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Synthesis and Antimicrobial Evaluation of Novel 4-Methyl-7-((5-Aryl-4*H*-1,2,4-Triazol-3-Yl)Methoxy)-2*H*-Benzopyran-2-Ones

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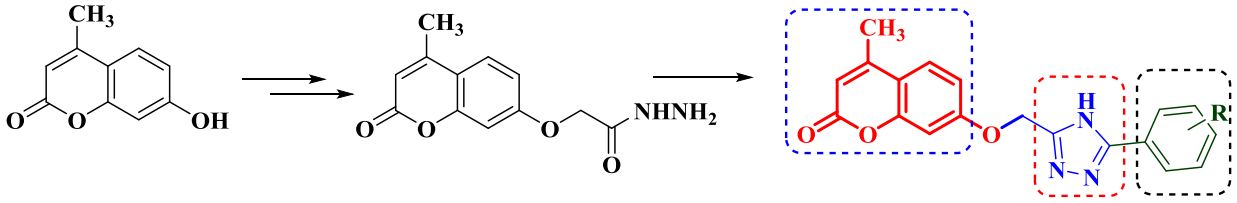
ABSTRACT

A new series of 4-methyl-7-triazolylmethoxy-2H-benzopyran-2-ones were synthesized using 7-hydroxy-4-methyl-2H-benzopyran-2-one as the starting material. It was first converted into 7-yloxyacetohydrazide by functional group inter conversion strategies and then reacted with aromatic or heterocyclic aldehydes and ammonium acetate in acetic acid to give the titled compounds. All the synthesized compounds were evaluated for their in vitro antibacterial and antifungal activities against seven strains of bacteria and four strains of fungi. MIC results of most of the synthesized compounds (14-23, 26-27 and 29) have shown them to possess promising to comparable activities while compounds 15, 19, 21, 26, 27 and 29 were found to be better antifungal agents than the standard drugs in vitro and can serve as lead molecules to discover a new antimicrobial in future to combat the growing drug resistance and infections developed by various microorganisms.



Keywords: 7-Hydroxy-4-methyl-2*H*-benzopyran-2-one, 1,2,4-Triazole, Antibacterial, Antifungal, Cup-plate method.

INTRODUCTION

The growing antimicrobial resistance has threatened the humanity all around the world to discover new molecules which can effectively prevent and treat an ever increasing range of infections caused by bacteria, parasites, viruses and fungi. Over the last three decades, no major new types of antibiotics have been developed which can fight infectious diseases like tuberculosis, malaria, urinary tract infections and even HIV. Globally, over 700000 deaths each year are attributed to drug resistance. Survey has concluded that in India alone, an additional two million lives can be lost due to drug resistance. So it is foremost important to discover new small molecules to combat these infections before it is too late.

2*H*-Benzopyran-2-ones commonly known as coumarins are widely spread in nature and have been reported to possess various biological and/or pharmacological properties such as antibacterial [1], anti-oxidant [2,3], anti-inflammatory [4], antiviral [5], anticoagulant [6], anticancer [7], cytotoxic [8], antimicrobial [9], antileucemic [10], anti-hepatitis and hepatoprotective [11], analgesic and anti-pyretic activities [12]. 7-Hydroxy-4-methyl-2*H*-benzopyran-2-ones that belongs to an old class of naturally occurring 2*H*-benzopyran-2-ones were also known to possess activities like antifungal [13], anti-inflammatory [14], antiplatelet [15], antiproliferative [16], inhibitors of DNA polymerase [17], anti-ache agent [18] and potent anti-estrogen both *in vitro* and *in vivo* models of breast cancer [19]. Our group, in the past few years, has been actively engaged in studying the chemistry and biological effects of 2*H*-benzopyran-2-one [20-23].

On the other hand, 1,2,4-triazoles were also well recognized as 5-lipoxygenase inhibitors [24], besides possessing anticancer [25,26], antitumor [27,28] and antifungal activity [29]. The triazole moiety is an important part of various drugs such as fluconazole [30], ravuconazole [31], voriconazole [32-34], itraconazole [35] and posaconazole [36].

In view of above, both 2*H*-benzopyran-2-one especially 7-hydroxy-4-methyl-2*H*-benzopyran-2-ones and 1,2,4-triazole individually act as an important pharmacophore and exhibit promising biological activities. As a part of our ongoing search for the discovery of new bioactive heterocycles and in view of our recent experience working on 1,2,4-triazoles [37,38], we aimed now to synthesize novel molecules, incorporating the structural features of both 2*H*-benzopyran-2-one and 1,2,4-triazole, on the promise that the two biodynamic moieties, present in tandem will contribute significantly to prevent various infections caused by bacteria, parasites, viruses and fungi.

MATERIALS AND METHODS

Experimental

Ethyl-(4-methyl-2H-benzopyran-2-one-7-yloxy)acetate (2)

7-Hydroxy-4-methyl-2*H*-benzopyran-2-one (1) (5.0 g, 2.8 mmol) was dissolved in 50 ml of dry acetone and anhyd. K_2CO_3 (11.590 g, 4.4 mmol) added to it. To the mixture ethylbromoacetate (4.740 g, 2.8 mmol) was added. The reaction mixture was refluxed for 6 h and progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was filtered and the solvent was removed under reduced pressure using rotary evaporator and contents subjected to column chromatography. Elution with 10% ethyl acetate/petroleum ether gave ethyl-(4-methyl-2*H*-benzopyran-2-one-7-yloxy)acetate (2) as a white solid. Yield 7.120g (95%), M. P. 86°C-88°C, R_f=0.38 in petroleum ether/ethyl acetate (90: 10) as developing solvent system; IR (KBr) ν_{max}: 3077, 2923, 1758, 1710, 1611, 1426, 1269, 1197, 1061, 843, 750 cm^{-1}; ^{1}H-NMR (δ, $CDCl_3$, 400 MHz): 7.49 (d, 1H, J=8.8 Hz), 6.87 (d, 1H, J=8.8 Hz), 6.75 (s, 1H), 6.13 (s, 1H), 4.66 (s, 2H, -OCH_2-), 4.26 (q, 2H, -OCH_2CH_3), 2.37 (s, 3H, -CH_3), 1.29 (t, 3H, J=1.5 Hz, -CH_2CH_3); ^{13}C-NMR (δ, $CDCl_3$, 100 MHz): 167.8 (>C=O), 160.9, 160.4, 154.8, 152.3, 125.6, 114.2, 112.3, 112.2, 101.5, 65.1, 61.5, 18.5, 14.0. Mass spectral data, TOF ES^+ m/z (%): 263 (M^++1). Molecular formula: $C_{14}H_{14}O_5$ Elemental Anal. Calcd.: C, 64.12; H, 5.38; Found: C, 64.09; H, 5.42.

(4-Methyl-2H-benzopyran-2-one-7-yloxy)acetohydrazide (3)

A mixture of ethyl-(4-methyl-2*H*-benzopyran-2-one-7-yloxy)acetate (2) (6.0 g, 2.2 mmol) and hydrazine hydrate (1.140 g, 2.2 mmol) in ethanol was refluxed for a period of 4 h. Progress of the reaction was monitored by TLC. After completion of the reaction, contents were cooled and the solid that separated was filtered to give (4-methyl-2*H*-benzopyran-2-one-7-yloxy)acetohydrazide (3) as white solid. Yield 4.270g (75%), M. P. 196°C-198°C, R_f=0.34 in chloroform/methanol (98: 2) as developing solvent system; IR (KBr) ν_{max}: 3266, 3083, 1731, 1676, 1509, 1439, 1284, 1153, 1074, 815, 740 cm^{-1}; ^{1}H-NMR (δ, $CDCl_3$, 400 MHz): 8.55 (brs, 1H, D_2O exchangeable, >NH), 7.43 (d, 1H, J=8.8 Hz), 6.81 (d, 1H, J=8.8 Hz), 6.75 (s, 1H), 6.05 (s, 1H), 4.51 (s, 2H, -OCH_2-), 2.47 (brs, 2H, D_2O exchangeable, -NH_2) 2.30 (s, 3H, -CH_3); ^{13}C-NMR (δ, $CDCl_3$, 100 MHz): 171.9 (>C=O), 161.8, 159.4, 139.8, 128.8, 125.3, 123.4, 112.3, 111.7, 101.5, 66.5 (-OCH_2-), 18.0 (-CH_3). Mass spectral data, TOF ES^+ m/z (%): 249 (M^++1). Molecular formula: $C_{12}H_{12}N_2O_4$ Elemental Anal. Calcd.: C, 58.06; H, 4.87; N, 11.29; Found: C, 58.10; H, 4.88; N, 11.26.

General procedure for the synthesis of 1,2,4-triazole. A mixture of (4-methyl-2*H*-benzopyran-2-one-7-yloxy)acetohydrazide (3) (1.0 mmol) and aromatic aldehydes/heteroaromatic aldehydes (1.0 mmol) was dissolved in 10 ml of glacial acetic acid. To the mixture ammonium acetate was added. The reaction mixture was stirred for a period of 6-8 h at room temperature. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was poured into ice cold water and neutralize with ammonia. The solid that separated was filtered, washed with water to give the desired product. R_f was recorded in chloroform/methanol (95: 5) as developing solvent system.

4-Methyl-7-((5-phenyl-4H-1,2,4-triazol-3-yl)methoxy)-2H-benzopyran-2-one (14)

White solid, Yield: 96%, M. P. 220°C-222°C; IR (KBr) ν_{max}: 3421, 3075, 1711, 1687, 1511, 1435, 1272, 1161, 1085, 836, 688 cm^{-1}; ^{1}H-NMR (δ, DMSO-d_6, 400 MHz): 11.63 (brs, 1H, D_2O exchangeable, >NH), 7.64 (d, 2H, J=7.0 Hz), 7.57 (d, 1H, J=8.8 Hz), 7.37-7.35 (m, 3H), 6.94-6.92 (m, 1H), 6.85 (s, 1H), 6.09 (s, 1H), 5.20 (s, 2H, -OCH_2-), 2.37 (s, 3H, -CH_3); ^{13}C-NMR (δ, DMSO-d_6, 100 MHz): 168.0 (>C=O), 161.0, 159.5, 154.3, 152.6, 147.7, 144.0, 133.3, 129.8, 128.4, 126.7, 126.3, 125.7, 113.0, 111.8, 111.2, 101.2, 64.8 (-OCH_2), 18.0 (-CH_3). Mass spectral data, TOF ES^+ m/z (%): 333 (M^+). Molecular formula: $C_{19}H_{15}N_3O_3$ Elemental Anal. Calcd.: C, 68.46; H, 4.54; N, 12.61; Found: C, 68.44; H, 4.58; N, 12.58.

4-Methyl-7-((5-(4-methylphenyl)-4H-1,2,4-triazol-3-yl)methoxy)-2H-benzopyran-2-one (15)

White solid, Yield: 90%, M. p. 222°C-224°C; IR (KBr) ν_{max}: 3402, 2925, 1713, 1689, 1617, 1414, 1272, 1158, 1083, 835, 663 cm^{-1}; ^{1}H-NMR (δ, DMSO-d_6, 400 MHz): 11.60 (brs, 1H, D_2O exchangeable, >NH), 8.30-8.17 (m, 1H), 7.79 (s, 1H), 7.78-7.59 (m, 2H), 7.34-7.23 (m, 2H), 6.99-6.90 (m, 1H), 6.16 (s, 1H), 5.25 (s, 2H, -OCH_2-), 2.53 (s, 3H, -CH_3), 2.36 (s, 3H, -CH_3); ^{13}C-NMR (δ, DMSO-d_6, 100 MHz): 168.0 (>C=O), 160.7, 154.4, 152.5, 147.5, 144.2, 138.9, 130.6, 129.1, 126.7, 125.6, 113.3, 111.8, 111.2, 101.1, 64.7, 20.9, 18.0. Mass spectral data, TOF ES^+ m/z (%): 348 (M^++1). Molecular formula: $C_{20}H_{17}N_3O_3$ Elemental Anal. Calcd.: C, 69.15; H, 4.93; N, 12.10; Found: C, 69.18; H, 4.94; N, 12.08.

4-Methyl-7-((5-(4-methoxyphenyl)-4H-1,2,4-triazol-3-yl)methoxy)-2H-benzopyran-2-one(16)

White solid, Yield: 95%, M. P. 242°C-244°C; IR (KBr) ν_{max}: 3315, 3037, 2918, 1686, 1628, 1608, 1541, 1509, 1304, 1257, 1153, 1085, 1021, 834, 810, 578 cm^{-1}; ^{1}H-NMR (δ, DMSO-d_6, 400 MHz): 11.54 (brs, 1H, D_2O exchangeable, >NH), 7.95 (s, 1H), 7.67-7.65 (m, 3H), 7.01-6.98 (m, 3H), 6.21 (s, 1H), 5.26 (s, 2H, -OCH_2-), 3.79 (s, 3H, -OCH_3), 2.39 (s, 3H, -CH_3); ^{13}C-NMR (δ, DMSO-d_6, 100 MHz): 168.2 (>C=O), 161.2, 160.3, 159.9, 154.5, 153.1, 143.3, 129.1, 128.4, 126.8, 126.1, 125.9, 114.3, 113.3, 112.2, 110.9, 101.2, 66.5 (-OCH_2-), 54.9, 17.5 (-CH_3). Mass spectral data, TOF ES^+ m/z (%): 364 (M^++1). Molecular formula: $C_{20}H_{17}N_3O_4$ Elemental Anal. Calcd.: C, 66.11; H, 4.72; N, 11.56; Found: C, 66.12; H, 4.70; N, 11.57.

4-Methyl-7-((5-(4-fluorophenyl)-4H-1,2,4-triazol-3-yl)methoxy)-2H-benzopyran-2-one (17)

White solid, Yield: 96%, M. P. 254°C-256°C; IR (KBr) ν_{max}: 3422, 3085, 2927, 1708, 1685, 1393, 1272, 1162, 1087, 839 cm^{-1}; ^{1}H-NMR (δ, DMSO-d_6, 400 MHz): 11.65 (brs, 1H, D_2O exchangeable, >NH), 8.00 (s, 1H), 7.77 (t, 2H, J=8.8 Hz), 7.67 (d, 1H, J=8.8 Hz), 7.28 (t, 2H, J=8.8 Hz), 6.97 (s, 1H), 6.21 (s, 1H), 5.29 (s, 2H, -OCH_2-), 2.35 (s, 3H, -CH_3); ^{13}C-NMR (δ, DMSO-d_6, 100 MHz): 167.9 (>C=O), 161.4, 159.8, 154.5, 153.0, 148.2, 142.1, 133.6, 133.2, 130.6, 128.7, 126.8, 126.1, 115.7, 113.0, 111.0, 101.6, 65.0 (-OCH_2-), 17.8 (-CH_3). Mass spectral data, TOF ES^+ m/z (%): 352 (M^++1). Molecular formula: $C_{19}H_{14}N_3O_3F$. Elemental Anal. Calcd.: C, 64.95; H, 4.02; N, 11.96; Found: C, 64.98; H, 4.00; N, 11.95.

4-Methyl-7-((5-(3,4-dimethoxyphenyl)-4H-1,2,4-triazol-3-yl)methoxy)-2H-benzopyran-2- one (18)

White solid, Yield: 92%, M. P. 172°C-174°C; IR (KBr) ν_{max}: 3448, 3089, 1715, 1683, 1510, 1392, 1148, 1081, 841, 759 cm^{-1}; ^{1}H-NMR (δ, DMSO-d_6, 400 MHz): 11.53 (brs, 1H, D_2O exchangeable, >NH), 7.94 (s, 1H), 7.58 (d, 1H, J=8.8 Hz), 7.15 (d, 1H, J=8.8 Hz), 6.96-6.91(m, 2H), 6.84 (s, 1H), 6.11 (s, 1H), 5.23 (s, 2H, -OCH_2-), 3.90 (brs, 6H, 2×-OCH_3), 2.42 (s, 3H, -CH_3); ^{13}C-NMR (δ, DMSO-d_6, 100 MHz): 168.5 (>C=O), 161.4, 160.2, 154.9, 152.8, 149.1, 147.9, 145.6, 125.8, 125.7, 121.9, 115.3, 112.8, 112.5, 111.7, 108.9, 101.7, 65.2 (-OCH_2-), 55.8, 49.6, 17.8 (-CH_3). Mass spectral data, TOF ES^+ m/z (%): 394 (M^++1). Molecular formula: $C_{21}H_{19}N_3O_5$ Elemental Anal. Calcd.: C, 64.12; H, 4.87; N, 10.68; Found: C, 64.14; H, 4.84; N, 10.70.

4-Methyl-7-((5-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazol-3-yl)methoxy)-2H-benzopyran-2-one (19)

White solid, Yield: 98%, M. P. 148°C-150°C; IR (KBr) ν_{max}: 3446, 2922, 1720, 1686, 1502, 1390, 1153, 1122, 1085, 838 cm^{-1}; ^{1}H-NMR (δ, DMSO-d_6, 400 MHz): 11.48 (brs, 1H, D_2O exchangeable, >NH), 7.79 (s, 1H), 7.47-7.44 (m, 1H), 6.84 (d, 1H, J=7.3 Hz), 6.78 (s, 1H), 6.72 (s, 1H), 6.00 (s, 1H), 5.11 (s, 2H, -OCH_2-), 3.77 (brs, 6H, 2×-OCH_3), 3.71 (s, 3H, -OCH_3), 2.30 (s, 3H, -CH_3); ^{13}C-NMR (δ, DMSO-d_6, 100 MHz): 168.3 (>C=O), 161.5, 160.6, 155.1, 153.2, 151.9, 144.8, 139.3, 129.2, 126.6, 125.7, 124.2, 113.7, 111.4, 101.2, 65.6 (-OCH_2-), 60.8, 55.9, 18.5 (-CH_3). Mass spectral data, TOF ES^+ m/z (%): 424 (M^++1). Molecular formula: $C_{22}H_{21}N_3O_6$ Elemental Anal. Calcd.: C, 62.41; H, 5.00; N, 9.92; Found: C, 62.38; H, 5.02; N, 9.94.

4-Methyl-7-((5-(4-hydroxyphenyl)-4H-1,2,4-triazol-3-yl)methoxy)-2H-benzopyran-2-one (20)

White solid, Yield: 98%, M. P. 248°C-250°C; IR (KBr) ν_{max}: 3305, 2915, 1702, 1664, 1550, 1390, 1273, 1153, 1083, 833 cm^{-1}; ^{1}H-NMR (δ, DMSO-d_6, 400 MHz): 11.42 (brs, 1H, D_2O exchangeable, >NH), 9.82 (brs, 1H, D_2O exchangeable, -OH), 7.91 (s, 1H), 7.62 (d, 1H, J=8.8 Hz), 7.53 (t, 2H, J=8.8 Hz), 6.97 (s, 1H), 6.87-6.81 (m, 2H), 6.13 (s, 1H), 5.21 (s, 2H, -OCH_2-), 2.41 (s, 3H, -CH_3); ^{13}C-NMR (δ, DMSO-d_6, 100 MHz): 168.1 (>C=O), 161.4, 160.2, 159.2, 154.7, 152.7, 144.9, 129.0, 128.6, 125.8, 124.7, 115.6, 113.6, 112.3, 111.9, 111.5, 101.5, 65.1 (-OCH_2-), 18.3 (-CH_3). Mass spectral data, TOF ES^+ m/z (%): 350 (M^++1). Molecular formula: $C_{19}H_{15}N_3O_4$ Elemental Anal. Calcd.: C, 65.32; H, 4.33; N, 12.03; Found: C, 65.29; H, 4.36; N, 12.02.

4-Methyl-7-((5-(4-hydroxy-3-methoxyphenyl)-4H-1,2,4-triazol-3-yl)methoxy)-2H-benzopyran-2-one (21)

White solid, Yield: 97%, M. P. 190°C-192°C; IR (KBr) ν_{max}: 3450, 3294, 2924, 1689, 1508, 1389, 1152, 1081, 809, 748 cm^{-1}; ^{1}H-NMR (δ, DMSO-d_6, 400 MHz): 11.46 (brs, 1H, D_2O exchangeable, >NH), 9.52 (brs, 1H, D_2O exchangeable, -OH), 7.89 (s, 1H), 7.68 (t, 1H, J=8.8 Hz), 7.26 (d, 1H, J=8.0 Hz), 7.08-7.04 (m, 1H), 6.99-6.92 (m, 1H), 6.79 (d, 1H, J=8.0 Hz), 6.19 (s, 1H), 5.27 (s, 2H, -OCH_2-), 3.79 (s, 3H, -OCH_3), 2.41 (s, 3H, -CH_3); ^{13}C-NMR (δ, DMSO-d_6, 100 MHz): 167.9 (>C=O), 162.7, 161.1, 159.6, 154.3, 153.5, 149.0, 148.1, 144.6, 126.4, 125.5, 120.8, 113.5, 112.1, 109.6, 109.0, 101.6, 64.8 (-OCH_2-), 55.1, 18.1 (-CH_3). Mass spectral data, TOF ES^+ m/z (%): 380 (M^++1). Molecular formula: $C_{20}H_{17}N_3O_5$ Elemental Anal. Calcd.: C, 63.32; H, 4.52; N, 11.08; Found: C, 63.30; H, 4.48; N, 11.12.

4-Methyl-7-((5-(4-chlorophenyl)-4H-1,2,4-triazol-3-yl)methoxy)-2H-benzopyran-2-one (22)

White solid, Yield: 90%, M. P. 262°C-264°C; IR (KBr) ν_{max}: 3421, 3067, 1710, 1687, 1391, 1272, 1159, 1086, 978, 834, 615 cm^{-1}; ^{1}H-NMR (δ, DMSO-d_6, 400 MHz): 11.65 (brs, 1H, D_2O exchangeable, >NH), 7.98 (s, 1H), 7.69 (d, 2H, J=8.8 Hz), 7.64 (t, 1H, J=8.8 Hz), 7.42 (d, 2H, J=8.8 Hz), 6.96-6.91 (m, 1H), 6.14 (s, 1H), 5.24 (s, 2H, -OCH_2-), 2.38 (s, 3H, -CH_3); ^{13}C-NMR (δ, DMSO-d_6, 100 MHz): 168.5 (>C=O), 165.0, 161.0, 159.5, 154.4, 152.6, 142.7, 133.9, 130.6, 128.8, 128.0, 125.8, 125.6, 113.3, 111.8, 110.9, 101.0, 64.7 (-OCH_2-), 17.5 (-CH_3). Mass spectral data, TOF ES^+ m/z (%): 369 (M^++1). Molecular formula: $C_{19}H_{14}N_3O_3Cl$. Elemental Anal. Calcd.: C, 62.05; H, 3.84; N, 11.43; Found: C, 62.10; H, 3.79; N, 11.40.

4-Methyl-7-((5-(2-hydroxyphenyl)-4H-1,2,4-triazol-3-yl)methoxy)-2H-benzopyran-2-one (23)

White solid, Yield: 91%, M. P. 278°C-280°C; IR (KBr) ν_{max}: 3284, 3101, 2915, 1724, 1684, 1538, 1393, 1276, 1079, 842, 748 cm^{-1}; ^{1}H-NMR (δ, DMSO-d_6, 400 MHz): 11.82 (brs, 1H, D_2O exchangeable, >NH), 11.02 (brs, 1H, D_2O exchangeable, -OH), 8.51 (s, 1H), 7.68-7.61 (m, 1H), 7.26-7.19 (m, 1H), 7.03-6.93 (m, 2H), 6.90-6.82 (m, 2H), 6.14 (s, 1H), 4.79 (s, 2H, -OCH_2-), 2.38 (s, 3H, -CH_3); ^{13}C-NMR (δ, DMSO-d_6, 100 MHz): 167.7 (>C=O), 162.9, 160.8, 160.4, 159.5, 158.6, 157.5, 154.2, 149.2, 131.4, 129.1, 125.4, 118.9, 116.0, 112.1, 111.2, 101.5, 65.0 (-OCH_2-), 17.7 (-CH_3). Mass spectral data, TOF ES^+ m/z (%): 350 (M^++1). Molecular formula: $C_{19}H_{15}N_3O_4$ Elemental Anal. Calcd.: C, 65.32; H, 4.33; N, 12.03; Found: C, 65.29; H, 4.38; N, 12.01.

4-Methyl-7-((5-(2-thiophenyl)-4H-1,2,4-triazol-3-yl)methoxy)-2H-benzopyran-2-one (26)

White solid, Yield: 92%, M. P. 232°C-234°C; IR (KBr) ν_{max}: 3421, 3084, 2923, 1712, 1685, 1390, 1272, 1160, 1085, 838, 767 cm^{-1}; ^{1}H-NMR (δ, DMSO-d_6, 400 MHz): 11.49 (brs, 1H, D_2O exchangeable, >NH), 8.05 (s, 1H), 7.46 (d, 1H, J=9.5 Hz), 7.29 (s, 1H), 7.16 (s, 1H), 6.95 (s, 1H), 6.84 (s, 1H), 6.00 (s, 1H), 5.04 (s, 2H, -OCH_2-), 2.31 (s, 3H, -CH_3); ^{13}C-NMR (δ, DMSO-d_6, 100 MHz): 168.1 (>C=O), 161.4, 159.2, 154.7, 152.1, 144.9, 129.0, 128.6, 125.8, 124.7, 115.6, 113.6, 112.3, 111.5, 101.5, 65.1 (-OCH_2-), 18.3 (-CH_3). Mass spectral data, TOF ES^+ m/z (%): 340 (M^++1). Molecular formula: $C_{17}H_{13}N_3O_3S$. Elemental Anal. Calcd.: C, 60.17; H, 3.86; N, 12.38; Found: C, 60.20; H, 3.84; N, 12.35.

4-Methyl-7-((5-(2-furanyl)-4H-1,2,4-triazol-3-yl)methoxy)-2H-benzopyran-2-one (27)

White solid, Yield: 94%, M. P. 202°C-204°C; IR (KBr) ν_{max}: 3447, 3056, 2926, 1709, 1686, 1275, 1162, 1084, 845, 795 cm^{-1}; ^{1}H-NMR (δ, DMSO-d_6, 400 MHz): 11.52 (brs, 1H, D_2O exchangeable, >NH), 7.82 (s, 1H), 7.74-7.65 (m, 1H), 7.54-7.49 (m, 1H), 6.94-6.86 (m, 1H), 6.74-6.66 (m, 1H), 6.43 (s, 1H), 6.03 (s, 1H), 5.08 (s, 2H, -OCH_2-), 2.33 (s, 3H, -CH_3); ^{13}C-NMR (δ, DMSO-d_6, 100 MHz): 169.4 (>C=O), 163.8, 161.0, 153.7, 149.6, 148.1, 143.7, 138.3, 133.7, 129.8, 126.4, 122.1, 121.1, 111.3, 109.7, 64.7 (-OCH_2-), 17.8 (-CH_3). Mass spectral data, TOF ES^+ m/z (%): 324 (M^++1). Molecular formula: $C_{17}H_{13}N_3O_4$ Elemental Anal. Calcd.: C, 63.16; H, 4.05; N, 13.00; Found: C, 63.15; H, 4.04; N, 13.02.

4-Methyl-7-((5-(4-oxo-4H-chromen-3-yl)-4H-1,2,4-triazol-3-yl)methoxy)-2H-benzopyran-2-one (29)

White solid, Yield: 94%, M. P. 270°C-272°C; IR (KBr) ν_{max}: 3422, 3067, 2921, 1719, 1677, 1613, 1510, 1440, 1388, 1286, 1205, 1154, 1077, 1014, 809, 748, 583 cm^{-1}; ^{1}H-NMR (δ, DMSO-d_6, 400 MHz): 11.68 (brs, 1H, D_2O exchangeable, >NH), 8.09 (t, 1H, J=7.3 Hz), 7.75 (t, 1H, J=8.8 Hz), 7.57 (d, 2H, J=8.8 Hz), 7.44 (t, 1H, J=8.8 Hz), 6.94-6.92 (m, 1H), 6.87-6.85 (m, 1H), 6.09-6.04 (m, 2H), 4.55 (s, 2H, -OCH_2-), 2.36 (s, 3H, -CH_3); ^{13}C-NMR (δ, DMSO-d_6, 100 MHz): 191.4 (>C=O), 165.2 (>C=O), 160.2, 159.8, 154.1, 151.9, 134.3, 130.2, 125.8, 122.2, 121.1, 120.1, 120.0, 118.8, 117.1, 113.4, 112.4, 112.2, 111.6, 101.4, 65.7 (-OCH_2-), 18.1 (-CH_3). Mass spectral data, TOF ES^+ m/z (%): 402 (M^++1). Molecular formula: $C_{22}H_{15}N_3O_5$ Elemental Anal. Calcd.: C, 65.83; H, 3.77; N, 10.47; Found: C, 65.86; H, 3.78; N, 10.44.

RESULTS AND DISCUSSION

To develop a lead molecule for future, a series of new 4-methyl-7-triazolylmethoxy-2*H*-benzopyran-2-ones 14-23, 26-27 and 29 have been synthesized. All the synthesized compounds along with intermediates were fully characterized by their detailed spectral studies such as Infrared (IR), Proton Nuclear Magnetic Resonance (^{1}H-NMR), Carbon-13 Nuclear Magnetic Resonance (^{13}C-NMR) and Mass.

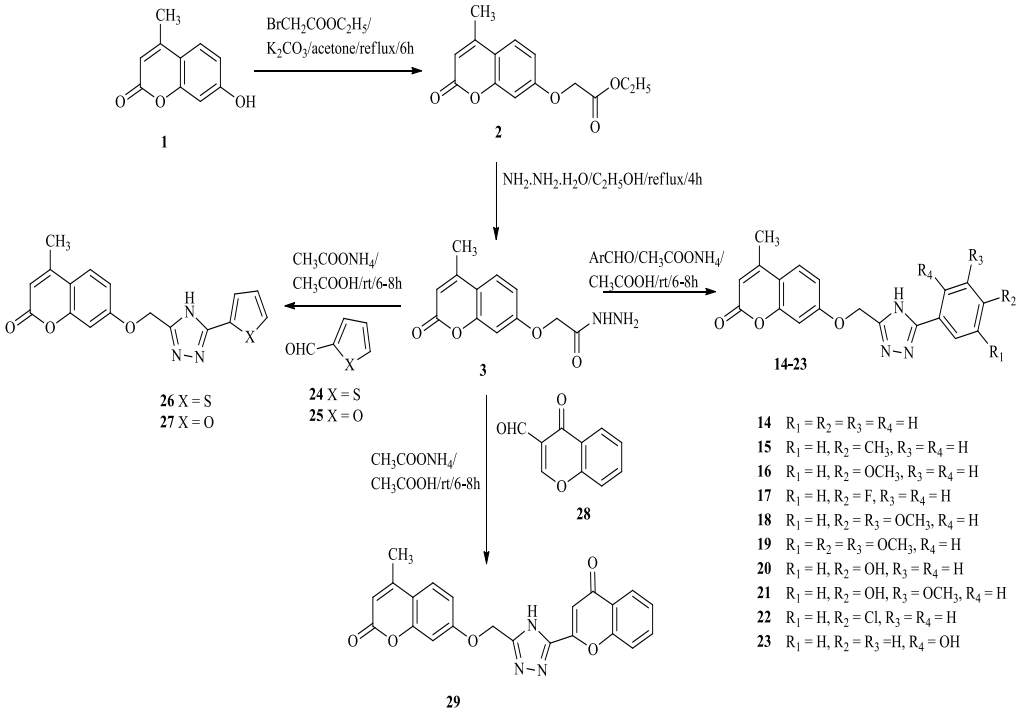
7-Hydroxy-4-methyl-2*H*-benzopyran-2-one (1) was chosen as the starting material and was reacted with ethyl bromoacetate in the presence of anhydrous potassium carbonate in acetone under refluxing conditions to give ethyl-(4-methyl-2H-benzopyran-2-one-7-yloxy)acetate (2) as a

white solid having M. P. 86°C-88°C. Its IR spectrum showed absorption band at 1758 cm^{-1}, characteristic for an ester carbonyl. Further its ^{1}H-NMR showed a quartet of two protons at δ (ppm)=4.26 and triplet for three protons at δ (ppm)=1.29 and its ^{13}C-NMR displayed peaks at δ (ppm)=61.5, 14.0 confirming the formation of an ester. Thus benzopyran (1) and ethyl bromoacetate have coupled together with the loss of HBr. The methylene flanked between benzopyran and an ester moiety appeared at δ (ppm)=4.66 as singlet for two protons in ^{1}H-NMR and at δ (ppm)=65.1 in ^{13}C-NMR. Finally mass spectrum confirmed compound 2 to be ethyl-(4-methyl-2H-benzopyran-2-one-7-yloxy)acetate as showed M^++1 at m/z 263 in TOF ES^+ corresponding to molecular formula $C_{14}H_{14}O_5$. 2 was then refluxed with equimolar amount of hydrazine hydrate in ethanol to give a white solid having M. P. 196°C-198°C and characterized as (4-methyl-2*H*-benzopyran-2-one-7-yloxy)acetohydrazide (3). It showed the absence of protons for the ester ethyl group but instead displayed D_2O exchangeable broad singlet's at δ (ppm)=8.55 (1H) and δ (ppm)=2.47 (2H) corresponding to -$NHNH_2$ in its ^{1}H-NMR spectrum. Its IR spectrum showed absorption band at 3266 & 3083 cm^{-1} and at 1676 cm^{-1} which may correspond to -$NHNH_2$ and hydrazidic carbonyl respectively. This indicated that ester has been converted into hydrazide and compound 3 was characterized as (4-methyl-2*H*-benzopyran-2-one-7-yloxy)acetohydrazide, which was also confirmed by its mass spectrum, showing M^++1 at m/z 249 in TOF ES^+ corresponding to molecular formula $C_{12}H_{12}N_2O_4$.

In the final step, (4-methyl-2*H*-benzopyran -2-one-7-yloxy)acetohydrazide (3) was reacted with equimolar amount of benzaldehyde (4) in the presence of ammonium acetate in acetic acid at room temperature to yield 4-methyl-7-((5-phenyl-4*H*-1,2,4-triazol-3-yl)methoxy)-2*H*-benzopyran-2-one (14) as white solid in 96% yield, having M. P. 220°C-222°C. Its IR spectrum showed absence of bands for hydrazidic carbonyl and –$NHNH_2$ indicating that hydrazide has reacted to form triazole ring as indicated by absorption band at 3421 cm^{-1} corresponding to >NH of the triazole ring. The >NH was further confirmed by a D_2O exchangeable broad singlet at δ (ppm)=11.63 for one proton in its ^{1}H-NMR. Further its ^{1}H-NMR showed usual peaks for benzopyran ring at δ (ppm)=7.57 as doublet for one proton, δ (ppm)=6.85 as singlet for one proton and at δ (ppm)=6.09 as singlet for one proton along with a doublet at δ (ppm)=7.64 for two protons and multiplets at δ (ppm)=7.37-7.35 for three protons and also multiplet at δ (ppm)=6.94-6.92 for one proton. A singlet at δ (ppm)=5.20 for two protons in ^{1}H-NMR corresponded to the -OCH_2 flanked between benzopyran and triazole which also appeared at δ (ppm)=64.8 in ^{13}C-NMR. Its ^{13}C-NMR displayed peaks at δ (ppm)=168.0, 161.0, 159.5, 154.3, 152.6, 147.7, 144.0, 133.3, 129.8, 128.4, 126.7, 126.3, 125.7, 113.0, 111.8, 111.2 and 101.2 in the aromatic region indicating the presence of benzopyran, phenyl and triazole moieties. Finally, in its TOF ES^+ it showed M^+ at m/z 333 corresponding to the molecular formula $C_{19}H_{15}N_3O_3$, confirming that acetohydrazide and benzaldehyde have reacted together and underwent intermolecular cyclization to give 14, characterized as 4-methyl-7-(5-phenyl-4*H*-1,2,4-triazol-3-yl)methoxy)-2*H*-benzopyran-2-one on the basis of above spectral details.

The above reaction was similarly carried out taking (4-methyl-2*H*-benzopyran-2-one-7-yloxy)acetohydrazide (3) and substituted aromatic aldehydes (4-13) and heteroaromatic aldehydes (24-25 and 28) separately to obtain the corresponding novel 1,2,4-triazolylbenzopyran-2-ones (14-23, 26-27 and 29).

The synthetic schemes are outlined below:



Antimicrobial activity

The antibacterial and antifungal activities of all the newly synthesized compounds were evaluated *in vitro* using Cup-plate method [39-41] against three Gram-positive bacterial strains *Staphylococcus aureus* (MTCC 096), *Bacillus subtilis* (MTCC 441), *Staphylococcus epidermis* (MTCC 435), four Gram-negative bacterial strains *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 424), *Salmonella typhi* (MTCC 733) and *Klebsiella pneumoniae* (MTCC 432) and four fungal strains *Aspergillus niger* (MTCC 282), *Aspergillus fumigates* (MTCC 343), *Aspergillus flavus* (MTCC 277) and *Candida albicans* (MTCC 227). Both antibacterial and antifungal activities were assessed by Minimum Inhibitory Concentration (MIC) by serial dilution method [42] and by measuring zone of inhibition [43]. Each compound was tested at various concentrations (100, 50, 25, 12.5 and 6.25 µg/ml) by serially diluted Dimethyl sulfoxide (DMSO) from the stock solution and procedure used from reported method. Clinically antimicrobial drugs Ciprofloxacin and Miconazole were used as the positive control and DMSO was used for blank. The experiments were repeated three times, and the average values are presented in Tables 1 and 2 with their graphical representation in Figures 1-4.

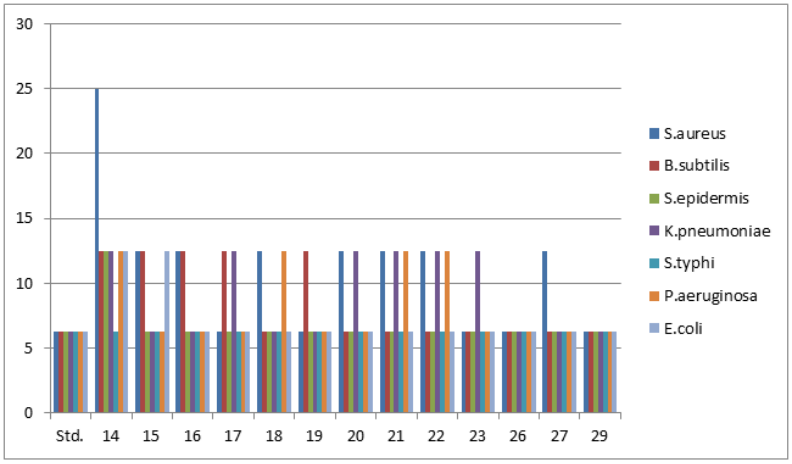


Figure 1: MIC Value for antibacterial activities

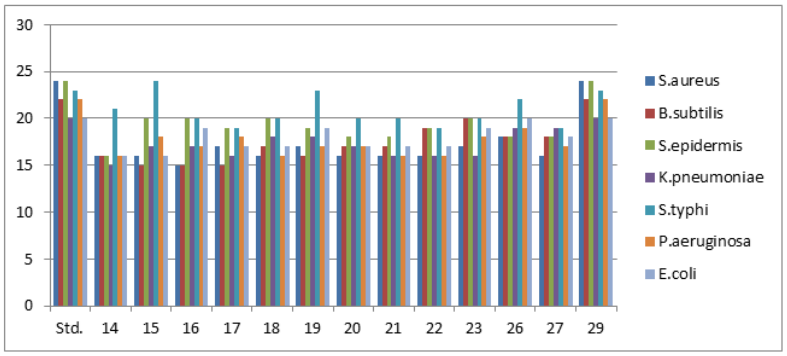


Figure 2: Zone of inhibition for antibacterial activities

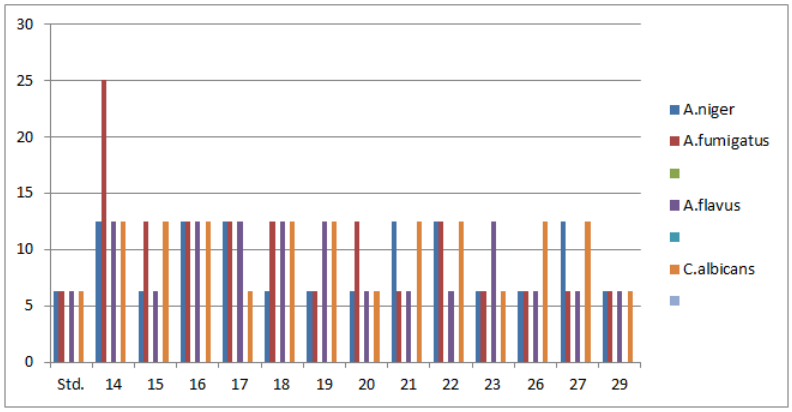


Figure 3: MIC Value for antifungal activities

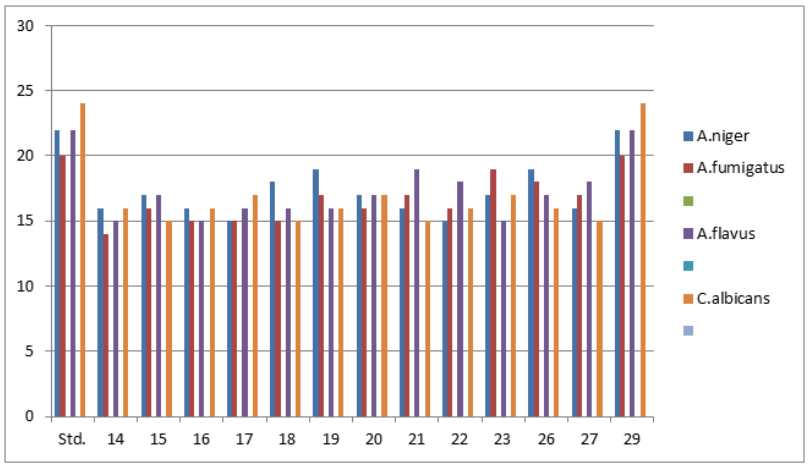


Figure 4: Zone of inhibition for antifungal activities

The obtained results, depicted in Tables 1 and 2, revealed that:

In antibacterial studies, all tested compounds have shown moderate to significant antibacterial activity against *S. aureus* while these compounds possessed moderate antibacterial activity against *B. subtilis*. Against *S. epidermis*, tested compounds have shown significant to good antibacterial activity. All compounds have shown promising antibacterial activity against *K. pneumonia* and these compounds possessed significant to good antibacterial activity against *S. typhi* while all tested compounds possessed almost promising to very good antibacterial activity against *P. aeruginosa*. All tested compounds possessed moderate to good antibacterial.

In antifungal studies, all tested compounds have shown significant to promising antifungal activity against *A. niger* and compounds 15, 18-20, 26 and 29 possessed nearly comparable antifungal activity. All compounds have shown promising to very good antifungal activity against *A. fumigates*. All tested compounds showed moderate to significant antifungal activity against *A. flavus* while compounds 15, 21-22 and 26, 27 and 29 possessed promising antifungal activity. Most of the compounds have shown promising antifungal activity against *C. albicans*.

Table 1: MIC Value (Zone of inhibition) for the antibacterial activity

S. No.	Sample Code	Gram +ve Bacteria			Gram -ve Bacteria			
		Staphylococcus aureus	*Bacillus subtilis*	*Staphylococcus epidermis*	*Klebsiella pneumoniae*	*Salmonella typhi*	*Pseudomonas aeruginosa*	*Escherichia coli*
1	Std.	6.25 (22-24)	6.25 (20-22)	6.25 (22-24)	6.25 (18-20)	6.25 (21-23)	6.25 (20-22)	6.25 (18-20)
2	14	25 (12-14)	12.5 (14-16)	12.5 (14-16)	12.5 (13-15)	6.25 (19-21)	12.5 (14-16)	12.5 (14-16)
3	15	12.5 (14-16)	12.5 (13-15)	6.25 (18-20)	6.25 (15-17)	6.25 (22-20)	6.25 (16-18)	12.5 (14-16)
4	16	12.5 (13-15)	12.5 (13-15)	6.25 (18-20)	6.25 (15-17)	6.25 (18-20)	6.25 (15-17)	6.25 (17-19)
5	17	6.25 (15-17)	12.5 (13-15)	6.25 (17-19)	12.5 (14-16)	6.25 (17-19)	6.25 (16-18)	6.25 (15-17)
6	18	12.5 (14-16)	6.25 (15-17)	6.25 (18-20)	6.25 (16-18)	6.25 (18-20)	12.5 (14-16)	6.25 (15-17)
7	19	6.25 (15-17)	12.5 (14-16)	6.25 (17-19)	6.25 (16-18)	6.25 (21-23)	6.25 (15-17)	6.25 (17-19)
8	20	12.5 (14-16)	6.25 (15-17)	6.25 (16-18)	12.5 (15-17)	6.25 (18-20)	6.25 (15-17)	6.25 (15-17)
9	21	12.5 (14-16)	6.25 (15-17)	6.25 (16-18)	12.5 (14-16)	6.25 (18-20)	12.5 (14-16)	6.25 (15-17)
10	22	12.5 (14-16)	6.25 (17-19)	6.25 (17-19)	12.5 (14-16)	6.25 (17-19)	12.5 (14-16)	6.25 (15-17)
11	23	6.25 (15-17)	6.25 (18-20)	6.25 (18-20)	12.5 (14-16)	6.25 (18-20)	6.25 (16-18)	6.25 (17-19)
12	26	6.25 (16-18)	6.25 (16-18)	6.25 (16-18)	6.25 (17-19)	6.25 (20-22)	6.25 (17-19)	6.25 (18-20)
13	27	12.5 (14-16)	6.25 (16-18)	6.25 (16-18)	6.25 (17-19)	6.25 (17-19)	6.25 (15-17)	6.25 (16-18)
14	29	6.25 (22-24)	6.25 (20-22)	6.25 (22-24)	6.25 (18-20)	6.25 (21-23)	6.25 (20-22)	6.25 (18-20)

Standard drug for bacteria: Ciprofloxacin; *Zone of Inhibition (Internal diameter: 6 mm)

Table 2: MIC Value (Zone of inhibition) for the Antifungal activity

S. No.	Sample Code	Fungi			
		Aspergillus niger	*Aspergillus fumigatus*	*Aspergillus flavus*	*Candida albicans*
1	Std.	6.25 (20-22)	6.25 (18-20)	6.25 (20-22)	6.25 (22-24)
2	14	12.5 (14-16)	25 (12-14)	12.5 (13-15)	12.5 (14-16)
3	15	6.25 (15-17)	12.5 (14-16)	6.25 (15-17)	12.5 (13-15)
4	16	12.5 (14-16)	12.5 (13-15)	12.5 (13-15)	12.5 (14-16)
5	17	12.5 (13-15)	12.5 (13-15)	12.5 (14-16)	6.25 (15-17)
6	18	6.25 (16-18)	12.5 (13-15)	12.5 (14-16)	12.5 (13-15)
7	19	6.25 (17-19)	6.25 (15-17)	12.5 (14-16)	12.5 (14-16)
8	20	6.25 (15-17)	12.5 (14-16)	6.25 (15-17)	6.25 (15-17)
9	21	12.5 (14-16)	6.25 (15-17)	6.25 (17-19)	12.5 (13-15)
10	22	12.5 (13-15)	12.5 (14-16)	6.25 (16-18)	12.5 (14-16)
11	23	6.25 (15-17)	6.25 (17-19)	12.5 (13-15)	6.25 (15-17)
12	26	6.25 (17-19)	6.25 (16-18)	6.25 (15-17)	12.5 (14-16)
13	27	12.5 (14-16)	6.25 (15-17)	6.25 (16-18)	12.5 (13-15)
14	29	6.25 (20-22)	6.25 (18-20)	6.25 (20-22)	6.25 (22-24)

Standard drug for fungi: Miconazole; * Zone of Inhibition (Internal diameter: 6 mm)

CONCLUSIONS

A new series of 4-methyl-7-triazolylmethoxy-2H-benzopyran-2-ones were synthesized. All synthesized compounds were evaluated for their *in vitro* antibacterial and antifungal activity using Cup-plate method against seven strains of bacteria and four strains of fungi. The MIC results of all the synthesized compounds (14-23, 26,27 and 29) have shown promising to comparable activities to that of standards. Some of the compounds behaves like the known drugs towards various stains *in vitro* and hence are good candidates for trial in future *in vivo* to develop a pharmacophore to prevent various infections caused by bacteria, parasites, viruses and fungi.

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