorobenzylidene)amino)-5-mercapto-4H-1,2,4-triazol-3-yl)methoxy)-4-methyl-2H-chromen-2-one 8a.

IR (KBr, cm^{-1}) 3310 (NH), 1712 (C=O), 1601 (C=N), 1490 (C=C Ar), 1150 (C-O-C), 819. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ 2.37 (s, 3H, CH_3), 5.42 (s, 2H, OCH_2), 6.22 (s, 1H, coumarin-H), 7.02 (dd, *J* = 8.8, 2.5 Hz, 1H, coumarin-H), 7.15 (d, *J* = 2.5 Hz, 1H, coumarin-H), 7.58 (m, 2H, Ar-H), 7.65 (d, *J* = 8.8 Hz, 1H, coumarin-H),7.87-7.79 (m, 2H, Ar-H), 8 (s, 1H, HC=N), 10.03 (s, 1H, SH, D_2O exchangeable). **^{13}C-NMR** (100 MHz, DMSO-*d*6) 18.1, 60.1, 102.2, 111.6, 112.7, 113.9, 126.5, 129.2, 130.2, 130.8, 137.4, 146.6, 153.2, 154.4, 159.9, 160.5, 162.1, 162.2. HRESI-MS **(m/z)**: calcd. $[M+H]^+$ for $C_{20}H_{15}ClN_4O_3S$: 427.0626, found: 427.0614. Yield: (70%); m.p. 212-214 ^oC.

2.1.3.2. (E)-7-((4-((4-bromobenzylidene)amino)-5-mercapto-4H-1,2,4-triazol-3-yl)methoxy)-4-methyl-2H-chromen-2-one 8b.

IR (KBr, cm^{-1}) 3375 (NH), 1715 (C=O), 1600(C=N), 1493 (C=C Ar), 1144 (C-O-C), 807. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ 2.36 (s, 3H, CH_3), 5.40 (s, 2H, OCH_2), 6.25 (s, 1H, coumarin-H), 7.02 (dd, *J* = 8.8, 2.5 Hz, 1H, coumarin-H), 7.14 (d, *J* = 2.5 Hz, 1H, coumarin-H), 7.28 (m, 2H, Ar-H), 7.67 (d, *J* = 8.3 Hz, 1H, coumarin-H), 7.87-7.79 (m, 2H, Ar-H), 7.95 (s, 1H, HC=N), 9.86 (s, 1H, SH, D_2O exchangeable). HRESI-MS **(m/z)**: calcd. $[M+H]^+$ for $C_{20}H_{15}BrN_4O_3S$: 471.0121, found: 471.0114. Yield: (83%); m.p. 224-226 ^oC.

2.1.3.3. (E)-7-((4-((1-(2-fluorophenyl)ethylidene)amino)-5-mercapto-4H-1,2,4-triazol-3 yl)methoxy)-4-methyl-2H-chromen-2-one 8c.

IR (KBr, cm^{-1}) 3415 (NH), 1705 (C=O), 1598(C=N), 1490 (C=C Ar), 1152 (C-O-C), 760. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ 2.27 (s, 3H, N=C($C\underline{H}_3$)), 2.39 (s, 3H, CH_3), 5.40 (s, 2H, OCH_2), 6.21 (s, 1H, coumarin-H), 6.89 (m, 2H, Ar-H), 7.08 (dd, *J* = 8.8, 2.6 Hz, 1H, coumarin-H), 7.15 (d, *J* = 2.6 Hz, 1H, coumarin-H), 7.21-7.31 (m, 2H, Ar-H), 7.45 (d, *J* = 7.4 Hz, coumarin-H), 11.01 (s, 1H, SH, D_2O exchangeable). **^{13}C-NMR** (100 MHz, DMSO-*d*6) 16.1, 18.1, 59.9, 101.9, 111.8, 112.3, 112.4, 114.2, 124.2, 126.7, 131.1, 132.0, 153.3, 153.4, 154.5, 159.0, 159.9, 160.0, 169.1, 178.1. HRESI-MS **(m/z)**: calcd. $[M+H]^+$ for $C_{21}H_{17}FN_4O_3S$: 425.1078, found: 425.1075. Yield: (85%); m.p. 230-232 ^oC.

2.1.3.4. (E)-7-((4-((3-bromobenzylidene)amino)-5-mercapto-4H-1,2,4-triazol-3-yl)methoxy)-4-methyl-2H-chromen-2-one 8d.

. **IR** (KBr, cm^{-1}) 3415 (NH), 1700 (C=O), 1600 (C=N), 1492 (C=C Ar), 1151 (C-O-C), 779, 714. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ 2.40 (s, 3H, CH_3), 5.32 (s, 2H, OCH_2), 6.22 (s, 1H, coumarin-H), 6.96-7.08 (m, 3H, Ar-H), 7.41 (dd, *J* = 7.9, 4.2 Hz, 1H, coumarin-H), 7.62 (d, *J* = 7.4 Hz, 1H, coumarin-H), 7.71 (d, *J* = 6.2 Hz, coumarin-H), 8 (s, 1H, Ar-H), 8.31 (s, 1H, HC=N), 11.77 (s, 1H, SH, D_2O exchangeable). **^{13}C-NMR** (100 MHz, DMSO-*d*6) 18.5, 65.7, 102.0, 111.9, 112.8, 113.7, 122.6, 126.7, 129.4, 129.6, 131.3, 132.9, 136.8, 142.7, 146.6, 153.8, 155.0, 160.5, 161.8, 169.1 HRESI-MS **(m/z)**: calcd. $[M+H]^+$ for $C_{20}H_{15}BrN_4O_3S$: 471.0121, found: 471.0109. Yield: (80%); m.p. 214-216 ^oC.

2.1.3.5. (E)-7-((4-((3-chlorobenzylidene)amino)-5-mercapto-4H-1,2,4-triazol-3-yl)methoxy)-4-methyl-2H-chromen-2-one 8e.

IR (KBr, cm^{-1}) 3420 (NH), 1698 (C=O), 1601 (C=N), 1493 (C=C Ar), 1152 (C-O-C), 782, 725. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ 2.40 (s, 3H, CH_3), 5.33 (s, 2H, OCH_2), 6.22 (s, 1H, coumarin-H), 6.99 (d, *J* = 8.9 Hz, 1H, coumarin-H), 7.00 (s, 1H, coumarin-H), 7.48 (t, *J* = 5.3 Hz, 3H, Ar-H), 7.71 (d, *J* = 6.1 Hz, coumarin-H), 7.81 (s, 1H, Ar-H), 8.31 (s, 1H, HC=N), 11.76 (s, 1H, SH, D_2O exchangeable). **^{13}C-NMR** (100 MHz, DMSO-*d*6) 18.1, 65.3, 101.6, 111.2, 112.4, 113.1, 125.9, 126.1, 126.3, 129.6, 130.6, 133.6, 136.1, 142.3, 153.3, 154.5, 161.3, 163.8, 168.6. HRESI-MS **(m/z)**: calcd. $[M+H]^+$ for $C_{20}H_{15}ClN_4O_3S$: 427.0626, found: 427.0617. Yield: (75%); m.p. 225-227 ^oC.

2.1.3.6. (E)-7-((4-(benzylideneamino)-5-mercapto-4H-1,2,4-triazol-3-yl)methoxy)-4-methyl-2H-chromen-2-one 8f.

IR (KBr, cm^{-1}) 3415 (NH), 1697 (C=O), 1600 (C=N), 1493 (C=C Ar), 1152 (C-O-C), 746, 698. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ 2.39 (s, 3H, CH_3), 5.30 (s, 2H, OCH_2), 6.22 (s, 1H, coumarin-H), 6.94 (d, *J* = 8.8 Hz, 1H, coumarin-H), 7.09 (s, 1H, coumarin-H), 7.44 (m, 5H, Ar-H), 7.71 (d, *J* = 6.1 Hz, coumarin-H), 8.33 (s, 1H, HC=N), 11.65 (s, 1H, SH, D_2O exchangeable). **^{13}C-NMR** (100 MHz, DMSO-*d*6) 18.3, 65.2, 101.6, 111.2, 112.3, 113.3, 126.3, 126.5, 126.9, 128.7, 128.8, 130.2, 133.9, 143.9, 153.3, 154.5, 154.5, 161.3, 163.6, 168.4. HRESI-MS **(m/z)**: calcd. $[M+H]^+$ for $C_{20}H_{16}N_4O_3S$: 393.1016, found: 393.1004. Yield: (79%); m.p. 223-225 ^oC.

2.1.3.7. (E)-7-((4-((1-(4-fluorophenyl)ethylidene)amino)-5-mercapto-4H-1,2,4-triazol-3-yl)methoxy)-4-methyl-2H-chromen-2-one 8g.

IR (KBr, cm^{-1}) 3495 (NH), 1711 (C=O), 1605 (C=N), 1500 (C=C Ar), 1154 (C-O-C), 806. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ 2.24 (s, 3H, N=C($C\underline{H}_3$)), 2.43 (s, 3H, CH_3), 5.33 (s, 2H, OCH_2), 6.22 (s, 1H, coumarin-H), 6.92-7.03 (m, 2H, coumarin-H),7.24 (dd, *J* = 8.9, 1.8 Hz, 2H, Ar-H), 7.68 (d, *J* = 8.9 Hz, coumarin-H), 7.84-7.93 (m, 2H, Ar-H), 10.92 (s, 1H, SH, D_2O exchangeable). **^{13}C-NMR** (100 MHz, DMSO-*d*6) 13.6, 18.1, 65.6, 101.5, 111.1, 112.3, 113.2, 115.1, 115.3, 126.3, 128.4, 128.5, 147.5, 153.3, 154.5, 160.1, 161.4, 169.2. HRESI-MS **(m/z)**: calcd. $[M+H]^+$ for $C_{21}H_{17}FN_4O_3S$: 425.1078, found: 425.1075. Yield: (74%); m.p. 243-245 ^oC.

2.1.3.8. (E)-7-((5-mercapto-4-((4-methoxybenzylidene)amino)-4H-1,2,4-triazol-3-yl)-methoxy)-4-methyl-2H-chromen-2-one 8h.

^{1}H-NMR (400 MHz, DMSO-d_6) δ 2.40 (s, 3H, CH_3), 3.80 (s, 3H, OCH_3), 5.27 (s, 2H, OCH_2), 6.2 (s, 1H, coumarin-H), 6.93-7.8 (m, 7H, Ar-H), 8.30 (s, 1H, HC=N), 11.53 (s, 1H, SH, D_2O exchangeable). **^{13}C-NMR** (100 MHz, DMSO-*d*6) 18.1, 55.3, 65.2, 101.6, 111.4, 112.3, 113.3, 114.2, 114.3, 126.3, 126.5, 128.5, 128.7, 143.8, 153.3, 154.5, 154.5, 160.9, 161.3, 163.3, 168.1. **IR** (KBr, cm^{-1}) 3285 (NH), 1678 (C=O), 1605 (C=N), 1495 (C=C Ar), 1146 (C-O-C), 826. **HRESI-MS (m/z)**: calcd. $[M+H]^+$ for $C_{21}H_{18}N_4O_4S$: 423.1122, found: 423.1107. Yield: (83%); m.p. 193-195 ^oC.

2.1.3.9. (E)-7-((5-mercapto-4-((1-(4-methoxyphenyl)ethylidene)amino)-4H-1,2,4-triazol-3-yl)methoxy)-4-methyl-2H-chromen-2-one 8i.

IR (KBr, cm^{-1}) 3280 (NH), 1712 (C=O), 1600 (C=N), 1496 (C=C Ar), 1155 (C-O-C), 807. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ 2.26 (s, 3H, N=C($C\underline{H}_3$)), 2.45(s, 3H, CH_3), 3.79 (s, 3H, OCH_3), 5.31 (s, 2H, OCH_2), 6.18 (s, 1H, coumarin-H), 6.91-7.03 (m, 2H, coumarin-H), 7.70 (dd, *J* = 11.2, 7.2 Hz, 2H, Ar-H), 7.73-7.82 (m, 3H, Ar-H), 10.81 (s, 1H, SH, D_2O exchangeable). **HRESI-MS (m/z)**: calcd. $[M+H]^+$ for $C_{22}H_{20}N_4O_4S$: 437.1278, found: 437.1265. Yield: (82%); m.p. 205-207 ^oC.

2.1.3.10. (E)-7-((4-((4-fluorobenzylidene)amino)-5-mercapto-4H-1,2,4-triazol-3-yl)methoxy)-4-methyl-2H-chromen-2-one 8j.
IR (KBr, cm^{-1}) 3370 (NH), 1698 (C=O), 1605 (C=N), 1495 (C=C Ar), 1154 (C-O-C), 830. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ 2.39 (s, 3H, CH_3), 5.30 (s, 2H, OCH_2), 6.23 (s, 1H, coumarin-H), 7 (d, *J* = 8.6 Hz, C_6 of coumarin), 7.12 (s, 1H, coumarin-H), 7.63-7.84 (m, 4H, Ar-H), 7.7 (d, *J* = 8.8 Hz, 1H, coumarin-H), 8.33 (s, 1H, HC=N), 11.65 (s, 1H, SH, D_2O exchangeable). **^{13}C-NMR** (100 MHz, DMSO-*d*6) 18.1, 65.2, 101.5, 111.2, 112.4, 115.7, 115.9, 126.3, 126.5, 129.1, 129.2, 142.8, 153.4, 154.5, 160.1, 161.3, 162.2, 168.4 HRESI-MS **(m/z)**: calcd. $[M+H]^+$ for $C_{20}H_{15}FN_4O_3S$: 411.0922, found: 411.0910. Yield: (82%); m.p. 213-215 ^oC.

2.1.3.11. (E)-7-((4-((4-(dimethylamino)benzylidene)amino)-5-mercapto-4H-1,2,4-triazol-3-yl)methoxy)-4-methyl-2H-chromen-2-one 8k.
. IR (KBr, cm^{-1}) 3460 (NH), 1711 (C=O), 1601 (C=N), 1483 (C=C Ar), 1329 (C-N), 1150 (C-O-C), 819. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ 2.37 (s, 3H, CH_3), 3.00 (s, 6H, $N(C\underline{H}_3)_2$), 5.33 (s, 2H, OCH_2), 6.21 (s, 1H, coumarin-H), 6.67-6.74 (m, 2H, Ar-H), 7.01 (dd, *J* = 8.8, 2.7 Hz, 1H, coumarin-H), 7.14 (d, *J* = 2.6 Hz, 1H, coumarin-H), 7.53-7.61 (m, 2H, Ar-H), 7.65 (d, *J* = 8.8 Hz, 1H, coumarin-H), 9.36 (s, 1H, HC=N), 14.3 (s, 1H, SH, D_2O exchangeable). **^{13}C-NMR** (100 MHz, DMSO-*d*6) 18.1, 39.5, 60.1, 102.2, 111.3, 111.6, 112.7, 113.8, 118.3, 126.5, 130.4, 146.3, 153.1, 153.2, 154.4, 160.0, 160.6, 165.5. HRESI-MS **(m/z)**: calcd. $[M+H]^+$ for $C_{22}H_{21}N_5O_3S$: 436.1438, found: 436.1425. Yield: (80%); m.p. 201-203 ^oC.

2.1.3.12. (E)-7-((4-((4-isopropylbenzylidene)amino)-5-mercapto-4H-1,2,4-triazol-3-yl)methoxy)-4-methyl-2H-chromen-2-one 8l.
IR (KBr, cm^{-1}) 3320 (NH), 1687 (C=O), 1597 (C=N), 1468 (C=C Ar), 1159 (C-O-C), 832. **^{1}H-NMR** (400 MHz, DMSO-d_6) δ 1.19 (d, *J* = 6.9 Hz, 6H, $CH(C\underline{H}_3)_2$), 2.42 (s, 3H, CH_3), 2.92 (p, *J* = 6.9 Hz, 1H, $C\underline{H}(CH_3)_2$), 5.4 (s, 2H, OCH_2), 6.27 (s, 1H, coumarin-H), 7.05 (ddd, *J* = 18.8, 8.8, 2.6 Hz, 2H, Ar-H), 7.15 (d, *J* = 2.6 Hz, coumarin-H), 7.34 (d, *J* = 8.1 Hz, 1H, coumarin-H), 7.65 (d, *J* = 8.8 Hz, 1H, coumarin-H), 7.71 (d, *J* = 8.2 Hz, 2H, Ar-H), 9.87 (s, 1H, HC=N), 14.31 (s, 1H, SH, D_2O exchangeable). **^{13}C-NMR** (100 MHz, DMSO-*d*6) 18.1, 23.4, 33.5, 60.1, 101.9, 111.8, 112.4, 113.4, 126.5, 126.7, 127.0, 128.7, 129.5, 146.5, 153.2, 153.2, 153.7, 154.4, 159.9, 160.6, 164.0**.** HRESI-MS **(m/z)**: calcd. $[M+H]^+$ for $C_{23}H_{22}N_4O_3S$: 435.1485, found: 435.1473. Yield: (77%); m.p. 215-217 ^oC.

2.2. Anti-inflammatory screening
Male adult albino rats (120–150 g) were obtained from the animal house (Faculty of Medicine, Assiut University, Egypt). Animals were housed in separate cages five animals each, in room temperature at 25 ± 2 ^oC. Animals were allowed free access to rodent chow and water and maintained at a 12 h light/dark cycle. Work was conducted in accordance with the internationally accepted principles for laboratory animals' use and care as found in the European Community Guidelines [26] and Institutional Ethical Committee Approval was obtained. The test compounds and the reference drug were suspended in 1% NaCMC in normal saline. Suspensions of the test compounds, reference drug and 1% NaCMC-saline solution (negative control) were injected i.p. (1 ml each).
The anti-inflammatory activity of the test compounds was evaluated according to the carrageenan induced paw edema method [27] in comparison to indomethacin as a reference drug. The test is based on pedal inflammation in rat paws induced by subplantar injection of carrageenan suspension (0.2 ml of 1% solution in normal saline) into the right hind paw of the rats. Male adult albino rats were divided into groups of five animals each. The rat paw thickness was measured with a Vernier caliper (SMIEC, Shangahai, China) before and 1 h after carrageenan injection to detect the carrageenan induced inflammation. The test compounds and indomethacin, at a dose of 28 µmol/kg, were injected i.p. to 18 different groups of rats 1 h after carrageenan injection. In addition, a control group received the vehicle 1% NaCMC solution in normal saline. The difference between the thicknesses of the two paws was taken as a measure of edema inhibition. The measurement was carried out at 0.5, 1, 2, 3, 4 and 5 h after injection of the test compounds, reference drug and control. The results are listed in Table I.

2.3. Analgesic activity
The analgesic activity of some selected compounds **(6a**, **8g**, **8i**, **6k** and **8l)** was determined in mice using the hot plate method [28], in comparison to indomethacin as a reference drug. In this method, the time taken by the mouse to lick its feet or to jump within a plexiglass cylinder placed on a hot plate surface (55 ^oC) was determined. This reaction time was taken as the end and the increase in hot plate latency was taken as a measure of the analgesic activity. Male adult albino mice (20–25 g) were divided into groups, each of five animals. Solutions of the test

compounds and the reference drug in (1% sodium carboxymethylcellulose (NaCMC) solution in normal saline) were injected i.p. at a dose level of 10 mg/kg into mice. Control animals were similarly treated with (1% sodium carboxymethylcellulose (NaCMC) solution in normal saline). The reaction time was evaluated directly after 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 h of injection. Results of analgesic activity of test compounds and indomethacin were displayed in Table II.

2.4. Ulcerogenic effect

The ulcerogenecity of some selected compounds with regards to their anti-inflammatory and analgesic activities **(6a**, **8d** and **8l)** was carried out on adult male albino rats [29]. Male albino rats were fasted for 24 h. The test compounds and indomethacin drug were administered orally at doses of 10, 30, and 60 mg/kg to groups of rats each of five animals. After 6 h, the animals were sacrificed; the stomachs were removed and washed with saline. Stomachs of each group and gastric lesions on the mucosa were examined grossly by naked eye or under a binocular magnifier. Stomachs were kept in 10% w/v formalin solution. After 24 h, the surface of stomachs was reexamined by naked eye and binocular magnifier. The results were cited in Table III.

2.5. Acute toxicity (LD_{50})

The median lethal dose (LD_{50}) of the relatively most active compounds **6a**, **8k** and **8l** was determined in mice [30]. Groups of male adult albino mice, each of five animals (25–30 g), were injected i.p. with graded doses of the test compounds. The percentage mortality in each group of animals was determined 24 h later to injection.

2.6. Antimicrobial screening

2.6.1. Antibacterial activity

2.6.1.1. Organisms and culture conditions

The used bacterial cultures were obtained from Assiut University Mycological Center (AUMC), Assiut University, Assiut. The antibacterial activity of compounds was determined according to the agar disc diffusion method [31].

Staphylococcus aureus (AUMC B71) was used to test the antibacterial activity of the target compounds as representatives of Gram positive strains, while the Gram negative strains were represented by *Escherichia coli* (AUMC B69).

MATERIALS AND METHODS

Cell suspension of bacterial strains was prepared from 48 h old cultures grown on nutrient agar (NA) in sterilized water [31]. One ml suspension was added to Petri dishes of 9 cm in diameter and then 15 ml of NA was poured into the plates. Plates were shaken gently to homogenize the inocula.

Sterile 5 mm filter paper disc (Whatman) was saturated with 10 µL solutions of the test compounds or ciprofloxacin as a reference drug (53 µmol-mL^{-1} in DMSO). In addition, other disks were impregnated with the solvent (DMSO) and served as a negative control. The discs were then dried for 1 h and placed in the center of each plate. The seeded plates were incubated at 35±2 °C for 24-48 h. The radii of inhibition zones (in mm) were measured in triplicate and the results are given in Table IV.

2.6. Antifungal activity

2.6.1. Organisms and culture conditions

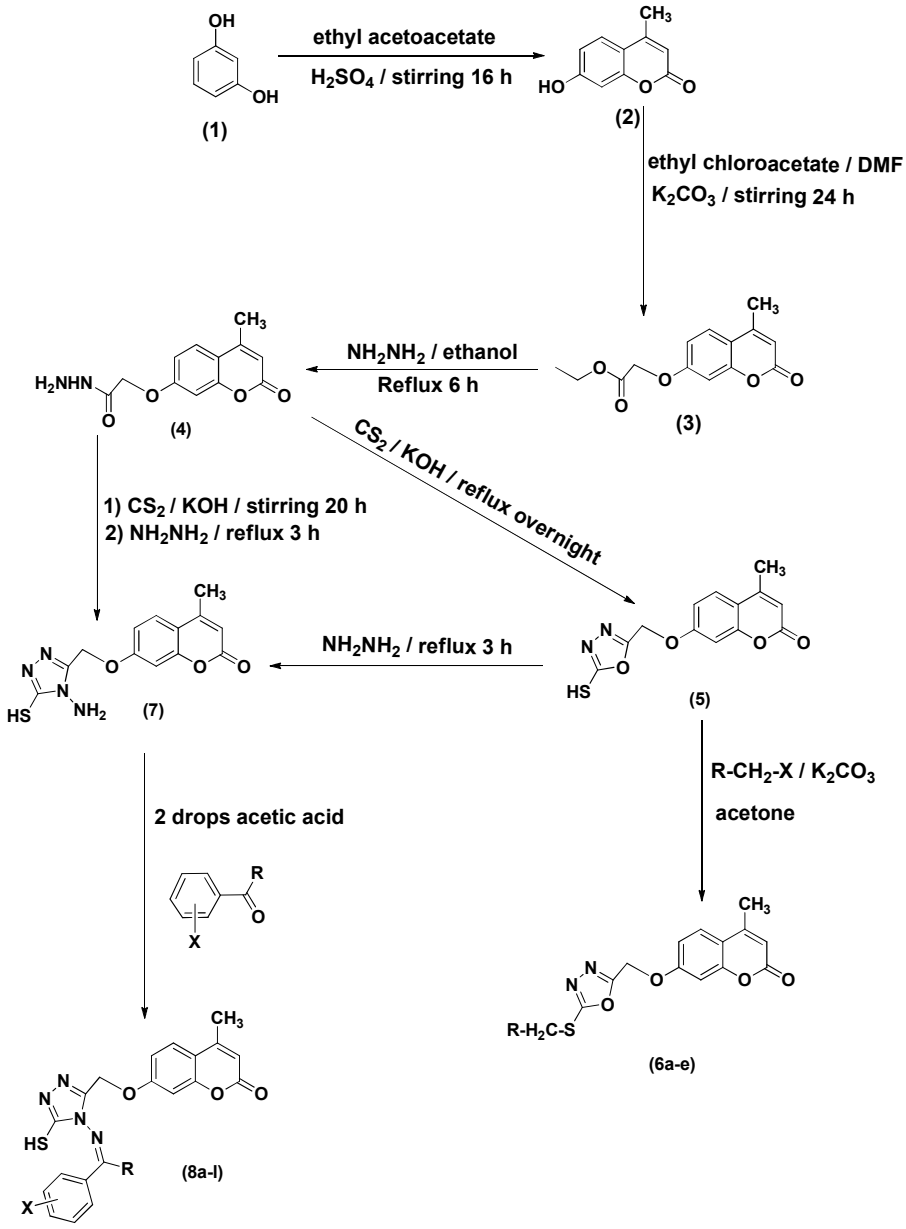
The used Sabouraud Agar (SA) media were prepared in Assiut University Mycological Center (AUMC), Assiut University, Assiut. The antifungal activity of compounds was determined according to the agar disc diffusion method [31].

Candida albicans (Robin) Berkhout AUMC 421, was used to test the antifungal activity of the target compounds.

2.6.2. Materials and method

Spore suspension in sterile distilled water was prepared from 2-5 days old culture of the test fungi growing on Sabouraud agar (SA) medium[31]. The final spore concentration was nearly 5×10^4 spores/mL. About 15 ml of growth medium was introduced on sterilized Petri dishes of 9 cm diameter and inoculated with 1 mL of spore suspension. Plates were shaken gently to homogenize the inocula. Antifungal activity of the tested compounds **7** and **8a-8j** was performed by the standard agar disk diffusion method as follows.

Sterile 5-mm filter paper disc (Whatman) was saturated with 10 µL solutions of the test compound or ketoconazole (40 µmol-mL^{-1} in DMSO). In addition, other disks were impregnated with the solvent (DMSO) and served as a negative control. The disks were then dried for 1 h and placed in the center of each plate. The seeded plates were incubated at 28±2 °C for 7 days. The radii of inhibition zones (in mm) of triplicate sets were measured at successive intervals during the incubation period and results are presented in Table IV.



Scheme 1: Synthetic route of compounds 6a-6e, 7 and 8a-8l

RESULTS AND DISCUSSION

3.1. Chemistry

The starting2-(4-methyl-2-oxo-2*H*-chromen-7-yloxy) acetohydrazide **4** was prepared by refluxing ethyl 2-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)acetate **3** with hydrazine hydrate in presence of ethanol as a solvent[24]. Structure of compound **4** was confirmed by comparison of its physical and spectral data with the reported ones [24].

The intermediate 7-((5-mercapto-1,3,4-oxadiazol-2-yl)methoxy)-4-methyl-2*H*-chromen-2-one **5** was prepared by refluxing hydrazide **4** with carbon disulphide in alcoholic potassium hydroxide solution. Structure of compound **5** was confirmed by comparison of its physical and spectral data with the reported ones [25] as illustrated in scheme 1.

Treatment of 7-((5-mercapto-1,3,4-oxadiazol-2-yl)methoxy)-4-methyl-2*H*-chromen-2-one **5** with an appropriate alkyl or aralkyl halide in presence of potassium carbonate and acetone as a solvent affords compounds **6a-e**. The structures of compounds **6a-e** were confirmed by IR, ^{1}H, and ^{13}C NMR as well as High Resolution Mass Spectroscopy.

The **IR** spectra of compounds **6a-e** showed bands at 1253, 1195 cm^{-1} for the (C-S-C) moiety and one band at 1140 cm^{-1} for (C-O-C), in addition to strong band around 1715 cm^{-1} due to carbonyl function group.

^{1}H NMR spectra of compounds **6a-e** displayed additional signals due to alkyl groups, and the signals belonging to **NH** or **SH** groups disappeared. **^{1}H NMR** of compound **6a** showed a triplet signal at δ 1.37 ppm and quartet at δ 3.25 ppm corresponding for ethyl group while compound **6c** showed a singlet signal at δ 4.25 ppm for benzyl protons in addition to signals of original nucleus.
Compound **6a** was further more confirmed by **HRESI-MS**, it showed a molecular ion peak at m/z 319.0739 $[M+H]^{+}$, which correlated with the calculated value for the formula **$C_{15}H_{14}N_2O_4S$** (319.0747 $[M+H]^{+}$).

Refluxing compound **5** with hydrazine hydrate gives the new key 7-((4-amino-5-mercapto-4*H*-1,2,4-triazol-3-yl)methoxy)-4-methyl-2*H*-chromen-2-one**7** or it may prepared directly from **4** over two stage reactions (**Scheme 1**). Structure of compound **7** was confirmed by spectral methods of analysis.

IR spectrum of compound **7** showed two bands at 3400, 3370 cm^{-1} due to (NH_2) stretching and 3220cm^{-1} for (NH) stretching, bands at 1680, 1603 $cm^{-1}$for (C=O) and (C=N) functions respectively. **^{1}H NMR** spectrum of compound **7** displayed singlet signal at δ 13.78 ppm corresponding to SH group and singlet signal at δ 4.5 ppm corresponding to NH_2, the two peak were exchangeable by D_2O, in addition to characteristic patterns of coumarin aromatic protons. The structure of **7** has been further confirmed by **HRESI-MS**, which showed a peak at m/z 305.0709 for $[M+H]^{+}$ consistent with the calculated value of its molecular formula **$C_{13}H_{12}N_4O_3S$** (305.0703).

The target compounds **8a-8l** were synthesized by condensation of compound **7** with an appropriate benzaldehydes or acetophenone **(Scheme 1)**. The structures of compounds **8a-8l** were confirmed by spectral methods of analyses. The **IR** spectra of compounds **8a-8l** displayed no absorption bands belonging to NH_2 group, instead new bands for aryl substitutions. Also **^{1}H NMR** spectra of compounds **8a-8l** confirm the disappearance of NH_2 signal, and displaying an exchangeable signal at δ 10 ppm for SH. This confirm the formation of Schiff bases expense NH_2.

^{1}H NMR spectra of compounds **8c, 8i** and **8g** showed additional singlet signal at δ 2.27 ppm corresponding to acetophenone methyl group, while **8h** shows a singlet one at δ 3.8 for *p*-methoxy protons. Also **8k** showed (6H) singlet signal at δ 3 ppm for 4-$N(CH_3)_2$ protons. Furthermore, **HRESI-MS** analysis also confirms the suggested structures of compounds **8a-8l**. Compound **8a** illustrated a peak at m/z 427.0614 $[M+H]^{+}$ consistent with the molecular formula **$C_{20}H_{15}ClN_4O_3S$,** while **8l** gave a peak at m/z 435.1473 $[M+H]^{+}$ consistent with the molecular formula **$C_{23}H_{22}N_4O_3S$** which correlated with the desired product

3.2. Anti-inflammatory activity
Results of anti-inflammatory activity for the test compounds **6a-6e, 7** and **8a-8l**, Table I, revealed that all compounds showed a gradual increase of the anti-inflammatory activity up to its maximum after 3-4 h except compounds **6d, 6e, 8i, 8k** and **8l.** Compounds **6d, 6e** and **8i** showed maximum activity after 2 h. while compounds **8k** and **8l** shows there highest results after 5 h. Compounds **6a, 7, 8a, 8f, 8k** and **8l** were the most active compounds showing 80–117 % anti-inflammatory activity of indomethacin.

Moreover, compounds **6a** and **8k** exhibited almost comparable activities to those of indomethacin, furthermore, compound **8l** was more active than indomethacin giving 117 % anti-inflammatory activity at 5 h.

Table I. Percentage of edema inhibition of compounds 6a-6e, 7, 8a-8l and indomethacin

Compound No.	Percentage of edema inhibition ± SE					
	30 min	1hr	2hr	3hr	4hr	5hr
Control	-	-	-	-	-	-
Indomethacin	22.22 ± 0.29	33.33 ± 0.22	67.39 ± 0.23	78.72 ± 0.14	78.72 ± 0.20	79.16 ± 0.22
6a	24.22 ± 0.39	25.11 ± 0.42	48.04 ± 0.80	54.04 ± 0.76	74.46 ± 0.60	47.91 ± 0.55
6b	18.88 ± 0.49	28.88 ± 0.59	48.04 ± 1.10	45.10 ± 1.20	57.44 ± 0.80	41.66 ± 0.70
6c	1.55 ± 0.69	17.77 ± 0.33	23.40 ± 0.55	17.02 ± 0.68	10.42 ± 0.74	46.87 ± 0.82
6d	14.66 ± 1.11	33.33 ± 0.37	58.69 ± 0.95	51.06 ± 0.82	42.76 ± 0.90	48.33 ± 1.30
6e	12.00 ± 1.15	15.33 ± 1.10	44.13 ± 0.93	39.78 ± 0.88	36.59 ± 0.71	35.00 ± 81
7	25.77 ± 1.14	34.44 ± 1.17	52.17 ± 1.01	63.61 ± 0.91	50.00 ± 0.44	52.91 ± 0.57
8a	30.44 ± 1.22	36.88 ± 1.32	53.47 ± 0.39	58.29 ± 0.42	63.19 ± 0.70	66.66 ± 0.50
8b	10.22 ± 1.21	16.00 ± 1.31	52.82 ± 0.83	53.61 ± 0.72	41.27 ± 0.66	55.00 ± 0.74
8c	17.11 ± 0.98	24.66 ± 0.78	43.61 ± 0.69	50.00 ± 0.75	62.98 ± 0.87	47.70 ± 1.42
8d	15.55 ± 1.21	26.66 ± 1.10	43.69 ± 1.15	55.10 ± 1.81	40.42 ± 1.32	25.00 ± 1.17
8e	14.66 ± 0.22	30.44 ± 0.29	46.30 ± 0.93	56.60 ± 0.94	36.38 ± 0.33	16.45 ± 0.27
8f	22.44 ± 1.10	28.66 ± 1.08	58.26 ± 1.00	63.19 ± 0.27	71.91 ± 0.33	67.29 ± 0.42
8g	8.88 ± 0.31	17.11 ± 0.62	39.56 ± 1.43	40.42 ± 0.88	24.47 ± 0.76	10.41 ± 0.60
8h	22.44 ± 0.92	30.44 ± 0.57	40.21 ± 1.12	42.76 ± 1.52	52.55 ± 0.38	45.62 ± 0.88
8i	9.55 ± 0.87	16.88 ± 1.41	38.91 ± 1.21	32.55 ± 1.34	32.98 ± 0.67	8.33 ± 1.00
8j	11.77 ± 0.45	24.44 ± 1.10	43.04 ± 1.14	54.90 ± 0.89	24.04 ± 1.18	20.41 ± 1.31
8k	17.77 ± 0.55	45.77 ± 0.92	56.52 ± 0.74	59.78 ± 0.97	71.48 ± 1.04	75.83 ± 0.44
8l	25.11 ± 0.60	38.88 ± 0.98	65.43 ± 0.72	74.46 ± 0.56	79.78 ± 0.86	92.50 ± 0.89

3.3. Analgesic activity

Results of analgesic activity for some selected test compounds **6a, 8g, 8i, 8k** and **8l,** table II, revealed that all compounds exhibited a gradual decreased of the analgesic activity after first half hour. Compound **8l**, is the only one which showed gradual increase in its activity up to 4 h. In addition**, 8i** was the most active one and gave maximum activity (126% comparable to indomethacin) after 30 min. Most of tested compounds exhibited moderate to good activities comparable to the reference drug.

Table II. Analgesic activity of compounds 6a, 8g, 8i, 8k, 8l and indomethacin

Compound	The average reaction time (s) at different time intervals					
	30 min	1hr	2hr	3hr	4hr	5hr
Negative control	15.60 ± 0.30	15.30 ± 0.22	16.00 ± 0.35	15.30 ± 0.24	16.60 ± 0.80	14.60 ± 0.74
Indomethacin	16.60 ± 0.27	27.00 ± 0.34	46.30 ± 0.68	64.00 ± 0.79	59.30 ± 0.30	58.30 ± 0.46
6a	56.00 ± 0.90	38.00 ± 0.82	28.00 ± 0.98	20.00 ± 0.85	15.00 ± 0.84	15.00 ± 0.49
8g	39.20 ± 1.10	30.00 ± 0.77	30.00 ± 0.38	38.00 ± 0.72	43.50 ± 0.68	40.70 ± 0.67
8i	81.20 ± 1.15	54.50 ± 0.68	39.50 ± 1.18	38.00 ± 0.57	37.00 ± 1.42	35.50 ± 0.75
8k	40.00 ± 0.88	39.00 ± 0.77	13.00 ± 1.00	12.00 ± 0.48	12.00 ± 1.20	10.00 ± 1.09
8l	14.90 ± 0.76	33.00 ± 0.65	33.00 ± 1.20	35.00 ± 0.23	35.00 ± 1.32	33.00 ± 1.22

3.4. Ulcerogenicty

The test was carried out according reported method. Compounds **6a, 8d** and **8l**were selected for this test. Results of ulcerogenic effect of three compounds **6a, 8d** and **8l** revealed that all of them showed superior safety profile when compared to indomethacin since they gave 100% protection in the population of the test animals at oral doses 10, 30 and 60 mg/kg as shown in table III.

Table III. Ulceorgenic effects of compounds 6a, 8d, 8l and indomethacin

Comp. No.	Dose mg/kg	Ratio of ulcerated animals	Ulcer index
6a	10 30 60	0/4 0/4 0/4	0.00
8d	10 30 60	0/4 0/4 0/4	0.00
8l	10 30 60	0/4 0/4 0/4	0.00
Indomethacin	10 30	3/4 4/4	1.2 mm 1.5 mm

3.5. Acute toxicity (LD_{50})

Acute toxicity (LD_{50}) study revealed that the median lethal doses (LD_{50}) of compounds **6a** and **8k** were found non toxic up to 240 mg/kg. Compound **8l** showed more safe profile up to 300 mg/kg whereas the LD50 of indomethacin equals to 13 mg/kg (i.p.) [32].

3.6. Antibacterial activity

The synthesized compounds **7** and **8a-8j** were tested for their *in vitro* antibacterial activity against *Staphylococcus aureus*as a representative of Gram positive strains and *Escherichia coli* as a Gram negative strain using ciprofloxacin as a reference drug.

The result revealed that most of the newly synthesized compounds exhibited moderated to good antibacterial activities if compared to that of ciprofloxacin against the test organisms Table IV. Compound **8d** exhibited significant antibacterial activity nearly against *E.Coli* (*MIC* = 12.5 µmol mL^{-1}), however it showed less activity against *Staphylococcus aureus* (*MIC* = 25 µmol mL^{-1}). Also, the key intermediate compound **7** showed good antibacterial activity against *E.Coli* (*MIC* = 12.5 µmol mL^{-1}), and it also revealed activity *Staphylococcus aureus* (*MIC* = 50 µmol mL^{-1}).

Table IV. Antimicrobial activity of tested compounds (expressed as the inhibition zone diameter and as MIC µM/mL).andd references drugs

Comp.	E-coli Inhibition zone (mm), MIC (µM/ml)	Staph. Aureus Inhibition zone (mm), MIC (µM/ml)	Candida Inhibition zone (mm), MIC (µM/ml)
7	(30), (**12.5**)	(20), (**50**)	(10), ---
8a	(20), (**50**)	-----	-----
8b	(18), (**50**)	-----	(20), (**25**)
8c	------	-----	(19), (**50**)
8d	(30), (**12.5**)	(20), (**25**)	(20), (**25**)
8e	(20), (**50**)	(25), (**25**)	(10), ---
8f	(11), ---	(20), (**50**)	-----
8g	(24), (**25**)	-----	-----
8h	(28), (**12.5**)	(18), (**50**)	-----
8i	(26), (**26**)	-----	(10), ---
8j	(20), (**50**)	(18), (**50**)	-----
Cipro.	(40), (**1.75**)	(40), (**1.75**)	-----
Flucon.	-----	-----	(40), (**1.85**)

3.7. Antifungal activity

The new derivatives **7** and **8a-8j** were tested for antifungal activities against *Candida albicans*using using fluconazole as a reference drug Table IV.

The results revealed that *Candida albicans* showed high resistance for most of tested group. Only compounds **8b, 8c, and 8d** showed antifungal activities (*MIC* = 25, 50, 25 μmol mL^{-1}) respectively.

CONCLUSION

A new series of coumarin derivatives linked triazoles and oxadiazole moieties were design, synthesized, and structurally elucidated using IR, ^{1}H NMR and ^{13}C NMR as well as high resolution mass analysis. All compounds were tested for their possible anti-inflammatory activities and they shows a significance results especially compound **8l** which showed higher results than Indomethacin, Some selected compounds **6a, 8g, 8i, 8k** and **8l** were tested for their analgesic properties, the results were very good especially compound **8i** which showed 126% comparable to the reference drug after only 30 min. Furthermore results of ulcerogenic effect of three compounds **6a, 8d** and **8l** regarding their anti-inflammatory and analgesic activities revealed that all of them showed superior GI safety profile. Selected compounds **7** and **8a-8j** were tested for antibacterial and antifungal activities. The results revealed that most of the newly synthesized compounds exhibited moderated to very good antibacterial activities comparable to ciprofloxacin against the test organisms especially gram-negative bacteria than the positive ones, and weak to moderate activities against fungal infection.

REFERENCES

[1] D. H. Lee, M. J. Lee, H. M. Song, B. J. Song, K. D. Seo, M. Pastore, Anselmi, C. S. Fantacci, F. De Angelis, M. K. Nazeeruddin, *Dyes and Pigments* **2011,** *91*, 192.
[2] N. Mishra, V. Patil, A. Asrondkar, A. S. Bobade, A. Chowdhary, *Journal of Applied Chemistry (IOSR-JAC)*, **2014,** *7*, 48.
[3] S. K. Deokar, *Der Chemica Sinica* **2014,** *5*, 74.
[4] D. Patel, S. Prasad, R. Kumar, S. Hemalatha, *Asian Pacific journal of tropical biomedicine* **2012,** *2*, 320.
[5] G. Borges Bubols, D. da Rocha Vianna, A. Medina-Remon, G. von Poser, R. Maria Lamuela-Raventos, V. Lucia Eifler-Lima, S. Cristina Garcia, *Mini reviews in medicinal chemistry* **2013,** *13*, 318
[6] M. Valan, A. Britto, R. Venkataraman, *Int J Chem Sci* **2010,** *8*, 1421.
[7] R. Choure, K. Pitre, *Can. J. Chem. Eng. Technol* **2010,** *1*, 7.
[8] D. Završnik, S. Muratović, D. Makuc, J. Plavec, M. Cetina, A. Nagl, E. D. Clercq, J. Balzarini, M. Mintas, *Molecules* **2011,** *16*, 6023.
[9] Y. Chen, S. Wang, X. Xu, X. Liu, M. Yu, S. Zhao, S. Liu, Y. Qiu, T. Zhang, B-F. Liu, *Journal of medicinal chemistry* **2013,** *56*, 4671.
[10] B. Nikhil, B.Shikha, P. Anil, N. B. Prakash, *Inter Res J Pharm* **2012,** *3*, 29.
[11] Y. Bansal, P. Sethi, G. Bansal, *Medicinal Chemistry Research* **2013,** *22*, 3049.
[12] C. L. Freel Meyers, M. Oberthür, H. Xu, L. eide, D. Kahne, C. T. Walsh, *Angewandte Chemie International Edition* **2004,** *43*, 67.
[13] L. Heide, *Biotechnology advances* **2009,** *27*, 1006.
[14] B. Musicki, A. M. Periers, L. Piombo, P. Laurin, M. Klich C. Dupuis-Hamelin, P. Lassaigne, A. Bonnefoy, *Tetrahedron letters* **2003,** *44*, 9259.
[15] S. S. Sahoo, S. Shukla, S. Nandy, H. B. Sahoo, *European Journal of Experimental Biology* **2012,** *2*, 899.
[16] H. N. Raundal, R. P. Jadhav, A. A. Patil, V. D. Bobade, *Journal of Chemical and Pharmaceutical Research* **2014,** *6*, 102.
[17] J. H. Jacob, F. I. Irshaid, Y.A. Al-Soud, *Advanced Studies in Biology* **2013,** *5*, 291.
[18] D. Bharathi, S. Hemalatha, G. Devadass, P. Kumar, P. Shanmugasundaram, M. Aanandhi, *Int J Chem Tech Res* **2010,** *2*, 1867.
[19] R. Kumar, M. S. Yar, B. Srivastava, A. Rai, *Der Pharma Chemica* **2014,** *6*, 137.
[20] A. Almasirad, Z. Mousavi, M. Tajik, M. J. Assarzadeh, A. Shafiee, *DARU Journal of Pharmaceutical Sciences* **2014,** *22*, 22.
[21] Y. Durusty, H. Karakus, M. Z. Yavuz, A. A. Gepdiremen, *Turkish Journal of Chemistry* **2014,** *38*, 739.

[22] A. Pankaj, D. Sanjib, A. Namita, G. Ashish, U. Baghel, *International Journal of Pharmacy & Life Sciences* **2010,** *1*, 113.
[23] B. G. M. Youssif, *bulletin of pharmaceutical sciences* **2013,** *36*, 104.
[24] D. P. Kardile, M. R. Holam, A. S. Patel, S. B. Ramani, *Rasayan J. Chem.* **2011,** *4*, 66.
[25] O. M. Abdelhafez, K. M. Amin, H. I. Ali, M. M. Abdalla, R. Z, Batran, *Journal of medicinal chemistry* **2012,** *55*, 10424.
[26] B. Tan *Naional Advisory Committee for Laboratory Animal Research* **2004**.
[27] L. Nargund, G. Reddy, V. Hariprasad, *Journal of pharmaceutical sciences* **1994,** *83*, 246.
[28] E. A. Boyle, F. R. Mangan, R. E. Markwell, S. A. Smith, M. J. Thomson, R. W. Ward, P. A. Wyman, *Journal of medicinal chemistry* **1986,** *29*, 894.
[29] H. Ikuta, H. Shirota, S. Kobayashi, Y. Yamagishi, K. Yamada, I. Yamatsu, K. Katayama, *Journal of medicinal chemistry* **1987,** *30*, 1995.
[30] F. Sztaricskai, I. E. Takács, F. Pusztai, G. Szabó, I. Csípõ, *Archiv der Pharmazie* **1999,** *332*, 321.
[31] H. William, Microbiological Assay, An Introduction to Quantitative Principles and Evaluation. *Academic Press, New York*, **1977**, 1-86.
[32] A. F. Mohammed, S. G. Abdel-Moty, M. A. Hussein, A. M. Abdel-Alim *Arch. Pharm. Res* **2013,** *36*, 1465.