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RESEARCH ARTICLE

Synthesis and Characterization of Novel Oxadiazole and Pyrazole Hybrids as Potential Antimicrobial Agents

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Abstract: As a part of our ongoing research in the exploration of novel antimicrobial agents, we report herein novel synthesis of hybrid molecules 4-methyl-7-(2-oxo-2-(5-(substitutedthio)-1,3,4-oxadiazol-2-yl)ethoxy)-2*H*-chromen-2-ones and 4-substitutedbenzylidene-3-methyl-1-(2-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)acetyl)-1*H*-pyrazol-5(4*H*)-ones by combining coumarin ring with 1,3,4-oxadiazole and pyrazole scaffolds respectively. All the synthesized compounds have been characterized by chemical analysis, IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy. All the compounds have been evaluated for their *in vitro* antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and fungi *Aspergillus niger* using serial broth dilution method. All the synthesized compounds displayed antimicrobial activity. Among all the compounds **7c** and **7d** have been emerged as highly potent molecules with 75 µg/mL potency.

Keywords: Oxadiazoles, Pyrazoles, Knoevenegel condensation, Coumarin derivatives, Antimicrobial activity

Introduction

Over the years, a wide range of antimicrobial agents have been developed which prolonged the lifespan and eased the affliction of million peoples. But the evolution of antibiotic resistance strains is of principally severe concern due to the biochemical fickleness of several bacteria and the over use of many of these antibiotics. Multidrug resistant bacteria have become a major public health crisis because existing antibiotics are no longer effective in many cases. Considering the rapid advance of multidrug resistance to the existing variety of marketed antibiotics, new approaches are of an immediate need.

It was observed from the literature that certain five membered heterocyclic compounds possess interesting biological activity. Among them the compounds bearing 1,3,4-oxadiazole and pyrazole nucleus have wide applications in medicinal chemistry. A number of oxadiazole derivatives were reported to possess varied biological activities such as anti-

inflammatory[1], antibacterial[2,3], fungicidal[4,5], analgesic, muscle relaxant and tranquilising[6] properties. In the last 30 years pyrazole ring has attracted much attention as it has become fairly accessible and shows diverse properties. Pyrazoles and several *N*-substituted pyrazoles are known to possess numerous chemical, biological and medicinal applications because of their versatile biological activities such as antitumour[7], antileukemia[8], antidepressant[9,10] and antitubercular[11]. A typical model of the pyrazole containing diaryl-heterocyclic template that is known to selectively inhibit cyclooxygenase enzyme COX-2[12], Celecoxib is a safe anti-imflammatory and analgesic agent.

Coumarins owe their class name to 'Coumarous', the vernacular name of the tonka bean (*Dipteryxodorata* Willd., Fabaceae), from which coumarin itself was isolated in 1820[13]. Coumarins are found at high levels in some essential oils, particularly cinnamon bark oil, cassia leaf oil and lavender oil. The coumarins are of great interest due to their biological properties. In particular, their physiological, bacteriostatic and antitumour activity makes these compounds attractive for further backbone derivatization and screening as novel therapeutic agents. Both coumarin and coumarin derivatives have shown promise as potential inhibitors of cellular proliferation in various carcinoma cell lines[14-17]. Coumarin and its derivatives are biologically active[18] compounds and widely occur in nature. The coumarin heterocyclic ring is a common feature of various bioactive compounds such as calanolides[19] and lipid lowering agents[20]. Recent studies have revealed that coumarin and its derivatives exhibit several other medicinal applications[21] such as anticoagulants, antifungal, insecticidal, anthelminths, hypnotics, photoalexins, HIV protease inhibitors and AChE inhibitors[22].

Fascinated by the varied biological activity of coumarin, 1,3,4-oxadiazole and pyrazole derivatives it was contemplated to synthesize a new series of 1,3,4-oxadiazoles and pyrazoles carrying coumarin scaffold with a view to kill multidrug resistant bacteria. In our earlier paper we have reported[23] the synthesis and biological evaluation of 2-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)acetohydrazide, 7-((5-mercapto-1,3,4-oxadiazol-2-yl)methoxy)4-methyl-2*H*-chromen-2-one and 3-methyl-1-(2-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy) acetyl)-1*H*-pyrazol-5(4*H*)-one. As an extension to this we report herein the synthesis and biological evaluation of 4-methyl-7-(2-oxo-2-(5-(substitutedthio)-1,3,4-oxadiazol-2-yl) ethoxy)-2*H*-chromen-2-ones and 4-(substitutedbenzylidene)-3-methyl-1-(2-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)acetyl)-1*H*-pyrazol-5(4*H*)-ones.

Experimental

All chemicals and reagents were procured from Merck India limited. All reactions except those of aqueous media were carried out by standard techniques with the exclusion of moisture. Melting points were determined in open capillaries on a Mel-Temp apparatus and are uncorrected. The homogeneity of the compounds was checked by TLC (silica gel H, BDH, ethyl acetate-hexane, 3:5). Column chromatography over silica gel (Merck, 70- 230 and 230- 400 mesh ASTH for flash chromatography) was applied when necessary to isolate and purity the reaction products. The IR spectra were recorded on Perkin-Elmer spectrum 100 FT-IR spectrometer as KBr pellets. The wave numbers are given in cm^{-1}. The ^1H NMR spectra were recorded in $CDCl_3$/DMSO-d_6 on a jeol JNM λ-400 MHz machine. The ^{13}C NMR spectra were recorded in $CDCl_3$/DMSO-d_6 on a Jeol JNM spectrometer operating at 100 MHz. All chemical shifts are reported in δ (ppm) using TMS as an internal standard. The mass spectra were recorded on VG 7070H mass spectrometer. The micro analyses were performed on Perkin-Elmer 240 C elemental analyzer.

Synthetic methods and spectroscopic details

A solution of equimolar quantities of resorcinol and ethyl acetoacetate was added drop wise to concentrated sulfuric acid and allowed to stand for 2 h. Then the reaction mixture was poured into a mixture of ice and water with continuous stirring and then neutralized with 40% sodium hydroxide solution. The precipitate formed was filtered, washed with water and recrystallized from ethanol to give **7**-hydroxy-4-methyl-2*H*-chromen-2-one **1**. Equimolar quantities of **7**-hydroxy-4-methyl-2*H*-chromen-2-one **1** and ethyl chloroacetate were allowed to react in presence of potassium carbonate for 8 h in dimethylformamide medium to get ethyl 2-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)acetate **2**. Compound **2** was further refluxed with hydrazine hydrate in methanol for 8 h to furnish 2-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)acetohydrazide **3**.

*2-((4-Methyl-2-oxo-2H-chromen-7-yl)oxy)acetohydrazide, (**3**)*

Yield 76%; m.p.:192-195 ºC; IR (KBr, cm^{-1}) : 3332 (N-H stretching), 3083, 3060 (Aromatic C-H stretching), 2980, 2908 (C-H stretching in CH_3/CH_2), 1730 (C=O stretching coumarin), 1676 (C=O stretching hydrazide), 1284 (sp^2 C-O stretching), 1074 (sp^3 C-O stretching); LCMS: *m/z* 249.08 [M+H] (248.08). Anal. Calcd for $C_{12}H_{12}N_2O_4$: C, 58.06; H, 4.87; N, 11.29. Found C, 57.96; H, 4.79; N, 11.21%.

*Synthesis of 7-((5-mercapto-1,3,4-oxadiazol-2-yl)methoxy)-4-methyl-2H-chromen-2-one **(4)***

A mixture of 0.01 mol of 2-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)acetohydrazide **3** and 0.01 mol (0.56 g) of KOH and 10 mL of carbon disulphide were refluxed in 50 mL of 95% ethanol for 12 h. The resultant mixture was concentrated and cooled to room temperature. Then it was acidified with dil. HCl. The solid mass thus separated out was filtered, dried and purified by recrystallized from ethanol.

Yield 80%; m. p.:198-200 ºC; IR (KBr, cm^{-1}): 3075 (C-H stretching in aromatics), 2925 (C-H stretching in CH_3/CH_2), 2766 (S-H stretching in thiols), 1678 (C=O stretching coumarin), 1606 (C=N stretching), 1279, 1076 (sp^2/sp^3 C-O stretching). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.41 (s, 3H, coumarin-CH_3), 3.32 (broad, 1H, SH), 5.22 (s, 2H, CH_2), 6.18 (s, 1H, H3), 7.68 (d, 1H, H5), 7.61 (dd, 1H, H6), 7.01 (d, 1H, H8). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 18.6 (coumarin-CH_3), 161.2 (C-2), 112.6 (C-3), 154.8 (C-4), 125.7 (C-5), 112.6 (C-6), 160.5 (C-7), 102.1 (C-8), 152.4 (C-9), 113.8 (C-10) (coumarin ring), 65.8 (OCH_2), 165.3, 164.9 (oxadiazole ring); MS *m/z*: 290 [M^+] (290). Anal. Calcd for $C_{13}H_{10}N_2O_4S$: C, 53.79; H, 3.47; N, 9.65. Found: C, 52.75; H, 3.35; N, 9.49 %.

*Synthesis of 4-methyl-7-(2-oxo-2-(5-(yridine-4-ylthio)-1,3,4-oxadiazol-2-yl)ethoxy)-2H-chromen-2-one **(5a)***

Equimolar quantities of 7-((5-mercapto-1,3,4-oxadiazol-2-yl)methoxy)-4-methyl-2*H*-chromen-2-one **4** and 4-chloropyridine were refluxed in 95% ethanol for 2 h. The reaction mixture was monitored by TLC until the disappearance of starting materials. The resultant solution was concentrated under reduced pressure. The product was dissolved in ethyl acetate and the organic phase was washed successively with 5% HCl, 5% Na_2CO_3 solution, water (2x40 mL) and the organic layer was collected, washed with brain solution, dried over Anhy. Na_2SO_4 and ethyl acetate decanted off. The ethyl acetate was then concentrated under reduced pressure. The solid mass separated out was collected, dried and recrystallized from ethanol to obtain **5a**. Compounds **5b-d** were similarly obtained, substituting 4-chloropyridine with different chloro compounds.

4-Methyl-7-(2-oxo-2-(5-(pyridin-4-ylthio)-1,3,4-oxadiazol-2-yl)ethoxy)-2H-chromen-2-one ***(5a)***

Yield 72%; m.p.:244-246 ºC; IR (KBr, cm^{-1}): 3070 (C-H stretching in aromatics), 2918 (C-H stretching in CH_3/CH_2), 1765 (C=O stretching coumarin), 1616 (C=N stretching), 1275, 1081 (sp^2/sp^3 C-O stretching). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.40 (s, 3H, coumarin- CH_3), 5.16 (s, 2H, -OCH_2), 6.20 (s, 1H, coumarin H3), 7.70 (d, 1H, coumarinH5), 7.63 (d, 1H, coumarinH6), 6.82 (d, 1H, coumarinH8), 7.83, 7.85 (d, 2H, pyridine), 8.80, 8.82 (d, 2H, pyridine) ppm.^{13}C NMR (100 MHz, DMSO- d_6): δ 18.5(coumarin CH_3), 161.4 (C-2), 112.4 (C-3), 153.8 (C-4), 125.4 (C-5), 112.8 (C-6), 160.4 (C-7), 101.9 (C-8), 152.5 (C-9), 113.6 (C-10) (coumarin ring), 65.1 (OCH_2), 165.4, 165.9 (oxadiazole ring), 141.3, 124.9 (2), 151.2 (2) (aromatic ring); LCMS: *m/z* 368.06 [M+H] (367.06). Anal.Calcd for $C_{18}H_{13}N_3O_4S$: C, 58.85; H, 3.57; N, 11.44. Found C, 58.64; H, 3.46; N, 11.35%.

4-Methyl-7-(2-oxo-2-(5-(pyridin-2-ylthio)-1, 3, 4-oxadiazol-2-yl)ethoxy)-2H-chromen-2-one ***(5b)***

Yield 70%, m.p.:255-257 ^{o}C; IR (KBr, cm^{-1}): 3071 (C-H stretching in aromatics), 2930 (C-H stretching in CH_3/CH_2), 1685 (C=O stretching coumarin), 1602 (C=N stretching), 1268, 1072 (sp^2/sp^3 C-O stretching); ^{1}H NMR (400 MHz, DMSO- d_6): δ 2.42 (s, 3H, coumarin-CH_3), 5.24 (s, 2H, -OCH_2), 6.18 (s, 1H, comarinH3), 7.66 (d, 1H, comarin H5), 7.58 (d, 1H, comarinH6), 6.85 (s, 1H, comarin H8), 8.32 (d, 1H, pyridine-H), 7.20 (t, 1H, pyridine-H), 7.64 (t, 1H, pyridine-H), 7.35 (d, 1H, pyridine-H); ^{13}C NMR (100MHz, DMSO- d_6): δ 18.6 (coumarin CH_3), 161.0 (C-2), 112.7 (C-3), 154.2 (C-4), 125.4 (C-5), 112.3 (C-6), 160.6 (C-7), 102.4 (C-8), 151.5 (C-9), 113.5 (C-10) (coumarin ring), 65.3 (OCH_2), 164.6, 165.3 (oxadiazole ring), 153.2, 149.3, 122.5, 136.6, 121.4 (aromatic ring); MS: *m/z* 368.08, [M+H] (367.06); Anal. Calcd for $C_{18}H_{13}N_3O_4S$: C, 58.85; H, 3.57; N, 11.44; Found C, 58.62; 3.46; 11.29%.

7-(2-(5-((4-Aminophenyl)thio)-1,3,4-oxadiazol-2-yl)-2-oxoethoxy)-4-methyl-2H-chromen-2-one ***(5c)***

Yield 71%, m.p.:268-269 ºC; IR (KBr, cm^{-1}): 3350 (N-H stretching), 3065 (C-H stretching in aromatics), 2920 (C-H stretching in CH_3/CH_2), 1685 (C=O stretching coumarin), 1605 (C=N stretching), 1260, 1075 (sp^2/sp^3 C-O stretching); ^{1}H NMR (400 MHz, DMSO- d_6): δ 2.43 (s, 3H, coumarin-CH_3), 5.18 (s, 2H, -OCH_2), 6.25 (s, 2H, Ar- NH_2), 6.20 (s, 1H, coumarin-H3), 7.65 (d, 1H, coumarin-H5), 7.56 (d, 1H, coumarin-H6), 6.85 (s, 1H, coumarin-H8), 6.55, 6.57 (d,2H, Ar-H), 7.09, 7.11 (d, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO- d_6): δ 18.6 (coumarin CH_3), 161.1 (C-2), 112.8 (C-3), 154.2 (C-4), 125.8 (C-5), 112.4 (C-6), 160.2 (C-7), 102.4 (C-8), 152.4 (C-9), 113.2 (C-10) (coumarin ring), 65.7 (OCH_2), 164.5,165.5 (oxadiazole ring), 126.9, 128.2(2), 115.8(2), 143.9 (aromatic ring); MS: *m/z* 382.07 [M+H] (381.07). Anal. Calcd for $C_{19}H_{15}N_3O_4S$: C, 59.83; H, 3.96; N, 11.02; Found C, 59.68; H, 3.84, N, 10.92%.

*7-(2-(5-((2-Aminophenyl)thio)-1,3,4-oxadiazol-2-yl)-2-oxoethoxy)-4-methyl-2H-chromen-2-one(****5d****)*

Yield 71%, m.p.: 276-278 ºC; IR (KBr, cm^{-1}): 3355 (N-H stretching), 3075 (C-H stretching in aromatics), 2922 (C-H stretching in CH_3/CH_2), 1680 (C=O stretching coumarin), 1604 (C=N stretching), 1270, 1072 (sp^2/sp^3 C-O stretching); ^{1}H NMR (400 MHz, DMSO- d_6): δ 2.40 (s, 3H, Coumarin-CH_3), 5.21 (s, 2H, -OCH_2), 6.20 (s, 2H, NH_2), 6.16 (s, 1H, coumarin-H3), 7.69 (d, 1H, coumarin-H5), 7.60 (d, 1H, coumarin-H6), 6.80 (s, 1H, coumarin-H8), 6.52

(d, 1H, Ar-H), 6.71 (t, 1H, Ar-H), 7.05 (t, 1H, Ar-H), 7.25 (d, 1H, Ar-H); ^{13}C NMR (100 MHz, DMSO- d_6): δ 18.4 (coumarin CH_3), 161.5 (C-2), 112.4 (C-3), 154.6 (C-4), 125.1 (C-5), 112.6 (C-6), 160.4 (C-7), 102.2 (C-8), 152.1 (C-9), 113.9 (C-10) (coumarin ring), 65.4 (OCH_2), 164.8, 165.6 (oxadiazole ring), 118.6, 145.1, 115.6, 128.5, 124.8, 128.7 (aromatic ring); MS: *m/z* 382.02 [M+H] (381.07). Anal. Calcd for $C_{19}H_{15}N_3O_4S$: C, 59.83; H, 3.96; N, 11.02. Found: C, 59.68; H, 3.82; N, 10.89%.

*Synthesis of 3-methyl-1-(2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetyl)-1H-pyrazol-5(4H)-one(**6**)*

A mixture of ethyl acetoacetate (0.01 mol) and 2-((4-methyl-2-oxo-2*H*-chromen-7-yl) oxy)acetohydrazide (3) (0.02 mol) in ethanol (20 mL) was heated under reflux for 8 h on a water bath. After completion of the reaction, ethanol was evaporated. The residue was dissolved in water, neutralized with $NaHCO_3$ and extracted with ether. Then the ether solution was evaporated under reduced pressure to furnish the pure compound. It was recrystallized from ethanol.

Yield75%; m.p.**:**304-308 ºC; IR (KBr, cm^{-1}): 3055 (C-H stretching in aromatics), 2977, 2815 (C-H stretching in CH_3/CH_2), 1712 (C=O stretching coumarin), 1627 (C=O stretching pyrazolin-5-one ring), 1604 (C=N stretching amide), 1218, 1064 (sp^2/sp^3 C-O stretching). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.39 (s, 3H, pyrazolin-5-one CH_3), 2.49 (s, 3H, coumarin-CH_3), 3.31 (s, 2H, CH_2 pyrazolin-5-one ring), 4.76 (s, 2H, -OCH_2), 6.23 (s,1H, coumarin-H3), 7.71 (d, 1H, coumarin-H5), 7.02 (dd, 1H, coumarin-H6),6.99 (d, 1H, coumarin-H8); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 18.5 (coumarin CH_3), 27.3 (pyrazolin-5-one CH_3), 161.0 (C-2), 113.0(C-3), 154.9(C-4), 126.9(C-5), 111.9(C-6), 160.4(C-7), 102.1(C-8), 153.8(C-9), 114.1(C-10) (coumarin ring), 66.6 (OCH_2), 159.8 (C=O amide), 41.8, 161.4, 166.6 (pyraoline-5-one ring); MS: *m/z*: 314 [M^+] (314). Anal. Calcd for $C_{16}H_{14}N_2O_5$: C 61.14, H 4.49, N 8.91. Found: C, 60.52; H, 4.30; N, 8.62%.

*Synthesis of 4-benzylidene-3-methyl-1-(2-((4-methyl-2-oxo-2H-chromen-7-yl) oxy) acetyl)-1H-pyrazol-5(4H)-one(**7a**)*

3-Methyl-1-(2-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)acetyl)-1*H*-pyrazol-5(4*H*)-one **6** (0.01 mol) and benzaldehyde (0.01 mol) suspended in dry toluene were taken in a flask equipped with a Dean-Stark apparatus fitted with a calcium chloride guard tube. Then catalytic amount of piperidine (0.5 mL) was added and the reaction mixture was refluxed with stirring for about 8 h. The progress of the reaction was monitored by TLC until the disappearance of starting materials. The product precipitated on cooling was washed with methanol and purified by recrystallization from a mixture of ethanol and chloroform (1:1). Compounds **7b-e** were prepared similarly, taking appropriate substituted aldehyde in place of benzaldehyde.

*4-Benzylidene-3-methyl-1-(2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetyl)-1H-pyrazol-5(4H)-one (**7a**)*

Yield 63%; m.p.: 226-228 ºC; IR (KBr, cm^{-1}): 3060 (C-H stretching in aromatics), 2960 (C-H stretching in CH_3/CH_2), 1729 (C=O stretching coumarin), 1668 (C=O stretching pyrazolin-5-one ring), 1624 (C=N stretching amide), 1203, 1075 (sp^2/sp^3 C-O stretching); ^{1}H NMR (400 MHz, DMSO- d_6): δ 2.38 (s, 3H, pyrazolin-5-one CH_3), 2.46 (s, 3H, coumarin- CH_3), 4.72 (s, 2H, -OCH_2), 6.80 (s, 1H, =CH), 6.21 (s, 1H, coumarin H3), 7.71 (d, 1H, coumarin H5), 7.06 (d, H, coumarin H6), 7.03 (s, 1H, coumarin H8), 7.72 (d, 2H, Ar- H), 7.59 (d, 2H, Ar- H), 7.45 (t, 1H, Ar- H); ^{13}C NMR (100 MHz, DMSO- d_6): δ 18.9

(coumarin CH_3), 27.6 (pyrazolin-5-one CH_3), 161.3 (C-2), 113.4 (C-3), 155.4 (C-4), 127.1 (C-5), 112.8 (C-6), 160.8 (C-7), 102.5 (C-8), 153.4 (C-9), 114.5 (C-10) (coumarin ring), 66.2 (OCH_2), 165.7 (C=O amide), 159.2, 137.5, 163.0 (pyrazolin-5-one ring), 154.1 (=CH), 134.6, 129.2 (2), 129.3 (2), 128.3 (aromatic ring); LCMS: *m/z* 403.42 [M+H] (402.12). Anal. Calcd for $C_{23}H_{18}N_2O_5$: C, 68.65; H, 4.51; N, 6.96. Found: C, 68.12; H, 4.38; N, 6.72%.

*4-(4-Chlorobenzylidene)-3-methyl-1-(2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy) acetyl)-1H-pyrazol-5(4H)-one(**7b**)*

Yield 68%; m.p.: 246-248 ºC; IR (KBr cm^{-1}): 3052 (C-H stretching in aromatics), 2930 (C-H stretching in CH_3/CH_2), 1715 (C=O stretching coumarin), 1625 (C=O stretching pyrazolin-5-one ring), 1603 (C=N stretching amide), 1220, 1060 (sp^2/sp^3 C-O stretching); ^{1}H NMR (400 MHz, DMSO- d_6): δ 2.36 (s, 3H, pyrazolin-5-one CH_3), 2.40 (s, 3H, coumarin-CH_3), 4.68 (s, 2H, -OCH_2), 6.86 (s, 1H, =CH), 6.20 (s, 1H, coumarin H3), 7.69 (d, 1H,coumarin H5), 7.06 (d, 1H, coumarin H6), 7.02 (s, 1H, coumarin H8), 7.54, 7.57 (d, 2H, Ar-H), 7.78, 7.81(d, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO- d_6): δ 18.5 (coumarin CH_3), 27.3 (pyrazolin-5-one CH_3), 161.7 (C-2), 113.8 (C-3), 154.6 (C-4), 126.7 (C-5), 113.2 (C-6), 161.4 (C-7), 102.8 (C-8), 155.2 (C-9), 114.1 (C-10) (coumarin ring), 66.4 (OCH_2), 165.8 (C=O amide), 158.6, 136.8, 162.6 (pyrazolin-5-one ring), 156.3 (=CH), 131.2, 136.5 (2), 129.2 (2) 133.7 (aromatic ring); LSMS: *m/z* 437.12 [M+H] (436.08). Anal. Calcd for $C_{23}H_{17}ClN_2O_5$: C, 63.24; H, 3.92; N, 6.41. Found: C, 62.69; H, 3.70; N, 6.28%.

*4-(4-Hydroxybenzylidene)-3-methyl-1-(2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetyl)-1H-pyrazol-5(4H)-one(**7c**)*

Yield 62%; m.p.: 251-253 ºC; IR (KBr cm^{-1}): 3436 (O-H stretching), 3045 (C-H stretching in aromatics), 2920 (C-H stretching in CH_3/CH_2), 1715 (C=O stretching coumarin), 1668 (C=O stretching pyrazolin-5-one ring), 1624 (C=N stretching amide), 1267, 1081 (sp^2/sp^3 C-O stretching); ^{1}H NMR (400 MHz, DMSO- d_6): δ 2.32 (s, 3H, pyrazolin-5-one CH_3), 2.41 (s, 3H, coumarin-CH_3), 4.65(s, 2H, -OCH_2), 5.81(s, 1H, OH), 6.82 (s, 1H, =CH), 6.16 (s, 1H, coumarin H3),7. 66 (d, 1H, coumarin H5), 7.08 (d, 1H, coumarin H6), 7.04 (s, 1H, coumarin H8), 6.96, 6.98 (d, 2H, Ar-H), 7.78, 7.80 (d, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO- d_6): δ 18.8 (coumarin CH_3), 27.3 (pyrazolin-5-one CH_3), 161.2 (C-2), 113.5 (C-3), 155.0 (C-4), 127.4 (C-5), 112.6 (C-6), 160.5 (C-7), 102.8 (C-8), 152.6 (C-9), 114.2 (C-10) (coumarin ring), 66.4 (OCH_2), 165.2 (C=O amide), 158.8, 136.9, 162.2 (pyrazolin-5-one ring), 153.6 (=CH), 126.4, 128.8(2), 115.4(2), 160.8 (aromatic ring); LCMS: *m/z* 419.42 [M+H] (418.11). Anal. Calcd for $C_{23}H_{18}N_2O_6$: C, 66.02; H, 4.34; N, 6.70. Found: C, 65.82; H, 4.21; N, 6.54%.

*4-(2-Hydroxybenzylidene)-3-methyl-1-(2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy) acetyl)-1H-pyrazol-5(4H)-one(**7d**)*

Yield 65%; m.p.: 236-238 ºC; IR (KBr cm^{-1}): 3430 (O-H stretching), 3050 (C-H stretching in aromatics), 2972, 2860 (C-H stretching in CH_3/CH_2), 1714 (C=O stretching coumarin), 1628 (C=O stretching pyrazolin-5-one ring), 1603 (C=N stretching amide), 1220, 1062 (sp^2/sp^3 C-O stretching); ^{1}H NMR (400 MHz, DMSO- d_6): δ 2.35 (s, 3H, pyrazolin-5-one CH_3), 2.43 (s, 3H, coumarin- CH_3), 4.70 (s, 2H, -OCH_2), 5.75 (s, 1H, OH), 6.85 (s, 1H, =CH), 6.18 (s, 1H, coumarin H3),7.70 (d, 1H, coumarin H5), 7.04 (d, 1H, coumarin H6), 7.01 (s, 1H, coumarin H8), 6.92 (d, 1H, Ar-H), 7.01 (t, 1H, Ar-H), 7.48 (t, 1H,Ar-H), 7.55 (d, 1H, Ar-H); ^{13}C NMR (100 MHz, DMSO- d_6): δ 18.5 (coumarin CH_3), 27.2 (pyrazolin-5-one CH_3), 161.6 (C-2), 113.6 (C-3), 155.2 (C-4), 127.6 (C-5), 113.4 (C-6), 160.5 (C-7), 102.4

(C-8), 153.8 (C-9), 114.6 (C-10) (coumarin ring), 66.2 (OCH_2), 165.2 (C=O amide), 158.6, 136.9, 163.5 (pyrazolin-5-one ring), 153.7 (=CH), 120.5, 160.8, 117.2, 133.5, 121.3, 136.8 (aromatic ring); LCMS: *m/z* 419.42 [M+H] (418.11). Anal. Calcd for $C_{23}H_{18}N_2O_6$: C, 66.02; H, 4.34; N, 6.70. Found: C, 65.63; H, 4.19; N, 6.52%.

*4-(2-Methoxybenzylidene)-3-methyl-1-(2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy) acetyl)-1H-pyrazol-5(4H)-one(**7e**)*

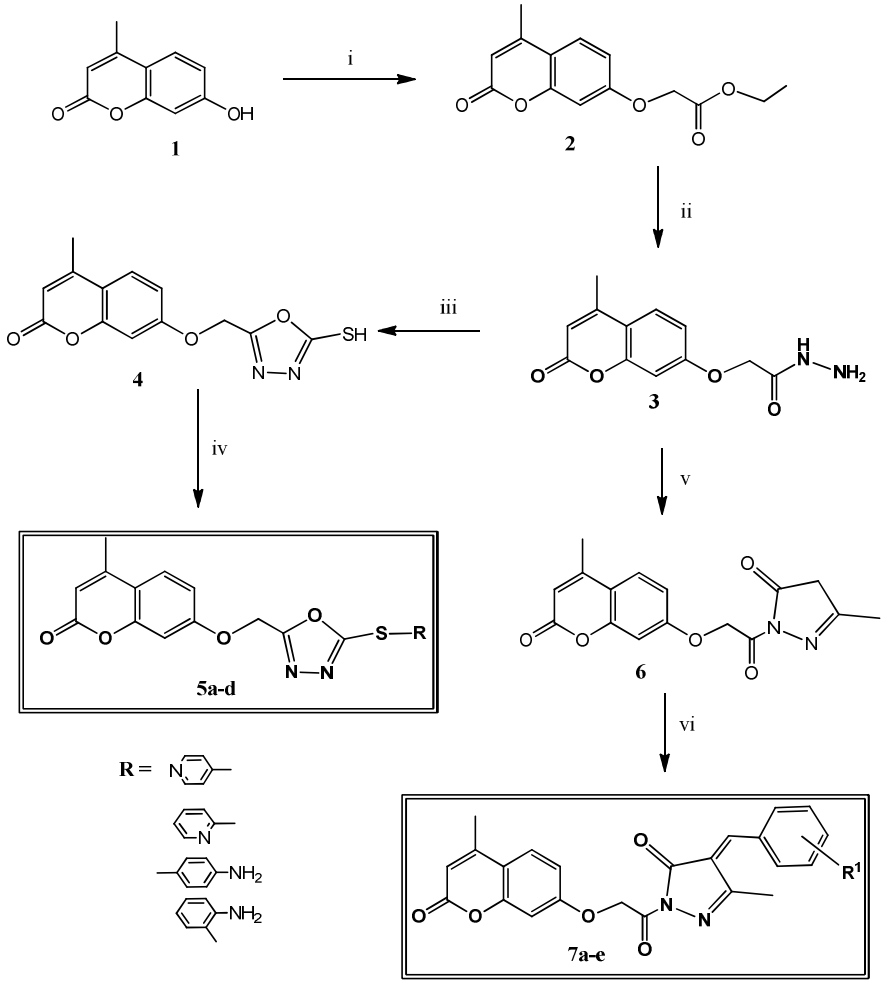
Yield 66%; m.p.: 241-243 ºC; IR (KBr cm^{-1}): 3052 (C-H stretching in aromatics), 2965, 2845 (C-H stretching in CH_3/CH_2), 1715 (C=O stretching coumarin), 1628 (C=O stretching pyrazolin-5-one ring), 1602 (C=N stretching amide), 1216, 1053 (sp^2/sp^3 C-O stretching); ^{1}H NMR (400 MHz, DMSO- d_6): δ 2.36 (s, 3H, pyrazolin-5-one CH_3), 2.40 (s, 3H, coumarin-CH_3), 3.76 (s, 3H, Ar-OCH_3), 4.72 (s, 2H, -OCH_2), 6.82 (s, 1H, =CH), 6.17 (s, 1H, coumarin H3), 7.66 (d, 1H, coumarin H5), 7.12 (d, 1H, coumarin H6), 7.08 (s, 1H, coumarin H8), 7.14 (d, 1H, Ar-H), 7.18 (t, 1H, Ar-H), 7.36 (t,1H, Ar-H), 7.52(d, 1H, Ar-H); ^{13}C NMR (100 MHz, DMSO- d_6): δ 18.8 (coumarin CH_3), 27.4 (pyrazolin-5-one CH_3), 55.4 (OCH_3), 161.6 (C-2), 113.3 (C-3), 154.8 (C-4), 127.4 (C-5), 112.6 (C-6), 160.2 (C-7), 102.4 (C-8), 155.6 (C-9), 114.2 (C-10) (coumarin ring), 66.5 (OCH_2), 165.4 (C=O amide), 159.0, 137.2, 162.5 (pyrazolin-5-one ring), 153.4 (=CH), 122.6, 160.4, 112.1, 130.2, 120.8, 136.8 (aromatic ring); LCMS: *m/z* 433.42 [M+H] (432.13). Anal. Calcd for $C_{24}H_{20}N_2O_6$: C, 66.66; H, 4.66; N, 6.48. Found: C, 66.12; H, 4.47; N, 6.29%.

Results and Discussion

The synthetic strategies adopted to obtain the target compounds are depicted in Scheme 1. One of the precursors 7-((5-mercapto-1,3,4-oxadiazol-2-yl)methoxy)4-methyl-2*H*-chromen-2-one, **4** was obtained by adopting a simple one pot procedure that involves reacting 2-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)acetohydrazide **3** with carbon disulfide under strong basic conditions followed by acidification with dilute hydrochloric acid. The thiol group was further suitably condensed with different aromatic halo compounds to give the corresponding oxadiazole derivatives 4-methyl-7-(2-oxo-2-(5-(substitutedthio)-1,3,4-oxadiazol-2-yl)ethoxy)-2*H*-chromen-2-ones **5a-d**. Another precursor 3-methyl-1-(2-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)acetyl)-1*H*-pyrazol-5(4*H*)-one **6** was obtained by condensing 2-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)acetohydrazide **3** with ethyl acetoacetate in ethanol and refluxing for 8 h. The next step involves Knoevenegel condensation of compounds **6** containing active methylene group with various substituted aromatic aldehydes in presence of catalytic amount of piperidine to yield 4-substitutedbenzylidene-3-methyl-1-(2-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)acetyl)-1*H*-pyrazol-5(4*H*)-one **7a-e**.

All the synthesized compounds were characterized by chemical analysis, IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy. Compound **3** showed a characteristic absorption band at 3332 cm^{-1} corresponding to NH stretching and the mass spectrum showed a peak at *m/z* 249.08 in LCMS due to [M+H] ion corresponding to molecular formula $C_{12}H_{12}N_2O_4$. Compound **4** showed a characteristic absorption band at 2766 cm^{-1} corresponding to SH stretching in thiols. The proton NMR spectrum of the compound showed a broad signal at δ 3.32 characteristic of SH proton along with signals for other protons. The absence of these characteristic signal of SH group in compounds **5a-d** confirm the successful condensation of compound **4** with aromactic halogeno compounds to furnish the desired target molecules. The proton NMR spectrum of compound **6** showed a signal δ 3.31 corrresponding to the methylene proton of heterocyclic ring. The disappearance of this characteristic signal of

methylene group of compound **6** and the appearance of new signal at around δ 6.80 corresponding to the methine proton of Knovenegel adducts along with other characteristic peaks confirms the successful formation of the adducts **7a-e**.



R^{1}=7a -H, 7b 4-Cl, 7c 4-OH, 7d 2-OH, 7e 2-OCH_3

i) Ethyl chloroacetate, Anhy, K_2CO_3, DME ii) Hydrazine hydrate, ethanol iii) CS_2, KOH, iv) Ethanol v) Ethyl acetoacetate, ethanol vi) Piperidine, Tolune

Scheme 1. Synthetic route

Antimicrobial activity

Following common standard strains were used for screening of antibacterial and antifungal activities: *Staphylococcus aureus*, *Escherichia coli* and fungi *Aspergillus niger*. DMSO was used as diluent to get desired concentration of synthesized compounds to test upon standard bacterial strains. Each synthesized compound was diluted for obtaining 2000 µg/mL concentration, as a stock solution. In primary screening 1000 µg/mL concentrations of the synthesized compounds were taken. The synthesized compounds found active in this primary screening were further tested in a second set of dilution against all microorganisms.

The compounds found active in primary screening were similarly diluted to obtain 500, 200, 100, 87.5, 75, 62.5, 50, 37.5, 25, 12.5, 6.25, 3.13, 1.56, 0.78, 0.39, 0.19 and 0.09 µg/Ml and 2 Ml of these solutions were taken in test tubes. The highest dilution showing at least 99% inhibition zone was taken as MIC. The results of this were much affected by the size of the inoculums. The test mixture should contain 10^8 microorganism/Ml. The minimum inhibitory concentration[19-20] of the compounds was determined by broth dilution method. The respective clinical strain was spread separately on the Mueller-Hinton broth[21] medium for antibacterial activity and Sabouraud dextrose agar (SDA) broth for antifungal activity. Then 2 µL of test organism suspension was added and incubated at 37 ºC for 24 h for bacteria and 48 h for fungi studies. The drugs Gentamycin and Nystatin were used as standards for comparison of antibacterial and antifungal activities respectively. The minimum inhibitory concentration (MIC) was the lowest concentration of test compound that inhibit the visible growth of the organism and was determined in triplicates. The results are tabulated in Table 1.

Table 1. Minimum Inhibitory Concentration (MICs) of the compounds synthesized

Minimum inhibitory concentration, Concentration in µg/Ml				
Compound	R	Bacteria organisms[a]		Fungi
		Gram-positive	Gram-negative organisms[a]	*Aspergillus niger*
		Staphylococcus aureus	*Escherichia coli*	
3	-	100	87.5	100
4	-	100	87.5	100
5[a]	4-pyridyl	87.5	75	87.5
5b	2-pyridyl	75	75	87.5
5c	4-$NH_2C_6H_4$	100	87.5	100
5d	2-$NH_2C_6H_4$ $NH_2C_6H_4$aminophenyl	100	87.5	87.5
6	-	100	100	100
7[a]	-H	87.5	75	87.5
7b	4-Cl	87.5	87.5	87.5
7c	4-OH	75	75	75
7d	2-OH	75	75	75
7e	2-OCH_3	75	75	87.5
Gentamycin		0.39	0.09	-
Nystatin		-	-	100

From the results presented in Table 1, it is clear that all the synthesized coumarin derivatives have displayed antimicrobial activity *in vitro* against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria and fungi (*Aspergillus niger*), the MIC raging between 75-100 µg/Ml.

The lowest MIC was 75 µg/Ml for compounds **5b, 7c, 7d** and **7e** against *Staphylococcus aureus* and the highest being 100 µg/Ml for compounds **3, 4, 5c, 5d** and **6**. The MIC for compounds **5a,7a** and **7b** was 87.5 µg/Ml.

A lowest MIC of 75 µg/Ml was observed with compounds **5a, 5b, 7a, 7c, 7d** and **7e** against *Escherichia coli* and the highest MIC was 100 µg/Ml for compound **6**. The MIC for compounds **3, 4, 5c, 5d** and **7b** was 87.5 µg/Ml.

In testing with *Aspergillus niger* a lowest MIC of 75 µg/mL was observed for compounds **7c, 7d**. The highest MIC being 100 µg/mL for compounds **3, 4, 5c** and **6**. The MIC for compounds **5a, 5b, 5d, 7a, 7b** and **7e** was 87.5 µg/mL.

Of all the compounds studied, **7c** and **7d** are found more active exhibiting a MIC of 75 µg/mL against all the strains. The antimicrobial activities are not significantly influenced by variation of substituent in the aromatic rings. The result reveal that the Knovenegel adducts with electron releasing OH group in the phenyl ring exhibit high activity compared to the other compounds under investigation in both antibacterial and antifungal activities. But at the same time all the compounds are found to be less active than the standard compound, Gentamycin. All the compounds show antifungal activity comparable with the standard compound, Nystatin.

Conclusion

The compounds 4-methyl-7-(2-oxo-2-(5-(substitutedthio)-1,3,4-oxadiazol-2-yl)ethoxy)-2*H*-chromen-2-ones and 4-substitutedbenzylidene-3-methyl-1-(2-((4-methyl-2-oxo-2*H*-chromen-7-yl)oxy)acetyl)-1*H*-pyrazol-5(4*H*)-ones were synthesized by combining coumarin ring with 1,3,4-oxadiazole and pyrazole scaffolds respectively. The spectral data are consistent with the structure of the newly synthesized compounds. The minimum inhibitory concentration (MIC) of the synthesized compounds was studied using broth dilution method. The results revealed that all the compounds synthesized exhibited moderate to good antimicrobial activity.

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