

## Supporting Information

### Simple Tetrahydroisoquinolines are Potent and Selective Kappa Opioid Receptor Antagonists

Chad M. Kormos, Pauline W. Ondachi, Scott P. Runyon, James B. Thomas, S. Wayne Mascarella, Ann  
M. Decker, Hernán A. Navarro, and F. Ivy Carroll\*

Research Triangle Institute, PO Box 12194, Research Triangle Park, NC 27709-2194, United States

#### Table of Contents:

Material and Methods .....	S2
[ <sup>35</sup> S]GTP $\gamma$ S Assay.....	S3
Calculated Physiochemical Properties.....	S4
Docking Studies and Calculations.....	S4
References.....	S4
Figure S1.....	S6

## Materials and Methods

Melting points were determined using a capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance ( $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR) spectra were obtained on a 500 MHz NMR spectrometer or a 300 MHz NMR spectrometer. Chemical shifts are reported in parts per million (ppm) with reference to internal solvent. Mass spectra (MS) were conducted on a mass spectrometer equipped with an ESI (turbo spray) source. Elemental analyses were performed by an external laboratory. The purity of the compounds (>95%) was established by elemental analysis. Optical rotations were measured on an automatic polarimeter. Analytical thin-layer chromatography (TLC) was carried out using silica gel 60 F<sub>254</sub> TLC plates. TLC visualization was achieved with a UV lamp, in an iodine chamber, or with ninhydrin stain. Flash column chromatography was done on a system using prepacked silica gel columns or using silica gel 60 (230–400 mesh). Solvent system: DMA80 80:18:2  $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{conc. NH}_4\text{OH}$ . Unless otherwise stated, reagent-grade chemicals were obtained from commercial sources and were used without further purification. All moisture- and air-sensitive reactions and reagent transfers were carried out under dry nitrogen.

**(2S)-3-Methyl-1-oxo-1-piperidin-1-ylbutan-2-amine (4).** Piperidine (1.0 mL, 10 mmol) was slowly added to a solution of Boc-L-valine (0.52 g, 2.3 mmol) and HBTU (0.88 g, 2.3 mmol) in  $\text{CH}_3\text{CN}$  (10 mL) at room temperature. After 12 h, the reaction was concentrated then partitioned between  $\text{Et}_2\text{O}$  and aq.  $\text{NaHCO}_3$ . The ether layer was washed with dilute acid,  $\text{NaHCO}_3$  and then brine. The dried organic layer ( $\text{Na}_2\text{SO}_4$ ) was concentrated to afford an oil (0.49 g) which consisted of tetramethylurea, ethyl acetate, and the desired Boc-protected amide (**4**):  $^1\text{H}$  NMR (300 MHz,  $\text{CHLOROFORM-d}$ )  $\delta$  5.25 – 5.53 (m, 1H), 4.48 (dd,  $J = 5.27, 9.04$  Hz, 1H), 3.51 – 3.66 (m, 2H), 3.38 – 3.51 (m, 2H), 1.91 (d,  $J = 5.46$  Hz, 1H), 1.49 – 1.76 (m, 6H), 1.39 – 1.48 (m, 9H), 0.96 (d,  $J = 6.78$  Hz, 3H), 0.87 (d,  $J = 6.78$  Hz, 3H). This crude mixture was dissolved in  $\text{CH}_3\text{OH}$  (10 mL) and HCl (6 M, 10 mL). After 1 h, the reaction was concentrated to a residue, which was purified by chromatography on silica gel eluting with a gradient up to 33% DMA80 in  $\text{CH}_2\text{Cl}_2$  to afford 1.25 g (67% over two steps) of the desired amine (**4**):  $^1\text{H}$  NMR (300 MHz,  $\text{CHLOROFORM-d}$ )  $\delta$  3.30 – 3.72 (m, 5H), 1.39 – 1.96 (m, 9H), 0.72 – 1.13 (m, 6H).

**(2S)-3-Methyl-1-piperidin-1-ylbutan-2-amine (5).** A solution of **4** (275 mg, 1.5 mmol) in THF (10 mL) was treated with  $\text{BH}_3\cdot\text{SMe}_2$  (10 M, 2 mL) and heated to reflux overnight. The reaction was quenched with the addition of methanol, then acidified with HCl (6 M, 10 mL). The reaction mixture was concentrated and the residue subjected to silica gel chromatography eluting with a gradient of DMA80 in  $\text{CH}_2\text{Cl}_2$  to afford 216 mg (85%) of the desired amine **5**:  $^1\text{H}$  NMR (300 MHz,  $\text{CHLOROFORM-d}$ )  $\delta$  3.59 – 3.80 (m, 1H), 2.22 – 3.28 (m, 6H), 1.79 – 2.07 (m, 3H), 1.37 – 1.75 (m, 6H), 0.80 – 1.11 (m, 6H); MS (ESI)  $m/z$  171.3 ( $\text{M} + \text{H}$ )<sup>+</sup>.

**(3R)-7-Hydroxy-N-[(1S)-2-methyl-1-(piperidin-1-ylmethyl)propyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide Dihydrochloride (1).** The amine **5** (216 mg, 1.3 mmol) was dissolved in  $\text{CH}_2\text{Cl}_2$  (5 mL) and added to a solution of 7-hydroxy-Boc-D-Tic(OH) (100 mg, 0.34 mmol), EDC·HCl (100 mg, 0.52 mmol) and catalytic HOBT in  $\text{CH}_2\text{Cl}_2$  (5 mL). The reaction stirred overnight, then was concentrated and subjected to chromatography on silica gel using a gradient of DMA80 in  $\text{CH}_2\text{Cl}_2$ . The product containing fractions were concentrated then treated with and concentrated from  $\text{CH}_3\text{OH}$  (5 mL) and HCl (6 M, 5 mL). The concentrated residue was subjected to chromatography on silica gel eluting with a gradient of DMA80 in  $\text{CH}_2\text{Cl}_2$  to afford the free base of compound **1**:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.07 (d,  $J = 9.80$  Hz, 1H), 6.86 (d,  $J = 8.10$  Hz, 1H), 6.55 (dd,  $J = 2.35, 8.19$  Hz, 1H), 6.39 (d,  $J = 2.07$  Hz, 1H), 4.24 (t,  $J = 10.64$  Hz, 1H), 3.52 – 3.76 (m, 2H), 3.20 (dd,  $J = 5.18, 11.59$  Hz, 1H), 2.89 (dd,  $J = 5.09, 16.58$  Hz, 1H), 2.55 – 2.76 (m, 3H), 2.38 (br. s., 2H), 2.16 – 2.31 (m, 2H), 1.83 (dd,  $J = 6.78, 11.68$  Hz, 1H), 1.65 (d,  $J = 4.71$  Hz, 4H), 1.47 (d,  $J = 5.46$  Hz, 2H), 0.83 – 0.99 (m, 6H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  173.4, 155.0, 137.5, 130.7, 125.1, 113.7, 112.1, 60.2, 56.6, 54.6, 49.4, 48.4, 31.9, 29.2, 25.0, 24.0, 18.9, 18.1; MS (ESI)  $m/z$  346.2 ( $\text{M}$

+ H)<sup>+</sup>. The free base was converted to a white powder as the dihydrochloride salt (48.4 mg): mp 138–142 °C (fusion); [ $\alpha$ ]<sub>D</sub><sup>25</sup> = +66.5 (c 0.36, CH<sub>3</sub>OH). Anal. (C<sub>20</sub>H<sub>33</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>2</sub>•2.25 H<sub>2</sub>O) C, H, N.

***N*-[(1*E*)-2-Cyclohexylethylidene]-2-methylpropane-2-sulfonamide (7).** Magnesium sulfate (5.0 g, 42 mmol) was added to a solution of cyclohexylacetaldehyde (2.1 g, 17 mmol), *R*-(*tert*-butyl)sulfonamide (1.06 g, 8.7 mmol), and pyridinium *p*-toluenesulfonate (100 mg, 0.4 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (14 mL). After 12 h, the suspension was filtered and concentrated and the residue subjected to chromatography on silica gel eluting with a gradient up to 25% EtOAc in hexanes to afford 1.47 g (74%) of compound **7**: <sup>1</sup>H NMR (300 MHz, CHLOROFORM-*d*)  $\delta$  8.07 (t, *J* = 5.27 Hz, 1H), 2.42 (t, *J* = 6.03 Hz, 2H), 1.61 – 1.82 (m, 6H), 1.15 – 1.42 (m, 12H), 1.04 (br. s., 2H); <sup>13</sup>C NMR (75 MHz, CHLOROFORM-*d*)  $\delta$  169.3, 56.3, 43.5, 35.3, 33.0, 33.0, 25.9, 25.8, 22.2.

***N*-[(1*R*)-1-(Cyclohexylmethyl)-2-methylpropyl]-2-methylpropane-2-sulfonamide (8).** A solution of **7** (1.47 g, 6.4 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (35 mL) at –78 °C was treated with *i*PrMgCl (6 mL, 2.0 M in THF). The solution was allowed to warm to room temperature overnight, then was quenched by the addition of NH<sub>4</sub>Cl (sat.). The organic layer was washed with NaHCO<sub>3</sub> (sat.) and dried (Na<sub>2</sub>SO<sub>4</sub>). The concentrated residue was subjected to chromatography on silica gel eluting with a gradient of EtOAc in hexanes to afford 0.92 g (53%) of the desired sulfonamide (**8**): <sup>1</sup>H NMR (300 MHz, CHLOROFORM-*d*)  $\delta$  3.20 (br. s., 1H), 3.05 (d, *J* = 8.10 Hz, 1H), 2.00 (dtd, *J* = 3.58, 6.88, 13.75 Hz, 1H), 1.57 – 1.81 (m, 5H), 1.32 – 1.45 (m, 1H), 1.09 – 1.30 (m, 13H), 0.74 – 1.02 (m, 8H); <sup>13</sup>C NMR (75 MHz, CHLOROFORM-*d*)  $\delta$  59.3, 56.0, 39.3, 34.3, 34.1, 32.8, 32.4, 26.6, 26.4, 26.1, 22.7, 18.2, 17.4.

**(2*R*)-1-Cyclohexyl-3-methylbutan-2-amine (9).** The sulfonamide **8** (0.92 g 3.4 mmol) was dissolved in methanol (1.7 mL) and treated with HCl in dioxane (1.7 mL, 4 N). After 30 minutes at room temperature, the solution was concentrated to afford 0.67 g (97%) of **9** a fluffy white solid: <sup>1</sup>H NMR (300 MHz, CHLOROFORM-*d*)  $\delta$  8.33 (br. s., 2H), 3.17 (br. s., 1H), 1.94 – 2.23 (m, 1H), 1.49 – 1.91 (m, 9H), 0.72 – 1.48 (m, 10H); Ms (ESI) *m/z* 170.3 (M + H)<sup>+</sup>.

**(3*R*)-*N*-[(1*R*)-1-(Cyclohexylmethyl)-2-methylpropyl]-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (2).** A solution of dicyclohexylcarbodiimide (DCC) (105 mg, 0.51 mmol) in THF (0.9 mL) was added to a solution of **9** (100 mg, 0.49 mmol), NEt<sub>3</sub> (0.14 mL, 1.0 mmol), HOBT (69 mg, 0.51 mmol) and 7-hydroxy-Boc-D-Tic(OH) (150 mg, 0.51 mmol) in THF (3 mL) at 0 °C. The solution was allowed to warm to room temperature overnight, forming a suspension. The solids were filtered, and the filtrate concentrated to a residue, which dissolved in methanol (3 mL) and treated with 6 N HCl (3 mL). After 12 h, the solution was concentrated and the residue was subjected to chromatography on silica gel eluting with a gradient up to 25% DMA80. The cleanest fraction was re-subjected to chromatography on silica gel eluting with a gradient up to 100% EtOAc in hexanes to afford 41.9 mg (25% over two steps) of the free base **2**: <sup>1</sup>H NMR (300 MHz, CHLOROFORM-*d*)  $\delta$  7.02 (d, *J* = 9.80 Hz, 1H), 6.95 (d, *J* = 8.29 Hz, 1H), 6.69 (dd, *J* = 2.26, 8.10 Hz, 1H), 6.56 (d, *J* = 2.07 Hz, 1H), 3.82 – 4.02 (m, 3H), 3.58 (dd, *J* = 5.09, 10.17 Hz, 1H), 3.14 (dd, *J* = 5.09, 16.01 Hz, 1H), 2.74 (dd, *J* = 10.17, 16.01 Hz, 1H), 1.46 – 1.87 (m, 6H), 1.03 – 1.39 (m, 7H), 0.67 – 1.00 (m, 9H); <sup>13</sup>C NMR (75 MHz, CHLOROFORM-*d*)  $\delta$  172.9, 154.9, 136.3, 129.9, 125.2, 114.3, 112.3, 56.8, 51.2, 47.3, 39.7, 34.6, 34.2, 32.6, 32.4, 30.6, 26.5, 26.3, 26.1, 19.1, 17.7. The free base was converted into a white powder as the hydrochloride salt: MS (ESI) *m/z* 345.3 (M + H)<sup>+</sup>; m.p. 258–262 °C (fusion); [ $\alpha$ ]<sub>D</sub><sup>25</sup> = +99 (c 0.10, CH<sub>3</sub>OH). Anal. (C<sub>21</sub>H<sub>33</sub>ClN<sub>2</sub>O<sub>2</sub>•0.25 H<sub>2</sub>O) C, H, N.

**[<sup>35</sup>S]GTP $\gamma$ S Assay.** The [<sup>35</sup>S]GTP $\gamma$ S assays were conducted using the methods previously reported.<sup>S1,S2</sup> Briefly, the binding of the GTP analogue [<sup>35</sup>S]-GTP $\gamma$ S to membranes was determined in a volume of 500  $\mu$ L. The assay mixture contained 50 mM HEPES (pH 7.4), 100 mM NaCl, 5 mM MgCl<sub>2</sub>, 1 mM EDTA•2H<sub>2</sub>O, 1 mM dithiothreitol, 0.1% BSA, 10  $\mu$ M GDP, test compounds, and approximately 132,000 cpm of [<sup>35</sup>S]-GTP $\gamma$ S (0.1 nM). Human kappa (20  $\mu$ g), mu (10  $\mu$ g), or delta (7  $\mu$ g) opioid receptor-expressing CHO cell membrane protein was added to each tube. For the kappa receptor assays, eight-

point concentration response curves of the agonist U69,593 (31.6  $\mu$ M, 10  $\mu$ M, 3.16  $\mu$ M, 316 nM, 100 nM, 31.6 nM, 3.16 nM, 0.316 nM final) were prepared and the dilutions were co-incubated with a single concentration of each test compound. For the mu and delta receptors, the agonists were DAMGO (31.6  $\mu$ M, 10  $\mu$ M, 3.16  $\mu$ M, 316 nM, 100 nM, 31.6 nM, 3.16 nM, 0.316 nM final) and DPDPE (10  $\mu$ M, 3.16  $\mu$ M, 1  $\mu$ M, 100 nM, 10 nM, 1 nM, 0.1 nM, 0.01 nM final), respectively. Following a 60 min shaking incubation at 22°C, the assay was terminated by filtration under vacuum on a Brandel (Gaithersburg, MD, USA) 96-well harvester using pre-soaked Perkin-Elmer GF/B glass fiber filters. The filters were rinsed three times with 1 mL washes of ice-cold wash buffer (20 mM HEPES, pH 7.4, 100 mM NaCl, 10 mM MgCl<sub>2</sub>). Filter plates were dried for 1 hr at 55°C. Microscint 20 (50  $\mu$ L) was added to each well and filter-bound radioactivity was counted on a Packard TopCount NXT microplate scintillation and luminescence counter. Total binding (TB) was determined in the absence of compounds and nonspecific binding (NSB) was determined in the presence of 10  $\mu$ M unlabeled GTP $\gamma$ S. Percent specific bound (SB) was calculated using the equation % SB = (SB/MB) x 100 where maximal binding (MB) is calculated by subtracting NSB from TB. Percent SB was plotted against the log of compound concentration. Data were fit to a three-parameter logistic curve to generate EC<sub>50</sub> values (Prism, version 6.0, GraphPad Software, Inc., San Diego, CA). K<sub>e</sub> values were calculated using the equation  $K_e = [L]/((EC_{50}^+/EC_{50}^-) - 1)$  where [L] is the concentration of test compound, EC<sub>50</sub><sup>+</sup> is the EC<sub>50</sub> of the control agonist with test compound, and EC<sub>50</sub><sup>-</sup> is the EC<sub>50</sub> of control agonist alone. K<sub>e</sub> values were considered valid when the EC<sub>50</sub><sup>+</sup>/EC<sub>50</sub><sup>-</sup> ratio was at least 4.

**Calculated Physiochemical Properties.** The topological polar surface area (TPSA) and calculated lipophilicity (clogP) values were calculated using the ChemAxon Instant JChem package. Predictions of the logarithm of the *in-vivo* blood-brain ratio (logBB) were based on the Clark and Pickett model.<sup>S3</sup>

**Docking Studies and Calculations.** The docking studies and calculation studies were conducted using procedures analogous to that reported in Reference S4.<sup>S4</sup>

## Analysis Appendix

Cmpd	Formula	Calculated			Found		
		C	H	N	C	H	N
<b>1</b>	C <sub>20</sub> H <sub>33</sub> Cl <sub>2</sub> N <sub>3</sub> O <sub>2</sub> • 2.25 H <sub>2</sub> O	52.34	8.24	9.16	52.11	7.85	9.10
<b>2</b>	C <sub>21</sub> H <sub>33</sub> Cl <sub>2</sub> N <sub>2</sub> O <sub>2</sub> • 0.25 H <sub>2</sub> O	65.44	8.76	7.27	65.37	8.79	7.24

## References

- (S1) Kormos, C. M.; Cueva, J. P.; Gichinga, M. G.; Runyon, S. P.; Thomas, J. B.; Brieady, L. E.; Mascarella, S. W.; Gilmour, B. P.; Navarro, H. A.; Carroll, F. I. Effect of the 3- and 4-methyl groups on the opioid receptor properties of N-substituted trans-3,4-dimethyl-4-(3-hydroxyphenyl)piperidines. *J. Med. Chem.* **2014**, *57*, 3140-7.
- (S2) Kormos, C. M.; Gichinga, M. G.; Maitra, R.; Runyon, S. P.; Thomas, J. B.; Brieady, L. E.; Mascarella, S. W.; Navarro, H. A.; Carroll, F. I. Design, synthesis, and biological evaluation of (3R)-1,2,3,4-tetrahydro-7-hydroxy-N-[(1S)-1-[(3R,4R)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl]-2-methylpropyl]-3-isoquinolinecarboxamide (JDTic) analogues: in vitro pharmacology and ADME profile. *J. Med. Chem.* **2014**, *57*, 7367-81.
- (S3) Clark, D. E.; Pickett, S. D. Computational methods for the prediction of 'drug-likeness'. *Drug Discov. Today* **2000**, *5*, 49-58.

- (S4) Kormos, C. M.; Jin, C.; Cueva, J. P.; Runyon, S. P.; Thomas, J. B.; Brieady, L. E.; Mascarella, S. W.; Navarro, H. A.; Gilmour, B. P.; Carroll, F. I. Discovery of N-{4-[(3-Hydroxyphenyl)-3-methylpiperazin-1-yl]methyl-2-methylpropyl}-4-phenoxybenzamide Analogues as Selective Kappa Opioid Receptor Antagonists. *J. Med. Chem.* **2013**, 56, 4551-4367.

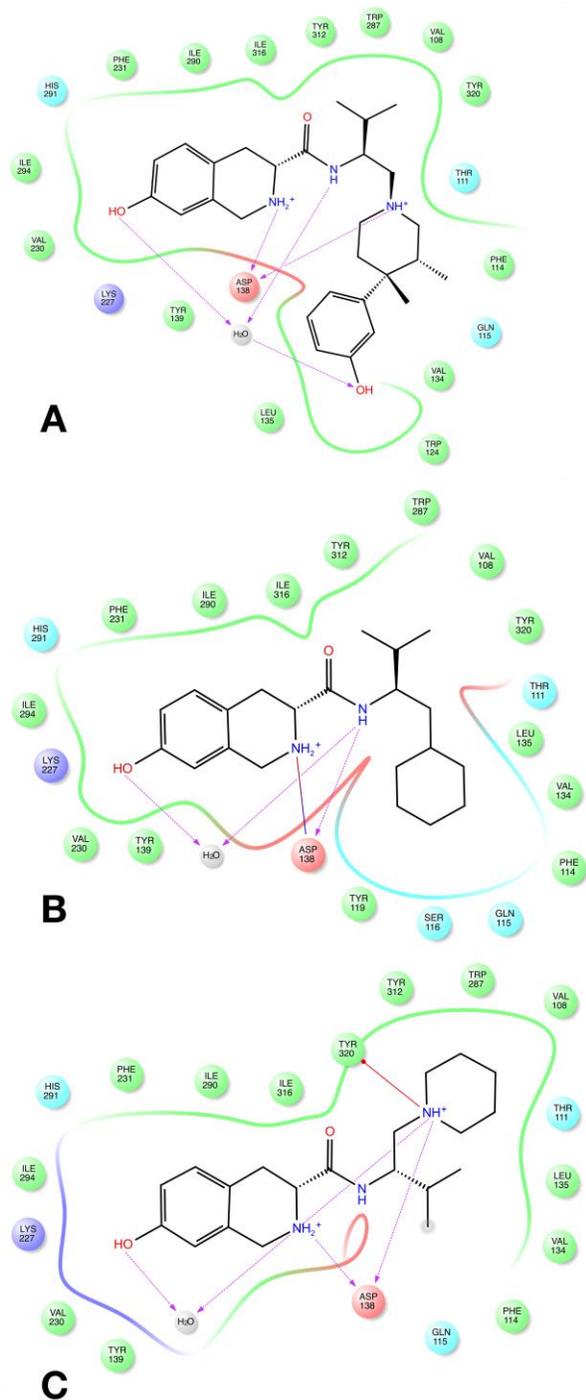


Figure S1. Two-dimensional interaction diagrams illustrating the steric, hydrogen-bond, and cation- $\pi$  aromatic interactions between the ligands JDtic (A, observed), Compound **2** (B, calculated), Compound **1** (C, calculated) and the kappa opioid receptor. Receptor residues are color-coded to indicate the predominate interaction type: negative charge (red), positive charge (dark blue), hydrophobic (green), polar (light blue). Hydrogen bonds are indicated as purple arrows. Cation- $\pi$  aromatic interactions are indicated by solid red lines.