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Drug efficacy of novel 3-O-methoxy-4-halo disubstituted 5,7-dimethoxy chromans; evaluated via DNA gyrase inhibition, bacterial cell wall lesion and antibacterial prospective

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ABSTRACT

In this study, novel 3-O-methoxy-4-halo, disubstituted-5,7-dimethoxy chromans with bacterial cell wall degrading potentials were synthesized, characterized and evaluated as DNA gyrase inhibitors and antibacterial agents. Compounds were showed a broad spectrum of antimicrobial activity against both Gram^{+ve} bacteria (*S. aureus* (MTCC 3160), *C. diphtheriae* (MTCC 116), *S. pyogenes* (MTCC 442)) and Gram^{-ve} bacteria (*E. coli* (MTCC 443), *P. aeruginosa* (MTCC 424), *K. pneumoniae* (MTCC 530)). Further, a molecular docking study was carried out to get more insight into the binding mode of present study compounds to target proteins (PDB ID: 2XCT (*S. aureus* DNA gyrase A), PDB ID: 3G75 (*S. aureus* DNA gyrase B), PDB ID: 3L7L (Teichoic acid polymerase). In the results, 14 > 20 > 24 > 12 > 18 > 17 were found as the most active against almost all executed activities in this study. The predicted Lipinski's filter scores, SAR, pharmacokinetic/pharmacodynamics, and ADMET properties of these compounds envisioned the druggability prospects and the necessity of further animal model evaluations of 3-O-methoxy-4-halo disubstituted 5,7-dimethoxy chromans to establish them as an effective and future antibiotics.

1. Introduction

"Medicine is a science of uncertainty and an art of probability" – William Osler. Antibiotics are uniquely societal drugs because individual use affects others in the community and environment." – Dr. Stuart Levy. After reading these quotes, we have started to think about most probable and safer antibiotics, why because the bacterial resistant to the available antibiotics gradually increasing day by day. Chroman and coumarins are the highly demanded small molecule chemical entities in heterocyclic chemistry, especially in novel antibiotic designing and development. For example, Novobiocin and Clorobiocin are coumarin based anti-microbial drugs.[1–4] In recent decades, coumarins based compounds have been recognized and are established as an effective antioxidant,[5] anticancer,[6,7] anti-inflammatory,[7] antineurotic[8], antifungal,[9] analgesic and anticonvulsant agents.[10] Most significantly, a number of Chroman compounds have been identified from natural sources (Plants) such as *Melicope pteleifolia*,[11] *Evodia lepta*[12]and *Melicope stipitata*.[13] Aiming to obtain an effective, therapeutically potential chroman or coumarin based antibiotic, it was highly necessitated to understand the physiologically and genetically significant targets of bacteria. In such condition, our present study's main objective is to target both physical (cell wall) and genetically important (DNA gyrase) as markers to develop the chroman or coumarin compounds as a new class of antibiotics.

In the bacterial pathogenicity, bacterial cell wall playing a vital role and being the most responsive and foremost part and also causing atrocious effects in host system.[14–16] Peptidoglycan (PG) and teichoic acids are fabricating the thick cell wall of Gram^{+ve} bacteria to maintain the structural association and the shape of the cell.[17] Composed with polyol-phosphate units, Teichoic acid polymers are playing a key constitutive role in the Gram^{+ve} bacterial cell wall construction and this cell wall is amalgamated by monotopic membrane proteins of the TagF (Teichoic acid polyglycerol phosphate) polymerase family.[18] This TagF polymerase is accomplished by the elongation substrate CDP-glycerol which is acting as an originator substrate in the cell wall synthesis.[19] With this, the CDP-glycerol: poly glycerol phosphotransferase (CGPTase) enzyme is also constantly involved in the teichoic acid development like the teichoic acid polymerases.[20] Apart from the al cell wall pathogenicity, pathogenic bacterial genetic material (DNA) is considered as uncontrolled to multiply into double, triple… and so on

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in a quick time period. This makes the bacterial pathogens very tough to eliminate them from their host environments especially from the humans.

In the bacterial genetic system, DNA gyrase (Type II topoisomerase), an essential bacterial enzyme that catalyzes the ATP-dependent negative super-coiling of double-stranded closed-circular DNA, involving in the formation of supercoils by cutting the two ends of single circular DNA and get twisted around each other.[21–23] Therefore, either degrading the cell wall constitution or inhibiting DNA gyrase would be the precise way to compete against bacterial toxicity. Considering all the above discussed points, in this study, the 3-O-methoxy-4-halo disubstituted 5,7-dimethoxy chromans along with a family of novel cis-2,2dihydroxy-5,7dimethoxy-6-acetyl-2,2dimethyl chromans (10, 11) and 6-acetyl-5-hydroxy-7-methoxy-2,2-dimethyl chroman-4-ones that were supplemented with a mixture of electron donating or withdrawing substituents was efficiently synthesized and validated as DNA gyrase inhibitors (*in vitro*) as well as the cell wall teichoic acid polymerase inhibitors (*in silico*) along with their antibacterial effects.

2. Materials and methods

2.1. Chemistry

All chemicals and solvents were procured from Sigma-Aldrich, Merck, and Himedia, AD-mix-alpha, AD-mix-beta (USA), and were used as received without doing further purification. Thin-Layer chromatography (TLC) was executed using pre-coated silica plates (Merck-silica gel 60 F254). Merck silica gel 60 (230–400 mesh) was used for flash column chromatography. Melting point (mp) were checked using an OptiMelt automated melting point system and are uncorrected. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on Bruker-400 MHz instrument. Mass spectra of all compounds were recorded on Agilent ion trap MS and Infrared (IR) spectra were recorded on Perkin Elmer FT-IR spectrometer.

2.1.1. Procedure for the preparation 5,7-dimethoxy-2,2-dimethylchroman-4-one (1)

A mixture of 5,7-dihydroxy-2,2-dimethyl chroman-4-one 5.0 g (0.10 mol), MeI 8.52 g (0.25 mol), K_2CO_3 4.98 g (0.15 mol) in acetone (20.0 ml) was stirred at 60 °C for 20 h. After completion of the reaction, as indicated by TLC, the solvent was distilled out under vaccum. The resulting residue was dissolved in ethyl acetate and water. The obtained crude product was extracted with ethyl acetate (2 × 20 ml) and washed with water (20 ml) followed by treated with saturated sodium chloride solution (20 ml) and dried over (Na_2SO_4). The solvent was distilled off and the resulting product was purified by diethyl ether and diisopropyl ether mixture (5 ml:15 ml) to obtain off white solid (5.0 g, 89%). Melting point: 103.3–104.4 °C: IR (KBr) ϒmax: 2981, 2968, 1678, 1572, 1427, 1383, 1368, 1316, 1244, 1117, 1065, 1031, 1001, 936, 880, 815, 730, 659, 587. ^{1}H NMR: NMR (400 MHz, $CDCl_3$) δ ppm: 5.80 (s, 2H), 3.64 (s, 3H), 3.59 (s, 3H), 2.41 (s, 2H), 1.20 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 189.46, 165.77, 163.25, 161.72, 104.98, 93.64, 92.16, 78.63, 55.90, 55.35, 49.89, 26.35. MS (EI, *m/z*): 237.0 (M +). Analysed and calculated for $C_{13}H_{16}O_4$: C, 66.09; H, 6.83; O, 27.08%. Found: C, 66.05; H, 6.85; O, 27.10%.

2.1.2. Procedure for the preparation of 5,7-dimethoxy-2,2-dimethylchroman-4-ol (2)

To a solution of 5,7-dimethoxy-2,2-dimethylchroman-4-one (**1**) 5.0 g (0.1 mol) in methanol (20 ml) at 0–5 °C, and $NaBH_4$ (0.80 g, 0.1 mol) was added. The reaction mixture was stirred for 2–3 h at 25–30 °C. The pH was adjusted to 7.0–8.0 using acetic acid (2.0 ml). After completion of the reaction, as indicated by TLC, the solvent was distilled out under vaccum. The resulting residue was dissolved in ethyl acetate and water. Product was extracted with ethyl acetate (2 × 20 ml) and washed with water (20 ml) followed by treating with saturated sodium chloride solution (20 ml) and dried over (Na_2SO_4). Further, the solvent was distilled off and the resulting syrup product **2** (5.0 g, 5,7-dimethoxy-2,2-dimethylchroman-4-ol) was recovered. IR (KBr) ϒmax: 3438, 3102, 2982, 2968, 1678, 1464, 1427, 1368, 1118, 1031, 1001, 815, 729, 658 cm^{-1}. ^{1}H NMR: NMR (400 MHz, $CDCl_3$) δ ppm: 6.05 (s, 2H), 5.91 (s, 1H), 4.72 (s, 1H), 4.46 (s, 1H), 3.77 (s, 3H), 3.73 (s, 3H), 2.30 (m, 1.90–1.77), 1.34 (S, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 160.76, 159.90, 154.81, 106.08, 93.87, 91.35, 74.52, 58.17, 55.90, 55.78, 41.52, 29.05, 26.52. Analysed and calculated for $C_{13}H_{18}O_4$: C, 65.53; H, 7.61; O, 26.86%. Found: C, 65.51; H, 7.62; O, 26.87%.

2.1.3. Procedure for the preparation of 5,7-dimethoxy-2,2-dimethyl-2H-chromene (3)

The compound 5,7-dimethoxy-2,2-dimethylchroman-4-ol (**2**) was dissolved in 20 ml of DMSO. With this, 5.0 ml of 1.5 N HCl was added at 25–30 °C under stirring condition. Then the mass was stirred for 2 h at 25–30 °C. After completion of the reaction, as indicated by TLC, pH was adjusted to 7.0–8.0 using 10% $NaHCO_3$ solution. The product was further extracted using ethyl acetate (20 ml × 2) in water. The product layer (Ethyl acetate layer) washed with 20.0 ml water again followed by treating with saturated sodium chloride solution (20 ml) and dried over (Na_2SO_4). The solvent was distilled off and the resulting product was purified by column chromatography using ethyl acetate and n-hexane to get pure syrup product (**3**) (4.4 g, 88.6%). IR (KBr) ϒmax: 2964, 2874, 1633, 1606, 1575, 1494, 1462, 1423, 1389, 1376, 1345, 1303, 1247, 1168, 1002, 934, 881, 867, 822, 704, 638, 616 cm^{-1}. ^{1}H NMR: (400 MHz, $CDCl_3$) δ ppm: 6.528–6.503 (d, 1H, J = 10.0 Hz), 5.97 (s,1H), 5.363–5.33(d, 1H, J = 9.6 Hz), 3.71(s, 3H), 3.69 (s, 3H), 1.34 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 161.01, 156.14, 154.67, 125.86, 116.72, 104.18, 93.98, 91.44, 55.50, 55.30, 27.77. MS (EI, *m/z*): 221.1 (M+). Analysed and calculated for $C_{13}H_{16}O_3$: C, 70.89; H, 7.32; O, 21.79%. Found: C, 70.90; H, 7.34; O, 21.76%.

2.1.4. Preparation of 1-(5,7-dimethoxy-2,2-dimethyl-2H-chromen-6-yl) ethanone (4a) and 1-(5,7-dimethoxy-2,2-dimethyl-2H-chromen-8-yl) ethanone (4b) using Friedel-Crafts acylation (Scheme 1)

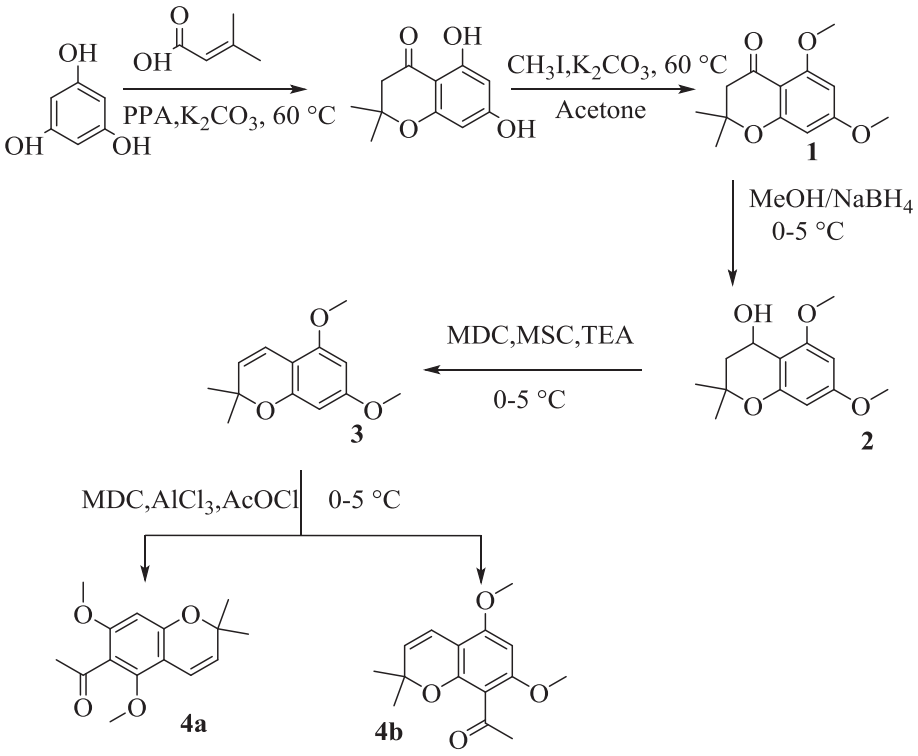
To a solution of 5,7-dimethoxy-2,2-dimethyl-2H-chromene (**3**) 4.0 g (0.1 mol) in MDC, $AlCl_3$ 4.84 g (0.2 mol) was added at 0–5 °C. To this reaction mixture acetyl chloride 2.14 g (0.15 mol) was added slowly at 0–5 °C and stirring was continued for about 1–2 h at 25–30 °C. After completion of the reaction, as indicated by TLC, the reaction mass was cooled and water was added slowly under stirring condition. Product was extracted in MDC (12 × 2) and washed with water followed by treating with saturated sodium chloride solution (20 ml) and dried over (Na_2SO_4). The solvent was distilled off and the resulting product was purified by column chromatography using ethyl acetate and n-hexane to get pure solids of **4a** (25%) and **4b** (40%).

2.1.4.1. 1-(5,7-dimethoxy-2,2-dimethyl-2H-chromen-6-yl)ethanone (4a).

Pale yellow solid, Melting point: 93.7–94.2 °C. IR (KBr) ϒmax: 3048, 2947, 2937, 1695, 1634, 1608, 1478, 1330, 1155, 1101, 888, 808, 691 cm^{-1}. ^{1}H NMR: NMR (400 MHz, DMSO) δ ppm: 6.442–6.418 (d, 1H, J = 9.6), 5.663–5.638(d, 1H, J = 10.0), 6.34 (s, 1H), 3.74 (s, 3H), 3.65 (s, 3H), 2.37 (s, 3H), 1.37 (s, 6H). ^{13}C NMR (100 MHz, DMSO) δ ppm: 200.14, 157.07, 155.21, 153.25, 128.08, 117.94, 115.74, 107.19, 96.09, 76.50, 63.05, 55.87, 32.26, 27.43. NOESY (400 MHz DMSO): Aromatic proton at (C8) shows correlation with C-7 attached O-methoxy functional group only. MS (EI, *m/z*): 263.1 (M+). Analysed and calculated for $C_{15}H_{18}O_4$: C, 68.68; H, 6.92; O, 24.40%. Found: C, 68.69; H, 6.90; O, 24.41%.

2.1.4.2. 1-(5,7-dimethoxy-2,2-dimethyl-2H-chromen-8-yl)ethanone (4b).

Off white solid, Melting point: 105.3–107.5 °C. IR (KBr) ϒmax: 3002, 2985, 2964, 2944, 1705, 1637, 1469, 1334, 1156, 1114, 880, 794, 684 cm^{-1}. ^{1}H NMR: NMR (400 MHz, DMSO) δ ppm: 6.49–6.47 (d, 1H, J = 8.0 MHz), 5.55–5.53 (d, 1H, J = 8.0 MHz), 6.26 (s, 1H), 3.83



Scheme 1. Route of synthesis of compounds **4a** and **4b** (approach – 1).

(s, 3H), 3.77 (s, 3H), 2.33 (s, 3H), 1.33 (s, 6H). ^{13}C NMR (100 MHz, DMSO) δ ppm: 199.91, 157.63, 156.59, 150.85, 127.34, 116.43, 113.50, 103.69, 89.29, 76.91, 56.27, 32.94, 27.58. NOESY (400 MHz DMSO): Aromatic proton attached to C6 carbon shows correlation with both C-5 and C-7O-methoxy functional groups. C-8 acetyl group shows correlation with C-7 attached O-methoxy functional group. MS (EI, *m/z*): 263.0 (M+). Analysed and calculated for $C_{15}H_{18}O_4$: C, 68.68; H, 6.92; O, 24.40%. Found: C, 68.69; H, 6.90; O, 24.41%.

2.1.5. Preparation of 6-acetyl-5,7-dimethoxy-2,2-dimethylchroman-4-one (5)

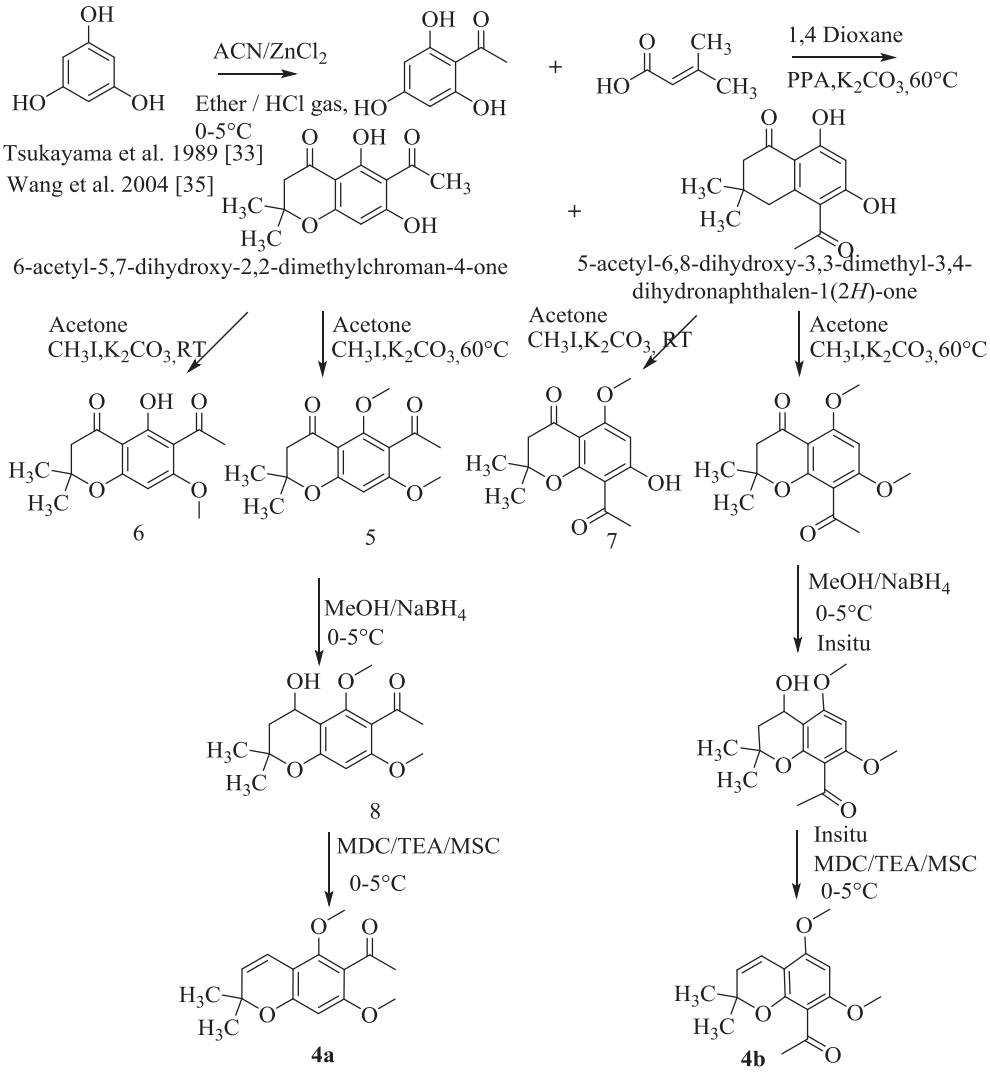
A mixture of 6-acetyl-5,7-dihydroxy-2,2-dimethylchroman-4-one 15.0 g (0.10 mol), MeI 34.0 g (0.4 mol), K_2CO_3 12.40 g (0.15 mol) in acetone 75.0 ml was stirred at 60 °C for 20 h. After the completion of reaction, as indicated by TLC, solvent was distilled out under vaccum. The resulting residue was dissolved in ethyl acetate and water. Product was extracted with ethyl acetate (2 × 20 ml) and washed with water (20 ml) followed by treating with saturated sodium chloride solution (20 ml) and dried over (Na_2SO_4). The solvent was distilled off and the resulting product was purified by diethyl ether and diisopropyl ether mixture (5 ml:15 ml) to obtain off white solid (14.67 g, 88.0%). Melting point: 153.5–155.1 °C. IR (KBr) ϒmax: 2983, 2969, 2958, 2849, 1704, 1597, 1474, 1447, 1395, 1186, 1131, 1061,885, 806, 666 cm^{-1}. ^{1}H NMR: NMR (400 MHz, $CDCl_3$) δ ppm: 5.98 (s, 1H), 3.87 (s, 3H), 3.80 (s, 3H), 2.58 (s, 2H), 2.9 (s, 3H), 1.36 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 200.43, 189.35, 162.82, 162.04, 159.13, 113.19, 104.81, 87.97, 79.58, 56.19, 55.87, 49.98, 32.41, 26.42. MS (EI, *m/z*): 279.1 (M+). Analysed and calculated for $C_{15}H_{18}O_5$: C, 64.74; H, 6.52; O, 28.74%. Found: C, 64.73; H, 6.51; O, 28.76%.

2.1.6. Preparation of 6-acetyl-5-hydroxy-7-methoxy-2,2-dimethylchroman-4-one (6)

A mixture of 6-acetyl-5,7-dihydroxy-2,2-dimethylchroman-4-one (Note: This compound was synthesized according to Tsukyama et al. 1989[33]; Wang et al. 2004.[35] Refer Scheme 2) 15.0 g (0.10 mol), MeI 17.0 g (0.2 mol), K_2CO_3 12.40 g (0.15 mol) in acetone 75.0 ml was stirred 25–30 °C for 20 h. After the completion of reaction, as indicated by TLC, solvent was distilled out under vaccum. The crude reaction mixture was then dissolved in ethyl acetate and water. The final product was extracted with ethyl acetate (2 × 20 ml) and washed with water (20 ml) followed by treating with saturated sodium chloride solution (20 ml) and dried over (Na_2SO_4). The solvent was distilled off and the resulting product was purified by diethyl ether and diisopropyl ether mixture (5 ml:15 ml) to obtain off white solid (14.40 g, 95.0%). Melting point: 197.7–199.4 °C. IR (KBr) ϒmax: 3432, 2926, 1701, 1676, 1630, 1598, 1452, 1374, 1263, 1228, 1110, 982, 914, 877, 829, 639 cm^{-1}. ^{1}H NMR: NMR (400 MHz, $CDCl_3$) δ ppm: 12.64 (s, 1H, D_2O exchangeable), 6.20 (s, 1H), 3.83 (s, 3H), 2.85 (s, 2H), 2.39 (s, 3H), 1.42 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 198.89, 196.15, 164.36, 162.63, 159.92, 110.71, 101.25, 91.90, 80.00, 56.44, 46.76, 32.24, 26.01. MS (EI, *m/z*): 265.1 (M+). Analysed and calculated for $C_{14}H_{16}O_5$: C, 63.63; H, 6.10; O, 30.27%. Found: C, 63.64; H, 6.11; O, 30.25%; HRMS Calculated [M+] *m/z* 264.2770, Found 264.2770.

2.1.7. Preparation of 8-acetyl-7-hydroxy-5-methoxy-2,2-dimethylchroman-4-one (7)

A mixture of 8-acetyl-5,7-dihydroxy-2,2-dimethylchroman-4-one (Note: This compound was synthesized according to Tsukyama et al. 1989[33]; Wang et al. 2004.[35] Refer Scheme 2) 15.0 g (0.10 mol), MeI 17.0 g (0.2 mol), K_2CO_3 12.40 g (0.15 mol) in acetone 75.0 ml was stirred at 25–30 °C for 20 h. After the completion of reaction, as indicated by TLC, solvent was distilled out under vaccum. The resulting residue was dissolved in ethyl acetate and water. Product was extracted with ethyl acetate (2 × 20 ml) and washed with water (20 ml) followed by treating with saturated sodium chloride solution (20 ml) and dried over (Na_2SO_4). The solvent was distilled off and the resulting product was purified by diethyl ether and diisopropyl ether mixture (5 ml:15 ml) to obtain off white solid (13.0 g, 84.0%). Melting point: 80.6–82.6 °C. IR (KBr) ϒmax: 3430, 3018, 3001, 2971, 1697, 1645, 1585, 1465, 1386, 1372, 1258, 1230, 1098, 1011, 980, 910, 885, 831, 628 cm^{-1}. ^{1}H NMR: NMR (400 MHz, $CDCl_3$) δ ppm: 12.32 (s, 1H, D_2O exchangeable), 6.21 (s, 1H), 3.80 (s, 3H), 2.84 (s, 2H), 2.35 (s, 3H),



Scheme 2. Route of synthesis of compounds **4a** and **4b** (approach – 2).

1.39 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 198.47, 196.75, 163.97, 163.77, 156.87, 111.78, 101.15, 92.31, 80.08, 56.37, 46.57, 32.25, 25.98. MS (EI, *m/z*): 265.1 (M+). Analysed and calculated for $C_{14}H_{16}O_5$: C, 63.63; H, 6.10; O, 30.27%. Found: C, 63.64; H, 6.11; O, 30.25%.

*2.1.8. Preparation of 1-(4-hydroxy-5,7-dimethoxy-2,2-dimethylchroman-6-yl)ethanone (**8**)*

To the 6-acetyl-5,7-dimethoxy-2,2-dimethylchroman-4-one (**5**) 15.0 g (0.1 mol) in methanol (60 ml), $NaBH_4$ (2.45 g, 0.12 mol) was added at 0–5 °C. The pH was adjusted to 7.0–8.0 using acetic acid (2.0 ml). Further, the reaction mixture was stirred for 2–3 h at 25–30 °C. After completion of the reaction, as indicated by TLC, solvent was distilled out under vaccum. The resulting residue was dissolved in ethyl acetate and water. Product was extracted with ethyl acetate (2 × 20 ml) and washed with water (20 ml) followed by treating with saturated sodium chloride solution (20 ml) and dried over (Na_2SO_4). The solvent was distilled off and the resulting solid purified using diethyl ether and n-hexane to obtain a white colour solid (14.05 g, 93.0%). Melting point: 99.6–101.5 °C. IR (KBr) Ymax: 3504, 2981, 2942, 2887, 2843, 1692, 1602, 1467, 1439, 1347, 1183, 1154, 1111, 1048, 884, 793, 616.6 cm^{-1}; ^{1}H NMR (400 MHz, DMSO) δ ppm: 6.22 (s, 1H), 5.05 (s, 1H, D_20 exchangeable) 3.80 (s, 3H), 3.72 (s, 3H), 1.80–1.90 (m, 2H), 2.28 (s, 3H), 1.32 (s, 3H), 1.24 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ ppm: 200.66, 160.41, 157.11, 151.42, 113.02, 106.35, 88.93, 75.28, 58.37, 56.23, 41.20, 32.83, 29.39, 26.45. MS (EI, *m/z*): 281.1 (M+). Analysed and calculated for $C_{15}H_{20}O_5$: C, 64.27; H, 7.19; O, 28.54 Found: C, 64.29; H, 7.16; O, 28.55%; HRMS Calculated [M+] *m/z* 280.3200, Found 280.3210.

*2.1.8.1. Preparation of 1-(5,7-dimethoxy-2,2-dimethyl-2H-chromen-6-yl) ethanone (**4a**)*. To a solution of 1-(4-hydroxy-5,7-dimethoxy-2,2-dimethyl chroman-6-yl)ethanone (**8**) 10 g (0.1 mol) in MDC (40 ml), TEA (7.60 g, 0.2 mol) was added at 0–5 °C. To this, methane sulphonyl chloride 4.30 g (0.1 mol) dissolved in MDC (10.0 ml) was slowly added at 0–5 °C. The was mass stirred for 15–30 min at 0–5 °C. After the completion of the reaction, as indicated by TLC, the reaction mixture was quenched with water (20.0 ml) and the product was extracted in MDC (20 ml × 2). The product layer (MDC) washed with water 20.0 ml followed by treating with saturated sodium chloride solution (20 ml) and dried over (Na_2SO_4). The solvent was distilled off and the resulting product was purified by diethyl ether and n-hexane to obtain pale yellow color solid (**4a**) 8.4 g, 90%.

*2.1.8.2. Preparation of 1-(5,7-dimethoxy-2,2-dimethyl-2H-chromen-8-yl) etha-none (**4b**)*. A mixture of the 8-acetyl-5,7-dihydroxy-2,2-dimethyl chroman-4-one (Scheme 2) 15.0 g (0.10 mol), MeI 34.0 g (0.4 mol), K_2CO_3 12.40 g (0.15 mol) in acetone 75.0 ml was stirred at 60 °C for 20 h. After completion of the reaction, as indicated by TLC, the solvent was distilled out under vacuum. The resulting residue was dissolved in

ethyl acetate and water. The product was extracted with ethyl acetate (2 × 20 ml) and washed with water (20 ml) followed by treating with saturated sodium chloride solution (20 ml) and dried over (Na_2SO_4). The solvent was distilled off and the resulting product 8-acetyl-5,7-dimethoxy-2,2-dimethyl chroman-4-one (Scheme 2) without isolation was taken to next step. A mixture of the 6-acetyl-5,7-dimethoxy-2,2-dimethyl chroman-4-one (**5**) 15.0 g (0.1 mol) in methanol (60 ml) at 0–5 °C, $NaBH_4$ (2.45 g, 0.12 mol) was added. Further, the reaction mixture was stirred for 2–3 h at 25–30 °C. After completion of the reaction as indicated by TLC, adjusted the pH to 7.0–8.0 using acetic acid (2.0 ml), solvents was distilled out under vacuum.

Further, the resulting residue was dissolved in ethyl acetate and water. The product was extracted with ethyl acetate (2 × 20 ml) and washed with water (20 ml) followed by treating with saturated sodium chloride solution (20 ml) and dried over (Na_2SO_4). The solvent was distilled off and the resulting product [1-(4-hydroxy-5,7-dimethoxy-2,2-dimethylchroman-8-yl)ethanone] without isolation was taken to next step. The above material dissolved in MDC (40 ml) at 0–5 °C, TEA (7.60 g, 0.2 mol) was added. Methane sulphonyl chloride 4.30 g (0.1 mol) was dissolved in MDC (10.0 ml) was added slowly to the reaction mass at 0–5 °C and stirred the mass for 15–30 min at 0–5 °C. After completion of the reaction, the reaction mass was quenched with water (20.0 ml). Extracted the product in MDC (20 ml × 2). The product layer (MDC) washed with water 20.0 ml followed by treating with saturated sodium chloride solution (20 ml) and dried over (Na_2SO_4). The solvent was distilled off and the resulting product was purified by diethyl ether and n-hexane to obtain off-white color solid (**4b**) 14.15 g, 90%.

2.1.9. Preparation of 1-(5-hydroxy-7-methoxy-2,2-dimethyl-2H-chromen-8-yl)ethanone (9)

A solution of 1-(5,7-dimethoxy-2,2-dimethyl-2H-chromen-8-yl) ethanone 10 g (**4b**) (0.10 mol) in MDC (60 ml) was cooled to −70 to −80 °C under nitrogen atmosphere. Boron tribromide 0.95 g (0.1 mol) was dissolved in MDC (10.0 ml) was added slowly to the reaction mass at −75 to −80 °C. Further, the mass was stirred for 30 min at −75 to −80 °C. After completion of the reaction as indicated by TLC, the reaction mixture was quenched with methanol (10.0 ml) and the product was extracted in MDC (30 ml × 2). Then the product layer (MDC layer) was washed with water (30.0 ml) followed by treating with saturated sodium chloride solution (30 ml) and dried over (Na_2SO_4). Finally, the solvent was distilled off and the resulting product was purified by column chromatography to obtain pale yellow color solid (**6**). Melting point: 62.6–64.5 °C. IR (KBr) ϒmax: 3522, 3051 2969, 2926, 2858, 1611, 1586, 1491, 1330, 1172, 1107, 986, 876, 818, 690 cm^{-1}. ^1H NMR: NMR (400 MHz, DMSO) δ ppm: 13.72 (s, 1H, D_2O exchangeable), 6.46–6.43 (d, 1H, J = 12.0), 5.55–5.52 (d, 1H, J = 12.0), 6.09 (s, 1H), 3.81 (s, 3H), 3.2.59 (s, 3H), 1.43 (s, 6H). ^{13}C NMR (100 MHz, DMSO) δ ppm: 203.38, 165.87, 161.05, 156.01, 125.82, 116.20, 105.80, 102.75, 92.87, 78.32, 56.50, 33.32, 27.65. MS (EI, *m/z*): 263.1 (M+). Analysed and calculated for $C_{14}H_{16}O_4$: C, 67.73; H, 6.50; O, 25.78%. Found: C, 67.75; H, 6.51; O, 25.74%; HRMS Calculated [M+] *m/z* 248.1049, Found 248.1049.

2.2. General procedure for the preparation of 3, 4 dihydroxy chromans

Procedure-1: A mixture of chiral ligand α or β – AD-mix [$(DHQD)_2PHAL$] 3.45 g (0.110 mol) in a mixture of 5.0 ml tertiary butanol and 5 ml water, stirred to get a clear solution at room temperature (RT). Methanesulfonamide 0.416 g (0.10 mol) was added and stirred at RT for 5 min. The mixture was cooled to 0–2 °C. 1.0 g (0.1 mol) of 1-(5,7-dimethoxy-2,2-dimethyl-2H-chromen-6-yl) ethanone was added and the mass were stirred at 0–2 °C for 20–24 h. After the reaction completion, as indicated by TLC, 3.5 g of solid sodium sulfite was added at 0–2 °C and this mass was stirred for 1 h at RT. The product was extracted with ethyl acetate (2 × 20 ml) and washed with 2 N KOH solution (10 ml), water (20 ml) followed by treating with saturated sodium chloride solution (20 ml) and dried over (Na_2SO_4). The solvent was distilled off and the resulting solid was purified by flash chromatography (silica gel, ethyl acetate/n-hexane). In this solvent system, the ligand does not move and so that we obtained 1.02 g of an off-white color solid (90%).

Procedure-2: A Mixture of Chiral ligand α or β – AD-mix [$(DHQD)_2PHAL$] 3.45 g (0.110 mol) in MDC 5.0 ml, OsO_4 1.12 g (0.11 mol) was added and stirred at RT for 5 min. The mass was stirred for 60 min at −20 °C, and then a solution of 1-(5,7-dimethoxy-2,2-dimethyl-2H-chromen-6-yl)ethanone (**4a**) 1.0 g (0.1 mol) in MDC 3.0 ml was added slowly in the period of 15–20 min, the again the mass was stirred for 32–36 h at −20 to −18 °C. After the reaction completion, as indicated by TLC, the reaction mass was kept in RT. To this, 10% sodium sulfite (8.0 ml, pH ~9.0) and 10% sodium bi sulfite (8.0 ml, pH ~4.0) solution was added. This mass was stirred for 2 h followed by the addition of the solvents THF/Ethyl acetate (1:4, 50 ml). Further, the temperature was raised up to 50–55 °C and the stirring was continued for 2–4 h. Finally, the mass was filtered at RT. The final product was extracted in Ethyl actete, combined organic layers were washed with 0.1 N HCl followed by brine solution and dried over sodium sulphate and concentrated under vaccum to remove solvents completely. The resulting solid was purified by flash chromatography (silica gel, ethyl acetate/n-hexane) In this solvent system the ligand does not move and so that we obtained 1.0 g of an off-white color solid (88.5%).

2.2.1. 1-((3S,4S)-3,4-dihydroxy-5,7-dimethoxy-2,2-dimethylchroman-6-yl)ethanone (10)

The Chiral Ligand used in this reaction is β-AD-mix [$(DHQD)_2PHAL$], off white powder with 85% of yield. Melting point: 136.2–138.2 °C: IR (KBr) ϒmax: 3504, 3416, 2989, 2964, 2936, 1697, 1607, 1473, 1198, 1138, 1102, 990, 880, 830, 687 cm^{-1}; ^1H NMR: (400 MHz, DMSO) δ ppm: 6.23 (s, 1H), 4.97–4.96 (d, 1H, D_2O exchangeable), 4.85–4.84 (d, 1H, D_2O exchangeable), 4.695–4.674 (d, 1H, J = 4.0), 3.75 (s, 3H), 3.72 (s, 3H), 3.544–3.518 (t, 1H, J = 4.8), 2.39 (s, 3H), 1.33 (s, 6H). ^{13}C NMR (100 MHz, DMSO) δ ppm: 200.63, 157.55, 157.12, 154.97, 117.95, 110.32, 95.63, 77.89, 71.62, 63.10, 60.93, 55.73, 32.34, 26.89, 21.21. MS (EI, *m/z*): 296.5 (M+1). Analysed and calculated for $C_{15}H_{20}O_6$: C, 60.80; H, 6.80; O, 32.40%. Found: C, 60.81; H, 6.78; O, 32.41%; HRMS Calculated [M+] *m/z* 296.3190, Found 296.3200.

2.2.2. 1-((3R,4R)-3,4-dihydroxy-5,7-dimethoxy-2,2-dimethylchroman-6-yl)ethanone (11)

The Chiral Ligand used in this reaction is α-AD-mix [$(DHQD)_2PHAL$], off white powder with 88% of yield. Melting point: 124.2–126.4 °C. IR (KBr) ϒmax: 3504, 3416, 2989, 2964, 2937, 1697, 1608, 1474, 1199, 1139, 1102, 990, 880, 831, 688 cm^{-1}; ^1H NMR: (400 MHz, DMSO) δ ppm: 6.22 (s, 1H), 4.98–4.97 (d, 1H, D_2O exchangeable), 4.86–4.85 (d, 1H, D_2O exchangeable), 4.67–4.66 (d, 1H, J = 4.4), 3.73 (s, 3H), 3.71 (s, 3H), 3.52–3.51 (d, 1H, J = 5.2), 2.38 (s, 3H), 1.32 (s, 6H). ^{13}C NMR (100 MHz, DMSO) δ ppm: 200.72, 157.56, 157.13, 154.99, 117.96, 110.36, 95.64, 77.93, 71.63, 63.14, 60.94, 55.76, 32.39, 26.94, 21.25. NOESY (400 MHz DMSO): Aromatic proton at (C8) shows correlation with C-7 attached O-methoxy functional group only. MS (EI, *m/z*): 297.1 (M+1). Analysed and calculated for $C_{15}H_{20}O_6$: C, 60.80; H, 6.80; O, 32.40%. Found: C, 60.81; H, 6.78; O, 32.41%; HRMS Calculated [M+] *m/z* 296.3190, Found 296.3200.

2.2.3. 1-((3S,4S)-3,4-dihydroxy-5,7-dimethoxy-2,2-dimethylchroman-8-yl)ethanone (12)

The Chiral Ligand used in this reaction is β – AD-mix [$(DHQD)_2PHAL$], off white powder with 90% of yield. Melitng point: 119.6–121.8 °C. IR (KBr) ϒmax: 3337, 3007, 2975, 2938, 2914, 2842, 1682, 1606, 1478, 1174, 1136, 1098, 995, 870, 857, 677 cm-1; 1H NMR: (400 MHz, DMSO) δ ppm: 6.26 (s, 1H), 4.83–4.81 (d, 1H, D_2O exchangeable), 4.75–4.74 (d, 1H, D_2O exchangeable), 4.65–4.64 (d, 1H,

J = 4.0), 3.83 (s, 3H), 3.76 (s, 3H), 3.51–3.48 (t, 1H, J = 5.2), 2.35 (s, 3H), 1.29 (s,6H). ^{13}C NMR (100 MHz, DMSO) δ ppm: 200.05, 160.17, 156.97, 150.35, 112.28, 105.74, 88.47, 77.88, 71.53, 60.40, 55.79, 55.67, 32.30, 27.04, 20.58. NOESY (400 MHz DMSO): Aromatic proton (C6) shows correlation with both C-5 and C-7O-methoxy functional groups. MS (EI, *m/z*): 297.0 (M + 1). Analysed and calculated for $C_{15}H_{20}O_6$: C, 60.80; H, 6.80; O, 32.40%. Found: C, 60.79; H, 6.79; O, 32.42%, HRMS Calculated [M +] *m/z* 296.3190, Found 296.3200.

2.2.4. 1-((3R,4R)-3,4-dihydroxy-5,7-dimethoxy-2,2-dimethylchroman-8-yl)ethanone (13)

The Chiral Ligand used in this reaction is α – AD-mix [$(DHQD)_2PHAL$], off white powder with 90% of yield Melting point: 109.3–111.3 °C. IR (KBr) ϒmax: 3337, 3006, 2975, 2938, 2913, 2842, 1682, 1606, 1467, 1162, 1098, 998, 869, 857, 613 cm^{-1}; ^{1}H NMR: (400 MHz, DMSO) δ ppm: 6.26 (s, 1H), 4.80–4.78 (d, 1H, D_2O exchangeable), 4.73–4.72 (d, 1H, D_2O exchangeable), 4.658–4.637 (t, 1H, *J* = 4.0 Hz), 3.83 (s, 3H), 3.75 (s, 3H), 3.509–3.481 (t, 1H, J = 5.2), 2.32 (s, 3H), 1.28 (s, 6H). ^{13}C NMR (100 MHz, DMSO) δ ppm: 200.02, 160.19, 156.99, 150.37, 112.32, 105.75, 88.47, 79.15, 77.89, 71.54, 60.43, 55.79, 55.67, 32.29, 27.02, 20.59. MS (EI, *m/z*): 297.1 (M + 1). Analysed and calculated for $C_{15}H_{20}O_6$: C, 60.80; H, 6.80; O, 32.40%. Found: C, 60.81; H, 6.79; O, 32.40%; HRMS Calculated [M +] *m/z* 296.3190, Found 296.3200.

2.2.5. Synthesis of 8-acetyl-7-methoxy-2,2-dime-thyl-2H-chromen-5-yltrifluoromethanesulfonate (14)

To a solution of 1-(5-hydroxy-7-methoxy-2,2-dimethyl-2H-chromen-8-yl)ethanone (**9**), 1.0 g (0.10 mol) in MDC 50 ml, TEA (0.25 mol) was added to the reaction mass and cooled to 0–5 °C. With this mass, Triflic anhydride 1.4 g (0.11) was added slowly for 30 min, then the temperature was raised to 37 °C (RT). After stirred for 60 min at RT, the reaction was completed as indicated by TLC. The mass was then quenched with water and stirred for 30 min. The final product was extracted in MDC, then washed with water, NaCl solution and dried over Na_2SO_4. The remaining solvent in the reaction mixture was distilled out under vacuum to get syrup. The product was purified by column chromatography using EA – n-hexane system to obtain pale yellow color solid 1.45 g (95%). Melting point: 79.9–81.3 °C, IR (KBr) ϒmax: 3031, 2957, 2926, 2853, 1700, 1637, 1595, 1467, 1443, 1379, 1319, 1186, 1173, 1106, 980, 933, 751, 690 cm^{-1}. ^{1}H NMR: (400 MHz, DMSO) δ ppm: 6.58 (s, 1H), 6.56–6.54 (d, 1H, *J* = 8.0 Hz), 5.83–5.81(d, 1H, *J* = 8.0 Hz), 3.86 (s, 3H), 3.36 (s, 1H), 1.44 (s, 6H). ^{13}C NMR (100 MHz, DMSO) δ ppm: 196.80, 156.79, 152.48, 146.14, 131.02, 120.14, 116.96, 116.73, 115.11, 110.53, 98.95, 78.67, 56.84, 32.39, 27.59. MS (EI, *m/z*): 381.1 (M + 1). Analysed and calculated for $C_{15}H_{15}F_3O_6S$: C, 47.37; H, 3.98; F, 14.99; O, 25.24; S, 8.43%. Found: C, 47.38; H, 3.95; F, 14.97; O, 25.25; S, 8.45%; HRMS Calculated [M +] *m/z* 380.3342, Found 380.3344.

2.2.6. Preparation of 1-(7-methoxy-2,2-dimethyl-2H-chromen-8-yl) ethanone (15)

A mixture of 8-acetyl-7-methoxy-2,2-dimethyl-2H-chromen-5-yl trifluoromethanesulfonate (**14**) 0.5 g (0.10 mol) in DMF 5 ml, $Pd(OAC)_2$ (1.47 mg, 0.05 mol), Pd-DPPF (5.35 mg, 0.05 mol) was degased with HCOOH and then TEA was added at RT and the final mass was flushed with nitrogen. Further the temperature was raised to 70–75 °C and stirred for 20 h. After the reaction completion, as indicated by TLC, the mass was quenched with water (10 ml) and the product was extracted in ethyl acetate (10 ml × 2). The obtained organic layer was washed with water (10.0 ml), brine (10 ml) solution and dried over Na_2SO_4. The solvent was distilled to obtain a final syrup product (**15**) (0.27 g, 90%). IR (KBr) ϒmax: 3373, 1662, 1582, 1358, 1267, 1111, 1094, 1046, 1023, 825, 763 cm^{-1}. ^{1}H NMR: (400 MHz, DMSO) δ ppm: 7.54–7.52 (d, 1H, J =), 6.57–6.55 (d, 1H, J =), 6.53–6.50 (d, 1H, J =), 5.67–5.65 (d, 1H, J =), 3.77 (s, 3H), 2.44 (s, 3H), 1.36 (s, 6H). ^{13}C NMR (100 MHz, DMSO) δ ppm: 195.70, 158.08, 153.48, 130.55, 129.15, 120.01, 115.94, 109.78, 103.76, 76.68, 55.77, 31.83, 27.18. MS (EI, *m/z*): 232.7 (M + 1). Analysed and calculated for $C_{14}H_{16}O_3$: C, 72.39; H, 6.94; O, 20.66%. Found: C, 72.40; H, 6.95; O, 20.65%; HRMS Calculated [M +] *m/z* 232.2790, Found 232.2790.

2.3. General procedure for the preparation of methoxy halogenation of chromans

To a solution of 2.0 g (0.10 mol) 1-(5,7-dimethoxy-2,2-dimethyl-2H-chromen-8-yl)ethanone (**4b**) in methanol 10.0 ml, Oxone 5.16 g (0.11 mol) was added at 0.5 °C. NH_4 halides (X = F, C, Br, I) (0.11 mol) was added and the mass was stirred for 2–3 h at 0–5 °C. The reaction was completed as indicated by TLC, the reaction mass quenched with 10% sodium thiosulphate solution (5.0 ml) and then 15.0 ml ethyl acetate was added, the product was extracted then washed with water, NaCl solution and dried over Na_2SO_4. Solvents were distilled out under vacuum to get crude product. Product purified by using diethyl ether or column chromatography to obtain the pure product.

2.3.1. Preparation of 1-(4-bromo-3,5,7-trimethoxy-2,2-dimethylchroman-8-yl)ethanone (16)

To a solution of 2.0 g (0.10 mol) 1-(5,7-dimethoxy-2,2-dimethyl-2H-chromen-8-yl)ethanone (**4b**) in methanol 10.0 ml, Oxone 5.16 g (0.11 mol) was added at 0.5 °C. Then, 0.814 g (0.11 mol) NH_4Br was added and the mass was stirred for 2–3 h at 0–5 °C. Reaction was completed as indicated by TLC and the reaction mass was quenched with 10% sodium thio sulphatesolution (5.0 ml) then ethyl acetate (20.0 ml) was added and the product was extracted in ethyl acetate layer and washed with water, NaCl solution and dried over Na_2SO_4. Remaining solvent was distilled out under vacuum to get crude product. The crude product was purified using diethyl ether to obtain the pure product (2.27 g, 80%). Melting point: 105.40–107.4 °C, IR (KBr) ϒmax: 2987, 2938, 2840, 1852, 1752, 1734, 1653, 1601, 1494, 1460, 1383, 1337, 1185, 1117, 1007, 968, 936, 887, 864, 733, 655, 573 cm^{-1}. ^{1}H NMR: (400 MHz, DMSO) δ ppm: 6.35 (s, 1H), 4.86 (s, 1H), 4.51 (s, 1H), 3.87 (s, 3H), 3.79 (s, 3H), 3.33 (s, 3H), 2.31 (s, 3H), 1.47 (s, 6H). ^{13}C NMR (100 MHz, DMSO) δ ppm: 199.64, 160.73, 157.45, 149.98, 112.19, 100.52, 89.03, 75.83, 75.36, 57.04, 56.0, 55.85, 54.59, 32.32, 29.26, 23.51. MS (EI, *m/z*): 373.2 (M + 2). Analysed and calculated for $C_{16}H_{21}BrO_5$: C, 51.49; H, 5.67; Br, 21.41; O, 21.43% Found: C, 51.50; H, 5.66; Br, 21.40; O, 21.44%; HRMS Calculated [M +] *m/z* 373.2430, Found 373.2430.

2.3.2. Preparation of 1-(4-chloro-3,5,7-trimethoxy-2,2-dimethylchroman-8-yl) ethanone (17)

To a solution of 1-(5,7-dimethoxy-2,2-dimethyl-2H-chromen-8-yl) ethanone (**4b**) 2.0 g (0.10 mol) in methanol 10.0 ml, Oxone 5.16 g (0.11 mol) was added at 0.5 °C. Then, 0.45 g (0.11 mol) NH_4Cl was added and the mass was stirred for 2–3 h at 0–5 °C. Reaction was completed as indicated by TLC and the reaction mass then quenched with 10% sodium thio sulphatesolution (5.0 ml) and 20.0 ml of ethyl acetate was added. Then the product was extracted in ethyl acetate layer, washed with water, NaCl solution and dried over Na_2SO_4. Remaining solvent was distilled out under vacuum to get crude product. Further, the product was purified by column chromatography using ethyl acetate and pet ether to obtain a final syrup product (2.0 g, 80%). IR (KBr) ϒmax: 2923, 2850, 1696, 1605, 1585, 1462, 1436, 1413, 1368, 1348, 1268, 1158, 1156, 1111, 1076, 965, 922, 883, 796, 779, 603, 567 cm^{-1}. ^{1}H NMR: (400 MHz, DMSO) δ ppm: 6.34 (s, 1H), 4.64 (s, 1H), 4.34 (s, 1H), 3.92 (s, 3H), 3.87 (s, 3H), 3.45 (s, 3H), 2.31 (s, 3H), 1.43 (s, 6H). ^{13}C NMR (100 MHz, DMSO) δ ppm: 199.60, 160.80, 157.47, 149.92, 112.21, 100.59, 89.00, 76.04, 74.82, 60.05, 57.11, 55.96, 55.82, 32.29, 26.90, 23.71. MS (EI, *m/z*): 328.8 (M + 2). Analysed and calculated for $C_{16}H_{21}ClO_5$: C, 58.45; H, 6.44; Cl, 10.78; O, 24.33%, Found: C, 58.46; H, 6.43; Cl, 10.76; O, 24.35%; HRMS

Calculated [M +] *m/z* 328.7890, Found 328.7892.

*2.3.3. Preparation of 1-(4-fluoro-3,5,7-trimethoxy-2,2-dimethylchroman-8-yl) ethanone (**18**)*

To a solution of 2.0 g (0.10 mol) 1-(5,7-dimethoxy-2,2-dimethyl-2H-chromen-8-yl)ethanone (**4b**) in methanol 10.0 ml, Oxone 5.16 g (0.11 mol) was added at 0.5 °C. NH_4F 0.31 g (0.11 mol) was added and the mass was stirred for 2–3 h at 0–5 °C. Reaction was completed as indicated by TLC and the reaction mass was quenched with 10% sodium thio sulphatesolution (5.0 ml). Then ethyl acetate 20.0 ml was added and the product was extracted in ethyl acetate layer and washed with water, NaCl solution and dried over Na_2SO_4. Solvent was distilled out under vacuum to get crude product. The product obtained is purified by column chromatography using ethyl acetate and pet ether to obtain syrup (1.52 g, 64%). IR (KBr) ϒmax: 2954, 2923, 2852, 1716, 1624, 1585, 1463, 1445, 1430, 1393, 1364, 1327, 1276, 1216, 1179, 1145, 1115, 1094, 1044, 1025, 961, 816, 580 cm^{-1}. ^1H NMR: (400 MHz, DMSO) δ ppm: 8.58 (s, 1H), 5.23 (s, 1H), 4.11 (s, 1H), 3.84 (s, 3H), 3.75 (s, 3H), 3.46 (s, 3H), 2.30 (s, 3H), 1.19 (s, 6H). ^{13}C NMR (100 MHz, DMSO) δ ppm: 200.50, 149.85, 146.09, 141.97, 136.65, 77.09, 75.00, 69.22, 61.44, 60.20, 57.85, 120.42, 111.80, 32.31, 24.29, 23.58. Analysed and calculated for $C_{16}H_{21}FO_5$: C, 61.53; H, 6.78; F, 6.08; O, 25.61%, Found: C, 61.52; H, 6.76; F, 6.09; O, 25.63%; HRMS Calculated [M +] *m/z* 312.3374, Found 312.3378.

*2.3.4. Preparation of 1-(4-bromo-5-hydroxy-3,7-dimethoxy-2,2-dimethylchroman-8-yl)ethanone (**19**)*

To a solution of 1.0 g (0.10 mol) 1-(5-hydroxy-7-methoxy-2,2-dimethyl-2H-chromen-8-yl) ethanone (**9**) in methanol 5.0 ml, Oxone 2.72 g (0.11 mol) was added at 0.5 °C. 0.43 g (0.11 mol) NH_4Br was added and the mass was stirred for 2–3 h at 0–5 °C. Reaction was completed as indicated by TLC and the reaction mass was quenched with 10% sodium thio sulphate solution (3.0 ml) then ethyl acetate 10.0 ml was added, the product was extracted in ethyl acetate layer and then washed with water, NaCl solution and dried over Na_2SO_4. Solvents were distilled out under vacuum to get crude product. The product was purified by column chromatography using ethyl acetate and pet ether to obtain syrup (1.0 g, 70%). ^1H NMR: (400 MHz, DMSO) δ ppm:14.0 (s, 1H, D_2O exchangable), 6.03 (s, 1H), 4.54 (s, 1H), 4.26 (s, 1H), 3.77 (s, 3H), 3.47 (s, 3H), 2.56 (s, 3H), 1.54 (s, 6H). ^{13}C NMR (100 MHz, DMSO) δ ppm: 202.3, 166.51, 163.98, 154.28, 118.89, 104.61, 98.42, 75.67, 75.07, 55.06, 54.84, 52.60, 32.26, 28.64, 23.62. MS (EI, *m/z*): 359.3 (M + 2). Analysed and calculated for $C_{15}H_{19}BrO_5$: C, 50.15; H, 5.33; Br, 22.24; O, 22.27%, Found: C, 50.16; H, 5.31; Br, 22.24; O, 22.29%; HRMS Calculated [M +] *m/z* 359.2160, Found 359.2161.

*2.3.5. Preparation of 1-(4-chloro-5-hydroxy-3,7-dimethoxy-2,2-dimethylchroman-8-yl)ethanone (**20**)*

To a solution of 1.0 g (0.10 mol) 1-(5-hydroxy-7-methoxy-2,2-dimethyl-2H-chromen-8-yl) ethanone (**9**) in methanol 5.0 ml, Oxone 2.72 g (0.11 mol) was added at 0.5 °C. 0.23 g (0.11 mol) NH_4Cl was added, the mass was stirred for 2–3 h at 0–5 °C. Reaction was completed as indicated by TLC and the reaction mass was quenched with 10% sodium thio sulphatesolution (5.0 ml). Then ethyl acetate 10.0 ml was added and the product was extracted in ethyl acetate layer and then washed with water, NaCl solution and dried over Na_2SO_4. Solvents were distilled out under vacuum to get crude product. The product was purified by column chromatography using ethyl acetate and pet ether to obtain solid (1.0 g, 79.3%). Melting point: 72.60–74.60 °C; IR (KBr) ϒmax: 3436, 2997, 2982, 2932,2927, 2827, 1616, 1592, 1492, 1432,1386, 1327, 1292, 1211, 1183, 1132, 1117, 1026, 960, 889, 823, 661, 635, 589 cm^{-1}.^1H NMR: NMR (400 MHz, $CDCl_3$) δ ppm:14.0 (s, 1H, D_2O exchangable), 6.07 (s, 1H), 4.50 (d, 1H), 4.42 (d, 1H), 3.86 (s, 3H), 3.53 (s, 3H), 2.61 (s, 3H), 1.43(s, 3H), 1.32 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 203.24, 167.53, 165.07, 155.26, 105.62, 99.63, 93.05, 77.41, 75.54, 60.29, 57.63, 56.03, 33.262, 26.92, 24.79. MS (EI, *m/z*): 315.1 (M + 2) Analysed and calculated for $C_{15}H_{19}ClO_5$: C, 57.24; H, 6.08; Cl, 11.26; O, 25.42%; Found: C, 57.25; H, 6.09; Cl, 11.23; O, 25.43%; HRMS Calculated [M +] *m/z* 314.7620, Found 314.7622.

*2.3.6. Preparation of 1-(4,6-dibromo-3,5,7-trimethoxy-2,2-dimethylchroman-8-yl)ethanone (**21**)*

To a solution of 2.0 g (0.10 mol) 1-(5,7-dimethoxy-2,2-dimethyl-2H-chromen-8-yl)ethanone (**4b**) in methanol 10.0 ml, Oxone 10.31 g (0.22 mol) was added at 0.5 °C. 1.63 g (0.22 mol) NH_4Br was added and the mass was stirred for 10–12 h at 25–30 °C. Reaction was completed as indicated by TLC and the reaction mass quenched with sodium thio sulphate solution (15.0 ml). Then ethyl acetate 20.0 ml was added and the product was extracted in ethyl acetate layer, washed with water, NaCl solution and dried over Na_2SO_4. Solvent was distilled out under vacuum to get crude product. The crude product was purified by column chromatography using diethyl ether to obtain pure product (2.75 g, 80%). Melting point: 119.8–121.7 °C, ^1H NMR: (400 MHz, DMSO) δ ppm: 5.04 (s, 1H), 4.63 (s, 1H), 3.86 (s, 3H), 3.74 (s, 3H), 3.52 (s, 3H), 2.50 (s, 3H), 1.58 (s, 3H), 1.48 (s, 3H). MS (EI, *m/z*): 451.0 (1:2:1 ratio). Analysed and calculated for $C_{16}H_{20}Br_2O_5$: C, 42.50; H, 4.46; Br, 35.35; O, 17.69%, Found: C, 42.48; H, 4.45; Br, 35.37; O, 17.70%; HRMS Calculated [M +] *m/z* 452.1390, Found 452.1391.

*2.3.7. Preparation of 1-(4,6-dibromo-5-hydroxy-3,7-dimethoxy-2,2-dimethylchroman-8-yl)ethanone (**22**)*

To a solution of 1.0 g (0.10 mol) 1-(5-hydroxy-7-methoxy-2,2-dimethyl-2H-chromen-8-yl) ethanone (**9**) in methanol 5.0 ml, Oxone 5.44 g (0.22 mol) was added at 0.5 °C. 0.86 g (0.22 mol) NH_4Br was added and the mass was stirred for 10–12 h at 25–30 °C. Reaction was completed as indicated by TLC and the reaction mass was quenched with 10% sodium thio sulphate solution (3.0 ml). Then ethyl acetate 10.0 ml was added and the product was extracted in ethyl acetate layer, washed with water, NaCl solution and dried over Na2SO4. Solvent was distilled out under vacuum to get crude product. The crude product was purified by column chromatography using ethyl acetate and pet ether to obtain solid (1.23 g, 70%). Melting point: 127–129 °C, IR (KBr) ϒmax: 2994, 2946, 2825, 1618, 1570, 1462, 1420, 1388, 1369, 1354, 1304, 1280, 1245, 1174, 1126, 1091, 1076, 1050, 1028, 981, 957, 925, 884, 842, 797, 771, 740, 684, 604, 578, 555 cm^{-1}; ^1H NMR: (400 MHz, DMSO) δ ppm: 14.0 (s, 1H, D_2O exchangable), 5.08–5.07 (d, 1H, J = 4.0), 4.63–4.62 (d, 1H, J = 4.0), 3.95 (s, 3H), 3.39 (s, 3H), 2.68 (s, 3H), 1.66 (s, 3H), 1.62 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ ppm: 203.94, 163.07, 161.46, 154.39, 108.55, 106.36, 97.38, 77.58, 75.56, 61.48, 56.64, 53.05, 33.30, 29.35, 23.55. MS (EI, *m/z*): 439.0 (M + 2, 1:2:1 ratio). Analysed and calculated for $C_{15}H_{18}Br_2O_5$: C, 41.12; H, 4.14; Br, 36.48; O, 18.26%; Found: C, 41.10; H, 4.16; Br, 36.48; O, 18.28%; HRMS Calculated [M +] *m/z* 438.1120, Found 438.1121.

*2.3.8. Procedure for the preparation of 1-(5-hydroxy-4,6-diiodo-3,7-dimethoxy-2,2-dimethyl chroman-8-yl)ethanone (**23**)*

To a solution of 1.0 g (0.10 mol) 1-(5-hydroxy-7-methoxy-2,2-dimethyl-2H-chromen-8-yl)ethanone (**9**) in methanol 5.0 ml, Oxone 5.44 g (0.22 mol) was added at 0.5 °C. Then, 2.40 g (0.44 mol) of NH_4I was added and the mass was stirred for 10–12 h hours at 25–30 °C. Reaction was completed as indicated by TLC and the reaction mass was quenched with 10% sodium thio sulphatesolution (5.0 ml). Then ethyl acetate 10.0 ml was added and the product was extracted in ethyl acetate layer, washed with water, NaCl solution and dried over Na_2SO_4. Solvent was distilled out under vacuum to crude product. The crude product was purified by column chromatography using ethyl acetate and pet ether to obtain off-white solid (1.28 g, 60.1%). Melting point: 151.9–153.9 °C; IR (KBr) ϒmax: 3432, 2988, 2939, 2822, 1610, 1578, 1560, 1457, 1415, 1402, 1367, 1351, 1300, 1275, 1240, 1192, 1168, 1106, 1087, 1073, 1041, 978, 921, 881, 870, 836, 790, 757, 711, 676, 601, 575, 549 cm^{-1}. ^1H NMR: (400 MHz, DMSO) δ ppm: 14.30 (s, 1H,

D_2O exchangable), 5.15 (s, 1H), 4.79 (s, 1H), 3.86 (s, 3H), 3.48 (s, 3H), 2.58 (s, 3H), 1.57 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 203.91, 166.13, 163.99, 155.96, 108.08, 106.44, 77.98, 77.78, 74.42, 61.52, 56.34, 35.62, 33.61, 33.15, 22.24. MS (EI, *m/z*): 532.9 (M + 1). Analysed and calculated for $C_{15}H_{18}I_2O_5$: C, 33.86; H, 3.41; I, 47.70; O, 15.03%; Found: C, 33.84; H, 3.38; I, 47.73; O, 15.05%; HRMS Calculated [M +] *m/z* 532.1129, Found 532.1130.

*2.3.9. Preparation of 1-(6-bromo-5,7-dimethoxy-2,2-dimethyl-2H-chromen-8-yl)ethanone (**24**)*

To a solution of 1-(5,7-dimethoxy-2,2-dimethyl-2H-chromen-8-yl) ethanone (**4b**) 2.0 g (0.10 mol) in ethanol 10.0 ml, Oxone 5.11 g (0.11 mol) was added at 0.5 °C. Then, 0.814 g (0.11 mol) NH_4Br was adde and the mass was stirred for 1–2 h at 25–30 °C. Reaction was completed as indicated by TLC and the reaction mass was quenched with sodium thio sulphate solution (5.0 ml). Then ethyl acetate 20.0 ml was added and the product was extracted in ethyl acetate layer, washed with water, NaCl solution and dried over Na_2SO_4. Solvent was distilled out under vacuum to crude product. The crude product was purified by using diethyl ether to obtain pure product (2.21 g, 85%). Melting point: 119.7–121.7 °C, IR (KBr) ϒmax: 2990, 2965, 2843, 1701, 1653, 1603, 1498, 1470, 1416, 1383, 1364, 1337, 1236, 1206, 1185, 1168, 1118, 1006, 962, 935, 887, 869, 794, 688,656, 576, 562, 501 cm^{-1}. ^1H NMR: (400 MHz, DMSO) δ ppm: 6.86 (s, 1H), 6.33 (s, 1H), 3.85 (s, 3H), 3.79 (s, 3H), 2.34 (s, 3H), 1.44 (s, 6H). ^{13}C NMR (100 MHz, DMSO) δ ppm: 198.82, 157.74, 155.41, 148.82, 119.88, 119.71, 113.02, 104.01, 89.55, 79.82, 56.03, 56.03, 32.41, 25.76, 24.76. ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 200.26, 158.12, 155.85, 150.31, 120.53, 119.94, 113.57, 105.16, 88.30, 80.54, 55.90, 55.71, 32.61, 26.34, 21.77. MS (EI, *m/z*): 341.3 (M + 2). Analysed and calculated for $C_{15}H_{17}BrO_4$: C, 52.80; H, 5.02; Br, 23.42; O, 18.76%; Found: C, 52.81; H, 5.03; Br, 23.42; O, 18.74%; HRMS Calculated [M +] *m/z* 341.2010, Found 341.2013.

*2.3.10. Preparation of 1-(6-chloro-5,7-dimethoxy-2,2-dimethyl-2H-chromen-8-yl)ethanone (**25**)*

To a solution of 2.0 g (0.10 mol) 1-(5,7-dimethoxy-2,2-dimethyl-2H-chromen-8-yl)ethanone (**4b**) in ethanol (10.0 ml), Oxone 5.16 g (0.11 mol) was added at 0.5 °C. Then, 0.45 g (0.11 mol) NH_4Cl was added and the mass was stirred for 1–2 h at 25–30 °C. Reaction was completed as indicated by TLC and the reaction mass was quenched with sodium thio sulphate solution (5.0 ml). Then ethyl acetate (20.0 ml) was added and the product was extracted in ethyl acetate layer, washed with water, NaCl solution and dried over Na_2SO_4. Solvent was distilled out under vacuum to get crude product. The crude product was purified by column chromatography using ethyl acetate and pet ether to obtain pure solid (1.87 g, 82.7%). Melting point: 104.3–106.2 °C. ^1H NMR: (400 MHz, DMSO) δ ppm: 6.66 (s, 1H), 6.34 (s, 1H), 3.85 (s, 3H), 3.79 (s, 3H) 2.35 (s, 3H), 1.50 (s, 6H). ^{13}C NMR (100 MHz, DMSO) δ ppm: 198.85, 157.60, 155.50, 148.53, 128.79, 115.46, 113.04, 103.46, 89.63, 79.33, 74.95, 57.15, 56.00, 32.39, 24.91. MS (EI, *m/z*): 296.8 (M + 2). Analysed and calculated for $C_{15}H_{17}ClO_4$: C, 60.71; H, 5.77; Cl, 11.95; O, 21.57%; Found: C, 60.72; H, 5.75; Cl, 11.97; O, 21.56%; HRMS Calculated [M +] *m/z* 296.7470, Found 296.7476.

*2.3.11. Preparation of 1-(6-iodo-5,7-dimethoxy-2,2-dimethyl-2H-chromen-8-yl)ethanone (**26**)*

To a solution of 1.0 g (0.10 mol) 1-(5,7-dimethoxy-2,2-dimethyl-2H-chromen-8-yl)ethanone (**4b**) in ethanol (5.0 ml), Oxone 2.72 g (0.11 mol) was added at 0.5 °C. To this, 1.2 g (0.22 mol) NH_4I was added and the mass was stirred for 1–2 h at 25–30 °C. Reaction was completed as indicated by TLC, the reaction mass was quenched with 10% sodium thio sulphatesolution (5.0 ml). Then, ethyl acetate (10.0 ml) was added and extracted the product in ethyl acetate layer washed with water, NaCl solution and dried over Na_2SO_4. Solvent was distilled out under vacuum to get crude product. The crude product purified by column chromatography using ethyl acetate and pet ether to obtain yellow color syrup (1.0 g, 79.3%). Melting point: 127.4–129.8 °C; ^1H NMR: NMR (400 MHz, DMSO) δ ppm: 6.53–6.50 (d, 1H, J = 12), 5.81–5.79 (d, 1H, J = 10.0), 3.71 (s, 3H), 3.67 (s, 3H), 2.43 (s, 3H), 1.45 (s, 6H). ^{13}C NMR (100 MHz, DMSO) δ ppm: 199.54, 156.51, 156.12, 150.93, 130.91, 122.29, 116.05, 115.52, 112.0, 80.20, 77.37, 62.43, 61.59, 32.05, 29.37, 27.52. MS (EI, *m/z*): 389.0 (M + 1). Analysed and calculated for $C_{15}H_{17}IO_4$: C, 46.41; H, 4.41; I, 32.69; O, 16.49%; Found: C, 46.43; H, 4.42; I, 32.68; O, 16.47%; HRMS Calculated [M +] *m/z* 388.2015, Found 388.2018.

*2.3.12. Procedure for the preparation of 1-(4-bromo-3-hydroxy-5,7-dimethoxy-2,2-dimethyl chroman-8-yl)ethanone (**27**)*

To a solution of 1.0 g (0.10 mol) 1-(5,7-dimethoxy-2,2-dimethyl-2H-chromen-8-yl)ethanone (**4b**) in acetonitrile 4.0 ml, water 2.0 ml, Oxone 2.72 g (0.11 mol), NH_4 Br 0.407 g (0.11 mol) was added at 0–5 °C. The mass was stirred for 1 h at 0–5 °C. Reaction was completed as indicated by TLC, and the reaction mass was quenched with 10% sodium thio sulphate solution (5.0 ml). Then ethyl acetate (10.0 ml) was added, the product was extracted in ethyl acetate layer, washed with water, NaCl solution and dried over Na_2SO_4. Solvent was distilled out under vacuum to get crude product. The crude product purified by using diisopropyl ether to obtain off white solid (0.96 g, 70%). Melting point: 125.4–127.3 °C, IR (KBr) ϒmax: 3518, 3000, 2981, 2939, 2846, 1778, 1706, 1685, 1585, 1492, 1462, 1370, 1347, 1293, 1264, 1156, 1116, 1095, 1025, 923, 911, 885, 805, 761, 744, 708, 686, 621, 568, 486; ^1H NMR: (400 MHz, DMSO) δ ppm: 6.32 (s,1H), 5.81 (s, 1H, D_2O exchangable), 4.93 (s,1H), 4.41 (s,1H), 3.84 (s,3H), 3.77 (s,3H), 2.311 (s,3H), 1.51 (s,3H), 1.43 (s,3H). ^{13}C NMR (100 MHz, DMSO) δ ppm: 199.78, 160.40, 157.05, 149.91, 112.27, 102.97, 89.08, 76.23, 65.55, 60.00, 55.85, 55.77, 32.36, 27.94, 24.78. MS (EI, *m/z*): 359.1 (M + 2). Analysed and calculated for $C_{15}H_{19}BrO_5$: C, 50.15; H, 5.33; Br, 22.24; O, 22.27%; Found: C, 50.13; H, 5.31; Br, 22.24; O, 22.32%; HRMS Calculated [M +] *m/z* 359.2160, Found 359.2160.

2.4. Antibacterial activity studies

Antimicrobial activity potentials of present study compounds was determined by the method described by European Committee for Antimicrobial Susceptibility Testing.[24] Commercially available Clorobiocin and Novobiocin were used as reference drugs. 1% Dimethyl sulphoxide (DMSO) was used as a control without mixing the test compounds. Further, compounds were tested at various concentrations against $Gram^{+ve}$ *Staphylococcus aureus* (MTCC 96), *Corynebacterium diphtheriae* (MTCC – 116), and *Streptococcus pyogenes* (MTCC 442) and $Gram^{-ve}$ bacteria *Klebsiella pneumoniae* (MTCC 530), *Escherichia coli* (MTCC 443), and *Pseudomonas aeruginosa* (MTCC 424).

2.5. Molecular docking studies

Autodock 4.2.6 and Autodock Tools (ADT) 1.5.6 were used for the docking studies. All strategies and parameters were used and adjusted according to the present study's necessity with our earlier reports.[25–28] The three-dimensional structures of targeted enzymes/proteins (PDB ID: 2XCT (*S. aureus* DNA gyrase A), PDB ID: 3G75 (*S. aureus* DNA gyrase B), PDB ID: 3L7L (Teichoic acid polymerase) was retrieved from www.rcsb.org/pdb/.

2.6. DNA gyrase inhibition activity

DNA gyrase A (*S.aureus*) inhibition assay of present study compounds was carried out as reported earlier.[29] *S. aureus* was cultured in medium B (2 g yeast extract, 10 g polypeptone, 1.2 g $(NH_4)_2SO_4$, 8 g Na_2HPO, 2 g KH_2PO_4, 0.2 g $MgSO_4$, 4 g glucose in 1L distilled water). DNA gyrase purification, Supercoiling, and decatenation were executed

as reported by F. Blanche et al. 1996.[30] Extracted DNA was treated with DNA gyrase and untreated DNA samples were determined the DNA gyrase inhibition via Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) technique.

2.7. Gram^{+ve} bacterial cell wall surface degradation examinations

C. diphtheriae was inoculated in Hardy Diagnostics Loeffler (HDL) Medium (5 ml) and grown overnight. Cells were harvested by centrifuging at 10,000 × *g* for 10 min. Pellet was resuspended in 2 ml of 1 × Wash Buffer (Guanidine hydrochloride in ethanol). 1 ml of this live culture was added to 5 ml of 1 × Wash Buffer. To achieve a 'dead' standard, 1 ml of this live culture was suspended with 5 ml of 70% isopropanol. Both samples were incubated at RT for 1-h mingling frequently by overturn. After incubation, samples were centrifuged at 10,000 × *g* for 10 min and both pellets were resuspended in 5 ml of 1 × Wash Buffer each. Centrifuge at 10,000 × *g* for 10 min. Further, the pellet was resuspended in 1 ml of 1X Wash Buffer. Finally, 1 μL Total Cell Stain and 1 μL Dead Cell Stain was added to each sample tube (1 ml).

2.8. Estimation of viable and non-viable bacterial cells

The experiment was carried out in a 96 well plate as per the reported instruction.[31] A blank solution containing 1 ml 1 × Wash Buffer and 1 μL total cell stain and dead cell stain was used for the assay. This mixture was further incubated for 1 h under dark at room temperature. The experiment was done in triplicate. 200 μL of each sample and the blank solution was pipetted out and transferred into separate wells of 96-well plate. The fluorescence was measured at Ex 490 nm/EM 525 nm and Ex 536 nm/EM 617 nm used for reading 1 & 2 respectively for all wells. The percentage of dead cells or cell viability is calculated using the formula:

$$\text{Percentage Viability} = 100-([\text{Number of red cells}/\text{Number of green cells}] \times 100)$$

2.9. Cell wall degradation assessment after treating the compounds

S. aureus culture was examined for their hourly physiological changes or cell wall damage after treating the coumarin/chroman compounds in optimized concentrations. Gradual changes in bacterial physiological conditions, especially the changes that were occured on bacterial cell wall, was measured by estimating dead cells by means of estimating debris level at every 30 min. The experiment was continued until the whole bacterial cell death or cell wall damaged. Amount of final cell debris determines the total ability of degrading/killing potency of tested compounds in a particular time period.

2.10. HRBC membrane protection potentials evaluations

To determine the cell membrane fortification/mortification effects against the human cellular system, the Human Red Blood Cells (HRBC) membrane stabilization potential of the title compounds (chromans/coumarins) at various concentrations (n = 4) was assessed in a 96 well plate using Diclofenac as the standard.[32] The percentage hemolysis was calculated by assuming the hemolysis generated in presence of distilled water at 100%. The percentage of HRBC membrane stabilization was calculated using the following formula,

$$\%\text{inhibition of hemolysis} = 100 \times [(OD_1-OD_2)/OD_1]$$

where OD_2 = optical density of sample OD_1 = optical density of control.

2.11. Cytotoxicity evaluations – MTT assay

Early passage of breast cancer (MCF-7) and normal breast epithelial cells (MCF-10) were used for the cytotoxicity assessment of present study chromans coumarins compounds. The cytotoxicity of compounds **9**, **13**, **14**, **18** and **20** on different cancer cell lines was assessed by the MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide) assay by referring our earlier reports.[28] Doxorubicin was used as the standard drug. The cell viability was calculated using the following formula:

$$\text{Cell viability}(\%) = \text{Mean OD}/\text{Control OD} \times 100\%$$

2.12. Statistical analysis

All results were expressed as a percentage increase or decrease with respect to control values. All results were compared by performing one-way ANOVA with Dunnett's post-test. GraphPad Prism version 7.1 for Windows, GraphPad Software, USA (www.graphpad.com) was used for statistical analysis. A difference was considered statistically significant if $p \leq 0.05$. The 50% inhibitory concentration (IC_{50}) was calculated from the dose–response curve obtained by plotting percentage inhibition versus concentration.

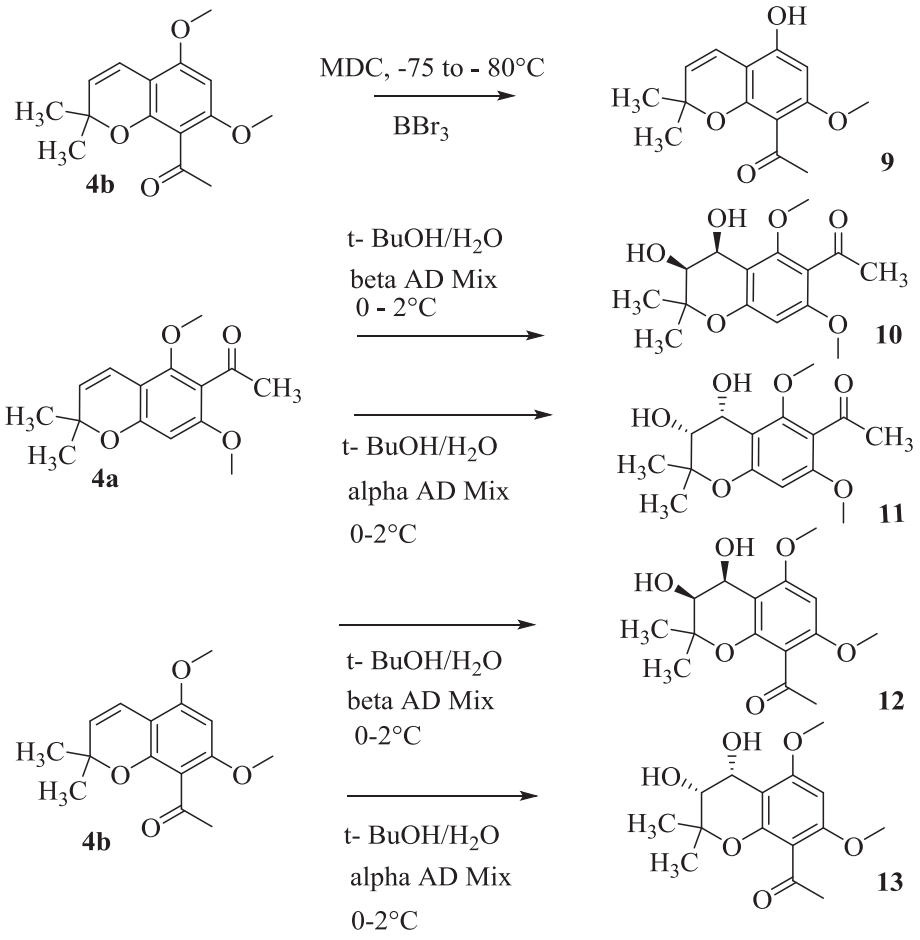
3. Results

3.1. Synthesis of proposed 3-O-methoxy-4-halo disubstituted 5,7-dimethoxy chromans

The parent compound 5,7-dihydroxy-2,2-dimethyl chroman-4-one was achieved as per the instructions of Tsukiyama et al. 1989 and Alex and Ross 2004.[33,34] Compound **1** was prepared by treating 5,7-dihydroxy-2,2-dimethyl chroman-4-one with methyl iodide in presence of potassium carbonate, further, compound **1** undergoes reduction using sodium borohydride in methanol medium to achieve **2**. Compound **2** was treated with methane sulphonyl chloride to get **3**, and finally compound **3** undergoes Friedel-Crafts acylation to achieve **4a** and **4b**. In the Friedel-Crafts acylation reaction experiment (Scheme 1), the anticipated yield was relatively observed to be very less. We have used the NOESY experiment to determine the position of acetyl groups at 6 (**4a**) and 8 **(4b)**.

In **4a,** the aromatic proton at C8 has a correlation with the O-methoxy group at C7, this confirms the presence of an acetyl group at the C6 position. Simultaneously, in **4b** the aromatic proton at C6 has a correlation with both C5 and C7 of O-methoxy groups. 6-acetyl-5,7-dihydroxy-2,2-dimethyl chroman-4-one and 8-acetyl-5,7-dihydroxy-2,2-dimethyl chroman-4-one (Scheme 2) are also achieved as per the previously reported instructions by Tsukiyama et al. 1989 and Alex and Ross 2004.[33,34] Further, the above mentioned starting materials were methoxylated to achieve **5, 6** and **7**. These starting materials were reduced to achieve **8** and dehydrated to get **4a** & **4b** in affordable yeild. All the reaction were carried out without purifying the intermediates to achieve better yield. Compound **9** was achieved by demethoxylation reaction (Scheme 3), by treating **4b** with BBr_3 at −75 to −80 °C.[35] The *Cis* dihydroxy compounds **10**, **11** and **12**, **13** were prepared from **4a** and **4b** respectively by using shapeless asymmetric dihydroxylation reaction.[33,36,37] Here the position of the acetyl group at 6 (**10**, **11**) and 8 **(12**, **13)** was confirmed by NOESY experiment.

The derivatives **14** and **15** were prepared by using 1-(5-hydroxy-7-methoxy-2,2-dimethyl-2H-chromen-8-yl)ethanone (**9**) as mentioned in Scheme 4. Compound **9**'s hydroxyl group was protected with triflate to achieve **14**, further, it was reduced using palladium catalysts $Pd(OAc)_2$ and Pd-DPPF[38] to get **15**. Both the reactions were carried out carefully by not having any moisture contamination. Compounds **16–26** (Scheme 5) was prepared by using ammonium salts (NH_4 F, NH_4 Cl, NH_4 Br, NH_4 I) as a halogen source and Oxone as an oxidant.[39,40] The alkoxy



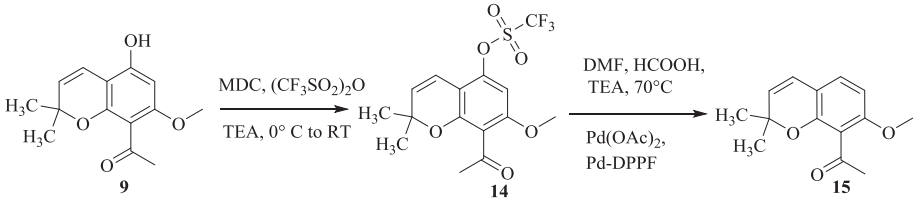
Scheme 3. Route of Synthesis of **9**, **10**, **11**, **12** and **13** from **4a** & **4b**.

halogenation was thoroughly studied for the formation of mono and di-substituted product with reference to temperature and equivalence of the reagent. The reaction was thermodynamically controlled to achieve methoxy halogenation predominantly in the olefin functional group. Aromatic halogenation was carried out using ethanol as a solvent medium instead of methanol. Because, due to steric hindrance, ethanol could not provide the ethoxy brominated product. Compound **27**, bromohydrin, was synthesized with NH_4Br and Oxone system using acetonitrile and water as a solvent medium.

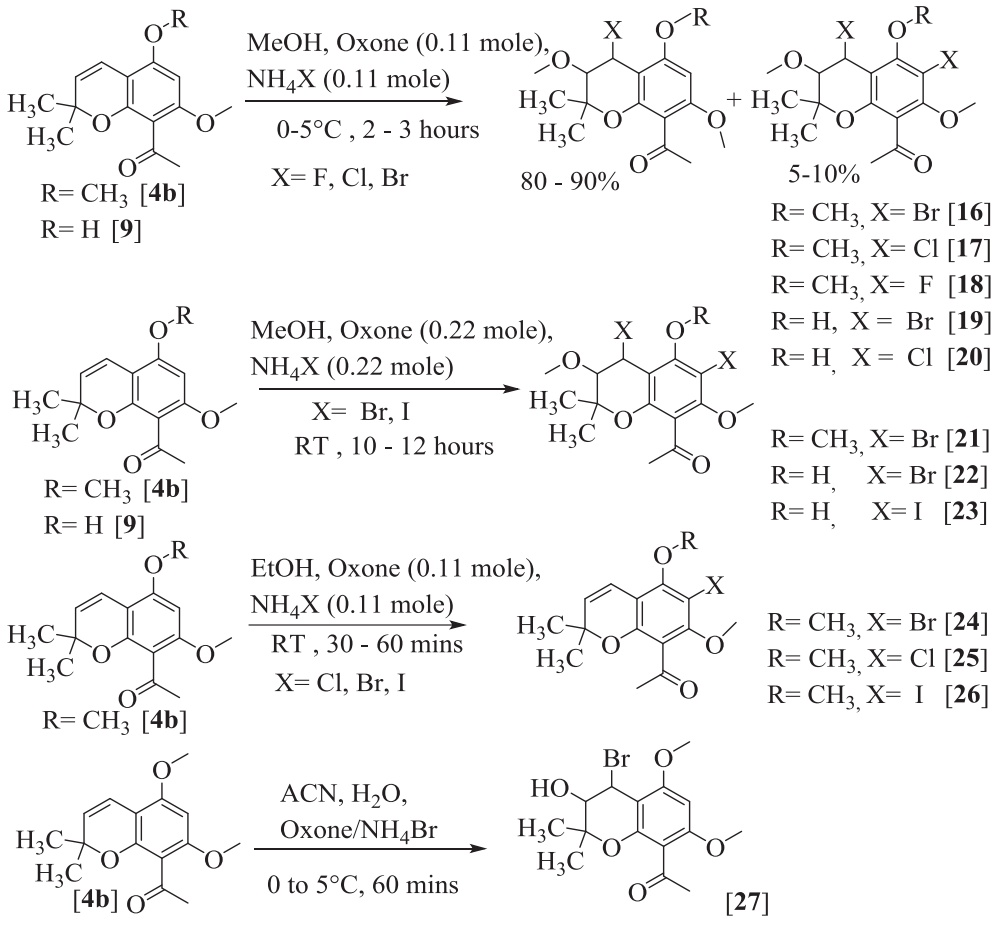
3.2. Molecular docking results

Drug efficacy of present study compounds was determined through the molecular mechanistic values and best-docked poses that were obtained from molecular docking studies. The best binding affinity and ligand-receptor interaction details of the compounds was assessed. The expected binding free energy for *S. aureus* DNA gyrase A (PDB ID: 2XCT) was obtained in a range between −7.26 and −28.09 kcal/mol. It was −7.85 to −41.87 kcal/mol for DNA gyrase B (PDB ID: 3G75) and for TAP (Teichoic Acid Polymerase) (PDB ID: 3L7L) it was −6.35 to −37.23 kcal/mol. These binding free energy values indicates that the chromans/coumarins are selectively exposed the binding affinity towards DNA gyrase B than DNA gyrase A. Astonishingly, remarkable binding affinity was found against TAP. As per the Table 1 statistical estimations, the calculated cumulative binding affinity was calculated as −339.31, −613.14, −547.10 kcal/mol for DNA gyrase A, DNA gyrase B, and TAP respectively. Thus, a DNA gyrase B inhibition activity was considered to be evaluated as the most effective anticipated bioactivity of coumarin/chroman compounds. Interestingly, the overall distinguished chemotherapy efficacy of these compounds was also recognized from the obtained mean values of molecular mechanistic results of DNA gyrase A, DNA gyrase B and TAP (-16.60, −28.72 and −25.86 kcal/mol respectively).

To specify a compounds drug efficacy towards the proposed activities here, the lowest binding energy, inhibitory constant (*ki*) and best interactions were assessed. In the assessment, compound **14** was found to have most efficient binding affinity with a least average binding energy (-35.73 kcal/mol, *ki* = 42 pM). At the same time, compound **20** (-31.72 kcal/mol, *ki* = 58 pM), compound **24** (−30.75 kcal/mol, *ki* = 82 pM), compound **12** (−29.30 kcal/mol, *ki* = 90 pM), compound **18** (−28.20 kcal/mol, *ki* = 96 pM), and compound **17** (−27.69 kcal/mol, *ki* = 98 pM) were also had comparable values to **14**. Later, these



Scheme 4. Synthesis of **14** and **15** from **9**.



Scheme 5. Synthesis route of compounds **16–27**.

Table 1
Molecular docking and molecular validation of 3-O-methoxy-4-halo disubstituted5,7-dimethoxy chromans.

Molecular Docking Studies					Druggability analysis		
#Ligand	2xct	3 g75	3l7l	ABA*	LogP	S + logD	**Ro5**
6	−14.41	−30.32	−23.76	−22.83	0.42	1.7	< None >
9	−21.38	−28.73	−26.01	−25.37	2.12	3.53	< None >
10	−17.01	−30.73	−29.13	−25.62	−0.08	−2.52	< None >
11	−22.48	−28.71	−28.69	−26.63	0.13	0.98	< None >
12	−19.82	−36.66	−24.41	−26.96	0.13	0.98	< None >
13	−17.06	−30.73	−29.19	−25.64	0.13	0.98	< None >
14	−28.09	−41.87	−37.23	−35.73	2.53	1.18	< None >
15	−13.13	−26.46	−25.57	−21.72	2.52	3.37	< None >
16	−8.54	−7.851	−6.35	−7.58	1.84	3.19	< None >
17	−16.18	−35.96	−30.34	−27.49	1.72	3.06	< None >
18	−18.97	−36.6	−35.08	−30.22	0.93	2.87	< None >
19	−6.74	−30.36	−12.07	−16.39	1.09	1.62	< None >
20	−19.71	−40.43	−35.03	−31.72	1.45	3.18	< None >
21	−19.63	−15.29	−10.57	−15.16	2.66	3.55	< None >
22	−17.32	−28.8	−29.64	−25.25	2.41	2.45	< None >
23	−13.73	−19.8	−24.51	−19.35	3.06	0.85	Mw
24	−20.26	−33.34	−33.06	−28.89	1.84	3.19	< None >
25	−20.41	−21.64	−21.55	−21.2	2.27	3.45	< None >
26	−11.76	−24.25	−25.64	−20.55	1.96	3.6	< None >
27	−14.81	−21.27	−22.24	−19.44	1.47	2.33	< None >
Sum	−348.63	−603.1	−543.1	–	32.43	46.73	–
Mean	−16.6	−28.72	−25.86	–	1.55	2.22	–

3-O-methoxy-4-halo disubstituted 5,7-dimethoxy chromans.

* ABA – Average Binding Affinity; Crystal structures of PDB ID: 2XCT – DNA gyrase A, 3G75 – DNA gyrase B, 3L7L – Teichoic acid polymerase. Component of Lipinski's Rule of 5 (Ro5): LogP – lipophilicity, the partition coefficient of a molecule between an aqueous and lipophilic phase, usually octanol and water. S + logD – The distribution coefficient, it is the ratio of the sum of the concentrations of all forms of the compound.

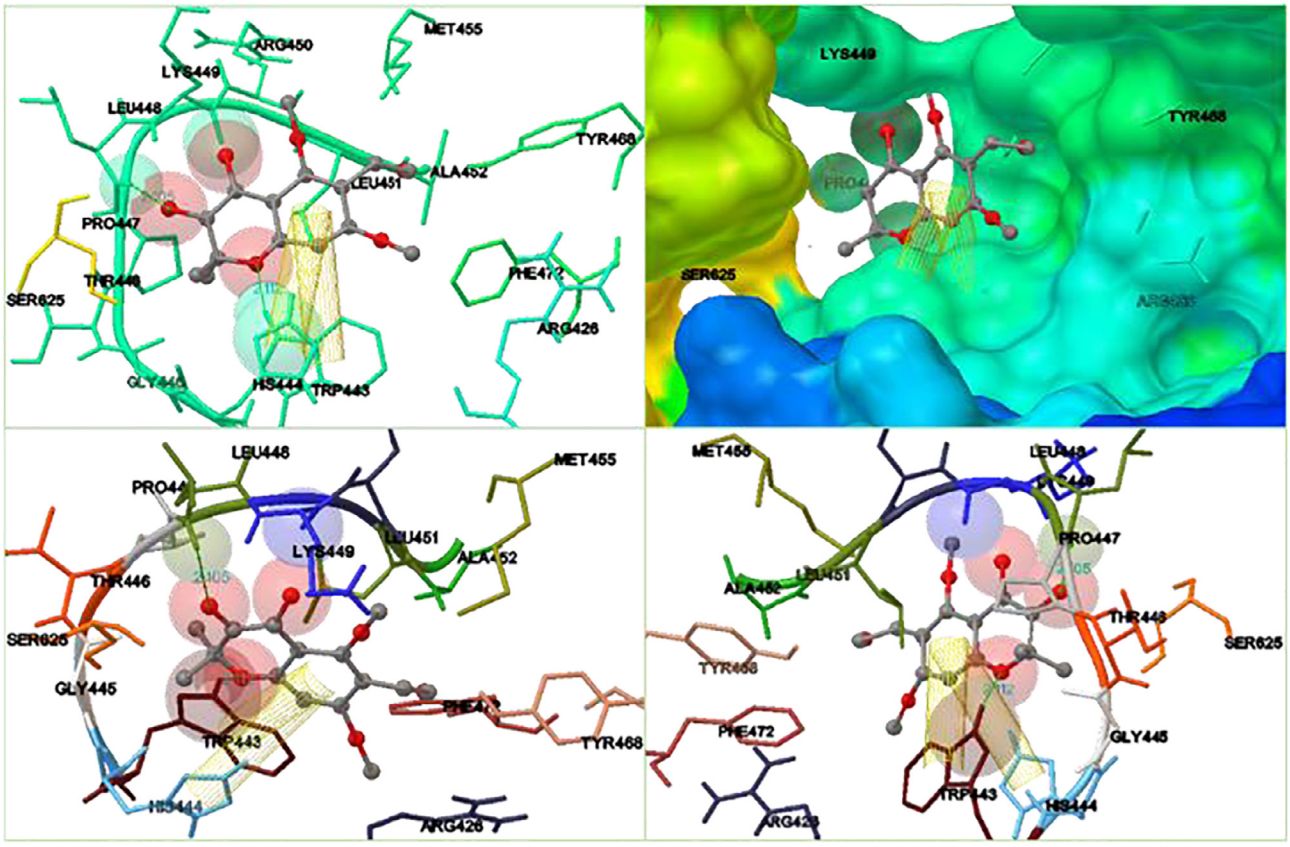


Fig. 1. A representative illustration of best-docked poses or interactions of 14 into the active binding site of DNA gyrase B (PDB ID: 3G75). Note: Stick and Balls (Ligand), Sticks only (Amino acid residues), Spherical (H-bonds), Cylindrical (π-π interactions) and Conical (Cation-π interactions).

compounds were screened for further DNA gyrase decatenation studies. Apart from these, compounds **9–13**, **16**, **22** were also found to have a significant binding energy values (range of −60 to −63 kcal/mol). Fig. 1 depicts the interaction details of compound **14** with DNA gyrase B (PDB ID: 3G75). Established non-covalent (π-π and π-cation interactions) and hydrogen bonding interactions of compound **14** confirms the anticipated best docked ligand-receptor binding interactions as well as the most probable therapeutic output against the proposed *in vitro* or *in vivo* studies. Usually, Hydrogen bonds are facilitating the solubility of a small molecule and the non-covalent interactions (π-π and cation-π interactions) are involving in the exchanging of positive charge of a cation associated to the electrons in a π-system of a molecule.

3.3. DNA gyrase inhibition studies

Eleven compounds that were screened based on the obtained binding affinity (moderate to excellent) values, from molecular docking studies were tested for their inhibition potential against DNA gyrase of *S. aureus*. Fig. 2 illustrates the considerable IC_{50} values that were calculated from the inhibition assay studies against DNA gyrase of *S. aureus*. An IC_{50} range of 1.86–24.02 nM with a calculated relative percentage activity range of 78–94% was obtained as overall result. Clorobiocin (standard drug) showed an IC_{50} of 8.5 nM (86% inhibition). Meanwhile, comparatively dominant results over Clorobiocin was exhibited by the compounds **14** and **20** (IC_{50} 2.5 (94% inhibition and 3.5 nM 90% inhibition respectively). Compound **9** (IC_{50} 7.5 nM), **18** (IC_{50} 9.5 nM) and **13** (IC_{50} 10 nM) were also there in that list. In the mechanism, DNA gyrase alleviating a bacterial strain's life by protecting their double-stranded DNA from the helicase wounding. Helicase is the main causative agents of bacterial DNA's negative supercoiling as well as the relaxation of positive supercoils. In this study, the effectiveness of selected compounds for inhibition of DNA gyrase from

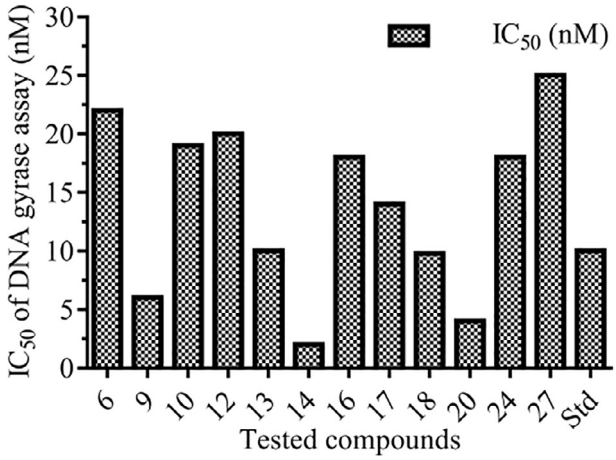


Fig. 2. IC_{50} and the relative activity of Chroman compound to demonstrate *S. aureus* DNA gyrase A inhibition. Note: All IC_{50} values (μM) are the mean of duplicate or triplicate measurements.

S. aureus was examined by assessing the transformation in DNA mobility passing through the electrophoresis gel. Normally the supercoiled form DNA of plasmid migrates quicker on the agarose gel due to its confirmation. Due to nicking on either relaxed or circular form DNA, they migrate bit by bit and appearing on top of agarose gel. pBR322 which was incubated with *S. aureus* DNA gyrase along with and without proposed compounds and Novobiocin was used as positive control. The compounds and Novobiocin were used at a concentration of 1.5 μg in the reaction amount.

From the excellent active group (screened from docking studies) **9**, **14**, **18**, **20** (lane 5–8); from moderate active group **10**, **24** (lane 9 & 10);

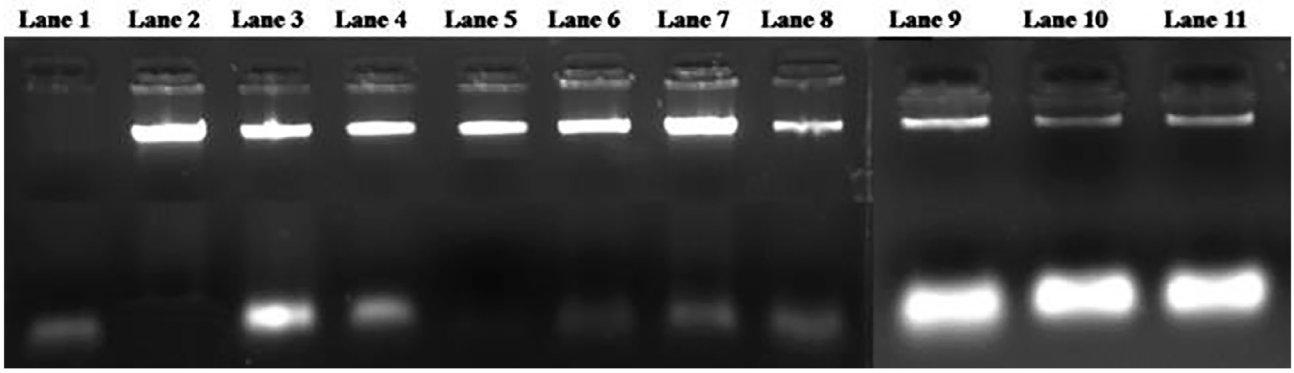


Fig. 3. DNA Gyrase inhibition of chroman derivatives. Lane 1 = pBR322 alone, Lane 2 (positive control) = pBR322 + gyrase; Lanes 3 (negative control) = 1% DMSO; Lanes 4 (standard drug) = gyrase + pBR322 + Novobiocin; Lanes 5–8 = pBR322 + gyrase + active group (**9**, **14**, **18**, **20**); Lanes 9–11 = pBR322 + gyrase + moderate active group (**10**, **24**, **27**). Note: Compounds were observed at a concentration of 2 μg in a final reaction volume of 20 μL.

from the inactive group, **27** (lane 11) were tested respectively as representative compounds from each group. Fig. 3 depicts the *S. aureus* DNA gyrase inhibition ability of 3-O-methoxy-4-halo disubstituted 5,7-dimethoxy chromans. Surprisingly, lane 5 loaded with **9** was exactly showed very less supercoiled band which indicates the excellent DNA gyrase inhibition activity of **9**. Lane 4′s supercoil intensity (Clorobiocin (standard) loaded) is relatively higher than the lane 6–8 which were loaded with excellent active group **14**, **18** and **20** respectively are also can be considered as effective DNA gyrase inhibitors. The intensity of the compounds **10**, **24** and **27** supercoiled bands was stronger which suggests that these compounds only possess lesser inhibitory potential than the standard and other most potential compounds **9**, **14**, **18** and **20**.

3.4. Antibacterial activity results

Table 2 depicts the MIC values calculated to determine the antibacterial potentials of 3-O-methoxy-4-halo disubstituted 5,7-dimethoxy chromans against both Gram^{+ve} and Gram^{-ve} bacteria. The best antibacterial activity was obtained against Gram^{+ve} bacterium (*S. aureus*) over Gram^{-ve} bacteria evidenced from an MIC range of 0.02–1.54 μg mL^{-1}. With lowest average MIC values, compound **14** (0.474 ± 0.18 μg mL^{-1}), **20** (0.636 ± 0.14 μg mL^{-1}) and **17** (0.696 ± 0.20 μg mL^{-1}) were the most active and therapeutically potent (n = 5) (R^2 = 0.9927). Also, with an average MIC range of 0.764 ± 0.17–2.304 ± 0.67 μg mL^{-1}, remaining compounds were also exhibited good antibacterial activity, except **10** (2.991 ± 0.72 μg mL^{-1}), and significantly, these values were only had a slight variation with the MIC value of the standard Novobiocin (2.434 ± 0.73 μg mL^{-1}).

3.4.1. Estimation of viable and non-viable bacterial cells via cell wall degradation assessment

The anticipated cell wall damage, perhaps, due to the Teichoic Acid Polymerase (the enzyme which is involving the cell wall construction function of all Gram^{+ve} bacteria) inhibition by the present study compounds, 3-O-methoxy-4-halo disubstituted 5,7-dimethoxy chromans, was analyzed. Enumeration of bacterial cell viability was carried out by estimating the absorbed dye by the living cells and dead cells in a 96 well plate. Earlier, the bacterial samples were cultured in corresponding broth media and an average % of Living/Dead cells was measured along with the average time required for the survival or death. Mainly this execution was intended to analyze (or) to estimate the viable and non-viable bacterial cells due to cell wall degradation. A graph (Fig. 4) was plotted with the % live/dead cells versus the time that needs to exterminate the total organisms in broth media. In the overall results, the total destruction of bacterial cell was required an average of 160 min. In the microscopic cell wall damage observations, *C. diphtheriae* culture was examined for their hourly cell damage after treating the test compounds in optimized concentrations. Gradual

Table 2
Antibacterial activity of 3-O-methoxy-4-halo disubstituted 5,7-dimethoxy chromans against selected pathogens.

Entry	Minimum inhibitory concentration (μg mL^{-1})						^MIC ± SEM
	Gram^{-ve}			Gram^{+ve}			
	KP	*EC*	*PA*	*SA*	*CD*	*SP*	
6	2.24	0.99	1.67	1.00	1.02	3.12	1.561 ± 0.41
9	0.90	0.88	1.02	0.10	0.82	1.00	0.764 ± 0.17
10	3.88	4.1	3.01	1.54	1.28	5.02	2.991 ± 0.72
12	0.92	0.84	1.01	0.64	0.9	1.02	0.882 ± 0.07
13	2.18	2.24	4.52	0.92	0.99	2.85	2.304 ± 0.67
14	0.64	0.64	0.84	0.02	0.04	0.83	0.474 ± 0.18
16	2.02	1.08	1.26	0.98	1.12	2.98	1.484 ± 0.38
17	0.96	1.00	1.02	0.18	0.21	1.07	0.696 ± 0.20
18	0.76	0.92	0.94	0.16	0.92	0.94	0.776 ± 0.15
20	0.72	0.68	0.87	0.08	0.68	0.87	0.636 ± 0.14
24	1.99	2.13	3.12	0.99	1.08	2.04	1.872 ± 0.40
Std*	0.75	4.94	1.75	0.92	1.32	3.24	2.434 ± 0.73
Ref**	0.50 [41]	5.00 [42]	100 [43]	0.78 [44]	–	–	–
Control*	–	–	–	–	–	–	–

*Control-DMSO (Dimethylsulfoxide), *Standard-Novobiocin, *Ref – Reference; ^Average MIC of both Gram^{+ve} and Gram^{-ve} bacterial tested (n = 5) (r^2 = 0.9927); *KP-Klebsiella pneumoniae, EC – E.coli, PA-Pseudomonas aeruginosa, SA-Staphylococcus aureus, CD-Corynebacterium diphtheriae, SP-Streptococcus pyogenes.*

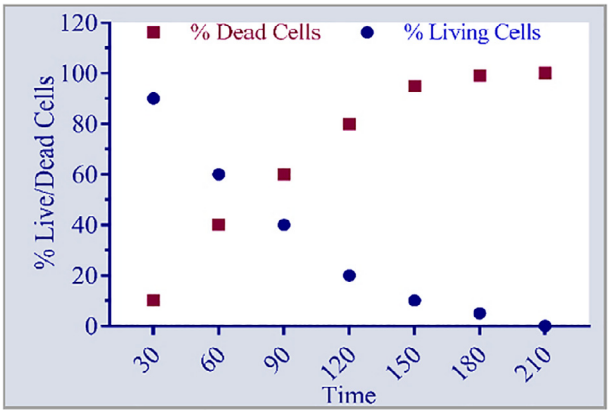


Fig. 4. Cell viability results. Values are the mean of six different experiments (3 Gram^{+ve} + 3 Gram^{-ve} organism).

changes in bacterial cell wall was measured by estimating dead cells or cell debris level at every 30 min until whole bacterial death (Fig. 5). Amount of final cell debris indicates the total cell wall damage/mortality of the bacterial cells so as the ability of 3-O-methoxy-4-halo disubstituted 5,7-dimethoxy chromans compounds within a particular time period.

3.5. HRBC cell wall protection of 3-O-methoxy-4-halo disubstituted 5,7-dimethoxy chromans

The results of HRBC membrane stabilization effects of 3-O-methoxy-4-halo disubstituted 5,7-dimethoxy chromans is depicted in Table 3. The obtained, remarkable, HRBC membrane protection results of these chemical compounds indicating the non-harmf nature of them against human cellular subjects (average protection range 92%). This remarkable cell wall protection and degradation difference exhibited by these compounds, perhaps, due to the variation between prokaryotic and eukaryotic cells (bacteria and HRBC respectively). According to the measured relative % protection and IC_{50} values, except compounds **6** and **10**, all compounds were found as most active as the standard, Indomethacin.

3.6. Cytotoxicity study of compounds 9, 13, 14, 18 and 20

Cytotoxicity of the best DNA gyrase inhibitors of this study was assessed against epithelial type breast cancer cell lines and normal cell lines (MCF-7 and MCF-10 respectively) by MTT assay (n = 4). In the activity, there was no change in the normal cell lines (MCF-10) even after 8 h from the compound treatment. Meanwhile, a gradual, dose depended cell death was observed against MCF-7 cell lines. The cell viability was assessed with a control (Dimethylsulphoxide (DMSO)) and a standard (Doxorubicin). An average of 20% cancer cell viability was observed after 4 h from the compound treatment. Meanwhile it was reduced as 16% at the end of 6 h. The efficient cytotoxicity against cancer cell lines by the compounds was recognized from this. The relative % activity calculated for all compounds was far better from Doxorubicin, the standard drug. Fig. 6, shows the ups and down of cytotoxic activity exhibited by **9**, **13**, **14**, **18** and **20**. Among all the compounds, **14** was dominated for the activity with a maximum relative % activity up to 90% and lowest IC_{50} of 10 nM.

Table 3
HRBC cell wall protection effects of 3-O-methoxy-4-halo disubstituted 5,7-dimethoxy chromans.*

Entry	Mean Absorbance ± SD*	% HRBC cell wall Protection	IC_{50} µg/mL
12	0.4046 ± 0.025	88.52	0.0094
13	0.3888 ± 0.038	85.31	0.8802
24	0.3634 ± 0.038	86.16	0.1241
9	0.4624 ± 0.064	90.45	0.0182
18	0.3947 ± 0.021	85.15	0.0922
14	0.4852 ± 0.012	98.84	0.0012
17	0.3659 ± 0.022	86.37	0.2224
20	0.4242 ± 0.018	93.25	0.0102
6	0.3841 ± 0.042	80.65	1.2406
16	0.3731 ± 0.007	85.55	0.1184
10	0.3611 ± 0.009	80.88	1.0016
Indomethacin	0.3834 ± 0.012	85.64	0.0860
Control#	0.1868 ± 0.033	No inhibition	–

Distilled water.
* S.D. = Standard deviation (Average of four determinations) (n = 4).

3.7. Structure-activity relationships

In the Structure-Activity Relationship (SAR), substituted 3-O-methoxy-4-halo disubstituted 5,7-dimethoxy chromans with various electron withdrawing and electron discharging groups were synthesized, analyzed and compared with the DNA gyrase inhibition and antibacterial activity. The interaction details with corresponding receptors that were highly associated with the antibacterial activities was unveiled through molecular docking studies. While discussing about the

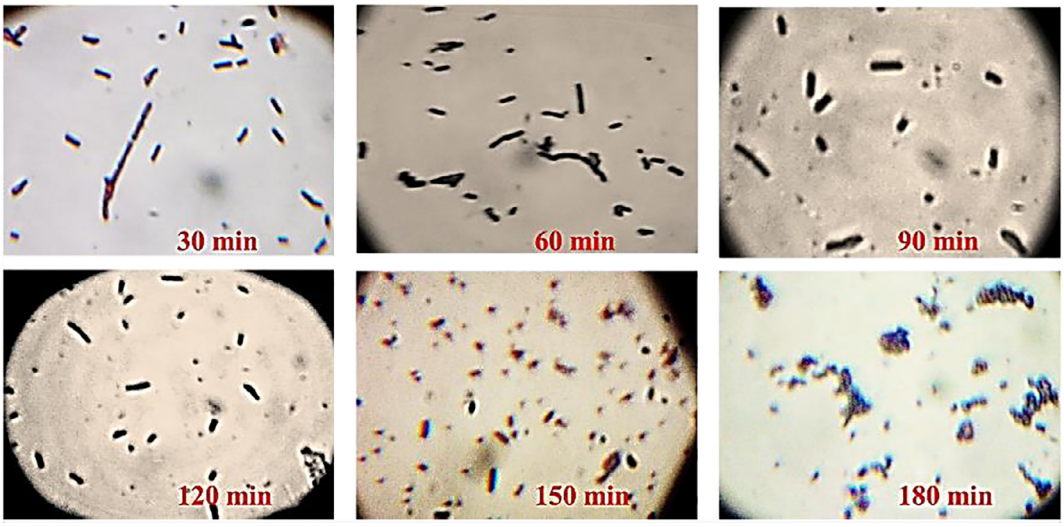


Fig. 5. Physiological observations of *C. diphtheriae* cell wall degradation/cell death analysis.

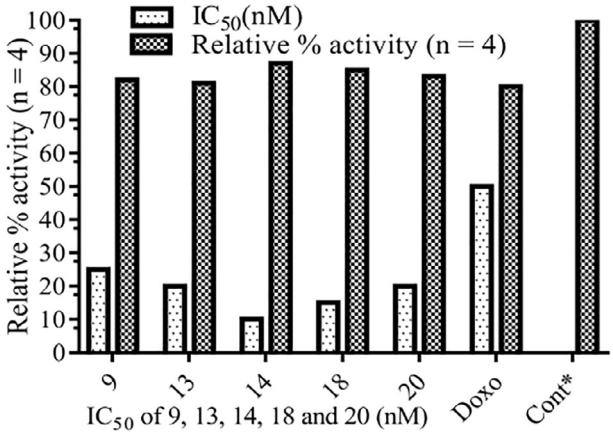


Fig. 6. Cytotoxicity analysis of compounds 9, 13, 14, 18 and 20. Doxo – Doxorubicin; Cont* – Control (DMSO). Values are the mean of four different experiments.

functional components of present study compounds, the functional scaffolds such as aromatic arene, azaarene, benzene ring, phenol and CHO-containing 1,2-diol, alkanol, carbonyl, ether, ketone/aldehyde, halogen-containing leaving groups and S-containing sulfonate ester were found as the most commonly substituted entities. In the molecular docking ligand-receptor analysis, almost all compounds were interacted through these substituents with the targeted receptors by remarkably establishing an average of 5 hydrogen bonds and at least with a non-covalent interaction (π-π and cation-π interactions). Druggability of these compounds was also estimated with Lipinski's filter and Ro5 (Rule of 5) analysis (Table 1). With the help of Lipinki's filter results, the solubility potential or lower lipophilicity was recognized (mean LogP 1.55 (n = 21) value should be < 5). The average distribution coefficient (Simulated LogD) was only 2.22 (anticipated value < 5). This favorable values suggesting the anticipated ADME (pharmacokinetics (PK)) feasibility of 3-O-methoxy-4-halo disubstituted 5,7-dimethoxy chromans.

In the present study, the utility of inversely substituted 21 chroman/coumarins has been scrutinized as the DNA gyrase inhibitors as well as an efficient anti-bacterial drug candidates. With the established efficacious therapeutic values from all proposed activity studies, 3-O-methoxy-4-halo disubstituted 5,7-dimethoxy chromans could be the future antibiotics against both Gram^{+ve} as well as Gram^{-ve} organisms (broad spectrum). The MIC values obtained for the present study compounds (Table 2) are comparatively better than the compounds which are reported by various reserachers.[41–44] In fact, between the MICs and the IC_{50} values (DNA gyrase inhibition), there was a noteworthy relationship was found (R^2 = 0.9942).

4. Conclusion

In summary, antibacterial medicinal values of well established 3-O-methoxy-4-halo disubstituted 5,7-dimethoxy chromans were evaluated by means of DNA gyrase inhibition, bacterial cell wall degradation and *in vitro* anti bacterial assessment. The average binding affinity calculated from molecular docking studies (Table 1) helped us to screen most potent compounds among all 21 3-O-methoxy-4-halo disubstituted 5,7-dimethoxy chromans. In addition to all these valuable findings, to uphold the activity capabilities of these compounds, we are also suggesting the necessity of further substitution of electron donor/withdrawal groups in the functional component of chroman/coumarin scaffolds.

Acknowledgments

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2018.05.016.

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