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Synthesis and antimicrobial studies of tetrazol-5-yl-methoxy-8,9-dihydropyrano[2,3-*f*]chromene-2,10-diones and their coumarin derivatives

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Twelve new coumarin-tetrazole derivatives, tetrazol-5-yl(methoxy)-8,9-dihydropyrano[2,3-*f*] chromene-2,10-diones, were synthesized by 6-((2H-tetrazol-5-yl)methoxy)-4-methyl-8,9-dihydropyrano[2,3-f]chromene-2,10-dione derivatives treated with alkyl bromides in dry acetone and anhydrous potassium carbonate. The structures of all the newly synthesized molecules were assigned by spectral data and elemental analysis. The synthesized compounds were screened for their antimicrobial activities strains using the diffusion plate method. Most of the compounds showed moderate to good activity against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa.* Out of two strains of fungi, these compounds showed moderate to good activity against *Aspergillus fumigatus* and *Aspergillus niger*.

Keywords: Tetrazole, coumarin, antibacterial, antifungal, zone of inhibition

INTRODUCTION

Coumarins are an important class of natural and synthetic compounds that possess a wide spectrum of biological and pharmacological activities [1]. The coumarin skeleton is found in many natural products and is used as an important synthetic intermediate for the preparation of numerous heterocyclic compounds which show unique physical properties [2]. Furthermore, a large number of pharmaceutical drug products like novobiocin, warfarin, contain 7-hydroxy-4-methyl-2-coumarin as an important structural element [2, 3]. Coumarins containing heterocyclic moieties have a noteworthy medicinal value due to their high potential pharmacological activities such as antibacterial [3-12], antifungal [13-15], anti-tuberculosis activities [16-19], etc. Tetrazole derivatives are well known for their various potential biological activity [20]. Moreover, they are also observed as biologically comparable to the carboxylic acid group [21]. In fact, it has been also noticed that the toxic properties of a drug can be decreased by incorporation of a tetrazole ring into the molecule [22]. Generally, synthesis of tetrazoles can be achieved most commonly *via* the formal [2 + 3] cycloaddition of azides and nitriles. However, various reports in the literature describe that the mechanism of the reaction is different for diverse azide species. When an organic azide is used as the dipole, only certain highly activated nitriles are competent dipolarophiles [23]. In these cases, the reaction is region- selective, and only the 1-alkylated product is observed [24-32]. In this study we report the synthesis and characterization of twelve new coumarin derivatives by incorporation of tetrazole moiety for better biological and pharmacological activities.

EXPERIMENTAL

All reagents were purchased commercially (SD fine, India) and used without further purification. Melting points were determined by the open capillary method. The IR spectra (in KBr pellets) were recorded on a Perkin-Elmer FTIR spectrophotometer. ^{1}H NMR (300 MHz, DMSO-D6) and ^{13}C NMR (75 MHz, DMSO-D6) spectra were recorded on a Bruker Avance 300 spectrometer, TMS as internal standard (chemical shifts and ppm). Mass spectra were recorded on a VG micromass70-70H instrument.

A mixture of benzene-1,2,4-triol (5 mmol) **(1)** and ethyl 3-oxobutanoate (20 mL) **(2a-b)** in conc. H_2SO_4 (20 mL) was stirred for 10-12 h at room temperature (TLC monitoring) to obtain the desired product 6,7-dihydroxy-4-methyl-2H-chromen-2-one **(3a-b)** (Scheme 1) [3,11, 12, 33]. The product **(3a-b)** reacts with Ac_2O and HCl in presence of pyridine after reflux (TLC monitoring) to obtain the desired product 8-acetyl-6,7-dihydroxy-4-methyl-2H-chromen-2-one **(4a-b).** The latter reacts with HCHO and ethanol in presence of K_2CO_3 and pyridine after reflux (TLC monitoring) to obtain the

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desired product 6-hydroxy-4-methyl-8,9-dihydropyrano [2,3-f] chromene-2,10-dione **(5a-b).** The latter product and chloroacetonitrile (**6**) were dissolved in dry acetone and refluxed over anhydrous potassium carbonate for 3 h on a water bath (TLC monitoring). The excess solvent was evaporated under reduced pressure and the obtained residue was treated with petroleum ether (60–85%). The solid product was filtered off and washed with petroleum ether (60–85%) to obtain the desired product 2-((4-methyl-2,10-dioxo-2,8,9,10-tetrahydropyrano[2,3-f] chromen-6-yl) oxy) acetonitrile **(7a-b).**

General procedure for the synthesis of 6-((2H-tetrazol-5-yl) methoxy)-4-methyl-8,9-dihydropyrano[2,3-f]chromene-2,10-dione, 9(a-b)

A mixture of **7(a-b)** (3 g, 0.013 mol), sodium azide **(8)** (1 g, 0.014 mol) and NH_4C1 (0.85 g, 0.016 mol) in 75 ml of DMF was heated for 8 h at 120 °C (Scheme 2). The reaction mixture was cooled to room temperature and after crushed ice was added, a light cream colour precipitate was obtained. It was collected by filtration and washed with water, dried at 50°C to get the crude compound which was purified by recrystallization in methanol to furnish the pure compound **9(a)** as light cream solid. IR (KBr): *v* NH at 3222cm^{-1}, C=O at 1722 cm^{-1}, and O=C of coumarin at 1573 cm^{-1}. Anal. calcd. for $C_{15}H_{12}N_4O_5$: C, 54.88; H, 3.68; N, 17.07; O, 24.37. Found: C, 54.87; H, 3.69; N, 17.09; O, 24.35. ^{1}H NMR (300 MHz, DMSO-D6) δ 2.44 (s, 3H), 2.62 (d, *J* = 10.2 Hz, 2H), 4.71 (d, *J* = 10.2 Hz, 2H), 5.10 (s, 1H), 5.60 (s, 2H), 6.43 (s, 1H), 10.13 (s, 1H). ^{13}C NMR (75 MHz, DMSO-D6) δ 191.2, 161.1, 154.7, 154.1, 152.2, 148.5, 142.1, 120.7, 118.7, 111.2, 108.1, 63.6, 61.2, 37.1 and 19.0. Mass (ES): *m/z* = 328. M.P =146°C, Yield: 76%.

*6-((2H-Tetrazol-5-yl) methoxy)-3,4-dimethyl-8,9-dihydropyrano[2,3-f]chromene-2,10-dione, **(9b):*** Similar reaction conditions of **9a**, were employed for **9b**. The spectral data for **9b** are as follows: IR (KBr): *u* NH at 3213cm^{-1} and C=O at 1715 cm^{-1}, O=C of coumarin at 1566 cm^{-1}. Anal. calcd. for $C_{16}H_{14}N_4O_5$: C, 56.14; H, 4.12; N, 16.37; O, 23.37. Found: C, 56.16; H, 4.10; N, 16.38; O, 23.36.^{1}H NMR (300 MHz, DMSO-D6) δ 2.01 (3H, s), 2.42 (3H, s), 2.78 (2H, d, *J* = 10.2 Hz), 4.46 (2H, d, *J* = 10.2 Hz), 5.10 (2H, s), 7.16 (1H, s), 10.05 (s, 1H). ^{13}C NMR (75 MHz, DMSO-D6) δ 192.2, 162.4, 157.7, 157.4, 154.0, 149.5, 142.7, 121.8, 119.8, 111.4, 109.2, 64.6, 62.6, 37.6, 35.1 and 21.7.Mass (ES): *m/z*= 342. Yield: 79%.

General procedure for the synthesis of 4-methyl-6-((2-methyl-2H-tetrazol-5-yl) methoxy)-8,9-dihydropyrano[2,3-f]chromene-2,10-dione, 11(a-l)

6-((2H-Tetrazol-5-yl) methoxy)-4-methyl-8,9-dihydropyrano[2,3-f]chromene-2,10-dione, **9a** (1 g, 0.003 mol) and methyl bromide, **10a** (0.35 ml, 0.003 mol) were dissolved in 50 ml of dry acetone, refluxed at 70 °C over anhydrous potassium carbonate for 3 h on a water bath (Scheme 1). The acetone was removed under reduced pressure and crushed ice was added to the residue. The product **(11a)** was filtered and washed with plenty of water, yield 80%. The spectral data for **11a** are as follows: mp: 131°C, IR (KBr): *v* C=O at 1723 cm^{-1}, O=C of coumarin at 1567 cm^{-1}. Anal. calcd. for $C_{16}H_{14}N_4O_5$: C, 56.14; H, 4.12; N, 16.37; O, 23.37. Found: C, 56.12; H, 4.13; N, 16.38; O, 23.37. ^{1}H NMR (300 MHz, DMSO-D6) δ 2.43 (3H, s), 2.68 (2H, d, *J* = 10.2 Hz), 3.90 (3H, s), 4.77 (2H, d, *J* = 13.2 Hz), 5.11 (2H, s), 5.89 (1H, s), 7.46 (1H, s). ^{13}C NMR (75 MHz, DMSO-D6) δ 199.2, 159.3, 153.7, 153.3, 152.9, 149.2, 145.2, 116.8, 113.8, 111.4, 105.8, 69.5, 67.0, 38.8, 37.5, 19.1. ESI-HRMS: m/z $[M + H]^+$ = 342.10 (Calcd. M^+ = 342.31)

Similar reaction conditions of **11a** were employed for all the other compounds **11(b-l)** and the spectral data are as follows:

6-((2-Ethyl-2H-tetrazol-5-yl) methoxy)-4-methyl-8,9-dihydropyrano[2,3-f]chromene-2,10-dione **(11b):** Yield: 80%. mp: 139°C, IR (KBr): *v* C=O at 1724 cm^{-1}, O=C of coumarin at 1566 cm^{-1}. Anal. Calcd for $C_{17}H_{16}N_4O_5$: C, 57.30; H, 4.53; N, 15.72; O, 22.45. Found: C, 57.32; H, 4.52; N, 15.72; O, 22.44. ^{1}H NMR (300 MHz, DMSO-D6) δ 1.15 (3H, t), 2.41 (3H, s), 2.63 (2H, d, J = 2.5 Hz), 4.25 (2H, q), 4.75 (2H, d, J = 10.2 Hz), 5.02 (2H, s), 5.29 (1H, s), 6.76 (1H, s). ^{13}C NMR (75 MHz, DMSO-D6) δ 196.1, 159.2, 153.4, 153.1, 151.9, 148.1, 145.3, 114.8, 113.3, 110.9, 106.5, 69.2, 67.3, 38.6, 37.9, 19.5, 10.2, ESI-HRMS: m/z $[M + H]^+$ = 356.11 (Calcd. M^+ = 356.33)

*4-Methyl-6-((2-propyl-2H-tetrazol-5-yl) methoxy)-8,9-dihydropyrano[2,3-f]chromene-2,10-dione **(11c)***: Yield: 82%. mp: 151°C, IR (KBr): *v* C=O at 1738 cm^{-1}, O=C of coumarin at 1569 cm^{-1}. Anal. Calcd for $C_{18}H_{18}N_4O_5$: C, 58.37; H, 4.90; N, 15.13; O, 21.60. Found: C, 58.38; H, 4.90; N, 15.12; O, 21.60. ^{1}H NMR (300 MHz, DMSO-D6) δ 0.92 (3H, t), 1.80 (2H), 2.43 (3H, s), 2.60 (2H, d, *J* = 10.5 Hz), 4.42 (2H, t), 4.75 (2H, d, *J* = 10.2 Hz), 5.02 (2H, s), 5.66 (1H, s), 7.06 (1H, s). ^{13}C NMR (75 MHz, DMSO-D6) δ 197.3, 158.5, 153.7, 152.4, 151.2, 148.7, 145.6, 116.2, 114.2, 108.9, 106.6, 68.8, 67.8, 37.6, 34.9, 20.1, 19.2, 11.1, ESI-HRMS: m/z $[M + H]^+$ = 369. 13 (Calcd. M^+ = 370.36).

6-((2-Butyl-2H-tetrazol-5-yl)methoxy)-4-methyl-8,9-dihydropyrano[2,3-f]chromene-2,10-dione **(11d):** Yield: 86%. mp: 158°C, IR (KBr): *v* C=O at 1732 cm^{-1}, O=C of coumarin at 1563 cm^{-1}. Anal. calcd. for $C_{19}H_{20}N_4O_5$: C, 59.37; H, 5.24; N, 14.58; O, 20.81. Found: C, 59.39; H, 5.23; N, 14.59; O, 20.82. ^{1}H NMR (300 MHz, DMSO-D6) δ 0.81 (3H, t), 1.23 (2H), 1.76 (2H, t), 2.41 (3H, s), 2.61 (2H, d, *J* = 3.2 Hz), 4.22 (2H, t), 4.65 (2H, d, *J* = 10.2, Hz), 5.01 (2H, s), 5.80 (1H, s), 7.01 (1H, s). ^{13}C NMR (75 MHz, DMSO-D6) δ 195.3, 156.5, 152.9, 151.1, 150.8, 147.0, 144.1, 116.2, 115.1, 111.5, 106.4, 68.2, 67.3, 41.2, 33.2, 30.1, 20.6, 19.8, 11.4, ESI-HRMS: m/z $[M + H]^+$ = 384.14 (Calcd. M^+ = 384.39)

4-Methyl-6-((2-pentyl-2H-tetrazol-5-yl) methoxy)-8,9-dihydropyrano[2,3-f]chromene-2,10-dione **(5e):** Yield: 90%. mp: 166°C, IR (KBr): *v* C=O at 1733 cm^{-1}, O=C of coumarin at 1573 cm^{-1}. Anal. calcd. for $C_{20}H_{22}N_4O_5$: C, 60.29; H, 5.57; N, 14.06; O, 20.08. Found: C, 60.27; H, 5.59; N, 14.07; O, 20.07. ^{1}H NMR (300 MHz, DMSO-D6) δ 0.84 (3H, t), 1.24-1.33 (4H, t), 1.66 (2H, t), 2.43 (3H, s), 2.54 (2H, d, *J* = 10.5 Hz), 4.16 (2H, t), 4.66 (2H, d, *J* = 13.2 Hz), 5.22 (2H, s), 5.55 (1H, s), 6.76 (1H, s). ^{13}C NMR (75 MHz, DMSO-D6) δ 195.1, 153.5, 152.7, 151.4, 150.2, 146.7, 141.6, 112.9, 110.8, 109.1, 104.9, 64.7, 63.6, 44.9, 32.8, 30.1, 22.7, 20.6, 19.3, 11.4, ESI-HRMS: m/z $[M + H]^+$ = 398.16 (Calcd. M^+ = 398.41)

6-((2-Isobutyl-2H-tetrazol-5-yl) methoxy)-4-methyl-8,9-dihydropyrano[2,3-f]chromene-2,10-dione **(11f):** Yield: 89%. mp: 160°C, IR (KBr): *v* C=O at 1732 cm^{-1}, O=C of coumarin at 1563 cm^{-1}. Anal. Calcd for $C_{19}H_{20}N_4O_5$: C, 59.37; H, 5.24; N, 14.58; O, 20.81. Found: C, 59.38; H, 5.25; N, 14.59; O, 20.80. ^{1}H NMR (300 MHz, DMSO-D6) δ 0.90 (6H, d, *J* = 6.8 Hz), 2.07 (1H), 2.40 (3H, s), 2.58 (2H, d, *J* = 14.5 Hz), 4.29 (2H, d, *J* = 6.4 Hz), 4.70 (2H, d, *J* = 10.2 Hz), 5.11 (2H, s), 5.69 (1H, s), 6.88 (1H, s). ^{13}C NMR (75 MHz, DMSO-D6) δ 195.6, 156.1, 152.9, 151.1, 150.1, 147.1, 144.2, 115.1, 114.3, 111.1, 106.2, 68.1, 66.2, 40.1, 38.1, 20.2, 19.1,19.0, 11.2, ESI-HRMS: m/z $[M + H]^+$ = 384.14 (Calcd M^+ = 384.39)

3,4-Dimethyl-6-((2-methyl-2H-tetrazol-5-yl) methoxy)-8,9-dihydropyrano[2,3-f]chromene-2,10-dione **(11g)***:* Yield: 70%. mp: 141°C, IR (KBr): v C=O at 1733 cm^{-1}, O=C of coumarin at 1567 cm^{-1}. Anal. Calcd for $C_{17}H_{16}N_4O_5$: C, 57.30; H, 4.53; N, 15.72; O, 22.45. Found: C, 57.32; H, 4.51; N, 15.71; O, 22.45. ^{1}H NMR (300 MHz, DMSO-D6) δ 2.13 (3H, t), 2.43 (3H, s), 2.54 (2H, d, *J* = 10.2 Hz), 3.67 (3H, s), 4.44 (2H, d, *J* = 10.2 Hz), 5.22 (2H, s), 6.78 (1H, s), ^{13}C NMR (75 MHz, DMSO-D6) δ 197.6, 159.1, 152.7, 151.3, 150.9, 147.2, 145.2, 116.2, 113.3, 111.2, 105.7, 69.6, 67.3, 38.5, 37.3, 19.2, 13.4, ESI-HRMS: m/z $[M + H]^+$ = 356.11 (Calcd M^+ = 356.33)

6-((2-Ethyl-2H-tetrazol-5-yl)methoxy)-3,4-dimethyl-8,9-dihydropyrano[2,3-f]chromene-2,10-dione **(11h):** Yield: 80%. mp: 148°C, IR (KBr): *v* C=O at 1723 cm^{-1}, O=C of coumarin at 1575 cm^{-1}. Anal. calcd. for $C_{18}H_{18}N_4O_5$: C, 58.37; H, 4.90; N, 15.13; O, 21.60. Found: C, 58.39; H, 4.91; N, 15.13; O, 21.61. ^{1}H NMR (300 MHz, DMSO-D6) δ 1.09 (3H, t), 2.01 (3H, s), 2.34 (3H, s), 2.56 (2H, d, *J* = 10.5 Hz), 4.24 (2H, q), 4.73 (2H, d, *J* = 10.2 Hz), 5.10 (2H, s), 6.66 (1H, s). ^{13}C NMR (75 MHz, DMSO-D6) δ 197.6, 158.1, 152.7, 151.9, 151.2, 148.2, 144.2, 115.2, 112.3, 110.2, 104.7, 68.6, 65.3, 48.5, 37.2, 19.3, 13.1, 10.1, ESI-HRMS: m/z $[M + H]^+$ = 370.13 (Calcd. M^+ = 370.36)**.**

3,4-Dimethyl-6-((2-propyl-2H-tetrazol-5-yl) methoxy)-8,9-dihydropyrano[2,3-f]chromene-2,10-dione **(11i):** Yield: 86%. mp: 157°C, IR (KBr): *v* C=O at 1724 cm^{-1}, O=C of coumarin at 1573 cm^{-1}. Anal. calcd. for $C_{19}H_{20}N_4O_5$: C, 59.37; H, 5.24; N, 14.58; O, 20.81. Found: C, 59.39; H, 5.25; N, 14.58; O, 20.82. ^{1}H NMR (300 MHz, DMSO-D6) δ 0.94 (3H, t), 1.93 (2H, t), 2.11 (3H, s), 2.43 (3H, s), 2.74 (2H, d, *J* = 14.5 Hz), 4.22 (2H, t), 4.46 (2H, d, *J* = 13.2 Hz), 5.02 (2H, s), 7.02 (1H, s). ^{13}C NMR (75 MHz, DMSO-D6) δ 195.6, 159.7, 154.2, 152.1, 150.1, 148.5, 145.1, 116.1, 114.5, 109.6, 103.1, 65.1, 63.1, 51.6, 41.1, 20.5, 19.1, 13.1, 10.1 , ESI-HRMS: m/z $[M + H]^+$ = 384.14 (Calcd. M^+ = 384.39)

6-((2-Butyl-2H-tetrazol-5-yl) methoxy)-3,4-dimethyl-8,9-dihydropyrano[2,3-f]chromene-2,10-dione **(11j):** Yield: 88%. mp: 167°C, IR (KBr): *v* C=O at 1728 cm^{-1}, O=C of coumarin at 1563 cm^{-1}. Anal. calcd. for $C_{20}H_{22}N_4O_5$: C, 60.29; H, 5.57; N, 14.06; O, 20.08. Found: C, 60.28; H, 5.58; N, 14.07; O, 20.07. ^{1}H NMR (300 MHz, DMSO-D6) δ 0.82 (3H, t), 1.43 (2H, t), 1.58 (2H, t), 2.34 (3H, s), 2.64 (3H, s), 2.88 (2H, d, *J* = 14.5 Hz), 4.58 (2H, t), 4.96 (2H, d, *J* = 10.2 Hz), 5.40 (2H, s), 7.01 (1H, s). ^{13}C NMR (75 MHz, DMSO-D6) δ 194.9, 156.7, 153.2, 152.3, 150.1, 146.2, 142.1, 113.1, 110.1, 107.2, 101.1, 66.4, 62.1, 50.1, 40.2, 30.2, 20.1, 19.2, 13.2, 10.1 , ESI-HRMS: m/z $[M + H]^+$ = 398.16 (Calcd. M^+ = 398.41)

3,4-Dimethyl-6-((2-pentyl-2H-tetrazol-5-yl) methoxy)-8,9-dihydropyrano[2,3-f]chromene-2,10-dione **(11k):** Yield: 91%. mp: 171°C, IR (KBr): *v* C=O at 1725 cm^{-1}, O=C of coumarin at 1573 cm^{-1}. Anal. calcd. for $C_{21}H_{24}N_4O_5$: C, 61.15; H, 5.87; N, 13.58; O, 19.40. Found: C, 61.17; H, 5.88; N, 13.57; O, 19.41. ^{1}H NMR (300 MHz, DMSO-D6) δ 0.81 (3H, t), 1.22-1.35 (4H, t), 1.79 (2H, t), 2.01 (3H, s),

2.41 (3H, s), 2.69 (2H, d, *J* = 14.5 Hz), 4.36 (2H, t), 4.70 (2H, d, *J* = 10.2 Hz), 5.22 (2H, s), 7.11 (1H, s). ^{13}C NMR (75 MHz, DMSO-D6) δ 195.9, 158.2, 156.1, 152.1, 151.1, 147.2, 140.5, 112.1, 110.1, 107.2, 100.1, 67.2, 61.1, 55.2, 41.1, 32.2, 23.1, 21.1, 18.4, 11.5, 10.3, ESI-HRMS: m/z $[M + H]^+$ = 412.17 (Calcd. M^+ = 412.44)

6-((2-Isobutyl-2H-tetrazol-5-yl) methoxy)-3,4-dimethyl-8,9-dihydropyrano[2,3-f]chromene-2,10-dione **(11l):** Yield: 88%. mp: 170°C, IR (KBr): *v* C=O at 1728 cm^{-1}, C=C of coumarin at 1567 cm^{-1}. Anal. calcd. for $C_{20}H_{22}N_4O_5$: C, 60.29; H, 5.57; N, 14.06; O, 20.08. Found: C, 60.28; H, 5.58; N, 14.07; O, 20.07. ^{1}H NMR (300 MHz, DMSO-D6) δ 0.89 (6H, d, *J* = 6.8 Hz), 2.04 - 2.13 (4H, m), 2.43 (3H, s), 2.62 (2H, d, *J* = 14.5 Hz), 4.04 (2H, d, *J* = 6.4 Hz), 4.65 (2H, d, *J* = 10.2 Hz), 5.19 (2H, s), 6.99 (1H, s). ^{13}C NMR (75 MHz, DMSO-D6) δ 196.9, 158.1, 155.7, 154.1, 152.1, 147.1, 142.5, 112.3, 110.4, 108.7, 104.2, 66.2, 63.8, 48.6, 40.1, 30.7, 20.9, 20.1, 19.1, 10.2, ESI-HRMS: m/z $[M + H]^+$ = 398.16 (Calcd. M^+ = 398.41).

All synthesized compounds were screened for their antimicrobial and anti-fungal activities by using the diffusion plate method [23-30]. A filter paper sterilized disk saturated with the measured quantity (25 μL) of the sample (1 mg/mL) was placed on a plate (9 cm diameter) containing a solid bacterial medium (nutrient agar) or a fungal medium (potato dextrose agar) that was seeded with the spore suspension of the test organism. After incubation at 37°C for 24 h for bacteria (in case of fungi, at 25°C for 72 h), the diameter of the clear zone of inhibition surrounding the sample was taken as a measure of the inhibitory power of the sample against the particular test organism (% inhibition = sample inhibition zone (cm)/plate diameter × 100). All measurements were done in methanol as a solvent that has zero inhibition activity. The antimicrobial activity of the new compounds was examined against two Gram-positive bacteria (*Staphylococcus aureus*, MTCC 096 and *Bacillus subtilis*, MTCC 441) and two Gram-negative bacteria (*Escherichia coli*, MTCC 443 and *Pseudomonas aeruginosa*, MTCC 424), whereas, for antifungal studies two fungi (*Aspergillus niger*, MTCC 282 and *Aspergillus fumigates*, MTCC 343) were taken. The obtained results are compared with the reference antibiotics purchased from Hyderabad chemicals center. Table 2 contains data of the antibacterial and antifungal testing.

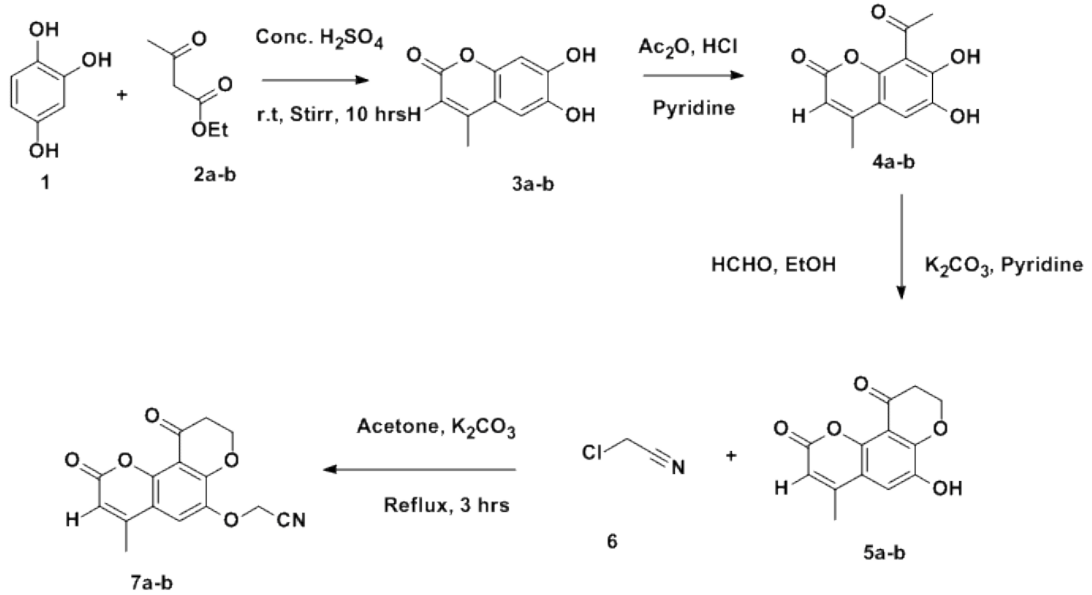
RESULTS AND DISCUSSION

Chemistry

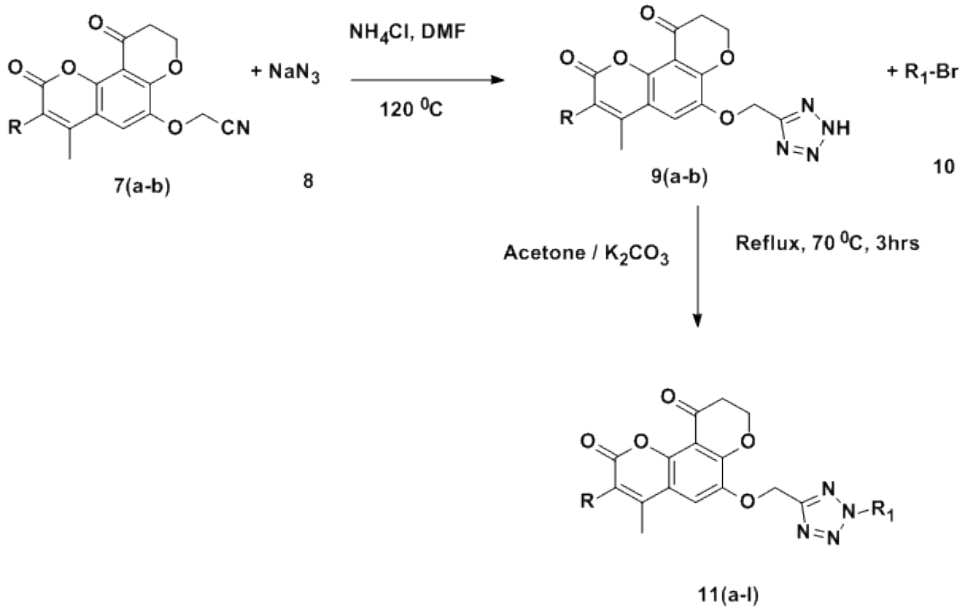
Benzene-1,2,4-triol **(1)** with ethyl 3-oxobutanoate **(2a-b)** undergo conventional cyclocondensation reaction in presence of conc. H_2SO_4, and by stirring for 10-12 h at room temperature 6,7-dihydroxy-4-methyl-2H-chromen-2-one **(3a-b)** was prepared in 80% yield. The compound **(3a-b)** was treated with Ac_2O and HCl in presence of pyridine after reflux to obtain the product (78% yield) 8-acetyl-6,7-dihydroxy-4-methyl-2H-chromen-2-one **(4a-b).** The latter reacted with HCHO and ethanol in presence of K_2CO_3 and pyridine after reflux to obtain 6-hydroxy-4-methyl-8,9-dihydropyrano[2,3-f] chromene-2,10-dione **(5a-b)** (65% yield). Compound **(5a-b)** was treated with chloroacetonitrile (**6**) dissolved in dry acetone and was refluxed over anhydrous potassium carbonate for 3 h on a water bath. After purification the desired product 2-((4-methyl-2,10-dioxo-2,8,9,10-tetrahydropyrano [2,3-f] chromen-6-yl) oxy) acetonitrile **(7a-b)** (68% yield) was obtained**.**

Finally, a mixture of **7(a)**, sodium azide **(8)** and NH_4C1 in DMF was heated for 8 h at 120 °C (Scheme 2). The crude compound, purified by recrystallization, furnished the pure compound **9(a)** as light cream solid with 76% yield. In the IR spectrum, amine at 3222 cm^{-1}, carbonyl at 1722 cm^{-1}, and O=C of coumarin at 1573 cm^{-1} are seen. Compound **9a** was treated with methyl bromide, **10a** was dissolved in dry acetone, and was refluxed at 70 °C over anhydrous potassium carbonate for 3 h on a water bath (Scheme 2). The acetone was removed under reduced pressure and crushed ice was added to the residue. The product **(11a)** was filtered and washed with plenty of water. The yield was 80%, observed mp: 181°C, IR (KBr): *v* C=O at 1723 cm^{-1}, O=C of coumarin at 1567 cm^{-1}. The ^{1}H NMR spectrum showed a typical singlet signal attributable to 2.43, 3.90, 5.11, 5.89 7.46. Compound **11a** was also confirmed from its mass spectrum which revealed the parent ion peak at m/z $[M + H]^+$ = 342.10 that coincided well with the suggested calcd. M^+ = 342.31. A methyl group-containing **7(b)**, sodium azide **(8)** and NH_4C1 in DMF were heated for 8 h at 120 °C (Scheme 2), the purified compound **9(b)** yielded 79%. In the IR spectrum amine at 3213 cm^{-1}, carbonyl at 1715 cm^{-1}, and O=C of coumarin at 1566 cm^{-1} are seen. The ^{1}H NMR spectrum showed a typical singlet signal attributable to 2.01, 2.42, 5.10, 7.16 and 10.05.

Compound **9b** was also confirmed from its mass spectrum which revealed the parent ion peak at m/z = 342. After compound **9b** was treated with methyl bromide, **10a** was dissolved in dry acetone, refluxed at 70 °C over anhydrous potassium carbonate for 3 h on a water bath (Scheme 2). The acetone was removed under reduced pressure and crushed ice was added to the residue. The product **(11b)** was filtered and washed with plenty of water. The yield was 70%, observed mp: 141°C, IR (KBr): *ν* C=O at 1733 cm^{-1}, O=C of coumarin at 1567 cm^{-1}. The ^1H NMR spectrum showed a typical singlet signal attributable to 2.13, 2.43, 3.67, 5.22, 6.78. Compound **11a** was also confirmed from its mass spectrum which revealed the parent ion peak at m/z $[M + H]^+$ = 356.11 that coincided well with the suggested calcd. M^+ = 356.33. Also, in our present research work we observed that if methyl groups are present in the title compounds, their yield is low, for example, **11a** compound contains R=H, R_1= CH_3, its yield is 80% and **11g** compound contains R= CH_3, R_1= CH_3, its yield is only 70%. Increasing carbon chain on the title compounds increases yield, e.g., **11e** compound contains R=H, R_1= n-C_5H_{11}, its yield is 90% and **11g** compound contains R= CH_3, R_1= n-C_5H_{11}, its yield is 91%.



Scheme 1. Reagents and conditions for synthesis of 2-((4-methyl-2,10-dioxo-2,8,9,10-tetrahydropyrano[2,3-f]chromen-6-yl)oxy)acetonitrile (7a-b)



Scheme 2. Reagents and conditions for synthesis of the title compounds (11a-l)

Table. 1. R, R_1 substituents and yield of title compounds, 11(a-l).

Product	R	R_1	Yield (%)
11a	H	CH_3	80
11b	H	C_2H_5	80
11c	H	n-C_3H_7	82
11d	H	n-C_4H_9	86
11e	H	n-C_5H_{11}	90
11f	H	iso-C_4H_9	89
11g	CH_3	CH_3	70
11h	CH_3	C_2H_5	80
11i	CH_3	n-C_3H_7	86
11j	CH_3	n-C_4H_9	88
11k	CH_3	n-C_5H_{11}	91
11l	CH_3	iso-C_4H_9	88

Bioactivity

Go through the biological assay of the novel coumarin-tetrazole derivatives shows that most of the compounds were highly to moderately potent against the specific strains (Table 3). In general, most of the tested derivatives displayed better activity against Gram-positive bacterial strains as compared to Gram-negative bacterial strains. The new compounds were screened to determine their antimicrobial activity *in vitro* against two pathogenic Gram-positive bacteria, *viz*. *Staphylococcus aureus* MTCC 096, and *Bacillus subtilis*, MTCC 441, two pathogenic Gram-negative bacteria, *viz*. *Escherichia coli*, MTCC 443 and *Pseudomonas aeruginosa*, MTCC 424, and fungal cultures of *Aspergillus niger*, MTCC 282 and *Aspergillus fumigates*, MTCC 343. The reference drugs were ciprofloxacin for antibacterial and miconazole for antifungal tests, respectively.

Based on the values of the inhibition zone diameter shown in Table 2, it could be concluded that most of the evaluated tetrazole coumarin derivatives displayed moderate to significant broad-spectrum antimicrobial activity comparing to the used reference drugs. The parent tetrazole-coumarin compound exhibited moderate antibacterial and fungal activity, while its conversion to the compounds **11a–l** affected the potency.

Table. 2. Antimicrobial activity studies of the title compounds, **11(a-l)**, using Gram-positive bacteria, Gram-negative bacteria and two fungi in inhibition zone diameter mm/mg.

Compound	Gram-positive bacteria		Gram-negative bacteria		Fungi	
	Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Aspergillus niger*	*Aspergillus fumigatus*
9a	10	12	12	15	10	20
9b	15	20	15	20	15	20
11a	20	15	10	10	15	15
11b	10	20	20	0	15	15
11c	25	30	15	10	25	20
11d	15	10	10	20	15	10
11e	10	10	25	15	10	20
11f	0	10	15	15	30	30
11g	10	15	10	20	35	25
11h	20	15	10	25	30	30
11i	15	10	20	25	35	30
11j	20	25	15	10	10	15
11k	15	30	10	15	15	20
11l	10	15	10	20	30	25
Ciprofloxacin	25	35	30	25		
Miconazole	–	–	–	–	40	35

Inhibition zone diameter units in mm/mg

It was found that the tetrazole-coumarin derivative **11c** exhibited potent antibacterial activity against the Gram-positive *S. aureus* and *B. subtilis* and the Gram-negative *E. coli*, and *P. aeruginosa* of inhibition zones 15, 20 mm/mg, respectively, *vs* 30, 25 mm/mg of the reference drug ciprofloxacin, while its antifungal potency was moderate, producing inhibition zone 25, 20 mm/mg when compared to that of the reference antifungal drug 40, 35 mm/mg. It was found that the tetrazole-coumarin derivative **11g** exhibited low potent antibacterial activity against the two Gram-positive *S. aureus* and *B. subtilis* and the Gram-negative *E. coli*, and *P. aeruginosa* of inhibition zones 10, 15, 10, 20 mm/mg, respectively, *vs* 25, 30, 30, 25 mm/mg of the reference drug ciprofloxacin, while its antifungal potency was high, producing inhibition zone 35, 25 mm/mg when compared to that of the reference antifungal drug 40, 35 mm/mg. The antifungal potency of **11h** was high, producing inhibition zone 30, 30 mm/mg when compared to that of the reference antifungal drug 40, 35 mm/mg. The antifungal potency of **11f** was high, producing inhibition zone 30, 30 mm/mg when compared to that of the reference antifungal drug 40, 35 mm/mg but its antibacterial activity was very low. The antifungal potency of **11i** was high, producing inhibition zone 35, 30 mm/mg when compared to that of the reference antifungal drug 40, 35 mm/mg. Iso butyl group-containing 11f and 11l compounds showed high antifungal potency, producing inhibition zone 25, 30 mm/mg when compared to that of the reference antifungal drug 40, 35 mm/mg but the least anti-bacterial activity.

In antibacterial studies, all the compounds tested were found with moderate to good activity towards Gram-positive and Gram-negative strains. Most of the compounds showed moderate to good activity against Gram-positive *Staphylococcus aureus* bacteria. Compounds **11a, 11c, 11h** and **11j** showed good antibacterial activity against *Staphylococcus aureus.* Some of the compounds showed moderate to good activity against Gram-positive *Bacillus subtilis* bacteria. Compounds **11c, 11j** and **11k** showed good antibacterial activity against *Bacillus subtilis.* **11b, 11e** and **11i** showed good antibacterial activity against *Escherichia coli.* **11h** and **11i** showed good antibacterial activity against *Pseudomonas aeruginosa.* Out of two strains of fungi, all these compounds showed moderate to good activity against *Aspergillus niger and Aspergillus fumigatus.* Compounds **11g, 11h, 11i** and **11l** possessed high antifungal activity against *Aspergillus niger;* **11f, 11h** and **11i** possessed high antifungal activity against *Aspergillus fumigatus*, which was almost similar to that of the standard drug. Further modification and optimization are needed to get more significant antimicrobial activity against various types of bacteria and fungi.

CONCLUSION

We have successfully synthesized tetrazol-5-yl) methoxy)-8,9-dihydropyrano[2,3-*f*]chromene-2,10-diones by 6-((2H-tetrazol-5-yl)methoxy)-4-methyl-8,9-dihydropyrano[2,3-f]chromene-2,10-dione derivatives treated with alkyl bromide in good yields. The structures of all compounds were assigned by their spectral data and CHN analysis. All newly synthesized compounds were screened for their zone of inhibition against two strains of bacteria. Most of the compounds showed moderate to good antimicrobial activities, whereas some compounds showed promising antifungal properties. These were further used to determine the minimum bacterial concentration (MBC) and minimum fungal concentration (MFC) against some selected strains of bacteria and fungi. This study will provide a road map to design new coumarin derivatives which can be used as antibacterial and antifungal drugs.

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Supplementary material: *supportive/supplementary material containing ^{1}h-nmr, and ^{13}c-nmr for all 8,9-dihydropyrano 6- [(2-alkyl-2h-tetrazol-5-yl) methoxy]-4-methyl-2h-cromen-2-ones (11a-l) is provided.*

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