Supplementary Materials for

Structure-guided design of partial agonists at an opioid receptor

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Supplementary Materials and Methods

Chemical Synthesis

Synthesis of bitopics cores (Supplementary Fig. 2)

(4bS,8R,8aS,14bR)-7-allyl-5,6,7,8,14,14b-hexahydro-4,8-methanobenzofuro[2,3-a]pyrido [4,3-b]carbazole-1,8a(9H)-diol, Nalindole (1): For the synthesis of indole core a modified Fisher synthesis was employed. Naloxone.HCl (363.84, 1.0 mmol, 1.0 equiv) and phenylhydrazine (120 mg, 110 µl, 1.1 mmol, 1.1 equiv) were dissolved in trifluoroethanol (5 ml) and one drop of 10 M HCl was added (presence of more water adversely affects the reaction). The reaction mixture was heated in microwave at 120 °C for 1 minute for phenylhydrazine or, if necessary, until completion of the reaction. The mixture was quenched with sat. $NaHCO_3$ and extracted with DCM. Purification on silica using 0.5% to 6% MeOH in DCM gave the title compound as light yellow solid, 380 mg (95%). ^{1}H NMR (400 MHz, cd_3od) δ 7.36 (d, *J* = 7.6 Hz, 1H), 7.32 (d, *J* = 8.3 Hz, 1H), 7.10 (ddd, *J* = 8.3, 7.1, 1.2 Hz, 1H), 6.96 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 1H), 6.66 (s, 2H), 6.03 – 5.88 (m, 1H), 5.70 (s, 1H), 5.72 – 5.59 (m, 2H), 3.99 – 3.89 (m, 1H), 3.85 – 3.75 (m, 3H), 3.46 (d, *J* = 19.8 Hz, 1H), 3.28 – 3.16 (m, 2H), 2.92 (d, *J* = 16.2 Hz, 1H), 2.71 (dt, *J* = 13.3, 6.8 Hz, 1H), 2.64 (d, *J* = 16.1 Hz, 1H), 1.92 (d, *J* = 13.7 Hz, 1H), three protons (OH x 2 and NH) were not observed. ^{13}C NMR (100 MHz, cd_3od) δ 144.75, 141.89, 138.73, 130.09, 130.00, 127.73, 127.63, 126.47, 123.47, 122.35, 120.30, 119.84, 119.28, 119.24, 112.18, 109.22, 85.01, 73.49, 63.89, 56.61, 47.80, 47.28, 29.97, 29.62, 24.37.

(4bS,8R,8aS,15bR)-7-allyl-5,6,7,8,9,15b-hexahydro-8aH-4,8-methanobenzofuro[3,2-c]pyrido[3,4-b]acridine-1,8a-diol, Allylquino (3): Naloxone.HCl (728 mg, 2.0 mmol, 1.0 equiv), 2-aminobenzaldehyde (480 mg, 4.0 mmol, 2.0 equiv) and p-TsOH.H_2O (340 mg, 1.77 mmol, 0.89 equiv) were mixed in trifluoroethanol and refluxed for 14 h. After cooling the mixture was suspended in DCM, and the product was extracted into 1M NaOH. If an emulsion forms, that can be broken by the addition of a few drops of MeOH. The aqueous layer was washed with DCM, which was checked for the presence of product. To the aqueous layer 1M HCl was added until pH 8-9 was reached and the product was extracted into DCM. After drying over Na_2SO_4 and removal of the solvent, the product is usually sufficiently pure for deallylation, but also can be further purified on silica using 0.5% to 6% MeOH in DCM, 644 mg (78%).

^{1}H NMR (400 MHz, cdcl$_3$) δ 7.83 (dd, *J* = 7.9, 1.8 Hz, 1H), 7.61 (s, 1H), 7.52 (dd, *J* = 7.8, 1.8 Hz, 1H), 7.38 – 7.26 (m, 2H), 6.72 (d, *J* = 8.1 Hz, 1H), 6.57 (d, *J* = 8.1 Hz, 1H), 5.79 (ddt, *J* = 16.6, 10.0, 6.4 Hz, 1H), 5.60 (s, 1H), 5.20 (dd, *J* = 17.2, 1.7 Hz, 1H), 5.15 (dd, *J* = 10.1, 1.7 Hz, 1H), 3.17 (d, *J* = 18.7 Hz, 1H), 3.15 – 3.11 (m, 2H), 3.08 (d, *J* = 6.3 Hz, 1H), 2.75 (dd, *J* = 15.9, 1.6 Hz, 1H), 2.69 (d, *J* = 3.8 Hz, 1H), 2.64 (dd, *J* = 12.6, 6.1 Hz, 1H), 2.61 – 2.49 (m, 1H), 2.36 – 2.20 (m, 2H), 1.78 – 1.66 (m, 1H), two protons (OH x 2) were not observed. ^{13}C NMR (100 MHz, cdcl$_3$) δ 154.09, 146.80, 144.24, 139.28, 136.50, 135.32, 130.51, 128.76, 128.61, 128.08, 127.78, 126.83, 126.69, 124.47, 119.20, 118.06, 117.86, 90.43, 71.64, 61.79, 57.88, 47.18, 43.30, 36.16, 31.86, 23.15.

Deallylation

1,3-dimethylbarbituric acid (286 mg, 1.83 mmol) freshly purified (suspended in cold methanol, filtered and dried under vacuum) catalyst Pd(PPh$_3$)$_4$ (176 mg, 0.15 mmol) were added to a flame dried vial containing the corresponding allyl derivative (3.05 mmol) and a magnetic stirring bar. The vial was sealed, flushed with argon, 30 mL anhydrous DCM was added, and the yellow reaction mixture was heated to reflux at 40 °C overnight. If the amount of precipitate withing the first few hours precluded stirring, more DCM was added, and the mixture was sonicated briefly. The reaction mixture was cooled to room temperature, 2 equivalents of 4 M HCl in dioxane was added and the mixture was filtered. The residue was washed several times with DCM and dried. The products were usually sufficiently pure for further reactions, but further purification can be achieved on a short silica pad, eluting the nor compounds using 20% MeOH (containing 0.35 N NH$_3$) in DCM.

(4bS,8R,8aS,14bR)-5,6,7,8,14,14b-hexahydro-4,8-methanobenzofuro[2,3-a]pyrido[4,3-b]carba-zole-1,8a(9H)-diol, (**2**): 1037 mg, quantitative. ^{1}H NMR (400 MHz, cd$_3$od) δ 7.38 (d, *J* = 7.8 Hz, 1H), 7.34 (d, *J* = 8.3 Hz, 1H), 7.11 (t, *J* = 7.8 Hz, 1H), 6.97 (t, *J* = 7.7 Hz, 1H), 6.66 (d, *J* = 2.5 Hz, 2H), 5.70 (s, 1H), 3.89 (d, *J* = 6.5 Hz, 1H), 3.53 – 3.42 (m, 1H), 3.28 – 3.16 (m, 2H), 3.05 – 2.89 (m, 1H), 2.95 (d, *J* = 15.6 Hz, 1H), 2.71 (dd, *J* = 12.7, 4.3 Hz, 1H), 2.65 (d, *J* = 16.0 Hz, 1H), 1.90 (d, *J* = 13.5 Hz, 1H), four protons (OH x 2 and NH x 2) were not observed. ^{13}C NMR (100 MHz, cd$_3$od) δ 144.79, 141.76, 138.62, 130.23, 130.00, 127.65, 123.47, 122.71, 120.22, 119.86, 119.34, 119.18, 112.28, 109.40, 85.30, 72.35, 59.16, 47.90, 37.97, 29.73, 29.69, 28.45.

(4bS,8R,8aS,15bR)-5,6,7,8,9,15b-hexahydro-8aH-4,8-methanobenzofuro[3,2-c]pyrido[3,4-b]ac-ridine-1,8a-diol, (4): 1131 mg, quantitative. ^{1}H NMR (400 MHz, cd_3od) δ 7.80 (d, *J* = 8.2 Hz, 1H), 7.72 (s, 1H), 7.61 (dd, *J* = 8.3, 1.4 Hz, 1H), 7.48 (ddd, *J* = 8.4, 6.9, 1.4 Hz, 1H), 7.37 (ddd, *J* = 8.1, 6.8, 1.2 Hz, 1H), 6.80 – 6.65 (m, 2H), 5.58 (s, 1H), 3.97 (d, *J* = 6.1 Hz, 1H), 3.42 (d, *J* = 19.4 Hz, 1H), 3.37 – 3.35 (m, 1H), 3.30 – 3.21 (m, 1H), 3.03 (td, *J* = 13.3, 4.2 Hz, 1H), 2.91 (d, *J* = 16.6 Hz, 1H), 2.78 – 2.67 (m, 1H), 2.69 (d, *J* = 16.9 Hz, 1H), 1.86 (dd, *J* = 13.5, 3.9 Hz, 1H), three protons (OH x 2 and NH) were not observed. ^{13}C NMR (100 MHz, cd_3od) δ 154.38, 147.68, 145.20, 141.58, 138.89, 130.66, 130.05, 128.96, 128.38, 128.24, 128.16, 127.68, 122.86, 121.09, 119.60, 90.39, 70.94, 58.48, 47.13, 37.84, 36.55, 29.62, 28.89.

Preparation of di-BOC-protected guanidino alcohols (Supplementary Fig. 3)

The corresponding amino-alcohol (10 mmol, 1.0 equiv), N,N′-Di-Boc-1H-pyrazole-1-carboxamidine (3.4 g, 11 mmol, 1.1 equiv) and triethyl amine (2.0 g, 20 mmol, 2.0 equiv) were mixed in MeOH (50 mL) and stirred overnight. MeOH was removed under vacuum, the residue was redissolved in DCM and washed 3 times with 0.01 M HCl or until the aqueous layer reached pH 3. Finally, the organic layer was washed with diluted K_2CO_3 solution, dried over Na_2SO_4 and the solvent was removed on rotary evaporator. The product was used without further purification in the next step, analytical samples were obtained by purification using 20% to 50% EtOAc in hexane on silica.

N,N'-Di-Boc-1-(3-hydroxypropyl)guanidine, (**5**): 2.76 g (87%). ^{1}H NMR (400 MHz, $cdcl_3$ with a few drops of cd_3od) δ 3.62 (t, *J* = 5.7 Hz, 2H), 3.52 (t, *J* = 6.4 Hz, 2H), 1.81 – 1.70 (m, 2H), 1.52 (s, 9H), 1.49 (s, 9H)^{13}C NMR (100 MHz, $cdcl_3$) δ 167.91, 161.80, 157.98, 88.61, 84.67, 63.35, 42.27, 37.18, 33.07, 32.89.

N,N'-Di-Boc-1-(4-hydroxybutyl)guanidine, (**7**): 3.01 g (91%). ^{1}H NMR (400 MHz, $cdcl_3$) δ 11.46 (s, 1H), 8.39 (t, *J* = 5.4 Hz, 1H), 3.66 (t, *J* = 6.0 Hz, 2H), 3.43 (td, *J* = 7.0, 5.4 Hz, 2H), 1.72 – 1.53 (m, 4H), 1.47 (d, *J* = 1.9 Hz, 18H). ^{13}C NMR (100 MHz, $cdcl_3$) δ 163.29, 156.20, 153.32, 83.31, 79.59, 62.10, 40.56, 29.53, 28.34, 28.14, 25.67.

N,N'-Di-Boc-1-(5-hydroxypentyl)guanidine, (**9**): 3.31 g (96%). ^{1}H NMR (400 MHz, $cdcl_3$ with a few drops of cd_3od) δ 3.59 (t, *J* = 6.6 Hz, 2H), 3.39 (d, *J* = 7.1 Hz, 2H), 1.69 – 1.56 (m, 4H), 1.51 (d, *J* = 5.8 Hz, 18H), 1.43 (tdd, *J* = 9.2, 6.2, 3.4 Hz, 2H). ^{13}C NMR (100 MHz, $cdcl_3$) δ 168.37, 161.15, 158.17, 88.44, 84.57, 66.89, 45.78, 37.08, 33.82, 33.16, 32.92, 28.08.

N,N'-Di-Boc-1-(6-hydroxyhexyl)guanidine, (**11**): 3.58 g (quantitative). ^{1}H NMR (400 MHz, cdcl$_3$ with a few drops of cd$_3$od) δ 3.58 (t, *J* = 6.6 Hz, 2H), 3.38 (t, *J* = 7.2 Hz, 2H), 1.59 (dt, *J* = 13.0, 6.6 Hz, 2H), 1.51 (s, 9H), 1.50 (s, 9H), 1.49 (s, 1H), 1.46 – 1.34 (m, 5H). ^{13}C NMR (100 MHz, cdcl$_3$) δ 163.14, 155.87, 152.94, 83.19, 79.32, 61.86, 40.52, 32.10, 28.69, 27.93, 27.69, 26.41, 25.18.

N,N'-Di-Boc-1-(7-hydroxyheptyl)guanidine, (**13**): 3.55 g (95%). ^{1}H NMR (400 MHz, cdcl$_3$) δ 11.47 (s, 1H), 8.26 (t, *J* = 5.1 Hz, 1H), 3.60 (t, *J* = 6.6 Hz, 2H), 3.37 (td, *J* = 7.2, 5.0 Hz, 2H), 1.58 – 1.50 (m, 4H), 1.46 (d, *J* = 3.7 Hz, 18H), 1.39 – 1.29 (m, 2H), 1.32 (s, 5H). ^{13}C NMR (100 MHz, cdcl$_3$) δ 164.83, 157.29, 154.52, 84.21, 80.42, 64.05, 42.07, 33.81, 30.16, 30.04, 29.49, 29.26, 27.94, 26.73.

Preparation of N-Boc-8-aminooctanol (15) (Supplementary Fig. 3)

8-Aminooctanol (1.45 g, 10 mmol, 1.0 equiv), di-tert-butyl dicarbonate (Boc$_2$O) (2.18 g, 10 mmol, 1.0 equiv), and triethylamine (2.02 g, 20 mmol, 2.0 equiv) were dissolved in dichloromethane (DCM, 50 mL) and stirred overnight at room temperature. After completion of the reaction, the solvent was removed under reduced pressure. The residue was redissolved in DCM (50 mL) and washed three times with 0.01 M HCl until the aqueous layer reached pH 3. The organic layer was then washed with dilute K_2CO_3 solution to neutralize any remaining acid, dried over anhydrous Na_2SO_4, and the solvent was removed using a rotary evaporator. The product was used without further purification in the next step. Analytical samples were obtained by purification using 20% to 50% ethyl acetate (EtOAc) in hexane on silica gel, 2.21 g (90%). ^{1}H NMR (400 MHz, cdcl$_3$) δ 3.54 (q, *J* = 5.5 Hz, 2H), 3.02 (q, *J* = 6.8 Hz, 2H), 1.55 – 1.43 (m, 2H), 1.43 – 1.38 (m, 2H), 1.37 (s, 9H), 1.31 – 1.21 (m, 8H), two protons (OH and NH were not observed).^{13}C NMR (100 MHz, cdcl$_3$) δ 157.25, 80.13, 63.82, 41.72, 33.83, 31.15, 30.48, 30.38, 29.57, 27.86, 26.84.

Preparation of di-BOC protected guanidino aldehydes (Supplementary Fig. 3)

The corresponding guanidino alcohol (2.0 mmol, 1.0 equiv) and triethyl amine (7.5 mmol, 3.75 equiv) were dissolved in DCM (20 mL) and cooled in an ice bath. Dess-Martin periodinate (2.5 mmol, 1.25 equiv) was dissolved in DCM (20 mL) and added slowly. The mixture was allowed to warm to room temperature and was stirred for 3 hours or until the completion of the reaction. Saturated K_2CO_3 solution (3 mL) was added followed by powdered K_2CO_3 (3 g) in a few minutes. The resulting mixture was stirred for 10 minutes, the solids removed by filtration and washed with

DCM. The organic layer was dried over Na_2SO_4 and the solvent was removed on rotary evaporator. The product was purified using 20% to 50% EtOAc in hexane on silica until at least 80% purity (by LC-MS) and used as such in the next step.

Reductive amination (Supplementary Figs. 4 and 5)

The corresponding nor compounds (0.1 – 0.2 mmol, 1 equiv.) and aldehydes (0.5 – 1.0 mmol, 5 equiv.) were dissolved in trifluoroethanol (2-3 mL) and stirred at 75 °C for 3 minutes. $NaBH_3CN$ (0.1 – 0.2 mmol, 1 equiv.) was added and the mixtures were heated at 75 °C for 2 more minutes. The reactions were checked on TLC and if necessary, a further equivalent of $NaBH_3CN$ was added and the mixture was heated for a further 15 minutes at most, checking for completion every 5 minutes to avoid partial deprotection of the di-boc guanidino group. A 4-gram Redisep silica column was equilibrated with hexanes and the reaction mixtures were loaded directly after cooling and eluted first using a gradient of 0% to 100% EtOAc in hexanes and then 0% to 20% MeOH in DCM. The desired compounds usually eluted in the MeOH/DCM phase. The fractions containing the product were combined and repurified on a 4-gram silica column using 1% to 5% MeOH in DCM as eluent.

N,N'-Di-Boc-1-(3-((4bS,8R,8aS,14bR)-1,8a-dihydroxy-5,6,8a,9,14,14b-hexahydro-4,8-methanobenzofuro[2,3-a]pyrido[4,3-b]carbazol-7(8H)-yl)propyl)guanidine (**17**): 0.1 mmol scale, 33 mg (49%). ^{1}H NMR (400 MHz, cdcl$_3$) δ 11.52 (s, 1H), 8.41 (dd, *J* = 12.2, 6.0 Hz, 2H), 7.39 (d, *J* = 7.8 Hz, 1H), 7.19 (d, *J* = 8.2 Hz, 1H), 7.10 (t, *J* = 7.6 Hz, 1H), 7.00 (t, *J* = 7.4 Hz, 1H), 6.55 (dd, *J* = 8.2, 1.5 Hz, 1H), 6.45 (d, *J* = 8.1 Hz, 1H), 5.69 (s, 1H), 3.48 (q, *J* = 6.4 Hz, 2H), 3.10 (d, *J* = 17.5 Hz, 1H), 3.10 (s, 1H), 2.88 (d, *J* = 15.7 Hz, 1H), 2.78 (dd, *J* = 18.6, 6.5 Hz, 1H), 2.61 (d, *J* = 15.7 Hz, 1H), 2.56 – 2.46 (m, 3H), 2.36 (td, *J* = 12.5, 4.8 Hz, 1H), 2.29 – 2.14 (m, 1H), 1.81 – 1.68 (m, 3H), 1.50 (s, 9H), 1.45 (s, 9H), two protons were not observed (2 x OH). ^{13}C NMR (100 MHz, cdcl$_3$) δ 163.63, 156.34, 153.49, 142.83, 139.12, 137.29, 130.76, 128.83, 126.71, 125.20, 122.83, 119.36, 119.18, 119.05, 117.33, 111.61, 111.43, 85.64, 83.41, 79.54, 72.99, 63.26, 52.07, 48.07, 43.71, 39.05, 31.28, 28.90, 28.35, 28.20, 27.32, 23.58.

N,N'-Di-Boc-1-(4-((4bS,8R,8aS,14bR)-1,8a-dihydroxy-5,6,8a,9,14,14b-hexahydro-4,8-methanobenzofuro[2,3-a]pyrido[4,3-b]carbazol-7(8H)-yl)butyl)guanidine (**18**): 0.1 mmol scale, 23 mg (34%). ^{1}H NMR (400 MHz, cdcl$_3$) δ 11.52 (s, 1H), 8.41 – 8.33 (m, 2H), 7.40 (d, *J* = 7.9 Hz, 1H), 7.26 (d, *J* = 8.4 Hz, 1H), 7.13 (t, *J* = 7.6 Hz, 1H), 7.02 (t, *J* = 7.5 Hz, 1H), 6.57 (d, *J*

= 8.1 Hz, 1H), 6.49 (d, *J* = 8.2 Hz, 1H), 5.68 (s, 1H), 3.44 (q, *J* = 6.6 Hz, 2H), 3.11 (d, *J* = 18.6 Hz, 1H), 3.09 (d, *J* = 6.6 Hz, 1H), 2.87 (d, *J* = 15.7 Hz, 1H), 2.79 (dd, *J* = 18.7, 6.5 Hz, 1H), 2.64 – 2.41 (m, 4H), 2.34 (d, *J* = 13.1 Hz, 1H), 2.24 (t, *J* = 11.8 Hz, 1H), 1.74 (d, *J* = 12.2 Hz, 1H), 1.67 – 1.40 (m, 4H), 1.49 (s, 9H), 1.45 (s, 9H), two protons were not observed (2 x OH). ^{13}C NMR (100 MHz, cdcl$_3$) δ 163.69, 156.36, 153.48, 142.93, 139.09, 137.30, 130.74, 128.87, 126.77, 125.29, 122.93, 119.43, 119.19, 119.11, 117.25, 111.67, 111.40, 85.64, 83.35, 79.53, 72.89, 63.29, 54.20, 48.12, 43.61, 40.75, 31.40, 28.87, 28.36, 28.21, 26.94, 24.95, 23.56.

N,N'-Di-Boc-1-(4-((4bS,8R,8aS,14bR)-1,8a-dihydroxy-5,6,8a,9,14,14b-hexahydro-4,8-methanobenzofuro[2,3-a]pyrido[4,3-b]carbazol-7(8H)-yl)pentyl)guanidine (**19**): 0.2 mmol scale, 80 mg (58%). ^{1}H NMR (400 MHz, cdcl$_3$) δ 11.50 (s, 1H), 8.40 – 8.24 (m, 2H), 7.38 (d, *J* = 7.8 Hz, 1H), 7.22 (d, *J* = 8.2 Hz, 1H), 7.08 (t, *J* = 7.6 Hz, 1H), 6.99 (d, *J* = 7.6 Hz, 1H), 6.53 (d, *J* = 8.0 Hz, 1H), 6.44 (d, *J* = 8.2 Hz, 1H), 5.68 (s, 1H), 3.63 – 3.22 (m, 2H), 3.11 (d, *J* = 17.6 Hz, 1H), 3.10 (s, 1H), 2.86 (d, *J* = 15.8 Hz, 1H), 2.83 – 2.72 (m, 1H), 2.60 (d, *J* = 15.8 Hz, 1H), 2.51 (d, *J* = 10.9 Hz, 1H), 2.48 – 2.40 (m, 2H), 2.32 (d, *J* = 12.1 Hz, 1H), 2.24 (d, *J* = 12.1 Hz, 1H), 1.71 (d, *J* = 12.1 Hz, 1H), 1.65 – 1.39 (m, 4H), 1.47 (s, 9H), 1.44 (s, 9H), 1.36 – 1.32 (m, 2H), two protons were not observed (2 x OH). ^{13}C NMR (100 MHz, cdcl$_3$) δ 163.65, 156.24, 153.41, 142.97, 139.30, 137.29, 130.76, 129.01, 126.71, 125.07, 122.67, 119.22, 118.99, 118.95, 117.30, 111.40, 111.35, 85.46, 83.19, 79.40, 72.89, 63.13, 54.40, 48.06, 43.59, 40.85, 31.45, 28.87, 28.83, 28.31, 28.12, 27.34, 24.59, 23.45.

N,N'-Di-Boc-1-(4-((4bS,8R,8aS,14bR)-1,8a-dihydroxy-5,6,8a,9,14,14b-hexahydro-4,8-methanobenzofuro[2,3-a]pyrido[4,3-b]carbazol-7(8H)-yl)hexyl)guanidine (**20**): 0.2 mmol scale, 64 mg, (46%). ^{1}H NMR (400 MHz, cdcl$_3$) δ 11.50 (s, 1H), 8.32 (d, *J* = 5.2 Hz, 1H), 8.30 (s, 1H), 7.40 (d, *J* = 7.9 Hz, 1H), 7.26 (d, *J* = 8.2 Hz, 1H), 7.12 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 1H), 7.01 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 1H), 6.55 (d, *J* = 8.1 Hz, 1H), 6.48 (d, *J* = 8.2 Hz, 1H), 5.69 (s, 1H), 3.41 (td, *J* = 7.3, 5.1 Hz, 2H), 3.15 (d, *J* = 19.2 Hz, 1H), 3.11 (s, 1H), 2.89 (d, *J* = 15.7 Hz, 1H), 2.85 – 2.76 (m, 1H), 2.60 (d, *J* = 15.7 Hz, 1H), 2.57 (dd, *J* = 11.2, 4.5 Hz, 1H), 2.49 (qd, *J* = 7.4, 5.5 Hz, 2H), 2.37 (td, *J* = 12.4, 4.8 Hz, 1H), 2.28 (td, *J* = 11.9, 3.3 Hz, 1H), 1.76 (dt, *J* = 11.4, 2.6 Hz, 1H), 1.68 – 1.43 (m, 4H), 1.49 (s, 9H), 1.49 (s, 9H), 1.36 – 1.30 (m, 4H), two protons were not observed (2 x OH). ^{13}C NMR (100 MHz, cdcl$_3$) δ 163.74, 156.27, 153.47, 142.82, 139.09, 137.30, 130.72, 128.87, 126.73, 125.22, 122.84, 119.37, 119.20, 119.09, 117.26, 111.58, 111.42,

85.66, 83.22, 79.46, 72.90, 63.10, 54.54, 48.12, 43.73, 41.00, 31.41, 29.02, 28.88, 28.39, 28.20, 27.57, 27.07, 26.81, 23.48.

N,N'-Di-Boc-1-(4-((4bS,8R,8aS,14bR)-1,8a-dihydroxy-5,6,8a,9,14,14b-hexahydro-4,8-methanobenzofuro[2,3-a]pyrido[4,3-b]carbazol-7(8H)-yl)heptyl)guanidine (**21**): 0.2 mmol scale, 27 mg (19%). ^{1}H NMR (400 MHz, cdcl$_3$) δ 11.51 (s, 1H), 8.36 (s, 1H), 8.31 (t, *J* = 5.2 Hz, 1H), 7.40 (d, *J* = 7.9 Hz, 1H), 7.24 (d, *J* = 8.0 Hz, 1H), 7.11 (t, *J* = 7.6 Hz, 1H), 7.00 (t, *J* = 7.4 Hz, 1H), 6.53 (d, *J* = 8.1 Hz, 1H), 6.45 (d, *J* = 8.2 Hz, 1H), 5.72 (s, 1H), 3.39 (q, *J* = 6.7 Hz, 2H), 3.14 (d, *J* = 19.0 Hz, 1H), 3.12 (s, 1H), 2.89 (d, *J* = 15.7 Hz, 1H), 2.80 (dd, *J* = 18.6, 6.6 Hz, 1H), 2.63 (d, *J* = 15.8 Hz, 1H), 2.56 (dd, *J* = 11.4, 4.3 Hz, 1H), 2.48 (t, *J* = 7.3 Hz, 2H), 2.37 (td, *J* = 12.3, 4.6 Hz, 1H), 2.31 – 2.17 (m, 1H), 1.74 (d, *J* = 12.0 Hz, 1H), 1.59 – 1.40 (m, 4H), 1.48 (apparent d, *J* = 2.9 Hz, 18H), 1.37 – 1.30 (m, 6H), two protons were not observed (2 x OH). ^{13}C NMR (100 MHz, cdcl$_3$) δ 163.73, 156.24, 153.44, 142.78, 139.03, 137.29, 130.76, 128.86, 126.73, 125.34, 122.82, 119.35, 119.18, 119.08, 117.21, 111.65, 111.38, 85.68, 83.18, 79.43, 72.91, 63.14, 54.65, 48.17, 43.62, 41.05, 31.48, 29.25, 29.02, 28.86, 28.38, 28.18, 27.73, 27.31, 26.92, 23.45.

N,N'-Di-Boc-1-(3-((4bS,8R,8aS,15bR)-1,8a-dihydroxy-5,6,8,8a,9,15b-hexahydro-7H-4,8-methanobenzofuro[3,2-c]pyrido[3,4-b]acridin-7-yl)propyl)guanidine (**22**): 0.2 mmol scale, 75 mg (56%). ^{1}H NMR (400 MHz, cdcl$_3$) δ 11.52 (s, 1H), 8.41 (t, *J* = 5.2 Hz, 1H), 8.09 (d, *J* = 8.5 Hz, 1H), 7.81 (s, 1H), 7.69 (d, *J* = 8.2 Hz, 1H), 7.61 (ddd, *J* = 8.4, 6.8, 1.4 Hz, 1H), 7.47 (ddd, *J* = 8.1, 6.8, 1.2 Hz, 1H), 6.68 (d, *J* = 8.1 Hz, 1H), 6.60 (d, *J* = 8.1 Hz, 1H), 5.68 (s, 1H), 4.74 (s, 1H), 3.62 – 3.43 (m, 2H), 3.23 (d, *J* = 18.6 Hz, 1H), 3.10 (d, *J* = 6.3 Hz, 1H), 2.86 (d, *J* = 15.8 Hz, 1H), 2.78 (d, *J* = 16.9 Hz, 1H), 2.75 – 2.56 (m, 3H), 2.49 – 2.36 (m, 2H), 1.87 – 1.76 (m, 3H), 1.48 (s, 9H), 1.46 (s, 9H). two protons were not observed (2 x OH). ^{13}C NMR (100 MHz, cdcl$_3$) δ 163.73, 156.33, 154.31, 153.55, 147.47, 143.79, 138.83, 136.54, 130.66, 129.24, 129.16, 128.41, 128.11, 127.07, 127.04, 125.02, 119.43, 117.21, 91.34, 83.40, 79.44, 71.79, 62.56, 52.17, 47.42, 43.80, 39.05, 36.46, 31.97, 28.40, 28.22, 27.17, 23.55.

N,N'-Di-Boc-1-(3-((4bS,8R,8aS,15bR)-1,8a-dihydroxy-5,6,8,8a,9,15b-hexahydro-7H-4,8-methanobenzofuro[3,2-c]pyrido[3,4-b]acridin-7-yl)pentyl)guanidine (**23**): 0.2 mmol scale, 78 mg (56%) .^{1}H NMR (400 MHz, cdcl$_3$) δ 11.48 (s, 1H), 8.31 (t, *J* = 5.2 Hz, 1H), 8.00 (q, *J* = 8.2 Hz, 1H), 7.77 (d, *J* = 4.7 Hz, 1H), 7.64 (dt, *J* = 8.6, 3.8 Hz, 1H), 7.54 (p, *J* = 7.5 Hz, 1H), 7.43 (dp, *J* = 7.4, 4.0 Hz, 1H), 6.68 (d, *J* = 8.1 Hz, 1H), 6.58 (d, *J* = 8.1 Hz, 1H), 5.63 (d, *J* = 3.7 Hz, 1H),

3.39 (q, *J* = 6.6 Hz, 2H), 3.20 (d, *J* = 18.6 Hz, 1H), 3.09 (s, 1H), 2.86 – 2.68 (m, 3H), 2.63 (s, 1H), 2.52 (s, 2H), 2.37 (s, 2H), 1.80 (s, 1H), 1.62 – 1.50 (m, 4H), 1.47 (s, 9H), 1.44 (s, 9H), 1.42 – 1.35 (m, 2H) two protons were not observed (2 x OH). ^{13}C NMR (100 MHz, cdcl$_3$) δ 163.74, 156.28, 154.25, 153.48, 147.32, 143.93, 138.97, 136.60, 130.52, 129.14, 129.06, 128.30, 128.06, 127.03 (broad, likely overlapping signals 2 x C), 124.83, 119.42, 117.44, 91.08, 83.22, 79.40, 71.67, 62.50, 54.42, 47.34, 43.72, 40.81, 36.35, 32.05, 29.83, 28.86, 28.43, 28.16, 24.58, 23.54.

N,N'-Di-Boc-1-(3-((4bS,8R,8aS,15bR)-1,8a-dihydroxy-5,6,8,8a,9,15b-hexahydro-7H-4,8-methano-benzofuro[3,2-c]pyrido[3,4-b]acridin-7-yl)hexyl)guanidine (**24**): 0.2 mmol scale, 57 mg (40%). ^{1}H NMR (400 MHz, cdcl$_3$) δ 11.51 (s, 1H), 8.31 (t, *J* = 5.2 Hz, 1H), 8.00 (dq, *J* = 8.5, 0.9 Hz, 1H), 7.76 (s, 1H), 7.68 – 7.62 (m, 1H), 7.56 (ddd, *J* = 8.5, 6.8, 1.5 Hz, 1H), 7.44 (ddd, *J* = 8.1, 6.9, 1.2 Hz, 1H), 6.69 (d, *J* = 8.1 Hz, 1H), 6.60 (d, *J* = 8.1 Hz, 1H), 5.67 (s, 1H), 3.41 (td, *J* = 7.2, 5.1 Hz, 2H), 3.23 (d, *J* = 18.6 Hz, 1H), 3.08 (d, *J* = 6.5 Hz, 1H), 2.85 (dd, *J* = 15.8, 1.7 Hz, 1H), 2.79 – 2.68 (m, 2H), 2.66 – 2.55 (m, 1H), 2.51 (tt, *J* = 8.8, 4.3 Hz, 2H), 2.44 – 2.32 (m, 2H), 1.88 – 1.79 (m, 1H), 1.63 – 1.51 (m, 4H), 1.50 (s, 9H), 1.49 (s, 9H), 1.41 – 1.35 (m, 4H), two protons were not observed (2 x OH). ^{13}C NMR (100 MHz, cdcl$_3$) δ 163.72, 156.22, 154.29, 153.44, 147.18, 143.98, 139.05, 136.56, 130.56, 129.04, 128.94, 128.29, 128.00, 126.95 (broad, likely overlapping signals 2 x C), 124.79, 119.34, 117.51, 90.85, 83.16, 79.37, 71.65, 62.45, 54.51, 47.37, 43.60, 40.96, 36.33, 32.13, 29.03, 28.40, 28.18, 27.58, 27.06, 26.81, 23.41.

N,N'-Di-Boc-1-(3-((4bS,8R,8aS,15bR)-1,8a-dihydroxy-5,6,8,8a,9,15b-hexahydro-7H-4,8-methano-benzofuro[3,2-c]pyrido[3,4-b]acridin-7-yl)heptyl)guanidine (**25**): 0.2 mmol scale, 55 mg (38%). ^{1}H NMR (400 MHz, cdcl$_3$) δ 11.44 (s, 1H), 8.24 (t, *J* = 5.2 Hz, 1H), 7.94 (d, *J* = 8.5 Hz, 1H), 7.71 (s, 1H), 7.58 (d, *J* = 8.2 Hz, 1H), 7.46 (t, *J* = 8.0 Hz, 1H), 7.36 (t, *J* = 7.5 Hz, 1H), 6.62 (d, *J* = 8.1 Hz, 1H), 6.52 (d, *J* = 8.1 Hz, 1H), 5.54 (s, 1H), 4.03 – 3.89 (m, 2H), 3.34 (q, *J* = 6.6 Hz, 2H), 3.15 (d, *J* = 18.6 Hz, 1H), 3.01 (d, *J* = 6.4 Hz, 1H), 2.78 (d, *J* = 15.9 Hz, 1H), 2.75 – 2.60 (m, 2H), 2.60 – 2.51 (m, 1H), 2.44 (td, *J* = 7.0, 2.8 Hz, 1H), 2.29 (dt, *J* = 8.1, 4.8 Hz, 2H), 1.75 (d, *J* = 9.4 Hz, 1H), 1.54 – 1.44 (m, 4H), 1.43 (s, 9H), 1.41 (s, 9H), 1.28 (s, 6H), two protons not observed (2 x OH). ^{13}C NMR (100 MHz, cdcl$_3$) δ 163.68, 156.17, 154.26, 153.38, 147.00, 144.06, 139.16, 136.53, 130.53, 128.93, 128.77, 128.24, 127.90, 126.88, 126.83, 124.63, 119.27, 117.63, 90.61, 83.09, 79.30, 71.61, 62.43, 54.54, 47.30, 43.52, 40.96, 36.28, 32.08, 29.17, 28.96, 28.36, 28.12, 27.59, 27.22, 26.87, 23.36

tert-butyl(6-((4bS,8R,8aS,15bR)-1,8a-dihydroxy-5,6,8,8a,9,15b-hexahydro-7H-4,8-methanobenzofuro[3,2-c]pyrido[3,4-b]acridin-7-yl)octyl)carbamate (**26**): 0.2 mmol scale, 36 mg (30%). ^{1}H NMR (400 MHz, cdcl$_3$) δ 7.98 (d, *J* = 8.5 Hz, 1H), 7.76 (s, 1H), 7.63 (d, *J* = 8.1 Hz, 1H), 7.52 (ddd, *J* = 8.5, 6.8, 1.5 Hz, 1H), 7.41 (t, *J* = 7.5 Hz, 1H), 6.65 (d, *J* = 8.1 Hz, 1H), 6.55 (d, *J* = 8.1 Hz, 1H), 5.62 (s, 1H), 3.19 (d, *J* = 18.6 Hz, 1H), 3.10 – 3.01 (m, 3H), 2.82 (d, *J* = 15.8 Hz, 1H), 2.73 (d, *J* = 15.4 Hz, 1H), 2.70 – 2.62 (m, 1H), 2.63 – 2.55 (m, 1H), 2.47 (td, *J* = 7.0, 2.9 Hz, 2H), 2.40 – 2.25 (m, 2H), 1.83 – 1.76 (m, 1H), 1.58 – 1.40 (m, 4H), 1.39 (s, 9H), 1.34 – 1.25 (m, 8H), two protons were not observed (2 x OH). ^{13}C NMR (100 MHz, cdcl$_3$) δ 156.12, 154.35, 147.23, 143.86, 139.06, 136.64, 130.54, 129.16, 128.92, 128.40, 128.07, 127.04, 127.02, 124.78, 119.40, 117.44, 90.86, 79.15 71.69, 62.51, 54.62, 47.42, 43.54, 40.70, 36.36, 32.19, 30.15, 29.52, 29.36, 28.54, 27.70, 27.34, 26.86, 23.41.

Boc deprotection (Supplementary Figs. 4 and 5)

The resulting boc protected guanidines and amines (0.03 – 0.1 mmol) were dissolved in 4M HCl in dioxane (2 mL) under argon and stirred for between 3h to 12h at room temperature. Diethyl ether (6 mL) was added, and the mixture was sonicated until a fine precipitate formed, the vials were centrifuged and the solvent decanted. This process was repeated three times, then the solids were dried at room temperature. Purity was checked by NMR and if necessary further purification was performed on a reverse phase chromatography using acetonitrile (0.05% TFA) and water (0.05% TFA) as eluent, 10% to 40% acetonitrile gradient. However, if the boc protected guanidine precursors were pure, no further purification of the final products was necessary. For biological testing the HCl salts were used, obtained as dihydrochlorides for the indole series and trihydrochlorides for the quino series. The TFA salts can be converted to the HCl salts by dissolution in minimal amount of methanol (about 0.3 mL on a 0.1 mmol scale), addition of at least 10 equivalents of HCl (4M HCl in dioxane) and precipitation by the addition of diethyl ether (6 mL). This process is repeated 3 times to obtain the HCl salts.

1-(3-((4bS,8R,8aS,14bR)-1,8a-dihydroxy-5,6,8a,9,14,14b-hexahydro-4,8-methanobenzofuro[2,3-a]pyrido[4,3-b]carbazol-7(8H)-yl)propyl)guanidine bis(trifluoroacetate) (**C3-Indole 2x TFA salt):** 0.03 mmol scale, isolated 16 mg dihydrochloride salt (quantitative), obtained 17 mg bis(trifluoroacetate) salt (81%) after HPLC. ^{1}H NMR (400 MHz, cd$_3$od) δ 7.33 (d, *J* = 2.7 Hz, 1H), 7.31 (d, *J* = 3.0 Hz, 1H), 7.09 (t, *J* = 7.6 Hz, 1H), 6.95 (t, *J* = 7.5 Hz, 1H), 6.70 – 6.61 (m, 2H),

5.66 (s, 1H), 3.85 (d, *J* = 6.2 Hz, 1H), 3.30 (apparent dd, *J* = 14.4, 7.7 Hz, 5H), 3.11 (t, *J* = 12.7 Hz, 2H), 2.91 (d, *J* = 16.0 Hz, 1H), 2.76 (d, *J* = 13.0 Hz, 1H), 2.66 (dd, *J* = 13.4, 4.4 Hz, 1H), 2.58 (d, *J* = 17.4 Hz, 1H), 2.12 (dq, *J* = 12.6, 6.2 Hz, 1H), 1.92 (dq, *J* = 12.3, 6.2 Hz, 1H), 1.77 (d, *J* = 13.4 Hz, 1H). (3), 7 protons were not observed (2 x OH, indole NH, guanidine NH, NH_2 and =NH). ^{13}C NMR (100 MHz, cd_3od) δ 158.83, 144.90, 142.03, 138.89, 130.25, 130.23, 127.82, 123.69, 122.50, 120.57, 120.07, 119.53, 119.47, 112.42, 109.43, 85.18, 73.59, 64.24, 51.93, 48.12, 47.82, 39.53, 30.18, 29.76, 24.96, 24.73. HRMS calcd for $C_{26}H_{29}N_5O_3$ (MH+) 460.234316; found 460.234129.

1-(3-((4bS,8R,8aS,14bR)-1,8a-dihydroxy-5,6,8a,9,14,14b-hexahydro-4,8-methanobenzofuro [2,3-a]pyrido[4,3-b]carbazol-7(8H)-yl)butyl)guanidine dihydrochloride (C4-Indole 2x HCl salt): 0.03 mmol scale, 16.5 mg (quantitative). ^{1}H NMR (400 MHz, cd_3od) δ 7.39 (d, *J* = 7.9 Hz, 1H), 7.35 (d, *J* = 8.2 Hz, 1H), 7.12 (ddd, *J* = 8.1, 7.0, 1.1 Hz, 1H), 7.02 – 6.91 (m, 1H), 6.68 (d, *J* = 1.4 Hz, 2H), 5.72 (s, 1H), 3.97 (d, *J* = 6.3 Hz, 1H), 3.53 – 3.27 (m, 5H), 3.25 – 3.13 (m, 2H), 3.00 (d, *J* = 16.1 Hz, 1H), 2.92 (d, *J* = 12.7 Hz, 1H), 2.75 (dd, *J* = 13.5, 4.7 Hz, 1H), 2.68 (d, *J* = 16.1 Hz, 1H), 2.06 – 1.88 (m, 2H), 1.84 – 1.64 (m, 3H). 7 protons were not observed (2 x OH, indole NH, guanidine NH, NH_2 and =NH). ^{13}C NMR (100 MHz, cd_3od) δ 158.64, 144.95, 142.06, 138.92, 130.32, 130.23, 127.85, 123.67, 122.59, 120.51, 120.04, 119.50, 119.43, 112.40, 109.45, 85.22, 73.64, 63.71, 53.93, 48.27, 47.94, 41.89, 30.30, 29.84, 26.88, 24.82, 22.75. HRMS calcd for $C_{27}H_{31}N_5O_3$ (MH+) 474.249966; found 474.24991.

1-(3-((4bS,8R,8aS,14bR)-1,8a-dihydroxy-5,6,8a,9,14,14b-hexahydro-4,8-methanobenzofuro [2,3-a]pyrido[4,3-b]carbazol-7(8H)-yl)pentyl)guanidine bis(trifluoroacetate) (C5-Indole 2x TFA salt): 0.1 mmol scale, obtained 55.5 mg (quantitative) dihydrochloride, yielding 59.2 mg (83%) bis(trifluoroacetate) salt after HPLC**.** ^{1}H NMR (400 MHz, cd_3od) δ 7.34 (d, *J* = 6.3 Hz, 1H), 7.32 (d, *J* = 5.2 Hz, 1H), 7.10 (t, *J* = 7.6 Hz, 1H), 6.96 (t, *J* = 7.5 Hz, 1H), 6.68 (s, 2H), 5.67 (s, 1H), 3.85 (d, *J* = 5.7 Hz, 1H), 3.38 – 3.27 (m, 2H), 3.25 – 3.15 (m, 3H), 3.02 (qd, *J* = 12.7, 4.5 Hz, 2H), 2.91 (d, *J* = 16.0 Hz, 1H), 2.75 (td, *J* = 12.7, 3.6 Hz, 1H), 2.65 (dd, *J* = 13.4, 4.6 Hz, 1H), 2.58 (d, *J* = 16.1 Hz, 1H), 1.84 (dq, *J* = 11.9, 5.7 Hz, 1H), 1.79 – 1.72 (m, 1H), 1.71 – 1.61 (m, 3H), 1.51 – 1.38 (m, 2H). 7 protons were not observed (2 x OH, indole NH, guanidine NH, NH_2 and =NH). ^{13}C NMR (100 MHz, cd_3od) δ 158.69, 144.90, 142.01, 138.89, 130.31, 130.27, 127.83, 123.69, 122.60, 120.61, 120.05, 119.54, 119.43, 112.43, 109.45, 85.18, 73.59, 63.66, 54.22, 47.99,

47.88, 42.08, 30.19, 29.76, 29.36, 24.96, 24.64, 24.62. HRMS calcd for $C_{28}H_{33}N_5O_3$ (MH+) 488.265616; found 488.265851.

1-(3-((4bS,8R,8aS,14bR)-1,8a-dihydroxy-5,6,8a,9,14,14b-hexahydro-4,8-methanobenzofuro [2,3-a]pyrido[4,3-b]carbazol-7(8H)-yl)hexyl)guanidine bis(trifluoroacetate) (C6-Indole 2x TFA salt): 0.07 mmol scale, obtained 55.0 mg (quantitative) dihydrochloride salt, resulting in 40.3 mg (79%) bis(trifluoroacetate) after HPLC. ^{1}H NMR (400 MHz, cd$_3$od) δ 77.37 (d, *J* = 7.9 Hz, 1H), 7.32 (d, *J* = 8.2 Hz, 1H), 7.10 (t, *J* = 7.6 Hz, 1H), 6.96 (t, *J* = 7.5 Hz, 1H), 6.65 (s, 2H), 5.69 (s, 1H), 3.89 (d, *J* = 6.4 Hz, 1H), 3.41 (d, *J* = 19.9 Hz, 1H), 3.32 (d, *J* = 6.7 Hz, 2H), 3.19 (t, *J* = 7.1 Hz, 3H), 3.09 (d, *J* = 12.3 Hz, 1H), 2.95 (d, *J* = 15.9 Hz, 2H), 2.73 (dd, *J* = 13.8, 4.7 Hz, 1H), 2.66 (d, *J* = 16.1 Hz, 1H), 1.95 – 1.81 (m, 2H), 1.67 – 1.62 (m, 3H), 1.47 (s, 4H). 7 protons were not observed (2 x OH, indole NH, guanidine NH, NH_2 and =NH). ^{13}C NMR (100 MHz, cd$_3$od) δ 158.66, 144.96, 142.08, 138.94, 130.33, 130.22, 127.84, 123.67, 122.53, 120.45, 120.04, 119.44 (broad, likely overlap of two peaks), 112.39, 109.42, 85.23, 73.60, 63.68, 54.41, 48.19, 47.97, 42.31, 30.27, 29.84, 29.71, 27.29, 27.25, 25.30, 24.66. HRMS calcd for $C_{29}H_{35}N_5O_3$ (MH+) 502.281266; found 502.281317.

1-(3-((4bS,8R,8aS,14bR)-1,8a-dihydroxy-5,6,8a,9,14,14b-hexahydro-4,8-methanobenzofuro [2,3-a]pyrido[4,3-b]carbazol-7(8H)-yl)heptyl)guanidine bis(trifluoroacetate) (C7-Indole 2x TFA salt): 0.038 mmol scale, yielded 32.2 mg (quantitative) dihydrochloride, resulting in 23.9 mg (85%) bis(trifluoroacetate) upon HPLC purification. ^{1}H NMR (400 MHz, cd$_3$od) δ 7.37 (d, *J* = 8.0 Hz, 1H), 7.32 (d, *J* = 8.2 Hz, 1H), 7.10 (t, *J* = 7.6 Hz, 1H), 6.96 (t, *J* = 7.5 Hz, 1H), 6.65 (s, 2H), 5.70 (s, 1H), 3.89 (d, *J* = 6.5 Hz, 1H), 3.42 (d, *J* = 19.9 Hz, 1H), 3.36 – 3.28 (m, 2H), 3.18 (t, *J* = 7.2 Hz, 3H), 3.08 (td, *J* = 11.9, 4.6 Hz, 1H), 3.02 – 2.87 (m, 3H), 2.74 (dd, *J* = 13.4, 4.9 Hz, 1H), 2.67 (d, *J* = 16.1 Hz, 1H), 1.92 (d, *J* = 13.9 Hz, 1H), 1.85 (s, 1H), 1.74 – 1.54 (m, 3H), 1.45 (s, 6H). 7 protons were not observed (2 x OH, indole NH, guanidine NH, NH_2 and =NH). ^{13}C NMR (100 MHz, cd$_3$od) δ 158.66, 144.98, 142.11, 138.96, 130.35, 130.23, 127.86, 123.68, 122.55, 120.45, 120.05, 119.45 (broad, likely overlap of two peaks), 112.40, 109.44, 85.25, 73.61, 63.67, 54.50, 48.21, 48.00, 42.43, 30.30, 29.95, 29.86, 29.82, 27.61, 27.56, 25.41, 24.67. HRMS calcd for $C_{30}H_{37}N_5O_3$ (MH+) 516.296917; found 516.297086.

1-(3-((4bS,8R,8aS,15bR)-1,8a-dihydroxy-5,6,8,8a,9,15b-hexahydro-7H-4,8-methano-benzofuro[3,2-c]pyrido[3,4-b]acridin-7-yl)propyl)guanidine trihydrochloride (C3-Quino 3x

HCl salt): 0.1 mmol scale, 53.9 mg (quantitative). ^{1}H NMR (400 MHz, cd3od) δ 8.95 (s, 1H), 8.30 (d, *J* = 8.6 Hz, 1H), 8.22 (d, *J* = 8.2 Hz, 1H), 8.15 (t, *J* = 7.8 Hz, 1H), 7.93 (t, *J* = 7.5 Hz, 1H), 6.82 (d, *J* = 8.1 Hz, 1H), 6.73 (d, *J* = 8.0 Hz, 1H), 6.05 (s, 1H), (4.15 (s, 1H), 3.61 – 3.49 (m, 2H), 3.46 – 3.35 (m, 6H), 3.12 (d, *J* = 12.2 Hz, 1H), 3.04 (d, *J* = 17.2 Hz, 1H), 2.89 (t, *J* = 12.8 Hz, 1H), 2.35 – 1.91 (m, 2H), 2.08 (d, *J* = 13.7 Hz, 1H), 6 protons were not observed (2 x OH, guanidine NH, NH_2 and =NH). ^{13}C NMR (100 MHz, cd_3od) δ 158.71, 151.46, 149.05, 144.52, 142.27, 140.30, 136.58, 131.57, 130.33, 129.90, 129.79, 128.72, 122.62, 122.58, 121.74, 120.13, 85.06, 72.01, 63.11, 52.26, 48.22, 47.30, 39.66, 36.30, 29.69, 25.28, 24.88. HRMS calcd for $C_{27}H_{29}N_5O_3$ (MH+) 472.234316; found 472.23434. HRMS calcd for $C_{29}H_{33}N_5O_3$ (MH+) 500.265616; found 500.265356.

1-(3-((4bS,8R,8aS,15bR)-1,8a-dihydroxy-5,6,8,8a,9,15b-hexahydro-7H-4,8-methano-benzofuro[3,2-c]pyrido[3,4-b]acridin-7-yl)pentyl)guanidine tris(trifluoroacetate) (C5-Quino 3x TFA salt): 0.1 mmol scale, 61 mg (quantitative) trihydrochloride, which was transformed to tris(trifluoroacetate) as described above yielding 81.5 mg (97%). ^{1}H NMR (400 MHz, cd_3od) δ 8.12 (s, 1H), 7.99 (d, *J* = 8.6 Hz, 1H), 7.81 (d, *J* = 8.3 Hz, 1H), 7.72 (ddd, *J* = 8.5, 6.8, 1.4 Hz, 1H), 7.56 (t, *J* = 7.6 Hz, 1H), 6.80 – 6.70 (m, 2H), 5.74 (s, 1H), 4.00 (d, *J* = 6.6 Hz, 1H), 3.52 (d, *J* = 20.0 Hz, 1H), 3.41 (td, *J* = 12.2, 5.1 Hz, 1H), 3.29 – 3.11 (m, 5H), 3.08 (d, *J* = 16.8 Hz, 1H), 3.01 (dd, *J* = 12.9, 3.9 Hz, 1H), 2.88 (d, *J* = 16.5 Hz, 1H), 2.81 (dt, *J* = 13.4, 6.8 Hz, 1H), 1.97 (dd, *J* = 13.4, 3.8 Hz, 1H), 1.90 (dt, *J* = 12.7, 6.5 Hz, 1H), 1.81 – 1.65 (m, 3H), 1.57 – 1.44 (m, 2H), 6 protons not observed (2 x OH, guanidine NH, NH_2 and =NH). ^{13}C NMR (100 MHz, cd_3od) δ 158.70, 153.87, 146.09, 145.04, 141.89, 141.51, 132.33, 129.64, 129.48, 129.21, 128.76, 128.28, 126.94, 122.52, 121.61, 119.88, 88.98, 72.08, 62.93, 54.47, 47.97, 47.18, 42.08, 36.84, 30.09, 29.38, 25.01, 24.63, 24.59.

1-(3-((4bS,8R,8aS,15bR)-1,8a-dihydroxy-5,6,8,8a,9,15b-hexahydro-7H-4,8-methanobenzofuro [3,2-c]pyrido[3,4-b]acridin-7-yl)pentyl)guanidine trihydrochloride (C5-Quino 3x HCl salt): ^{1}H NMR (400 MHz, cd_3od) δ 8.90 (s, 1H), 8.30 (d, *J* = 8.7 Hz, 1H), 8.21 (d, *J* = 8.4 Hz, 1H), 8.14 (ddd, *J* = 8.6, 7.0, 1.3 Hz, 1H), 7.92 (ddd, *J* = 8.3, 6.9, 1.1 Hz, 1H), 6.83 (d, *J* = 8.2 Hz, 1H), 6.74 (d, *J* = 8.2 Hz, 1H), 6.04 (s, 1H), 4.16 (d, *J* = 6.7 Hz, 1H), 3.59 (d, *J* = 20.0 Hz, 1H), 3.49 (ddd, *J* = 15.7, 10.8, 4.9 Hz, 1H), 3.41 (d, *J* = 17.2 Hz, 1H), 3.39 – 3.32 (m, 2H), 3.31 – 3.26 (m, 1H), 3.28 – 3.18 (m, 2H), 3.11 (dd, *J* = 13.1, 4.0 Hz, 1H), 3.03 (d, *J* = 16.7 Hz, 1H), 2.89 (td, *J* = 13.4, 5.0 Hz, 1H), 2.08 (dd, *J* = 13.6, 3.5 Hz, 1H),

1.96 (s, 1H), 1.80 – 1.69 (m, 2H), 1.66 – 1.47 (m, 3H),), 6 protons not observed (2 x OH, guanidine NH, NH_2 and =NH). HRMS calcd for $C_{29}H_{33}N_5O_3$ (MH+) 500.265616; found 500.265356.

1-(3-((4bS,8R,8aS,15bR)-1,8a-dihydroxy-5,6,8,8a,9,15b-hexahydro-7H-4,8-methano-benzofuro[3,2-c]pyrido[3,4-b]acridin-7-yl)hexyl)guanidine tris(trifluoroacetate) (C6-Quino 3x TFA salt): 0.05 mmol scale, 36 mg trihydrochloride (quantitative), which was transformed to tris(trifluoroacetate) as described above yielding 41 mg (96%). ^{1}H NMR (400 MHz, cd_3od) δ 8.18 (s, 1H), 8.05 (d, *J* = 8.6 Hz, 1H), 7.87 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.77 (ddd, *J* = 8.5, 6.9, 1.4 Hz, 1H), 7.60 (ddd, *J* = 8.2, 6.9, 1.1 Hz, 1H), 6.80 – 6.69 (m, 2H), 5.74 (s, 1H), 3.98 (d, *J* = 6.6 Hz, 1H), 3.52 (d, *J* = 19.9 Hz, 1H), 3.41 (td, *J* = 12.0, 5.0 Hz, 1H), 3.29 – 3.25 (m, 1H), 3.25 – 3.12 (m, 4H), 3.09 (d, *J* = 16.7 Hz, 1H), 3.03 (dd, *J* = 13.0, 4.0 Hz, 1H), 2.96 – 2.88 (m, 1H), 2.82 (td, *J* = 13.4, 4.9 Hz, 1H), 1.99 (dd, *J* = 14.0, 4.0 Hz, 1H), 1.95 – 1.85 (m, 1H), 1.76 – 1.60 (m, 3H), 1.56 – 1.45 (m, 4H) (9) 6 protons were not observed (2 x OH, guanidine NH, NH_2 and =NH). ^{13}C NMR (100 MHz, cd_3od) δ 158.68, 154.04, 146.43, 145.07, 141.90, 141.29, 132.26, 129.67, 129.57, 129.21, 128.78, 128.30, 127.23, 122.50, 121.55, 119.87, 89.15, 72.11, 62.95, 54.60, 48.01, 47.21, 42.28, 36.91, 30.12, 29.66, 27.25, 27.21, 25.29, 24.58.

1-(3-((4bS,8R,8aS,15bR)-1,8a-dihydroxy-5,6,8,8a,9,15b-hexahydro-7H-4,8-methano-benzofuro[3,2-c]pyrido[3,4-b]acridin-7-yl)hexyl)guanidine trihydrochloride (C6-Quino 3x HCl salt): ^{1}H NMR (400 MHz, cd_3od) δ 8.77 (s, 1H), 8.26 (d, *J* = 8.7 Hz, 1H), 8.16 (d, *J* = 8.3 Hz, 1H), 8.13 – 8.04 (m, 1H), 7.88 (t, *J* = 7.5 Hz, 1H), 6.81 (d, *J* = 8.2 Hz, 1H), 6.73 (d, *J* = 8.2 Hz, 1H), 5.96 (s, 1H), 4.09 (d, *J* = 6.7 Hz, 1H), 3.57 (d, *J* = 19.9 Hz, 1H), 3.47 (td, *J* = 12.0, 5.2 Hz, 1H), 3.37 – 3.27 (m, 2H), 3.26 –3.15 (m, 4H), 3.11 (dd, *J* = 13.4, 4.1 Hz, 1H), 3.04 (d, *J* = 16.9 Hz, 1H), 2.87 (td, *J* = 13.4, 5.0 Hz, 1H), 2.07 (d, *J* = 12.5 Hz, 1H), 1.96 – 1.91 (m, 1H), 1.81 – 1.64 (m, 3H), 1.55 – 1.51 (m, 4H), 6 protons were not observed (2 x OH, guanidine NH, NH_2 and =NH). HRMS calcd for $C_{30}H_{35}N_5O_3$ (MH+) 514.281266; found 514.280867.

1-(3-((4bS,8R,8aS,15bR)-1,8a-dihydroxy-5,6,8,8a,9,15b-hexahydro-7H-4,8-methano-benzofuro[3,2-c]pyrido[3,4-b]acridin-7-yl)heptyl)guanidine tris(trifluoroacetate) (C7-Quino 3x TFA salt): 0.07 mmol scale, yielding 44.3 mg (quantitative) trihydrochloride, which was transformed to tris(trifluoroacetate) as described above yielding 60 mg (99%). ^{1}H NMR (400 MHz, cd_3od) δ 8.15 (s, 1H), 8.04 (d, *J* = 8.6 Hz, 1H), 7.86 (d, *J* = 8.2 Hz, 1H), 7.76 (ddd, *J* = 8.5, 6.9, 1.4 Hz, 1H), 7.59 (t, *J* = 7.6 Hz, 1H), 6.74 (q, *J* = 8.2 Hz, 2H), 5.73 (s, 1H), 3.98 (d, *J* = 6.6 Hz, 1H), 3.52 (d, *J* = 19.9 Hz, 1H), 3.40 (td, *J* = 12.0, 5.0 Hz, 1H), 3.27 (d, *J* = 6.1 Hz, 1H), 3.24 –

3.12 (m, 4H), 3.08 (d, *J* = 16.7 Hz, 1H), 3.02 (dd, *J* = 13.0, 3.9 Hz, 1H), 2.92 (d, *J* = 16.6 Hz, 1H), 2.82 (td, *J* = 13.4, 5.0 Hz, 1H), 1.99 (dd, *J* = 13.7, 3.6 Hz, 1H), 1.91 – 1.85 (m, 1H), 1.73 – 1.68 (m, 1H), 1.68 – 1.57 (m, 2H), 1.51 – 1.40 (m, 6H), 6 protons not observed (2 x OH, guanidine NH, NH_2 and =NH). ^{13}C NMR (100 MHz, cd_3od) δ 158.67, 154.11, 146.59, 145.09, 141.89, 141.08, 132.14, 129.69, 129.55, 129.14, 128.75, 128.26, 127.37, 122.51, 121.52, 119.85, 89.25, 72.12, 62.92, 54.67, 48.00, 47.21, 42.38, 36.93, 30.13, 29.87, 29.75, 27.52, 27.48, 25.36, 24.57.

1-(3-((4bS,8R,8aS,15bR)-1,8a-dihydroxy-5,6,8,8a,9,15b-hexahydro-7H-4,8-methano-benzofuro[3,2-c]pyrido[3,4-b]acridin-7-yl)heptyl)guanidine trihydrochloride (C7-Quino 3x HCl salt): ^1H NMR (400 MHz, cd_3od) δ 8.79 (s, 1H), 8.26 (d, *J* = 8.6 Hz, 1H), 8.17 (d, *J* = 8.3 Hz, 1H), 8.10 (t, *J* = 7.7 Hz, 1H), 7.89 (t, *J* = 7.6 Hz, 1H), 7.42 (s, 1H), 6.80 (d, *J* = 8.2 Hz, 1H), 6.73 (d, *J* = 8.2 Hz, 1H), 5.96 (s, 1H), 4.08 (d, *J* = 6.7 Hz, 1H), 3.57 (d, *J* = 20.0 Hz, 1H), 3.45 (dd, *J* = 17.2, 7.7 Hz, 1H), 3.35 (s, 1H), 3.28 (s, 1H), 3.26 – 3.07 (m, 5H), 3.04 (d, *J* = 16.6 Hz, 1H), 2.86 (td, *J* = 13.5, 4.9 Hz, 1H), 2.08 (d, *J* = 12.7 Hz, 1H), 1.92 (s, 1H), 1.73 (s, 1H), 1.69 – 1.58 (m, 2H), 1.50 (s, 6H), 6 protons were not observed (2 x OH, guanidine NH, NH_2 and =NH). HRMS calcd for $C_{31}H_{37}N_5O_3$ (MH+) 528.296917; found 528.297076.

(4bS,8R,8aS,15bR)-7-(6-aminooctyl)-5,6,7,8,9,15b-hexahydro-8aH-4,8-methanobenzofuro [3,2-c]pyrido[3,4-b]acridine-1,8a-diol tris(trifluoroacetate) (C8-Quino Amine 3x TFA salt): 0.05 mmol scale, gave 30.3 mg (quantitative) trihydrochloride, which was transformed to tris(trifluoroacetate) as described above yielding 40.9 mg (97%) tris(trifluoroacetate). ^1H NMR (400 MHz, cd_3od) δ 8.18 (s, 1H), 8.08 (d, *J* = 8.6 Hz, 1H), 7.89 (d, *J* = 8.2 Hz, 1H), 7.80 (ddd, *J* = 8.4, 6.9, 1.4 Hz, 1H), 7.63 (ddd, *J* = 8.1, 6.9, 1.2 Hz, 1H), 6.75 (d, *J* = 8.2 Hz, 1H), 6.71 (d, *J* = 8.2 Hz, 1H), 5.72 (s, 1H), 3.97 (d, *J* = 6.6 Hz, 1H), 3.52 (d, *J* = 19.8 Hz, 1H), 3.41 (td, *J* = 12.2, 5.0 Hz, 1H), 3.30 – 3.00 (m, 5H), 2.96 (d, *J* = 7.9 Hz, 1H), 2.94 – 2.87 (m, 2H), 2.82 (td, *J* = 13.4, 5.0 Hz, 1H), 2.01 (d, *J* = 17.5 Hz, 1H), 1.88 (s, 1H), 1.72 – 1.65 (m, 3H), 1.49 – 1.41 (m, 8H), 4 protons were not observed (2 x OH and NH_2). ^{13}C NMR (100 MHz, cd_3od) δ 154.13, 146.88, 144.92, 141.68, 140.42, 131.73, 129.51, 129.37, 128.85, 128.50, 127.98, 127.60, 122.24, 121.21, 119.63, 89.31, 71.93, 62.78, 54.48, 47.83, 47.00, 40.48, 36.80, 29.95, 29.88, 29.86, 28.36, 27.32, 27.18, 25.20, 24.35.

(4bS,8R,8aS,15bR)-7-(6-aminooctyl)-5,6,7,8,9,15b-hexahydro-8aH-4,8-methanobenzofuro [3,2-c]pyrido[3,4-b]acridine-1,8a-diol trihydrochloride (C8-Quino Amine 3x HCl salt): ^1H NMR (400 MHz, cd_3od) δ 8.70 (s, 1H), 8.22 (d, *J* = 8.7 Hz, 1H), 8.12 (d, *J* = 8.4 Hz, 1H), 8.09 – 8.00 (m, 1H), 7.84 (t, *J* = 7.6 Hz, 1H), 6.77 (d, *J* = 8.2 Hz, 1H), 6.70 (d, *J*

= 8.2 Hz, 1H), 5.91 (s, 1H), 4.04 (d, *J* = 6.7 Hz, 1H), 3.53 (d, *J* = 19.9 Hz, 1H), 3.48 – 3.36 (m, 1H), 3.35 – 3.21 (m, 3H), 3.17 – 3.04 (m, 1H), 3.01 (d, *J* = 16.9 Hz, 1H), 2.96 – 2.85 (m, 3H), 2.82 (dd, *J* = 13.4, 5.0 Hz, 1H), 2.04 (d, *J* = 13.1 Hz, 1H), 1.89 (s, 1H), 1.68 – 1.62 (m, 4H), 1.48 – 1.41 (m, 6H)), 4 protons were not observed (2 x OH and NH_2). HRMS calcd for $C_{31}H_{37}N_3O_3$ (MH+) 500.290769; found 500.290843.

Preparation of C6-urea and C8 quinoline derivatives (Supplementary Fig. 6.)

phenyl (6-hydroxyhexyl)carbamate (**27**): 6-aminohexanol (1.17 g, 10 mmol, 1.0 equiv.) was dissolved in anhydrous THF (40 mL) under argon and cooled to 0 °C. Phenyl chloroformate (1.56 g, 10 mmol, 1.0 equiv.) was added dropwise and the mixture was allowed to warm to room temperature. After stirring for one hour, the mixture was cooled to -78 °C and quenched with the addition of few drops of 7M NH_3 in MeOH. The solvents were removed on rotary evaporator and the crude was purified on silica using 0.5% to 10% MeOH in DCM as eluent. 1.19 g, 74%. ^{1}H NMR (400 MHz, cdcl$_3$) δ 7.33 (t, *J* = 7.7 Hz, 2H), 7.17 (t, *J* = 7.4 Hz, 1H), 7.13 – 7.07 (m, 2H), 5.17 (t, *J* = 6.0 Hz, 1H), 3.60 (t, *J* = 6.5 Hz, 2H), 3.23 (q, *J* = 6.7 Hz, 2H), 1.54 (m, *J* = 6.8 Hz, 4H), 1.36 (hept, *J* = 3.7 Hz, 4H). ^{13}C NMR (100 MHz, cdcl$_3$) δ 155.94, 152.25, 130.46, 126.43, 122.80, 63.87, 42.26, 33.72, 30.96, 27.61, 26.52.

phenyl (6-oxohexyl)carbamate (**28**): 1.7 g of (23) was dissolved in DCM (50 mL) containing 5 g silica and pyridinium chlorochromate (PCC, 1.54 g, 7.2 mmol, 1.0 equiv.) was added as a solution in DCM at 0 °C. The mixture was then allowed to warm to room temperature and stirred for 3 hours. Excess PCC was consumed by the addition of isopropyl alcohol. The mixture was then filtered through a pad of celite, the solvent was removed on rotary evaporator and the crude product was run through a small silica column using 0.5% to 10% MeOH gradient as solvent to remove colored impurities. The mixture of aldehyde and alcohol was used without further purification.

phenyl(6-((4bS,8R,8aS,15bR)-1,8a-dihydroxy-5,6,8,8a,9,15b-hexahydro-7H-4,8-methano-benzofuro[3,2-c]pyrido[3,4-b]acridin-7-yl)hexyl)carbamate (**29**) was prepared using the reductive amination protocol on a 0.3 mmol scale, 108 mg (61%). ^{1}H NMR (400 MHz, cdcl$_3$) δ 8.01 (d, *J* = 8.4 Hz, 1H), 7.89 (d, *J* = 5.3 Hz, 1H), 7.73 (d, *J* = 8.1 Hz, 1H), 7.66 (t, *J* = 7.6 Hz, 1H), 7.51 (t, *J* = 7.5 Hz, 1H), 7.34 (t, *J* = 7.6 Hz, 2H), 7.19 (q, *J* = 7.9 Hz, 1H), 7.10 (d, *J* = 7.9 Hz, 2H), 6.64 (q, *J* = 8.1 Hz, 2H), 5.57 (s, 1H), 3.38 – 3.19 (m, 2H), 3.22(s, 1H), 3.17 (s, 1H), 2.84 (d, *J* = 6.2 Hz, 2H), 2.78 (d, *J* = 16.9 Hz, 1H), 2.67 (d, *J* = 6.9 Hz, 1H), 2.58 (t, *J* = 7.2 Hz, 2H), 2.41

(s, 2H), 1.84 (d, *J* = 9.6 Hz, 1H), 1.62 – 1.53 (m, 5H), 1.42 (d, *J* = 6.4 Hz, 4H). ^{13}C NMR (100 MHz, cdcl$_3$) 155.28, 154.02, 150.89, 146.49, 143.17, 139.06, 137.24, 129.84, 129.55, 129.07, 128.16, 127.91, 127.45, 127.15, 127.04, 125.09, 123.85, 121.48, 119.51, 117.37, 89.63, 71.43, 62.10, 54.10, 46.96, 43.43, 40.71, 35.77, 31.73, 29.43, 27.07, 26.62, 26.31, 23.10.

1-(6-((4bS,8R,8aS,15bR)-1,8a-dihydroxy-5,6,8,8a,9,15b-hexahydro-7H-4,8-methano-benzofuro[3,2-c]pyrido[3,4-b]acridin-7-yl)hexyl)urea (**30**): 58 mg (25) (0.98 mmol, 1.0 equiv)) was dissolved in methanol (0.5 mL) and to this solution NH_4Cl (17 mg, 0.3 mmol, 3.0 equiv) and 7 M NH_3 in methanol (7 mL) was added. The mixture was stirred at room temperature for 7 days or until completion of the reaction and then DCM (30 mL) was added, and the solids removed by filtration. The remainder of ammonium chloride was removed by passing the crude product through a short pad of silica using 15% MeOH in DCM as eluent, yielding 503 mg (quantitative) product. ^{1}H NMR (400 MHz, cd$_3$od) δ 8.20 (s, 1H), 8.06 (d, *J* = 8.6 Hz, 1H), 7.88 (d, *J* = 8.2 Hz, 1H), 7.78 (ddd, *J* = 8.5, 6.9, 1.4 Hz, 1H), 7.61 (t, *J* = 7.6 Hz, 1H), 6.78 – 6.66 (m, 2H), 5.72 (s, 1H), 3.95 (d, *J* = 6.7 Hz, 1H), 3.48 (s, 1H), 3.46 – 3.34 (m, 1H), 3.29 – 3.18 (m, 2H), 3.18 – 2.98 (m, 5H), 2.93 (d, *J* = 16.6 Hz, 1H), 2.80 (td, *J* = 13.4, 4.9 Hz, 1H), 2.04 – 1.95 (m, 1H), 1.86 (s, 1H), 1.69 (s, 1H), 1.57 – 1.42 (m, 6H). ^{13}C NMR (100 MHz, cd$_3$od) δ 162.36, 154.12, 146.58, 145.10, 141.93, 141.24, 132.27, 129.67, 129.62, 129.24, 128.79, 128.30, 127.35, 122.49, 121.53, 119.87, 89.20, 72.13, 62.89, 54.60, 48.10, 47.24, 40.55, 36.96, 30.93, 30.15, 27.21, 27.08, 25.28, 24.57. HRMS calcd for $C_{30}H_{34}N_4O_4$ (MH+) 515.265282; found 515.264807.

(4bS,8R,8aS,15bR)-7-octyl-5,6,7,8,9,15b-hexahydro-8aH-4,8-methanobenzofuro[3,2-c] pyrido[3,4-b]acridine-1,8a-diol (**31**) was prepared as described in the generic reductive amination protocol on a 0.2 mmol scale, yielding 43.5 mg (45%) product. ^{1}H NMR (400 MHz, cd$_3$od) δ 8.11 (s, 1H), 8.03 (d, *J* = 8.6 Hz, 1H), 7.84 (d, *J* = 8.2 Hz, 1H), 7.74 (ddd, *J* = 8.5, 6.8, 1.4 Hz, 1H), 7.58 (dd, *J* = 8.3, 6.8 Hz, 1H), 6.77 – 6.66 (m, 2H), 5.69 (s, 1H), 3.94 (d, *J* = 6.7 Hz, 1H), 3.47 (s, 1H), 3.39 (td, *J* = 12.2, 5.1 Hz, 1H), 3.30 – 3.17 (m, 2H), 3.17 – 2.97 (m, 3H), 2.92 (d, *J* = 16.6 Hz, 1H), 2.79 (td, *J* = 13.4, 5.0 Hz, 1H), 1.98 (dd, *J* = 13.5, 3.6 Hz, 1H), 1.86 (q, *J* = 7.9 Hz, 1H), 1.67 (s, 1H), 1.43 (m, 3H), 1.39 (dd, *J* = 17.6, 6.3 Hz, 4H), 1.32 (m, *J* = 4.9 Hz, 4H), 0.94 – 0.86 (m, 3H). ^{13}C NMR (100 MHz, cd$_3$od) δ 154.14, 146.94, 144.93, 141.68, 140.30, 131.65, 129.53, 129.33, 128.80, 128.47, 127.93, 127.65, 122.25, 121.21, 119.63, 89.36, 71.92, 62.74, 54.51, 47.85, 47.01, 36.81, 32.70, 30.05, 30.01, 29.97, 27.40, 25.23, 24.35, 23.49, 14.2. HRMS calcd for $C_{31}H_{36}N_2O_3$ (MH+) 485.279869; found 485.279342.

Definition of Polar Contacts in the MD Study

Polar contacts are defined as follows. We considered a protein sidechain is making direct interactions with the ligand, including direct salt bridge and/or direct hydrogen bond, when the closest distance among heavy atoms of the ligand and the protein sidechain is less than 3.2 angstrom, otherwise, the two moieties make no polar contact. Similarly, we considered a water-mediated interaction is formed when a water is bridging between the ligand and a protein sidechain, i.e. when the following conditions are simultaneously satisfied – (1) the oxygen of the water is located within 3.2 angstrom in closest distance to the ligand and (2) the oxygen of the water is located within 3.2 angstrom in closest distance to the protein sidechain. For simplicity, consistent with similar analysis in the literature [1], the angular requirement of a hydrogen bond is neglected. The direct interaction and the water-mediated interaction between the ligand and a specific protein sidechain is not mutually exclusive; further, the water bridge can be supported by another supplementary protein residue. (See Fig3C, Fig S15 A, B). To report systematically how the polar contact occurred, we categorize the interaction with 3 yes/no conditions for each protein residue in a group of residues, where "Self" means the specific sidechain (e.g. D95) and "NonSelf" means the rest of the residues in the group (e.g. N131/N310/S135/S311 in the sodium pocket).

(a) There are direct interaction(s) between the ligand and the specific protein sidechain.

(b) There are "Self" water-mediated interaction(s) between the ligand and the specific protein sidechain.

(c) There are "NonSelf" water-mediated interaction(s) between the ligand and the other protein sidechain(s) in the group.

The three yes/no conditions above can be divided into 8 fine categories (See Supplementary Table 8).

(I) No Polar Interaction (a:No & b:No & c:No)

(II) WaterMediated Only (NonSelf) (a:No & b:No & c:Yes)

(III) WaterMediated Only (Self) (a:No & b:Yes & c:No)

(IV) WaterMediated Only (Self+NonSelf) (a:No & b:Yes & c:Yes)

(V) Direct Only (a:Yes & b:No & c:No)

(VI) Direct + WaterMediated (Self) (a:Yes & b:Yes & c:No)

(VII) Direct + WaterMediated (NonSelf) (a:Yes & b:No & c:Yes)

(VIII) Direct + WaterMediated (Self+NonSelf) (a:Yes & b:Yes & c:Yes)

The 8 fine categories (I-VIII) above can be further grouped into 3 coarse categories. (See Supplementary Table 7).

- "No Polar Contact" includes fine category (I-II), meaning no polar interactions, direct nor water-mediated interactions, from the specific protein sidechain indicated as "Self" involved. i.e. condition (a) and (b) are all negative.

- "WaterMediated Only" includes fine categories (III-IV), meaning only water-mediated interactions with the specific protein sidechain indicated as "Self" is involved; there are no direct interaction with "Self" in this category. i.e. condition (a) is negative but condition (b) is positive

"Direct Contact" includes fine categories (V-VIII), meaning direct interactions with the specific protein sidechain indicated as "Self" is involved. i.e. condition (a) is positive.

Note that both "WaterMediated Only" interactions and "Direct Contact" are attributes of "Polar Contact" i.e. including fine category (III-VIII); "No Polar Contact" applies to situations where neither "WaterMediated Only" nor "Direct Contact" apply. The statistics of these categories were collected from all the trajectories available, where analysis was performed every 0.5 ns contributing 2000 frames per trajectory. We also calculated the occupancy of each water-mediated interaction to show the cumulative length (i.e. the total amount of time in the simulation) where a water-mediated interaction is found between the ligand and each specific protein residue. While a bridging water can exchange rapidly with the bulk solvent, water-mediated interaction can form again rapidly in some protein residues. To report the contiguity of these water-mediated interactions, we measured the autocorrelation function C(t), which quantifies the probability of finding any water molecule, regardless of identity, that bridges the ligand and a specific protein residue at an initial time t0 and a time t later. To give a proxy on the equilibrium probability C(0) to find at least one water bridge, we followed an example from the literature [1], and fitted the observed C(t) to a single exponential $C(t) = \rho e^{-t/\tau}$, with parameter correlation time τ and population ρ, using the scipy.optimize.curve_fit function, where the observed C(t) was bootstrapped for 500 replicates of individual trajectories drawn with replacement to show an

estimate of the standard deviation. The population ρ obtained is an estimate of C(0) and it is reported in percentage. Finally, the root mean square deviation (r.m.s.d.) of the ligand is calculated using the heavy atoms of the ligands from frames aligned using the C-alpha of the receptor excluding the ligand. Molecular graphics are visualized using PyMOL v2.4.1 [2].

Molecular Dynamics Checklist

Reliability and reproducibility checklist for molecular dynamics simulations ***All boxes must be marked YES by acceptance unless an N/A option is available**	**Yes**	**N/A**	**Response (Please state where this information can be found in the text)**
1. Convergence of simulations and analysis			
1a. Is an evaluation presented in the text to show that the property being measured has equilibrated in the simulations (*e.g.* time-course analysis)?	☒		It is stated in "Materials and Methods" section "Molecular Dynamics Simulations"
1b. Then, is it described in the text how simulations are split into equilibration and production runs and how much data were analyzed from production runs?	☒		It is stated in "Materials and Methods" section "Molecular Dynamics Simulations"
1c. Are there at least 3 simulations per simulation condition with statistical analysis?	☒		It is stated in "Results" section "CryoEM structures of δOR bound to bitopic ligands". It is also detailed in "Materials and Methods" section "Molecular Dynamics Simulations"
1d. Is evidence provided in the text that the simulation results presented are independent of initial configuration?	☒		It is stated in "Materials and Methods" section "Molecular Dynamics Simulations"
2. Connection to experiments			
2a. Are calculations provided that can connect to experiments (*e.g.* loss or gain in function from mutagenesis, binding assays, NMR chemical shifts, J-couplings, SAXS curves, interaction distances or FRET distances, structure factors, diffusion coefficients, bulk modulus and other mechanical properties, *etc*.)?	☒		It is stated in "Results" section "CryoEM structures of δOR bound to bitopic ligands"
3. Method choice			

3a. Is it described in the text what force field and water model are used and why?	☒		It is stated in "Materials and Methods" section "Molecular Dynamics Simulations"
3b. Do simulations contain membranes, membrane proteins, intrinsically disordered proteins, glycans, nucleic acids, polymers, or cryptic ligand binding?	☒	☐	Response not needed if **N/A**
If 3b is **YES**, are enhanced sampling methods used?	☐	☒	Response not needed if **N/A**
If enhanced sampling methods are used, are the convergence criteria clearly stated?	☐	N/A	Enhanced sampling was not used.
If 3b is **YES**, is it explained in the text why or why not enhanced sampling methods are used?	☐	N/A	No, it is not explained in the text why enhanced sampling are not used.
4. Code and reproducibility			
4a. Is a table provided describing the system setup, such as simulation box dimensions, total number of atoms, total number of water molecules, salt concentration, lipid composition (number of molecules and type)?	☒		It is stated in SI Table S7.
4b. Is it described in the text what simulation and analysis software and which versions are used?	☒		It is stated in "Materials and Methods" section "Molecular Dynamics Simulations"
4c. Are initial coordinate and simulation input files and a coordinate file of the final output provided as supplementary files or in a public repository?	☒		It is stated in "Data Availability" section
4d. Is there custom code or custom force field parameters?	☐	☒	Response not needed if **N/A**
If **YES**, are they provided as supplementary profiles or in a public repository?	☐	N/A	

LC-MS/MS Settings

LC (ExionLC AD - HPLC system):

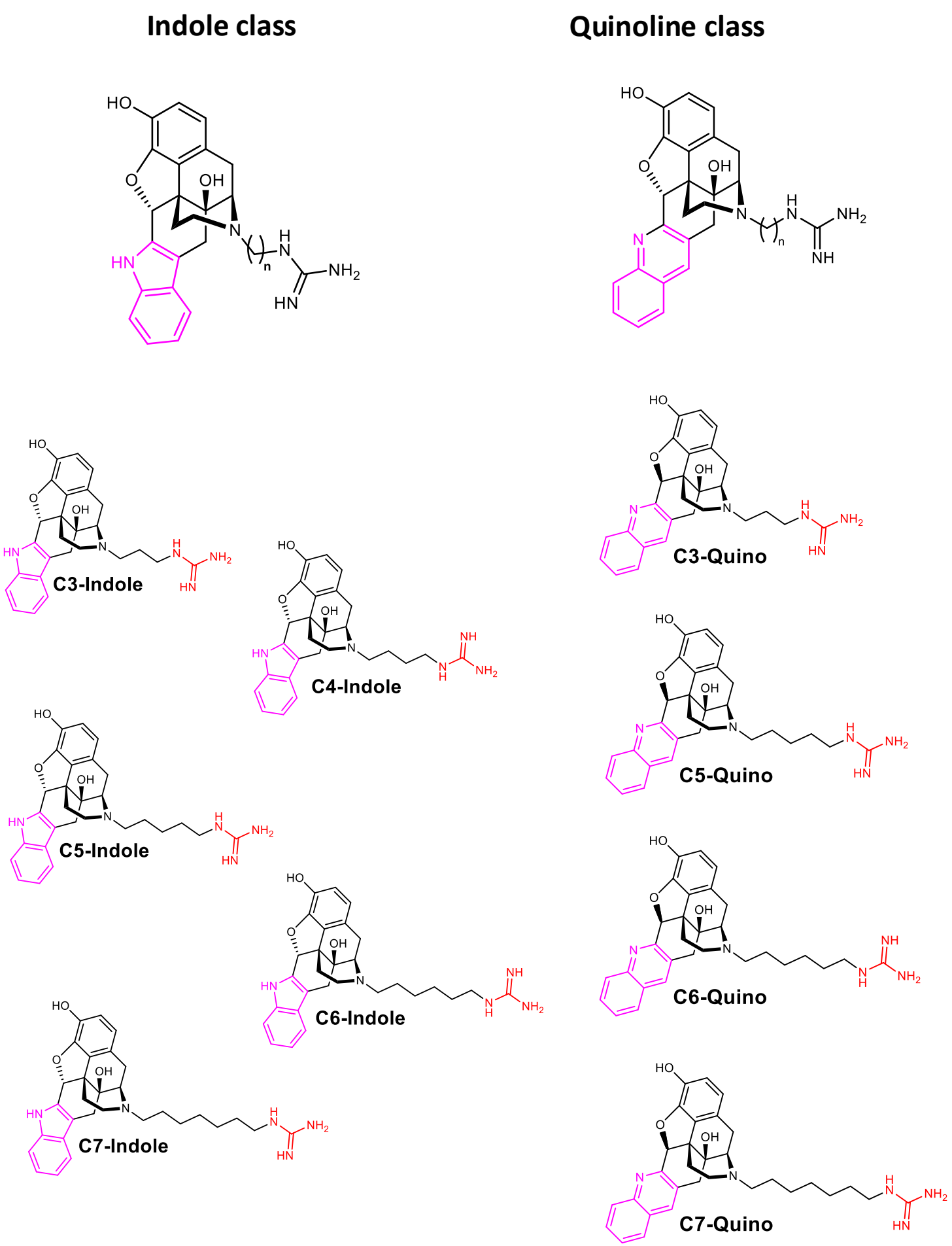
Column:	Armor C18 Analytical Column. 120Â, 5µm, 5cm x 2.1mm ID	Gradient elution conditions:	
Column Temperature (°C)	30	Time (min)	% MPB
Mobile Phase A	Water +0.1% Formic Acid	0.00	5
Mobile Phase B	Acetonitrile : Methanol (1:1) +0.1% Formic Acid	1.00	5

Flow (ml/min)	1	2.00	95
Run time total (min)	5.5	3.50	95
Injection volume(µl)	10	3.60	5
		5.50	5

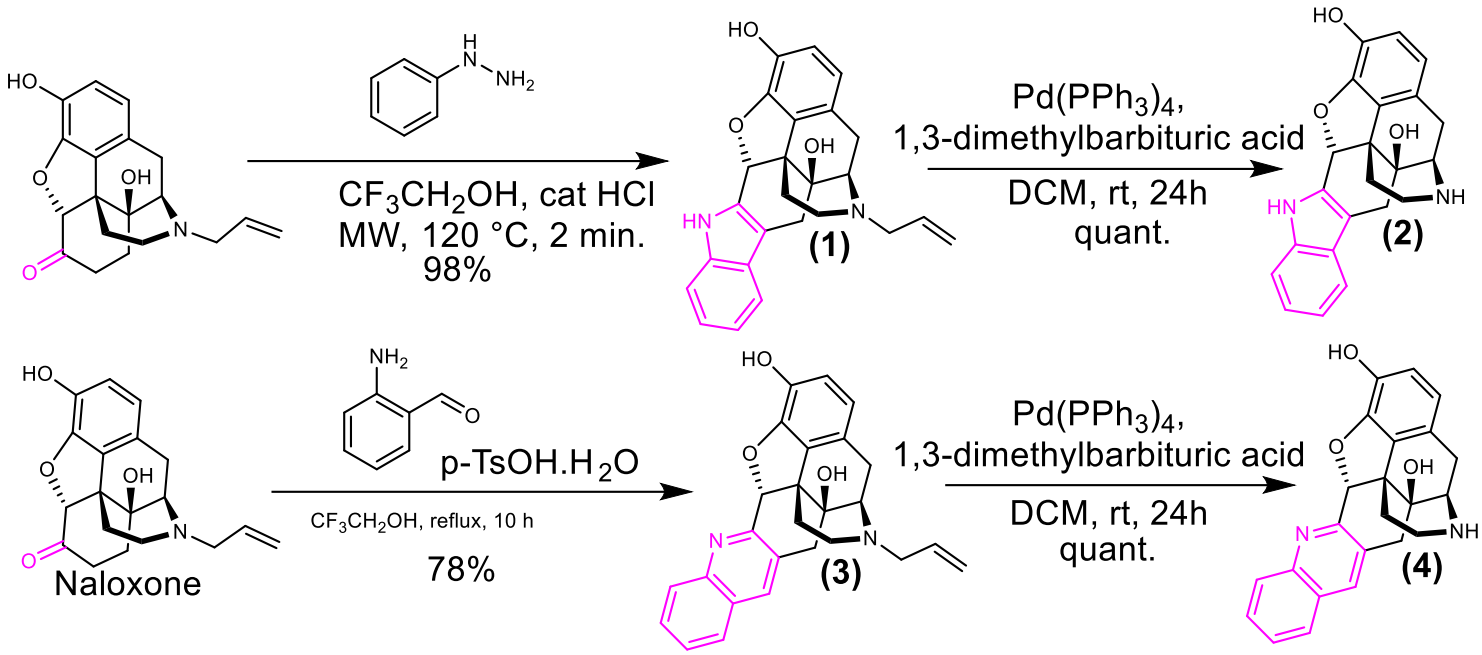
MS/MS (SCIEX Triple Quad 5500+ system – QTRAP Ready):

Ionization Method:	Electrospray	
Scan Type	MRM	
Polarity:	Positive	
Resolution:	Unit/Unit	
Source/Gas Parameter		
Curtain Gas (CUR)	30	
Collision Gas (CAD)	8	
IonSpray Voltage (IS)	1800	
Source Temperature (°C):	600	
Ion Source Gas1 (GS1)	50	
Ion Source Gas2 (GS2)	50	
Compound Parameters		
Compound:	C6-Quino	IS (Glyburide)
Transitions (m/z):	514.2/479.4 Da	494.1/369.1 Da
Declustering Potential (volts)	126	104
Entrance Potential (volts)	10	10
Collision Energy (volts)	47	23
Collision Cell Exit Potential (volts)	15	10

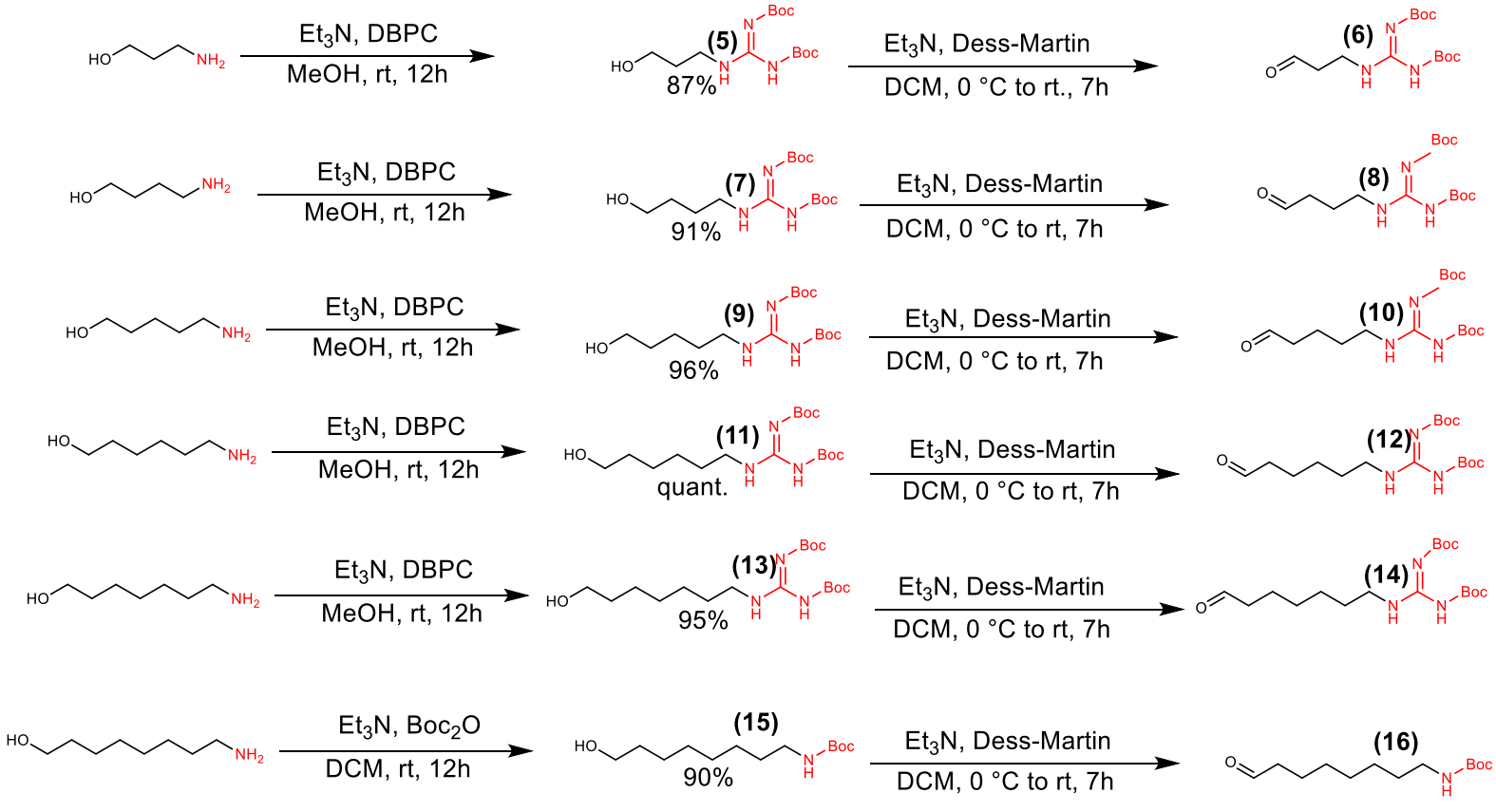
1. Cyphers, S., Ruff, E. F., Behr, J. M., Chodera, J. D. & Levinson, N. M. A water-mediated allosteric network governs activation of Aurora kinase A. *Nat Chem Biol* **13**, 402–408 (2017).
2. PyMOL, The PyMOL Molecular Graphics System, Version 2.4.1 Schrödinger, Inc.



Supplementary Fig. 1. Chemical structures of guano substituted compounds examined in this study

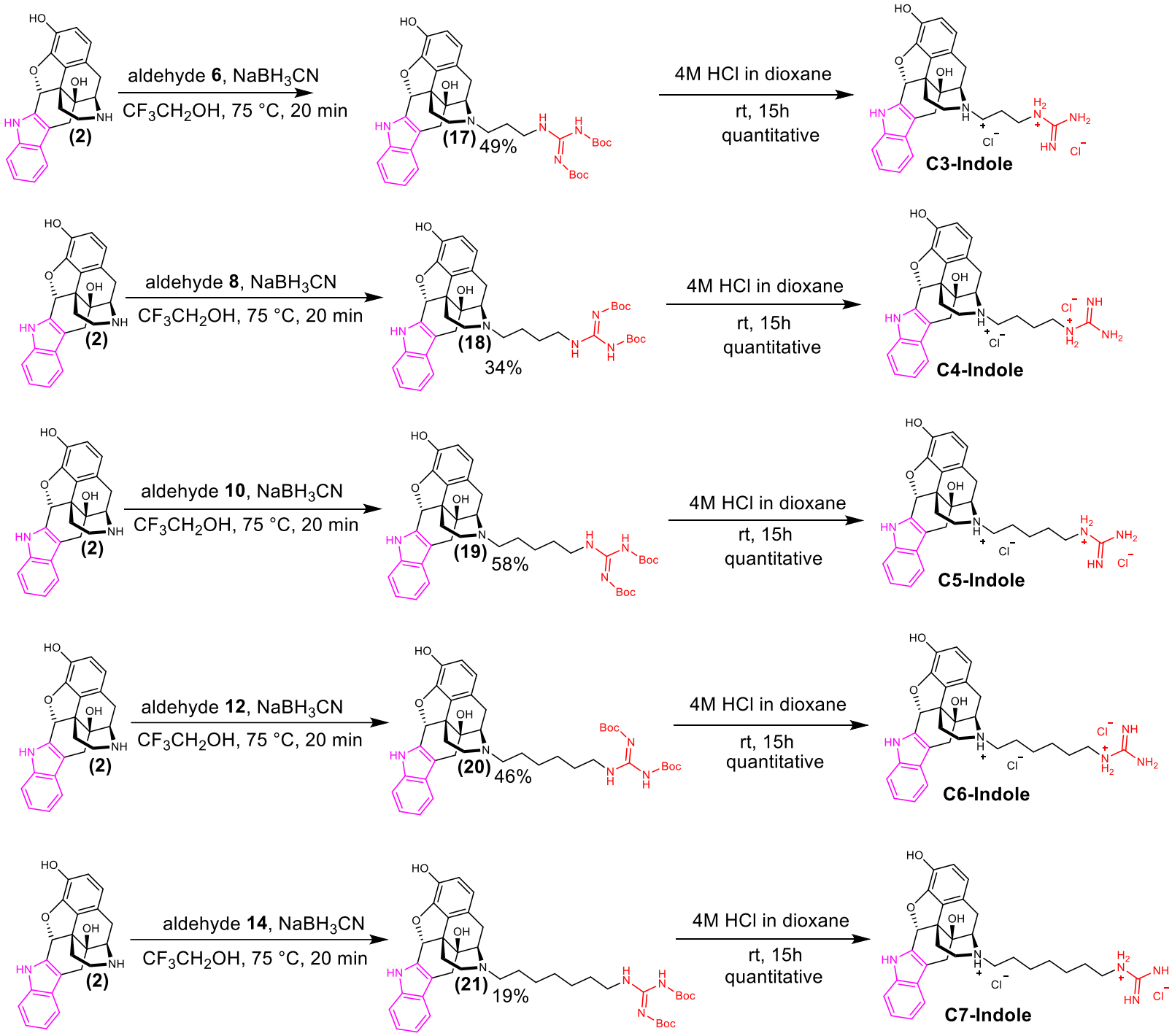


Supplementary Fig. 2. Synthesis of indole and quinoline cores from commercial naloxone.

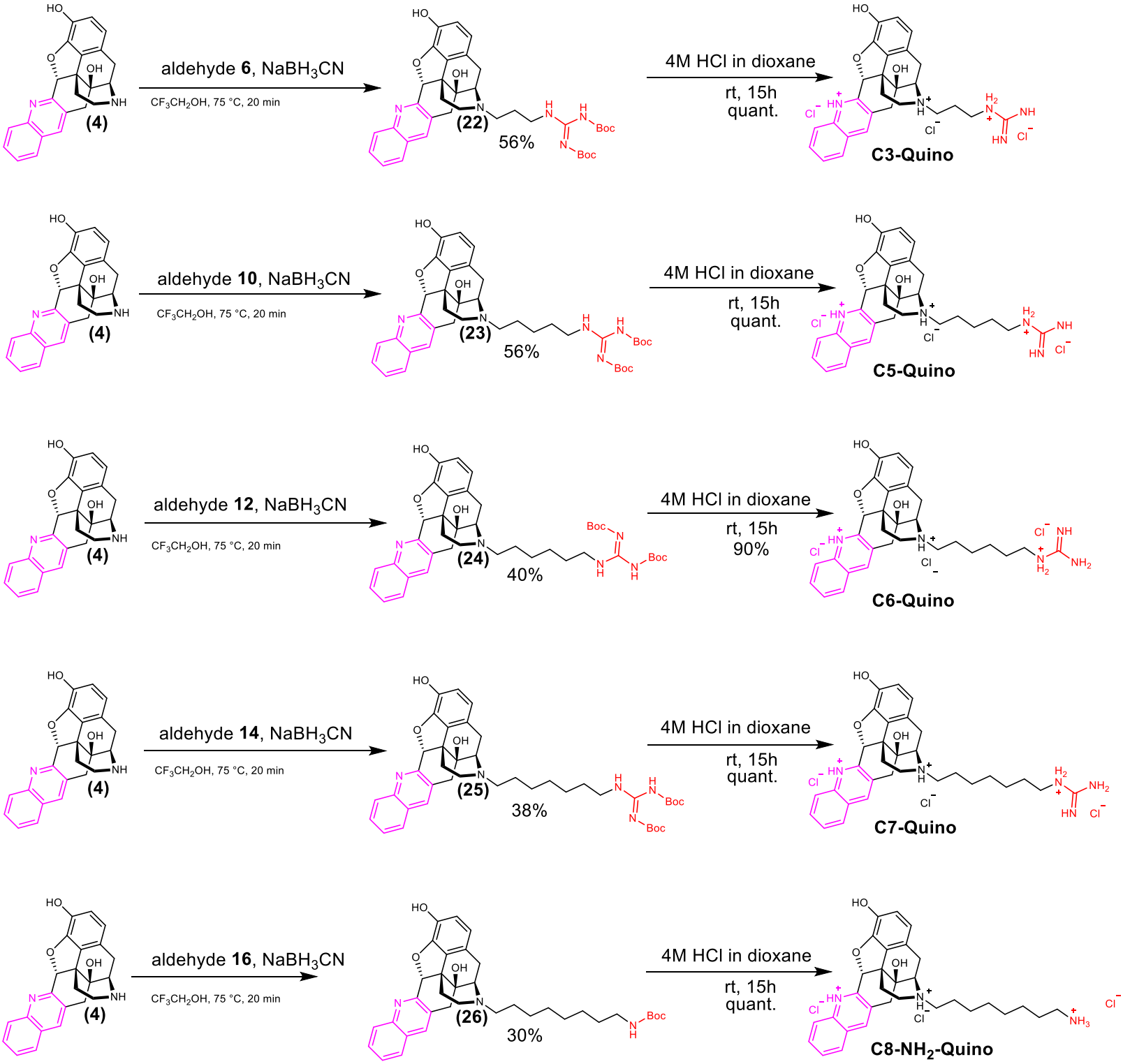


DBPC = N,N′-Di-Boc-1H-pyrazole-1-carboxamidine

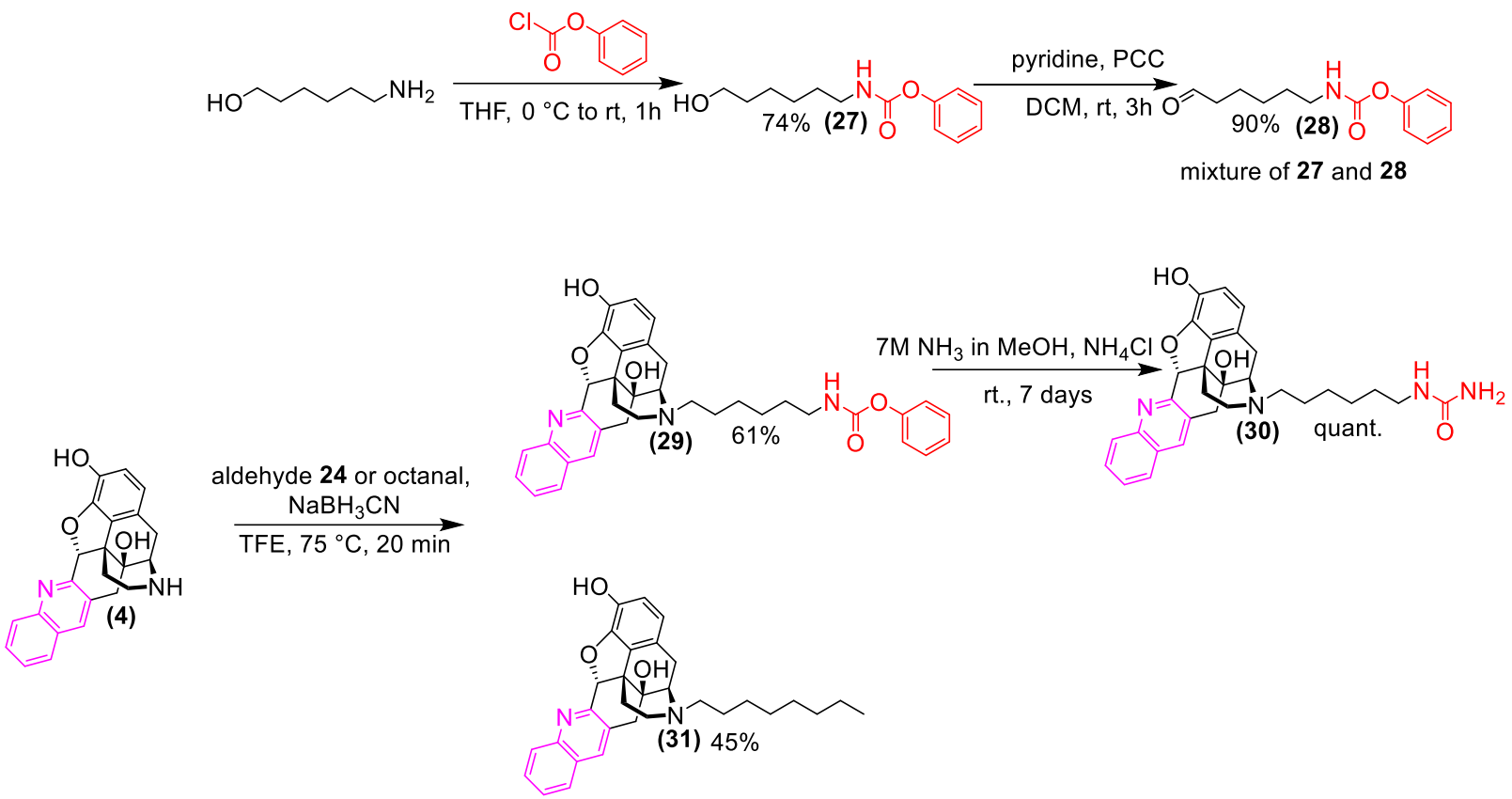
Supplementary Fig. 3. Synthesis of boc-protected aldehydes



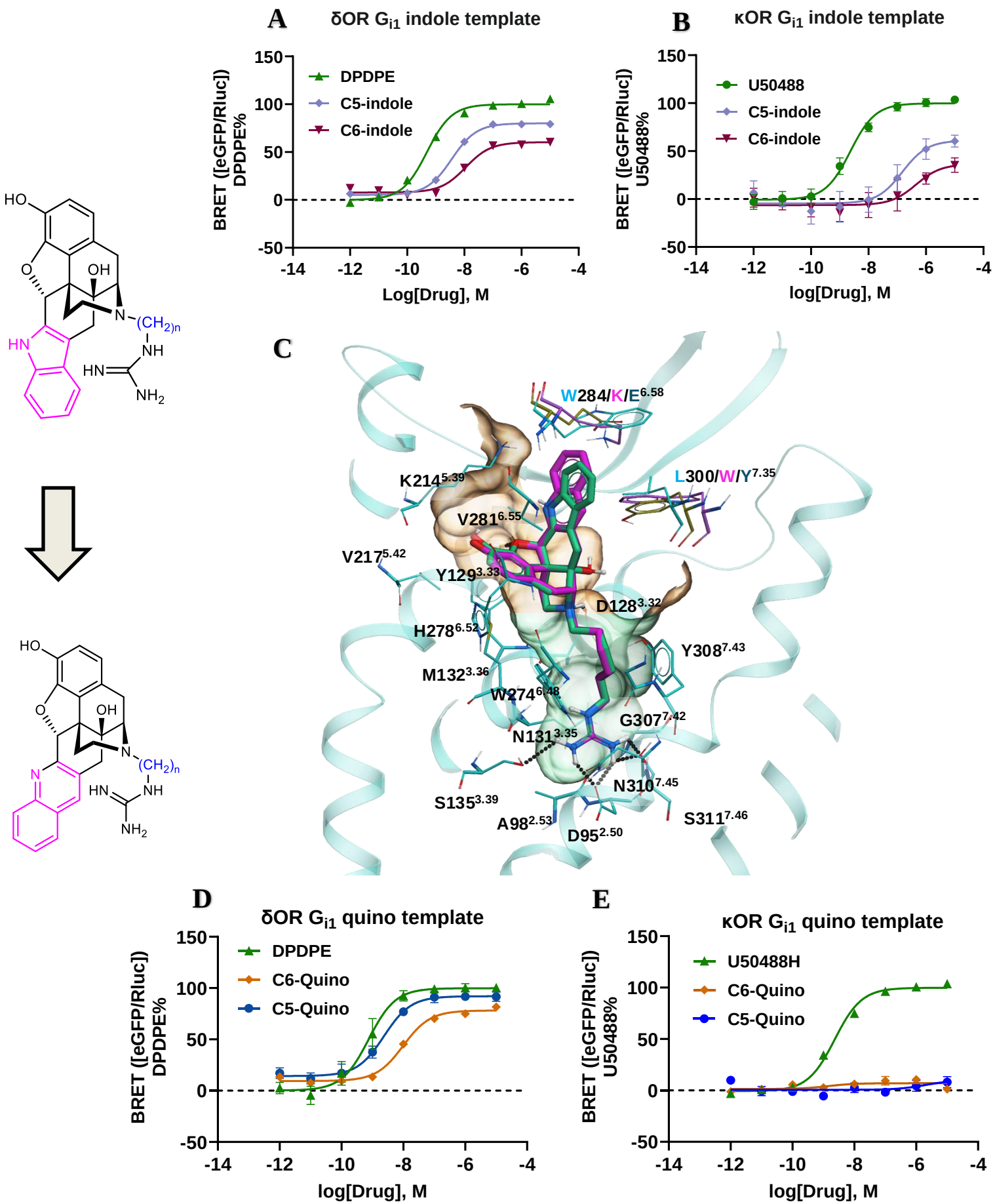
Supplementary Fig. 4. Synthesis of bitopic compounds on the indole core



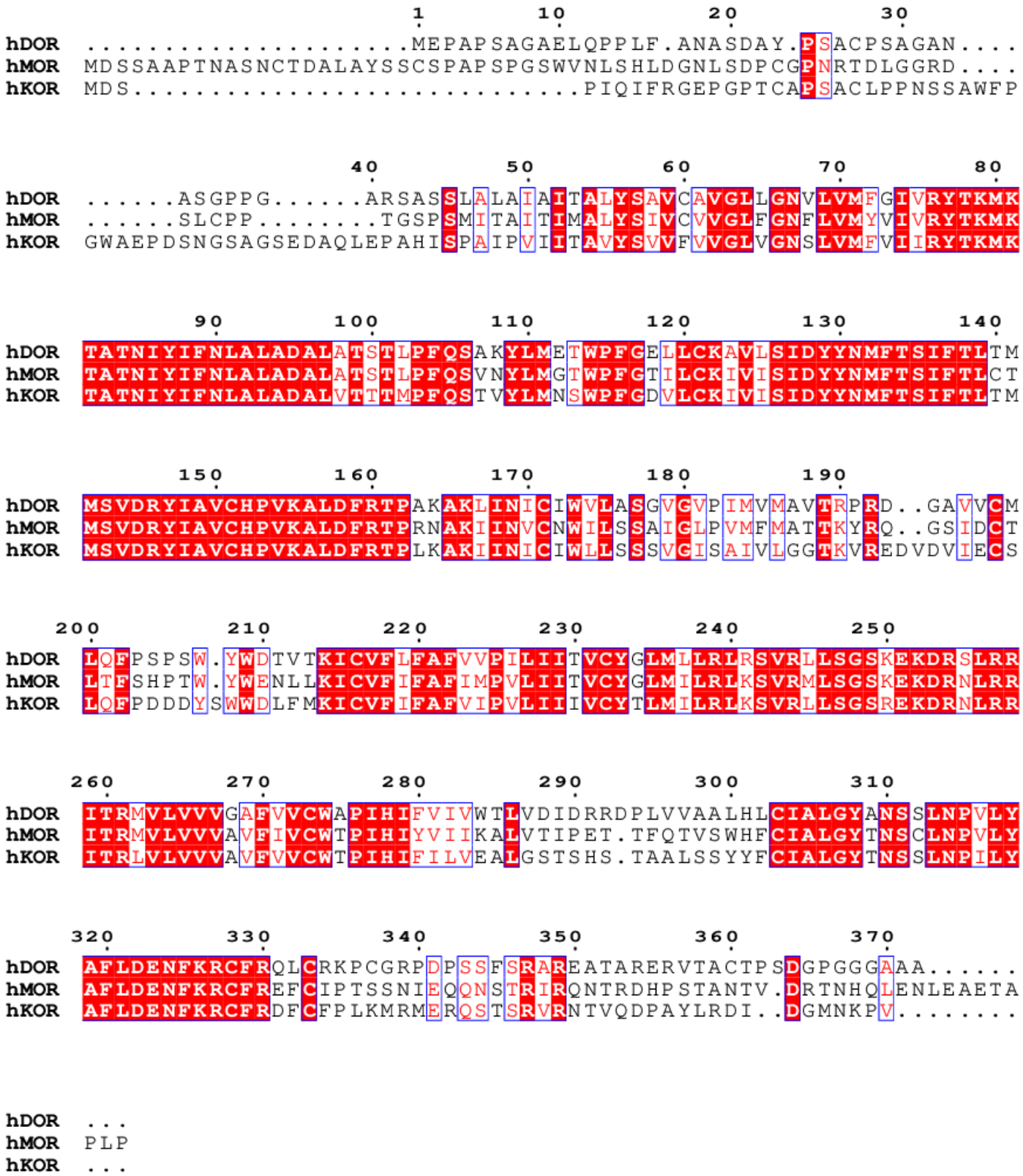
Supplementary Fig. 5. Synthesis of bitopic compounds on the quinoline core



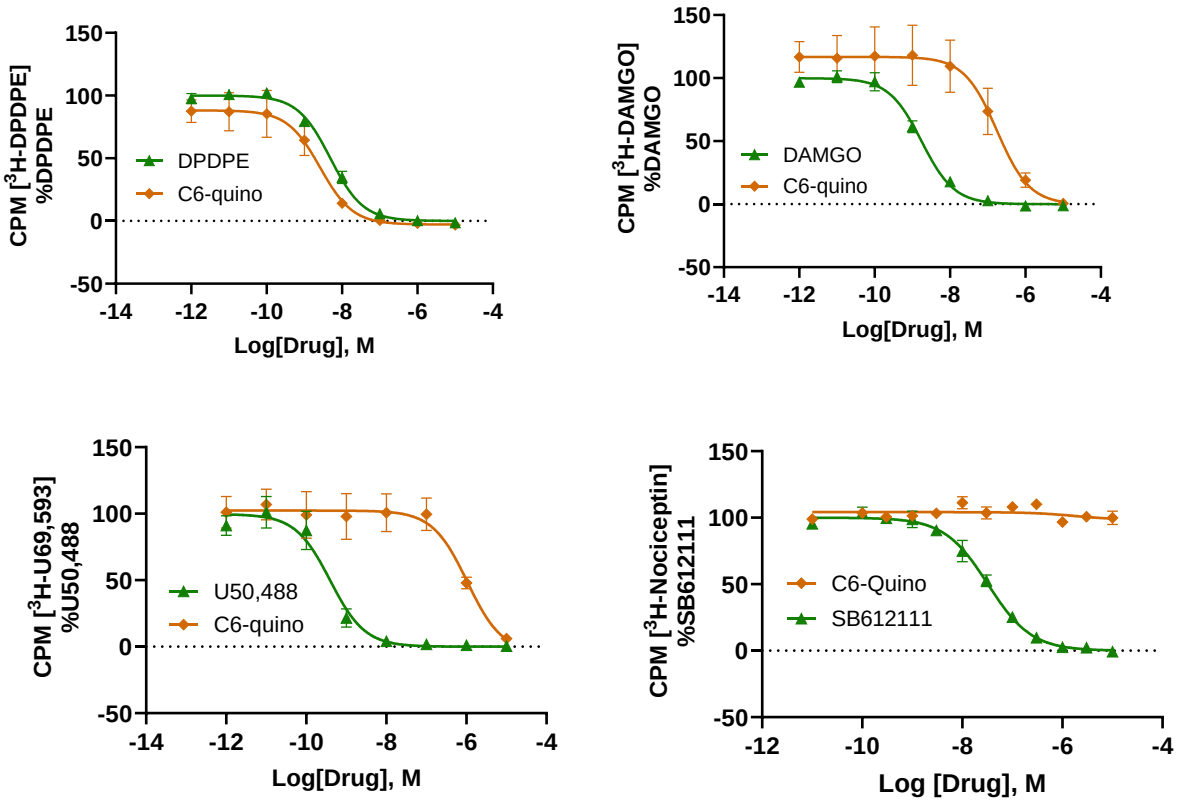
Supplementary Fig. 6. Synthesis of bitopics with C6-Urea and C8 “warheads”



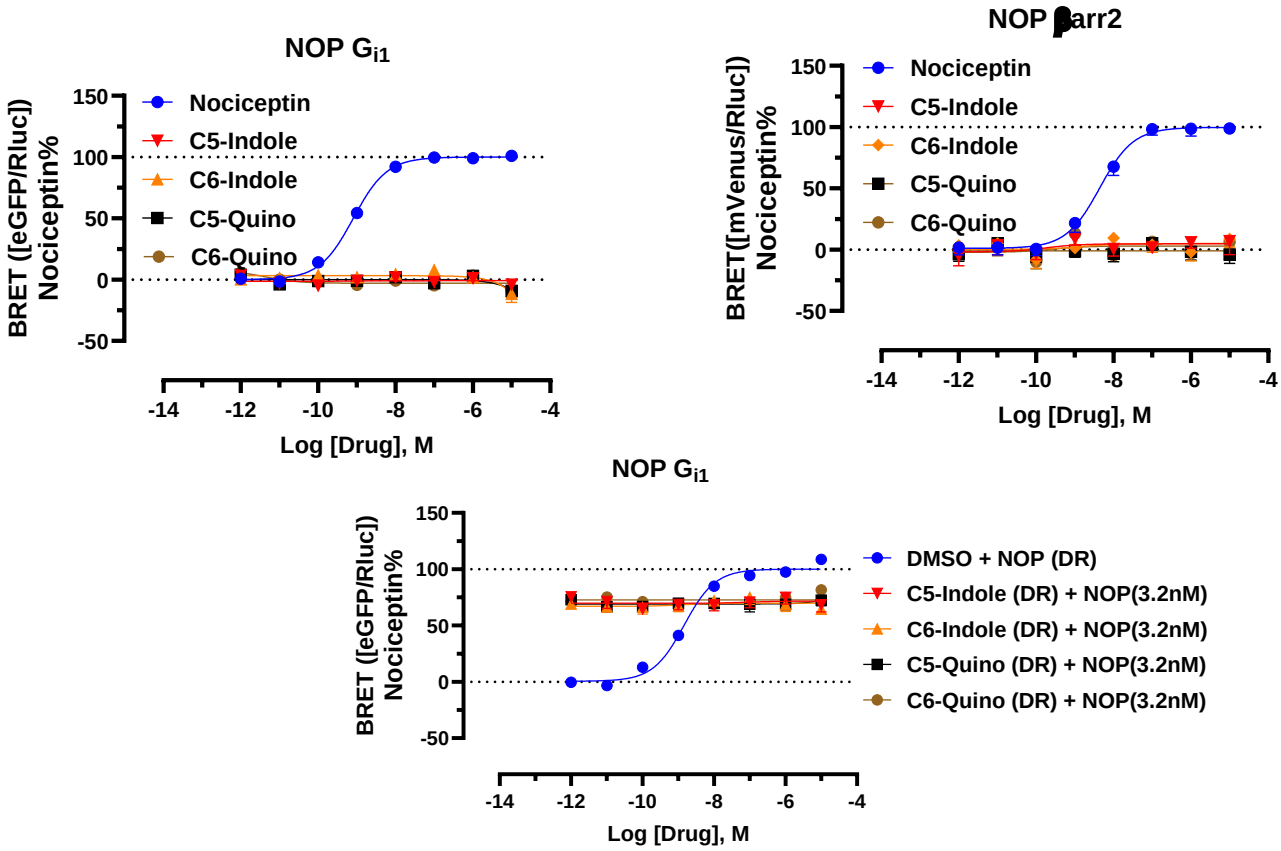
Supplementary Fig. 7. Gαi-1 signaling of C5 and C6 analogs at δOR and κOR using TRUPATH. (A-B) Gαi1 signaling of C5 and C6 indole analogs at δOR and κOR. (C) δOR interactions with **C6-Quino** and **C6-Indole** in the orthosteric binding pocket (top beige color) and the sodium binding site (lower green color). (D-E) Gαi-1 signaling of C5 and C6 Quino analogs at δOR and κOR. DPDPE and U50,488H were used as controls for δOR and κOR. Figures contain data ± SEM grouped from three independent biological replicates. Numerical values can be found in **Supplementary Table 1.**



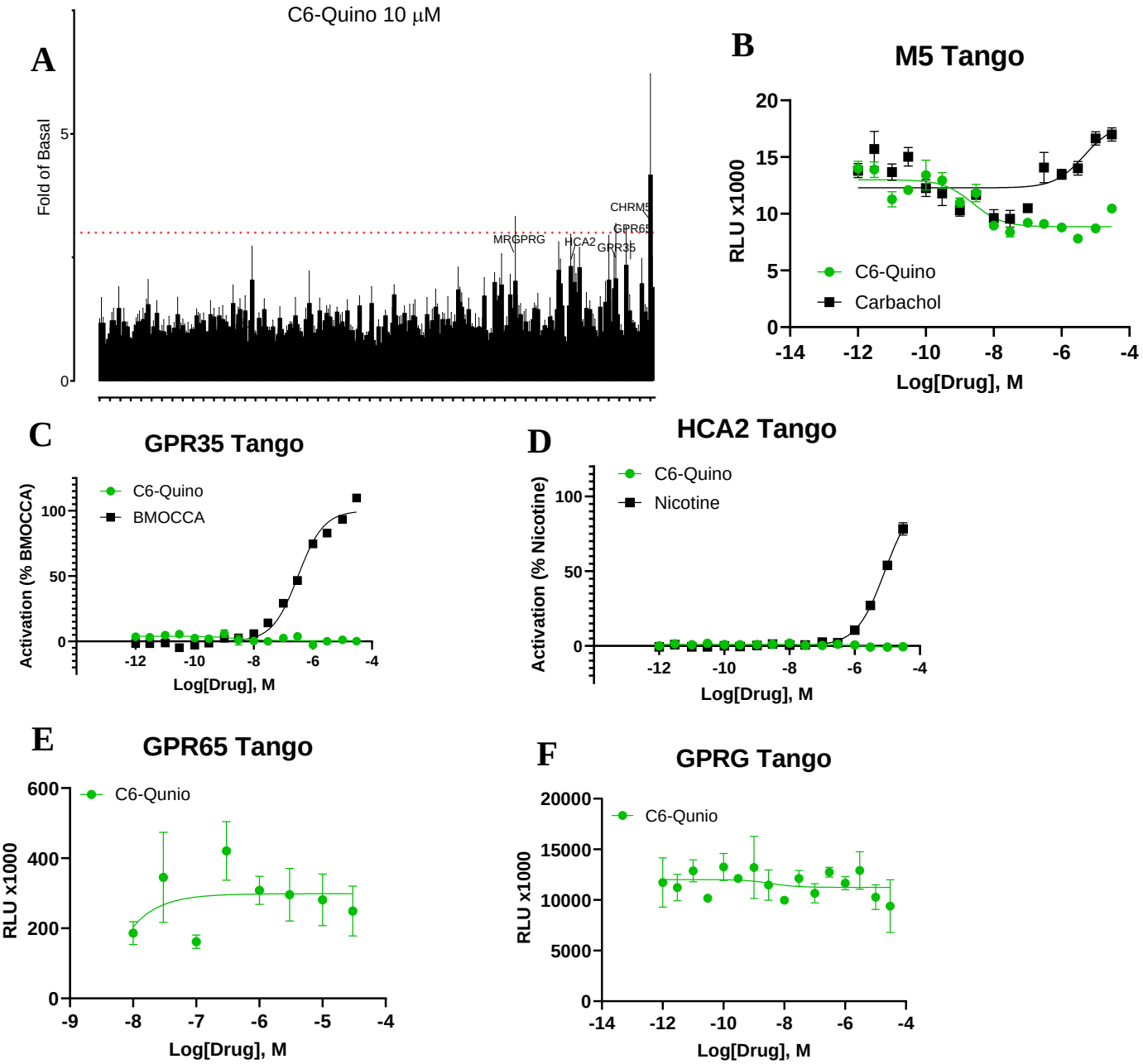
Supplementary Fig. 8. Sequence Comparison of Human δOR, μOR, and κOR. Alignment of the human δ-opioid receptor (δOR) with human μ-opioid receptor (μOR) and κ-opioid receptor (κOR) sequences.



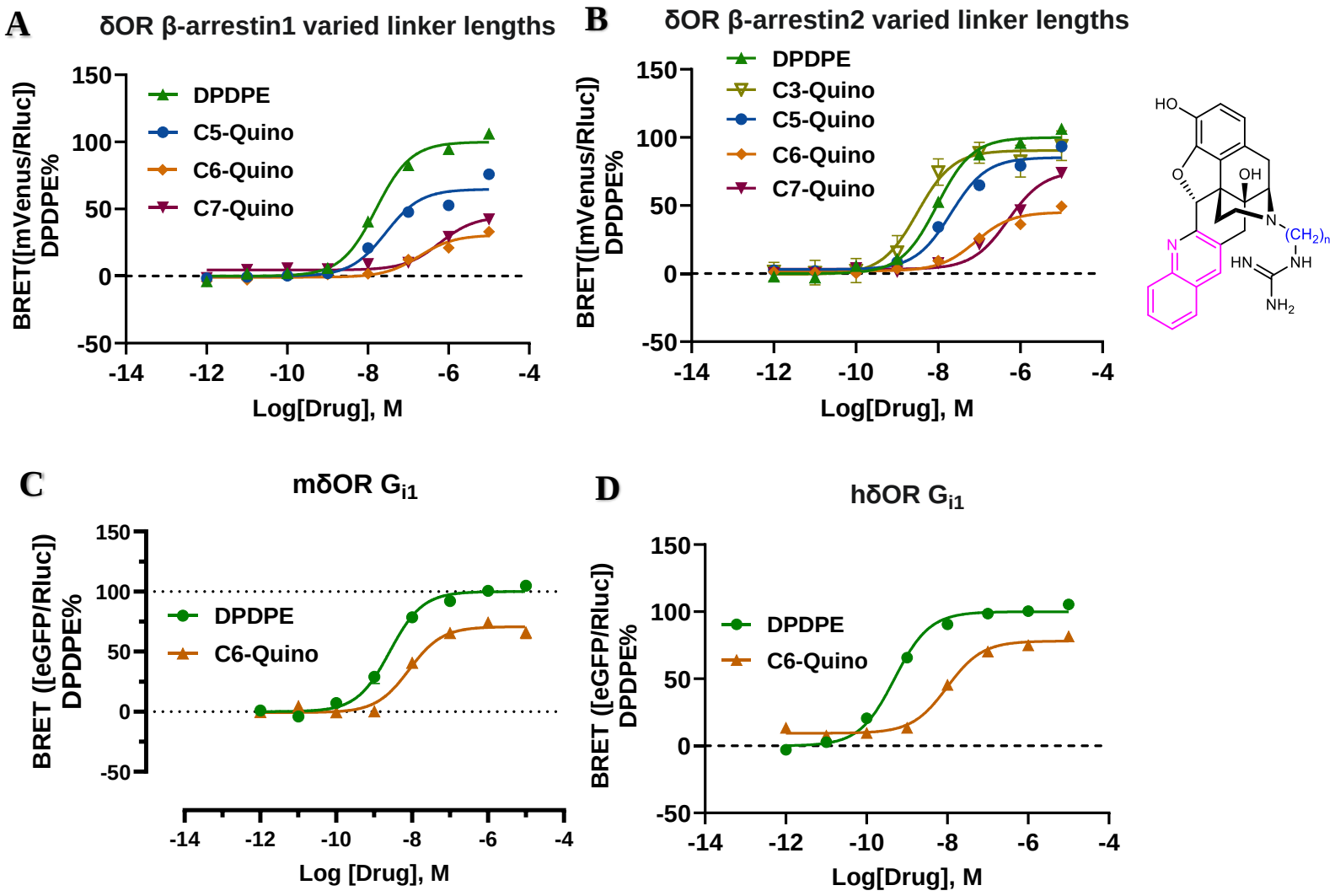
Supplementary Fig. 9. Selectivity of C6-quino binding at δOR, μOR, and κOR using radioligand competition binding. Figures contain data ± SEM grouped from three independent biological replicates. Quantification of data can be found in **Supplementary Table 2**.



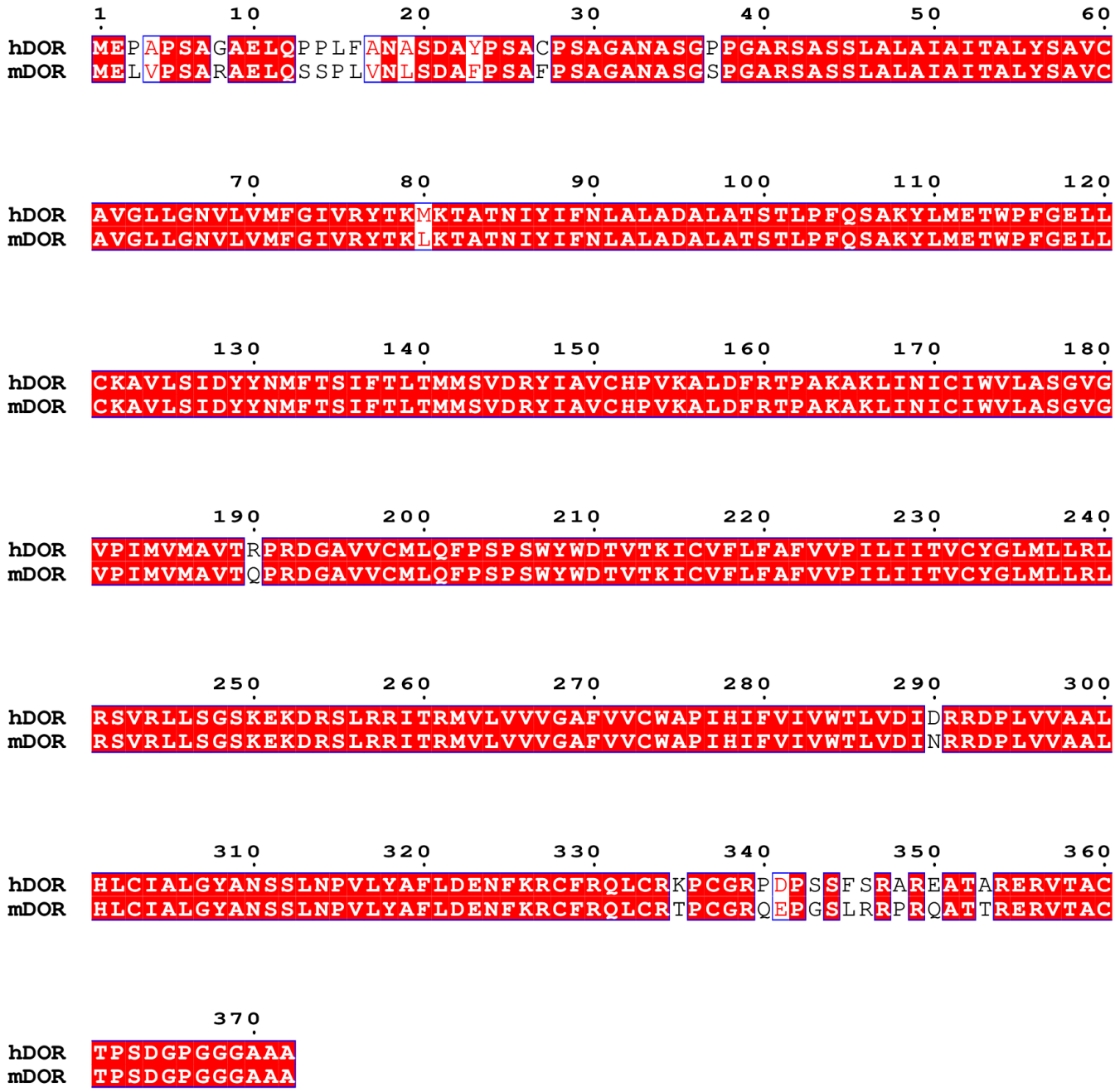
Supplementary Fig. 10. Functional selectivity of C6-quino at NOP. Figures contain data ± SEM grouped from three independent biological replicates.



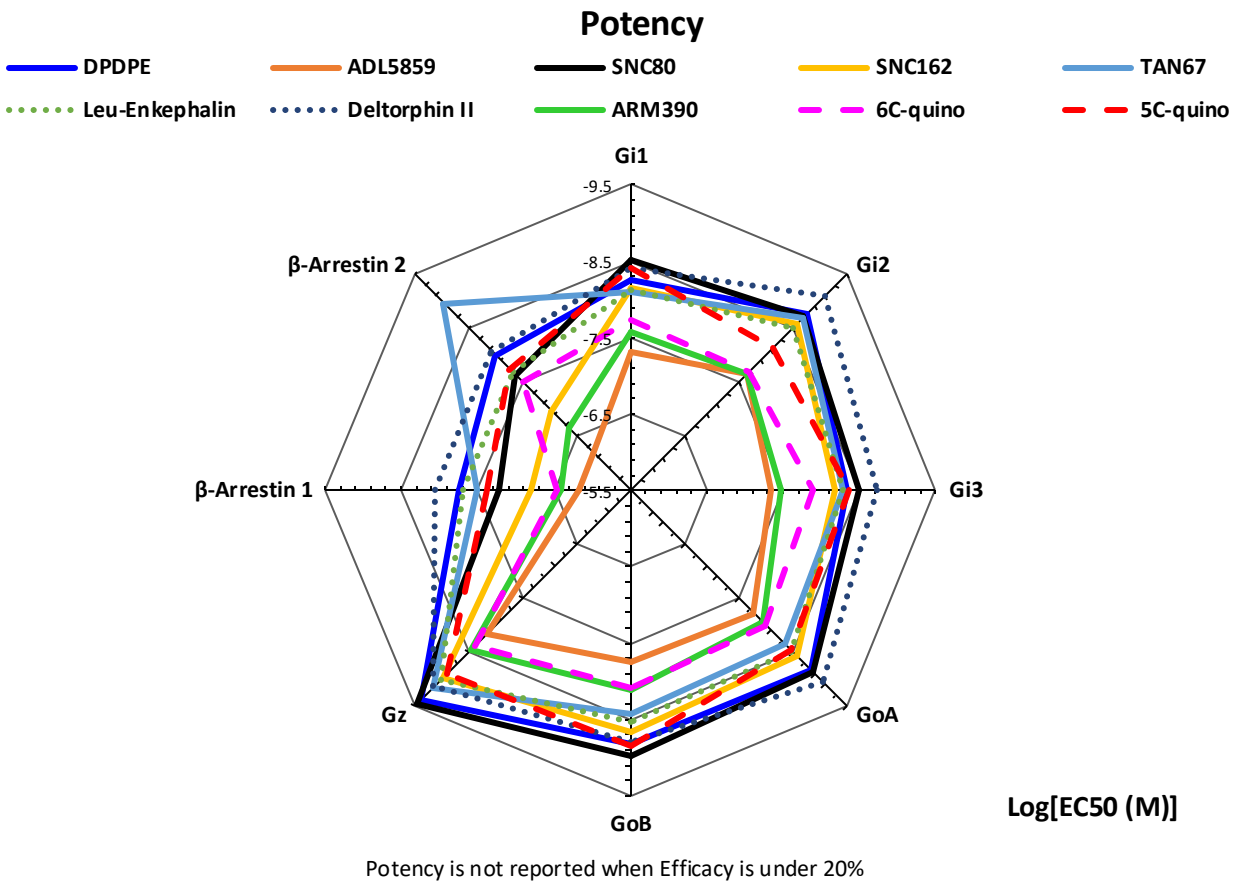
Supplementary Fig. 11. (A) C6-Quino screened across a ~317 target panel in the PRESTO TANGO assays using β-arrestin2 as the readout, with cutoff at 3-fold of basal levels. (B), (C), (D), (E), (F) Follow up study using Tango assays showing C6-Quino has no activity at the initial hits.



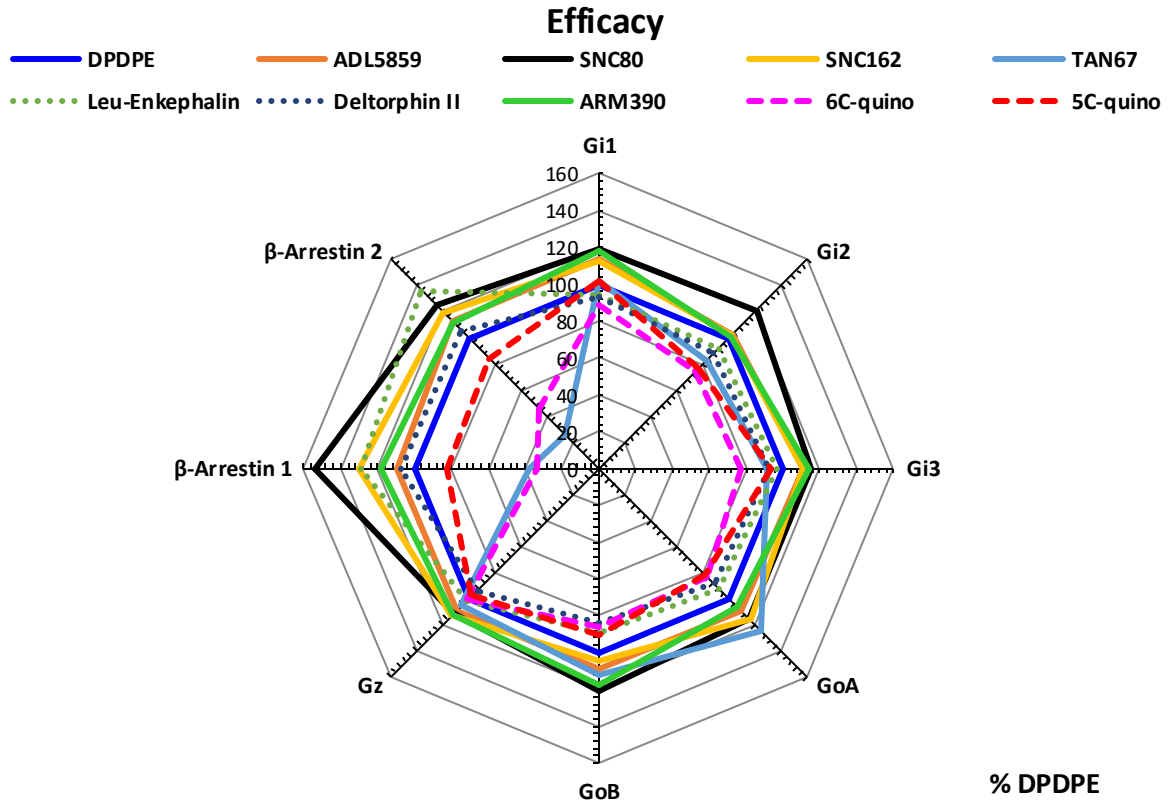
Supplementary Fig. 12. Profiling the signaling of C5, C6, and C7 analogs at δOR. (A) β-arrestin-1 signaling of C5, C6, and C7 Quino analogs at δOR. (B) β-arrestin-2 signaling of C3, C5, C6, and C7 Quino analogs at δOR. (C) and (D) G_{i1} signaling of C6-Quino on murine and human δOR, respectively. Figures contain data ± SEM grouped from three independent biological replicates. Efficacy and potency values are summarized in **Supplementary Table 3**.



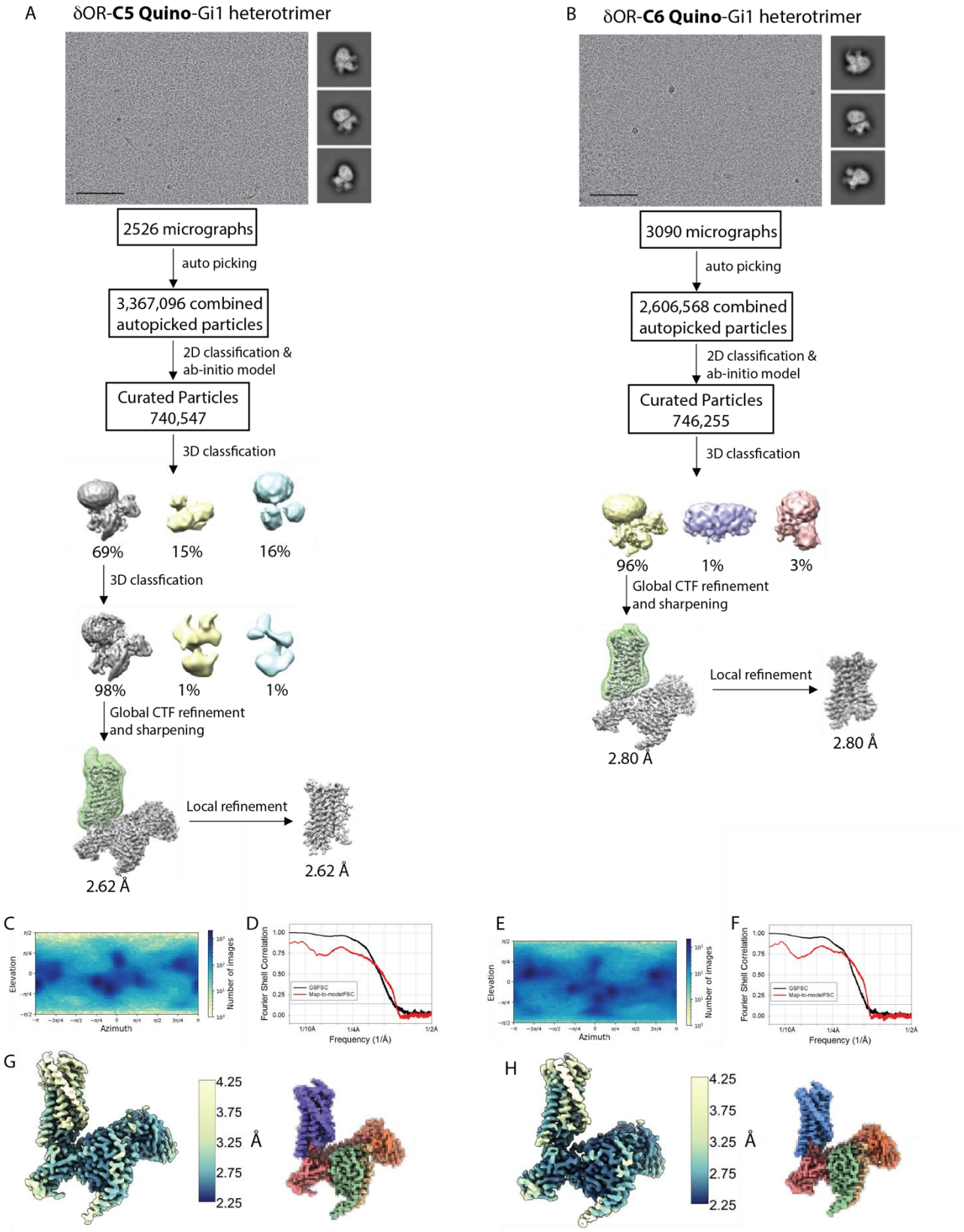
Supplementary Fig. 13. Comparison of human and murine δOR sequences. Sequence alignment of human and murine δOR showing no observed differences in the orthosteric binding pocket. Based on this comparison, we do not anticipate significant effects on functional readouts due to the sequence variation between species



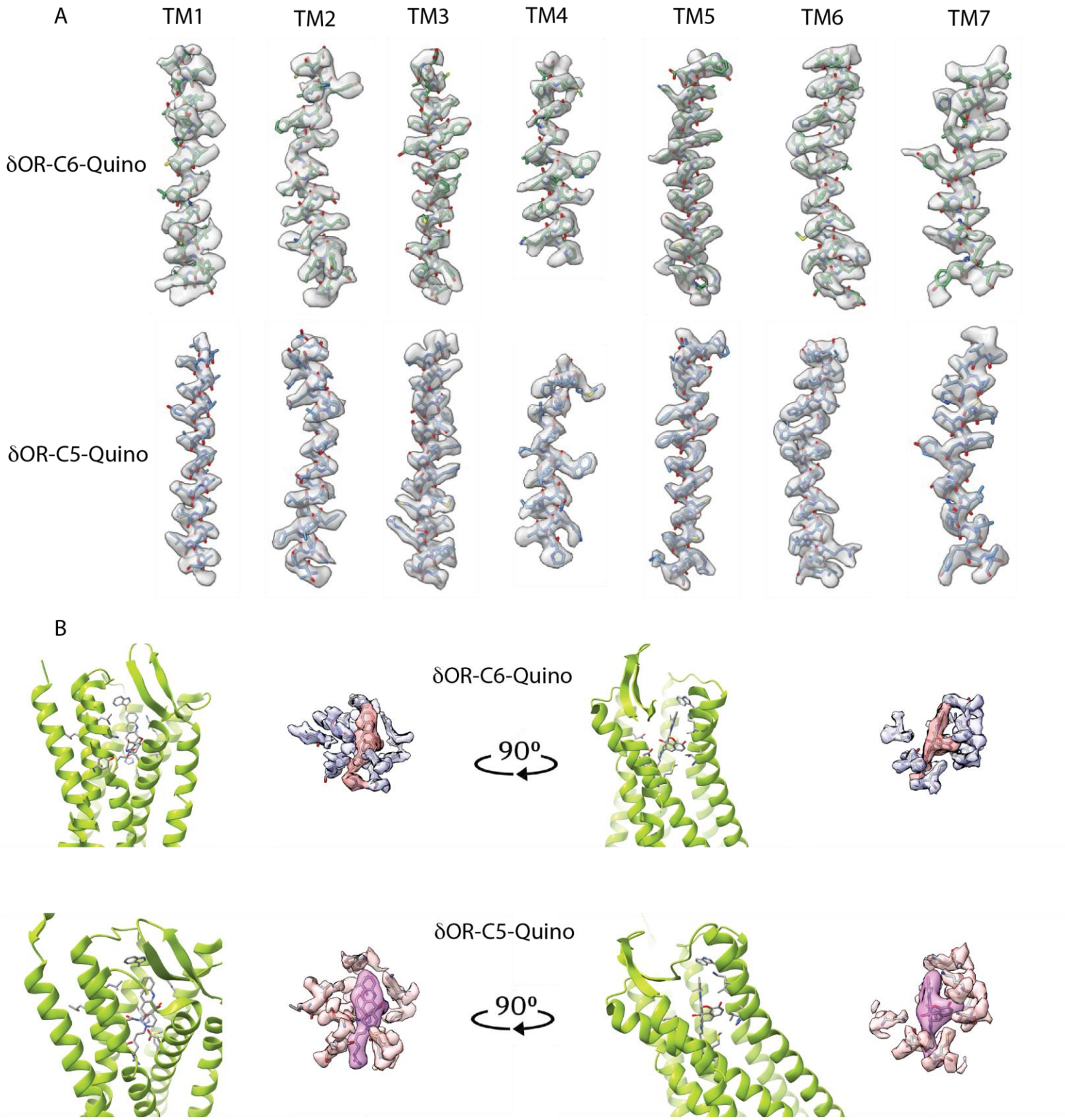
Potency is not reported when Efficacy is under 20%



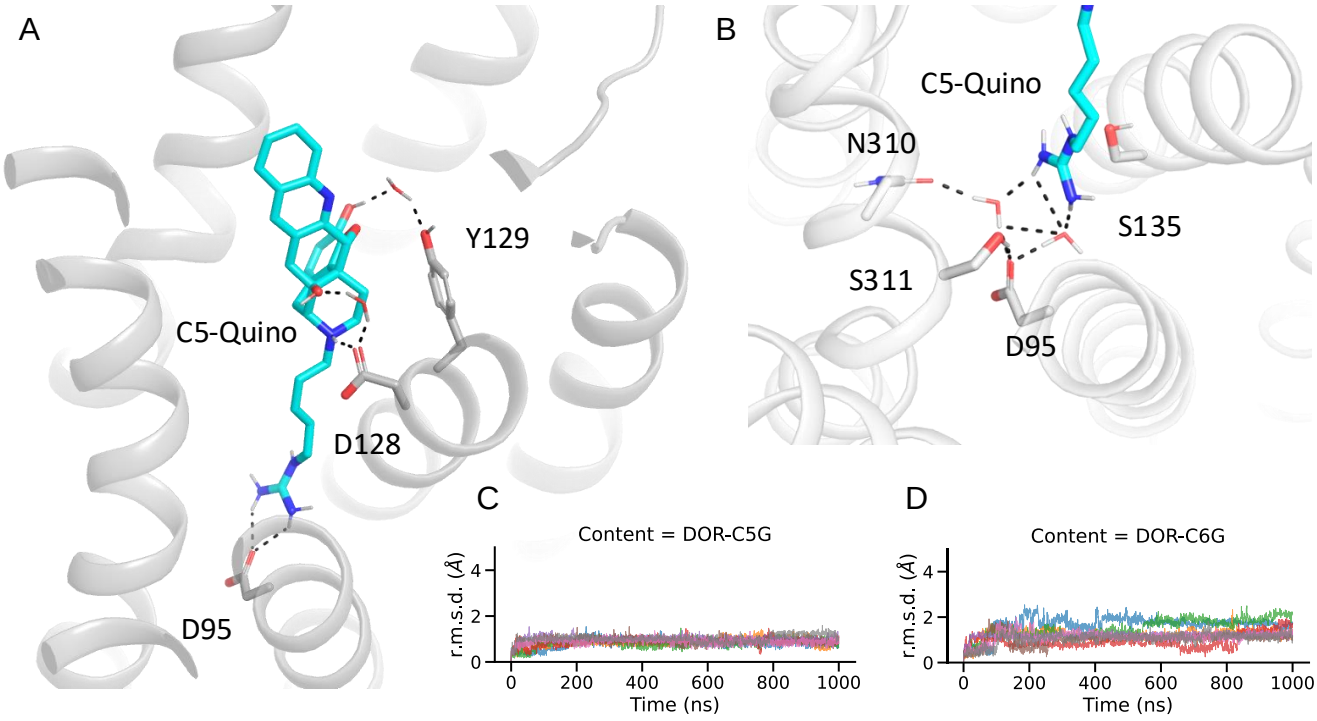
Supplementary Fig. 14. G protein signaling of C5, C6-Quino, and common δOR agonists at δOR using TRUPATH Gαβγ biosensors. Figures contain data ± SEM grouped from three independent biological replicates, displayed in **Supplementary Table 5**.



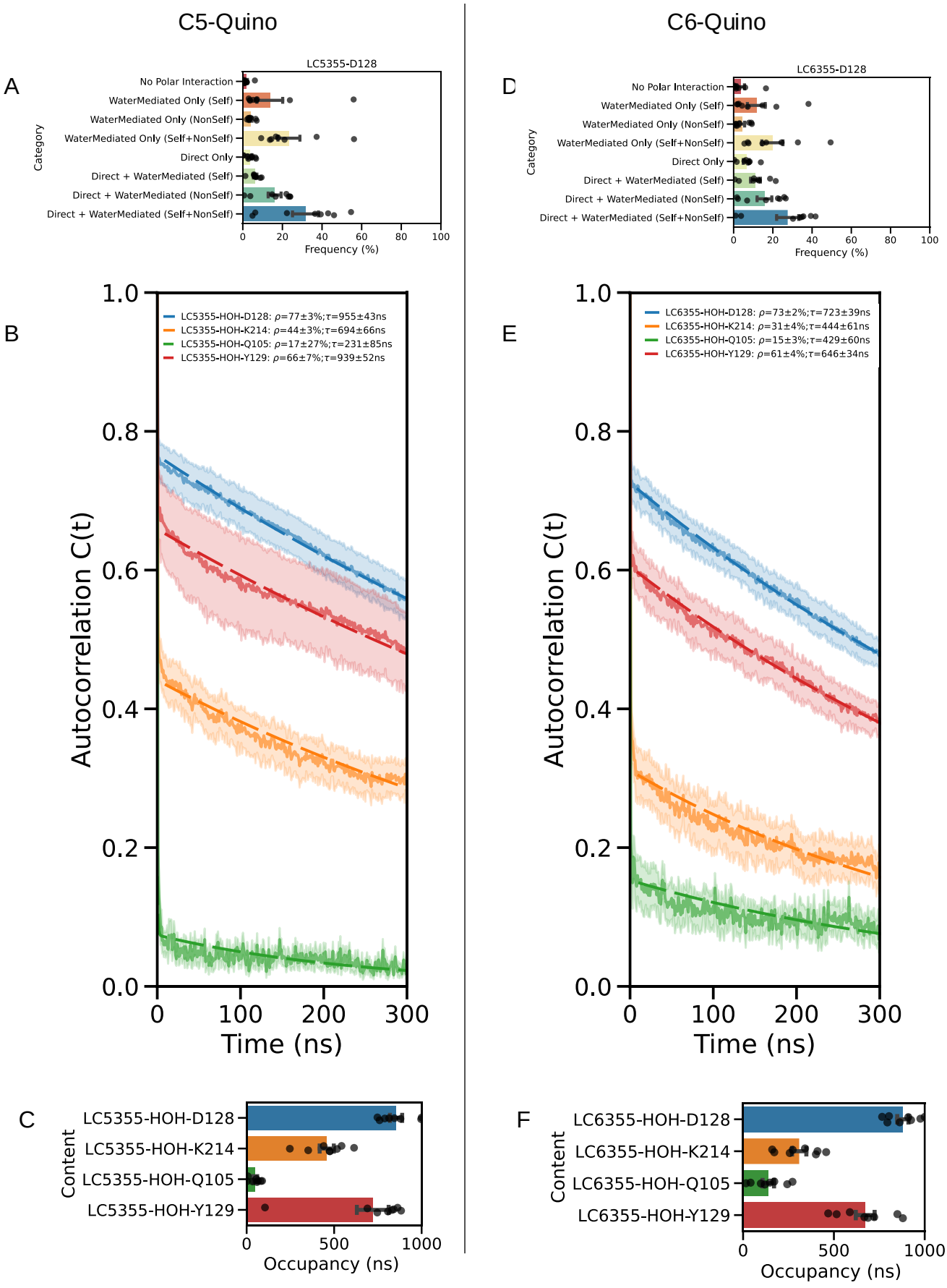
Supplementary Fig. 15: Flowchart of data processing for δOR-C5-Quino-G protein and δOR-C6-Quino-G protein structures. A local refined map on receptors was generated for each dataset. Respective agonist-bound δOR heterotrimeric complexes are shown (A and B). Of note, one round of heterogenous refinement was sufficient for the 2D curated δOR-C6-Quino-Gi1 particle stack. Orientational distribution heat map and plots of the gold-standard Fourier shell correlation (GSFSC) between half maps (black) and FSC between the model and the B-factor sharpened map for respective refined model (red) as calculated by Phenix_mtriage (C and D, E and F). Local resolution heat-map calculated using the local windowed FSC method (G and H).



Supplementary Fig. 16: Cryo-EM density of all seven transmembrane helices, ligands, and ligand-binding pocket residues. (A) Cryo-EM density of the seven transmembrane (TM) 1-7 and (**B**) density of ligands and key residues at different views for the structures of δOR bound to C6-Quino and C5-Quino. respectively.

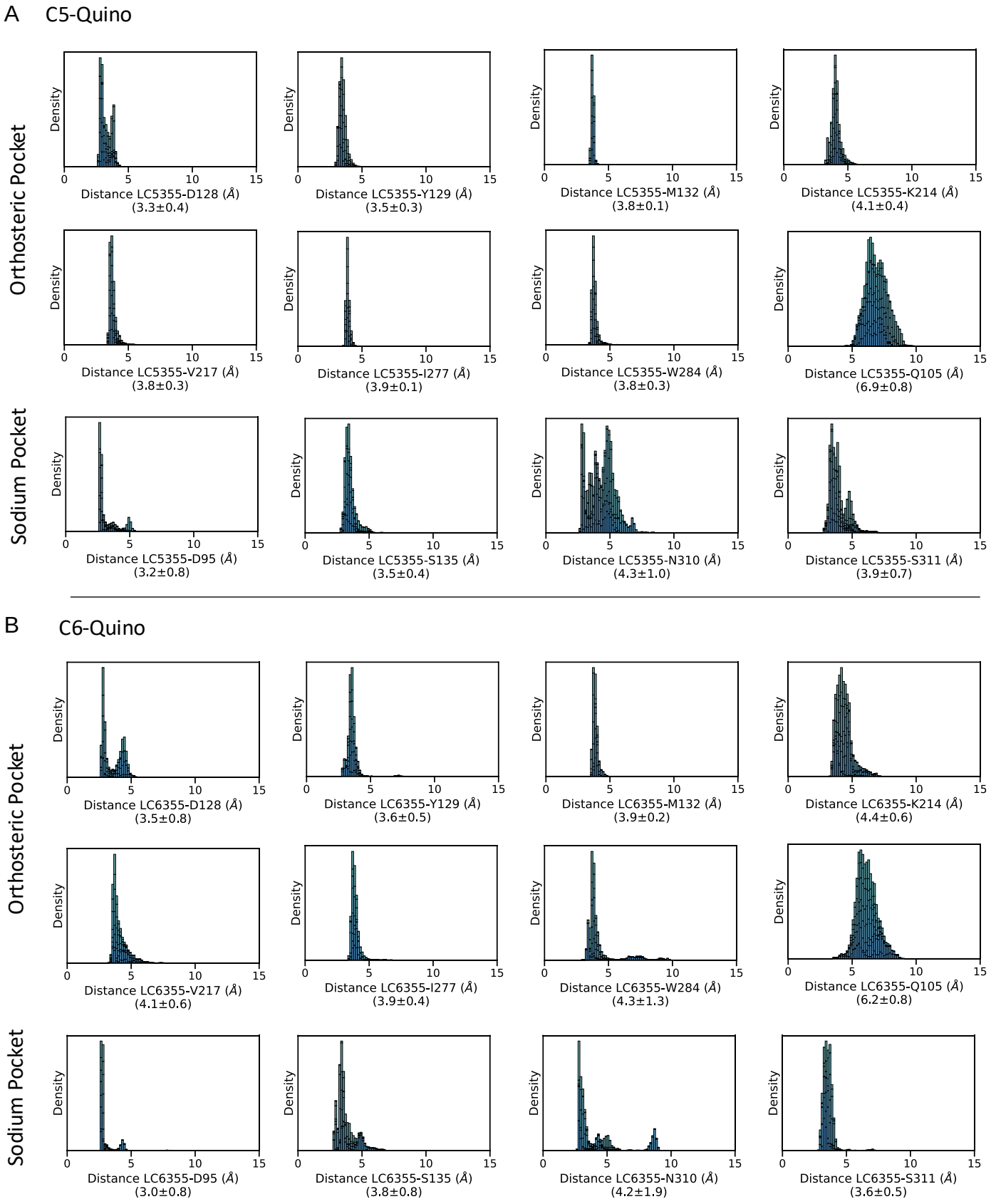


Supplementary Fig. 17 Overview on molecular dynamics simulations for C5- Quino and C6-Quino (A) Molecular graphics of a snapshot from a C5-Quino trajectory illustrating a direct interaction with D128$^{3.32}$ supplemented by a water bridge from the same protein residue, a water-mediated only interaction with Y129$^{3.33}$ and a direct only interaction with D95$^{2.50}$. (B) Molecular graphics of a snapshot from a C5-Quino trajectory illustrating a water-mediated only interaction at D95$^{2.50}$ and N310$^{7.45}$. Black dashed lines in (A) and (B) are polar contacts reported by PyMOL v2.4.1. Root mean square deviation (R.m.s.d.) of the ligand C5-Quino (C) and C6-Quino (D).

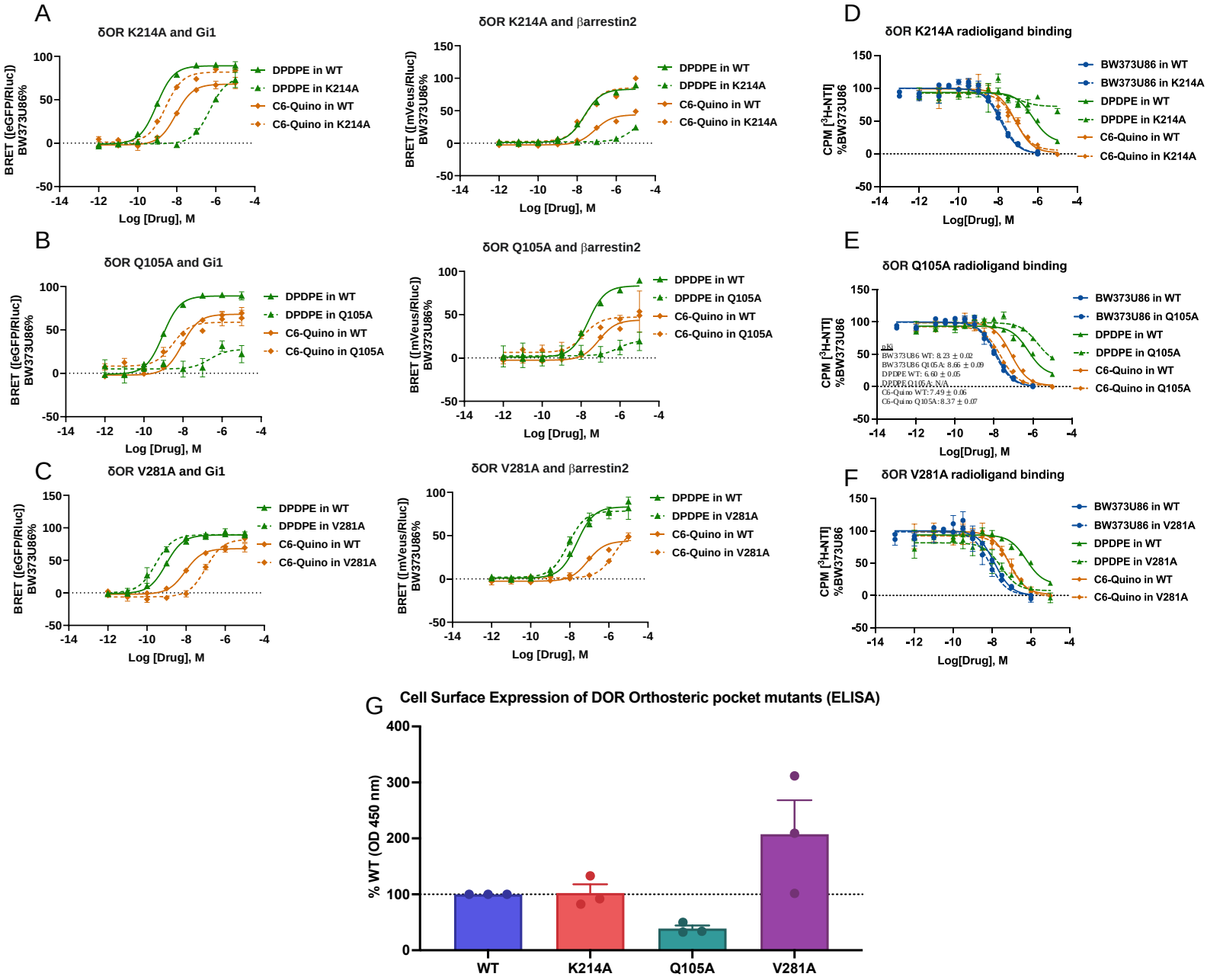


Supplementary Fig. 18

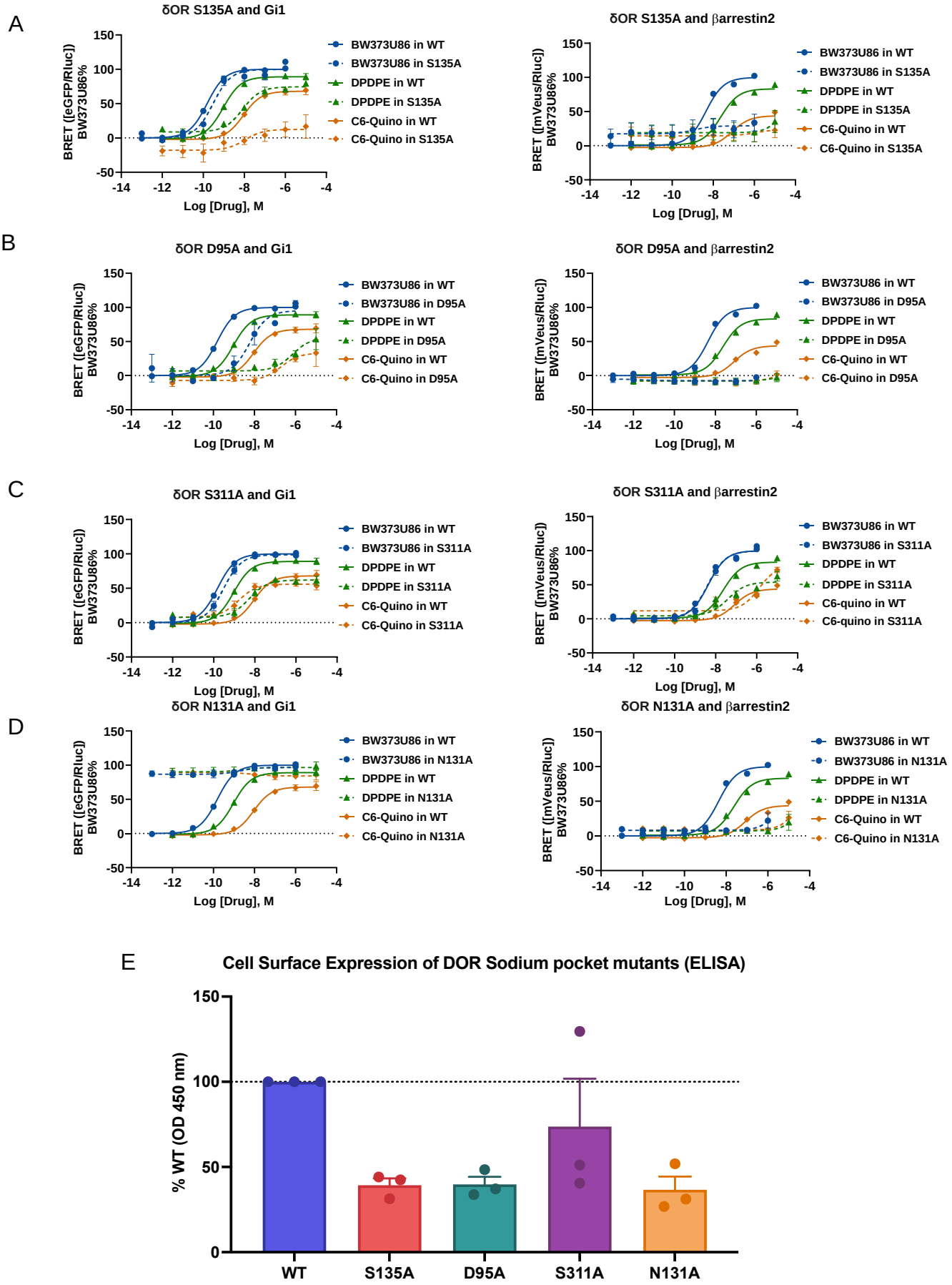
Supplementary Fig. 18. Statistics on Polar Contact between ligands and naltrindole pocket in Molecular Dynamics simulations. The naltrindole pocket residues includes D128, K214, Q105, Y129. (A-C) refers to C5-Quino; (D-F) refers to C6-Quino. Statistics on fine category of the polar contact are presented in (A) and (D). Here, the frequency in percentage refers to the number of frames in the trajectories where the categorized interaction occurred at the naltrindole pocket; the error bar presents standard error of means with n = 8 trajectories. For water mediated interactions, "Self" means D128 itself and "NonSelf" means the rest of the residues in the naltrindole pocket. Autocorrelation functions calculated for residues at naltrindole pocket (D128, K214, Q105, Y129) across all trajectories are presented in (B) and (E). A bootstrap over the trajectories were done to obtain mean (solid line) and standard deviation (shade) of the autocorrelations. Dashed lines are fitted to the exponential function to quantify long-lived populations. The two parameters in the exponential fitted to autocorrelation function, correlation time τ and population ρ are presented in the legend with error bound in standard deviation. Occupancy of each water-mediated interaction in nanosecond is presented in (C) and (F) with error bounded by standard error of the mean.



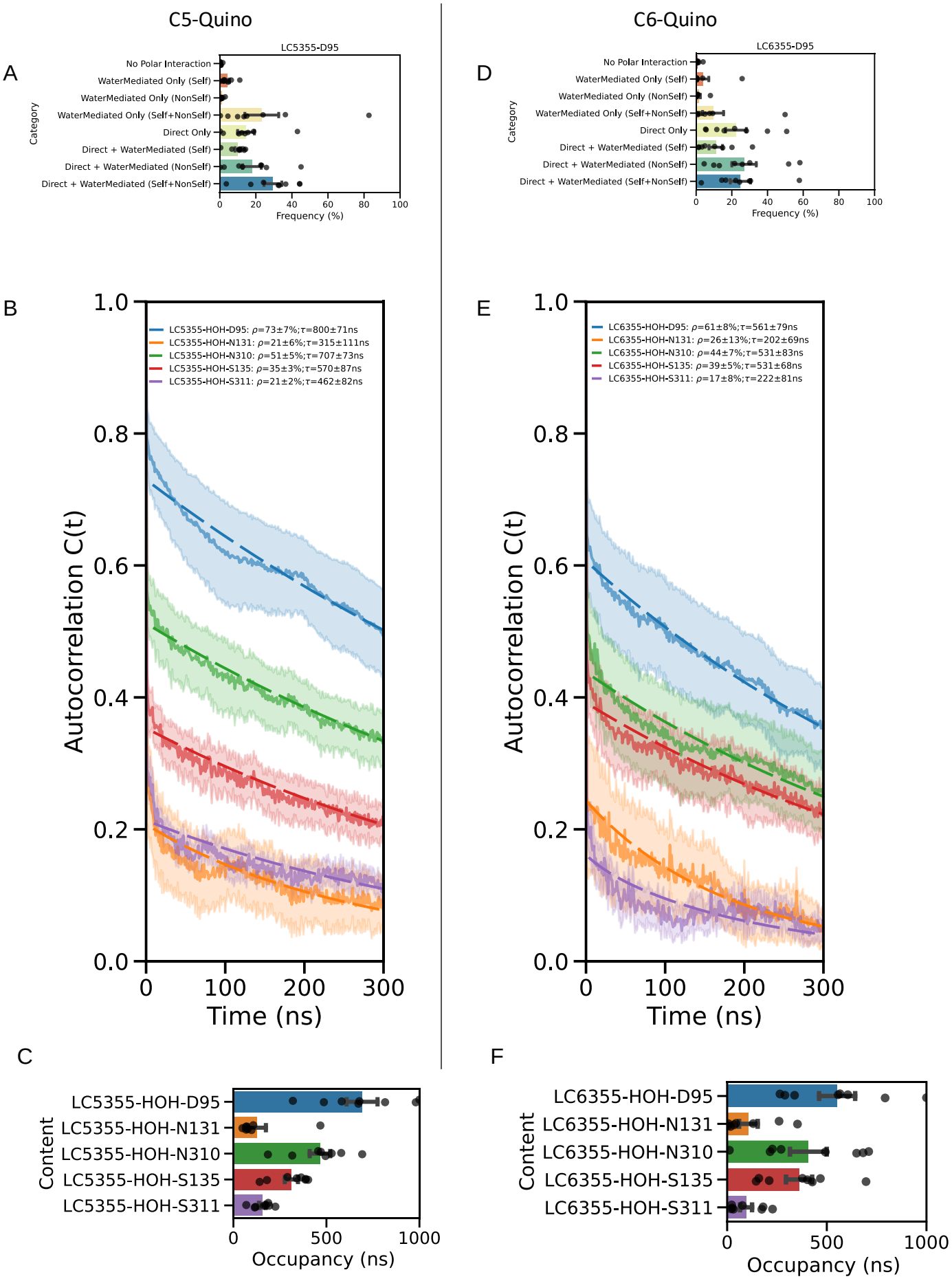
Supplementary Fig. 19. Closest distances between ligand and protein residues in molecular dynamics simulations. Shortest distance among the heavy atoms of sidechains in respective δOR residues to the heavy atoms of C5-Quino (A) and C6-Quino (B) were measured and presented in the histogram stacked by aggregating results from all trajectories. Numbers in parentheses are the mean and standard deviation of the respective distances in MD simulations. Residues in the orthosteric pocket and the sodium pocket were shown.



Supplementary Fig. 20. Effects of mutations in δOR orthosteric pocket contributing to C6-quino signaling. (A-B) BRET1 (β-arrestin2) or BRET2 (Gi1) was used to screen orthosteric pocket δOR mutations (A) K214A, (B) Q105A, and (C) V281A. Figures contain data ± SEM grouped from three independent biological replicates. Quantification of data can be found in **Supplementary Table 9**. (D-F) Radioligand competition binding for orthosteric pocket mutants (D) K214A, (E) Q105A, and (F) V281A. Figures contain data ± SEM grouped from three independent replicates. Inlet in panel (E) contains pKi values of BW373U86, DPDPE, and C6-Quino for WT and Q105A as the ^{3}H-NTI concentration used was not near the ^{3}H-NTI Kd for Q105A, distorting the curves. N/A, no activity. Full quantification of data can be found in **Supplementary Table 10**. (G) Cell surface expression of orthosteric pocket mutant K214A, Q105A, and V281A using ELISA normalized from baseline. Bar graph represents data ± SEM grouped from three independent biological replicates.

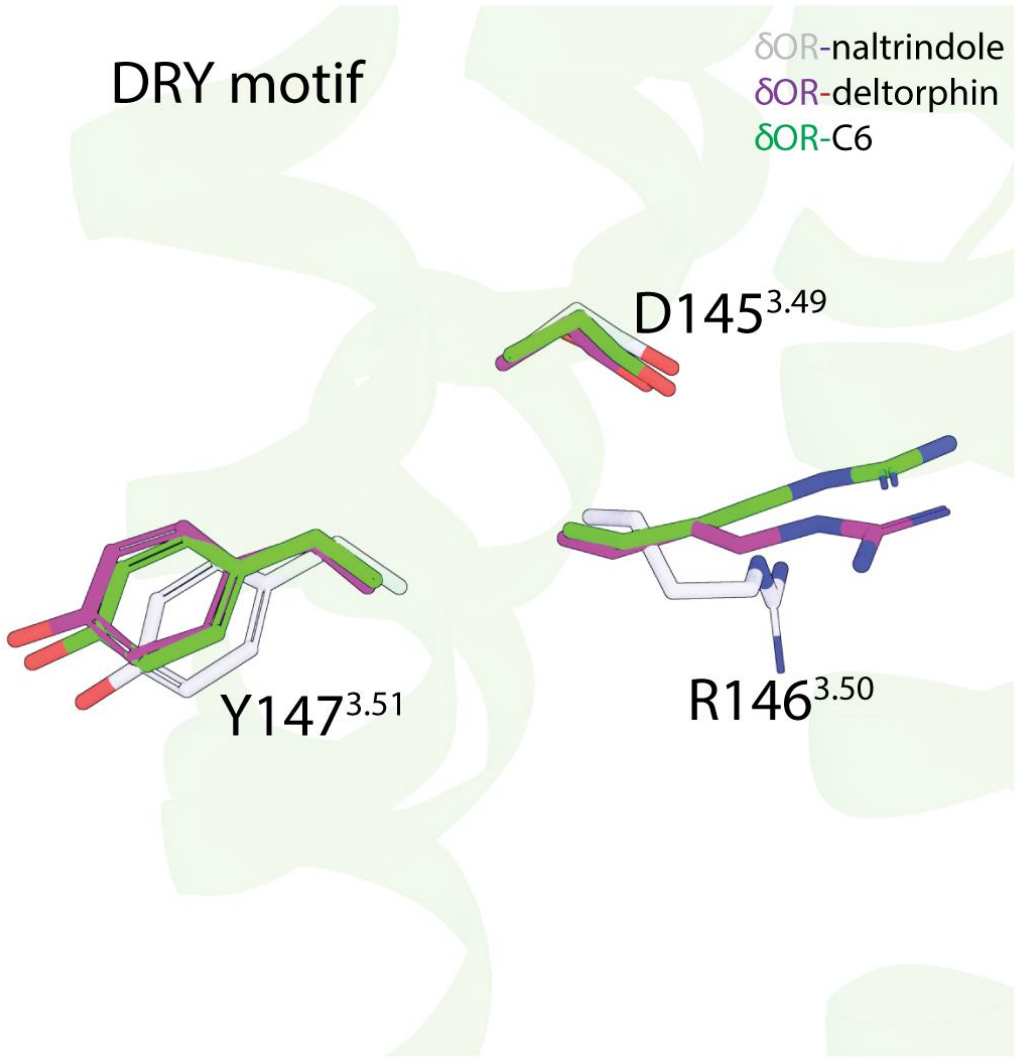


Supplementary Fig. 21. Effects of mutations in δOR sodium pocket to C6-quino signaling. (A-D) BRET1 (β-arrestin2) or BRET2 (Gi1) was used to screen sodium pocket δOR mutations (A) S135A, (B) D95A, (C) S311A, and (D) N131. Figures contain data ± SEM grouped from three independent biological replicates. Quantification of data can be found in **Supplementary Table 11**. (E) Cell surface expression of sodium pocket mutant S135A, D95A, S311A, and N131 using ELISA normalized from baseline. Bar graph represents data ± SEM grouped from three independent biological replicates.

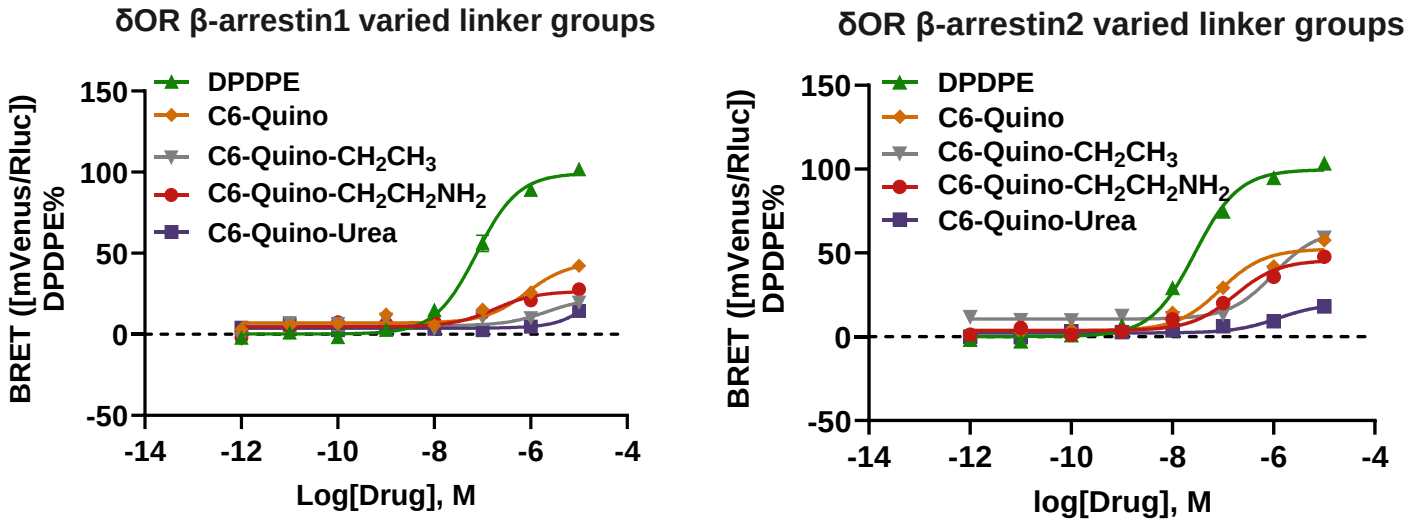


Supplementary Fig. 22

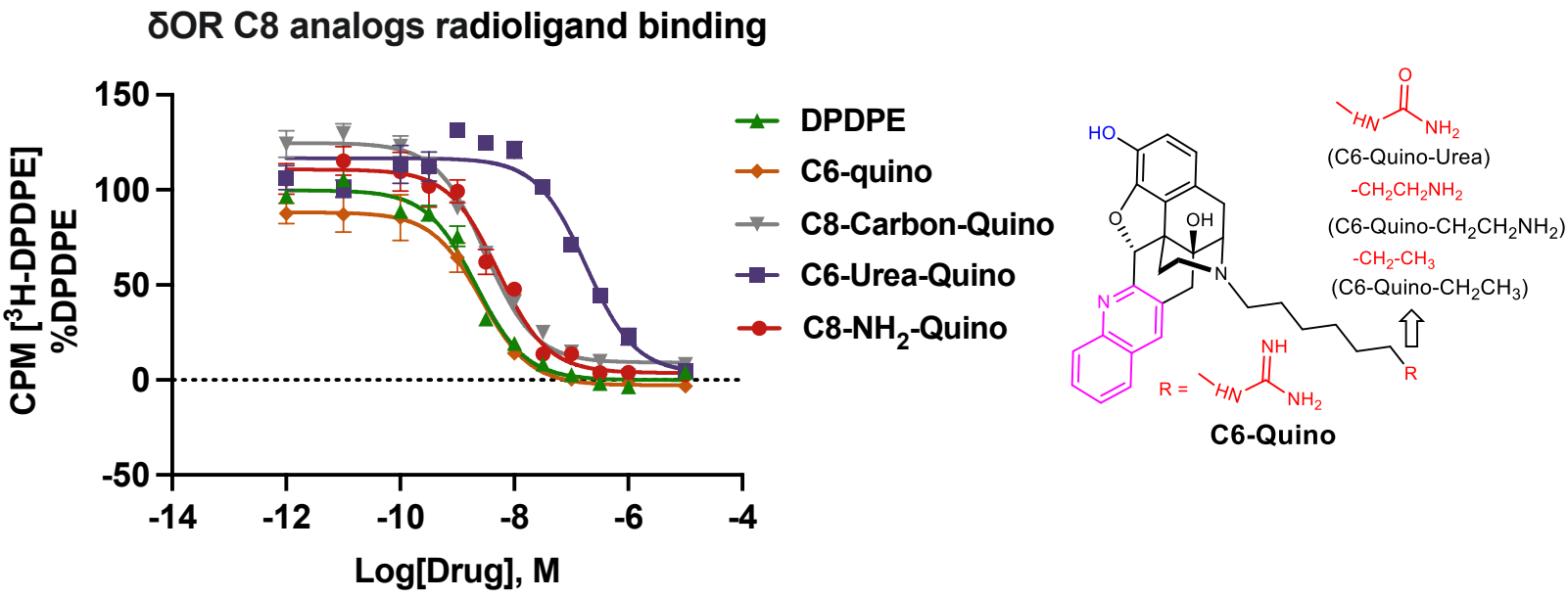
Supplementary Fig. 22. Statistics on Polar Contact between ligands and sodium pocket in Molecular Dynamics simulations. The sodium pocket residues includes D95, N131, N310, S135 and S311. (A-C) refers to C5-Quino; (D-F) refers to C6-quino. Statistics on fine category of the polar contact are presented in (A) and (D). Here, the frequency in percentage refers to the number of frames in the trajectories where the categorized interaction occurred at the sodium pocket; the error bar presents standard error of means with n = 8 trajectories. For water-mediated interactions, "Self" means D95 itself and "NonSelf" means the rest of the residues in the sodium pocket. Autocorrelation functions calculated for residues at sodium pocket (D95, N131, N310, S135 and S311) across all trajectories are presented in (B) and (E). A bootstrap over the trajectories were done to obtain mean (solid line) and standard deviation (shade) of the autocorrelations. Dashed lines are fitted to the exponential function to quantify long-lived populations. The two parameters in the exponential fitted to autocorrelation function, correlation time τ and population ρ are presented in the legend with error bound in standard deviation. Occupancy of each water-mediated interaction in nanosecond is presented in (C) and (F) with error bounded by standard error of the mean.



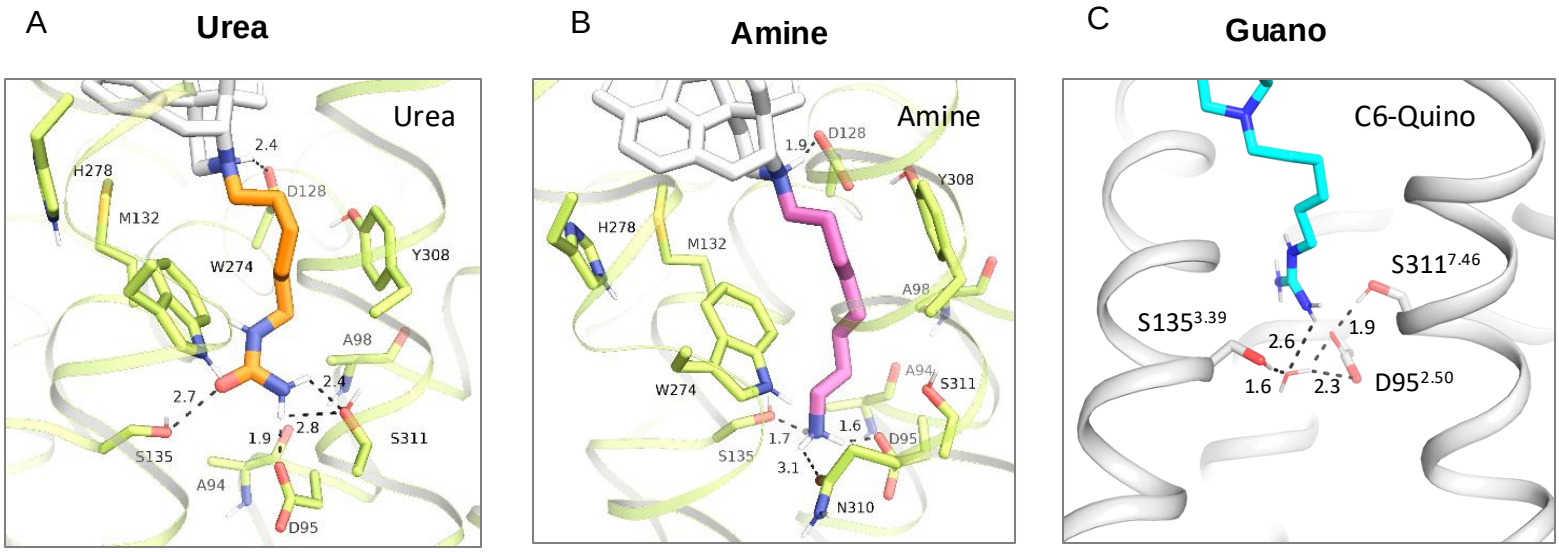
Supplementary Fig. 23. DRY motif from DOR structures with naltrindole (white), deltorphin (purple), and C6-quino (green)



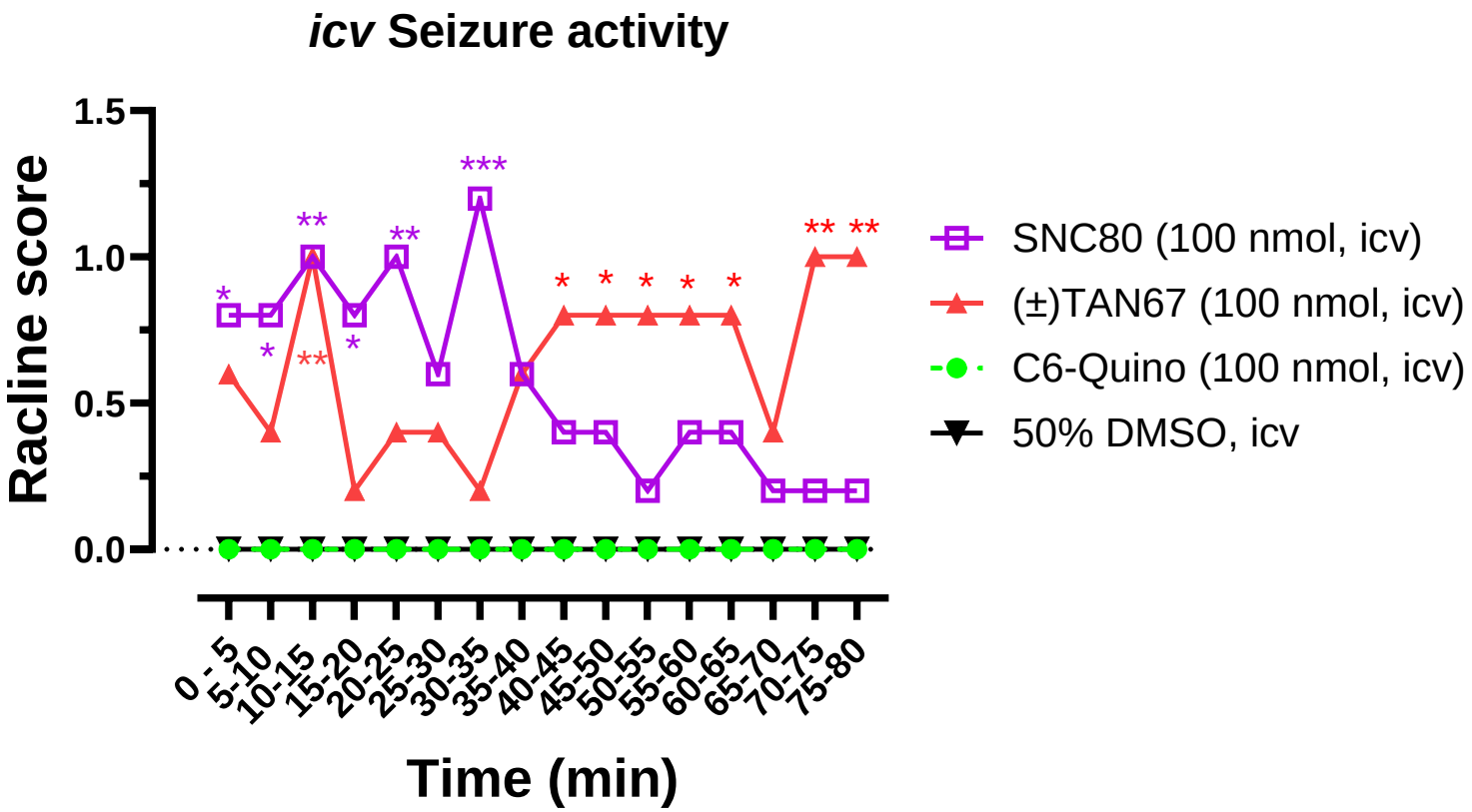
Supplementary Fig. 24. Profiling the β-arrestin signaling of quino compound derivatives with varied head group polarity. (A) β-arrestin-1 signaling of C6 guanidine (**C6-Quino**) and C6 urea substituted quinoline as well as C8 (all carbon) and C8 amine substituted quinoline. (B) β-arrestin-2 signaling of C6 and C6 urea substituted quinoline as well as C8 and C8 amine substituted quinoline. Figures contain data ± SEM grouped from three independent biological replicates summarized in **Supplementary Table 12**.



Supplementary Fig. 25. Radioligand binding of Quino compound derivatives with varied head group polarity. Figures contain data ± SEM grouped from three independent biological replicates. Quantification of data can be found in **Supplementary Table 13**.



Supplementary Fig. 26. Interactions in the sodium pocket (A) Neutral carbamide functionality reveals only hydrogen bonding with D95$^{2.50}$ in addition to the hydrogen bond network forming similarly to guanidine. (B) In the case of C6-Quino derivative with primary amine, in addition to the two hydrogen bonding interactions with S153$^{3.39}$ and N310$^{7.45}$ a salt bridge interaction between positively charged amine and D95$^{2.50}$ is also present. (C) Hydrogen bond network formed by guanidine in the sodium pocket



Supplementary Fig. 27. Supraspinal seizure activity of C6-Quino, SNC80 and (±)-**TAN67. Q**uantified seizures induced by C6-Quino, SNC80 and (±)-TAN67 over a 80 minutes timecourse. Statistical significance was determined by Two-way ANOVA with Dunnett's multiple comparison's test ($F(3,560)=44.29$, $p<0.0001$). C6-quino showed no seizures at any time interval. SNC80 was significantly different from saline at 0-5 (*p=0.0188), 5-10 (*p=0.0188), 10-15 (**p=0.0021), 15-20 (*p=0.0188), 20-25 (**p=0.0021), 30-35 (***p=0.0002) time intervals in minutes. (±)-TAN67 showed seizures at 10-15 (**p=0.0021), 40-45 (*p=0.0188), 45-50 (*p=0.0188), 50-55 (*p=0.0188), 55-60 (*p=0.0188), 60-65 (*p=0.0188), 70-75 (**p=0.0021), 75-80 (*p=0.0021).

BRET	δOR G_{i1}			μOR G_{i1}			κOR G_{i1}		
Ligand	Emax ± SEM (%)	pEC_{50} ± SEM	EC_{50}(nM)	Emax ± SEM (%)	pEC_{50} ± SEM	EC_{50}(nM)	Emax ± SEM (%)	pEC_{50} ± SEM	EC_{50}(nM)
DPDPE	100 ± 2	9.12 ± 0.07	0.75						
DAMGO				100 ± 2	8.42 ± 0.07	3.8			
U50,488							100 ± 2	8.63 ± 0.06	2.3
C5-Indole	80 ± 1	8.44 ± 0.05	3.6	NA	NA	NA	61 ± 4	6.82 ± 0.16	150
C6-Indole	60 ± 2	7.95 ± 0.10	11	NA	NA	NA	36 ± 5	6.35 ± 0.24	450
C5-Quino	92 ± 1	8.63 ± 0.06	2.4	NA	NA	NA	NA	NA	NA
C6-Quino	78 ± 1	8.01 ± 0.06	9.8	NA	NA	NA	NA	NA	NA

Supplementary Table 1. Opioid subtype selectivity of C5 and C6 analogs. Gαi-1 signaling of C5 and C6 indole and Quino analogs at δOR and κOR. DPDPE and U50,488H were used as controls for δOR and κOR.

Ligand	pKi ± SEM		
	δOR	**μOR**	**κOR**
DPDPE	8.60 ± 0.03	N/A	N/A
DAMGO	N/A	9.16 ± 0.04	N/A
U50,488	N/A	N/A	9.76 ± 0.09
C6-Quino	8.87 ± 0.05	7.16 ± 0.08	6.34 ± 0.10

Supplementary Table 2. Selectivity of C6-quino binding affinity at δOR, μOR, and κOR. Values represent pK_i ± SEM of radioligand competition binding with ^{3}H-DPDPE (δOR), ^{3}H-DAMGO (μOR), or ^{3}H-U69,593 (κOR) with three independent replicates. .

BRET	δOR G_{i1}			δOR β-arrestin1			δOR β-arrestin2		
Ligand	Emax ± SEM (%)	pEC_{50} ± SEM	EC_{50} (nM)	Emax ± SEM (%)	pEC_{50} ± SEM	EC_{50} (nM)	Emax ± SEM (%)	pEC_{50} ± SEM	EC_{50} (nM)
DPDPE	100 ± 1	9.31 ± 0.04	0.49						
C3-Indole	84 ± 2	8.74 ± 0.05	1.8						
C4-Indole	80 ± 5	8.46 ± 0.15	3.5						
C5-Indole	76 ± 3	8.55 ± 0.11	2.8						
C6-Indole	52 ± 2	8.23 ± 0.10	5.9						
C7-Indole	40 ± 2	7.28 ± 0.13	53						
DPDPE	100 ± 1	9.31 ± 0.04	0.49	99 ± 2	7.71 ± 0.07	20	100 ± 1	7.99 ± 0.04	9.3
C3-Quino	90 ± 3	8.94 ± 0.09	1.2				91 ± 8	8.50 ± 0.22	3.1
C5-Quino	78 ± 2	8.63 ± 0.06	2.4	65 ± 2	7.56 ± 0.12	28	82 ± 2	7.69 ± 0.07	20
C6-Quino	69 ± 2	8.01 ± 0.05	9.9	31 ± 2	6.73 ± 0.18	190	43 ± 2	7.09 ± 0.08	81
C7-Quino	78 ± 2	7.55 ± 0.06	28	45 ± 3	6.22 ± 0.15	600	72 ± 3	6.30 ± 0.07	500

Supplementary Table 3. Profiling the signaling of C5, C6, and C7 Quino-line analogs and C3 C5, C6, and C7 Indole analogs at δOR

Ligand	pKi ± SEM
DPDPE	8.60 ± 0.03
C5-Quino	9.25 ± 0.04
C6-Quino	8.87 ± 0.05
C7-Quino	8.52 ± 0.02

Supplementary Table 4. Binding affinity of Quino compounds with different linker lengths. Values represent pK_i ± SEM of radioligand competition binding with ^{3}H-DPDPE with three independent replicates.

DPDPE					ADL5859			
	E_{max} ± SEM (%)	pEC_{50} ± SEM	EC_{50} (nM)			E_{max} ± SEM (%)	pEC_{50} ± SEM	EC_{50} (nM)
Gi1	100 ± 3	8.25 ± 0.06	5.69		Gi1	114 ± 3	7.32 ± 0.06	47.6
Gi2	100 ± 8	8.77 ± 0.19	1.69		Gi2	102 ± 3	7.65 ± 0.08	22.2
Gi3	100 ± 3	8.33 ± 0.08	4.73		Gi3	111 ± 2	7.34 ± 0.05	45.5
GoA	100 ± 3	8.83 ± 0.07	1.49		GoA	109 ± 4	7.78 ± 0.08	16.5
GoB	100 ± 3	8.81 ± 0.06	1.54		GoB	109 ± 4	7.75 ± 0.09	17.7
Gz	100 ± 6	9.37 ± 0.15	0.43		Gz	109 ± 2	8.14 ± 0.04	7.23
β-arr1	100 ± 6	7.75 ± 0.14	17.8		β-arr1	110 ± 9	6.17 ± 0.14	681
β-arr2	100 ± 4	8.01 ± 0.1	9.88		β-arr2	112 ± 7	6.19 ± 0.11	650
SNC80					SNC162			
	E_{max} ± SEM (%)	pEC_{50} ± SEM	EC_{50} (nM)			E_{max} ± SEM (%)	pEC_{50} ± SEM	EC_{50} (nM)
Gi1	119 ± 2	8.53 ± 0.05	2.95		Gi1	113 ± 2	8.16 ± 0.04	6.96
Gi2	121 ± 4	8.71 ± 0.09	1.94		Gi2	102 ± 2	8.57 ± 0.07	2.68
Gi3	116 ± 2	8.48 ± 0.05	3.31		Gi3	111 ± 1	8.19 ± 0.04	6.49
GoA	115 ± 3	8.86 ± 0.07	1.37		GoA	116 ± 3	8.58 ± 0.07	2.66
GoB	121 ± 3	8.97 ± 0.06	1.07		GoB	105 ± 2	8.67 ± 0.07	2.12
Gz	111 ± 3	9.45 ± 0.06	0.36		Gz	113 ± 1	8.97 ± 0.03	1.06
β-arr1	154 ± 8	7.23 ± 0.12	58.6		β-arr1	130 ± 6	6.81 ± 0.10	156
β-arr2	125 ± 4	7.63 ± 0.08	23.3		β-arr2	120 ± 5	6.95 ± 0.09	111
TAN67					Leu-Enk			
	E_{max} ± SEM (%)	pEC_{50} ± SEM	EC_{50} (nM)			E_{max} ± SEM (%)	pEC_{50} ± SEM	EC_{50} (nM)
Gi1	97 ± 2	8.16 ± 0.06	6.86		Gi1	94 ± 2	8.12 ± 0.06	7.60
Gi2	83 ± 2	8.68 ± 0.08	2.08		Gi2	92 ± 3	8.5 ± 0.10	3.18
Gi3	91 ± 2	8.29 ± 0.05	5.17		Gi3	96 ± 2	8.27 ± 0.05	5.34
GoA	123 ± 4	8.36 ± 0.09	4.32		GoA	92 ± 4	8.47 ± 0.12	3.37
GoB	108 ± 4	8.49 ± 0.10	3.24		GoB	89 ± 4	8.54 ± 0.12	2.9
Gz	106 ± 1	9.14 ± 0.03	0.73		Gz	102 ± 2	9.02 ± 0.04	0.97
β-arr1	38 ± 6	7.52 ± 0.35	30.2		β-arr1	129 ± 6	7.69 ± 0.11	20.3
β-arr2	26 ± 3	8.96 ± 0.31	1.09		β-arr2	136 ± 6	7.68 ± 0.11	21.1
Deltorphin II					ARM390			
	E_{max} ± SEM (%)	pEC_{50} ± SEM	EC_{50} (nM)			E_{max} ± SEM (%)	pEC_{50} ± SEM	EC_{50} (nM)
Gi1	93 ± 3	8.42 ± 0.07	3.82		Gi1	118 ± 2	7.58 ± 0.05	26.1
Gi2	88 ± 4	9.11 ± 0.10	0.78		Gi2	101 ± 3	7.65 ± 0.09	22.5
Gi3	92 ± 2	8.74 ± 0.06	1.84		Gi3	114 ± 3	7.48 ± 0.06	33.3
GoA	89 ± 4	9.05 ± 0.12	0.9		GoA	106 ± 2	7.95 ± 0.06	11.2
GoB	83 ± 4	8.78 ± 0.13	1.66		GoB	118 ± 2	8.11 ± 0.05	7.68
Gz	93 ± 2	9.14 ± 0.05	0.73		Gz	112 ± 2	8.44 ± 0.05	3.62
β-arr1	107 ± 7	8.06 ± 0.16	8.72		β-arr1	118 ± 8	6.41 ± 0.14	388
β-arr2	105 ± 6	8.07 ± 0.13	8.52		β-arr2	111 ± 5	6.64 ± 0.09	228
C6-Quino					C5-Quino			
	E_{max} ± SEM (%)	pEC_{50} ± SEM	EC_{50} (nM)			E_{max} ± SEM (%)	pEC_{50} ± SEM	EC_{50} (nM)
Gi1	89 ± 2	7.72 ± 0.06	18.93		Gi1	102 ± 3	8.42 ± 0.07	3.81
Gi2	74 ± 4	7.68 ± 0.14	20.95		Gi2	76 ± 6	8.16 ± 0.20	7.00
Gi3	77 ± 2	7.89 ± 0.07	12.85		Gi3	94 ± 3	8.37 ± 0.07	4.25
GoA	83 ± 2	7.99 ± 0.07	10.22		GoA	82 ± 3	8.46 ± 0.09	3.47
GoB	86 ± 2	8.09 ± 0.08	8.14		GoB	90 ± 4	8.86 ± 0.10	1.39
Gz	101 ± 2	8.36 ± 0.06	4.33		Gz	97 ± 3	8.89 ± 0.07	1.28
β-arr1	34 ± 10	6.45 ± 0.60	354.1		β-arr1	82 ± 7	7.38 ± 0.21	42.0
β-arr2	45 ± 3	7.5 ± 0.20	31.3		β-arr2	84 ± 5	7.74 ± 0.14	18.3

Supplementary Table 5. TRUPATH data from **Supplementary Fig. 11**.

	DOR-G_{i1}-C5 Quino	DOR-G_{i1}-C6 Quino
	(EMD-45582) (PDB 9CGK)	(EMD-45581) (PDB 9CGJ)
Data collection and processing		
Magnification	45,000	45,000
Voltage (kV)	200	200
Electron exposure (e-/Å^2)	54.2	54.2
Number of movies used	2526	3090
Defocus mean (SD) µm	1.4 (0.3)	1.5 (0.2)
Pixel size (Å)	0.88	0.88
Symmetry imposed	C1	C1
Initial particle images (No.)	3,367,096	2,606,568
Final particle images (No.)	476,926	718,707
Map resolution (Å)	2.62	2.80
FSC threshold 0.143	0.143	0.143
Map resolution range (Å)	2.62-6.5	2.80-6.6
Refinement		
Initial model used (PDB code)	4N6H, 7DZP	4N6H, 7DZP
Model resolution (Å)	2.82	2.97
FSC threshold	0.5	0.5
Map sharpening B factor (Å^2)	-69.58	-48.19
Model composition		
Non-hydrogen atoms	8831	8804
Protein residues	1133	1130
Ligands	1	1
Protein - B factor (Å^2)	52.15	67.65
Ligands - B factor (Å^2)	86.79	109.33
r.m.s. deviations		
Bond lengths (Å)	0.005	0.004
Bond angles (°)	0.754	0.857
Validation		
MolProbity score	1.63	1.49
Clashscore	6.38	4.80
Poor rotamers (%)	0	0
Ramachandran plot		
Favored (%)	95.97	96.41
Allowed (%)	3.94	3.59
Disallowed (%)	0	0

Supplementary Table 6. Cryo-EM data collection, refinement and validation statistics.

Molecule	DOR-C5-Gi complex	DOR-C6-Gi complex
DOR protein	1	1
Gi1 protein	1	1
Gβ protein	1	1
Gγ protein	1	1
Ligand	1	1
CHL1	180	180
DPPC	330	330
DOPC	90	90
Sodium	188	172
Chloride	193	177
Water	69353	63183

Supplementary Table 7. Number of Molecules in the assembled system for Molecular Dynamics simulation

Fine Category	Condition (a)	Condition (b)	Condition (c)	Coarse Category
(I) No Polar Interaction	No	No	No	No Polar Contact
(II) WaterMediated Only (NonSelf)	No	No	Yes	No Polar Contact
(III) WaterMediated Only (Self)	No	Yes	No	WaterMediated Only
(IV) WaterMediated Only (Self+NonSelf)	No	Yes	Yes	WaterMediated Only
(V) Direct Only	Yes	No	No	Direct Contact
(VI) Direct + WaterMediated (Self)	Yes	Yes	No	Direct Contact
(VII) Direct + WaterMediated (NonSelf)	Yes	No	Yes	Direct Contact
(VIII) Direct + WaterMediated (Self+NonSelf)	Yes	Yes	Yes	Direct Contact

The conditions fulfilled by each category are the followings.

- Condition (a): There are direct interaction(s) between the ligand and the specific protein sidechain.
- Condition (b): There are "Self" water-mediated interaction(s) between the ligand and the specific protein sidechain.
- Condition (c): There are "NonSelf" water-mediated interaction(s) between the ligand and the other protein sidechain(s) in the group.

Supplementary Table 8. Definition of the categories of interactions between the ligand and the specific protein sidechain in the Molecular Dynamics simulations.

Receptor	$pEC_{50} \pm SEM$					
	BW373U86		DPDPE		C6-Quino	
	Gi1	β-arrestin2	Gi1	β-arrestin2	Gi1	β-arrestin2
δOR WT	9.81 ± 0.03	8.36 ± 0.07	9.03 ± 0.05	7.63 ± 0.06	8.04 ± 0.08	7.08 ± 0.13
δOR K214A	9.86 ± 0.05	8.39 ± 0.04	6.31 ± 0.11 * (p = 0.03)	N/A	8.57 ± 0.08 ** (p = 0.002)	7.57 ± 0.13 * (p = 0.02)
δOR Q105A	9.72 ± 0.09	8.75 ± 0.13 ** (p = 0.003)	6.63 ± 0.8	N/A	8.53 ± 0.22 ** (p = 0.003)	7.85 ± 0.42 ** (p = 0.002)
δOR V281A	9.34 ± 0.08 **** (p < 0.0001)	7.73 ± 0.02 *** (p = 0.0002)	9.55 ± 0.12	8.07 ± 0.13 ** (p = 0.003)	6.99 ± 0.10 **** (p < 0.0001)	N/A

Receptor	Emax ± SEM (%)					
	BW373U86		DPDPE		C6-Quino	
	Gi1	β-arrestin2	Gi1	β-arrestin2	Gi1	β-arrestin2
δOR WT	100 ± 1	100 ± 2	89 ± 1	83 ± 2	68 ± 2	44 ± 2
δOR K214A	100 ± 1	100 ± 1	76 ± 4	N/A	82 ± 2	85 ± 4
δOR Q105A	97 ± 2	101 ± 4	28 ± 7 *** (p = 0.0002)	N/A	59 ± 3	47 ± 6
δOR V281A	100 ± 2	99 ± 1	90 ± 3	78 ± 3	82 ± 4	N/A

Supplementary Table 9. Effects of mutations in δOR orthosteric pocket contributing to C6-Quino signaling. Values represent pEC_{50} ± standard error (top) or Emax ± standard error (bottom) of BRET1 (β-arrestin2) or BRET2 (Gi1) with three independent biological replicates. Mutant's potency and efficacy were compared to wildtype for significance analysis using one-way ANOVA with Dunnett's multiple-comparison test or unpaired two-tailed student's t-test (if only one mutant is compared). N/A, no activity. Asterisk (*) represents $p<0.05$, asterisk (**) represents $p<0.01$, asterisk (***) represents $p<0.001$, asterisk (****) represents $p<0.0001$. Exact p-value is listed in parentheses.

Receptor	pKi ± SEM		
	BW373U86	DPDPE	C6-Quino
δOR WT	8.23 ± 0.02	6.60 ± 0.05	7.49 ± 0.06
δOR K214A	8.09 ± 0.08	7.26 ± 0.92	7.42 ± 0.18
δOR Q105A	8.66 ± 0.09	N/A	8.37 ± 0.07
δOR V281A	8.41 ± 0.28	7.83 ± 0.23	7.29 ± 0.10

Supplementary Table 10. Binding affinity of C6-Quino at δOR orthosteric pocket mutations. Values represent pK_i ± SEM of radioligand competition binding with ^{3}H-NTI with three independent replicates. Mutant binding affinity was compared to wildtype for significance analysis one-way ANOVA with Dunnett's multiple-comparison test, but no significance was found. N/A, no activity.

Receptor	$pEC_{50} \pm SEM$					
	BW373U86		DPDPE		C6-Quino	
	Gi1	β-arrestin2	Gi1	β-arrestin2	Gi1	β-arrestin2
δOR wt	9.81 ± 0.03	8.36 ± 0.07	9.03 ± 0.05	7.63 ± 0.06	8.04 ± 0.08	7.08 ± 0.13
δOR S135A	9.52 ± 0.12	N/A	8.08 ± 0.11	N/A	7.96 ± 0.63	N/A
δOR D95A	8.23 ± 0.03 ** (p = 0.001)	N/A	6.15 ± 0.32 ** (p = 0.004)	N/A	6.69 ± 0.39 * (p = 0.03)	N/A
δOR S311A	9.52 ± 0.08	8.38 ± 0.08	8.28 ± 0.15	7.40 ± 0.20	8.79 ± 0.17	N/A
δOR N131A	N/A	N/A	N/A	N/A	N/A	N/A

Receptor	Emax ± SEM (%)					
	BW373U86		DPDPE		C6-Quino	
	Gi1	β-arrestin2	Gi1	β-arrestin2	Gi1	β-arrestin2
δOR WT	100 ± 1	100 ± 2	89 ± 1	83 ± 2	68 ± 2	44 ± 2
δOR S135A	100 ± 3	N/A	75 ± 2	N/A	12 ± 7 * (p = 0.04)	N/A
δOR D95A	95 ± 8	N/A	55 ± 9	N/A	33 ± 7	N/A
δOR S311A	99 ± 2	100 ± 3	62 ± 3	54 ± 4 ** (p = 0.004)	56 ± 2	N/A
δOR N131A	N/A	N/A	N/A	N/A	N/A	N/A

Supplementary Table 11. Effects of mutations in δOR sodium pocket to C6-quino signaling. Values represent pEC_{50} ± SEM (top) or Emax ± standard error (bottom) of BRET1 (β-arrestin2) or BRET2 (Gi1) with three independent biological replicates. Mutant's potency and efficacy were compared to wildtype for significance analysis using one-way ANOVA with Dunnett's multiple-comparison test or unpaired two-tailed student's t-test (if only one mutant is compared). N/A, no activity. Asterisk (*) represents $p<0.05$, asterisk (**) represents $p<0.01$.

BRET	δOR G_{i1}			δOR β-arrestin1			δOR β-arrestin2		
Ligand	Emax ± SEM (%)	pEC_{50} ± SEM	EC_{50} (nM)	Emax ± SEM (%)	pEC_{50} ± SEM	EC_{50} (nM)	Emax ± SEM (%)	pEC_{50}± SEM	EC_{50} (nM)
DPDPE	100 ± 1	8.88 ± 0.04	1.3	99 ± 2	7.12 ± 0.06	75	100 ± 2	7.55 ± 0.05	28
C8-NH_2-Quino	60 ± 2	7.59 ± 0.09	25	27 ± 2	6.75 ± 0.24	180	46 ± 2	6.77 ± 0.10	170
C8-Quino-Carbon	55 ± 2	6.41 ± 0.07	390	23 ± 5	5.66 ± 0.44	2.2 µM	64 ± 2	6 ± 0.08	1.0 µM
C6-Urea-Quino	54 ± 3	5.85 ± 0.09	1.4 µM	NA	NA	NA	20 ± 4	5.91 ± 0.33	1.2 µM

Supplementary Table 12. G-protein and β-arrestin signaling of amine, urea and 8C substituted quinoline

Receptor	pKi ± SEM				
	DPDPE	C6-Quino	C8-Carbon-Quino	C6-Urea-Quino	C8-NH_2-Quino
δOR	8.96 ± 0.08	8.87 ± 0.05	8.80 ± 0.08	7.03 ± 0.04	8.58 ± 0.03

Supplementary Table 13. Binding affinity of Quino compound deriva-tives with varied head group polarity. Values represent pK_i ± SEM of radioligand competition binding with with ^{3}H-DPDPE three independent replicates.

	Intrinsic Clearance	Microsome Stability	Plasma Stability	Plasma Protein Binding
Compound	Species (Clint - µl/min/mg)	half life (h)	half life (h)	% Bound
Sunitinib	25.6	27.1		
C6-Quino	< 6	> 2	> 8	88
Carbamezapine				65.4
Ritonavir				99.2

Supplementary Table 14. Intrinsic clearance, microsome stability for Sunitinib and 6C-Quino. Plasma stability of C6-Quino, and protein binding for C6-Quino, carbamezapine and ritonavir. Microsome stability was estimated from curve fitting six time points of one incubation. Timepoints are between 0 and 60 minutes. Plasma stability and plasma binding were estimated from the average of 2 replicates.

Figure no.	Panel title / Variable	Statistical Model / Comparisons	Test statistic	Number per group
5A	Anti-allodynia CCI	Repeated Measure Mixed-effects model (REML) followed by Tukey's multiple comparisons test	Time F (5.027, 225.0) = 52.70 **** p<0.0001 Treatment F (4, 46) = 22.84 **** p<0.0001 Time x Treatment F (32, 358) = 6.166 **** p<0.0001	
	20 min			
	Vehicle control (5% DMSO/95% saline) sc vs. C6-Quino (10 mg/kg, sc)	Tukey Post-hoc Analysis	*** p=0.0002	Vehicle (14) C6-Quino_10mg/kg (9)
	Vehicle control (5% DMSO/95% saline) sc vs. C6-Quino (30 mg/kg, sc)	Tukey Post-hoc Analysis	* p=0.0104	Vehicle (14) C6-Quino_30mg/kg (9)
	Vehicle control (5% DMSO/95% saline) sc vs. Gabapentin (50 mg/kg, IP)	Tukey Post-hoc Analysis	*** p=0.0007	Vehicle (14) Gabapentin_50mg/kg (11)
	40 min			
	Vehicle control (5% DMSO/95% saline) sc vs. C6-Quino (10 mg/kg, sc)	Tukey Post-hoc Analysis	** p=0.006	Vehicle (14) C6-Quino_10mg/kg (9)
	Vehicle control (5% DMSO/95% saline) sc vs. C6-Quino (30 mg/kg, sc)	Tukey Post-hoc Analysis	** p=0.007	Vehicle (14) C6-Quino_30mg/kg (9)
	Vehicle control (5% DMSO/95% saline) sc vs. Gabapentin (50 mg/kg, IP)	Tukey Post-hoc Analysis	*** p=0.0002	Vehicle (14) Gabapentin_50mg/kg (11)
	60 min			
	Vehicle control (5% DMSO/95% saline) sc vs. C6-Quino (10 mg/kg, sc)	Tukey Post-hoc Analysis	** p=0.0026	Vehicle (14) C6-Quino_10mg/kg (9)
	Vehicle control (5% DMSO/95% saline) sc vs. C6-Quino (30 mg/kg, sc)	Tukey Post-hoc Analysis	** p=0.0047	Vehicle (14) C6-Quino_30mg/kg (9)
	Vehicle control (5% DMSO/95% saline) sc vs. Gabapentin (50 mg/kg, IP)	Tukey Post-hoc Analysis	*** p=0.0005	Vehicle (14) Gabapentin_50mg/kg (11)
	80 min			
	Vehicle control (5% DMSO/95% saline) sc vs. C6-Quino (10 mg/kg, sc)	Tukey Post-hoc Analysis	* p=0.0121	Vehicle (14) C6-Quino_10mg/kg (9)
	Vehicle control (5% DMSO/95% saline) sc vs. C6-Quino (30 mg/kg, sc)	Tukey Post-hoc Analysis	**** p=<0.0001	Vehicle (14) C6-Quino_30mg/kg (9)
	Vehicle control (5% DMSO/95% saline) sc vs. Gabapentin (50 mg/kg, IP)	Tukey Post-hoc Analysis	** p=0.0044	Vehicle (14) Gabapentin_50mg/kg (11)
	100 min			
	Vehicle control (5% DMSO/95% saline) sc vs. C6-Quino (10 mg/kg, sc)	Tukey Post-hoc Analysis	* p=0.0257	Vehicle (14) C6-Quino_10mg/kg (9)
	Vehicle control (5% DMSO/95% saline) sc			
	PRE vs. POST	Tukey Post-hoc Analysis	**** p=<0.0001	14
	PRE vs. 20 min	Tukey Post-hoc Analysis	**** p=<0.0001	14
	PRE vs. 40 min	Tukey Post-hoc Analysis	**** p=<0.0001	14
	PRE vs. 60 min	Tukey Post-hoc Analysis	**** p=<0.0001	14
	PRE vs. 80 min	Tukey Post-hoc Analysis	**** p=<0.0001	14
	PRE vs. 100 min	Tukey Post-hoc Analysis	**** p=<0.0001	14
	PRE vs. 140 min	Tukey Post-hoc Analysis	**** p=<0.0001	14
	PRE vs. 180 min	Tukey Post-hoc Analysis	*** p=0.0001	14
	C6-Quino (5 mg/kg sc)			
	PRE vs. POST	Tukey Post-hoc Analysis	**** p=<0.0001	8
	PRE vs. 20 min	Tukey Post-hoc Analysis	**** p=<0.0001	8
	PRE vs. 40 min	Tukey Post-hoc Analysis	**** p=<0.0001	8
	PRE vs. 60 min	Tukey Post-hoc Analysis	**** p=<0.0001	8
	PRE vs. 80 min	Tukey Post-hoc Analysis	**** p=<0.0001	8
	PRE vs. 100 min	Tukey Post-hoc Analysis	**** p=<0.0001	8
	PRE vs. 140 min	Tukey Post-hoc Analysis	**** p=<0.0001	8
	PRE vs. 180 min	Tukey Post-hoc Analysis	**** p=<0.0001	8
	C6-Quino (10 mg/kg, sc)			
	PRE vs. POST	Tukey Post-hoc Analysis	** p=0.0016	9
	POST vs. 20 min	Tukey Post-hoc Analysis	** p=0.0015	9
	POST vs. 40 min	Tukey Post-hoc Analysis	** p=0.0094	9
	POST vs. 60 min	Tukey Post-hoc Analysis	** p=0.0019	9
	POST vs. 80 min	Tukey Post-hoc Analysis	* p=0.012	9
	POST vs. 100 min	Tukey Post-hoc Analysis	* p=0.034	9
	C6-Quino (30 mg/kg, sc)			
	PRE vs. POST	Tukey Post-hoc Analysis	**** p=<0.0001	9
	PRE vs. 180 min	Tukey Post-hoc Analysis	* p=0.0153	PRE (9) vs 180min (3)
	POST vs. 20 min	Tukey Post-hoc Analysis	* p=0.011	9
	POST vs. 40 min	Tukey Post-hoc Analysis	** p=0.0094	9
	POST vs. 60 min	Tukey Post-hoc Analysis	** p=0.0061	9
	POST vs. 80 min	Tukey Post-hoc Analysis	*** p=0.0002	9
	Gabapentin (50 mg/kg, IP)			
	PRE vs. POST	Tukey Post-hoc Analysis	*** p=0.0002	11
	PRE vs. 180 min	Tukey Post-hoc Analysis	* p=0.0474	11
	POST vs. 20 min	Tukey Post-hoc Analysis	** p=0.0016	11
	POST vs. 40 min	Tukey Post-hoc Analysis	*** p=0.0002	11
	POST vs. 60 min	Tukey Post-hoc Analysis	*** p=0.0006	11
	POST vs. 80 min	Tukey Post-hoc Analysis	** p=0.0094	11

Supplementary Table 15. Data showing the effects of different treatments on anti-allodynia as assessed in a chronic constriction injury (CCI) model. The statistical analysis was performed using a Repeated Measures Mixed-effects Model (REML), followed by Tukey's post-hoc multiple comparisons test. The test statistics for each comparison are presented with corresponding p-values. Comparisons include the vehicle control (5% DMSO/95% saline), C6-Quino (10 and 30 mg/kg, sc), and Gabapentin (50 mg/kg, IP), across various time points (20, 40, 60, 80, 100, 140, and 180 minutes). The number of animals per group is indicated for each comparison. Significant differences are denoted as follows: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, with exact P values displayed in the table.

Figure no.	Panel title / Variable	Statistical Model / Comparisons	Test statistic	Number per group
5B	CFA-induced pain	Two-way Repeated Measures ANOVA with Holm-Šídák's post-hoc multiple comparisons	Time F (1, 28) = 53.19 **** p<0.0001 Treatment F (3, 28) = 2.892 ns p=0.0530 Time x Treatment F (3, 28) = 4.795 ** p=0.0081	
	3 day post CFA			
	VEH vs. C6-Quino	Holm-Šídák multiple comparisons	ns p=0.9996	8
	VEH vs. SNC80	Holm-Šídák multiple comparisons	ns p=0.9943	8
	VEH vs. Gabapentin	Holm-Šídák multiple comparisons	ns p=0.9996	8
	C6-Quino vs. SNC80	Holm-Šídák multiple comparisons	ns p=0.9943	8
	C6-Quino vs. Gabapentin	Holm-Šídák multiple comparisons	ns p=0.9996	8
	SNC80 vs. Gabapentin	Holm-Šídák multiple comparisons	ns p=0.9943	8
	Post-drug			
	VEH vs. C6-Quino	Holm-Šídák multiple comparisons	** p=0.0033	8
	VEH vs. SNC80	Holm-Šídák multiple comparisons	*** p=0.0006	8
	VEH vs. Gabapentin	Holm-Šídák multiple comparisons	** p=0.0030	8
	C6-Quino vs. SNC80	Holm-Šídák multiple comparisons	ns p=0.8977	8
	C6-Quino vs. Gabapentin	Holm-Šídák multiple comparisons	ns p=0.9209	8
	SNC80 vs. Gabapentin	Holm-Šídák multiple comparisons	ns p=0.8977	8
	VEH			
	3 day post CFA vs. Post-drug C6-Quino	Holm-Šídák multiple comparisons	ns = 0.6415	8
	3 day post CFA vs. Post-drug SNC80	Holm-Šídák multiple comparisons	*** p=0.0002	8
	3 day post CFA vs. Post-drug Gabapentin	Holm-Šídák multiple comparisons	**** p<0.0001	8
	3 day post CFA vs. Post-drug	Holm-Šídák multiple comparisons	*** p=0.0001	8

Supplementary Table 16. Effects of various treatments on CFA-induced pain in a murine model. Data showing the effects of different treatments (Vehicle, C6-Quino, SNC80, and Gabapentin) on pain behavior following Complete Freund's Adjuvant (CFA) injection. A Two-way Repeated Measures ANOVA model was performed and comparisons between treatment 3 days post-CFA, and 1 hour after post-drug treatment were analyzed using Holm-Šídák's multiple comparisons test. The test statistics and corresponding p-values are shown for each comparison. Significant differences are indicated as follows: **p < 0.01, ***p < 0.001, and "ns" indicates no significant difference. The number of animals per group is indicated for each comparison.

Figure no.	Panel title / Variable	Statistical Model / Comparisons	Test statistic	Number per group
5C	NTG-cephalic mechanical thresholds	Three-way Repeated Measures ANOVA with Holm-Šídák's post-hoc multiple comparisons	Time F (1, 32) = 2.881 ns p=0.0993 SAL/NTG F (1, 32) = 39.30 **** p<0.0001 VEH/C6-Quino F (1, 32) = 4.482 * p=0.0421 Time x SAL/NTG F (1, 32) = 2.154 ns p=0.1519 Time x VEH/C6-Quino F (1, 32) = 3.447 ns p=0.0726 SAL/NTG x VEH/C6-Quino F (1, 32) = 1.675 ns p=0.2049 Time x SAL/NTG x VEH/DOR F (1, 32) = 4.434 * p=0.0432	
	Baselines			
	SAL/VEH vs. SAL/C6-Quino	Holm-Šídák multiple comparisons	ns p=0.9973	9
	NTG/VEH vs. NTG/C6-Quino	Holm-Šídák multiple comparisons	ns p=0.9973	9
	SAL/VEH vs. NTG/VEH	Holm-Šídák multiple comparisons	** p=0.0055	9
	SAL/C6-Quino vs. NTG/C6-Quino	Holm-Šídák multiple comparisons	*** p=0.0007	9
	Post-drug			
	SAL/VEH vs. SAL/C6-Quino	Holm-Šídák multiple comparisons	ns p=0.9973	9
	NTG/VEH vs. NTG/C6-Quino	Holm-Šídák multiple comparisons	** p=0.0049	9
	SAL/VEH vs. NTG/VEH	Holm-Šídák multiple comparisons	*** p=0.0009	9
	SAL/C6-Quino vs. NTG/C6-Quino	Holm-Šídák multiple comparisons	ns p=0.9709	9

Supplementary Table 17. Effect of C6-Quino on NTG-induced cephalic pain in a murine model. Data showing the effects of C6-Quino on pain behavior following chronic nitroglycerin (NTG) administration. A Three-way Repeated Measures ANOVA was performed and comparisons between treatments at baselines and post-drug timepoints were analyzed using Holm-Šídák's multiple comparisons test. The test statistics and corresponding p-values are shown for each comparison. Significant differences are indicated as follows: *p < 0.05, **p < 0.01, ***p < 0.001, and "ns" indicates no significant difference. The number of animals per group is indicated for each comparison.

Figure no.	Panel title / Variable	Statistical Model / Comparisons	Test statistic	Number per group
5D	Antagonism/PO activity CCI	Two-way ANOVA (Ordinary) followed by Dunnett's multiple comparisons test	Treatment: F(3,144) = 34.99, p < 0.0001	
	20 min Vehicle control (5% DMSO/95% saline) sc vs. C6-Quino (30 mg/kg, sc)	Dunnett's multiple comparisons test	*** p=0.0004	Vehicle (14) C6-Quino_30mg/kg (14)
	C6-Quino (30 mg/kg, sc) vs. NTB (3.2 mg/kg, sc)	Dunnett's multiple comparisons test	* p=0.0216	C6-Quino_30mg/kg (14) NTB 3.2 mg/kg (8)
	40 min Vehicle control (5% DMSO/95% saline) sc vs. C6-Quino (30 mg/kg, sc)	Dunnett's multiple comparisons test	**** p=<0.0001	Vehicle (14) C6-Quino_30mg/kg (14)
	NTB (3.2 mg/kg, sc) vs C6-Quino (30 mg/kg, sc)	Dunnett's multiple comparisons test	* *p=0.0092	C6-Quino_30mg/kg (14) NTB 3.2 mg/kg (8)
	60 min Vehicle control (5% DMSO/95% saline) sc vs. C6-Quino (30 mg/kg, sc)	Dunnett's multiple comparisons test	**** p=<0.0001	Vehicle (14) C6-Quino_30mg/kg (14)
	NTB (3.2 mg/kg, sc) vs C6-Quino (30 mg/kg, sc)	Dunnett's multiple comparisons test	****p<0.0001	C6-Quino_30mg/kg (14) NTB 3.2 mg/kg (8)
	Vehicle control (5% DMSO/95% saline) sc vs. C6-Quino (30 mg/kg, po)	Dunnett's multiple comparisons test	*p=0.0421	Vehicle (14) C6-Quino_30mg/kg (4)
	80 min Vehicle control (5% DMSO/95% saline) sc vs. C6-Quino (30 mg/kg, sc)	Dunnett's multiple comparisons test	**** p=<0.0001	Vehicle (14) C6-Quino_30mg/kg (14)
	NTB (3.2 mg/kg, sc) vs C6-Quino (30 mg/kg, sc)	Dunnett's multiple comparisons test	**** p<0.0001	C6-Quino_30mg/kg (14) NTB 3.2 mg/kg (8)
	Vehicle control (5% DMSO/95% saline) sc vs. C6-Quino (30 mg/kg, po)	Dunnett's multiple comparisons test	*p=0.0154	Vehicle (14) C6-Quino_30mg/kg (4)

Supplementary Table 18. Antagonism of C6-Quino's activity and oral activity in chronic constriction injury (CCI). Data show the effects of C6-Quino on pain behavior following CCI and the impact of NTB, a δOR antagonist. A Two-way ANOVA (Ordinary) was performed, followed by Dunnett's multiple comparisons test to compare treatment effects at different time points (20, 40, 60, and 80 minutes). The test statistics and corresponding p-values are shown for each comparison. Significant differences are indicated as follows: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, exact p values being displayed in the table and "ns" indicates no significant difference. The number of animals per group is indicated for each comparison.

Figure	Panel title / Variable	Statistical Model / Comparisons	Test statistic	Number per group
5F	Seizures	Two-way ANOVA (Ordinary) followed by Šídák's multiple comparisons test	Time: F(10, 231) = 6.99, p < 0.0001 Treatment: F(2, 231) = 79.04, p < 0.0001	
	3 min			
	Vehicle control (5% DMSO/95% saline, sc) vs. SNC80 (10mg,kg, sc)	Dunnett's multiple comparisons test	*** p=0.0006	SNC80 10mg,kg, sc (8); Vehicle (8)
	Vehicle control (5% DMSO/95% saline, sc) vs. C6-quino (30 mg/kg, sc)	Dunnett's multiple comparisons test	ns p=>0.9999	C6-quino 30 mg/kg,sc (8); Vehicle (8)
	6 min			
	Vehicle control (5% DMSO/95% saline, sc) vs. SNC80 (10mg,kg, sc)	Dunnett's multiple comparisons test	**** p=<0.0001	SNC80 10mg,kg, sc (8); Vehicle (8)
	Vehicle control (5% DMSO/95% saline, sc) vs. C6-quino (30 mg/kg, sc)	Dunnett's multiple comparisons test	ns p=>0.9999	C6-quino 30 mg/kg,sc (8); Vehicle (8)
	9 min			
	Vehicle control (5% DMSO/95% saline, sc) vs. SNC80 (10mg,kg, sc)	Dunnett's multiple comparisons test	**** p=<0.0001	SNC80 10mg,kg, sc (8); Vehicle (8)
	Vehicle control (5% DMSO/95% saline, sc) vs. C6-quino (30 mg/kg, sc)	Dunnett's multiple comparisons test	ns p=>0.9999	C6-quino 30 mg/kg,sc (8); Vehicle (8)
	12 min			
	Vehicle control (5% DMSO/95% saline, sc) vs. SNC80 (10mg,kg, sc)	Dunnett's multiple comparisons test	**** p=<0.0001	SNC80 10mg,kg, sc (8); Vehicle (8)
	Vehicle control (5% DMSO/95% saline, sc) vs. C6-quino (30 mg/kg, sc)	Dunnett's multiple comparisons test	ns p=>0.9999	C6-quino 30 mg/kg,sc (8); Vehicle (8)
	15 min			
	Vehicle control (5% DMSO/95% saline, sc) vs. SNC80 (10mg,kg, sc)	Dunnett's multiple comparisons test	**** p=<0.0001	SNC80 10mg,kg, sc (8); Vehicle (8)
	Vehicle control (5% DMSO/95% saline, sc) vs. C6-quino (30 mg/kg, sc)	Dunnett's multiple comparisons test	ns p=>0.9999	C6-quino 30 mg/kg,sc (8); Vehicle (8)
	18 min			
	Vehicle control (5% DMSO/95% saline, sc) vs. SNC80 (10mg,kg, sc)	Dunnett's multiple comparisons test	** p=0.0023	SNC80 10mg,kg, sc (8); Vehicle (8)
	Vehicle control (5% DMSO/95% saline, sc) vs. C6-quino (30 mg/kg, sc)	Dunnett's multiple comparisons test	ns p=>0.9999	C6-quino 30 mg/kg,sc (8); Vehicle (8)

Supplementary Table 19. Seizure-inducing effects of SNC80 and C6-Quino. Data showing seizure activity following administration of SNC80 (a δOR agonist) and C6-Quino. A Two-way ANOVA (Ordinary) was performed, followed by Dunnett's multiple comparisons test to compare treatment effects at different time points (3, 6, 9, 12, 15, and 18 minutes). The test statistics and corresponding p-values are shown for each comparison. Significant differences are indicated as follows: **p < 0.01, ***p < 0.001, ****p < 0.0001, exact p values being displayed in the table and "ns" indicates no significant difference. The number of animals per group is indicated for each comparison.

Figure no.	Panel title / Variable	Statistical Model / Comparisons	Test statistic	Number per group
5G	Locomotor	Two-way ANOVA (Ordinary) followed by Dunnett's multiple comparisons test	Time: F(8, 567) = 1.322, p = 0.2296; Treatment: FF(4, 567) = 540.7, p < 0.0001	
	20 min			
	Vehicle control (5% DMSO/95% saline, sc) vs. C6-quino (30 mg/kg, sc)	Dunnett's multiple comparisons test	ns, p = 0.9441	Vehicle (12); C6-quino (30 mg/kg, sc) (12)
	Saline vs. Morphine (10 mg/kg, sc)	Dunnett's multiple comparisons test	****, p < 0.0001	Saline (20); Morphine (10 mg/kg, sc) (12)
	Saline vs. Morphine (30 mg/kg, sc)	Dunnett's multiple comparisons test	****, p < 0.0001	Saline (20); Morphine (30 mg/kg, sc) (12)
	40 min			
	Vehicle control (5% DMSO/95% saline, sc) vs. C6-quino (30 mg/kg, sc)	Dunnett's multiple comparisons test	ns p=0.908	Vehicle (12); C6-quino (30 mg/kg, sc) (12)
	Saline vs. Morphine (10 mg/kg, sc)	Dunnett's multiple comparisons test	**** p=<0.0001	Saline (20); Morphine (10 mg/kg, sc) (12)
	Saline vs. Morphine (30 mg/kg, sc)	Dunnett's multiple comparisons test	****, p < 0.0001	Saline (20); Morphine (30 mg/kg, sc) (12)
	60 min			
	Vehicle control (5% DMSO/95% saline, sc) vs. C6-quino (30 mg/kg, sc)	Dunnett's multiple comparisons test	ns p=0.8988	Vehicle (12); C6-quino (30 mg/kg, sc) (12)
	Saline vs. Morphine (10 mg/kg, sc)	Dunnett's multiple comparisons test	**** p=<0.0001	Saline (20); Morphine (10 mg/kg, sc) (12)
	Saline vs. Morphine (30 mg/kg, sc)	Dunnett's multiple comparisons test	****, p < 0.0001	Saline (20); Morphine (30 mg/kg, sc) (12)
	80 min			
	Vehicle control (5% DMSO/95% saline, sc) vs. C6-quino (30 mg/kg, sc)	Dunnett's multiple comparisons test	ns p=0.9954	Vehicle (12); C6-quino (30 mg/kg, sc) (12)
	Saline vs. Morphine (10 mg/kg, sc)	Dunnett's multiple comparisons test	**** p=<0.0001	Saline (20); Morphine (10 mg/kg, sc) (12)
	Saline vs. Morphine (30 mg/kg, sc)	Dunnett's multiple comparisons test	****, p < 0.0001	Saline (20); Morphine (30 mg/kg, sc) (12)
	100 min			
	Vehicle control (5% DMSO/95% saline, sc) vs. C6-quino (30 mg/kg, sc)	Dunnett's multiple comparisons test	ns p=>0.9999	Vehicle (12); C6-quino (30 mg/kg, sc) (12)
	Saline vs. Morphine (10 mg/kg, sc)	Dunnett's multiple comparisons test	**** p=<0.0001	Saline (20); Morphine (10 mg/kg, sc) (12)
	Saline vs. Morphine (30 mg/kg, sc)	Dunnett's multiple comparisons test	****, p < 0.0001	Saline (20); Morphine (30 mg/kg, sc) (12)
	120 min			
	Vehicle control (5% DMSO/95% saline, sc) vs. C6-quino (30 mg/kg, sc)	Dunnett's multiple comparisons test	ns p=0.9999	Vehicle (12); C6-quino (30 mg/kg, sc) (12)
	Saline vs. Morphine (10 mg/kg, sc)	Dunnett's multiple comparisons test	**** p=<0.0001	Saline (20); Morphine (10 mg/kg, sc) (12)
	Saline vs. Morphine (30 mg/kg, sc)	Dunnett's multiple comparisons test	****, p < 0.0001	Saline (20); Morphine (30 mg/kg, sc) (12)
	140 min			
	Vehicle control (5% DMSO/95% saline, sc) vs. C6-quino (30 mg/kg, sc)	Dunnett's multiple comparisons test	ns p=>0.9999	Vehicle (12); C6-quino (30 mg/kg, sc) (12)
	Saline vs. Morphine (10 mg/kg, sc)	Dunnett's multiple comparisons test	**** p=<0.0001	Saline (20); Morphine (10 mg/kg, sc) (12)
	Saline vs. Morphine (30 mg/kg, sc)	Dunnett's multiple comparisons test	****, p < 0.0001	Saline (20); Morphine (30 mg/kg, sc) (12)
	160 min			
	Vehicle control (5% DMSO/95% saline, sc) vs. C6-quino (30 mg/kg, sc)	Dunnett's multiple comparisons test	ns p=0.9977	Vehicle (12); C6-quino (30 mg/kg, sc) (12)
	Saline vs. Morphine (10 mg/kg, sc)	Dunnett's multiple comparisons test	**** p=<0.0001	Saline (20); Morphine (10 mg/kg, sc) (12)
	Saline vs. Morphine (30 mg/kg, sc)	Dunnett's multiple comparisons test	****, p < 0.0001	Saline (20); Morphine (30 mg/kg, sc) (12)
	180 min			
	Vehicle control (5% DMSO/95% saline, sc) vs. C6-quino (30 mg/kg, sc)	Dunnett's multiple comparisons test	ns, p=0.9982	Vehicle (12); C6-quino (30 mg/kg, sc) (12)
	Saline vs. Morphine (10 mg/kg, sc)	Dunnett's multiple comparisons test	**** p=<0.0001	Saline (20); Morphine (10 mg/kg, sc) (12)
	Saline vs. Morphine (30 mg/kg, sc)	Dunnett's multiple comparisons test	****, p < 0.0001	Saline (20); Morphine (30 mg/kg, sc) (12)

Supplementary Table 20. Effects of C6-Quino and Morphine on Locomotor Activity. Data showing locomotor activity following administration of C6-Quino and Morphine. A Two-way ANOVA (Ordinary) was performed, followed by Dunnett's multiple comparisons test to compare treatment effects at different time points (20, 40, 60, 80, 100, 120, 140, and 160 minutes). The test statistics and corresponding p-values are shown for each comparison. Significant differences are indicated as follows: ****$p < 0.0001$, and "ns" indicates no significant difference. The number of animals per group is indicated for each comparison.

Figure no.	Panel title / Variable	Statistical Model / Comparisons	Test statistic	Number per group
5G	Respiration	Two-way ANOVA (Ordinary) followed by Dunnett's multiple comparisons test	Time: $F(9, 630) = 31.40$, $p < 0.0001$; Treatment: $F(4, 630) = 59.07$, $p < 0.0001$	
	20 min			
	Vehicle control (5% DMSO/95% saline, sc) vs. C6-quino (30 mg/kg, sc)	Dunnett's multiple comparisons test	ns, $p = 0.0529$	Vehicle (12); C6-quino (30 mg/kg, sc) (12)
	Saline vs. Morphine (30 mg/kg, sc)	Dunnett's multiple comparisons test	****, $p < 0.0001$	Saline (20); Morphine (30 mg/kg, sc) (12)
	40 min			
	Vehicle control (5% DMSO/95% saline, sc) vs. C6-quino (30 mg/kg, sc)	Dunnett's multiple comparisons test	ns, $p = 0.1144$	Vehicle (12); C6-quino (30 mg/kg, sc) (12)
	Saline vs. Morphine (30 mg/kg, sc)	Dunnett's multiple comparisons test	**** $p < 0.0001$	Saline (20); Morphine (30 mg/kg, sc) (12)
	60 min			
	Vehicle control (5% DMSO/95% saline, sc) vs. C6-quino (30 mg/kg, sc)	Dunnett's multiple comparisons test	ns, $p = 0.5508$	Vehicle (12); C6-quino (30 mg/kg, sc) (12)
	Saline vs. Morphine (30 mg/kg, sc)	Dunnett's multiple comparisons test	**** $p < 0.0001$	Saline (20); Morphine (30 mg/kg, sc) (12)
	80 min			
	Vehicle control (5% DMSO/95% saline, sc) vs. C6-quino (30 mg/kg, sc)	Dunnett's multiple comparisons test	ns, $p = 0.9925$	Vehicle (12); C6-quino (30 mg/kg, sc) (12)
	Saline vs. Morphine (30 mg/kg, sc)	Dunnett's multiple comparisons test	* $p = 0.0225$	Saline (20); Morphine (30 mg/kg, sc) (12)

Supplementary Table 21. Effects of C6-Quino and Morphine on Respiratory Function. Data showing respiratory changes following administration of C6-Quino and Morphine. A Two-way ANOVA (Ordinary) was performed, followed by Dunnett's multiple comparisons test to compare treatment effects at different time points (20, 40, and 60 minutes). The test statistics and corresponding p-values are shown for each comparison. Significant differences are indicated as follows: ****$p < 0.0001$, and "ns" indicates no significant difference. The number of animals per group is indicated for each comparison.