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Design, synthesis, spectral characterization and molecular docking studies of novel pyranoquinolinyl dihydropyridine carboxylates as potential antibacterial agents including *Vibrio cholerae* with minimal cytotoxity towards fibroblast cell line (L-929)

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ABSTRACT

Novel pyranoquinolinyl dihydropyridine carboxylate (PDC) derivatives were designed by incorporating the multi-drug resistance modulating effects of 1,4 dihydropyridines along with potential antibacterial activity of quinolines in the molecular design. The designed PDC derivatives were synthesized by multi-step synthesis involving Michael addition, reduction followed by inverse electro demand Diels-Alder reaction to produce pyranoquinolinyl dihydropyridine carboxylates in good yields. All the PDC derivatives were characterized by ^{1}H NMR, ^{13}C NMR, FT-IR, Mass spectral and CHN analysis. The Quinolinyl dihydropyridine carboxylate derivatives were evaluated for *in vitro* antibacterial activity by agar well diffusion method. Molecular docking studies revealed that the exo diethyl 4-(4aR,5S,10bR)-5-(4-chlorophenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-*c*] quinolin-8-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate diastereomer (5c) forms four hydrogen bonds with the cell wall protein of vibrio cholerae in comparison to the endo diethyl 4-((4aR,5R,10bR)-5-(4-chlorophenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-*c*]quinolin-8-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate diastereomer (4c) which forms two hydrogen bonds with the cell wall protein of vibrio cholerae and hence leading to better anchorage, enhanced gold score and relatively good antibacterial activity for the exo PDC derivatives. Minimum inhibitory concentration (MIC) of the active compounds was evaluated by macro dilution method. The mechanism of antibacterial action of the PDC derivatives was investigated by SEM studies. The cytotoxicity of PDC derivatives were evaluated against fibroblast cells (L-929).

1. Introduction

Research and development for finding new drugs and improved chemical entities for treatment and mitigation of various diseases associated with viruses such as Ebola virus, Corona virus, Avian influenza virus and carbapenem resistant strains of bacteria's such as Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacteriaceae is of utmost priority for chemists & scientists worldwide to ensure the very existence and survival of human beings [1–3]. According to WHO report (1995) [4], the major cause for children mortality in developing and under developed countries is cholera, contracted by ingesting food contaminated with vibrio cholerae. Drug resistant strains of Vibrio cholera [5] have further worsened the scenario posing a major challenge to medicinal practitioners. Due to the impact of cholera on infants and children [6], failure to develop new antibiotics, lack of treatment options for drug resistant strains, the development of new antibiotics to treat and mitigate cholera is highly desirable [7,8]. 1,4–dihydropyridines exhibit wide spectrum of biological activities such as vasodilator, bronchodilator, hepatoprotective, antitumor, geroprotoctive, antidiabetic agents [9–11] and calcium chennal blockers [12–14]. 1,4 dihyropyridines have been identified to bring about the reversal of multidrug resistance and are known to be multi-drug resistant modulators [15]. Pyrano and furano quinoline derivatives exhibit wide spectrum of biological activies such as anti-inflamatory [16], psychotropic [17], anti-allergic [18], antibacterial activity [19] and estrogenic activities [20]. Hence in an attempt to counter the problem of drug resistant bacteria, we have combined the multi-drug resistant modulating effect of 1,4 dihydropyridines along with strong

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antibacterial activity of quinolines to find a promising class of new chemical entities as potential antibacterial agents with minimal cytotoxicity.

Recently we have reported the novel pyranoquinolinyl acrylic acid diasteromers as potential α-Glucosidase inhibitors [21] and carbazolyl dihydropyrimidinones with good oral bioavailability [22]. In our search for finding new antibacterial agents we have recently reported design, synthesis, molecular docking and spectral studies of new class of Carbazolyl polyhydroquinolines derivatives as promising antibacterial agents with non-cytotoxicity towards human mononuclear cells (HMNC-PB) [23]. In continuation of our search for finding new chemical entities with potential antibacterial activity and minimal cytotoxicity, we now report the design, synthesis, spectral characterization, molecular docking and antibacterial studies of novel pyranoquinolinyl dihydropyridine carboxylates as potential antibacterial agents including vibrio cholerae.

2. Experimental section

2.1. Materials & methods

The AR grade solvents and chemicals were purchased from sigma Aldrich. The FT-IR spectrum of synthesized compounds were recorded by using Nicolet impact 400 FT-IR spectrometer using KBr pellets technique. ^{1}H NMR spectra were recorded on Bruker (400 MHz) in DMSO-d_6 or $CDCl_3$ solvent and chemical shifts are reported in δ values relative to the internal standard TMS and ^{13}C NMR spectra were recorded on 100 MHz in DMSO-d_6 or $CDCl_3$ solvent. Elemental analysis was determined by Yanagimoto MT3CHN recorder. Mass spectrum of all products were recorded by VG 70-70H mass spectrometer. Thin layer chromatography was performed on silica gel sheets (0.25 mm thickness with UV indicator PF-254, Merck, Darmstadt). Column chromatography was used with silica gel (100–200 mesh; SD Fine). SEM images of bacteria treated with quinolinyl dihydropyrine carboxylate derivatives were determined with VEGA/TESCAN electron microscope.

2.2. Synthetic procedure

2.2.1. General procedure for synthesis of pyranoquinolinyl dihydropyridine dicarboxylates derivatives

A mixture of diethyl-4-(4-aminophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (2) (0.01 mol), benzaldehyde (6a) (0.01 mol) and 3,4 dihydro-2H-pyran (7) (0.01 mol) were added into a round bottom flask containing acetonitrile as solvent (10.0 ml) in the presence of indiumtriflate (20.0 mol%) as catalyst and stirred at 25.0–27.0 °C for an appropriate time. The reaction was monitored by TLC. After complete conversion, the reaction mixture was concentrated under vacuum. Ethyl acetate [20 ml] and water [20 ml] was added to the reaction mixture. The organic layer was separated, concentrated under vacuum and the crude product was purified by column chromatography on silica gel (ethyl acetate/petroleum ether, 1:4) to give the products (diethyl-2,6-dimethyl-4-((4aR,5R,10bR)-5-phenyl-3,4,4a,5,6,10b-hexahydro–2H-pyrano[3,2-*c*]quinolin-8-yl)-1,4-dihydropyridine-3,5-dicarboxylate (4a) & diethyl 2,6-dimethyl-4-((4aR,5S,10bR)-5-phenyl-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-*c*]quinolin-8-yl)-1,4-dihydropyridine-3,5-dicarboxylate (5a) in 71.0% yield.

*2.2.1.1. Diethyl 2,6-dimethyl-4-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate **(1)**.* FT- IR (KBr) V_{max} cm^{-1}: 3338, 1686, 1645, 1247, 1119, 634; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.06–7.04 (d, 2H, J = 8 MHz), 6.54–6.52 (d, 2H, J = 8 MHz), 5.70 (brs, 1H, NH), 4.86 (s, 1H), 4.10–4.06 (q, 4H), 2.30 (s, 6H), 1.24–1.20 (t, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.8, 144.3, 143.4, 138.5, 128.9, 114.7, 104.4, 59.6, 38.6, 19.5, 14.2; MS: *m/z*: 374 (M^+); Anal. Calcd. for $C_{19}H_{22}N_2O_6$: C, 60.95; H, 5.92; N, 7.48; Found: C, 60.91; H, 5.99; N, 7.52.

*2.2.1.2. Diethyl 4-(4-aminophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate **(2)**.* FT- IR (KBr) V_{max} cm^{-1}: 3340, 1670, 1230, 1109, 652; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 10.17 (brs, 2H, NH_2), 8.09–8.07 (d, 2H, *J* = 8 Hz), 7.47–7.45 (d, 2H, J = 8 MHz), 6.18 (brs, 1H, NH), 5.10 (s, 1H), 4.11–4.08 (q, 4H), 2.35 (s, 6H), 1.24–1.20 (t, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.8, 155.1, 146.1, 144.9, 128.5, 123.0, 103.0, 59.8, 39.9, 19.4, 14.1; MS: *m/z*: 344 (M^+); Anal. Calcd. for $C_{19}H_{24}N_2O_4$: C, 66.26; H, 7.02; N, 8.13; Found: C, 66.31; H, 7.03; N, 8.09.

*2.2.1.3. Diethyl 2,6-dimethyl-4-((4aR,5R,10bR)-5-phenyl-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-c]quinolin-8-yl)-1,4-dihydropyridine-3,5-dicarboxylate **(4a)**.* FT- IR (KBr) V_{max} cm^{-1}: 3385, 2943, 1655, 1272, 1121, 625; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 9.72 (brs, 1H, NH), 7.38–7.27 (m, 5H), 6.98–6.96 (d, 1H, J = 8 MHz), 6.47–6.45 (d, 2H, J = 8 MHz), 5.28–5.27 (d, 1H, J = 4.00 MHz), 5.19 (s, 1H), 4.90 (s, 1H), 4.63 (brs, 1H, NH), 4.11–4.05 (q, 4H), 3.37–3.30 (t, 2H), 2.35–2.31 (d, 1H), 2.17 (s, 6H), 1.54–1.48 (m, 4H), 1.26–1.22 (t, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.66, 143.39, 141.26, 138.42, 128.22, 127.73, 127.41, 126.83, 118.98, 114.15, 104.36, 72.91, 61.84, 59.33, 39.90, 38.68, 30.94, 29.01, 25.44, 19.48, 14.33; MS: *m/z*: 516 (M^+); Anal. Calcd. for $C_{31}H_{36}N_2O_5$: C, 72.07; H, 7.02; N, 5.42; Found: C, 72.01; H, 6.99; N, 5.31.

*2.2.1.4. Diethyl 2,6-dimethyl-4-((4aR,5S,10bR)-5-phenyl-3,4,4a,5,6,10b-hexahydro-2H-pyrano [3,2-c]quinolin-8-yl)-1,4-dihydropyridine-3,5-dicarboxylate **(5a)**.* FT- IR (KBr) V_{max} cm^{-1}: 3365, 2946, 1660, 1253, 1118, 630; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 9.65 (brs, 1H, NH), 7.39–7.27 (m, 5H), 7.02–7.00 (d, 2H, J = 8 MHz), 6.61–6.59 (d, 1H, J = 8 MHz), 5.85 (brs, 1H, NH), 5.19 (s, 1H), 4.88 (s, 1H), 4.65–4.62 (d, 1H, *J* = 12 Hz), 4.10–4.05 (q, 4H), 3.46–3.41 (t, 2H), 2.36–2.30 (d, 1H), 2.17 (s, 6H), 1.48–1.41 (m, 4H), 1.29–1.23 (t, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.06, 143.58, 141.93, 135.77, 130.01, 128.48, 127.40, 126.78, 120.09, 115.83, 104.33, 68.11, 59.72, 54.73, 38.56, 31.49, 29.36, 25.91, 22.69, 19.62, 14.26; MS: *m/z*: 516 (M^+); Anal. Calcd. for $C_{31}H_{36}N_2O_5$: C, 72.07; H, 7.02; N, 5.42; Found: C, 72.09; H, 7.01; N, 5.39.

*2.2.1.5. Diethyl 2,6-dimethyl-4-((4aR,5R,10bR)-5-(4-nitrophenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-c]quinolin-8-yl)-1,4-dihydropyridine-3,5-dicarboxylate **(4b)**.* FT- IR (KBr) V_{max} cm^{-1}: 3345, 2987, 1676, 1239, 1109, 689; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 9.74 (brs, 1H, NH), 7.44–7.22 (m, 5H), 7.00 (s, 1H), 6.46 (d, 1H, J = 8 MHz), 5.80 (brs, 1H, NH), 5.22 (s, 1H), 4.88 (s, 1H), 4.10–4.07 (q, 4H), 3.76 (d, 1H, *J* = 4.00 Hz), 3.68 (t, 2H), 2.73 (d, 1H), 2.17 (s, 6H), 1.69–1.67 (d, 4H), 1.26–1.23 (t, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.78, 150.56, 146.12, 145.31, 139.89, 128.41, 124.51, 123.45, 116.22, 113.46, 104.66, 72.81, 69.54, 62.54, 61.27, 46.28, 43.27, 25.05, 22.72, 19.12, 14.43; MS: *m/z*: 561 (M^+); Anal. Calcd. for $C_{31}H_{35}N_3O_7$: C, 66.30; H, 6.28; N, 7.48; Found: C, 66.27; H, 6.29; N, 7.51.

*2.2.1.6. Diethyl 2,6-dimethyl-4-((4aR,5S,10bR)-5-(4-nitrophenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-c]quinolin-8-yl)-1,4-dihydropyridine-3,5-dicarboxylate **(5b)**.* FT- IR (KBr) V_{max} cm^{-1}: 3339, 2975, 1685, 1242, 1122, 701; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 9.45 (brs, 1H, NH), 7.42–7.27 (m, 5H), 7.08 (d, 1H, J = 12 MHz), 6.48 (d, 1H, J = 8 MHz), 5.75 (brs, 1H, NH), 4.90 (s, 1H), 4.52 (s, 1H), 4.11–4.08 (q, 4H), 3.75–3.73 (d, 1H, *J* = 8 Hz), 3.56–3.50 (t, 2H), 2.71–2.70 (d, 1H), 2.19 (s, 6H), 1.58–1.56 (d, 4H), 1.25–1.23 (t, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.00, 150.05, 146.45, 145.66, 139.13, 128.24, 124.45, 123.20, 116.32, 113.80, 104.89, 75.80, 69.83, 62.77, 61.73, 46.35, 43.68, 25.44, 22.01, 19.70, 14.05; MS: *m/z*: 561 (M^+); Anal. Calcd. for $C_{31}H_{35}N_3O_7$: C, 66.30; H, 6.28; N, 7.48; Found: C, 66.32; H, 6.21; N, 7.40.

2.2.1.7. Diethyl 4-((4aR,5R,10bR)-5-(4-chlorophenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-c]quinolin-8-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate ***(4c)***. FT- IR (KBr) V_{max} cm^{-1}: 3353, 2935, 1690, 1342, 1118, 704; ^1H NMR ($CDCl_3$, 400 MHz) δ: 9.64 (brs, 1H, NH), 7.36–7.25 (m, 4H), 7.00 (d, 1H, J = 8 MHz), 6.47 (d, 1H, J = 8 MHz), 6.20 (s, 1H), 5.25 (d, 1H, *J* = 4.00 Hz), 4.89 (s, 1H), 4.58 (brs, 1H, NH), 4.12–4.05 (q, 4H), 3.54 (d, 1H), 3.38–3.33 (t, 2H), 2.31–2.30 (d, 1H), 2.17 (s, 6H), 1.49–1.42 (m, 4H), 1.28–1.20 (t, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.95, 143.79, 143.13, 139.89, 138.71, 132.94, 128.41, 127.76, 126.80, 119.05, 114.29, 104.22, 72.75, 60.30, 59.64, 58.77, 39.11, 38.71, 30.89, 25.38, 19.34, 14.43; MS: *m/z*: 550 (M^+); Anal. Calcd. for $C_{31}H_{35}ClN_2O_5$: C, 67.56; H, 6.40; N, 5.08; Found: 67.50; H, 6.38; N, 5.10.

2.2.1.8. Diethyl 4-((4aR,5S,10bR)-5-(4-chlorophenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-c]quinolin-8-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate ***(5c)***. FT- IR (KBr) V_{max} cm^{-1}: 3351, 2938. 1691, 1342, 1115, 653; ^1H NMR ($CDCl_3$, 400 MHz) δ: 9.96 (brs, 1H, NH), 7.44–7.43 (m, 1H), 7.33 (m, 3H), 7.09 (s, 1H), 6.40 (d, 1H, J = 12 MHz), 6.12 (s, 1H), 5.19 (brs, 1H, NH), 4.87 (s, 1H), 4.62–4.59 (d, 1H, *J* = 12 Hz), 4.30 (s, 1H) 4.10–4.05 (q, 4H), 3.54–3.50 (t, 2H), 2.34–2.29 (d, 1H), 2.17 (s, 6H), 1.76–1.72 (m, 4H), 1.26–1.22 (t, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.66, 143.77, 142.77, 141.12, 137.50, 133.42, 128.71, 119.84, 113.80, 104.22, 74.60, 68.33, 61.73, 59.63, 54.36, 38.64, 30.90, 24.12, 19.39, 14.32; MS: *m/z*: 550 (M^+); Anal. Calcd. for $C_{31}H_{35}ClN_2O_5$: C, 67.56; H, 6.40; N, 5.08; Found: , 67.51; H, 6.43; N, 5.05.

2.2.1.9. Diethyl 2,6-dimethyl-4-((4aR,5R,10bR)-5-(p-tolylphenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-c]quinolin-8-yl)-1,4-dihydropyridine-3,5-dicarboxylate ***(4d)***. FT- IR (KBr) V_{max} cm^{-1}: 3348, 2942, 1656, 1207, 1116, 731; ^1H NMR ($CDCl_3$, 400 MHz) δ: 9.74 (brs, 1H, NH), 7.28 (d, 2H, J = 8 MHz), 7.17 (d, 2H, J = 8 MHz), 7.08 (s, 1H), 7.00 (d, 1H, J = 8 MHz) 6.37 (d, 1H, J = 8 MHz), 5.69 (brs, 1H, NH), 4.87 (s, 1H), 4.65 (d, 1H, *J* = 4.00 Hz), 4.30 (s, 1H), 4.10–4.03 (q, 4H), 3.70–3.64 (t, 2H), 2.59 (d, 1H), 2.35 (s, 3H), 2.32 (s, 6H) 1.62–1.48 (m, 4H), 1.27–1.22 (t, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.78, 143.43, 143.11, 139.51, 137.44 137.11, 130.05, 129.24, 127.73, 119.94, 113.59, 104.46, 74.84, 68.47, 59.61, 54.55, 38.91, 33.81, 24.27, 22.06, 21.14, 19.56, 14.12; MS: *m/z*: 530 (M^+); Anal. Calcd. for $C_{32}H_{38}N_2O_5$: C, 72.43; H, 7.22; N, 5.28; Found: C, 72.46; H, 7.21; N, 5.19.

2.2.1.10. Diethyl 2,6-dimethyl-4-((4aR,5S,10bR)-5-(p-tolylphenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-c]quinolin-8-yl)-1,4-dihydropyridine-3,5-dicarboxylate ***(5d)***. FT- IR (KBr) V_{max} cm^{-1}: 3364, 2925, 1686, 1232, 1132, 701; ^1H NMR ($CDCl_3$, 400 MHz) δ: 9.48 (brs, 1H, NH), 7.31 (m, 2H), 7.17 (d, 2H, J = 8 MHz), 6.99 (d, 1H, J = 8 MHz), 6.45 (d, 2H, J = 8 MHz), 5.85 (brs, 1H, NH), 5.26 (d, 1H, *J* = 12.00 Hz), 4.90 (s, 1H), 4.59 (s, 1H), 4.12–4.05 (q, 4H), 3.53–3.46 (t, 2H), 2.57–2.56 (d, 1H), 2.34 (s, 3H), 2.31 (s, 6H) 1.52–1.41 (m, 4H), 1.28–1.22 (t, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.64, 144.29, 143.37, 138.23, 137.05, 136.01, 128.89, 127.69, 126.73, 118.95, 113.92, 104.34, 72.93, 60.28, 59.00, 39.18, 38.69, 33.41, 25.48, 22.13, 21.08, 19.52, 14.32; MS: *m/z*: 530 (M^+); Anal. Calcd. for $C_{32}H_{38}N_2O_5$: C, 72.43; H, 7.22; N, 5.28; Found: C, 72.39; H, 7.19; N, 5.26.

2.2.1.11. Diethyl 2,6-dimethyl-4-((4aR,5R,10bR)-5-(4-methoxyphenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-c]quinolin-8-yl)-1,4-dihydropyridine-3,5-dicarboxylate ***(4e)***. FT- IR (KBr) V_{max} cm^{-1}: 3342, 2941, 1686, 1267, 1121, 675; ^1H NMR ($CDCl_3$, 400 MHz) δ: 9.55 (brs, 1H, NH), 7.34–7.28 (m, 3H), 7.04–7.02 (d, 1H, J = 8 MHz), 6.47 (d, 2H, J = 8 MHz), 6.20 (s, 1H), 5.24 (brs, 1H, NH), 4.91 (s, 1H), 4.58 (s, 1H), 4.12–4.04 (q, 4H), 3.77 (s, 3H), 3.64–3.61(t, 2H), 3.25–3.24 (d, 1H, *J* = 4.00 Hz), 2.17 (s, 6H), 2.06–2.01(d, 1H), 1.66–1.61 (m, 4H), 1.26–1.20 (t, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.10, 158.33, 150.42, 145.16, 139.85, 132.49, 127.59, 126.57, 117.73, 116.66, 114.35, 113.80, 104.00, 71.43, 69.13, 62.61, 61.67, 55.88, 44.93, 42.01, 25.44, 24.12, 19.39, 14.16; MS: *m/z*: 546 (M^+); Anal. Calcd. for $C_{32}H_{38}N_2O_6$: C, 70.31; H, 7.01; N, 5.12; Found: C, 70.29; H, 6.99; N, 5.10.

2.2.1.12. Diethyl 2,6-dimethyl-4-((4aR,5S,10bR)-5-(4-methoxyphenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-c]quinolin-8-yl)-1,4-dihydropyridine-3,5-dicarboxylate ***(5e)***. FT- IR (KBr) V_{max} cm^{-1}: 3361, 2936, 1681, 1255, 1130, 668; ^1H NMR ($CDCl_3$, 400 MHz) δ: 9.13 (brs, 1H, NH), 7.43 (s, 1H), 7.31 (m, 1H), 7.09 (s, 1H), 7.00 (m, 1H), 6.40 (d, 2H, J = 12 MHz), 6.12 (s, 1H), 5.19 (brs, 1H, NH), 4.87 (s, 1H), 4.64 (s, 1H), 4.10–4.02 (q, 4H), 3.80–3.76 (d, 1H, *J* = 16 Hz), 3.52 (s, 3H), 3.37–3.30 (t, 2H), 2.57 (s, 1H), 2.17 (s, 6H), 1.74–1.70 (m, 4H), 1.26–1.22 (t, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.93, 149.93, 146.56, 145.63, 139.07, 128.29, 124.94, 123.56, 117.27, 113.85, 104.07, 70.79, 68.86, 61.86, 61.27, 54.60, 49.29, 43.27, 28.07, 23.50, 19.72, 14.07; MS: *m/z*: 546 (M^+); Anal. Calcd. for $C_{32}H_{38}N_2O_6$: C, 70.31; H, 7.01; N, 5.12; Found: C, 70.28; H, 6.99; N, 5.14.

2.2.2. General procedure for synthesis of furoquinolinyl dihydropyridine dicarboxylate derivatives

A mixture of diethyl 4-(4-aminophenyl)-2, 6-dimethyl-1, 4-dihydropyridine-3, 5-dicarboxylate (2) (0.01 mol), benzaldehyde (6a) (0.01 mol) and 2, 3 dihydro-furan (0.01 mol) (8) was added into the round bottom flask containing acetonitrile as solvent (10.0 ml) in the presence of indium triflate (20.0 mol %) as catalyst and stirred at room temperature for an appropriate time (Table 2). After complete conversion the reaction mixture was concentrated under vacuum. Ethyl acetate [20 ml] and water [20 ml] was added to the reaction mixture. The ethylacetate layer was separated, concentrated under vacuum and the crude product was purified by column chromatography on silica gel (ethyl acetate/ petroleum ether, 1:4) to give the product diethyl 2,6-dimethyl-4-((3aR,4R,9bR)-4-phenyl-2,3,3a,4,5,9b-hexahydrofuro[3,2-*c*]quinolin-7-yl)-1,4-dihydropyridine-3,5-dicarboxylate (9a) & diethyl 2,6-dimethyl-4-((3aR,4S,9bR)-4-phenyl-2,3, 3a,4,5,9b-hexahydrofuro[3,2-*c*]quinolin-7-yl)-1,4-dihydropyridine-3,5-dicarboxylate (10a) in 69.0% yield.

2.2.2.1. Diethyl 2,6-dimethyl-4-((3aR,4R,9bR)-4-phenyl-2,3,3a,4,5,9b-hexahydrofuro[3,2-c]quinolin-7-yl)-1,4-dihydropyridine-3,5-dicarboxylate ***(9a)***. FT- IR (KBr) V_{max} cm^{-1}: 3358, 2945, 1672, 1231, 1124, 659; ^1H NMR ($CDCl_3$, 400 MHz) δ: 9.76 (brs, 1H, NH), 7.43–7.27 (m, 6H), 7.02 (d, 1H, J = 4 MHz), 6.46 (d, 1H, J = 8 MHz), 5.80 (brs, 1H, NH), 5.20–5.19 (d, 1H, *J* = 4.00 Hz), 4.88 (s, 1H), 4.64 (s, 1H), 4.11–4.08 (q, 4H), 3.70–3.67 (t, 2H), 2.32–2.31 (d, 1H), 2.15 (s, 6H), 1.91–1.88 (m, 2H), 1.26–1.23 (t, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.74, 143.24, 142.57, 139.25, 129.62, 128.64, 127.66, 126.61, 121.64, 114.71, 104.40, 76.17, 66.53, 59.23, 57.27, 45.98, 38.68, 28.67, 19.44, 14.05; MS: *m/z*: 502 (M^+); Anal. Calcd. for $C_{30}H_{34}N_2O_5$: C, 71.69; H, 6.82; N, 5.57; Found: C, 71.60; H, 6.89; N, 5.56.

2.2.2.2. Diethyl 2,6-dimethyl-4-((3aR,4S,9bR)-4-phenyl-2,3,3a,4,5,9b-hexahydrofuro[3,2-c]quinolin-7-yl)-1,4-dihydropyridine-3,5-dicarboxylate ***(10a)***. FT- IR (KBr) V_{max} cm^{-1}: 3351, 2939, 1658, 1240, 1110, 672; ^1H NMR ($CDCl_3$, 400 MHz) δ: 9.76 (brs, 1H, NH), 7.42–7.32 (m, 6H), 7.07 (d, 1H, J = 8 MHz), 6.48 (d, 1H, J = 8 MHz), 5.75 (s, 1H), 5.22 (brs, 1H, NH), 4.90 (s, 1H), 4.53 (d, 1H, *J* = 12.0 Hz), 4.14–4.03 (q, 4H), 3.79 (t, 2H), 2.33–2.30 (d, 1H), 2.19 (s, 6H), 1.69–1.64 (m, 2H), 1.28–1.26 (t, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.72, 143.51,141.94, 137.99, 130.65, 128.47, 126.28, 124.44, 123.60, 117.12, 114.01, 104.22, 64.94, 59.44, 57.69, 49.95, 43.37, 38.76, 28.76, 19.59, 14.26; MS: *m/z*: 502 (M^+); Anal. Calcd. for $C_{30}H_{34}N_2O_5$: C, 71.69; H, 6.82; N, 5.57; Found: C, 71.63; H, 6.79; N, 5.60

*2.2.2.3. Diethyl 2,6-dimethyl-4-((3aR,4R,9bR)-4-(4-nitrophenyl)-2,3,3a,4,5,9b-hexahydrofuro [3,2-c]quinolin-7-yl)-1,4-dihydropyridine-3,5-dicarboxylate **(9b)***. FT- IR (KBr) V_{max} cm^{-1}: 3347, 2942, 1659, 1256, 1125, 659; ^1H NMR ($CDCl_3$, 400 MHz) δ: 9.47 (brs, 1H, NH), 8.27–8.14 (m, 2H), 7.53 (d, 2H, J = 4 MHz), 7.08 (d, 2H, J = 8 MHz), 6.58 (s, 1H), 5.32 (brs, 1H, NH), 4.83 (s, 1H), 4.60 (s, 1H), 4.13–4.06 (q, 4H), 3.86–3.84 (d, 1H, *J* = 8.00 Hz), 3.73–3.70 (t, 2H), 2.78–2.77 (d, 1H), 2.21 (s, 6H), 1.94–1.91 (t, 2H), 1.29–1.27 (t, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.96, 149.93, 146.56, 145.63, 139.07, 128.29, 124.94, 123.56, 117.27, 113.85, 104.07, 74.79, 68.86, 61.86, 61.27, 49.29, 43.27, 28.07, 19.72, 14.07, MS: *m/z*: 547 (M^+); Anal. Calcd. for $C_{30}H_{33}N_3O_7$: C, 65.80; H, 6.07; N, 7.67; Found: C, 65.90; H, 6.06; N, 7.70.

*2.2.2.4. Diethyl 2,6-dimethyl-4-((3aR,4S,9bR)-4-(4-nitrophenyl)-2,3,3a,4,5,9b-hexahydro furo[3,2-c]quinolin-7-yl)-1,4-dihydropyridine-3,5-dicarboxylate **(10b)***. FT- IR (KBr) V_{max} cm^{-1}: 3342, 2939, 1649, 1233, 1110, 662; ^1H NMR ($CDCl_3$, 400 MHz) δ: 9.41 (brs, 1H, NH), 7.49–7.43 (m, 3H), 7.19 (s, 1H), 7.10 (m, 1H), 6.46 (d, 1H, J = 12 MHz), 6.20 (s, 1H), 5.21 (brs, 1H, NH), 4.90 (s, 1H), 4.62 (s, 1H), 4.16–4.13 (q, 4H), 3.81–3.78 (d, 1H, *J* = 12.00 Hz), 3.39–3.35 (t, 2H), 2.60–2.58 (d, 1H), 2.20 (s, 6H), 1.79–1.74 (t, 2H), 1.25–1.22 (t, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.77, 150.13, 146.75, 145.80, 139.34, 130.65, 124.44, 123.60, 117.12, 114.01, 104.11, 70.72, 68.23, 62.01, 61.53, 49.95, 43.37, 28.76, 19.59, 14.26; MS: *m/z*: 547 (M^+); Anal. Calcd. for $C_{30}H_{33}N_3O_7$: C, 65.80; H, 6.07; N, 7.67; Found: C, 65.85; H, 6.11; N, 7.71.

*2.2.2.5. Diethyl 4-((3aR,4R,9bR)-4-(4-chlorophenyl)-2,3,3a,4,5,9b-hexahydrofuro[3,2-c]quinolin-7-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate **(9c)***. FT- IR (KBr) V_{max} cm^{-1}: 3360, 2921, 1686, 1251, 1176, 686; ^1H NMR ($CDCl_3$, 400 MHz) δ: 9.75 (brs, 1H, NH), 7.42–7.32 (m, 4H), 7.26 (s, 1H), 7.08 (d, 1H, J = 12 MHz), 6.48–6.46 (d, 1H, J = 8 MHz), 5.66 (brs, 1H, NH), 5.20 (s, 1H), 4.90 (s, 1H), 4.53–4.52 (d, 1H, *J* = 4.00 Hz), 4.12–4.03 (q, 4H), 3.79–3.75 (t, 2H), 2.37–2.31 (m, 1H), 2.17 (s, 6H), 2.02–1.93 (m, 2H), 1.26–1.23 (t, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.80, 143.66, 143.36, 141.91,138.15, 130.60, 129.15, 128.59, 128.30, 128.06, 119.32, 114.00, 104.51, 65.12, 59.73, 57.81, 43.37, 38.75, 30.81, 28.50, 19.50, 14.10; MS: *m/z*: 536 (M^+); Anal. Calcd. for $C_{30}H_{33}ClN_2O_5$: C, 67.09; H, 6.19; N, 5.22; Found: C, 67.15; H, 6.16; N, 5.20.

*2.2.2.6. Diethyl 4-((3aR,4S,9bR)-4-(4-chlorophenyl)-2,3,3a,4,5,9b-hexahydrofuro[3,2-c]quinolin −7-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate **(10c)***. FT- IR (KBr) V_{max} cm^{-1}: 3346, 2985, 1645, 1238, 1121, 682; ^1H NMR ($CDCl_3$, 400 MHz) δ: 9.65 (brs, 1H, NH), 7.45–7.22 (m, 5H), 7.02 (d, 1H, J = 4 MHz), 6.46 (d, 1H, J = 8 MHz), 5.65 (brs, 1H, NH), 5.21–5.18 (d, 1H, *J* = 12.00 Hz), 4.88 (s, 1H), 4.62 (s, 1H), 4.11–4.01 (q, 4H), 3.89–3.85 (t, 2H), 2.37–2.27 (m, 1H), 2.14 (s, 6H), 1.91–1.84 (m, 2H), 1.28–1.19 (t, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.86, 143.55, 142.89, 140.95, 139.30, 133.15, 129.54, 128.86, 128.20, 127.73, 121.67, 114.64, 104.45, 76.05, 66.49, 59.56, 45.73, 38.82, 24.37, 19.57, 14.24; MS: *m/z*: 536 (M^+); Anal. Calcd. for $C_{30}H_{33}ClN_2O_5$: C, 67.09; H, 6.19; N, 5.22; Found: C, 67.15; H, 6.26; N, 5.20.

2.2.3. General procedure for synthesis of tetrahydrocyclopentaquinolinyl dihydropyridine dicarboxylate

Freshly cracked 1,3 cyclopentadiene was prepared by the procedure reported in literature [24]. Amine-diethyl 4-(4-aminophenyl)-2, 6-dimethyl-1, 4-dihydropyridine-3, 5-dicarboxylate (2) (0.01 mol), benzaldehyde (6a) (0.01 mol) and cyclopentadiene (0.01 mol) (11) was taken in the RB flask, in the presence of indium triflate (20.0 mol%) as a catalyst and acetonitrile as solvent and stirred at 25.0–27.0 °C. The reaction was monitored by TLC. After completion of the reaction, the solvent was removed by under vaccum. Ehyl acetate [20 ml] and water [20 ml] was added to the reaction mixture. The organic layer was separated and concentrated by high vaccum. The product was purified by column chromatography on silica gel (100–200 mesh, ethyl acetate/petroleum ether, 1:4) to give the product diethyl-2,6-dimethyl-4-((3aS,9bR)-4-phenyl-3a,4,5,9b-tetrahydro-3H-cyclopenta[*c*]quinolin-7-yl)-1,4-dihydropyridine-3,5-dicarboxylate (12a) in 51.0% yield.

*2.2.3.1. Diethyl-2,6-dimethyl-4-((3aS,4R,9bR)-4-phenyl-3a,4,5,9b-tetrahydro-3H-cyclopenta [c]quinolin-7-yl)-1,4-dihydropyridine-3,5-dicarboxylate **(12a)***. FT- IR (KBr) V_{max} cm^{-1}: 3343, 2989, 1675, 1472, 1225, 1070; ^1H NMR ($CDCl_3$, 400 MHz) δ: 9.61 (brs, 1H, NH), 8.10 (d, 1H, J = 8 MHz), 7.48–7.33 (m, 5H), 6.60 (d, 1H, J = 8 MHz), 6.49–6.42 (m, 1H), 5.65–5.61 (d, 2H), 5.26 (brs, 1H, NH), 4.90–4.87 (d, 1H), 4.10–4.05 (q, 4H), 3.69–3.66 (d, 1H), 3.09–3.05 (d, 1H), 2.60–2.57 (m, 1H), 2.48–2.45 (d, 2H), 2.26 (s, 6H), 1.29–1.25 (t, 6H);^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.78, 154.84, 145.58, 143.94, 138.58, 133.23, 129.91, 128.65, 127.29, 125.62, 115.69, 112.39, 104.40, 61.40, 59.48, 58.27, 45.87, 42.97, 31.58, 19.41, 14.05 ; MS: *m/z*: 498 (M^+); Anal. Calcd. for $C_{31}H_{34}N_2O_4$: C, 74.67; H, 6.87; N, 5.62; Found: C, 74.63; H, 6.81; N, 5.55.

*2.2.3.2. Diethyl 2,6-dimethyl-4-((3aS,9bR)-4-(4-nitrophenyl)-3a,4,5,9b-tetrahydro-3H-cyclopenta [c]quinolin-7-yl)-1,4-dihydropyridine-3,5-dicarboxylate **(12b)***. FT- IR (KBr) V_{max} cm^{-1}: 3351, 2998, 1677, 1491, 1260, 1012; ^1H NMR ($CDCl_3$, 400 MHz) δ: 9.32 (brs, 1H, NH), 8.26–8.21 (m, 2H), 7.52–7.48 (m, 2H), 6.98 (m, 1H), 6.60–6.58 (d, 1H, J = 8 MHz), 6.41 (s, 1H), 5.66–5.65 (m, 2H), 5.29 (brs, 1H, NH), 4.80–4.78 ((d, 1H), 4.26 (q, 4H), 3.88 (d, 1H), 3.54 (d, 1H), 2.89–2.87 (m, 1H), 2.34–2.31 (d, 2H), 2.22 (s, 6H), 1.34–1.29 (t, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 169.92, 155.06, 146.00, 143.63, 138.82, 133.85, 130.31, 129.55, 128.57, 127.82, 125.13, 115.43, 112.29, 104.05, 61.33, 59.87, 57.92, 46.02, 42.72, 30.86, 19.29, 14.13 ; MS: *m/z*: 543 (M^+); Anal. Calcd. for $C_{31}H_{33}N_3O_6$: C, 68.49; H, 6.12; N, 7.73; Found: C, 68.51; H, 6.11; N, 7.71.

*2.2.3.3. Diethyl 4-((3aS,9bR)-4-(4-chlorophenyl)-3a,4,5,9b-tetrahydro-3H-cyclopenta [c] quinolin-7-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate **(12c)***. FT- IR (KBr) V_{max} cm^{-1}: 3382, 2956, 1684, 1448, 1277, 1067; ^1H NMR ($CDCl_3$, 400 MHz) δ: 9.53 (brs, 1H, NH), 8.01–7.83 (m, 5H), 7.38–7.31 (m, 1H), 7.01 (m, 1H), 5.71–5.67 (d, 2H), 5.19 (brs, 1H, NH), 4.91–4.89 (d, 1H), 4.16–4.07 (q, 4H), 3.71–3.68 (d, 1H), 3.42–3.38 (d, 1H), 2.97–2.86 (m, 1H), 2.57–2.52 (d, 2H), 2.31 (s, 6H), 1.28–1.22 (t, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.38, 155.10, 145.96, 143.76, 138.90, 133.44, 130.11, 128.43, 127.00, 125.06, 115.34, 112.68, 103.99, 61.72, 58.49, 58.16, 45.94, 43.09, 29.66, 19.53, 14.30 ; MS: *m/z*: 532 (M^+); Anal. Calcd. for $C_{31}H_{33}ClN_2O_4$: C, 69.85; H, 6.24; N, 5.26; Found: C, 69.82; H, 6.26; N, 5.25.

*2.2.3.4. Diethyl 2,6-dimethyl-4-((3aS,9bR)-4-(p-tolyl)-3a,4,5,9b-tetrahydro-3H-cyclopenta [c] quinolin-7-yl)-1,4-dihydropyridine-3,5-dicarboxylate **(12d)***. FT- IR (KBr) V_{max} cm^{-1}: 3385, 2966, 1689, 1438, 1268, 1069; ^1H NMR ($CDCl_3$, 400 MHz) δ: 9.60 (brs, 1H, NH), 8.11–8.09 (d, 2H, J = 8 MHz), 7.48–7.33 (m, 3H), 6.61–6.42 (m, 2H), 5.76 (brs, 1H, NH), 5.66–5.60 (m, 2H), 4.91–4.88 (d, 1H), 4.12–4.05 (q, 4H), 3.68–3.65 (d, 1H), 3.10 (d, 1H), (2.59–2.57 (d, 1H), 2.39 (s, 3H), 2.33–2.30 (m, 2H), 2.17 (s, 6H), 1.29–1.23 (t, 6H);^{13}C NMR (100 MHz, $CDCl_3$) δ: 168.31, 155.10, 146.00, 143.07, 138.90, 129.00, 128.43, 127.13, 125.78, 114.52, 112.39, 104.43, 65.25, 59.69, 58.16, 46.53, 43.13, 30.94, 22.62, 19.62, 14.39 ; MS: *m/z*: 512 (M^+); Anal. Calcd. for $C_{32}H_{36}N_2O_4$: C, 74.97; H, 7.08; N, 5.46; Found: C, 74.87; H, 7.06; N, 5.51.

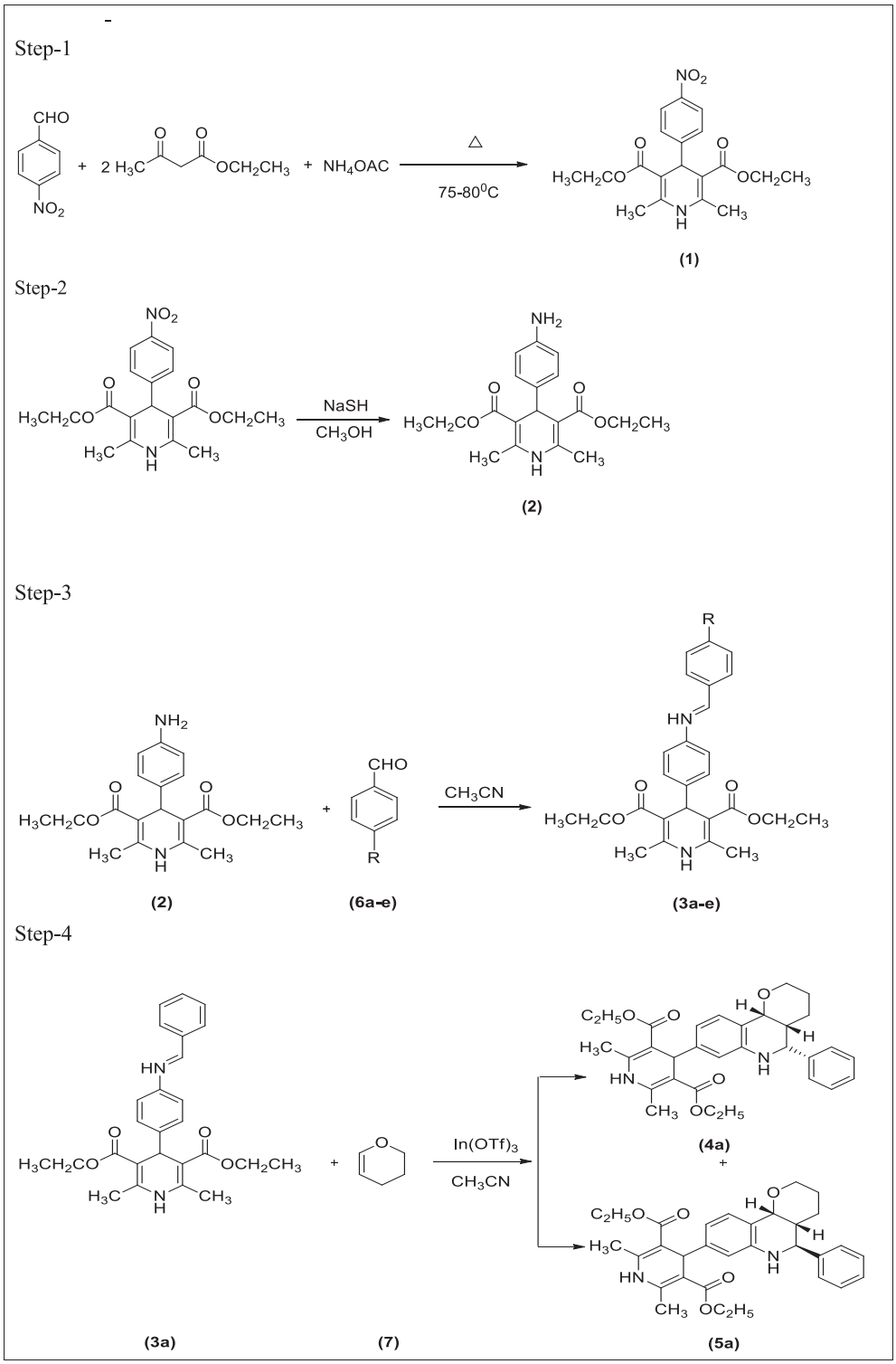
2.2.3.5. Diethyl 4-((3aS,9bR)-4-(4-hydroxyphenyl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c] quinolin-7-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-

dicarboxylate ***(12e)***. FT- IR (KBr) V_{max} cm^{-1}: 3372, 2950, 1679, 1467, 1279, 1072; ^1H NMR ($CDCl_3$, 400 MHz) δ: 9.45 (brs, 1H, NH), 7.48–7.28 (m, 3H), 6.60–6.58 (d, 2H, J = 8.0 MHz), 6.49–6.42 (m, 2H), 5.89 (s, 1H), 5.76 (brs, 1H, NH), 5.65–5.61 (d, 2H), 4.90–4.87 (d, 1H), 4.14–4.04 (q, 4H), 3.67 (d, 1H), 3.10 (d,1H), 2.59–2.57 (d, 1H), 2.40–2.29 (m, 2H), 2.17 (s, 6H), 1.31–1.26 (t, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 167.92, 155.04, 146.63, 143.94, 129.55, 128.65, 127.90, 126.04, 115.39, 114.52, 103.95, 59.69, 58.49, 47.00, 46.11, 38.70, 31.51, 19.57, 13.76 ; MS: *m/z*: 514 (M^+); Anal. Calcd. for $C_{31}H_{34}N_2O_5$: C, 72.35; H, 6.66; N, 5.44; Found: C, 72.37; H, 6.76; N, 5.39.

2.3. Biological assay

2.3.1. In vitro antibacterial assay

All synthesized compounds were evaluated for their *in vitro* antibacterial activity against pathogenic bacteria by well diffusion method [25,26]. The tested compounds were dissolved in acetic acid and DMSO solvent mixture. Test pathogens were spread on Mueller-Hinton agar



Scheme 1.

(MHA) plates. A well of diameter (6.0 mm) was made by using sterile cork borer and loaded with required concentration. The test plates were incubated for 24 h at 37 °C. Antimicrobial activity was evaluated by recording the zone of inhibition in mm against test microorganism and solvent. Acetic acid and DMSO solvent mixture was used as control. Ciprofloxacin was used as reference drug. The tests were carried out in triplicates.

2.3.2. Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of all the products were evaluated by using the broth micro dilution method using 96-well microplates reported in literature [27–29]. The test compounds in different concentrations of 500, 250, 125.25, 62.50, 31.25, 15.63, 7.81 μg/ml along with standardized microbial suspensions (1-2x10^{7}cfu/ml) were incubated for 18-24hrs at 37 °C. Ciprofloxacin was used as standard reference drugs. The lowest concentration of the test compounds that inhibited the bacterial growth completely was recorded as MIC in μg/mL. All experiments were performed in triplicate.

2.3.3. Molecular docking methodology.

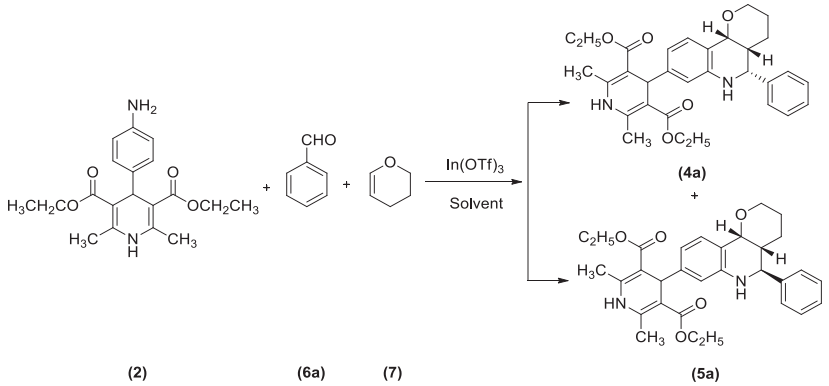
The first step in molecular docking studies is the preparation of Pyrano[3,2-*c*]quinolin-8-yl)-1,4-dihydro pyridine-3,5-dicarboxylates derivatives for docking with cell wall protein of vibrio cholera which includes structure optimization of the Pyrano[3,2-*c*]quinolin-8-yl)-1,4-dihydro pyridine-3,5-dicarboxylates derivatives. 3D conformer structures of the ligands were obtained using Chemsketch software after structure optimization and adding hydrogen bonds. They were converted to .mol2 format using BIOVIA Discovery Studio visualizer. Hydrogen bonds were added to all the ligands. The 3d crystal structure of vibrio cholerea was obtained from RSCB protein data bank (PDB code 5CXK). The water molecules were removed from the protein and hydrogen atoms were added to the protein by using discovery studio 2.5.5software The active sites of the protein were determined. The ligand binding site was defined as the binding site. The co-crystallized ligand and water molecules were deleted. Hydrogen bonds were added and the protein was saved in pdb format. CCDC GOLD was employed for calculating the docking modes of Pyrano[3,2-*c*]quinolin-8-yl)-1,4-dihydro pyridine-3,5-dicarboxylates derivatives with protein binding sites. CCDC GOLD was used for molecular docking. 20 Ã… units were set as the interaction diameter to allow better analysis of interactions with multiple chains and to allow free rotation of the ligands in the interaction sphere. In the analysis, the interactions of ligands were assessed according to the bonding interactions against active site of the protein. The Pyrano[3,2-*c*]quinolin-8-yl)-1,4-dihydro pyridine-3,5-dicarboxylates conformer and coordinates based on best fitness value for ligand was introduced into the protein using Discovery Studio Visualizer. The protein–ligand interactions were analyzed and the bonding type was noted. The fitness values of the screened drugs were noted.

3. Results and discussion

3.1. Chemistry

In the present investigation, we report the multistep synthesis of pyrano[3,2-c] quinolin-8-yl)-1,4-dihydropyridine-3,5-dicarboxylate derivatives. In the first step (scheme 1, step 1), nitro substituted Hantzsch dihydropyridine carboxylates were synthesized by condensation reaction of ethylacetoacetate, nitro benzaldehyde and ammonium acetate. The second step involves the reduction of the corresponding nitro group to amino group by sodium hydrosulfide in methanol (scheme 1, step 2). The schiffs base is generated *insitu* by reacting the amine; diethyl 4-(4-aminophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate with various substituted aromatic aldehydes (scheme 1, step 3). The last step (scheme 1, step 4) between *insitu* generated schiffs base with 3,4-dihydro-2H-pyran in acetonitrile solvent promoted by indium triflate

Table 1
Effect of temperature and solvent on synthesis pyrano[3,2-*c*]quinolin-8-yl)-1,4-dihydropyridine-3,5-dicarboxylates **(4a & 5a)** synthesis.



S. No	Solvent	Temperature (°C)	Time (h)	Yield (%)
1	Benzene	25–27	10.0	No reaction
2	Toluene	100–110	10.0	No reaction
3	Dichloromethane	25–27	10.0	No reaction
4	Ethyl acetate	60–70	8.0	10.0
5	Methanol	25–27	8.0	28.0
6	Ethanol	25–27	8.0	30.0
7	Ethanol	78–80	8.0	40.0
8	Acetonitrile	25–27	6.0	71.0
9	Acetonitrile	80–82	6.0	58.0

4a - Diethyl 2,6-dimethyl-4-((4aR,5R,10bR)-5-phenyl-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-c]quinolin-8-yl)-1,4-dihydropyridine-3,5-dicarboxylate
5a - Diethyl 2,6-dimethyl-4-((4aR,5S,10bR)-5-phenyl-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-c]quinolin-8-yl)-1,4-dihydropyridine-3,5-dicarboxylate
[a]All the reactions were carried out according to typical experimental procedure A.
[b]All the products were characterized by ^{1}HNMR, FT-IR & mass spectra.
[c]Isolated yield after purification.

as catalyst proceeds via inverse electron demand Diels Alder reaction (IED) at ambient temperature to afford the corresponding Pyrano[3,2-*c*] quinolin-8-yl)-1,4-dihydropyridine-3,5-dicarboxylate derivatives (4a-e, 5a-e) in moderate to good yields. The reaction is illustrated in scheme 1.

3.2. *Effect of temperature and solvent on Pyrano[3,2-c]quinolin-8-yl)-1,4-dihydro pyridine-3,5 dicarboxylates synthesis*

The reaction of diethyl-4-(4-aminophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (2) with benzaldehyde (6a) and 3,4-dihydro-2H-pyran (7) in the presence of indium triflate catalyst was chosen as a model reaction for studying the effect of temperature and solvent on pyrano[3,2-*c*]quinolin-8-yl)-1,4-dihydropyridine-3,5-dicarboxylates synthesis; the results are shown in Table 1. We have observed that in the less-polar solvents such as benzene, toluene etc the reaction did not progress at room temperature or elevated temperatures. The reaction afforded poor yields in solvents such as ethyl acetate, methanol and ethanol. However optimal yields and short reaction times were observed in acetonitrile solvent in the presence of indium triflate (20.0 mol%) as catalyst at room temperature. The results of the reaction are illustrated in table.1.

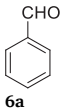
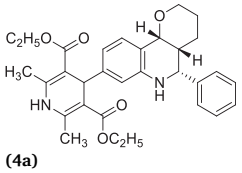
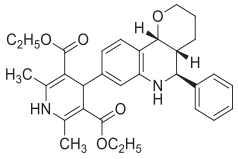
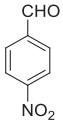
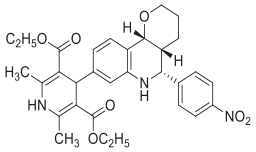
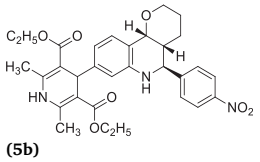
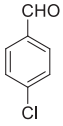
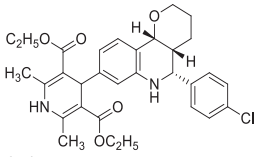
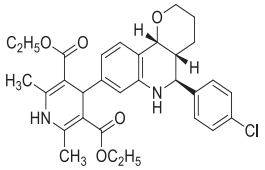
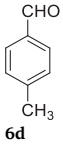
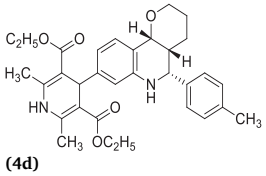
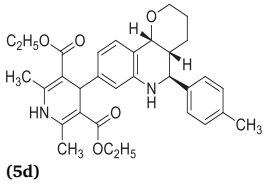
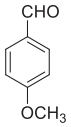
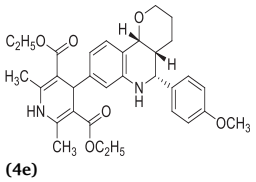
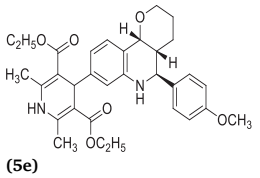
3.3. *The effect of substituents*

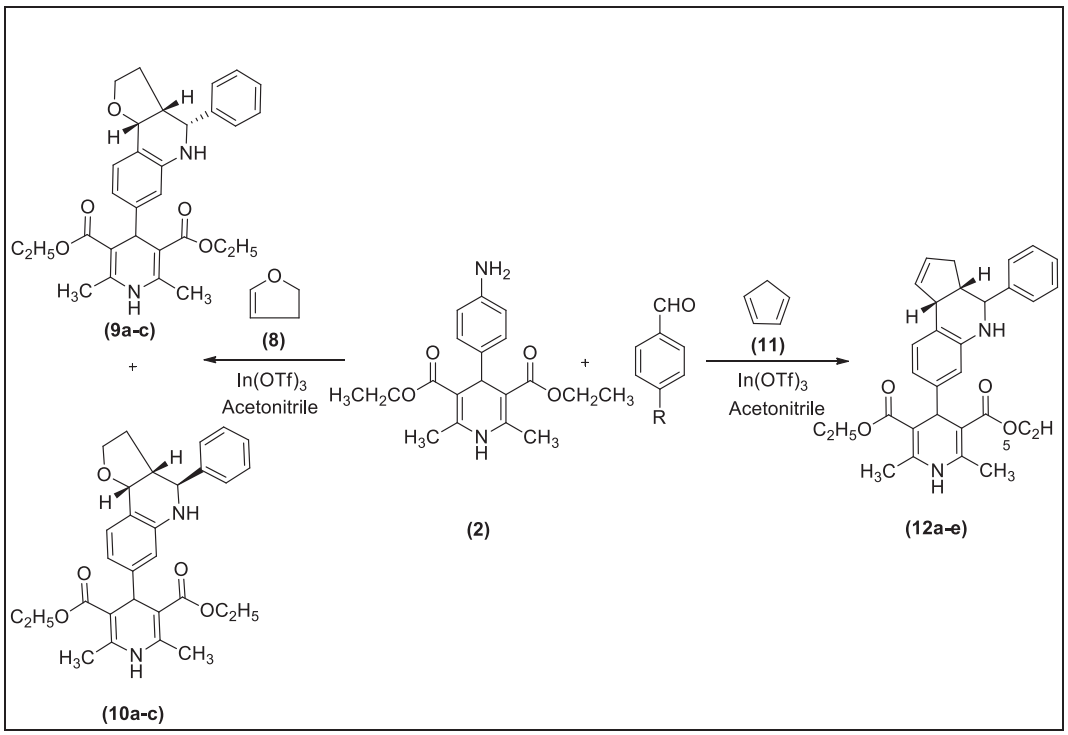
The reactions conditions were further extended for the synthesis of a series of pyrano[3,2-*c*]quinolin-8-yl)-1,4-dihydropyridine-3,5-dicarboxylates by reacting various other aromatic aldehydes with diethyl 4-(4-aminophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (2) and 3,4-dihydro-2H-pyran in the presence of indium triflate (20.0 mol%) as catalyst in acetonitrile as solvent at room temperature. The reaction proceeds smoothly with both electron donating and electron withdrawing substituents on the aromatic ring of the aldehydes affording the corresponding PDC derivatives in good to moderate yields. The results of the reaction are summarized in table 2 given below.

3.4. *Synthesis of furo[3,2-c]quinolin-7-yl)-1,4-dihydropyridine-3,5-dicarboxylate(FDC) and tetrahydro-3H-cyclopenta[c]qunolin-7-yl-1,4-dihydropyridine-3,5-dicarboxylate(CDC) derivatives*

After synthesizing a series of pyranoquinolinyl dihydropyridine carboxylate derivatives we focused our attention on extending the methodology for the synthesis of a series of furo[3, 2-*c*]quinolin-7-yl)-1,4-dihydropyridine-3,5-dicarboxylate and cyclopentaquinoline-1,4-dihydro pyridine-3,5-dicarboxylate derivatives. Furo[3,2-*c*]quinolin-7-yl)-1,4-dihydropyridine-3,5-dicarboxylates (9a-c & 10a-c) were formed in moderate yields when aromatic aldehydes (6a-c) were reacted with diethyl-4-(4-aminophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (2) and 2,3-dihydro-furan (8) in the presence of indium triflate (20.0 mol%) as catalyst in acetonitrile at room temperature to afford the corresponding furo[3,2-*c*]quinolin-7-yl)-1,4-dihydropyridine-3,5-dicarboxylates (9a-c & 10a-c). Cyclopentaquinoline dihydropyridine carboxylate derivatives (CDC) were formed in moderate yields when aromatic aldehydes (6a-e) were reacted with diethyl 4-(4-aminophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (2) and cyclopenta-1,3-diene (11) in acetonitrile as solvent and indium triflate (20.0 mol%) as catalyst at room temperature to afford the corresponding

Table 2
Effect of substituents on Pyrano[3,2-*c*]quinolin-8-yl)-1,4-dihydro pyridine-3,5-dicarboxylates synthesis.

S.No	Aldehyde	Time[a] (h/min)	Yield[b](%)	Endo:Exo ratio	Pyrano[3,2-*c*]quinolin-8-yl)-1,4-dihydropyridine-3,5-dicarboxylates	
1	 **6a**	6.0	71.0	48:52	 **(4a)**	 **(5a)**
2	 **6b**	13.5	65.0	42:58	 **(4b)**	 **(5b)**
3	 **6c**	12.0	67.0	37:63	 **(4c)**	 **(5c)**
4	 **6d**	11.0	68.0	44:56	 **(4d)**	 **(5d)**
5	 **6e**	10.0	70.0	35:65	 **(4e)**	 **(5e)**



Scheme 2.

CDC derivatives (12a-e) in good yields (scheme 2). Table 3 illustrates the scope of the reaction with respect to various substituted aldehydes and dienophiles.

3.5. Biological evaluation

3.5.1. Antibacterial activity

The newly synthesized pyranoquinolinyl dihydropyridine carboxylate (PDC), furanoquinolinyl dihydropyridine carboxylate (FDC) and cyclopentaquinolinyl dihydropyridine carboxylate (CPDC) derivatives were screened for antibacterial activity against seven strains of pathogenic bacteria shown in the table 4. The antibacterial activities of the quinolinyl dihydropyridine carboxylate derivatives were evaluated by employing agar well diffusion method. Among all the synthesized compounds screened for *invitro* antibacterial activity, the pyranoquinolinyl dihydropyridine carboxylate derivatives exhibited highest antibacterial activity in comparison to furanoquinolinyl dihydropyridine carboxylate derivatives or cyclopentaquinolinyl dihydropyridine carboxylate derivatives. Among the diastereomeric endo PDC derivatives (4a-e) and exo PDC derivatives (5a-e), it was found that the exo pyranoquinolinyl dihydropyridine carboxylate derivatives (5a-e) exhibited relatively superior antibacterial activity in comparison to the relatively less active endo pyranoquinolinyl dihydropyridine carboxylate derivatives (4a-e). However when the oxygen atoms on the quinolinyl dihydropyridine carboxylate derivatives were replaced by carbon atom as in cyclopentaquinolinyl dihydropyridine carboxylate derivatives (12a-e), diminished antibacterial activity was observed. Hence it is evident that the presence of oxygen heteroatom on the pyrano/furano quinolinyl dihydropyridine carboxylates is essential for binding with the cell wall protein of the pathogenic bacteria. Moreover the results of antibacterial studies is also supported by molecular docking studies which show that the oxygen atom on the both exo and endo PDC derivatives form strong hydrogen bonds with the amino acid residue CYS154:SG, CYS154:GSG and LEU155:N of vibrio cholerae which leads to better anchorage for the PDC derivatives leading to comparatively better activity of the PDC derivatives in comparison to CDC derivatives. However a reversal in activity was observed with the methoxy substituted PDC derivative wherein diethyl 4-((4aR,5R,10bR)-5-(4-methoxyphenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-*c*]quinolin-8-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (4e) exhibited relatively superior activity to diethyl 4-((4aR,5S,10bR)-5-(4-methoxyphenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-*c*]quinolin-8-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (5e). However all the compounds reported herein including PDC, FDC & CDC derivatives exhibited antibacterial activity against vibrio cholerae (zone of inhibition 8.0 mm-42 mm) at various concentrations in comparison to the reference drug ciprofloxacin. Hence these new class of compounds are promising candidates for treatment and mitigation of cholera after necessary pharmacological studies.

3.5.2. Mechanism of antibacterial action

The mode of action of the PDC derivatives diethyl 4-((4aR,5R,10bR)-5-(4-chlorophenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-*c*]quinolin-8-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (4c) & diethyl 4-((4aR,5S,10bR)-5-(4-chlorophenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-*c*]quinolin-8-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (5c) on pseudomonas aeruginosa and streptococcus mutans was studied by analyzing the changes in the cell morphology of pathogenic bacteria via scanning electron microscope analysis by following the procedure reported in literature [30,31]. Untreated pseudomonas aeruginosa and streptococcus mutans were used as control for studying the effect of these compounds on bacterial morphology. Pseudomonas aeruginosa exhibited rod shape (SEM image 1) before treatment with PDC derivatives whereas untreated streptococcus mutans exhibited round (spherical) shape (SEM image 4) as shown in the Fig. 1 below. The PDC derivatives (0.2 mM) were added to 2.0 ml of the standardized inoculums, incubated at 37 °C for 18.0 h. The bacterial cells were fixed with 2% glutaraldehyde in NA-cacodylate buffer. The

Table 3
Synthesis of furo [3, 2-c] quinolin-7-yl)-1, 4-dihydropyridine-3, 5-dicarboxylates and Tetrahydro-3H-cyclopenta[*c*]qunolin-7-yl-1,4-dihydropyridine-3,5-dicarboxylate.

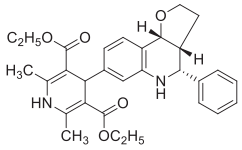
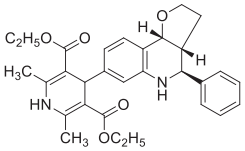
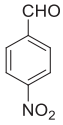
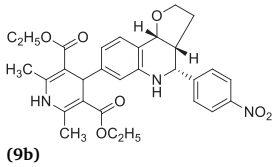
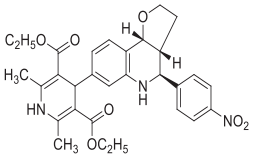
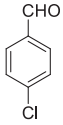
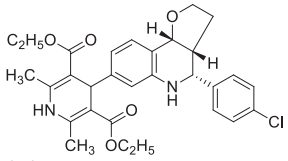
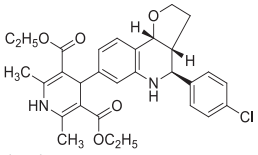
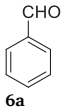
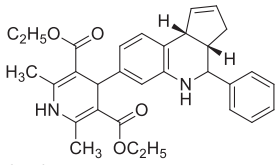
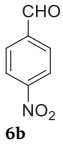
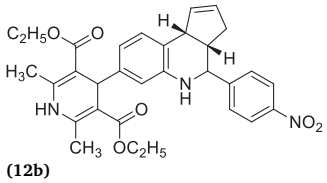
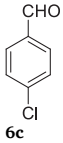
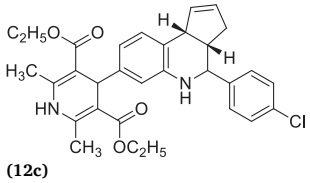
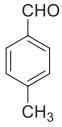
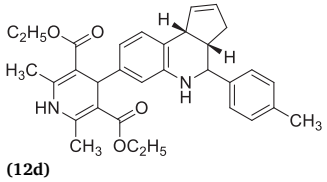
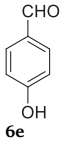
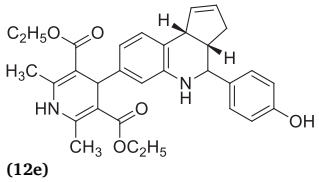
S.No	Aldehyde	Time[a] (h/min)	Yield[b](%)	Endo:Exo ratio	Furo[3,2-c]quinolin-7-yl)-1,4-dihydropyridine-3,5-dicarboxylate & Tetrahydro-3H-cyclopenta[c]qunolin-7-yl-1,4-dihydropyridine-3,5-dicarboxylate
1	 **6a**	6.5	69.0	47:53	 **(9a)**  **(10a)**
e2	 **6b**	14.0	59.0	38:62	 **(9b)**  **(10b)**
3	 **6c**	12.0	65.0	65:35	 **(9c)**  **(10c)**
4	 **6a**	17.0	51.0	–	 **(12a)**
5	 **6b**	20.0	45.0	–	 **(12b)**
6	 **6c**	21.0	47.0	–	 **(12c)**
7	 **6d**	19.0	52.0	–	 **(12d)**
8	 **6e**	20.0	54.0	–	 **(12e)**

Table 4
In vitro antibacterial activity studies of PDC, FDC and CDC derivatives.

Compounds	Positive Bacteria			Negative bacteria			
	B.substilis	S.aureus	S.mutans	E.coli	P.aeruginosa	K.pneumoniae	V.cholerae
4a	16	10	–	–	11	–	12
5a	12	12	–	–	10	11	16
4b	9	10	8	–	7	7	15
5b	25	14	35	18	19	21	37
4c	17	–	18	–	19	–	30
5c	30	24	46	40	38	34	42
4d	13	–	–	–	–	–	25
5d	12	–	–	–	12	–	11
4e	8	35	22	28	37	12	38
5e	10	8	8	–	–	7	16
9a	9	10	–	–	11	–	12
10a	12	10	–	–	10	11	18
9b	7	9	10	–	–	6	13
10b	15	10	14	8	12	–	12
9c	11	11	–	–	11	–	14
10c	12	10	11	–	13	–	20
12a	10	12	–	9	–	–	8
12c	11	–	–	–	10	–	14
Ciprofloxacin (Control)	30	35	45	42	36	30	36

*Diametre of zone of inhibition in mm

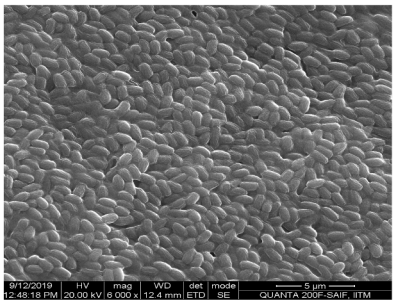
attached cells were fixed by immersion in 1% osmium tetroxide (OsO_4), dehydrated, viewed at 15 kV accelerating voltage in a scanning electron microscope and images were recorded at different intervals of incubation time to observe the changes in morphology of the bacterial cell. After 18.0 h of incubating the bacteria with PDC derivatives diethyl 4-((4aR,5R,10bR)-5-(4-chlorophenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-*c*]quinolin-8-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (4c) & diethyl 4-((4aR,5S,10bR)-5-(4-chlorophenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-*c*]quinolin-8-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (5c), the bacterial cell wall undergoes blubbing, budding and ruptures due to accumulation of cytoplasm leading cell death. The SEM inages (2, 3, 5 & 6) clearly show the cell wall rupture of pseudomonas aeruginosa and streptococcus mutans caused by PDC derivatives diethyl-4-((4aR,5R,10bR)-5-(4-chlorophenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-c] quinolin-8-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (4c) & diethyl 4-((4aR,5S, 10bR)-5-(4-chlorophenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-*c*]quinolin-8-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (5c) by blebbing, budding and cell wall lysis causing bacterial apoptosis due to cytoplasmic accumulation.

3.5.3. Minimum inhibitory concentration (MIC)

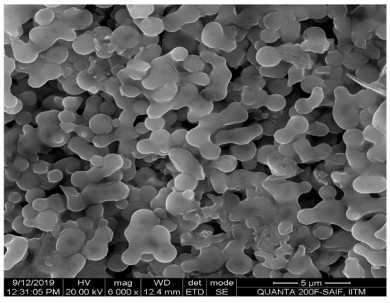
The minimum inhibitory concentration is the lowest concentration of the target compound that is required for inhibiting the visible growth of the microorganism under scrutiny. The MIC was determined by using the broth macro dilution method and the results are summarized in table 5 given below. Among the PDC derivatives screened for antibacterial activity, diethyl 4-((4aR,5S,10bR)-5-(4-chlorophenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-*c*]quinolin-8-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (5c) exhibited superior antibacterial activity against E.Coli, Pseudomonas aeruginosa, Klebsiella pneumonia and Vibrio cholerae in comparison to the reference drug Ciprofloxacin. Similarly diethyl 2,6-dimethyl-4-((4aR,5S,10bR)-5-(4-nitrophenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-*c*]quinolin-8-yl)-1,4-dihydropyridine-3,5-dicarboxylate (5b) exhibited potential antibacterial activity against Vibrio cholerae in comparison to the reference drug Ciprofloxacin with MIC value of 31.250 μg/mL. On comparing the antibacterial activity, MIC and zone of inhibition exhibited by both the exo and endo substituted PDC derivatives, it is evident from the table 5 that the exo substituted PDC derivatives exhibited relatively superior activity than the endo substituted PDC derivatives. However a reversal in activity was observed for diethyl 4-((4aR,5R,10bR)-5-(4-methoxyphenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-*c*]quinolin-8-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (4e) & diethyl 4-((4aR,5S,10bR)-5-(4-methoxyphenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-*c*]quinolin-8-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (5e) wherein diethyl 4-((4aR,5R,10bR)-5-(4-methoxyphenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-*c*]quinolin-8-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (4e) exhibited relatively better activity than diethyl 4-((4aR,5S,10bR)-5-(4-methoxyphenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-*c*]quinolin-8-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (5e) against Staphylococcus aureus and Vibrio cholerae in comparison to the reference drug ciprofloxacin. The MIC values for the various synthesized compounds against pathogenic strains of gram positive and gram negative bacteria is shown in table 5 given below

3.5.4. Molecular docking studies

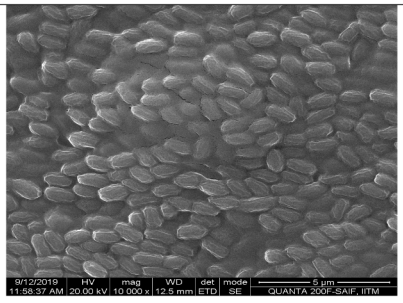
Inorder to understand the mode of binding between cell wall protein of vibrio cholerae and the exo & endo pyrano[3,2-*c*]quinolin-8-yl)-1,4-dihydropyridine-3,5-dicarboxylate derivatives, molecular docking studies were carried out by using Gold suite software [32]. The 3d crystal structure of vibrio cholerea was obtained from RSCB protein data bank (PDB code 5CXK) [33]. The water molecules were removed from the protein and hydrogen atoms were added to the protein by using discovery studio 2.5.5software [34].The energy minimized three dimensional structures of diethyl 4-((4aR,5R,10bR)-5-(4-chlorophenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-*c*]quinolin-8-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (4c) and diethyl 4-((4aR,5S,10bR)-5-(4-chlorophenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-*c*]quinolin-8-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (5c) was docked with the cell wall protein of the vibrio cholerae. The docking results showed that both the endo and exo diastereomers (4c & 5c) docked well within the binding pockets of vibrio cholerae. The major difference in the mode of binding of endo diasteromer diethyl 4-((4aR,5R,10bR)-5-(4-chlorophenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-*c*]quinolin-8-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (4c) and exo diastereomer diethyl 4-((4aR,5S,10bR)-5-(4-chlorophenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-*c*]quinolin-8-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-



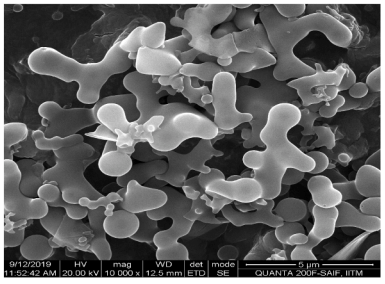
SEM image 1 of untreated pseudomonas aeruginosa



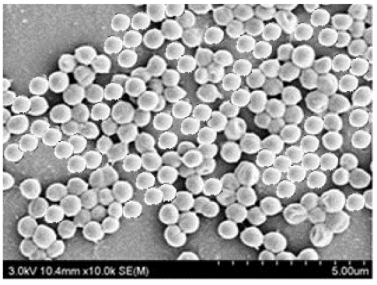
SEM image 2 of pseudomonas aeruginosa after treatment with endo PDC (4c)



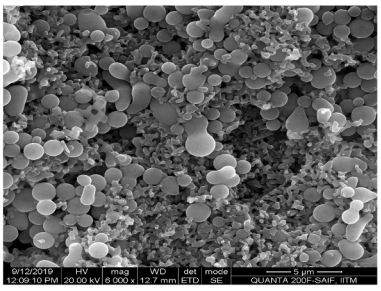
SEM image 1 of untreated pseudomonas aeruginosa



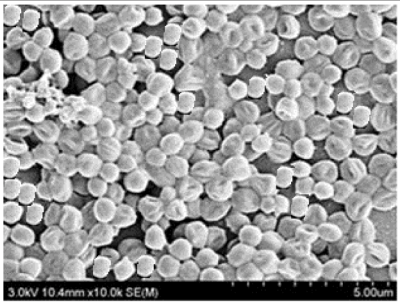
SEM image 3 of pseudomonas aeruginosa after treatment with exo PDC (5c)



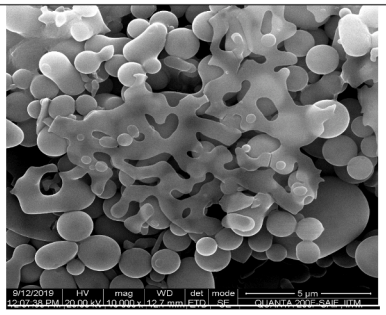
SEM image 4 of untreated Streptococcus Mutans



SEM image 5 of Streptococcus Mutans after treatment with endo PDC (4c)



SEM image 4 of untreated Streptococcus Mutans



SEM image 6 of Streptococcus Mutans after treatment with exo PDC (5c)

Fig. 1. SEM studies of the mode of action of PDC derivatives on pseudomonas aeruginosa and streptococcus mutans.

dicarboxylate (5c) is that the endo PDC diasteromer (4c) formed two hydrogen bonds with the target cell wall protein of V.cholera with a glide score of 12.68 whereas the exo PDC diastereomer (5c) formed four hydrogen bonds with the cell wall protein of V.cholera with a Goldsuite glide score of 19.74. The –NH group of endo diasteromer (4c) formed an hydrogen bond with GLU156 amino acid residue (bond length −2.746 A) while the oxygen atom present in the pyran ring of 4c formed another hydrogen bond with CYS154SG amino acid residue (bond length −2.368 A) where as one –NH group, one oxygen atom on pyran ring and the two ester carbonyl oxygen atoms of exo PDC diasteromer (5c) formed four hydrogen atom with the aminoacid residues GLU156 (bond length −2.746 A), CYS154 (bond length −2.746 A), LEU155 (bond length

Table 5
Minimum Inhibitory Concentration (MIC) evaluation of the PDC, FDC & CDC derivatives against different pathogenic strains of bacteria.

Compounds	Minimum inhibitory concentration (μg/mL) Bacterial strains						
	B.substilis	S.aureus	S.mutans	E.coli	P.aeruginosa	K.pneumoniae	V.cholerae
4a	125.0	500.0	N.D	N.D	500	N.D	250.0
5a	250.0	500.0	N.D	N.D	500	500	125.0
4b	N.D	250.0	500.0	N.D	N.D	N.D	125.0
5b	31.25	250.0	62.50	250.0	250.0	62.50	31.25
4c	125.0	N.D	125.25	N.D	62.50	N.D	62.50
5c	62.50	62.50	31.25	31.25	31.25	31.25	31.25
4d	250.0	N.D	N.D	N.D	N.D	N.D	62.50
5d	250.0	N.D	N.D	N.D	125.0	N.D	125.0
4e	N.D	31.25	125.0	62.50	31.25	250.0	31.25
5e	500.0	500.0	500.0	N.D	N.D	500.0	250.0
9a	N.D	500.0	N.D	N.D	500.0	N.D	250.0
10a	500.0	500.0	N.D	N.D	N.D	500.0	125.0
9b	N.D	N.D	N.D	N.D	N.D	N.D	250.0
10b	500.0	500.0	500.0	N.D	250.0	N.D	250.0
9c	250.0	500.0	N.D	N.D	250.0	N.D	250.0
10c	250.0	N.D	500.0	N.D	125.0	N.D	125.0
12a	500.0	250.0	N.D	500	500	N.D	500.0
12c	500.0	N.D	N.D	N.D	125.0	250.0	125.0
Ciprofloxacin (Control)	62.50	31.25	31.25	31.25	31.25	62.50	31.25

ND: Not Determined

−2.746 A) and LYS184 (bond length −2.746 A). The formation of four hydrogen bonds with the cell wall protein of Vibrio cholerae by exo PDC diasteromer (5c) results in relatively better anchorage for the diethyl 4-((4aR,5S,10bR)-5-(4-chlorophenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-*c*]quinolin-8-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (5c) and hence relatively better antibacterial activity in comparison the less active diethyl-4-((4aR,5R,10bR)-5-(4-chlorophenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-c] quinolin-8-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (4c) (Fig. 2).

3.5.5. Cytotoxicity

Many of the organic lead compounds developed for potential application as drugs fail because of toxicity, poor stability, difficult to scale up synthetic procedures etc. Hence in an effort to find a potential lead compound we further evaluated the pyrano[3,2-*c*]quinolin-8-yl)-1,4-dihydropyridine-3,5-dicarboxylate derivatives for cytotoxicity. The pyrano[3,2-*c*]quinolin-8-yl)-1,4-dihydropyridine-3,5-dicarboxylate derivatives were evaluated for cytotoxocity by MTT colorimetric assay against against L929 mouse fibroblasts cells. The L929 fibroblasts cells were treated with different concentrations of compounds (4a, 5a, 4c, 5c, 4e, 5e, 9a & 10a) in ethanol with a final maximum concentration of 100 μM for test compounds. All the compounds evaluated for cytotoxicity exhibited minimal cytotoxicity (<10%) at concentration of 25.0 μM and moderate cytotoxicity at concentrations of 50.0–100.0 μM towards L929 mouse fibroblasts cells as shown in Table. 6.

4. Conclusion

In conclusion the present work reports the synthesis of new class of pyrano[3,2-*c*]quinolin-8-yl)-1,4-dihydropyridine-3,5-dicarboxylate derivatives which combines the antibacterial activity of quinolines along with the multidrug resistance modulating effect of dihydropyrimidines. The designed pyrano[3,2-*c*]quinolin-8-yl)-1,4-dihydropyridine-3,5-dicarboxylate derivatives were synthesized by multi-step synthesis involving Michael addition and inverse electro demand Alder reaction (IED) to produce pyranoquinolinyl dihydropyridine carboxylates in good yields. All the products were characterized by ^{1}H NMR, ^{13}C NMR, FT-IR, Mass spectral and CHN analysis. All the synthesized pyranoquinolinyl dihydropyridine carboxylate (PDC), furanoquinolinyl dihydropyridine carboxylate (FDC) and cyclopentaquinolinyl dihydropyridine carboxylate (CPDC) derivatives were screened for antibacterial activity against pathogenic strains of gram positive and gram negative bacteria. The antibacterial results revealed that pyranoquinolinyl dihydropyridine carboxylate (PDC) derivatives exhibited superior antibacterial activity in comparison to furanoquinolinyl dihydropyridine carboxylate (FDC) and cyclopentaquinolinyl dihydropyridine carboxylate (CDC) derivatives with ciprofloxacin as a reference drug. Among the PDC derivatives, exo pyranoquinolinyl dihydropyridine carboxylate diastereomers (5a-e) exhibited relatively superior activity in comparison to the relatively less active endo pyranoquinolinyl dihydropyridine carboxylate diastereomers (4a-e). The molecular docking results showed that the diethyl 4-((4aR,5S,10bR)-5-(4-chlorophenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-*c*]quinolin-8-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate derivatives against Vibrio cholera (5c)

formed four hydrogen bonds with the amino acid residues of the bacterial cell wall of *Vibrio cholerae* while diethyl 4-((4aR,5R,10bR)-5-(4-chlorophenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-*c*]quinolin-8-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (4c) formed only two hydrogen bonds with amino acid residues of cell wall of the target enzyme thereby leading to better anchorage and increased activity for diethyl 4-((4aR,5S,10bR)-5-(4-chlorophenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-*c*]quinolin-8-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate derivatives against Vibrio cholera (5c). However all the compounds reported herein including PDC, FDC & CDC derivatives exhibited antibacterial inhibitory activity against vibrio cholerae (zone of inhibition 8.0 mm-42 mm) at various concentrations in comparison to the reference drug ciprofloxacin, Hence these compounds are a promising class of lead molecules for treatment and mitigation of cholera after necessary pharmacological evaluations. Based on the SEM analysis it was found that the PDC derivatives cause bacterial apoptosis by blebbing, budding and cell wall lysis. Moreover, the cytotoxic assay of the PDC derivatives revealed minimal cytotoxicity towards L929 mouse fibroblasts cells at 25 μM concentration wherein the % Cell viability was found to be greater than 90.0% and moderate cytotoxicity at concentrations of at 50.0–100.0 μM. Hence in conclusion, this paper reports a new class of PDC derivatives with simple synthetic procedures, superior antibacterial activity including vibrio cholerae and

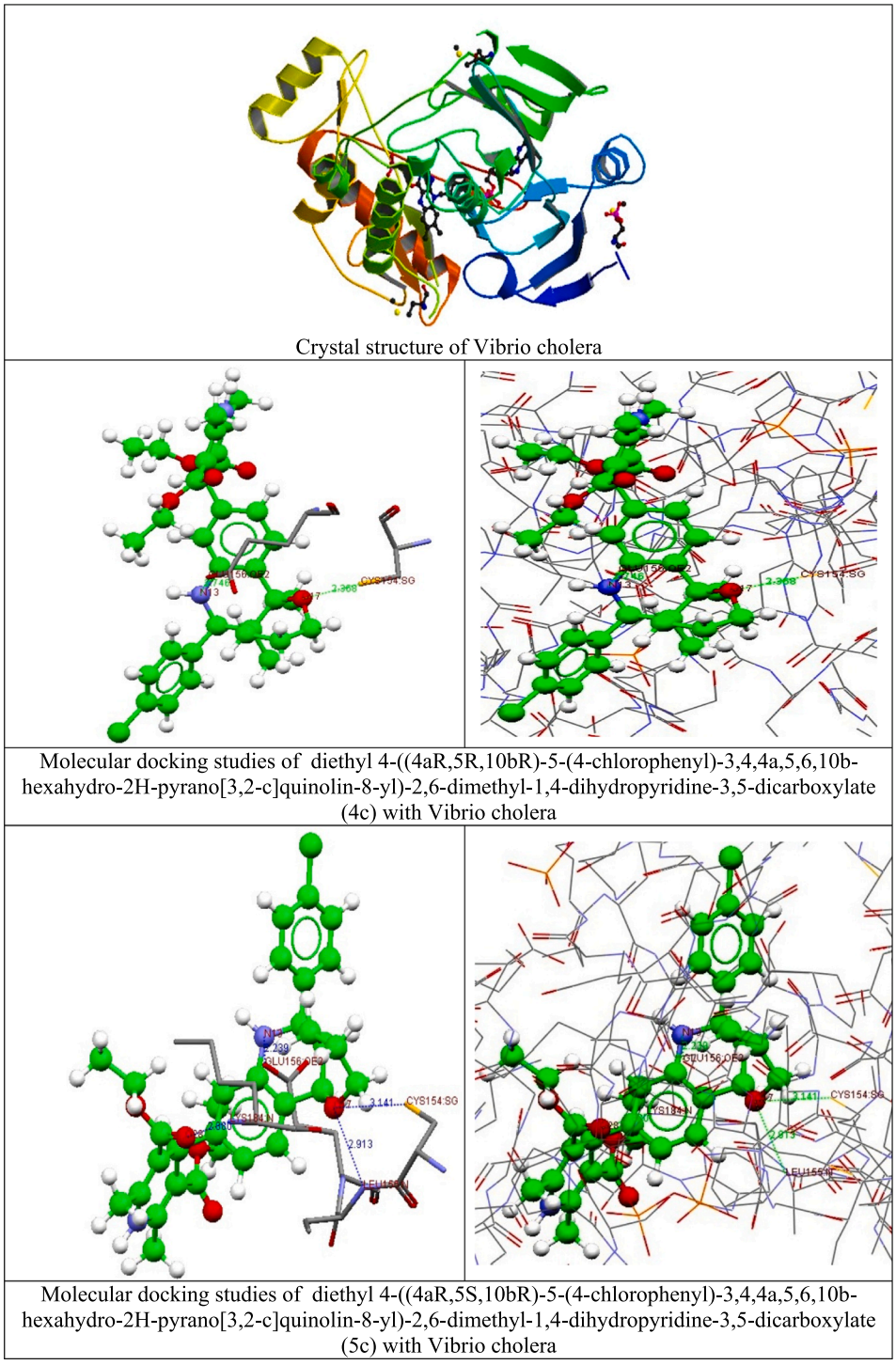


Fig. 2. Molecular docking of (diethyl 4-((4aR,5R,10bR)-5-(4-chlorophenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-*c*]quinolin-8-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (4c) & diethyl 4-((4aR,5S,10bR)-5-(4-chlorophenyl)-3,4,4a,5,6,10b-hexahydro-2H-pyrano[3,2-*c*]quinolin-8-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate derivatives against Vibrio cholera (5c).

Table 6
Cytotoxicity of PDC, FDC and CDC derivatives against L929 mouse fibroblasts cells.

S.No	Compound	Concentration (μM)	% of cell viability (triplicate values)		
1	4a	25	91.20 ± 0.12	92.08 ± 0.08	90.62 ± 0.27
		50	82.11 ± 0.13	86.51 ± 1.02	85.63 ± 0.08
		100	72.43 ± 0.46	70.38 ± 0.55	70.67 ± 0.12
2	5a	25	87.98 ± 0.30	91.50 ± 0.11	95.89 ± 0.30
		50	77.71 ± 0.27	79.47 ± 0.97	76.25 ± 0.47
		100	68.33 ± 0.59	65.98 ± 0.76	70.38 ± 0.37
3	4c	25	98.24 ± 0.08	99.71 ± 0.65	96.77 ± 0.56
		50	85.04 ± 0.06	88.27 ± 0.81	85.92 ± 0.21
		100	83.58 ± 0.76	79.18 ± 0.32	71.55 ± 0.34
4	5c	25	98.24 ± 0.20	98.53 ± 0.99	94.13 ± 0.24
		50	85.04 ± 0.27	86.51 ± 0.23	87.39 ± 0.36
		100	75.07 ± 0.58	73.61 ± 0.15	72.43 ± 0.25
5	4e	25	90.10 ± 1.01	91.81 ± 0.29	90.73 ± 0.16
		50	81.71 ± 2.31	85.82 ± 0.21	84.73 ± 0.14
		100	73.32 ± 0.48	72.01 ± 0.19	73.16 ± 0.32
6	5e	25	88.79 ± 0.52	92.05 ± 0.34	91.98 ± 0.45
		50	78.91 ± 0.32	80.71 ± 0.26	79.51 ± 0.61
		100	69.11 ± 0.51	68.18 ± 0.01	70.45 ± 0.32
7	9a	25	98.24 ± 0.32	99.71 ± 0.29	96.77 ± 0.21
		50	85.04 ± 0.43	88.27 ± 0.45	85.92 ± 0.11
		100	83.58 ± 0.38	79.18 ± 0.21	71.55 ± 0.21
8	10a	25	96.43 ± 0.68	97.31 ± 0.07	95.30 ± 0.45
		50	86.41 ± 0.71	87.19 ± 0.12	85.90 ± 0.32
		100	74.42 ± 0.68	75.11 ± 0.31	73.37 ± 0.41

moderate cytoyoxicity towards L929 mouse fibroblasts cells.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2020.104582.

References

[1] A. Hantzsch, Justus Liebigs, Uelm die Synthese pyridinartiger Verbin dungen %us Acotessigiither und Aldehydainmcniak, Ann. Chem. 215 (1882) 1–82.
[2] A.G. Atanasov, B. Waltenberger, E. Pferschy-Wenzig, T. Linder, C. Wawrosch, P. Uhrin, V. Temml, L. Wang, S. Schwaiger, E.H. Heiss, J.M. Rollinger, D. Schuster, J.M. Breuss, V. Bochkov, M.D. Mihovilovic, B. Kopp, R. Bauer, V.M. Dirsch, H. Stuppner, Discovery and resupply of pharmacologically active plant-derived natural products: A review. 33 (2015) 1582–1614.
[3] R.A. Khan, Natural products chemistry: The emerging trends and prospective goals, Saudi Pharm. J. 26 (2018) 739–753.
[4] WHO (1995).
[5] P. Garg, S. Sinha, R. Chakraborty, S.K. Bhattacharya, G.B. Nair, T. Ramamurthy, Y. Takeda, Emergence of fluoroquinolone resistant strains of vibrio cholerae O1 biotype El Tor among hospitalized patients with cholera in Calcutta, India. Antimicrob Agents Chemother. 45 (2001) 1605–1606.
[6] R.A. Maier, I.L. Pepper, Environmental Microbiology, 2nd edn., Elsevier Academic Press, Amsterdam and London, 2009.
[7] M.J. Finch, J.G. Jr, J. Morris, W. Kaviti, M.M.L. Kagwanja, Epidemiology of antimicrobial resistant cholera in Kenya and East Africa, Am J Trop Med Hyg. 39 (1988) 484–490.
[8] A. Ngandjio, M. Tejiokem, M. Wouafo, I. Ndome, M. Yonga, A. Guenole, Antimicrobial resistance and molecular characterization of *Vibrio cholerae* O1 during the 2004 and 2005 outbreak of cholera in Cameroon, Foodborne Pathog Dis. 6 (2009) 49–56.
[9] T. Godfraid, R. Miller, M. Wibo, Calcium antagonism and calcium entry blockade, Pharmacol Rev. 38 (1986) 321–416.
[10] A. Sausins, G. Duburs, Synthesis of 1,4-Dihydropyridines by Cyclocondensation Reactions, Heterocycles. 27 (1988) 269–289.
[11] P.P. Marger, R.A. Coburn, A.J. Solo, D.J. Triggle, H. Rothe, QSAR, diagnostic statistics and molecular modelling of 1,4-dihydropyridine calcium antagonists: a difficult road ahead, Drug Des Discov. 8 (1992) 273–289.
[12] R.A. Janis, D.J. Triggle, New developments in calcium ion channel antagonists, J Med Chem. 26 (1983) 775.
[13] R.H. Bocker, R.P. Guengerich, Oxidation of 4-aryl- and 4-alkyl-substituted 2,6-dimethyl-3,5-bis(alkoxycarbonyl)-1,4-dihydropyridines by human liver microsomes and immunochemical evidence for the involvement of a form of cytochrome P-450, J Med Chem. 29 (1986) 1596–1603.
[14] M.F. Gordeev, D.V. Patel, E.M. Gordon, Approaches to Combinatorial Synthesis of Heterocycles: A Solid-Phase Synthesis of 1,4-Dihydropyridines, J Org Chem. 61 (1996) 924–928.
[15] L. Capolongo, N. Amboldi, D. Ballinari, P. Cozzi, G. Melegaro, M. Ripamonti, F. Vaghi, M. Grandi, Reversal of multidrug resistance by new dihydropyridines with low calcium antagonist activity, Acta Oncol. 33 (1994) 787–791.
[16] J. Sharada, Y.R. Kumari, M. Kanakalingeswara Rao, Synthesis and biological activity of furoquinolines: 2-aroyl-4-methyl/4,6-dimethyl-3-phenyl-furo [3,2-c] quinolines, Indian Journal of Pharmaceutical Sciences 49 (1987) 17–21.
[17] K. Faber, H. Stueckler, T. Kappe, Non-steroidal antiinflammatory agents. 1. Synthesis of 4-hydroxy-2-oxo-1,2-dihydroquinolin-3-yl alkanoic acids by the wittig reaction of quinisatines, J Heterocycl Chem. 21 (1984) 1177–1184.
[18] I.N. Nesterova, L.M. Alekseeva, L.M. Andreeva, N.I. Andreeva, S.M. Golovina, V. G. Granic, Synthesis And Study The Pharmacological Activity Of Derivatives Of 5-Dimethylaminopyrano[3,2-C] Quinolin-2-Ones, Pharmaceutical Chemistry Journal. 29 (1995) 31.
[19] T. Kametani, H. Takeda, Y. Suzuki, T. Honda, Synthesis of quinoline derivatives by [4+ 2] cycloaddition reaction, Synth Commun. 15 (1985) 499–505.
[20] F. Hanawa, N. Fokialakis, A.L. Skaltsounis, Photo-activated DNA binding and antimicrobial activities of furoquinoline and pyranoquinolone alkaloids from rutaceae, Planta Med. 70 (2004) 531.
[21] G. Lavanya, K. Venkatapathy, C.J. Magesh, M. Ramanathan, R. Jayasudha, The first target specific, highly diastereoselective synthesis, design and characterization of pyranoquinolinyl acrylic acid diastereomers as potential α-glucosidase inhibitors, Bioorganic Chemistry. 84 (2019) 125–136.
[22] K. Venkatapathy, C.J. Magesh, G. Lavanya, P.T. Perumal, R. Sathishkumar, A nanocrystalline CdS thin film as a heterogeneous, recyclable catalyst for effective synthesis of dihydropyrimidinones and a new class of carbazolyl dihydropyrimidinones via an improved Biginelli protocol, New J. Chem. 43 (2019) 10989–11002.
[23] K. Venkatapathy, C.J. Magesh, G. Lavanya, P.T. Perumal, S. Prema, Design, synthesis, molecular docking and spectral studies of new class of Carbazolyl-polyhydroquinolines derivatives as promising antibacterial agents with non-cytotoxicity towards human mononuclear cells from Peripheral blood (HMNC-PB), J.heterocyclic chemistry. 57 (2020) 1936–1955.
[24] R.B. Moffett, "Cyclopentadiene and 3-Chlorocyclopentene". Organic Syntheses, Collective 4 (1962) 238.
[25] I.H.R.Tomi, A.H.R.Al-Daraji, R.R.T.,Al-Qaysi, M.M.Hasson, K.H.D.Al-Dulaimy, Synthesis, characterization and biological activities of some azo derivatives of aminothiadiazole derived from nicotinic and isonicotinic acids. Arab. J. Chem.. https://doi.org/10.1016/j.arabjc.2010.12.003.in press. (2010).

[26] B.V. Raman, A.S. Ramkishore, M.U. Maheswari, T. Radhakrisnan, Antibacterial activities of some folk medicinal plants of Eastern Ghats, J Pure Appl Microbiol. 3 (2009) 187–194.
[27] K. Wiegand, Hilpert, Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances, Nat. Protoc. 3 (2008) 163–175.
[28] R.K. Saini, A.S. Choudhary, Y.C. Joshi, P. Joshi, Solvent Free Synthesis of Chalcones and their Antibacterial Activities, E-Journal of Chemistry. 2 (4) (2005) 224–227.
[29] M.M.H.Bhuiyan, M.I.Hossain, Mahmud, M.M.Mohammad Al-Amin, Microwave-assisted Efficient Synthesis of Chalcones as Probes for Antimicrobial Activities. Chemistry Journal. 1 (2011) 21–28.
[30] A. Tailor, J.C. Waddington, X. Meng, B.K. Park, Detection of drug bioactivation in vivo:mechanism of nevirapine–albumin conjugate formation in patients, Chem. Res. Toxicol. 29 (2016) 1912–1935.
[31] L.B.R. Avila, Synthetic inhibitors of bacterial cell division targeting the GTP-binding site of FtsZ, ACS Chem. Biol. 8 (2013) 2072–2083.
[32] G. Jones, P. Willett, R.C. Glen, Development and validation of a genetic algorithm for flexible docking, J Mol Biol. 267 (1997) 727–748.
[33] V. Luca, D. Vullo, S. Prete, Cloning, characterization and anion inhibition studies of a gamma-carbonic anhydrase from the Antarctic bacterium Colwellia psychrerythraea, Bioorg Med Chem. 24 (2016) 835–840.
[34] Discovery Studio 2.5.5 Accelrys. Biovia, San Diego, CA; 2009. [cited 2019 Jun 13]. Available from: http://www.accelrys.com.