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Synthesis, Characterization and Cytotoxic Investigations of Novel C3-Dihydrofuran Substituted 1*H*-benzo[*g*]chromene-2,5,10-triones besides Antimicrobial study

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A series of novel C3-dihydrofuran substituted 1*H*-benzo[*g*]chromene-2,5,10-triones were synthesized and characterized by spectral analysis. The target molecules were screened for their antimicrobial and anticancer activities and structure and activity relationship (SAR) was investigated. Structure and activity relationship studies revealed that the compounds **6p**, **6q**, **6s**, **6t** were found to be more active in antimicrobial screening. Antiproliferative properties were evaluated against human cancer cell lines, namely, laryngeal carcinoma (Hep2), lung adenocarcinoma (A549) and cervical cancer (HeLa). The best among them, C3-dihydrofuran substituted 1*H*-benzo[*g*]chromene-2,5,10-trione with methoxy group substitution at ring A and B were selected for further structure activity relationship. Among the derivatives, (2*S*,3*S*)-propyl-4-acetyl-5-(7,9-dimethoxy-2,5,10-trioxo-5,10-dihydro-2*H*-benzo[*g*]chromen-3-yl)-3-(3,5-dimethoxyphenyl)-2,3-dihydrofuran-2-carboxylate (**6t**) showed most potent cytotoxic activity against all the three cancer cell lines. Toxicity studies revealed that dihydrofuran substituted 1*H*-benzo[*g*] chromene-2,5,10-triones (**6a-t**) are specifically target the cancer cell lines.

Keywords: Multicomponent, Antiprolifirative activity, Dihydrofuran substituted benzochromene trione, Antimicrobial.

INTRODUCTION

Naturally occurring chromenes show a broad spectrum of biological activities. This moity is core fragment of different natural products including pyranokunthone A and B, lambertellin B, β-lepachone C and α-xiloidone D [1-5]. Some derivatives of benzo[*g*]chromenes such as compounds **E** have been isolated from marine acetino mycete CNQ-525 bacteria (Fig. 1). Doxorubicin **F** commercially available anticancer drug which contains naphthoquinonechromene moity. These later materials have shown significant anticancer and antibacterial activities [6,7]. Because of broad pharmacological activities of benzo[*g*]chromenes, different synthetic methods have been introduced by various research groups [7-10]. Usually these synthetic approaches are including a multistep procedure. A semi synthetic method has also been introduced [11].

Within the previous decade, green chemistry has achieved the status of a major scientific discipline [12]. In that multicomponent reactions (MCRs) [13-18] have gained eminence as a synthetic tool for producing structurally complex molecular entities with attractive biological features through the establishment and cleavage of numerous carbon–carbon and carbon–heteroatom bonds in one pot [19,20]. It is becoming increasingly important both in academia and in industry to design less toxic and more environmentally friendly MCRs [21-24]. In the continuous efforts towards the synthesis of different biologically active heterocyclic molecules [25-27], we have developed a novel methodology for synthesis of C3-substituted dihydrofuran coumarin from one-pot four-component reaction of 2-hydroxy aromatic aldehydes, 6-methyl, 4-hydroxy pyranone, aromatic aldehyde and pyridinium ylide in the presence of tri-

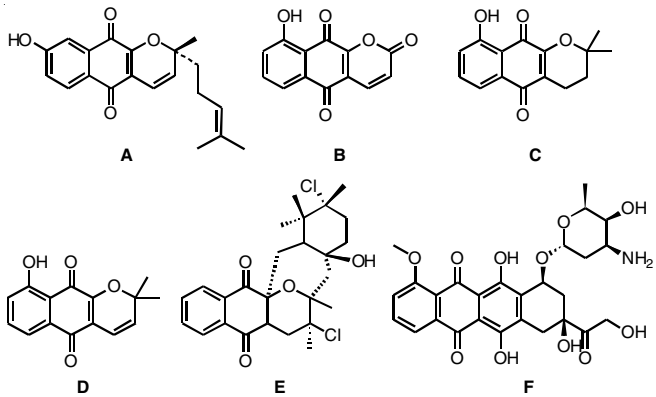


Fig. 1. Structures of pyranokunthone **A** and **B**, lambertellin **C**, β-lepachone **D** and α-xiloidone **E**, doxorubicin **F**

ethylamine under microwave-irradiation [28]. In continuation of our synthetic efforts towards C3-substituted dihydrofuran coumarin we contemplated the synthesis of dihydrofuran substituted 1*H*-benzo[*g*]-chromene-2,5,10-triones (Fig. 2). Such hybrid molecules represent both benzo[*g*] chromene-2,5,10-triones and dihydrofuran characteristics. Synthesis of such heterocyclic entities holds promise for integrating medicinal properties.

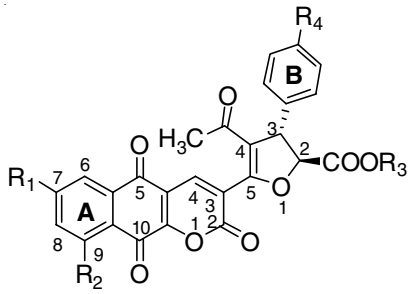


Fig. 2. Structure of dihydrofuran substituted benzochromene triones

EXPERIMENTAL

Melting points were recorded using open-ended capillary tubes on VEEGO VMP-DS instrument. The progression of all the reactions was monitored by TLC using a mixture of hexanes (60-80 °C boiling mixture) and ethyl acetate. Column chromatography was performed on silica gel (100-200 mesh, SRL Chemicals) using increasing percentage of ethyl acetate in hexanes. All microwave reactions were performed in a mono-mode Biotage Emery's Creator 300 Watt system with sample absorption set to "normal". ^{1}H NMR spectra (400 MHz) and ^{13}C NMR (100 MHz) and DEPT -135 spectra were recorded for $CDCl_3 + CCl_4$ (2:1) solutions on a Bruker-400 spectrometer with tetramethylsilane (TMS) as internal standard; *J* values are given in Hz. IR spectra were recorded as KBr solid solution on a Nicolet-6700 spectrometer. High resolution mass spectra were recorded on a Waters Micromass Q-TOF micro mass spectrometer using electron spray ionization mode. Organic solvents were distilled and dried before use. Melting points were measured in open capillary tubes and are uncorrected. Elemental analyses were performed on a Perkin Elmer 2400 Series II Elemental CHNS analyzer.

Synthesis of 3-formyl, 2-hydroxy napthaquinone derivatives (2a-j): A solution of 2-hydroxynaphthalene-1,4-dione (**1a**) (174 mg, 0.001 mol) dissolved in glacial acetic acid (5 mL), hexamethylenetetramine (560 mg, 0.004 mol) was added and the resulting solution was heated on a water bath for12 h. After cooling the solution at room temperature 4 mL of 6 N aq. HCl was added and then heated on a water bath for a further period for 0.5 h. The reaction mixture was then diluted with water (10 mL) and left for 1 h at 5 °C in the refrigerator. The reaction mixture was extracted with dichloromethane (2 × 10 mL). Combined organic solution was washed with water (20 mL), brine (20 mL) and dried over anhydrous Na_2SO_4. Column chromatographic purification on silica gel with increasing amount of ethyl acetate in hexanes provided **2a** as a free flowing solid in 70 % (141 mg) yield. Analytical samples were obtained by recrystallization from chloroform.

Synthesis of C3-dihydrofuran substituted 1*H*-benzo[*g*]-chromene-2,5,10-triones (6a-t)

(2*S*,3*S*)-Ethyl-4-acetyl-3-phenyl-5-(2,5,10-trioxo-5,10-dihydro-2*H*-benzo[*g*]chromen-3-yl)-2,3-dihydrofuran-2-carboxylate (6a): Mixture of one equivalent of 3-hydroxy-1,4-dioxo-1,4-dihydronaphthalene-2-carbaldehyde (**2a**) (329 mg, 0163 mmol), 6-methyl 4-hydroxy pyranone (**3**) (205 mg, 0.163 mmol), 1-(2-ethoxy-2-oxoethyl)pyridinium bromide (**4a**) (400 mg, 0.163 mmol), benzaldehyde (**5a**) (172 mg, 0.163 mmol) and trimethylamine (16 mg, 0.163 mmol) irradiated in the microwave oven at power level (300 W) for 3 min without solvent. The reaction mixture was purified through the column chromatography by eluting with hexanes in EtOAc (8:2). Analytical samples were obtained through the recrystallization from EtOH. Yield 670 mg (85 %). m.p.: 149 °C; Colourless solid; IR (KBr, ν_{max}, cm^{-1}): 3268, 3006, 1742, 1692, 1681, 1678, 1639, 1604, 1501, 1451, 1413, 1256, 1202, 1038, 940, 757; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (s, 1H), 7.84 (d, *J* = 8.0 Hz, 1H), 7.62 (t, *J* = 7.8 Hz, 1H), 7.41-7.34 (m, 3H), 7.23-7.18 (m, 2H), 6.90-6.87 (m, 2H), 5.51 (d, *J* = 4.4 Hz, 1H), 5.12 (d, *J* = 4.4 HZ, 1H), 4.32 (q, *J* = 6.0 Hz, 2H), 2.51 (s, 3H), 0.95 (t, *J* = 7.4 Hz, 3 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ191.3, 183.3, 181.1, 169.4, 166.9, 161.3, 155.1, 154.0, 139.4, 136.7, 133.3, 129.4, 126.76, 126.71, 124.6, 123.3, 121.7, 118.6, 117.3, 112.3, 105.1, 88.0, 62.7, 44.2, 26.7, 14.2 ppm; HRMS (ESI, *m/z*) 507.1050 calcd. for $C_{28}H_{20}O_8$ (M+Na) found 507.1048. Anal. calcd. for $C_{28}H_{20}O_8$; C, 69.42; H, 4.16; Found; C, 69.40; H, 4.15.

(2*S*,3*S*)-Propyl 4-acetyl-3-phenyl-5-(2,5,10-trioxo-5,10-dihydro-2*H*-benzo[*g*]chromen-3-yl)-2,3-dihydrofuran-2-carboxylate (6b): Mixture of one equivalent of 3-hydroxy-1,4-dioxo-1,4-dihydronaphthalene-2-carbaldehyde (**2a**) (329 mg, 0163 mmol), 6-methyl-4-hydroxy pyranone (**3a)** (205 mg, 0.163 mmol), 1-(2-oxo-2-propoxyethyl)pyridinium bromide (**4b**) (422 mg, 0.163 mmol), **5a** bezaldehyde (172 mg, 0.163 mmol) and trimethylamine (16 mg, 0.163 mmol) irradiated in the microwave oven at power level (300 W) for 3 min without solvent. The reaction mixture was purified through the column chromatography by eluting with hexanes in EtOAc (8:2). Analytical samples were obtained through the recrystallization from EtOH. Yield 690 mg (85 %). m.p.: 153 °C; Colourless solid; IR (KBr, ν_{max}, cm^{-1}): 3261, 2986, 1742, 1692, 1639, 1604, 1501, 1451, 1413, 1256, 1202, 1038, 940, 757; δ 8.52 (s, 1H), 7.84 (d, *J* = 8.0 Hz, 1H), 7.62 (t, *J* = 7.8 Hz, 1H), 7.42 (d, *J* = 8.0 Hz, 1H), 7.38-7.34 (m, 3H), 7.13-7.10 (m, 2H), 6.90-6.87 (m, 2H), 5.51 (d, *J* = 4.5 Hz, 1H), 5.12 (d, *J* = 4.5 HZ, 1H), 4.23-4.19 (m, 2H), 2.41 (s, 3H), 1.70 (q, *J* = 6.0 Hz, 2H), 0.93 (t, *J* = 7.4 Hz, 3 H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.3, 183.3, 181.1, 169.3, 167.7, 161.3, 155.4, 154.2, 137.0, 135.1, 129.2, 126.7, 126.0, 124.6, 123.5, 121.5, 118.3, 117.2, 112.3, 106.4, 88.0, 68.3, 44.1, 26.6, 21.6, 10.2 ppm; HRMS (ESI, *m/z*) 521.1207 calcd. for $C_{29}H_{22}O_8$ (M+Na) found 521.1205. Anal. calcd. for $C_{29}H_{22}O_8$; C, 69.87; H, 4.45; Found; C, 69.82; H, 4.41.

(2*S*,3*S*)-Propyl 4-acetyl-5-(7-chloro-2,5,10-trioxo-5,10-dihydro-2*H*-benzo[*g*]chromen-3-yl)-3-phenyl-2,3-dihydrofuran-2-carboxylate (6c): Yield (703 mg, 81 %), m.p.: 148 °C; Colourless solid; IR (KBr, ν_{max}, cm^{-1}): 3528,

2968, 2835, 1723, 1650, 1610, 1481, 1449, 1405, 1280, 1203, 1082, 1022, 929, 752; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (s, 1H), 8.00 (s, 1H), 7.85 (d, *J* = 7.8 Hz, 1H), 7.70 (t, *J* = 5.3 Hz, 1H), 7.66 (t, *J* = 8.0 Hz, 1H), 7.47 (d, *J* = 8.4 Hz, 1H), 7.40 (t, *J* = 7.5 Hz, 1H), 7.10 (d, *J* = 2.2 Hz, 1H), 6.54 (d, *J* = 8.6 Hz, 1H), 5.29 (d, *J* = 8.6 Hz, 1H), 5.15 (d, *J* = 4.6 Hz, 1H), 4.26-4.15 (m, 2H), 2.39 (s, 3H), 1.68 (q, *J* = 7.2 Hz, 2H), 0.93 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.3, 183.3, 181.1, 169.4, 167.02, 161.3, 155.1, 154.1, 141.5, 137.0, 133.2, 129.3, 126.7, 126.5, 124.6, 123.5, 121.5, 118.4, 117.2, 112.3, 106.0, 88.1, 68.1, 44.2, 26.7, 21.9, 10.3 ppm; HRMS (ESI, *m/z*) 555.0817 calcd. for $C_{29}H_{21}O_8Cl$ (M+Na) found 555.0815; Anal. calcd. for $C_{29}H_{21}O_8Cl$; C, 65.36; H, 3.97; Found; C, 65.35; H, 3.96.

(2*S*,3*S*)-Propyl 4-acetyl-5-(7-bromo-2,5,10-trioxo-5,10-dihydro-2*H*-benzo[*g*]chromen-3-yl)-3-phenyl-2,3-dihydrofuran-2-carboxylate (6d): Yield (743 mg, 79 %), m.p.: 151 °C; Colourless solid; IR (KBr, ν_{max}, cm^{-1}): 3423, 2968, 2880, 1727, 1689, 1648, 1587, 1574, 1493, 1456, 1408, 1325, 1272, 1178, 1095, 1030, 993, 931, 899, 855, 805, 755, 622; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (s, 1H), 7.83 (d, *J* = 7.8 Hz, 2H), 7.66 (t, *J* = 8.4 Hz, 1H), 7.47 (d, *J* = 8.4 Hz, 1H), 7.40 (t, *J* = 7.6 Hz, 1H), 7.02 (d, *J* = 8.4 Hz, 1H), 6.95 (s, 1H), 6.69 (d, *J* = 8.6 Hz, 1H), 5.37 (d, *J* = 8.6 Hz, 1H), 5.13 (d, *J* = 4.6 Hz, 1H), 4.26-4.16 (m, 2H), 2.26 (s, 3H), 1.68 (q, *J* = 7.0 Hz, 2H), 0.93 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.0, 183.1, 180.0, 169.0,168.1, 161.7, 156.3, 153.1, 141.7, 139.0, 133.6, 129.0, 127.6, 126.3, 125.6, 124.9, 123.8, 118.8, 117.1, 112.1, 104.1, 88.7, 68.7, 43.6, 26.1, 21.9, 10.4 ppm; HRMS (ESI, *m/z*) 599.0312 calcd. for $C_{29}H_{21}O_8Br$ (M+Na) found 599.0311; Anal. calcd. for $C_{29}H_{21}O_8Br$; C, 58.02; H, 3.53. Found;C, 58.01; H, 3.52.

(2*S*,3*S*)-Propyl 4-acetyl-5-(9-methoxy-2,5,10-trioxo-5,10-dihydro-2*H*-benzo[*g*]chromen-3-yl)-3-phenyl-2,3-dihydrofuran-2-carboxylate (6e): Yield (783 mg, 91 %), m.p.: 140 °C; Colourless solid; IR (KBr, ν_{max}, cm^{-1}): 3534, 2967, 1722, 1682, 1654, 1606, 1484, 1407, 1341, 1271, 1341, 1209, 1079, 1033, 933, 756; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (s, 1H), 7.80 (d, *J* = 8.4 Hz, 1H), 7.59 (t, *J* = 5.2. Hz,1H), 7.39 (d, *J* = 8.4 Hz, 1H), 7.32 (t, *J* = 7.6 Hz, 2H), 6.81 (dd, *J* = 6.2 Hz, 1.2 Hz, 2H), 6.73 (t, *J* = 6.0 Hz, 1H), 5.37 (d, *J* = 5.2 Hz, 1H), 5.08 (d, *J* = 5.2 Hz, 1H), 4.27-4.14 (m, 2H), 3.87 (s, 3H), 2.36 (s, 3H), 1.70 (q, *J* = 7.2 Hz, 2H), 0.95 (t, *J* = 7.6 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.4, 183.6, 180.1, 169.2, 166.8, 159.6, 155.4, 155.7, 146.9, 143.7, 142.4, 132.8, 124.7, 124.1, 123.2, 120.6, 120.1, 117.0, 112.4, 110.4, 104.1, 88.3, 67.7, 56.2, 45.4, 27.7, 22.0, 10.3 ppm; HRMS (ESI, *m/z*) 551.1313 calcd. for $C_{30}H_{24}O_9$ (M+Na) found 551.1310; Anal. calcd. for $C_{30}H_{24}O_9$; C, 68.18; H, 4.58; O, 27.25. Found; C, 68.16; H, 4.54; O, 27.24.

(2*S*,3*S*)-Propyl 4-acetyl-5-(9-ethoxy-2,5,10-trioxo-5,10-dihydro-2*H*-benzo[*g*]chromen-3-yl)-3-phenyl-2,3-dihydrofuran-2-carboxylate (6f): Yield (787 mg, 89 %), m.p.: 152 °C; Colourless solid; IR (KBr, ν_{max}, cm^{-1}): 3289, 2970, 2936, 2889, 1747, 1716, 1689, 1643, 1606, 1499, 1483, 1472, 1415, 1279, 1193, 1176, 1067, 1026, 949, 807; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (s, 1H), 7.80 (d, *J* = 7.6 Hz, 1H), 7.61 (t, *J* = 1.08 Hz, 1H), 7.39 (d, *J* = 8.4 Hz, 1H), 7.32 (t, *J* = 7.6 Hz, 1H), 6.79 (dd, *J* = 4.2, 1.2 Hz, 1H), 6.73 (m, 3H), 5.37 (d, *J* = 5.2 Hz, 1H), 5.06 (d, *J* = 5.2 Hz, 1H), 4.27-4.14 (m, 2H), 4.08 (q, *J* = 6.8 Hz, 2H), 2.21 (s, 3H), 1.73-1.68 (m, 2H), 1.41 (t, *J* = 6.9 Hz, 3H), 0.97 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.4, 183.1, 180.6, 169.2, 166.8, 159.6, 155.4, 146.2, 143.8, 139.6, 137.2, 132.8, 124.6, 124.1, 123.1, 120.6, 120.0, 117.0, 112.4, 111.2, 104.1, 88.4, 67.7, 64.7, 45.6, 27.1, 22.0, 14.9, 10.3 ppm; HRMS (ESI, *m/z*) 565.1469 calcd. for $C_{31}H_{26}O_9$(M+Na) found 565.1464; Anal. calcd. for $C_{31}H_{26}O_9$; C, 68.63; H, 4.83; O, 26.54. Found;C, 68.61; H, 4.82; O, 26.57.

(2*S*,3*S*)-Propyl 4-acetyl-5-(7-nitro-2,5,10-trioxo-5,10-dihydro-2*H*-benzo[*g*]chromen-3-yl)-3-phenyl-2,3-dihydrofuran-2-carboxylate (6g): Yield (643 mg, 73 %), m.p.: 146 °C; Colourless solid; IR (KBr, ν_{max}, cm^{-1}): 3310, 3079, 3120, 2978, 1746, 1691, 1642, 1564, 1591, 1525, 1497, 1437, 1340, 1287, 1245, 1156, 1049, 794, 755; ^{1}H NMR (400 MHz, $CDCl_3$) δ δ 8.48 (s, 1H), 8.06 (s, 1H), 7.95 (d, *J* = 7.8 Hz, 1H), 7.80 (t, *J* = 5.3 Hz, 1H), 7.76 (t, *J* = 8.0 Hz, 1H), 7.47 (d, *J* = 8.4 Hz, 1H), 7.40 (t, *J* = 7.5 Hz, 1H), 7.10 (d, *J* = 2.2 Hz, 1H), 6.64 (d, *J* = 8.6 Hz, 1H), 5.29 (d, *J* = 8.6 Hz, 1H), 5.15 (d, *J* = 4.6 Hz, 1H), 4.26-4.15 (m, 2H), 2.37 (s, 3H), 1.68 (q, *J* = 7.2 Hz, 2H), 0.93 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.3, 183.5, 181.5, 169.4, 167.0, 161.3, 155.1, 154.1, 143.5, 141.5, 137.0, 133.2, 129.3, 126.7, 126.5, 124.6, 123.5, 118.4, 117.2, 112.3, 106.0, 88.1, 68.1, 44.2, 26.7, 21.9, 10.3 ppm; HRMS (ESI, *m/z*) 486.1159 calcd. for $C_{29}H_{21}NO_{10}$ (M+Na) found 486.1156; Anal. calcd. for $C_{29}H_{21}NO_{10}$; C, 64.09; H, 3.89; N, 2.58. Found; C, 64.07; H, 3.88; N, 2.57.

(2*S*,3*S*)-Propyl 4-acetyl-5-(7,9-dichloro-2,5,10-trioxo-5,10-dihydro-2*H*-benzo[*g*]chromen-3-yl)-3-phenyl-2,3-dihydrofuran-2-carboxylate (6h): Yield (693 mg, 75 %), m.p.: 152 °C; Colourless solid; IR (KBr, ν_{max}, cm^{-1}): 3415, 2964, 2879, 1727, 1690, 1646, 1574, 1497, 1453, 1413, 1272, 1183, 1094, 1030, 990, 885, 756, 627; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (s, 1H), 7.96 (d, *J* = 8.2 Hz, 1H), 7.54 (t, *J* = 8.4 Hz, 1H), 7.30-7.28 (m, 3H), 7.00 (s, 1H), 6.84 (s, 1H), 5.45 (d, *J* = 8.7 Hz, 1H), 5.38 (d, *J* = 8.7 Hz, 1H), 4.17-4.13 (m, 2H), 2.29 (s, 3H), 1.67 (q, *J* = 6.8 Hz, 2H), 0.93 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.4, 183.0, 182.0, 171.6, 164.9, 163.0, 154.0, 153.0, 141.0, 138.3, 132.6, 131.1, 128.3, 126.4, 124.3, 123.8, 122.2, 116.8, 116.7, 115.5, 101.1, 83.9, 68.2, 43.6, 26.8, 21.8, 10.2 ppm; HRMS (ESI, *m/z*) 589.0427calcd. for $C_{29}H_{20}Cl_2O_8$ (M+Na) found 589.0424; Anal. calcd. for $C_{29}H_{20}O_8Cl_2$; C, 61.39; H, 3.55; Cl, 12.50; O, 22.56. Found; C, 61.37; H, 3.53; Cl, 12.49.

(2*S*,3*S*)-propyl-4-acetyl-5-(9-bromo-7-chloro-2,5,10-trioxo-5,10-dihydro-2*H*-benzo[*g*]chromen-3-yl)-3-phenyl-2,3-dihydrofuran-2-carboxylate (6i): Yield (698 mg, 70 %), m.p.: 165 °C; Colourless solid; IR (KBr, ν_{max}, cm^{-1}): 3534, 2967, 2846, 1722, 1694, 1653, 1609, 1484, 1407, 1346, 1272, 1210, 1078, 1034, 933, 893, 759, 697; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (s, 1H), 7.96 (d, *J* = 8.2 Hz, 1H), 7.54 (t, *J* = 8.4 Hz, 1H), 7.30-7.28 (m, 3H), 7.00 (s, 1H), 6.84 (s, 1H), 5.45 (d, *J* = 8.7 Hz, 1H), 5.38 (d, *J* = 8.7 Hz, 1H), 4.17-4.13 (m, 2H), 2.29 (s, 3H), 1.67 (q, *J* = 6.9 Hz, 2H), 0.88 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ196.1, 183.3, 182.2, 171.6, 164.9, 163.1, 154.3, 153.0, 141.0, 138.3, 132.7, 131.2, 128.2, 126.4, 124.3, 123.8, 122.0, 116.8, 116.7, 115.5,

101.8, 83.9, 68.3, 43.6, 26.8, 21.7, 10.3 ppm; HRMS (ESI, *m/z*) 610.0030 calcd. for $C_{29}H_{20}O_8BrCl$ (M+Na) found 610.0025; Anal. calcd. for $C_{29}H_{20}O_8BrCl$; C, 56.93; H, 3.29. Found; C, 56.91; H, 3.27.

(2*S*,3*S*)-Propyl-4-acetyl-5-(7,9-dibromo-2,5,10-trioxo-5,10-dihydro-2*H*-benzo[*g*]chromen-3-yl)-3-phenyl-2,3-dihydrofuran-2-carboxylate (6j): Yield (812 mg, 76 %), m.p.: 165 °C; Colourless solid; IR (KBr, ν_{max}, cm^{-1}): 3534, 2967, 2846, 1722, 1690, 1653, 1609, 1484, 1407, 1346, 1272, 1210, 1078, 1034, 933, 893, 759, 697; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36 (s, 1H), 7.96 (d, *J* = 8.2 Hz, 1H), 7.52 (t, *J* = 8.2 Hz, 1H), 7.30-7.28 (m, 3H), 7.00 (s, 1H), 6.85 (s, 1H), 5.45 (d, *J* = 4.4 Hz, 1H), 5.38 (d, *J* = 4.4 Hz, 1H), 4.19-4.15 (m, 2H), 2.27 (s, 3H), 1.67 (q, *J* = 6.9 Hz, 2H), 0.93 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 196.1, 183.0, 181.8, 171.2, 165.9, 163.6, 156.1, 153.0, 140.2, 139.0, 133.2, 132.4, 131.8, 125.6, 124.2, 124.0, 117.2, 116.7, 113.2, 103.0, 101.8, 83.5, 67.8, 43.8, 28.7, 21.8, 10.2 ppm; HRMS (ESI, *m/z*) 596.9519 calcd. for $C_{29}H_{20}O_8Br_2$ (M+Na) found 596.9517; Anal. calcd. for $C_{29}H_{20}O_8Br_2$; C, 53.07; H, 3.07; Found; C, 53.06; H, 3.05.

(2S,3S)-Propyl-4-acetyl-3-(4-chlorophenyl)-5-(2,5,10-trioxo-5,10-dihydro-2H-benzo[*g*]chromen-3-yl)-2,3-dihydrofuran-2-carboxylate (6k): Yield (703 mg, 81 %), m.p.: 148 °C; Colourless solid; IR (KBr, ν_{max}, cm^{-1}): 3321, 3012, 2835, 1735, 1666, 1618, 1489, 1469, 1412, 1289, 1233, 1090, 1021, 922, 760; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.40 (s, 1H), 8.26 (d, *J* = 7.8 Hz, 2H), 7.80 (d, *J* = 8.8 Hz, 1H), 7.77 (t, *J* = 8.3 Hz, 1H), 7.45 (d, *J* = 8.3 Hz, 2H), 7.42 (t, *J* = 7.5 Hz, 1H), 7.11 (s, 1H), 5.29 (d, *J* = 4.6 Hz, 1H), 5.15 (d, *J* = 4.6 Hz, 1H), 4.26-4.15 (m, 2H), 2.38 (s, 3H), 1.72 (q, *J* = 6.8 Hz, 2H), 0.93 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.6, 183.9, 181.4, 169.6, 168.9, 161.6, 155.4, 153.8, 139.1, 137.1, 133.6, 129.5, 127.5, 126.3, 125.6, 124.9, 123.4, 118.8, 117.3, 112.1, 104.1, 88.5, 68.1, 43.6, 26.1, 21.4, 10.3 ppm;HRMS (ESI, *m/z*) 555.0817 calcd. for $C_{29}H_{21}O_8Cl$ (M+Na) found 555.0815; Anal. calcd. for $C_{29}H_{21}O_8Cl$; C, 65.36; H, 3.97; Found; C, 65.35; H, 3.96.

(2*S*,3*S*)-Propyl-4-acetyl-3-(4-bromophenyl)-5-(2,5,10-trioxo-5,10-dihydro-2*H*-benzo[*g*]chromen-3-yl)-2,3-dihydrofuran-2-carboxylate (6l): Yield (743 mg, 79 %), m.p.: 151 °C; Colourless solid; IR (KBr, ν_{max}, cm^{-1}): 3316, 3011, 2840, 1733, 1658, 1621, 1480, 1467, 1410, 1283, 1240, 1092, 1019, 920, 756; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.48 (s, 1H), 8.10 (d, *J* = 7.8 Hz, 2H), 7.95 (d, *J* = 8.8 Hz, 1H), 7.78 (t, *J* = 8.3 Hz, 1H), 7.48 (d, *J* = 8.3 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 1H), 7.10 (d, *J* = 8.6 Hz, 1H), 5.29 (d, *J* = 4.6 Hz, 1H), 5.16 (d, *J* = 4.6 Hz, 1H), 4.21-4.13 (m, 2H), 2.34 (s, 3H), 1.67 (q, *J* = 6.8 Hz, 2H), 0.93 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.1, 183.9, 181.4, 169.0, 168.1, 161.7, 155.2, 153.1, 139.0, 137.6, 133.6, 129.0, 127.6, 126.3, 125.6, 124.9, 123.4, 118.8, 117.3, 112.1, 104.1, 88.5, 68.1, 43.6, 26.1, 21.9, 10.3 ppm; HRMS (ESI, *m/z*) 599.0312 calcd. for $C_{29}H_{21}O_8Br$ (M+Na) found 599.0311; Anal. calcd. for $C_{29}H_{21}O_8Br$; C, 58.02; H, 3.53. Found; C, 58.01; H, 3.52.

(2*S*,3*S*)-Propyl-4-acetyl-3-(4-nitrophenyl)-5-(2,5,10-trioxo-5,10-dihydro-2*H*-benzo[*g*]chromen-3-yl)-2,3-dihydrofuran-2-carboxylate (6m): Yield (672 mg, 76 %), m.p.: 154 °C; Colourless solid; IR (KBr, ν_{max}, cm^{-1}): 3520, 3120, 2951, 2832, 1729, 1663, 1610, 1487, 1449, 1400, 1287, 1203, 1088, 1027, 909, 757; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (s, 1H), 8.10 (d, *J* = 7.8 Hz, 2H), 7.95 (d, *J* = 8.8 Hz, 1H), 7.78 (t, *J* = 8.3 Hz, 1H), 7.48 (d, *J* = 8.3 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 1H), 7.10 (d, *J* = 8.6 Hz, 1H), 5.29 (d, *J* = 4.6 Hz, 1H), 5.16 (d, *J* = 4.6 Hz, 1H), 4.21-4.13 (m, 2H), 2.34 (s, 3H), 1.67 (q, *J* = 6.8 Hz, 2H), 0.93 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.4, 183.2, 181.1, 169.3, 168.6, 161.7, 155.9, 153.4, 139.0, 137.3, 133.1, 129.6, 127.6, 126.0, 125.6, 124.3, 123.6, 118.8, 117.3, 112.1, 104.1, 88.3, 68.1, 43.2, 26.2, 21.9, 10.3 ppm; HRMS (ESI, *m/z*) 566.1058 calcd. for $C_{29}H_{21}NO_{10}$ (M+Na) found 566.1054; Anal. calcd. for $C_{29}H_{21}NO_{10}$; C, 64.09; H, 3.89; N, 2.58; Found; C, 64.07; H, 3.88; N, 2.56;

(2*S*,3*S*)-Propyl 4-acetyl-3-[4-(dimethylamino)phenyl]-5-(2,5,10-trioxo-5,10-dihydro-2*H*-benzo[*g*]chromen-3-yl)-2,3-dihydrofuran-2-carboxylate (6n): Yield (706 mg, 80 %), m.p.: 155 °C; Colourless solid; IR (KBr, ν_{max}, cm^{-1}): 3520, 3120, 2951, 2832, 1729, 1663, 1610, 1487, 1449, 1400, 1287, 1203, 1088, 1027, 909, 757; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (s, 1H), 7.81 (d, *J* = 7.8 Hz, 1H), 7.61 (t, *J* = 7.8 Hz, 1H), 7.59 (d, *J* = 8.5 Hz, 2H), 7.40 (t, *J* = 7.8 Hz, 1H), 6.82 (d, *J* = 6.0 Hz, 2H), 6.74 (t, *J* = 6.0 Hz, 1H), 5.37 (d, *J* = 5.3 Hz, 1H), 5.08 (d, *J* = 5.3 HZ, 1H), 4.27-4.13 (m, 2H), 3. 33 (s, 6H), 2.33 (s, 3H), 1.71 (m, 2H), 0.97 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.4, 183.2, 180.6, 169.2, 166.8, 159.6, 155.4, 146.9, 143.7, 140.1, 139.2, 132.8, 127.6, 124.1, 123.1, 120.6, 120.1, 117.0, 112.4, 110.4, 104.1, 88.3, 67.7, 45.4, 41.4, 27.7, 22.0, 10.3 ppm; HRMS (ESI, *m/z*) 564.1629 calcd. for $C_{31}H_{27}NO_8$(M+Na) found 564.1623. Anal. calcd. for $C_{31}H_{27}NO_8$; C, 68.75; H, 5.03; N, 2.59; O, 23.64; Found; C, 68.75; H, 5.03; N, 2.59; O, 23.64.

(2*S*,3*S*)-Propyl 4-acetyl-3-(4-hydroxyphenyl)-5-(2,5,10-trioxo-5,10-dihydro-2*H*-benzo[*g*]chromen-3-yl)-2,3-dihydrofuran-2-carboxylate (6o): Yield (678 mg, 81 %), m.p.: 146 °C; Yellow Colour solid; IR (KBr, ν_{max}, cm^{-1}): 3361, 3205, 3009, 1723, 1672, 1647, 1628, 1486, 1408, 1325, 1277, 1364, 1221, 1072, 1036, 921, 749; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (s, 1H), 8.01 (d, *J* = 7.6 Hz, 2H), 7.70 (d, *J* = 8.8 Hz, 1H), 7.68 (t, *J* = 8.3 Hz, 1H), 7.48 (d, *J* = 8.3 Hz, 2H), 7.42 (t, *J* = 7.3 Hz, 1H), 7.10 (d, *J* = 8.6 Hz, 1H), 6.68 (s, 1H),5.29 (d, *J* = 4.6 Hz, 1H), 5.16 (d, *J* = 4.6 Hz, 1H), 4.26-4.15 (m, 2H), 2.39 (s, 3H), 1.72 (q, *J* = 6.8 Hz, 2H), 0.93 (t, *J* = 7.4 Hz, 3H) ppm;^{13}C NMR (100 MHz, $CDCl_3$) δ 193.2, 183.8, 180.7, 169.1, 166.8, 159.8, 155.4, 150.2, 146.9, 143.1, 142.0, 132.4, 124.4, 124.0, 123.2, 120.6, 120.1, 117.7, 112.9, 110.2, 103.4, 88.3, 67.5, 45.5, 27.8, 22.1, 10.4 ppm; HRMS (ESI, *m/z*) 551.1313 calcd. for $C_{29}H_{22}O_9$ (M+Na) found 537.1156; Anal. calcd. for $C_{29}H_{22}O_9$; C, 67.70; H, 4.31; Found; C, 67.68; H, 4.30.

(2*S*,3*S*)-Propyl 4-acetyl-3-(4-methoxyphenyl)-5-(2,5,10-trioxo-5,10-dihydro-2*H*-benzo[*g*]chromen-3-yl)-2,3-dihydrofuran-2-carboxylate (6p): Yield (783 mg, 91 %), m.p.: 140 °C; Colourless solid; IR (KBr, ν_{max}, cm^{-1}): 3361, 3009, 1724, 1676, 1657, 1608, 1486, 1400, 1321, 1273, 1354, 1201, 1073, 1031, 920, 746; ^{1}H NMR (400 MHz, $CDCl_3$) δ δ 8.46 (s, 1H), 8.00 (d, *J* = 7.8 Hz, 2H), 7.85 (d, *J* = 8.8 Hz, 1H), 7.78 (t, *J* = 8.3 Hz, 1H), 7.48 (d, *J* = 8.3 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 1H), 7.10 (d, *J* = 8.6 Hz, 1H), 5.29 (d, *J* = 4.6 Hz,

1H), 5.16 (d, *J* = 4.6 Hz, 1H), 4.26-4.15 (m, 2H), 3.86 (s, 3H), 2.39 (s, 3H), 1.69 (q, *J* = 6.8 Hz, 2H), 0.93 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.1, 183.7, 180.2, 168.3, 166.8, 159.1, 155.4, 150.7, 146.9, 143.2, 142.4, 132.8, 124.7, 124.1, 123.2, 120.6, 120.1, 117.0, 112.4, 110.4, 103.0, 88.4, 67.7, 56.1, 27.4, 22.0, 10.3 ppm; HRMS (ESI, *m/z*) 551.1313 calcd. for $C_{30}H_{24}O_9$ (M+Na) found 551.1310; Anal. calcd. for $C_{30}H_{24}O_9$; C, 68.18; H, 4.58; O, 27.25. Found; C, 68.16; H, 4.54; O, 27.24.

(2*S*,3*S*)-Propyl 4-acetyl-5-(9-methoxy-2,5,10-trioxo-5,10-dihydro-2*H*-benzo[*g*]chromen-3-yl)-3-(4-methoxyphenyl)-2,3-dihydrofuran-2-carboxylate (6q): Yield (691 mg, 76 %), m.p.: 161 °C; Yellow colour solid; IR (KBr, ν_{max}, cm^{-1}): 3312, 3104, 2959, 2834, 1718, 1666, 1613, 1489, 1450, 1408, 1299, 1203, 1088, 1020, 915, 761; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (s, 1H), 7.80 (d, *J* = 7.8 Hz, 1H), 7.59 (t, *J* = 7.2. Hz, 1H), 7.39 (d, *J* = 8.4 Hz, 1H), 7.32 (t, *J* = 7.6 Hz, 2H), 6.81 (dd, *J* = 7.7 Hz, 1.2 Hz, 2H), 5.37 (d, *J* = 5.2 Hz, 1H), 5.08 (d, *J* = 5.2 Hz, 1H), 4.27-4.14 (m, 2H), 3.87 (s, 6H), 2.39 (s, 3H), 1.73 (q, *J* = 7.2 Hz, 2H), 0.97 (t, *J* = 7.6 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.4, 183.6, 180.1, 169.2, 166.8, 159.6, 155.4, 150.7, 146.9, 143.2, 142.4, 132.8, 124.7, 124.1, 123.2, 120.6, 120.1, 117.0, 112.4, 110.4, 104.1, 88.3, 67.7, 56.24, 56.21, 45.4, 27.7, 22.0, 10.3 ppm; HRMS (ESI, *m/z*) 581.1418 calcd. for $C_{31}H_{26}O_{10}$ (M+Na) found 581.1414; Anal. calcd. for $C_{31}H_{26}O_{10}$; C, 66.66; H, 4.69; O, 28.65; Found; C, 66.64; H, 4.67; O, 28.69.

(2*S*,3*S*)-Propyl 4-acetyl-3-(3,5-dimethoxyphenyl)-5-(9-methoxy-2,5,10-trioxo-5,10-dihydro-2*H*-benzo[*g*]chromen-3-yl)-2,3-dihydrofuran-2-carboxylate (6r): Yield (691 mg, 76 %), m.p.: 168 °C; Yellow colour solid; IR (KBr, ν_{max}, cm^{-1}): 3520, 3120, 2951, 2832, 1729, 1663, 1610, 1487, 1449, 1400, 1287, 1203, 1088, 1027, 909, 757; δ 8.38 (s, 1H), 7.73-7.66 (m, 2H), 7.38 (s, 1H), 7.32 (m, 2H), 6.81 (d, *J* = 7.7 Hz, 1H), 5.37 (d, *J* = 5.2 Hz, 1H), 5.08 (d, *J* = 5.2 Hz, 1H), 4.27-4.14 (m, 2H), 3.86 (s, 9H), 2.35 (s, 3H), 1.73 (q, *J* = 7.2 Hz, 2H), 0.97 (t, *J* = 7.6 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.4, 183.5, 180.2, 169.1, 166.1, 158.2, 155.4, 150.7, 144.1, 143.2, 141.4, 131.8, 124.7, 124.1, 123.2, 120.6, 120.1, 117.4, 112.1, 110.8, 104.1, 88.2, 67.7, 56.1, 56.0, 45.4, 27.8, 22.2, 10.6 ppm; HRMS (ESI, *m/z*) 611.1524 calcd. for $C_{32}H_{28}O_{11}$ (M+Na) found 611.1521; Anal. calcd. for $C_{32}H_{28}O_{11}$; C, 65.30; H, 4.80; Found; C, 65.30; H, 4.80;

(2*S*,3*S*)-Propyl 4-acetyl-5-(7,9-dimethoxy-2,5,10-trioxo-5,10-dihydro-2*H*-benzo[*g*]chromen-3-yl)-3-(4-methoxyphenyl)-2,3-dihydrofuran-2-carboxylate (6s): Yield (651 mg, 68 %), m.p.: 164 °C; Yellow coloursolid; IR (KBr, ν_{max}, cm^{-1}): 3326, 3124, 2901, 2824, 1756, 1656, 1612, 1478, 1440, 1408, 1280, 1209, 1091, 1029, 901, 753; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (s, 1H), 7.61 (s, 1H), 7.38 (d, *J* = 8.4 Hz, 2H), 7.32 (d, *J* = 7.7 Hz, 2H), 6.82 (s, 1H), 5.37 (d, *J* = 5.3 Hz, 1H), 5.08 (d, *J* = 5.3 Hz, 1H), 4.27-4.14 (m, 2H), 3.88 (s, 9H), 2.37 (s, 3H), 1.73 (q, *J* = 7.4 Hz, 2H), 0.95 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ192.2, 183.6, 180.1, 169.4, 166.7, 158.6, 155.4, 150.4, 144.9, 143.2, 142.4, 132.8, 124.7, 124.1, 123.2, 120.6, 120.1, 117.8, 112.8, 110.4, 104.1, 88.4, 67.7, 56.27, 56.25, 56.20, 45.4, 27.7, 22.1, 10.4 ppm; HRMS (ESI, *m/z*) 566.1058 calcd. for $C_{32}H_{28}O_{11}$ (M+Na) found 566.1054; Anal. calcd. for $C_{32}H_{28}O_{11}$; C, 65.30; H, 4.80; O, 29.90; Found; C, 65.28; H, 4.79; O, 29.93.

(2*S*,3*S*)-Propyl 4-acetyl-5-(7,9-dimethoxy-2,5,10-trioxo-5,10-dihydro-2*H*-benzo[*g*]chromen-3-yl)-3-(3,5-dimethoxyphenyl)-2,3-dihydrofuran-2-carboxylate (6t): Yield (695 mg, 69 %), m.p.: 178 °C; Yellow solid; IR (KBr, ν_{max}, cm^{-1}): 3520, 3120, 2951, 2832, 1729, 1663, 1610, 1487, 1449, 1400, 1287, 1203, 1088, 1027, 909, 757; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (s, 1H), 7.61 (s, 1H), 7.40 (s, 1H), 7.34 (d, *J* = 2.4 Hz, 2H), 6.19 (s, 1H), 5.37 (d, *J* = 5.3 Hz, 1H), 5.08 (d, *J* = 5.3 Hz, 1H), 4.27-4.16 (m, 2H), 3.87 (s, 12H), 2.36 (s, 3H), 1.71 (q, *J* = 7 Hz, 2H), 0.95 (t, *J* = 7.4 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.4, 183.6, 180.1, 169.2, 166.8, 159.8, 155.4, 150.7, 146.9, 143.2, 142.4, 132.8, 124.7, 124.1, 123.2, 120.6, 120.1, 117.0, 112.4, 110.4, 104.1, 88.3, 67.7, 56.21, 56.20, 56.17, 45.4, 27.7, 22.0, 10.3 ppm; HRMS (ESI, *m/z*) 566.1058 calcd. for $C_{33}H_{30}O_{12}$ (M+Na) found 566.1054; Anal. calcd. for $C_{33}H_{30}O_{12}$; C, 64.07; H, 4.89; O, 31.04; Found; C, 64.05; H, 4.88; O, 31.07.

Antibacterial assay: 100 mL sterile conical flask of nutrient broth was inoculated with the test organisms and incubated at 37 °C overnight. By using a sterile pipette, 0.6 mL of the broth culture of each test organism was added to 60 mL of molten agar, mixed well and maintained at 45 °C. Sterile agar test plates of each test organism were prepared by pouring inoculated medium with uniform thickness. The agar was allowed to set and harden and wells of 4 mm diameter were cut at equidistant using a sterile cork borer. Agar plugs were removed. 100 µg/mL of test solutions (**6a-l**) were prepared in DMSO and were introduced into the wells using micropipette. The plates were kept at room temperature for 2 h for better diffusion of solution into the medium. The plates were incubated for 24 h at 37 °C. After incubation the diameter of inhibitory zones formed around each well was measured in millimetre (mm) using antibiotic zone scale. The assay was carried out in duplicate. DMSO was used as control and the antibacterial activity of the test compounds was compared with standard ''ciprofloxin''.

Antifungal assay: Sterile molten potato dextrose agar (PDA) medium was inoculated with 50 µL of fungal spore suspension aseptically and maintained at 45 °C temperature. The inoculated medium was mixed well and poured immediately in sterilized petri plates. Then five wells of 6 mm diameter were punched using sterile borer and filled with 100 µg/mL of test compounds **6a-q** as well as sterile DMSO 100 % as negative control. Plates were incubated for 24 h at 37 °C. Antifungal activity was determined by measuring the zone of inhibition. The zones produced by the test compounds were compared with the ketoconazole (standard).

Anticancer activity

MTT assay: Cell growth assays were carried out with the aid of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) reduction test. The MTT colorimetric assay was performed as described previously (Mossmann 1983). Briefly, Hep2, A549 and HeLa cells (5×10^3/well) were plated in 0.2 mL (DMEM with 10 % FBS) in 96-well plates in the presence of indicated concentration of the compounds in three

independent experiments. MTT was dissolved in phosphate buffered saline (PBS) at 5 mg/mL. After 48 h of incubation of Hep2, A549 and HeLa cells, MTT solution was added and the plate was incubated for 3 h and the cells were dissolved in 100 µL of DMSO. The conversion of MTT to formazan by metabolically viable cells was measured by the absorbance at 570 nm. The cell viability was expressed with the concentration that inhibits 50 % of growth (IC_{50}).

RESULTS AND DISCUSSION

The starting materials 2-formyl, 3-hydroxy napthaquinones (12 samples) could be derived from 2-Hydroxy napthaquinones were formylated with hexamethylenetetramine and acetic acid (Duff reaction) [29].

We have adopted the procedure for synthesis of **6a** from newly developed methodology by our lab for synthesis of novel C3-dihydrofuran substituted coumarin derivatives (**5**) from one-pot three-component reaction of *O*-hydroxy aromatic aldehydes, 6-methyl, 4-hydroxy pyran, aromatic aldehyde and pyridinium ylide in the presence of triethylamine under microwave-irradiation in solvent-free conditions (**Scheme-I**).

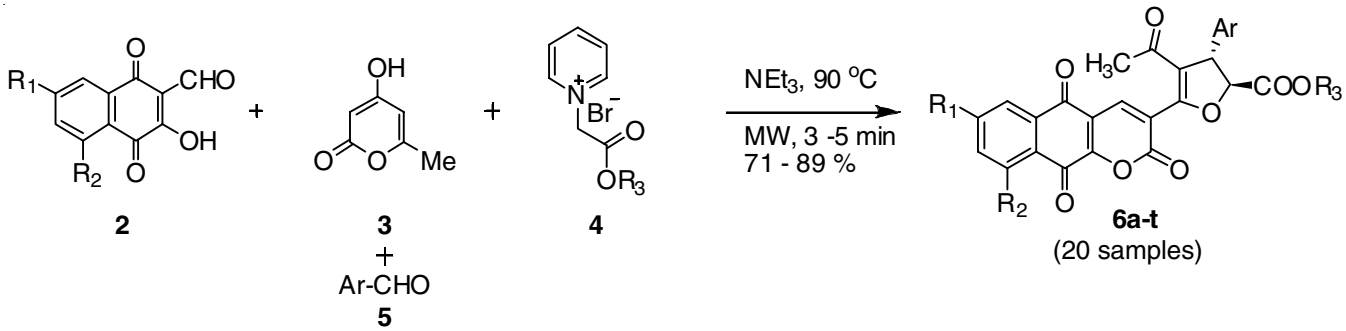
In the reaction protocol, when equimolar amounts of 3-formyl, 2-hydroxyl naphthalaldehyde (**2a**), 6-methyl, 4-hydroxy pyranone (**3**), aromatic aldehydes (**5**), ethyl ester pyridinium bromides (**4**) were reacted in the presence of 0.1 equivalents of Et_3N in a sealed vial under microwave-irradiation for 3 min at 90 °C leading to resulting product **6a** was isolated in 89 % yield. The structure of the compound was fully characterized by ^1H and ^{13}C NMR, MS and IR spectra and elemental analysis. In the ^1H NMR spectra, the two protons at 2,3-position of dihydrofuran ring display two doublets at 5.52 and 5.10 ppm with the *vicinal* coupling constant J = 4.4 and 4.4 Hz, respectively. It has been documented that in *cis*-2,3-dihydrofuran the *vicinal* coupling constant of the two methine protons J = 7-10 Hz, while in *trans*-2,3-dihydrofuran *vicinal* coupling constant J = 4-7 Hz. So we concluded that thermodynamically stable *trans* isomer of 2,3-dihydrofuran derivatives were formed. Further, it was confirmed from the analysis of the NOESY spectrum of the compound. The mass spectrum shows, a sharp distinguishable peak of compound **6a** at 507.1050 $[M+Na]^+$. In conclusion of this reaction, our reported methodology was successfully applied to 3-formyl, 2-hydroxy naphthaquininones.

Similarly, compounds **6a-t** was synthesized and characterized (Fig. 3). Newly prepared benzochromene trione derivatives (**6a-l**) was subjected to *in vitro* screening against three cancer cell lines namely Hep2 (Human laryngeal carcinoma), A549 (Human lung adenocarcinoma) and HeLa (human cervical cancer). Out of which, the **6e** emerged as the most promising lead compound open for further structure activity relationship (SAR) studies (Table-1). Three domains in **6e** namely, the aromatic ring (ring A, Fig. 2) of benzochromanone, aromatic ring (of ring B, Fig. 2) on dihyrofuran and alkyl on the ester group (of ring B, Fig. 2) were agreeable for alteration with different substituents while keeping rest of the molecule intact. Then, the substrate scope of the reaction was explored by using various *ortho*-hydroxyl naphthaquinone aldehydes in the model reaction. In all cases the substitution reaction provided the product dihydrofuran substituted benzochomene triones (**6a-t**) without any difficulty. Based on the biological activity of **6e** and substitution pattern of natural flavonoids (Harborne and Baxter 1999) we changed different substituents on ring A (Fig. 2) to alter steric and electronic effects [30]. Electronic effects were also observed in the reaction process. The electron-donating group (EDG) at 2, 5 positions of the *ortho*-hydroxyl benzaldehyde required less reaction time to give comparatively high yields of the product while stronger EWG-substituted

TABLE-1
ZONE OF INHIBITION (mm)[a] OF COMPOUNDS **6a-t** AGAINST TESTED BACTERIAL STRAINS

Entry	Compd.	Bacterial strains			
		B. subtilis	*E. coli*	*K. pneumonia*	*P. auregenosa*
1	**6a**	10	8	8	10
2	**6b**	9	10	9	10
3	**6c**	10	11	11	9
4	**6d**	8	8	9	8
5	**6e**	8	9	8	10
6	**6f**	8	11	12	11
7	**6g**	12	9	10	10
8	**6h**	11	10	10	9
9	**6i**	9	11	11	8
10	**6j**	11	10	12	11
11	**6k**	11	10	10	12
12	**6l**	11	11	10	11
13	**6m**	10	8	8	9
14	**6n**	9	9	10	9
15	**6o**	10	9	8	10
16	**6p**	11	9	9	10
17	**6q**	10	11	10	11
18	**6r**	13	14	14	14
19	**6s**	13	14	13	15
20	**6t**	16	17	15	14
21	Standard	24	27	22	26

Ciprofloxacin was used as standard.
[a]100 µg/mL of compound in each well



Scheme-I: Synthesis of C3-dihydrofuran substituted 1*H*-benzo[*g*]chromene-2,5,10-trione

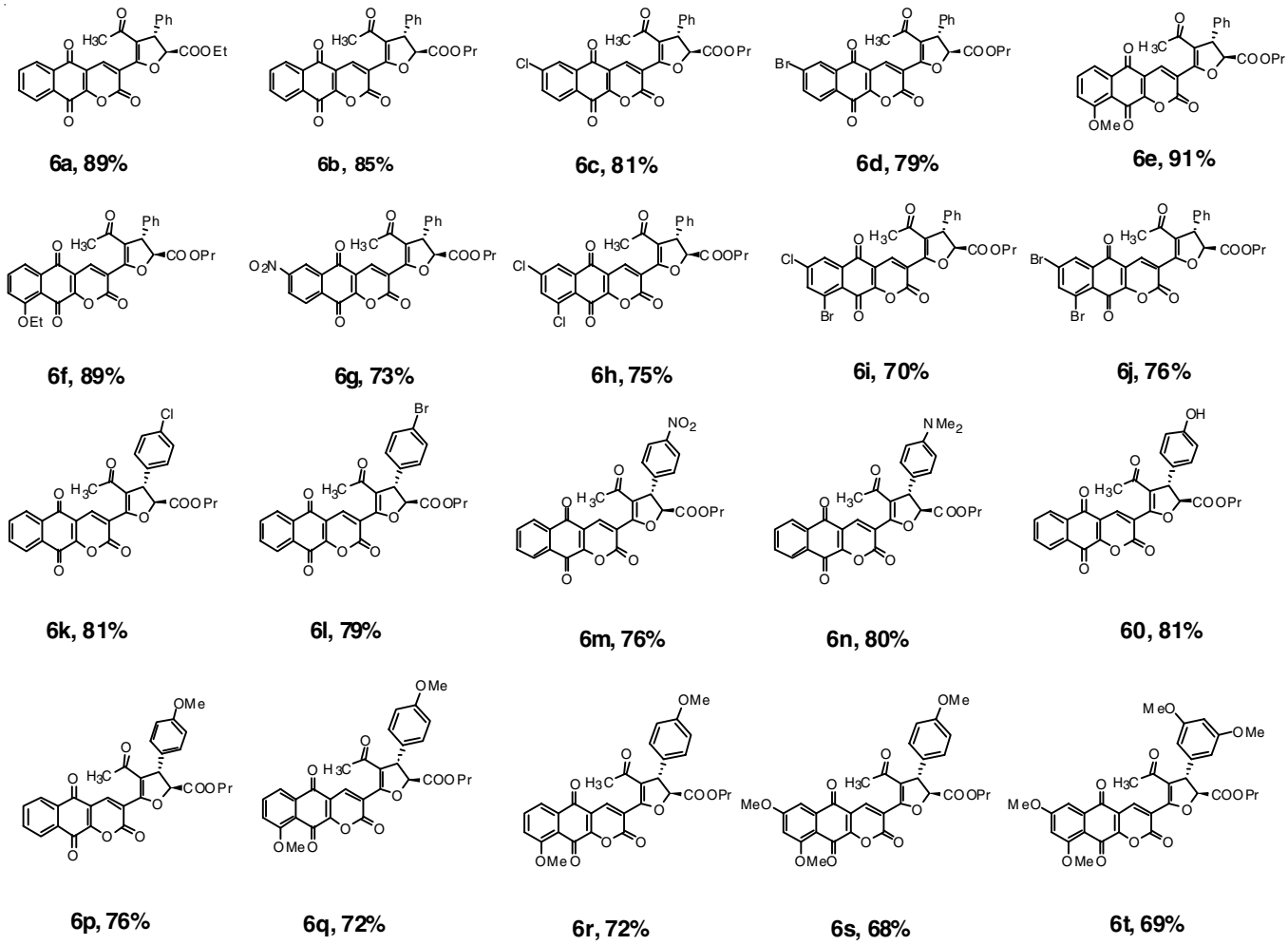


Fig. 3. Synthesis of structure and activity relationship directed a combinatorial library of 1*H*-benzo[*g*]chromene-2,5,10-triones

ones gave evidently poor yields. The electronic properties of the substituents of aromatic aldehydes significantly affect the reactivity. The electron-donating group (NMe_2) at the *para* position of the aldehyde to give comparatively high yield of the product while stronger EWG-substituted one (NO_2) gave evidently poor yield. Next, we changed the substitution on the ester group from methyl to ethyl and propyl, in the starting benzochomene triones and realized good yield of benzochromene derivatives (**6a-b**). Spectral (IR, ^{1}H NMR, ^{13}C NMR and DEPT) and analytical (ESI-MS HRMS) data of the all the derivatives (**6b-t**) agreed well with the assigned structures. We gathered structures of all the benzochromenones (**6a-t**) along with the time taken for the substitution reaction and yield of the product in Fig. 3 to provide overall picture of the substitution pattern and to discern structure and activity relationship results. The reaction did not provide even a trace of another isomer. List of synthetic compounds used for *in silico* and *in vitro* studies were given in the Fig. 3.

Biological evaluation

Antimicrobial activity: The newly synthesized and well characterized compounds (**6a-t**) were screened for *in vitro* antibacterial activity against Gram-positive bacteria (*Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*) and antifungal activity against *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans* using agar well diffusion assay and zones of inhibition of the test compounds were expressed in mm.

For biological tests 20 of three types of compounds are selected: (a) Benzochormene trione containing substituent groups at 3, 5 positions of ring A; (b) Benzochormene trione containing substituent groups at 3, 4, 5 positions of ring B; (c) Benzochormene trione containing substituent groups at methoxy substituents at C3, C5 of ring A and C3, C4, C5 of ring B (based on structure and activity relationship studies).

Antibacterial activity: The antibacterial activity of the test compounds (**6a-t**) was compared with standard ''ciprofloxacin'' and the results are presented in Table-1. As indicated in Table-1, most of the synthesized compounds generally showed potent antibacterial activity against all the tested bacterial strains. Compound **6t** shows good antibacterial activity against *B. subtilis*, *E. coli*, *K. pneumonia* and *P. aeruginosa* with zones of inhibition of 10, 8, 8 and 10 mm, respectively. In case of *E. coli*, compound **6t** is more active with a zone of inhibition of 17 mm. On bacterial strains, compound **6t** is potent against *P. auregenosa* with a zone of 14 mm.

Antifungal activity: The antifungal activity of tested compounds (**6a-t**) were compared with ketoconazole (standard) and the results are tabulated in Table-2 infer that compound **6t** was found to be interesting molecule with good antifungal

TABLE-2
ZONE OF INHIBITION (mm)[a] OF COMPOUNDS **6a-t** AGAINST TESTED FUNGAL STRAINS

Entry	Compound	*A. flavus*	*A. niger*	*C. albicans*
1	**6a**	12	11	8
2	**6b**	12	13	9
3	**6c**	11	12	7
4	**6d**	13	11	9
5	**6e**	11	10	8
6	**6f**	10	8	8
7	**6g**	8	7	10
8	**6h**	9	6	11
9	**6i**	7	7	7
10	**6j**	9	8	9
11	**6k**	9	11	9
12	**6l**	11	11	10
13	**6m**	10	10	9
14	**6n**	11	12	11
15	**6o**	10	9	9
16	**6p**	11	12	10
17	**6q**	11	10	9
18	**6r**	12	13	12
19	**6s**	14	14	11
20	**6t**	15	16	12
21	Standard	21	23	19

Ketoconazole was used as standard.
[a]100 µg/mL compound in each well.

activity against *A. flavus*, *A. niger*, *C. albicans* with zone of inhibition of 15, 16 and 16 mm, respectively.

Antiproliferative activity: Determination of antiproliferative activity of dihydrofuran substituted 1*H*-benzo[*g*]chromene-2,5,10-triones.In the first phase, antiproliferative activity of ten1*H*-benzo[*g*]chromene-2,5,10-triones (**6a-j**, Fig. 2) was evaluated *in vitro* against a panel of three human cancer cell lines, namely human laryngeal carcinoma (Hep2), human lung adenocarcinoma (A549) and human cervical cancer (HeLa) cells and the results for inhibitory concentration (IC_{50}) values are gathered in Table-3. The studies reveal that methoxy group substituted C3-dihydrofuran substituted 1*H*-benzo[*g*]chromene (**6e**) showed significant cytotoxic activity in comparison with other derivatives in all the three cancer cell lines (entry 5, Table-1). For the next batch of six 1*H*-benzo[*g*]chromene-2,5,10-triones (**6k-p**) substitution was kept intact and changes were made in ring B. Antiproliferative evaluation of **6k-p** revealed that C3-dihydrofuran substituted 1*H*-benzo[*g*]chromene (**6p**) (entry 16) which has methoxy group at aromatic ring B displays better activity compared to others. Interestingly, C3-dihydrofuran substituted 1*H*-benzo[*g*]chromene (**6q**) which has methoxy group at C8 in ring A and C4 in ring B displayed markedly shows better activity than other. For next batch of three C3-dihydrofuran substituted 1*H*-benzo[*g*]chromene (**6r-t**) changes were made ring A and in ring B with methoxy grops. Antiproliferative evaluation of **6r-t** revealed that 4-aryl-4*H*-chromene (**6t**) which has four methoxy groups' displays better activity compared to others (entry 20). In summary *in vitro* evaluation revealed that C3-dihydrofuran substituted 1*H*-benzo[*g*]-chromene (**6t**) is the most potent molecule within the batch of **6a-t**. In order to determine the cytotoxic effects, all the twenty compounds were subjected to *in vitro* cytotoxicity assay using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide

TABLE-3
in vitro ANTIPROLIFERATIVE ACTIVITY OF 1*H*-BENZO[*g*] CHROMENE-2,5,10-TRIONESDERIVATIVES (**6a-t**) AGAINST Hep2, A549, HeLa HUMAN CANCER CELLS BY MMT ASSAY EXPRESSED IN IC_{50} (µM)[a]

Entry	Compd.	Hep 2[b]	A549[c]	HeLa[d]
1	**6a**	50 ± 0.91	>100	>100
2	**6b**	50 ± 1.85	>100	>100
3	**6c**	45 ± 0.52	35 ± 0.84	50 ± 0.96
4	**6d**	45 ± 1.32	30 ± 1.78	50 ± 1.12
5	**6e**	25 ± 2.5	20 ± 1.72	35 ± 4.78
6	**6f**	30 ± 1.65	35 ± 2.5	35 ± 0.96
7	**6g**	40 ± 0.80	>100	>100
8	**6h**	50 ± 3.63	24 ± 1.85	35 ± 2.8
9	**6i**	50 ± 4.78	10 ± 0.95	>100
10	**6j**	35 ± 0.62	>100	>100
11	**6k**	24 ± 1.65	35 ± 2.5	15 ± 0.96
12	**6l**	2.5 ± 0.20	24 ± 1.85	18 ± 1.31
13	**6m**	100 ± 9.83	25 ± 1.37	>100
14	**6n**	35 ± 2.8	60 ± 5.7	12 ± 1.0
15	**6o**	30 ± 1.45	35 ± 1.25	35 ± 1.90
16	**6p**	20 ± 0.52	20 ± 0.84	35 ± 0.96
17	**6q**	6 ± 0.24	8 ± 0.52	14 ± 0.82
18	**6r**	4 ± 0.85	7 ± 0.28	12 ± 0.78
19	**6s**	2.5 ± 0.20	6 ± 0.85	10 ± 1.31
20	**6t**	2.2 ± 0.06	4 ± 0.35	9 ± 0.73
18	Doxorubicin	10 ± 0.8	0.65 ± 0.04	1.54 ± 0.08
19	Paclitaxel	1.8 ± 0.12	0.175 ± 0.01	0.26 ± 0.01

[a]Results are the average of three independent experiments; [b]Hep2-Human laryngeal carcinoma; [c]A549-Human lung adenocarcinoma; [d]HeLa-Human cervical cancer.

(MTT) reduction test with the panel of three cancer cell lines for 48 h. All the compounds exhibited minimal cytotoxicity on 'human peripheral blood mononuclear cell' (hPBMC), which indicates that benzochromene triones (**6a-t**) are selectively toxic towards cancer cell lines.

Conclusion

We have synthesized a combinatorial library of benzo chromene triones among the novel series benzo chromene triones (**6a-t**) compound **6t** (*B. subtilis, K. pneumonia*, *P. aeruginosa* and *E. coli*) potent for antibacterial activity and similarly compound **6t** (*Aspergillus sps*, *C. albicans*) exhibit good antifungal activity. Evaluated their antiproliferative activity against human laryngeal carcinoma (Hep2), lung adenocarcinoma (A549) and cervical cancer (HeLa). Among these, compounds dihydrofuran substituted benzochromene trione having four methoxy groups displayed the most potent antiproliferative activity against the three-cell lines uniformly. Toxicity studies revealed that the dihydrofuran substituted 1*H*-benzo[*g*] chromene-2,5,10-triones (**6a-t**) are specifically target the cancer cell lines. Thus we have discovered (**6t**) as the most potential anticancer molecule.

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REFERENCES

1. A.J.M. da Silva, C.D. Buarque, F.V. Brito, L. Aurelian, L.F. Macedo, L.H. Malkas, R.J. Hickey, D.V.S. Lopes, F. Noël, Y.L.B. Murakami, N.M.V Silva, P.A. Melo, R.R.B Caruso, N.G. Castro and P.R.R Costa, *Bioorg. Med. Chem.*, **10**, 2731 (2002).
2. B.G. Qabaja and G.B. Jones, *J. Org. Chem.*, **65**, 7187 (2000).
3. M. Rueping, E. Sugiono and E. Merino, *Angew. Chem. Int. Ed.*, **47**, 3046 (2008).
4. P.M. Brown, V. Krishnamoorthy, J.W. Mathieson and R.H. Thomson, *J. Chem. Soc. C*, 109 (1970).
5. M.A. Keniry and G.A. Poulton, *Magn. Reson. Chem.*, **29**, 46 (1991).
6. I.E. Soria-Mercado, A. Prieto-Davo, P.R. Jensen and W. Fenical, *J. Nat. Prod.*, **68**, 904 (2005).
7. M.P.S. Ishar, G. Singh, S. Singh, K.K. Sreenivasan and G. Singh, *Bioorg. Med. Chem. Lett.*, **16**, 1366 (2006).
8. J.Y. Goujon, F. Zammattio, S. Pagnoncelli, Y. Boursereau and B. Kirschleger, *Synlett*, 322 (2002).
9. B. Kesteleyn, N. De Kimpe and L. Van Puyvelde, *J. Org. Chem.*, **64**, 1173 (1999).
10. S. Claessens, G. Verniest, J. Jacobs, E. Van Hende, P. Habonimana, T. Nguyen Van, L. Van Puyvelde and N. De Kimpe, *Synlett*, 829 (2007).
11. B. Kesteleyn, N. De Kimpe and L. van Puyvelde, *Synthesis*, 1881 (1999).
12. T.B. Machado, A.V. Pinto, M.C.F.R. Pinto, I.C.R. Leal, M.G. Silva, A.C.F. Amaral, R.M. Kuster and K.R. Netto-dos Santos, *Int. J. Antimicrob. Agents*, **21**, 279 (2003).
13. S.L. Schreiber, *Science*, **287**, 1964 (2000).
14. L.A. Thompson, *Curr. Opin. Chem. Biol.*, **4**, 324 (2000).
15. B.M. Trost, *Angew. Chem. Int. Ed. Engl.*, **34**, 259 (1995).
16. L.F. Tietze, *Chem. Rev.*, **96**, 115 (1996).
17. I. Ugi, *Pure Appl. Chem.*, **73**, 187 (2001).
18. A. Dömling, *Chem. Rev.*, **106**, 17 (2006).
19. D.J. Ramon and M. Yus, *Angew. Chem. Int. Ed.*, **44**, 1602 (2005).
20. A. Dömling and I. Ugi, *Angew. Chem. Int. Ed.*, **39**, 3168 (2000).
21. J.D. Sunderhaus, C. Dockendorff and S.F. Martin, *Org. Lett.*, **9**, 4223 (2007).
22. F. Liéby-Muller, T. Constantieux and J. Rodriguez, *J. Am. Chem. Soc.*, **127**, 17176 (2005).
23. C. Haurena, E. Le Gall, S. Sengmany, T. Martens and M. Troupel, *J. Org. Chem.*, **75**, 2645 (2010).
24. B. Willy and T.J.J. Muller, *Eur. J. Org. Chem.*, 4157 (2008).
25. V.S. Tangeti, G.V. Siva Prasad, J. Panda and K.R. Varma, *Synth. Commun.*, **46**, 878 (2016).
26. H.S.P. Rao and V.S. Tangeti, *J. Chem. Sci.*, **125**, 777 (2013).
27. H.S.P. Rao, V.S. Tangeti and L. Adigopula, *Res. Chem. Intermed.*, **42**, 7285 (2016).
28. V.S. Tangeti, R. Varma K, G.V. Siva Prasad and K.V.V.V. Satyanarayana, *Synth. Commun.*, **46**, 613 (2016).
29. J.C. Duff and E.J. Bills, *J. Chem. Soc.*, 1987 (1932).
30. J.B. Harborne and H. Baxter, The Handbook of Natural Flavonoids, John Wiley & Sons, Chichester, UK (1999).