Supplemental Materials for:

Molecular pharmacodynamics of emixustat in protection against retinal degeneration

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Supplemental Methods

Chemicals and syntheses – Unless otherwise stated, solvents and reagents were purchased from Sigma (St. Louis, MO). Synthesis of Ret-NH_2 was performed by previously described procedures (1, 2). Because atRAL is much more stable than Ret-NH_2 or retinol, QEA-B-001 was synthesized and stored in the aldehyde form and then converted to a primary amine just prior to experiments as described for Ret-NH_2. The synthesis of racemic emixustat was achieved by following reported methods (3). The route for obtaining enantio-pure emixustat is described below and shown in Supplemental Figure 3, and is partly based on reported methods (4-6). Separation of emixustat enantiomers and their derivatives was achieved by normal phase HPLC (Daicel Chiralcel OD column, 250 x 4.60 mm) with 10% *i*-propanol/hexanes as the eluent. The flow rate was 1 mL/min. The signal was detected by UV absorbance at 272 nm. ^1H and ^{13}C-NMR spectra were recorded on a Varian Inova spectrometer (at the Department of Chemistry, Case Western Reserve University) operating at 400 MHz and 100 MHz for the ^1H and ^{13}C-NMR spectra, respectively. The internal references were TMS (δ 0.00) and $CDCl_3$ (δ 77.2) for ^1H and ^{13}C spectra, respectively.

Synthesis of enantio-pure emixustat (Supplemental Fig. 3)

3-Hydroxy-3-(3-hydroxyphenyl)propanenitrile (2): To a solution of anhydrous THF (150 ml) and acetonitrile (6.4 ml, 114.2 mmol), a solution of potassium *tert*-butoxide (28.4 g, 253.2 mmol) in THF (300 ml) was added at -45 ºC under nitrogen. After stirring for 45 min, 3-hydroxy-benzaldehyde (13.8 g, 112.8 mmol) in THF (60 ml) was added dropwise. The reaction was allowed to warm to -15 ºC over a 4 h period, and then was quenched by slow addition of saturated aq. NH_4Cl (200 ml). The layers were partitioned and the aqueous phase was extracted with EtOAc (2 x 100 ml). The combined organic layers were washed with water twice, brine, dried over anhydrous $MgSO_4$, and concentrated under vacuum. The crude compound was purified by column chromatography on silica gel and eluted with 50% EtOAc/hexanes to obtain **2** (14.7 g, 80%) ^1H NMR ($CDCl_3$, 400 MHz) δ 7.26 (t, 1H, *J* = 8 Hz), 6.95-6.91 (m, 2H), 6.82 (ddd, 1H, *J* = 1.2, 2.4, 8 Hz), 5.01 (t, 1H, *J* = 6.4 Hz), 2.77 (d, 2H, *J* = 6 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 156.5, 143.0, 130.4, 117.7, 117.4, 116.0, 112.5, 68.1, 25.7.

3-(3-Amino-1-hydroxypropyl) phenol (3): To a solution of nitrile **2** (10 g, 61.28 mmol) in dry THF (100 ml) at room temperature, borane methyl sulfide complex (5 M in ether, 36.8 ml) was slowly added. This reaction mixture was refluxed for 4 h. The reaction was quenched with methanol at 0 ºC, and the resulting solution was concentrated under vacuum. The residue was purified by silica gel column chromatography and eluted with $NH_4OH/MeOH/CH_2Cl_2$ (6 : 30 : 70) to afford **3** (9.2 g, 90%). ^{1}H NMR (DMSO-*d*$_6$, 400 MHz) δ 7.07 (t, 1H, *J* = 7.6 Hz), 6.74-6.72 (m, 1H), 6.70 (d, 1H, *J* = 7.6 Hz), 6.58 (ddd, 1H, *J* = 0.8, 2.4, 8 Hz), 4.55 (dd, 1H, *J* = 7.2, 7.6 Hz), 2.65-2.57 (m, 2H), 1.65-1.57 (m, 2H). ^{13}C NMR (DMSO-*d*$_6$, 100 MHz) δ 157.2, 148.2, 128.9, 116.4, 113.5, 112.6, 71.3, 48.7.

***tert*-Butyl-3-hydroxy-3-(3-hydroxyphenyl)propylcarbamate (4):** Di-*tert*-butyl dicarbonate (25 ml, 110 mmol) was slowly added into a mixture of amine **3** (20.0 g, 109.9 mmol) and potassium carbonate (15.3 g, 110.64 mmol) in dioxane (1 L). The resulting mixture was stirred at room temperature for 2 h. Then the reaction was quenched with water. The resulting mixture was extracted with EtOAc. The organic layer was washed with water, brine, dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated under vacuum. The crude compound was purified by silica gel column chromatography and eluted with 20% EtOAc/hexanes to afford **4** (18.5 g, 63%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.10 (t, 1H, *J* = 7.6 Hz), 6.82-6.78 (m,1H), 6.75 (d, 1H, *J* = 7.6 Hz), 6.71 (ddd, 1H, *J* = 0.8, 2.4, 8 Hz), 4.57 (t, 1H, *J* = 6.8 Hz), 3.30-3.12 (m, 1H), 3.12-3.10 (m, 1H), 1.82-1.73 (m, 2H), 1.42 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 157.1, 156.5, 145.7, 129.7, 117.6, 114.8, 112.8, 80.0, 71.9, 39.1, 37.7, 28.5.

***tert*-Butyl-3-(3-hydroxyphenyl)-3-oxopropylcarbamate (5):** Alcohol **4** (18.5g, 69 mmol) in dichloromethane (100 ml) was slowly added to the reaction mixture of celite (22.6g) and PCC (22.4g, 104 mmol) in dichloromethane (200 ml) over 15 min at 0 ºC. The reaction mixture was stirred at room temperature for 2 h. Then it was filtered through a pad of celite, and the filter bed was washed with dichloromethane. The filtrate was concentrated under vacuum. The resulting residue was purified by silica gel column chromatography and eluted with 40% EtOAc/hexanes to afford **5** (12.7 g, 46%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.50-7.43 (m, 2H), 7.31 (t, 1H, *J* = 8 Hz), 7.09 (dd, 1H, *J* = 2.4, 8 Hz), 5.23(brs, 1H), 3.53 (t, 2H, *J* = 5.6 Hz), 3.16 (t, 2H, *J* = 5.6 Hz), 1.43 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 199.5, 156.8, 156.5, 138.0, 130.1, 121.0, 120.3, 114.8, 79.9, 38.9, 35.7, 28.5.

2-(3-(3-Hydroxyphenyl)-3-oxopropyl)isoindoline-1,3-dione (6): To a solution of ketone **5** (13.38 g, 50.4 mmol) at 0 ^{o}C in anhydrous dichloromethane (55 ml) was added trifluoroacetic acid (55 ml). The solution was stirred at room temperature for 2 h. Then the solvent was removed under vacuum. The residue was triturated with toluene, then dissolved in acetonitrile/toluene (1 : 3; 168 ml) at 0 ºC. Then *N,N*-diisopropylethylamine (DIPEA) (20.9 ml, 120 mmol) and phthalic anhydride (7.14 g, 48.2 mmol) were serially added into the reaction solution and the mixture was refluxed for 2 h with a Dean-Stark assembly. After completion of the reaction, the solvent was removed under vacuum and the residue was dissolved in dichloromethane. The resulting solution was washed with water, saturated NH_4Cl, and saturated $NaHCO_3$ separately, dried over anhydrous $MgSO_4$, and filtered. The filtrate was concentrated under vacuum. The residue was purified by silica gel column chromatography with 75% EtOAc/hexanes as eluent to afford product **6** as a white solid (13.0 g, 80%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.88-7.82 (m, 2H), 7.76-7.68 (m, 2H), 7.50-7.43 (m, 2H), 7.30 (t, 1H, *J* = 8 Hz), 7.06 (d, 1H, *J* = 0.8, 2.8, 8 Hz), 6.11 (bs, 1H), 4.13 (t, *J* = 7.6 Hz, 2H), 3.39 (t, 2H, *J* = 7.2 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 197.5, 168.5, 156.4, 137.8, 134.2, 132.1, 130.1, 123.5, 120.9, 120.7, 114.6, 37.1, 33.7.

(*S*)-2-(3-Hydroxy-3-(3-hydroxyphenyl)propyl)isoindoline-1,3-dione (7a): A solution of (-)-diisopinocampheylchloroborane ((-)-Ipc_2B-Cl) (1.5 M in pentane, 17.8 ml, 26.8 mmol) was added into ketone **6** (2 g, 6.7 mmol) in dry THF (15 ml) under nitrogen at room temperature. The resulting solution was stirred at room temperature for 3.5 h. Then the reaction was quenched and partitioned with 25% aq. NH_4Cl (200 ml). The aqueous layer was additionally extracted with EtOAc (2 x 100 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, and concentrated under vacuum. The residue was purified by silica gel column chromatography with 40% EtOAc/hexanes as eluent to afford product **7a** as a white solid (1.84 g, 92%, *enantiomeric excess* (ee) 82%). The product was further purified by repeated crystallization in acetone until ee reached a value above 95%. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 7.87-7.79 (m, 4H), 7.06 (t, 1H, *J* = 8 Hz), 6.76-6.70 (m, 2H), 6.57 (dd, 1H, *J* = 1.2, 2.4, 8 Hz), 4.51 (t, 1H, *J* = 6.8 Hz), 3.73-3.57 (m, 2H), 1.91-1.84 (m, 2H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 167.9, 157.0, 147.1, 134.3, 131.8, 129.0, 122.9, 116.3, 113.6, 112.4, 70.2, 37.3, 35.0.

(*R*)-2-(3-Hydroxy-3-(3-hydroxyphenyl)propyl)isoindoline-1,3-dione (7b): A solution of (+)-diisopinocampheylchloroborane ((+)-Ipc_2B-Cl) (1.5 M in hexanes, 26.9 ml, 40.4 mmol) was added to ketone **6** (3 g, 10.1 mmol) in dry THF (15 ml). As described for the synthesis of **7a, a** white solid **7b** (2.78 g, 92%, ee 88%) was obtained. The product was further purified by repeated crystallization in acetone until the ee value reached above 95%. ^{1}H NMR (DMSO-d_6, 400MHz) δ 9.27(s, 1H), 7.86-7.77 (m, 4H), 7.06 (t, 1H, *J* = 7.6 Hz), 6.77-6.74 (m, 1H), 6.73 (d, 1H, *J* = 7.6 Hz),6.58 (ddd, 1H, *J* = 1.2, 2.4, 8 Hz), 5.26 (d, 1H, *J* = 4.4 Hz), 4.52 (t, 1H, *J* = 6.4 Hz), 3.72-3.57 (m, 2H), 1.93-1.83 (m, 2H). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 168.0, 157.2, 147.2, 134.4, 131.8, 129.1, 123.0, 116.4, 113.8, 112.6, 70.4, 37.4, 35.1.

(*S*)-2-(3-(3-(Cyclohexylmethoxy)phenyl)-3-hydroxypropyl)isoindoline-1,3-dione (9a): A mixture of NaH (60% suspension in mineral oil, 95.3 mg, 2.38 mmol) and **7a** (700 mg, 2.35 mmol) in 15 ml anhydrous DMSO was stirred at room temperature until all the NaH dissolved. Then compound **8** (457 mg, 2.38 mmol) was added. The resulting mixture was stirred at 90 ºC for 2 days. The reaction was quenched with 25% aq. NH_4Cl, and the reaction mass was extracted with EtOAc (2 x 100 ml). The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$ and concentrated under vacuum. The residue was purified by silica gel column chromatography with 25% EtOAc/hexanes as eluent to afford **9a** (184 mg, 21%, ee 96%). ^1H NMR ($CDCl_3$, 400 MHz) δ 7.81-7.76 (m, 2H), 7.70- 7.65 (m, 2H), 7.14 (t, 1H, *J* = 8 Hz), 6.89-6.83 (m, 2H), 6.68 (ddd, 1H, *J* = 0.8, 2.4, 8.4 Hz), 4.64 (dd, 1H, *J* = 7.2, 7.6 Hz), 3.86 (t, 2H, *J* = 6.4 Hz), 3.68 (d, 2H, *J* = 6.4 Hz), 2.1-2.0 (m, 2H), 1,87-1.63 (m, 6H), 1.32-1.12 (m, 3H), 1.08-0.95 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.8, 159.4, 145.3, 134.0, 132.0, 129.4, 123.3, 117.7, 113.6, 111.6, 73.4, 71.3, 37.8, 37.5, 35.0, 29.9, 26.6, 25.9.

(*R*)-2-(3-(3-(Cyclohexylmethoxy)phenyl)-3-hydroxypropyl)isoindoline-1,3-dione (9b): As described for the synthesis of **9a**, a colorless oil **9b** (150 mg, 19%), ee 99.6% was obtained. ^1H NMR ($CDCl_3$, 400 MHz) δ 7.86-7.81 (m, 2H), 7.76- 7.69 (m, 2H), 7.17 (t, 1H, *J* = 8 Hz), 6.90-6.85 (m, 2H), 6.71 (ddd, 1H, *J* = 0.8, 2.4, 8 Hz), 4.65 (dd, 1H, *J* = 7.6, 8 Hz), 3.9 (t, 2H, *J* = 6.4 Hz), 3.71 (d, 2H, *J* = 6.4 Hz), 2.1-2.0 (m, 2H), 1.90-1.65 (m, 6H), 1.35-1.15 (m, 3H), 1.09-0.97 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.9, 159.6, 145.3, 134.2, 132.1, 129.6, 123.4, 117.8, 113.8, 111.7, 73.5, 71.4, 37.9, 37.7, 35.0, 30.1, 26.7, 26.0.

(*S*)-3-Amino-1-(3-(cyclohexylmethoxy)phenyl)propan-1-ol ((*S*)-emixustat): A solution of **9a** (205 mg, 0.53 mmol) in EtOH (10 ml) and $N_2H_4{\cdot}H_2O$ (128.4 µl, 2.64 mmol) was stirred at room temperature for 26 h and then concentrated under vacuum. The residue was purified by silica gel column chromatography with $NH_4OH/MeOH/CH_2Cl_2$ (4 : 30 : 70) as eluent to afford **(*S*)-emixustat** (104 mg, 75%). ^1H NMR (CD_3OD, 400 MHz) δ 7.21 (t, *J* = 8 Hz, 1H), 6.94-6.87 (m, 2H), 6.77 (ddd, 1H, *J* = 0.8, 2.4, 8 Hz), 4.69 (dd, 1H, *J* = 8, 7.6 Hz), 3.76 (d, 2H, *J* = 6.4 Hz), 2.81-2.68 (m, 2H), 1.94-1.83 (m, 4H), 1.82-1.67 (m, 4H), 1.39-1.17 (m, 3H), 1.15-1.03 (m, 2H). ^{13}C NMR (CD_3OD,100 MHz) δ 160.9, 147.9, 130.3, 119.0, 114.3, 112.9, 74.4, 73.4, 42.2, 39.5, 39.2, 31.0, 27.7, 27.0.

(*R*)-3-Amino-1-(3-(cyclohexylmethoxy)phenyl)propan-1-ol ((*R*)-emixustat): As described above, 150 mg of **9b** (0.38 mmol) gave **(*R*)-emixustat)** (75 mg, 75%). ^1H NMR (CD_3OD, 400 MHz) δ 7.21 (t, *J* = 8 Hz, 1H), 6.93-6.88 (m, 2H), 6.78 (ddd, 1H, *J* = 0.8, 2.4, 8 Hz), 4.62 (dd, 1H, *J* = 8, 7.6 Hz), 3.76 (d,2H, *J* = 6.4 Hz), 2.75-2.62 (m, 2H), 1.93-1.68 (m, 8H), 1.59-1.18

(m,3H), 1.14-1.03 (m, 2H). ^{13}C NMR (CD_3OD, 100 MHz) δ 160.8, 148.1, 130.3, 119.0, 114.2, 112.9, 74.4, 73.4, 42.8, 39.6, 39.2, 31.0, 27.7, 27.0.

Synthesis of QEA-B-001 (Supplemental Fig. 4)

(2*Z*,4*E*)-3-(*tert*-Butyl)-5-(cyclohex-1-en-1-yl)penta-2,4-dienenitrile (12): To a stirred suspension of NaH (435 mg, 18 mmol) in dry THF (10 ml) at room temperature, a solution of **11** (3.53 g, 136 mmol) in dry THF (10 ml) was added dropwise. This reaction mixture was stirred at room temperature for 1 h. Then a solution of **10** (1 g, 9.0 mmol) in dry THF (10 ml) was slowly added and the resulting solution was stirred at room temperature for 1 h before the reaction was quenched with a saturated NH_4Cl solution. The aqueous layer was extracted twice with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude compound was purified by silica gel column chromatography and eluted with 1% EtOAc/hexanes to obtain **12** (590 mg, 37%). $[M + H]^+$: 216.3.

(2*Z*,4*E*)-3-(*tert*-Butyl)-5-(cyclohex-1-en-1-yl)penta-2,4-dienal (13): To a solution of **12** (590 mg, 2.7 mmol) in dry toluene (5.9 ml) cooled to -60 ºC under nitrogen was slowly added diisobutylaluminium hydride (DIBAL-H) (1 M in toluene, 3.2 ml) from a syringe and the reaction mixture was stirred at -60 ºC for 30 min. The mixture was then poured into a saturated sodium potassium tartrate solution and extracted with EtOAc. The organic layer was washed with water, dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by silica gel column chromatography and eluted with 1.2% EtOAc/petroleum ether to obtain **13** (250 mg, 41.8%). $[M + H]^+$: 219.3.

(2*E*,4*E*,6*Z*,8*E*)-7-(*tert*-Butyl)-9-(cyclohex-1-en-1-yl)-3-methylnona-2,4,6,8-tetraenenitrile (15): To a stirred suspension of NaH (132 mg, 5.5 mmol) in dry THF at 0 ºC under nitrogen was added a solution of **14** (895 mg, 4.12 mmol) in dry THF (6 ml). This reaction solution was stirred at room temperature for 1 h and then cooled to 0 ºC. After a solution of **13** (600 mg, 2.75 mmol) in dry THF (6 ml) was slowly added, stirring was continued at room temperature for 2 h when the reaction was quenched with a saturated NH_4Cl solution. The resulting mixture was extracted with EtOAc, dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by column chromatography on silica gel and eluted with 1% EtOAc/petroleum ether to obtain **15** (660 mg, 85.3%). $[M + H]^+$: 282.4.

7-*tert*-Butyl-9-cyclohex-1-enyl-3-methyl-nona-2,4,6,8-tetraenal (QEA-B-001): To a stirred solution of **15** (660 mg, 2.35 mmol) in dry toluene at -70 ºC under nitrogen was added dropwise DIBAL-H (1 M in toluene, 2.76 ml). This reaction mixture was stirred at -70 ºC for 30 min. Wet silica gel was added to the reaction mixture at -30 ºC, and stirring was continued at the same temperature for 1 h. Then the mixture was filtered and the filtrate was extracted three times with ether. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by silica gel column chromatography and eluted with 1% EtOAc/petroleum ether to obtain QEA-B-001 (150 mg, 22.48%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 10.07 (d, 1H, *J* = 8 Hz), 7.13 (dd, 1H, *J* = 10.8 Hz, 15.6 Hz), 6.35 (d, 1H, *J* = 15.6 Hz), 6.19-6.04 (m, 3H), 5.93 (d, 1H, *J* = 8.4 Hz), 5.80 (t, 1H, *J* = 4 Hz), 2.24 (d, 3H, *J* = 1.2 Hz), 2.23-2.16 (m, 4H), 1.74-1.62 (m, 4H), 1.11 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 191.5, 157.4, 156.0, 140.1, 136.2, 135.9, 133.6, 131.5, 128.5, 122.7, 121.7, 36.8, 29.9, 26.3, 24.7, 22.7, 22.6, 13.5. $[M + H]^+$: 285.3.

Synthesis of MB-002 (Supplemental Fig. 5)

1-(3-(cyclohexylmethoxy)phenyl)ethan-1-one: To a solution of PPh_3 (13.9 g, 52.9 mmol) and diisoprorylazodicarboxylate (10.4 g , 52.9 mmol) in anhydrous THF (150 mL) under argon at 0 °C was added dropwise 3′-hydroxyacetophenone (6 g, 44.1 mmol) with stirring. Then cyclohexylmethanol (5 g, 44.1 mmol) were added to the reaction and the mixture was stirred at room temperature overnight. The reaction mixture was concentrated under reduced pressure to give yellow to brown oil. Purification by flash chromatography (0 to 20% EtOAc:hexanes gradient) gave ether **1** as a yellow oil. Yield (6.9 g, 67%): 1H NMR (400 MHz, $CDCl_3$) δ 7.49-7.53 (m, 1H), 7.45-7.48 (m, 1H), 7.35 (t, *J*=8 Hz 1H), 7.10 (ddd, *J*=0.8-1.2, 2.4-2.8, 8-8.4 Hz 1H), 3.80 (d, J= 6.4 Hz, 2H), 2.59 (s, 3H), 1.83-1.90 (m, 2H), 1.66-1.83 (m, 4H),1.15-1.36 (m, 3H), 1.01-1.11 (m, 2H) ; ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.2, 159.7, 138.5, 129.6, 120.9, 120.2, 113.2, 73.8, 37.8, 30.0, 26.9, 26.6, 25.9.

Ethyl 3-(3-(cyclohexylmethoxy)phenyl)-3-oxopropanoate: To a flamed-dried 100 mL three-necked flask equipped with, a condenser, and a magnetic stirrer was added sodium hydride 60 % dispersion in mineral oil (1.3 g, 32.3 mmol) and washed two times with hexanes. Hexane was discarded and toluene (50 mL), and diethyl carbonate (2.8 g, 23.7 mmol) were added. At room temperature, a solution of ketone (2.5 g, 10.76 mmol) in toluene (10 mL) was added dropwise from the syringe pump over 1 h. After the addition, the mixture was heated to reflux until grayish color (NaH color) change to brownish yellow and the evolution of hydrogen ceased (1-2 h). The reaction was cooled to room temperature, glacial acetic acid (4 mL) was added dropwise and a heavy, pasty solid appeared. Ice-water was added until the solid was dissolved completely. The toluene layer was separated, and the water layer was extracted with EtOAc. The combined organic solution was washed with water and brine (5 mL), and then dried over $MgSO_4$. After evaporation of the solvent, the mixture was purified by flash chromatography (15-20% Et2O: Hexanes) gave beta-keto ester **2** as brown to yellow oil. Yield (2.2 g, 67%): 1H NMR (400 MHz, $CDCl_3$) δ 7.44-7.5 (m, 2H), 7.36 (t, *J*=8 Hz, 1H), 7.12 (ddd, *J*=0.8, 2.4, 8.4 Hz 1H), 4.21 (q *J*=7.2 Hz, 2H), 3.97(s, 3H), 3.79 (d, J= 6.4 Hz, 2H), 1.82-1.91 (m, 2H), 1.64-1.82 (m, 4H),1.14-1.36 (m, 3H), 0.99-1.11 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ192.6, 167.7, 159.8, 137.4, 129.8, 121.0, 120.9, 113.3, 73.8, 61.6, 46.2, 37.7, 30.0, 26.6, 25.9, 14.2.

1-(3-(cyclohexylmethoxy)phenyl)propane-1,3-diol (MB-002): compound **2** (3 g, 9.9 mmol) was dissolved in methanol (21 mL), and sodium borohydride (1.125 g, 29.7 mmol) was added slowly to the mixture at room temperature. The mixture was stirred until the reaction was complete based on TLC monitoring (30 min). All of the methanol was evaporated, and ethyl acetate (50 mL) was added. The mixture was shaken with brine solution (50 mL). The aqueous layer was extracted using ethyl acetate. Organic phases were combined, washed with water and dried over $MgSO_4$. Purification by flash chromatography (70% EtOAc: 30% hexanes gradient) gave the diol **(MB-002)** as a pale yellow oil. Yield (2 g, 77%): 1H NMR (400 MHz, CDCl3) δ 7.22 (t, *J*=8Hz, 1H), 6.85-6.93 (m, 2H), 6.78 (dd, *J*=2.4, 8.4 Hz, 1H), 4.88 (dd, *J*=4, 8.4 Hz, 1H), 3.80(m, 2H), 3.73 (d, J= 6.4 Hz, 2H), 3.10-3.20 (bs, 2H), 1.82-2 (m, 4H), 1.65-1.81 (m, 4H), 1.12-1.35 (m, 3H), 0.97-1.11 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.6, 146.1, 129.6, 117.8, 113.7, 111.8, 74.4, 73.6, 61.6, 40.5, 37.8, 30.0, 26.6, 25.9. HRMS (FT-ICR) m/z: [M + Na]+ calculated for $C_{16}H_{24}O_3Na^+$ 287.16177 ; found 287.16178

Supplemental Figure Legends

Supplemental Figure 1. ***Crystal structures of RPE65 in complex with emixustat.*** (**A**) Structure obtained from racemic emixustat. (**B**) Structure obtained from enantio-pure (*R*)-emixustat. (**C**) Structure obtained from enantio-pure (*S*)-emixustat. In each panel emixustat is shown as orange sticks, palmitate as cyan sticks and residues within 4.5 Å of the two ligands are shown as grey sticks. Unbiased σA-weighted omit mFo-DFc electron density maps at 3 RMSD contour levels are shown as a green mesh within 3 Å of the bound ligands. The positioning of the phenoxy moiety is similar between the structures. The cyclohexyl moiety adopts a different conformation in A compared to B and C.

Supplemental Figure 2. ***Conformational flexibility in the cyclohexyl moiety of emixustat.*** (**A**) Conformation in the $P6_522$ crystal form obtained from racemic emixustat. (**B**) Conformation in the $P6_5$ crystal form obtained from enantiopure (*R*)-emixustat. The conformation of the cyclohexyl group in (*S*)-emixustat was very similar to that of the (*R*) isomer. Note the inward movement of Phe264 and the major conformational change that brings Phe196 in close proximity to the cyclohexyl moiety in (**B**) compared to (**A**).

Supplemental Figure 3. ***Synthesis of emixustat enantiomers.*** Abbreviations: Boc_2O, di-*tert*-butyl dicarbonate; DIPEA, *N,N*-diisopropylethylamine; DMSO, dimethyl sulfoxide; EtOH, ethanol; Ipc_2B-Cl, diisopinocampheylchloroborane; PCC, pyridinium chlorochromate; TFA, trifluoroacetic acid; THF, tetrahydrofuran.

Supplemental Figure 4. ***Synthesis of QEA-B-001.*** Abbreviations: DIBAL, diisobutylaluminium hydride.

Supplemental Figure 5. ***Reagents and conditions for the synthesis of MB-002 (7-9).***

References

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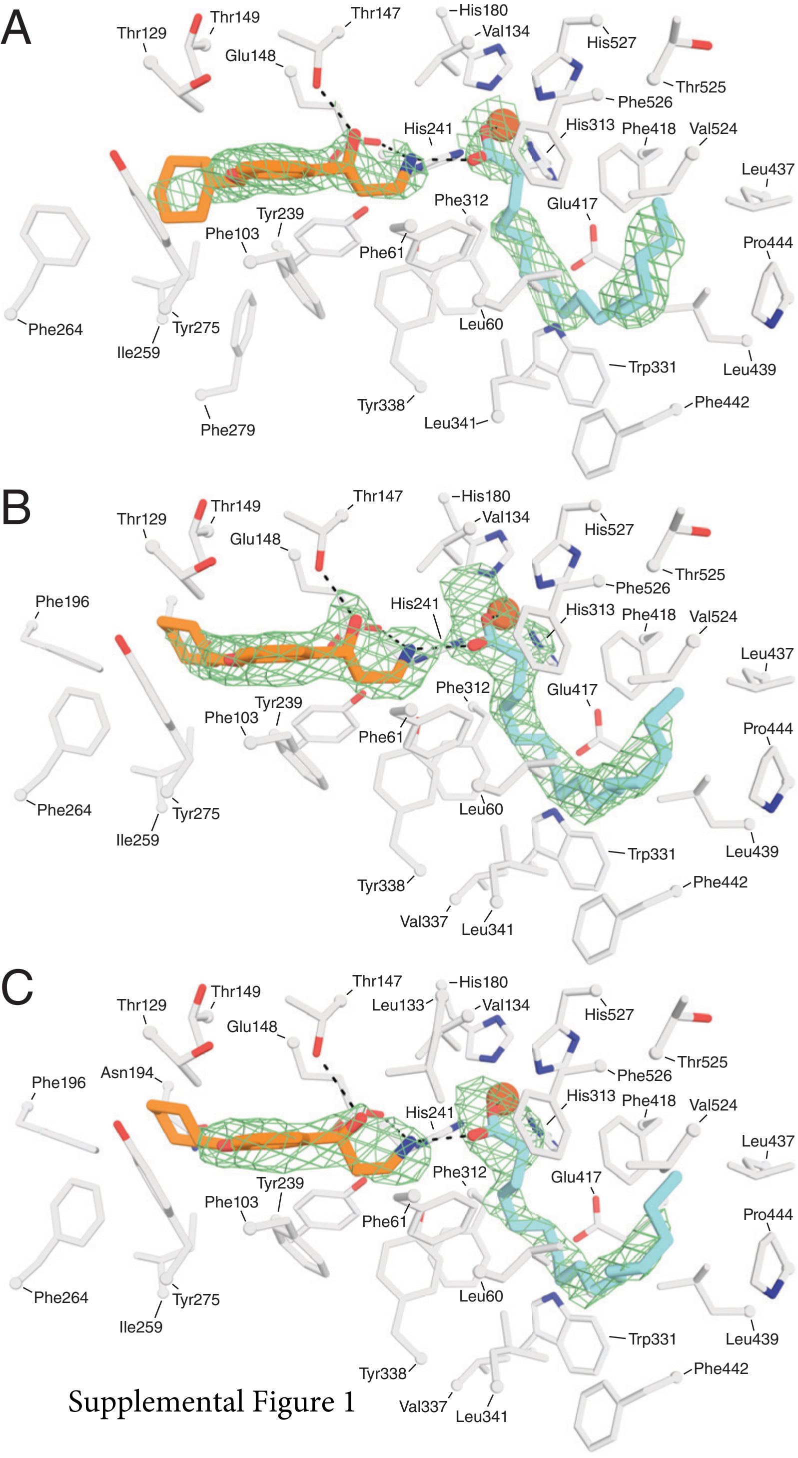
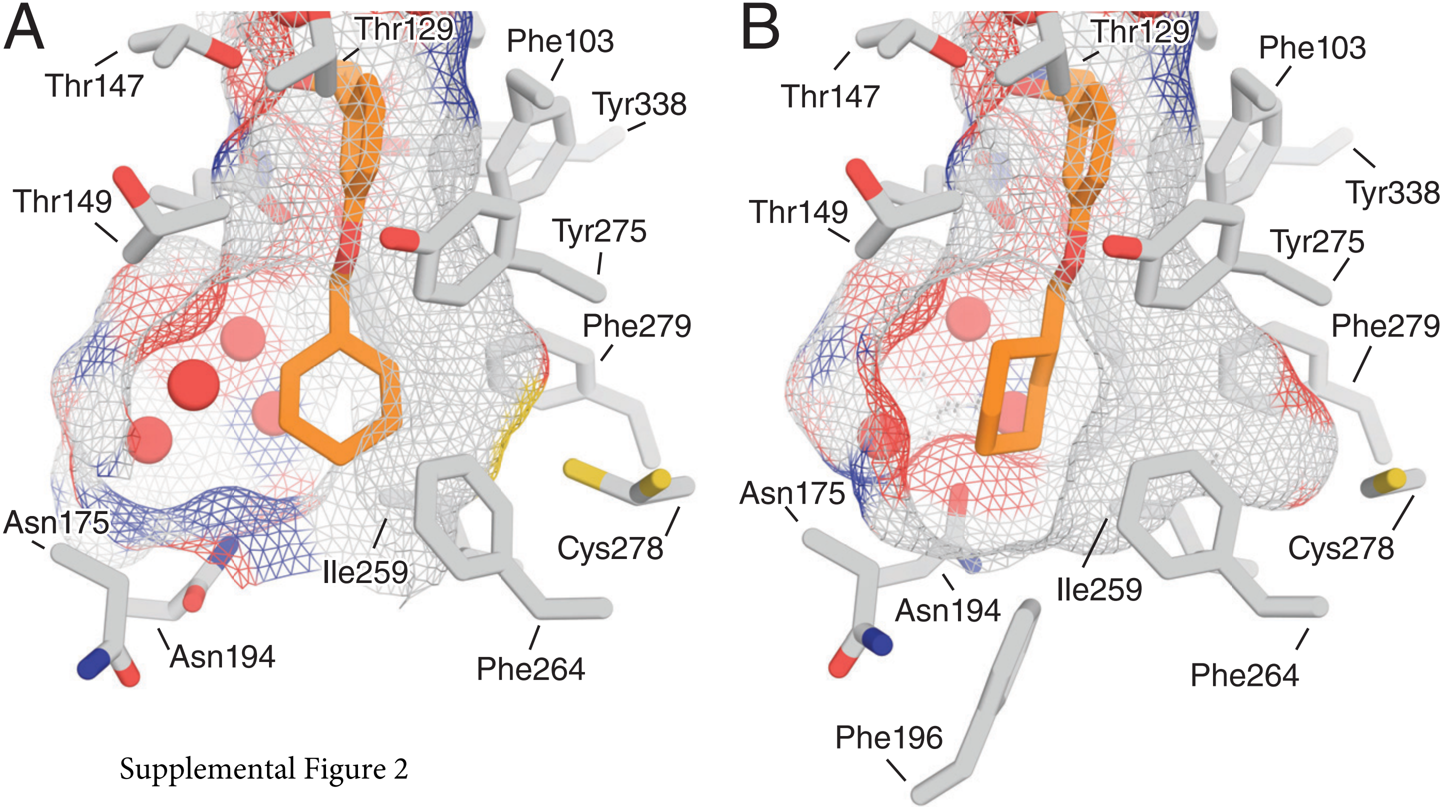
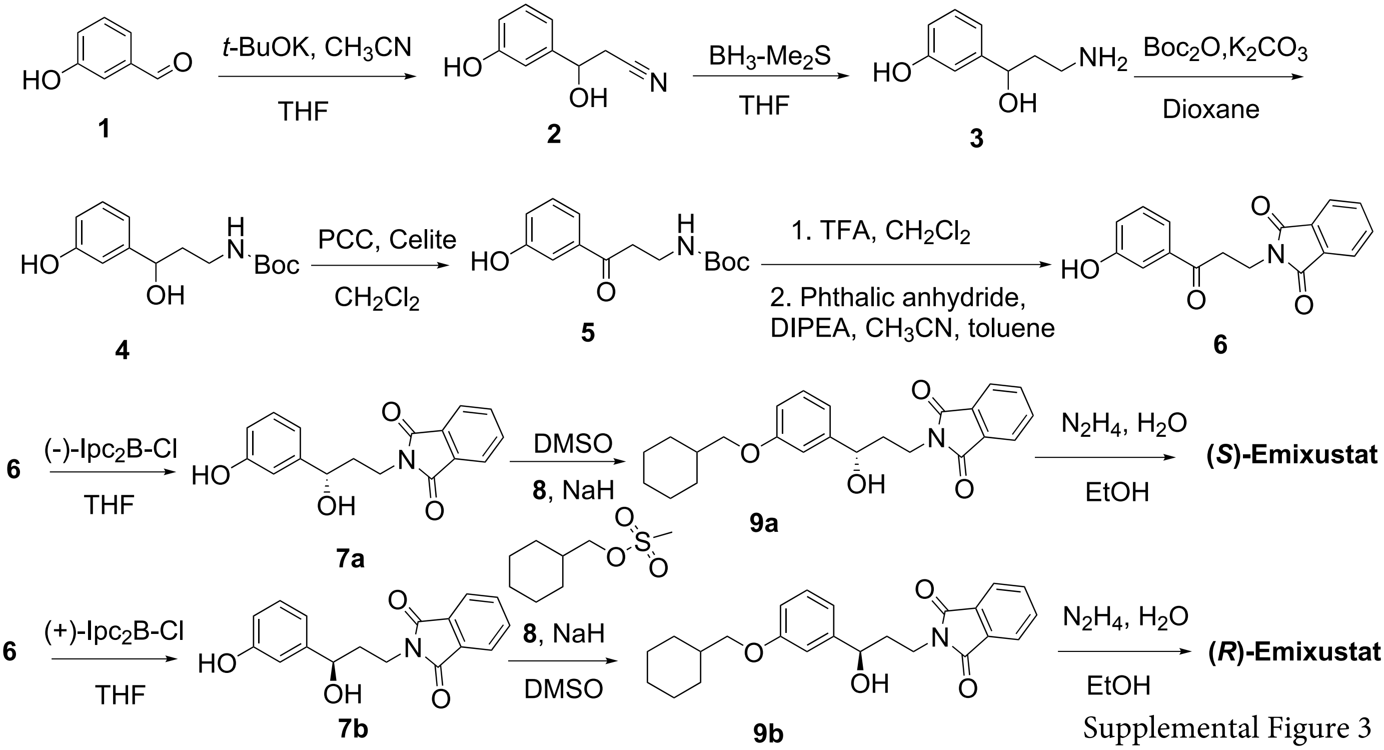


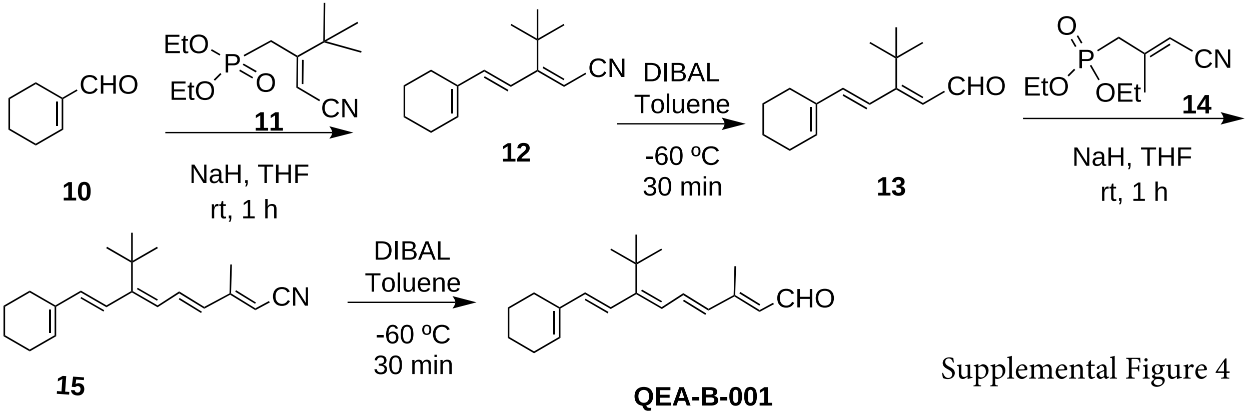
Supplemental Figure 1



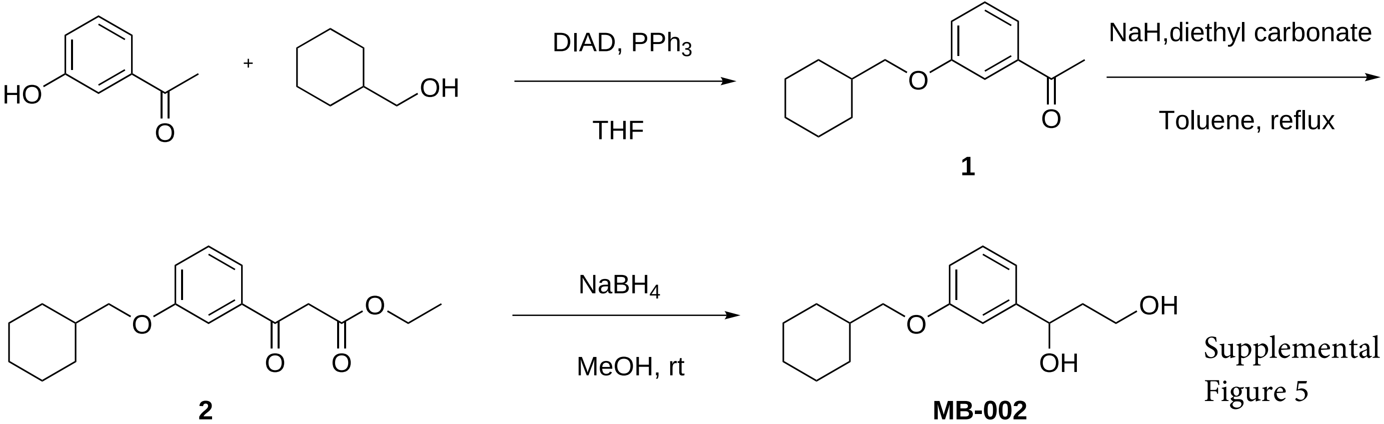
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5