Supplemental Material

LIGAND BINDING ENSEMBLES DETERMINE GRADED AGONIST EFFICACIES AT A G PROTEIN-COUPLED RECEPTOR

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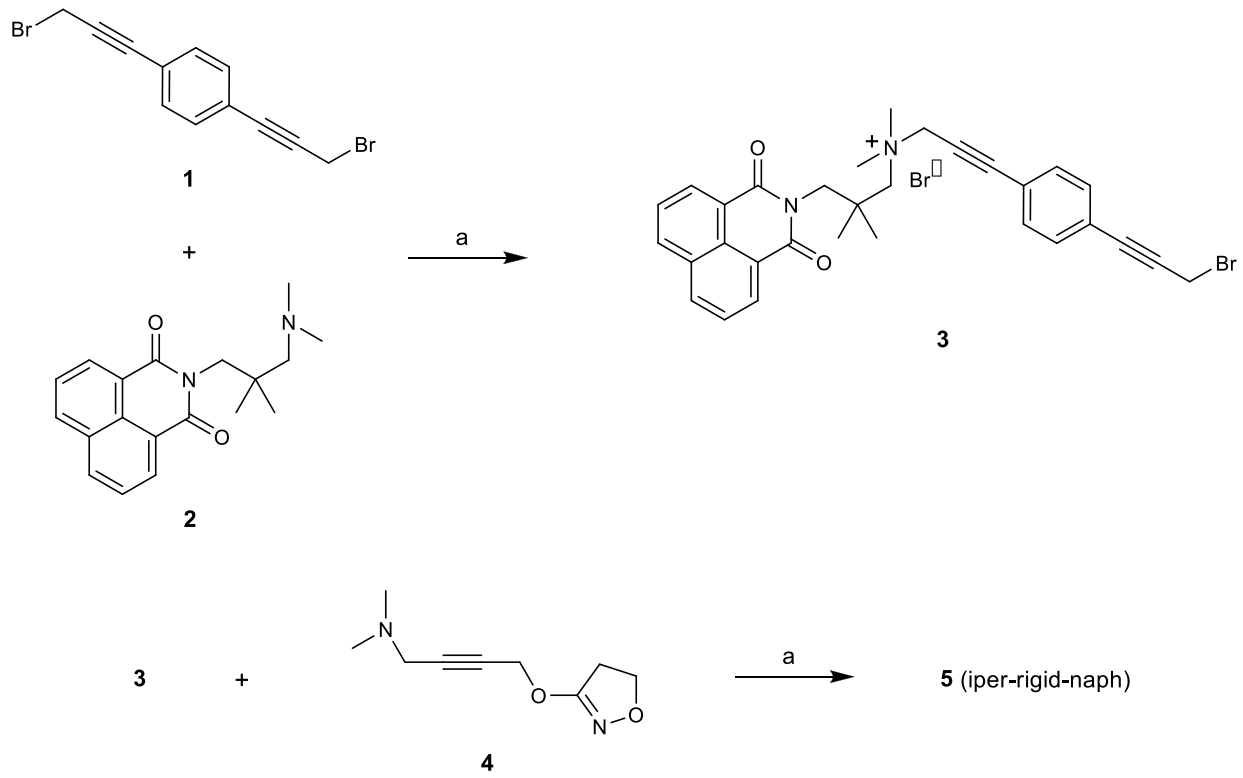
Supplementary Figures



Supplementary Figure 1: Synthesis of iper-rigid-naph. a) CH_3CN, reflux, 18 h.

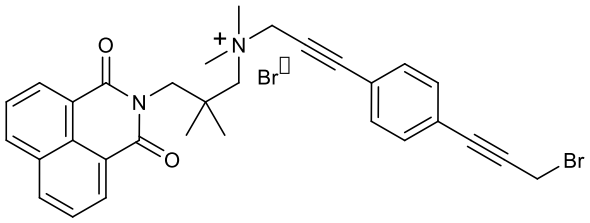
Syntheses and analyses of chemical probes

Melting points were determined on a model B 540 Büchi or a Sanyo Gallenkamp melting point apparatus and are uncorrected. TLC analyses were performed on commercial silica gel 60 F254 aluminum sheets; spots were further evidenced by an alkaline aqueous solution of potassium permanganate or an ethanol solution of phosphomolybdic acid. ^{1}H NMR and ^{13}C NMR were recorded with a Varian Mercury 300 (^{1}H, 300.063 MHz; ^{13}C, 75.451 MHz) spectrometer. The specific NMR assignments were unambiguously determined by using typical techniques including polarization transfer experiments (DEPT) and two-dimensional experiments, e.g. ^{1}H-^{1}H correlation (COSY) and ^{1}H-^{13}C-proton-carbon heteronuclear correlation (HMQC, HMBC). Chemical shifts (δ) are expressed in ppm and coupling constants (*J*) in Hertz. Abbreviations for data quoted are: s, singlet; d, doublet; dd, doublet of doublet; t, triplet; q, quartet; m, multiplet. ESI mass spectra of the new compounds were

recorded on a Varian 320 LC-MS/MS instrument. Data are reported as mass-to-charge ratio (m/z) of the corresponding positively charged molecular ions.

1,4-Bis-(3-bromo-1-prop-1yn-1y)benzene **1** was prepared following a published protocol (1). Tertiary amine **2** (2) and dimethylamino-Δ^2-isoxazoline **4** (3,4) were synthesized according to known procedures. Intermediate **3** was obtained in 87% yield by refluxing an acetonitrile solution containing **2** and a five-fold excess of dibromo-derivative **1**. Treatment of **3** with **4** in similar experimental conditions provided the dualsteric muscarinic ligand **5** (iper-rigid-naph) in 71% yield **(Supplementary Figure 1)**.

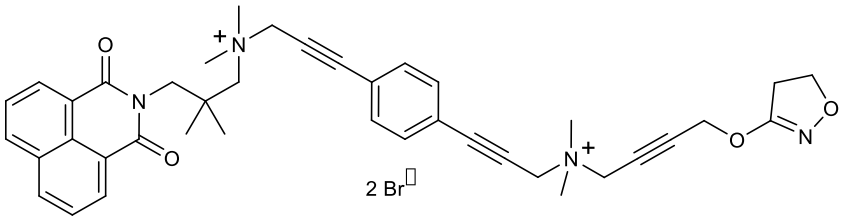
3-(4-(3-Bromoprop-1-yn-1-yl)phenyl)-*N*-(3-(1,3-dioxo-1*H*-benzo[de]isoquinolin-2(3*H*)-yl)-2,2-dimethylpropyl)-*N*,*N*-dimethylprop-2-yn-1-aminium Bromide [3]



A solution of 1,4-bis(3-bromoprop-1-yn-1-yl)benzene **1** (2.63 g, 8.44 mmol) and 2-(3-(dimethylamino)-2,2-dimethylpropyl)-1*H*-benzo[de]isoquinoline-1,3(2*H*)-dione **2** (524 mg, 1.69 mmol) in acetonitrile (35 mL) was refluxed for 18 h in a sealed glass reaction vessel (TLC in dichloromethane/methanol 9:1). The reaction mixture was then allowed to cool down to room temperature. After removing the clear supernatant containing most of the excess reagent, the precipitate was recrystallized twice from acetonitrile/diethyl ether to afford the desired product as a colorless solid (918 mg, 87% yield). Mp: 199-202 °C dec. ^{1}H NMR (300 MHz, CD_3OD): δ (ppm) 8.48 (dd, *J* = 7.3, 1.2 Hz, 2H, napht.), 8.36 (dd, *J* = 8.4, 1.2 Hz, 2H, napth.), 7.78 (dd, *J* = 8.3, 7.3 Hz, 2H, napht.), 7.24 (d, *J* = 8.3 Hz, 2H, arom.), 7.16 (d, *J* = 8.4 Hz, 2H, arom.), 4.66 (s, 2H, ≡C-CH_2Br), 4.36 (s, 2H, ≡C-CH_2N^+), 4.26 (s, 2H, C-CH_2N^+), 3.71 (s, 2H, C-CH_2N(napht.)), 3.40 (s, 6H, $(CH_3)_2N^+$), 1.44 (s, 6H, $(CH_3)_2$C). ^{13}C NMR (75 MHz, CD_3OD): δ (ppm) 166.71 (C=O, napht.), 135.83 (napht.), 133.09 (napht.), 132.74 (arom.), 132.56 (napht.), 129.17 (napht.), 128.23 (napth.), 125.09 (arom.), 123.34 (napht.), 121.59 (arom.), 92.75 (≡C-CH_2), 88.63 (≡C-CH_2), 86.08 (≡C-Ar), 79.47 (≡C-Ar),

73.10 (C-$\underline{C}H_2N^+$), 58.71 (C-$\underline{C}H_2N$(napht.)), 53.91 (≡C-$\underline{C}H_2N^+$), 50.79 (($\underline{C}H_3)_2N^+$), 40.53 (($\underline{C}H_3)_2C$), 26.01 ($(CH_3)_2\underline{C}$), 15.24 (≡C-$\underline{C}H_2Br$). MS (ESI) *m/z* $[M]^+$ Calcd for $C_{31}H_{30}BrN_2O_2^+$: 541.15. Found: 541.2.

4-((4,5-Dihydroisoxazol-3-yl)oxy)-*N*-(3-(4-(3-((3-(1,3-dioxo-1*H*-benzo[de]isoquinolin-2(3*H*)-yl)-2,2-dimethylpropyl)dimethylammonio)prop-1-yn-1-yl)phenyl)prop-2-yn-1-yl)-*N*,*N*-dimethylbut-2-yn-1-aminium Dibromide [5]



A solution of **4** (157 mg, 0.86 mmol) and **3** (448 mg, 0.72 mmol) in acetonitrile (7 mL) was refluxed for 18 h in a sealed glass reaction vessel (TLC in dichloromethane/methanol 9:1). The reaction mixture was then allowed to cool down to room temperature. After removing the clear supernatant, the yellowish precipitate was recrystallized from acetonitrile/diethyl ether to afford **5** (iper-rigid-naph) as a light yellow hygroscopic solid (411 mg, 71% yield). ^{1}H NMR (300 MHz, CD_3OD): δ (ppm) 8.54 (dd, *J* = 7.3, 1.0 Hz, 2H, napht.), 8.38 (dd, *J* = 8.3, 1.0 Hz, 2H, napht.), 7.81 (dd, *J* = 8.3, 7.3 Hz, 2H, napht.), 7.48-7.41 (m, 4H, arom.), 4.96 (s, 2H, -OCH_2-C≡), 4.74 (s, 2H, ≡C-CH_2N^+), 4.70 (s, 2H, ≡C-CH_2N^+), 4.58 (s, 2H, ≡C-CH_2N^+), 4.40 (t, *J* = 9.6 Hz, 2H, -OCH_2-, isox.), 4.32 (s, 2H, C-CH_2N^+), 3.74 (s, 2H, C-CH_2N(napht.)), 3.42 (s, 6H, $(CH_3)_2N^+$), 3.36 (s, 6H, $(CH_3)_2N^+$), 3.03 (t, *J* = 9.6 Hz, 2H, -CH_2-, isox.), 1.43 (s, 6H, $(CH_3)_2C$). ^{13}C NMR (75 MHz, CD_3OD): δ (ppm) 168.74 (-C=N-, isox.), 166.66 (C=O, napht.), 135.80 (napth.), 133.28 (napht.), 133.08 (arom.), 133.05 (arom.), 132.56 (napht.), 129.16 (napht.), 128.26 (napht.), 123.36 (napht.), 123.15 (arom.), 122.99 (arom.), 92.37 (≡$\underline{C}$-CH_2), 91.99 (≡$\underline{C}$-CH_2), 88.42 (≡$\underline{C}$-CH_2), 80.10 (≡$\underline{C}$-CH_2), 79.51 (≡$\underline{C}$-Ar), 76.37 (≡$\underline{C}$-Ar), 73.36 (C-$\underline{C}H_2N^+$), 71.21 (-OCH_2-, isox.), 58.90 (C-$\underline{C}H_2N$(napht.)), 58.33 (≡C-$\underline{C}H_2N^+$), 55.84 (≡C-$\underline{C}H_2N^+$), 55.37 (≡C-$\underline{C}H_2N^+$), 53.88 ($(CH_3)_2N^+$), 51.14 ($(CH_3)_2N^+$), 50.54 (-O$\underline{C}H_2$-C≡), 40.50 (($\underline{C}H_3)_2C$), 33.66 (-CH_2-, isox.), 26.15 ($(CH_3)_2\underline{C}$). MS (ESI) *m/z* $[M]^{2+}$ Calcd for $C_{40}H_{44}N_4O_4^{2+}$: 322.17. Found: 322.3.

Supplementary References

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