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Design, synthesis, and pharmacological evaluation of JDTic analogs to examine the significance of the 3- and 4-methyl substituents

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Abstract

The design and discovery of JDTic as a potent and selective kappa opioid receptor antagonist used the *N*-substituted *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine pharmacophore as the lead structure. In order to determine if the 3-methyl or 4-methyl groups were necessary in JDTic and JDTic analogs for antagonistic activity, compounds **4a–c**, and **4d–f** which have either the 3-methyl or both the 3- and 4-methyl groups removed, respectively, from JDTic and analogs were synthesized and evaluated for their *in vitro* opioid receptor antagonist activities using a [^{35}S]GTPγS binding assay. Other ADME properties were also assessed for selected compounds. These studies demonstrated that neither the 3-methyl or 3,4-dimethyl groups present in JDTic and analogs are required to produce potent and selective κ opioid receptor antagonists.

Graphical Abstract

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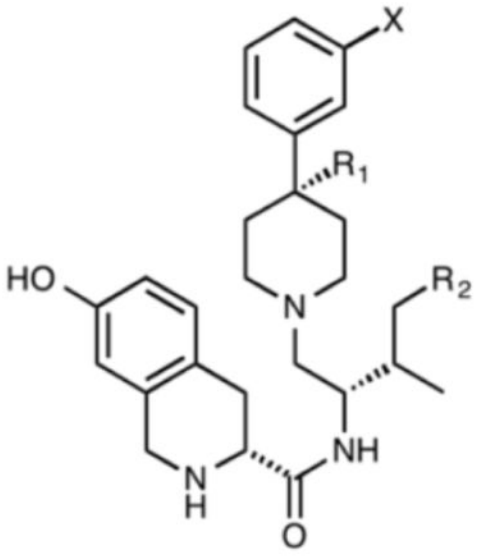

4a, X = OH, R_1 = CH_3, R_2 = H
b, X = F, R_1 = CH_3, R_2 = H
c, X = F, R_1, = R_2 = CH_3
d, X = OH, R_1 = R_2 = H
e, X = H, R_1 = R_2 = H
f, X = F, R_1 = R_2 = H

Keywords

JDTic; opioids; kappa antagonist; ADME properties

1. Introduction

The design and discovery of JDTic as a potent and selective kappa opioid receptor antagonist used the *N*-substituted *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine (**1**) pharmacophore as the lead structure.[1, 2] (Fig. 1) In a recent study we reported that *N*-phenylpropyl-4-methyl-4-(3-hydroxyphenyl)-piperidine (**2a**) where the 3-methyl group was removed from **1** and *N*-phenylpropyl-4-(3-hydroxyphenyl)-piperidine (**2b**), where both the 3- and 4-methyl groups were removed from **1** were still pure opioid receptor antagonists.[3] In another recent study we reported that the replacement of the hydroxy group in the 4-(3-hydroxyphenyl) moiety of JDTic with a hydrogen or fluoro group gave compounds **3a** and **3b** both of which were potent and selective kappa opioid receptor antagonists.[4] (Fig. 1) As a continuation of our structural activity relationship (SAR) studies directed toward the kappa opioid receptor as well as the development of potential pharmacotherapies for CNS disorders, compounds **4a–f** were synthesized and evaluated *in vitro* using a [^{35}S]GTPγS binding assay at the μ, δ, and κ opioid receptors and their ADME properties were established. (Fig. 1) A comparison of their *in vitro* antagonist activity and ADME properties

of selected compounds to those of JDTic and compounds **3a** and **3b** are herein presented. In addition, since the synthesis and [^{35}S]GTPγS *in vitro* properties of compounds **4d** were recently reported, we compare the results from the two studies.[5]

2. Chemistry

Compound **4a** was prepared according to Scheme 1. 3-Bromiosopropoxybenzene was prepared and distilled from the corresponding phenol and isopropyl bromide to be used as substrate for the lithium-halogen exchange. Addition of the resulting aryl lithium to *N*-methylpiperidione **5** gave the alcohol **6** in 42% yield. Dehydration of **6** with 2 eq. p-toluenesulfonic acid in toluene gave tetrahydropyridine **7** in 79% yield. Methylation according to the method reported by Werner et al.[6] was followed by $NaBH_4$ reduction of the enamine intermediate to afford **8**. Treatment of **8** with 1-chloroethyl chloroformate (ACE-Cl) in refluxing dichloroethane followed by methanolysis yielded piperidine **9**. Reductive amination of amine **9** with Boc-L-Valinal[7] using sodium cyanoborohydride in trifluoroethanol followed by Boc-deprotection afforded **10**. HBTU coupling of **10** with Boc-7-hydroxy-D-Tic-OH followed by deprotection with hydrogen bromide in acetic acid afforded **4a**.

Compound **4b** was prepared as outlined in Scheme 2. 1-Methyl-4-(3-fluorophenyl)-1,2,5,6-tetrahydropyridine (**11**) was prepared according to the method of Nagai et al.[8] The deprotonation and methylation proceeded analogously to the method described by Werner. [6] The resulting enamine was reduced with sodium borohydride in methanol. Demethylation with ACE-Cl followed by heating at reflux in methanol to hydrolyze the intermediate carbamate afforded 4-(3-fluorophenyl)-4-methylpiperidine (**12**). Piperidine **12** was coupled with Boc-L-valine using HBTU in acetonitrile. The *tert*-butyloxycarbonyl protecting group was removed with aqueous hydrogen chloride in methanol so that the amide could be reduced cleanly with borane dimethylsulfide at reflux in tetrahydrofuran. The resulting amine (**13**) was coupled with Boc-7-hydroxy-D-Tic-OH using 1-ethyl-3-(dimethylaminopropyl)carbodiimide hydrochloride (EDC • HCl) in dichloromethane followed by treatment with hydrogen chloride in dioxane and acetonitrile to afford **4b**.

Compound **4c** was prepared from **12** via a procedure analogous to that used for **4b** by substituting Boc-L-isoleucine for Boc-L-valine as outlined in Scheme 3. Piperidine **12** was first coupled to Boc-L-isoleucine using EDC and HOBt in acetonitrile followed by removal of the Boc protecting group present with aqueous hydrogen chloride in methanol so that the amide could be reduced cleanly with borane dimethylsulfide at reflux in tetrahydrofuran to give **14**. The resulting amine (**14**) was coupled with Boc-7-hydroxy-D-Tic-OH using EDC•HCl in dichloromethane followed by treatment with hydrogen chloride in dioxane and acetonitrile to afford **4c**.

The synthesis of **4f** is outlined in Scheme 4. Coupling of N-Boc-L-valine with 4-(3-fluorophenyl)piperidine **15** using HBTU in acetonitrile followed by cleavage of the *tert*-butyloxycarbonyl protecting group in intermediate *tert*-butyl [(1*S*)-1-{[4-(3-fluorophenyl)piperidin-1-yl]carbonyl}-2-methylpropyl]carbamate **16** using concentrated hydrochloric acid in methanol gave **17**. Reduction of **17** with diborane in tetrahydrofuran

gave **18**. Coupling of **18** with Boc-7-hydroxy-D-Tic-OH using EDC and catalytic amount of *N*-hydroxybenzotriazole (HOBt) in dichloromethane provided *tert*-butyl (3*R*)-3-{[(1*S*)-1-{[4-(3-fluorophenyl)piperidin-1-yl]methyl}-2-methylpropyl]carbamoyl}-7-hydroxy-3,4-dihydroisoquinoline-2(1*H*)-carboxylate **19**. Compound **4f** was obtained by treating **19** with concentrated hydrochloric acid in methanol.

Compounds **4d** and **4e** were synthesized by procedures analogous to the reported synthesis.[5]

3. Results and Discussion

In order to determine if the 3-methyl group on the piperidine ring of JDTic was required to obtain a pure antagonist, we synthesized and tested **4a** for its opioid antagonism properties. We found that **4a** had no agonist activity at 10 μM in the [^{35}S]GTPγS binding assay and was indeed a pure opioid receptor antagonist (Table 1). With K_e values of 67.5, 927, and 0.69 nM at the μ, δ, and κ opioid receptors, respectively, it was a potent and selective kappa opioid receptor antagonist. However, it was 35-times less potent than JDTic as a kappa opioid receptor antagonist (Table 1).

Replacement of the 3-OH in **4a** with a fluoro group gave **4b** which was also a pure opioid antagonist in the [^{35}S]GTPγS test (Table 1). Compound **4b**, which has a K_e = 0.18 nM at the κ receptor is only 9- and 18-fold less potent than JDTic and **3b** as a κ opioid antagonist (Table 1). With K_e values of 38 and 1480 nM at the μ and δ receptors, respectively, the compound **4b** remains highly selective for the κ relative to the μ and δ opioid receptors. Compound **4c**, which has the isopropyl group in **4b** replaced with an isobutyl group, has K_e values of 11.3, 903, and 0.033 nM at the μ, δ, and κ receptors, respectively. Thus, **4c** is 6-fold more potent than **4b** as a κ opioid receptor antagonist.

Compounds **4d**, **4e**, and **4f** have both the 3- and 4-methyl groups removed from the piperidine ring, relative to JDTic, **3a** and **3b**, respectively (Table 1). Compound **4d** with a K_e = 0.1 nM at the κ receptor is 5-times less potent than JDTic as a κ antagonist in this assay (Table 1). Having K_e values of 12.3 and 240 nM at the μ and δ receptors, respectively, **4d** is 123-fold and 2400-fold selective for the κ receptor relative to the μ and δ receptors, respectively. Compound **4e** also has the 3-OH removed. With a K_e = 0.051 nM at the κ receptor, this compound is only 2.6- and 2-times less potent as a κ antagonist than JDTic and **3a**, respectively. Compound **4e** has K_e values of 3.96 and 281 nM at the μ and δ receptors, respectively, and is 77- and 5500-fold selective for the κ receptor relative to the μ and δ receptors, respectively. Compound **4f** like **3b** has a 3-fluoro substituent in place of the 3-OH in JDTic and **4d**. Similar to JDTic, **4f** with a K_e = 0.023 nM at the κ receptor is a potent κ antagonist. Compound **4f** has a K_e = 3.55 and 128 nM at the μ and δ receptors, respectively. Thus, **4f** is 7- and 4-times more potent as a μ antagonist than JDTic and **3b**, respectively, and is 154- and 5600-fold selective for the κ receptor relative to the μ and δ receptors, respectively.

Compound **4d** has a structure identical to that of a compound named AT-076 reported by Zaveri and coworkers.[5] These authors report K_e values of 0.48, 24.95, and 4.3 nM at the μ,

δ, and κ opioid receptors for AT-076 compared to K_e values of 12.3, 240, and 0.1 nM determined in this study for **4d** using a [^{35}S]GTPγS binding assay. Although the Zaveri et al K_e values for the mu and delta receptors are lower than those for **4d**, both compounds show approximately the same relative selectivity for these receptors, but their K_e value is 43-fold higher for the κ receptor than that determined for **4d**. Differences in opioid receptor-effector coupling efficiency cannot explain this difference because the K_e is a ratiometric number that is calculated from the rightward shift in the agonist EC_{50} in the presence of antagonist. [9] However, differences in our K_e assay methods could affect receptor conformation altering the manner in which this ligand interacts with these receptors.

In order to determine if the compounds would be predicted to cross the blood/brain barrier, their topological polar surface area (TPSA), clogP, and derived logBB values were calculated and compared to JDTic, **3a** and **3b**. In general CNS compounds that have TPSA values less than 76Å^2[10], clogP values in the range of 2–4[11], and derived logBB values greater than −1[12] are predicted to cross the blood/brain barrier. Compounds **3b, 4f and 4c** have TPSA values less than 76Å^2. JDTic and **4a** with TPSA values of 84.83Å^2 are a little greater than 76Å^2. JDTic, **4a**, and **4b** have clogP values in the range of 2–4. Compounds **3b** and **4c** have clogP values a little above 4. All of the compounds have logBB values greater than −1 and thus, would be predicted to penetrate the brain.

Compound **4d** has a calculated TPSA value of 84.83 Å^2, which is identical to that of JDTic. Compounds **4e** and **4f** have TPSA values of 64.60 Å^2, which are identical to those of **3a** and **3b**. Compounds **4d**, **4e** and **4f** all have clogP values less than 4 and greater than 2. Compounds **4d**, **4e** and **4f** with logBB values of greater than −1 would be predicted to have good brain penetration.

ADME properties of these compounds were assessed (Table 2). Compounds that interact with the human ether-a-go-go gene (hERG) product, which is a potassium channel, can produce QT prolongation and cardiotoxic effects. The hERG K_i values of **4a–f** were compared to the K_i value for JDTic, **3a** and **3b (**Table 2). Compound **4a** had a K_i = >10 μM which is larger than the Ki values for JDTic (K_i = 8.820 μM). All the other compounds **(4b–f)** had K_i values lower than JDTic.

Compounds that have >5% permeability in the Madin Derby Canine Kidney cells stably expressing the human multidrug resistant gene (mdr1) product P-glycoprotein (MDCK-mdr1) monolayer permeability assay, >50% stability in the plasma and hepatic S9 stability assay, >25% transported in the Parallel Artificial Membrane Permeability Assay (PAMPA), and >20 μM solubility are considered desirable for further development by our group. The MDCK-mdr1 permeability, solubility, plasma, S9 stability and PAMPA permeability properties of **4a** were determined and compared to the properties of JDTic (Table 2). The permeability of **4a** is negligible across MDCK-mdr1 monolayers compared to 11% for JDTic. However, **4a** with solubility of 44 and 101 μM at pH 7.4 and 3, respectively, is more soluble than JDTic. With plasma and S9 stabilities of 62.5% following incubation in both assays, it is not as metabolically stable as JDTic but is above the 50% threshold considered to be desirable. The percent transported values of 1 and 2.6% at pH 7.4 and 5.5 in the PAMPA assay are well below the 26.8 and 57.9 percent values for JDTic. Due to the low K_i

values for **4b–f** in the hERG assay the ADME properties of the compounds were not determined.

4. Conclusions

In conclusion, JDTic analogs **4a–c** and **4d–f** which have the 3-methyl or both the 3- and 4-methyl groups removed, respectively, from JDTic and analogs were synthesized and evaluated for their ability to antagonize [^{35}S]GTPγS binding at the μ, δ, and κ opioid receptors. The data showed that neither the 3- or 3,4-dimethyl groups present in JDTic and analogs are required to obtain potent and selectivity kappa opioid receptor antagonists. Compound **4c** with a K_e = 0.033 nM at the κ receptor and 342- and 27,360-fold selectivity for the κ relative to the μ and δ receptors, respectively, was the most potent and κ selective compound having only the 3-methyl group removed. Compound **4f** with a K_e = 0.023 nM at the κ receptor and 154- and 5600-fold selectivity for the κ relative to the μ and δ receptors was the most potent and κ selective compound having both the 3- and 4-methyl groups removed. However, all of the compounds **4a–f** have subnanomolar potency for the κ receptor and are selective for the κ receptor relative to the μ and δ receptors and may be useful pharmacological tools for studying the κ opioid receptor.

5. Experimental

Melting points were determined using a MEL-TEMP II capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra were obtained on a Varian Avance DPX-300 MHz NMR spectrometer or a Bruker Unity Inova 500 MHz NMR spectrometer. Chemical shifts are reported in parts per million (ppm) with reference to internal solvent. Mass spectra (MS) were run on a Perkin-Elmer Sciex AP1 150 EX mass spectrometer equipped with APCI (atmospheric pressure chemical ionization) or ESI (turbospray) sources or on a Hewlett Packard 5989A instrument by electron impact. Elemental analyses were performed by Atlantic Microlab Inc., Atlanta, GA. Optical rotations were measured on an AutoPol III polarimeter, purchased from Rudolf Research. Analytical thin-layer chromatography (TLC) was carried out using EMD silica gel 60 F_{254} TLC plates. TLC visualization was achieved with a UV lamp or in an iodine chamber. Flash column chromatography was done on a CombiFlash Companion system using ISCO prepacked silica gel columns or using EM Science silica gel 60A (230–400 mesh). Solvent system: CMA80=80:18:2 $CHCl_3$-MeOH-conc. NH_4OH. 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.

(3*R*)-7-Hydoxy-*N*-[(1*S*)-1-{[4-(3-hydroxyphenyl)-4-methylpiperidin-1-yl]methyl}-2-methylpropyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (4a) dihydrochloride

The amine **10** and Boc-7-hydroxy-D-Tic-OH (1.1 eq.) were combined with HBTU (1.1 eq.) and NEt_3 (4 eq.) in DCM (30 mL). The reaction mixture was stirred overnight then concentrated. The residue was subjected to chromatography using EtOAc as the eluent to afford the protected product. The residue resulting from concentration of the desired fractions was refluxed in 1:1 48% HBr:glacial AcOH overnight. The resulting solution was

concentrated and the resulting residue was subjected to a gradient of CMA80:CH_2Cl_2 through silica gel to afford 128 mg of **4a** free base (21% over two steps): ^{1}H NMR ($CDCl_3$) δ 7.16 (t, 1H, *J* = 7.9 Hz), 6.95 (d, 1H, *J* = 8.1 Hz), 6.85 – 6.78 (m, 2H), 6.69 – 6.62 (m, 2H), 6.54 – 6.49 (m, 1H), 4.07 – 3.97 (m, 1H), 3.96 – 3.90 (m, 1H), 3.10 – 2.95 (m, 1H), 2.83 – 2.29 (m, 6H), 2.17 – 2.00 (m, 2H), 1.86 – 1.67 (m, 2H), 1.22 – 1.16 (m, 3H), 0.96–0.80 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 174.3, 173.7, 156.9, 155.1, 155.0, 136.3, 136.2, 130.2, 129.9, 129.5, 124.8, 124.7, 117.2, 114.0, 113.0, 112.7, 112.3, 112.1, 60.1, 59.8, 57.3, 56.9, 47.3, 47.1, 36.5, 36.4, 36.3, 36.0, 31.3, 31.2, 30.4, 19.8, 17.7, 17.5; MS (ESI) m/z 452.6 (M + H)$^+$; The free base was converted into the dihydrochloride salt: mp 180–184 °C (fusion); $[\alpha]^{25}_{D}$ +45.6° (c 0.50, CH_3OH). Anal. Calcd for $C_{27}H_{39}Cl_2N_3O_3$ • 1.5 H_2O: C, 58.80; H, 7.68; N, 7.62. Found: C, 59.08; H, 7.34; N, 7.34.

(3*R*)-*N*-[(1*S*)-1-{[4-(3-fluorophenyl)-4-methylpiperidin-1-yl]methyl}-2-methylpropyl]-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (4b) dihydrochloride

(2*S*)-1-[4-(3-Fluorophenyl)-4-methylpiperidin-1-yl]-3-methylbutan-2-amine (**13)** (274 mg, 0.98 mmol), Boc-7-hydroxy-D-Tic (300 mg, 1.02 mmol), hydroxybenzotriazole hydrate (20 mg, 0.12 mmol), and EDC • HCl(390 mg, 2.0 mmol) were combined in THF (10 mL). Diisopropylethylamine (0.75 mL, 4.3 mmol) was slowly added. The resulting solution was stirred at room temperature for 12 h then concentrated. The residue was dissolved in CH_2Cl_2 (15 mL) then washed with saturated aqueous $NaHCO_3$ (5 mL). The aqueous layer was extracted once with EtOAc (15 mL). The combined organic layers were washed with brine (5 mL), dried (Na_2SO_4), and concentrated. The residue was purified by chromatography on silica gel using a gradient up to 40% CMA80 in CH_2Cl_2. The product containing fractions were combined and concentrated then dissolved in acetonitrile (5 mL) to which HCl in dioxane (4 N, 5 mL) was added. The resulting solution was evaporated under a stream of N_2 to afford a white powder. The powder dissolved in a minimum of MeOH was purified by chromatography on silica gel using a gradient up to 50% CMA80 in CH_2Cl_2 to afford **4b** as the free base: ^{1}H NMR (300 MHz, $CDCl_3$) d 7.22 – 7.32 (m, 1H), 7.03 – 7.14 (m, 2H), 6.99 (td, *J* = 2.03, 11.21 Hz, 1H), 6.82 – 6.92 (m, 2H), 6.55 (dd, *J* = 2.35, 8.19 Hz, 1H), 6.42 (d, *J* = 2.26 Hz, 1H), 4.12 – 4.25 (m, 1H), 3.57 – 3.77 (m, 2H), 3.24 (dd, *J* = 5.18, 11.40 Hz, 1H), 2.78 – 2.98 (m, 2H), 2.46 – 2.69 (m, 3H), 2.06 – 2.43 (m, 5H), 1.73 – 1.91 (m, 3H), 1.19 (s, 3H), 0.84 – 0.96 (m, 6H); ^{19}F NMR (282 MHz, $CDCl_3$) d −112.84 (s, 1F); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.4, 163.1 (d, *J* = 245 Hz), 155.0, 151.2 (broad), 137.3, 130.7, 129.9 (d, *J* = 8.3 Hz), 125.1, 121.3 (d, *J* = 1.8 Hz), 113.8, 112.9 (d, *J* = 25.6 Hz), 112.6 (d, *J* = 24.8 Hz), 112.2, 59.9, 56.7, 50.5, 50.0, 49.8, 48.2, 36.2, 36.1, 31.6, 29.5, 19.0, 17.9. The free base was converted to the dihydrochloride salt, affording 142 mg (26% over two steps) of a white powder: MS (ESI) *m*/*z* 454.4 (M + H)$^+$, mp 205–209 °C (fusion), $[\alpha]^{25}_{D}$ +122 (c 0.1, CH_3OH). Anal. Calcd for $C_{27}H_{38}Cl_2FN_3O_2$•1.5H_2O: C, 58.59; H, 7.47; N, 7.59. Found: C, 58.73; H, 7.43, N, 7.30.

(3*R*)-*N*-[(1*S*,2*S*)-1-{[4-(3-Fluorophenyl)-4-methylpiperidin-1-yl]methyl}-2-methylbutyl]-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (4c) dihydrochloride

(2*S*,3*S*)-1-[4-(3-Fluorophenyl)-4-methylpiperidin-1-yl]-3-methylpentan-2-amine (**14)** (266 mg, 0.91 mmol), Boc-7-hydroxy-*D*-Tic (293 mg, 1.00 mmol), hydroxybenzotriazole hydrate (20 mg, 0.12 mmol), and EDC • HCl(390 mg, 2.0 mmol) were combined in 10 mL THF.

Diisopropylethylamine (0.75 mL, 4.3 mmol) was slowly added. The resulting solution was stirred at room temperature for 12 h then concentrated. The residue was dissolved in CH_2Cl_2 (15 mL) then washed with saturated aqueous $NaHCO_3$ (5 mL). The aqueous layer was extracted once with EtOAc (15 mL). The combined organic layers were washed with brine (5 mL), dried (Na_2SO_4), and concentrated. The residue was purified by chromatography on silica gel using a gradient up to 40% CMA80 in CH_2Cl_2. The product containing fractions were combined and concentrated then dissolved in acetonitrile (10 mL) to which aq. HCl (6 N, 2 mL) was added. The resulting solution was evaporated under a stream of N_2 to afford a powder. The powder dissolved in a minimum of MeOH was purified by chromatography on silica gel using a gradient up to 50% CMA80 in CH_2Cl_2 to afford **4c** as the free base: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22 – 7.33 (m, 1H), 7.14 (d, *J* = 9.04 Hz, 1H), 7.06 (d, *J* = 8.10 Hz, 1H), 6.98 (td, *J* = 2.03, 11.21 Hz, 1H), 6.83 – 6.92 (m, 2H), 6.50 (dd, *J* = 2.45, 8.29 Hz, 1H), 6.40 (d, *J* = 2.26 Hz, 1H), 4.26 (t, *J* = 9.98 Hz, 1H), 3.52 – 3.75 (m, 2H), 3.20 (dd, *J* = 5.18, 11.40 Hz, 1H), 2.89 (dd, *J* = 5.18, 16.48 Hz, 2H), 2.45 – 2.74 (m, 3H), 2.05 – 2.43 (m, 5H), 1.74 – 1.95 (m, 2H), 1.55 – 1.71 (m, 1H), 1.42 (ddd, *J* = 4.90, 7.54, 13.00 Hz, 1H), 1.17 – 1.23 (m, 3H), 1.04 – 1.17 (m, 1H), 0.83 – 0.96 (m, 6H); ^{19}F NMR (282 MHz, $CDCl_3$) δ −112.82 (s, 1F); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.2, 163.1 (d, *J* = 245 Hz), 154.9, 151.1 (broad), 137.5, 130.8, 129.9 (d, *J* = 8.4 Hz), 125.3, 121.3 (d, *J* = 2.0 Hz), 113.6, 112.9 (d, *J* = 21.9 Hz), 112.6 (d, *J* = 21.2 Hz), 112.1, 112.1, 58.8, 56.6, 50.5, 50.1, 48.8, 48.3, 38.3, 36.2, 36.1, 29.2, 25.7, 14.8, 11.9. The free base was converted to the dihydrochloride salt, affording 216 mg (38% over two steps) of a white powder: MS (ESI) *m*/*z* 468.3 $(M + H)^+$, mp 191–195 °C (fusion), $[\alpha]^{25}_D$ +65.2 (c 0.50, CH_3OH). Anal. Calcd for $C_{28}H_{40}Cl_2FN_3O_2 \cdot 1.25H_2O$: C, 59.73; H, 7.61; N, 7.46. Found: C, 59.76; H, 7.69; N, 7.21.

N-[(1*S*)-1-{[4-(3-Hydroxyphenyl)piperidin-1-yl]methyl}-2-methylpropyl]-3-methyl-4-(3-methylphenoxy)benzamide (4d) dihydrochloride

Compound **4d** was synthesized by a procedure analogous to the reported synthesis.[7] ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 6.94 – 7.11 (m, 2H), 6.75 (d, *J* = 8.29 Hz, 1H), 6.45 – 6.62 (m, 4H), 6.38 (s, 1H), 4.08 (d, *J* = 9.61 Hz, 1H), 3.75 (d, *J* = 16.58 Hz, 1H), 3.61 (d, *J* = 16.39 Hz, 1H), 3.33 (t, *J* = 7.06 Hz, 1H), 3.09 (d, *J* = 9.98 Hz, 1H), 2.86 (d, *J* = 10.36 Hz, 1H), 2.74 (br s, 1H), 2.58 (t, *J* = 11.59 Hz, 1H), 2.24 (d, *J* = 10.74 Hz, 2H), 2.08 (br s, 1H), 1.72 (dd, *J* = 6.31, 11.96 Hz, 2H), 1.60 (br s, 2H), 1.38 (br s, 2H), 0.86 (t, *J* = 6.69 Hz, 6H); ^{13}C NMR (75 MHz, CHLOROFORM-d) δ 173.4, 171.6, 156.7, 154.7, 147.7, 136.2, 132.7, 130.4, 129.5, 124.7, 119.0, 114.5, 113.5, 112.7, 60.2, 56.4, 52.4, 50.2, 49.3, 46.8, 42.0, 32.7, 32.1, 31.7, 29.5, 19.3, 17.9; ESI MS $(M + H)^+$ 438.6. The product was converted to the dihydrochloride salt by adding 2M HCl in ether to solution of the free base in dichloromethane: mp >220 °C (dec), $[\alpha]_D$ = + 58.5 (c 0.62, MeOH). Anal. Calcd for $C_{26}H_{37}Cl_2N_3O_3 \cdot 1.25H_2O$: C, 58.59; H, 7.47; N, 7.88. Found C, 58.45; H, 7.26; N, 7.78.

(3*R*)-7-Hydroxy-*N*-{(1*S*)-2-methyl-1-[(4-phenylpiperidin-1-yl]methyl]propyl}-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (4e) dihydrochloride

Compound **4e** was synthesized by the reported procedure.[7] ^{1}H NMR (300 MHz, CHLOROFORM-d) d 7.29 – 7.24 (m, 2H), 7.20 – 7.10 (m, 4H), 6.91 (d, *J* = 8.3 Hz, 1H), 6.61 (dd, *J* = 2.5, 8.2 Hz, 1H), 6.45 (d, *J* = 2.5 Hz, 1H), 4.26 (br. s., 1H), 3.71 (q, *J* = 15.5 Hz, 2H), 3.45 (d, *J* = 10.9 Hz, 1H), 3.26 (dd, *J* = 5.2, 11.2 Hz, 1H), 3.07 (d, *J* = 11.7 Hz,

1H), 2.94 (dd, *J* = 5.2, 16.5 Hz, 1H), 2.70 (t, *J* = 12.1 Hz, 1H), 2.61 – 2.48 (m, 1H), 2.40 – 2.17 (m, 3H), 2.06 (m, 1H), 1.99 – 1.76 (m, 5H), 0.95 (d, *J* = 7.0 Hz, 6H); ^{13}C NMR (75 MHz, CHLOROFORM-d) d 173.3, 154.8, 145.6, 137.6, 130.8, 128.5, 126.7, 126.3, 125.5, 113.7, 112.1, 60.0, 56.6, 56.2, 52.6, 49.8, 48.3, 42.2, 32.9, 32.4, 31.7, 29.4, 19.0, 18.0; ESI MS $(M + H)^+$ 422.5. The free base was converted to the dihydrochloride salt by adding 2M HCl in ether to solution of product in dichloromethane: $[\alpha]_D$ = + 62.7 (c 1.01, MeOH). Anal. Calcd for $C_{26}H_{37}Cl_2N_3O_2$•1.5H_2O: C, 59.88; H, 7.73; N, 8.06. Found C, 59.67; H, 7.40; N, 8.01.

(3*R*)-*N*-[(1*S*)-1-{[4-(3-Fluorophenyl)piperidin-1-yl]methyl}-2-methylpropyl]-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (4f) dihydrochloride

To a solution of *tert*-butyl (3*R*)-3-{[(1*S*)-1-{[4-(3-fluorophenyl)piperidin-1-yl]methyl}-2-methylpropyl]carbamoyl}-7-hydroxy-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (**19**) (150 mg, 0.28 mmol) in 4 mL methanol was added 4 mL conc. HCl and the reaction mixture stirred for overnight. Evaporation of solvents led to a white solid that was purified by chromatography on silica gel with CMA 80: chloroform (1:1) as the eluent to provide 98 mg (79%) of the title compound as a white solid. ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 7.19 (s, 1H), 7.08 – 7.16 (m, 1H), 7.03 (d, *J* = 9.61 Hz, 1H), 6.72 – 6.90 (m, 4H), 6.52 (dd, *J* = 2.45, 8.10 Hz, 1H), 6.37 (d, *J* = 2.07 Hz, 1H), 4.08 – 4.23 (m, 1H), 3.53 – 3.74 (m, 2H), 3.30 (d, *J* = 11.11 Hz, 1H), 3.20 (dd, *J* = 5.18, 11.21 Hz, 1H), 2.97 (d, *J* = 11.30 Hz, 1H), 2.86 (dd, *J* = 5.09, 16.58 Hz, 1H), 2.59 (t, *J* = 12.06 Hz, 1H), 2.38 – 2.52 (m, 1H), 2.21 – 2.36 (m, 2H), 2.06 – 2.20 (m, 1H), 1.90 – 2.03 (m, 1H), 1.60 – 1.88 (m, 5H), 0.87 (d, *J* = 6.78 Hz, 6H); ^{13}C NMR (75 MHz, CHLOROFORM-d) δ 173.4, 164.6, 161.3, 154.9, 148.4, 148.3, 137.3, 130.6, 129.9, 129.8, 125.4, 122.4, 122.3, 113.9, 113.8, 113.5, 113.2, 112.9, 112.2, 60.0, 56.7, 55.9, 52.7, 50.0, 48.1, 42.0, 32.7, 32.4, 31.5, 29.6, 19.1, 17.9; ESI MS $(M + H)^+$ 440.5. The free base was converted to the dihydrochloride salt by adding 2M HCl in ether to solution of product in dichloromethane: [mp 215 – 219 °C, $\alpha]_D$ = + 61.6 (c 1.04, MeOH). Anal. Calcd for $C_{26}H_{36}Cl_2FN_3O_2$•H_2O: C, 58.87; H, 7.22; N, 7.92. Found C, 59.20; H, 7.39; N, 7.70.

1-Methyl-4-[3-(1-methylethoxy)phenyl]piperidin-4-ol (6)

A solution of *n*-butyl lithium (2.5 M in hexanes, 8.7 mL, 22 mmol) was slowly added to a solution of 1-bromo-3-isopropoxybenzene (5.2 g, 24 mmol) in THF (14 mL) at −78 °C. After 30 minutes, *N*-methyl-4-piperidinone **(5)** (2.49 g, 22.0 mmol) was added dropwise. The solution warmed to r.t. overnight. Hydrochloric acid (6 M, 8 mL) was added and the resulting biphasic mixture was extracted with hexanes. The organic layer was discarded and the aqueous layer was adjusted to pH 10 with NH_4OH (2 M). Extraction with hexanes, drying with Na_2SO_4 and concentration to afford 2.29 g (42%) of crude **6**. ^{1}H NMR ($CDCl_3$) δ 7.25 (t, *J* = 7.9 Hz, 1H), 7.01 – 7.10 (m, 2H), 6.74 – 6.83 (m, 1H), 4.56 (spt, *J* = 6.0 Hz, 1H), 2.75 (d, *J* = 11.3 Hz, 1H), 2.38 – 2.54 (m, 2H), 2.35 (s, 3H), 2.17 (dt, *J* = 4.5, 13.0 Hz, 1H), 1.69 – 1.81 (m, 1H), 1.33 (d, *J* = 6.0 Hz, 6H).

1-Methyl-4-[3-(1-methylethoxy)phenyl]-1,2,3,6-tetrahydropyridine (7)

Crude **6** was refluxed in toluene (15 mL) with TsOH • H_2O (2 eq.) for 3 h. The product was extracted into water. The aqueous layer was adjusted to pH 10 with NaOH (2 M) and then extracted with hexanes. The combined organic layer was washed with NaOH (2 M) and dried (Na_2SO_4). Concentration afforded 1.67 g (79%) of crude **7**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.14 – 7.25 (m, 2H), 6.96 (d, *J* = 7.9 Hz, 1H), 6.87 – 6.93 (m, 1H), 6.77 (dd, *J* = 2.5, 8.1 Hz, 1H), 6.00 – 6.08 (m, 1H), 4.55 (spt, *J* = 6.1 Hz, 1H), 3.10 (q, *J* = 2.8 Hz, 2H), 2.62 – 2.70 (m, 2H), 2.51 – 2.62 (m, 2H), 2.40 (s, 3H), 2.36 (s, 1H), 1.67 (s, 1H), 1.33 (d, *J* = 6.0 Hz, 7H).

1,4-Dimethyl-4-[3-(1-methylethoxy)phenyl]piperidine (8)

Crude **7** in THF (18 mL) was treated at −15 °C with butyl lithium (2.5 M, 4.5 mL) to afford a blood-red solution. After cooling to −50 °C, dimethylsulfate (0.8 mL) was cautiously added. After 30 minutes, NH_4OH (2 M, 10 mL) was added and the resulting biphasic mixture was extracted with hexanes. The combined organic layer was washed with water, dried (Na_2SO_4), and concentrated to a residue which was dissolved in MeOH (20 mL) and treated at 0 °C with $NaBH_4$ (0.42 g). After warming to room temperature the solution was concentrated and subjected to silica gel chromatography using a gradient of CMA80 in CH_2Cl_2 as the eluent to afford 1.44 g (81%) of **8**. ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 7.20 – 7.31 (m, 1H), 7.01 – 7.10 (m, 2H), 6.73 – 6.83 (m, 1H), 4.56 (spt, *J* = 5.97 Hz, 1H), 2.75 (d, *J* = 11.30 Hz, 2H), 2.38 – 2.51 (m, 2H), 2.35 (s, 3H), 2.17 (dt, *J* = 4.52, 13.00 Hz, 2H), 1.68 – 1.81 (m, 2H), 1.33 (d, *J* = 6.03 Hz, 6H).

4-methyl-4-[3-(1-methylethoxy)phenyl]piperidine (9)

The *N*-methyl amine **8** was concentrated from toluene then dissolved in 1,2-dichloroethane (9 mL) and treated with freshly distilled 1-chloroethyl chloroformate (1.8 mL, 3.0 eq.). The resulting dark solution was refluxed 12 h then concentrated. The residue was dissolved in methanol (10 mL), refluxed 24 h and then concentrated. The residue was subjected to chromatography on silica gel using a gradient of CMA80 in CH_2Cl_2 as the eluent to afford 0.677 g (50%) of **9**. ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 7.14 – 7.31 (m, 1H), 6.96 (d, *J* = 7.91 Hz, 1H), 6.87 – 6.93 (m, 1H), 6.77 (dd, *J* = 2.45, 8.10 Hz, 1H), 5.99 – 6.10 (m, 1H), 4.55 (spt, *J* = 6.06 Hz, 1H), 3.10 (q, *J* = 2.83 Hz, 2H), 2.61 – 2.70 (m, 2H), 2.52 – 2.61 (m, 2H), 2.40 (s, 3H), 1.30 – 1.36 (m, 6H).

(2*S*)-3-Methyl-1-{4-methyl-4-[3-(1-methylethoxy)phenyl]piperidin-1-yl}butan-2-amine (10)

Boc-L-Valinal was prepared by LAH reduction of the Weinreb amide.[7] The aldehyde (319 mg, 1.6 mmol) was combined with amine **9** (572 mg, 2.5 mmol) in trifluoroethanol (12 mL) and stirred for 15 min prior to addition of $NaCNBH_3$ (154 mg, 2.5 mmol). The resulting mixture was stirred overnight, concentrated, and the residue partitioned between 2 M NH_4OH and EtOAc. The aqueous layer was extracted further with EtOAc. The dried (Na_2SO_4) combined organics were concentrated and the residue subjected to chromatography on silica gel using a gradient of CMA80 in CH_2Cl_2 as the eluent. ^{1}H NMR ($CDCl_3$) δ 7.23 (t, *J* = 8.0 Hz, 1H), 6.82 – 6.93 (m, 2H), 6.72 (dd, *J* = 1.9, 8.1 Hz, 1H), 4.74 (bs, 1H), 4.54 (spt, *J* = 6.0 Hz, 1H), 3.55–3.74 (m, 1H), 2.75 (bs, 1H), 2.58 (bs, 2H), 2.26 –

2.51 (m, 3H), 2.05 – 2.24 (m, 2H), 1.70 – 1.92 (m, 3H), 1.45 (s, 9H), 1.34 (d, *J* = 6.0 Hz, 6H), 1.21 (s, 3H), 0.81 – 0.99 (m, 6H). The isolated product was dissolved in CH_2Cl_2 (5 mL) and treated with TFA (10 mL) at r.t. for 12 h. The concentrated residue was partitioned between $CHCl_3$ and 2 M NH_4OH. The organic layer was dried (Na_2SO_4) and concentrated to afford 439 mg of **10** (1.38 mmol, 56% over two steps), which was used in the next step without further purifications.

4-Methyl-4-(3-fluorophenyl)piperidine (12)

A solution of *n*-BuLi (7.2 mL, 2.5 M in hexanes, 18 mmol) was added dropwise to a solution of **11** (2.46 g, 12.9 mmol) in THF (22 mL) maintained between −10 and −20 °C. After 15 min, the solution was cooled to −50 °C and dimethyl sulfate (1.75 mL, 18.5 mmol) was slowly and cautiously added. The reaction mixture was stirred an additional 30 min, then 2M NH_4OH (10 mL) was added. The resulting mixture was extracted with hexanes. The combined organic layer was washed with water, dried (Na_2SO_4), and concentrated to a residue. The residue was dissolved in CH_3OH (20 mL), cooled in an ice bath, and treated with an excess of $NaBH_4$ (0.5 g, 13 mmol). The reaction mixture was stirred 3 h at room temperature and then was quenched with the addition of acetone and saturated $NaHCO_3$. The concentrated residue was dissolved in water and EtOAc. The aqueous layer was extracted again with EtOAc before the combined organic layer was washed with water then concentrated to afford 1.72 g (8.3 mmol, 64%) of the 1,4-dimethylpiperidine intermediate. This residue was concentrated thrice from toluene then dissolved in 1,2-dichloroethane (12 mL). A freshly distilled aliquot of 1-chloroethyl chloroformate (1.3 mL, 12 mmol) was added under inert atmosphere and the resulting solution was heated at reflux overnight. The concentrated residue was then dissolved in CH_3OH and refluxed 1 h. The concentrated residue was dissolved in 2 M NaOH and extracted with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4), concentrated, and subjected to chromatography on silica gel using a gradient of CMA80 in DCM as the eluent to afford 0.74 g (30% from **11**) of **12**. ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 7.22 – 7.36 (m, 1H), 7.12 (d, *J* = 7.91 Hz, 1H), 7.04 (td, *J* = 2.14, 11.35 Hz, 1H), 6.88 (dt, *J* = 2.17, 8.05 Hz, 1H), 2.74 – 3.00 (m, 4H), 1.93 – 2.10 (m, 2H), 1.70 (ddd, *J* = 3.39, 7.25, 13.09 Hz, 3H), 1.20 – 1.30 (m, 3H).

(2*S*)-1-[4-(3-Fluorophenyl)-4-methylpiperidin-1-yl]-3-methylbutan-2-amine (13)

A solution of amine **12** (740 mg, 3.8 mmol) and Boc-L-valine (0.86 g, 4.0 mmol) in acetonitrile (20 mL) was cooled in an ice-bath, then treated with HBTU (1.51 g, 4.0 mmol) and diisopropylethylamine (2.1 mL, 12 mmol). The flask was removed from the ice bath, and the reaction mixture was stirred overnight. The solution was concentrated then partitioned between concentrated aqueous $NaHCO_3$ and EtOAc. The mixture was extracted three times with EtOAc (25 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered, and evaporated to leave a residue which was dissolved in acetonitrile (20 mL) and treated with HCl in dioxane (20 mL). The solvent was concentrated under a stream of nitrogen. The remaining residue was subjected to chromatography on silica gel using a gradient of CMA80 in DCM as the eluent to afford the intermediate amide. This amide was dissolved in THF (20 mL) and treated with borane dimethylsulfide (3 mL, 30 mmol). The solution was stirred at reflux overnight and then quenched with methanol. The residue was treated with HCl (6 M, 10 mL) and stirred for 1 h. Solid $NaHCO_3$ was

added to adjust the solution to a pH of 8, and the mixture was extracted with CH_2Cl_2 (3 × 25 mL), washed with brine, and dried over $MgSO_4$. The concentrated residue was subjected to chromatography on silica gel eluting with a gradient of CMA80 in DCM to afford 0.64 g (61% from **12**) of **13**: ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 7.22 – 7.33 (m, 1H), 7.11 (d, *J* = 8.10 Hz, 1H), 7.03 (td, *J* = 2.14, 11.35 Hz, 1H), 6.82 – 6.92 (m, 1H), 2.45 – 2.74 (m, 3H), 1.98 – 2.44 (m, 5H), 1.65 – 1.87 (m, 5H), 1.51 (qd, *J* = 6.66, 12.97 Hz, 1H), 1.18 – 1.23 (m, 3H), 0.83 – 0.95 (m, 6H); ^{19}F NMR (282 MHz, CHLOROFORM-d) δ −133.33.

(2*S*)-1-[4-(3-Fluorophenyl)-4-methylpiperidin-1-yl]-3-methylpentan-2-amine (14)

A solution of amine **12** (233 mg, 1.2 mmol) and Boc-L-isoleucine (275 g, 1.2 mmol) in acetonitrile (7 mL) was cooled in an ice-bath, then treated with HBTU (0.50 g, 1.3 mmol) and diisopropylethylamine (0.65 mL, 3.7 mmol). The flask was removed from the ice bath, and the reaction was stirred overnight. The solution was concentrated then partitioned between concentrated aqueous $NaHCO_3$ and EtOAc. The mixture was extracted three times with EtOAc (25 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered, and evaporated to leave a residue which was dissolved in methanol (10 mL) and treated with HCl (6 M, 10 mL). The solution was concentrated to a solid from toluene. The remaining residue was subjected to chromatography on silica gel using a gradient of CMA80 in DCM to afford the intermediate amide. This amide was dissolved in THF (15 mL) and treated with borane dimethylsulfide (2.5 mL, 25 mmol). The solution was stirred at reflux overnight, then quenched with and concentrated from methanol. The residue was treated with HCl (6 M, 10 mL) and methanol (10 mL), stirred for 1 h, and then concentrated. The residue was combined with 2 M NaOH (10 mL) and extracted with EtOAc (3 × 25 mL). The combined extracts were washed with brine, and dried ($MgSO_4$). The concentrated residue was subjected to chromatography on silica gel eluting with a gradient of CMA80 in DCM to afford 0.27 g (77% from **12**) of the intermediate amine **14**: ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 7.22 – 7.33 (m, 1H), 7.08 – 7.14 (m, 1H), 7.03 (td, *J* = 2.14, 11.35 Hz, 1H), 6.87 (ddt, *J* = 0.75, 2.45, 8.29 Hz, 1H), 2.71 – 2.84 (m, 1H), 2.44 – 2.67 (m, 2H), 1.96 – 2.43 (m, 5H), 1.57 – 1.83 (m, 6H), 1.48 (ddd, *J* = 3.96, 7.58, 12.95 Hz, 1H), 1.24 – 1.37 (m, 1H), 1.18 – 1.24 (m, 3H), 0.78 – 0.95 (m, 6H).

tert-Butyl [(1*S*)-1-{[4-(3-fluorophenyl)piperidin-1-yl]carbonyl}-2-methylpropyl]carbamate (16)

To a solution of 4-(3-fluorophenyl)piperidine (**15**) (5.0 mg, 27.9 mmol) in dry acetonitrile (150 mL) was added Boc-L-valine (6.5 g, 30 mmol) followed by 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (11.4 g, 30 mmol) and triethylamine (8. 3 g, 82.5 mmol). The reaction mixture was stirred overnight then concentrated. The resulting solid was treated with 50 mL saturated $NaHCO_3$. The aqueous layer was extracted with EtOAc (2 × 50 mL). Combined organic layers were dried (Na_2SO_4), filtered then concentated. The resulting residue was purified by column chromatography on silica gel with hexanes: ethyl acetate (1:1) to provide 7.93 g (75%) of the title compound. ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 7.14 – 7.24 (m, 1H), 6.78 – 6.92 (m, 2H), 5.32 (d, *J* = 9.04 Hz, 1H), 4.70 (d, *J* = 12.81 Hz, 1H), 4.44 (d, *J* = 6.97 Hz, 1H), 3.90 – 4.21 (m, 1H), 3.03 – 3.17 (m, 1H), 2.55 – 2.75 (m, 2H), 1.77 – 2.04 (m, 3H), 1.43 – 1.68 (m, 2H), 1.31 – 1.40 (m, 9H), 0.69 – 0.96 (m, 6H); ^{13}C NMR (75 MHz,

CHLOROFORM-d) δ 170.6, 164.7, 161.4, 155.9, 147.5, 130.1, 130.0, 122.4, 113.8, 113.7, 113.5, 113.3, 79.4, 54.8, 46.5, 46.1, 42.8, 42.6, 42.2, 33.7, 33.6, 32.8, 31.8, 31.5, 28.4, 19.8, 19.6, 17.2, 17.1; ESI MS $(M + H)^+$ 379.6.

(2*S*)-2-Amino-1-[4-(3-fluorophenyl)piperidin-1-yl]-3-methylbutan-1-one (17)

To a solution of *tert*-butyl [(1*S*)-1-{[4-(3-fluorophenyl)piperidin-1-yl]carbonyl}-2-methylpropyl]carbamate (**16**) (7.93 g, 21.0 mmol) in methanol (100 mL) was added conc. HCl (8 mL) and the reaction mixture stirred for 4 h at ambient temperature. Evaporation of solvents led to a white solid that was purified by chromatography on silica gel with CMA 80: chloroform (1:1) as the eluent to provide 6.05 g (92%) of the title compound as a white solid. ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 8.43 (br. s., 2H), 7.11 – 7.23 (m, 2H), 6.76 – 6.97 (m, 2H), 4.70 (d, *J* = 13.19 Hz, 1H), 4.51 (br. s., 1H), 3.76 – 4.12 (m, 1H), 3.09 (br. s., 1H), 2.67 (d, *J* = 9.04 Hz, 2H), 2.09 – 2.29 (m, 1H), 1.98 (s, 2H), 1.66 – 1.81 (m, 1H), 1.47 (br. s., 1H), 0.89 – 1.19 (m, 5H); ^{13}C NMR (75 MHz, CHLOROFORM-d) δ 167.4, 166.9, 164.6, 161.3, 147.7, 147.6, 147.3, 130.1, 130.0, 129.9, 122.8, 122.4, 114.1, 113.8, 113.7, 113.4, 113.1, 55.5, 55.2, 47.2, 46.4, 43.7, 43.0, 42.9, 41.8, 33.2, 32.5, 30.2, 30.1, 19.3, 18.9, 17.8, 17.5.

(2*S*)-1-[4-(3-Fluorophenyl)piperidin-1-yl]-3-methylbutan-2-amine (18)

To a cooled (0 °C) solution of (2*S*)-2-amino-1-[4-(3-fluorophenyl)-1-piperidin-1-yl]-3-methyl-butan-1-one (**17**) hydrochloride (6.05 g, 19.2 mmol) in THF (40 mL) was added borane dimethyl sulfide complex (3.8 mL, 38 mmol) (10 M as BH_3). The reaction mixture was warmed to ambient temperature and stirred overnight. The reaction mixture was refluxed for 3 h, cooled in ice, quenched with methanol and stirred for 1 h at ambient temperature. The reaction mixture was cooled in ice bath and treated with 2M HCl in ether and refluxed for 2 h. The solvent was evaporated and the resulting material purified by chromatography on silica gel using chloroform/CMA80 gradient to provide 2.5 g (49%) of the desired product as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68 (br. s, 2H), 7.17 – 7.25 (m, 1H), 6.93 – 7.02 (m, 2H), 6.82 – 6.89 (m, 1H), 3.01 – 3.12 (m, 3H), 2.44 – 2.66 (m, 3H), 2.31 – 2.37 (m, 1H), 2.03 – 2.23 (m, 2H), 1.75 – 1.93 (m, 4H), 1.17 (d, *J* = 7 Hz, 3H), 1.09 (d, *J* = 7 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.5, 161.3, 148.9, 148.8, 129.8, 129.7, 122.6, 122.5, 113.9, 113.6, 113.0, 112.7, 57.8, 55.5, 54.9, 53.1, 42.1, 33.3, 32.8, 29.3, 19.4, 18.5; ESI MS $(M + H)^+$ 265.5.

tert-Butyl (3*R*)-3-{[(1*S*)-1-{[4-(3-fluorophenyl)piperidin-1-yl]methyl}-2-methylpropyl]carbamoyl}-7-hydroxy-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (19)

To a solution of (2*S*)-1-[4-(3-fluorophenyl)piperidin-1-yl]-3-methylbutan-2-amine (70 mg, 0.27 mmol) (**18**) in dichloromethane (15 mL) was added Boc-7-hydroxy-D-Tic-OH (117 mg, 0.40 mmol) and triethylamine (80 mg, 0.8 mmol) followed by EDC • HCl (153 mg, 0.8 mmol) and HOBt (5.4 mg, 0.04 mmol). The reaction mixture was stirred overnight at ambient temperature. The reaction mixture was treated with saturated $NaHCO_3$ and extracted with dichloromethane. Combined organic layers were dried (Na_2SO_4), filtered then evaporated to obtain crude product. Purification of the crude product by chromatography on silica gel with CMA 80: chloroform (1:1) as the eluent provided 150 mg

(74%) of the title compound as a white solid. ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 7.09 – 7.31 (m, 2H), 6.68 – 6.97 (m, 4H), 6.37 – 6.65 (m, 2H), 5.66 – 5.95 (m, 1H), 4.55 – 4.94 (m, 1H), 4.25 – 4.53 (m, 2H), 3.76 (br. s., 1H), 3.15 (dd, *J* = 2.92, 15.35 Hz, 1H), 2.88 (dd, *J* = 6.03, 15.26 Hz, 1H), 2.66 (br. s., 2H), 2.22 – 2.43 (m, 1H), 1.90 – 2.21 (m, 2H), 1.69 – 1.90 (m, 2H), 1.49 – 1.70 (m, 3H), 1.26 – 1.49 (m, 9H), 0.76 (dd, *J* = 6.88, 17.61 Hz, 6H); ^{13}C NMR (75 MHz, CHLOROFORM-d) δ 171.4, 164.6, 161.3, 155.6, 149.0, 148.9, 129.8, 129.7, 129.3, 124.4, 122.5, 122.5, 114.9, 113.8, 113.5, 113.0, 112.7, 59.7, 54.5, 51.3, 44.8, 42.0, 33.3, 33.0, 30.3, 28.4, 19.1, 17.3.

[^{35}S]GTPγS Assay

The [^{35}S]GTPγS assays were conducted using the methods previously reported.[3, 4]

Calculated Pharmacokinetic 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 (equation 3).[13]

MDCK-mdr1 permeability assays

MDCK-mdr1 cells obtained from the Netherlands Cancer Institute were grown on Transwell type filters (Corning) for 4 days to confluence in DMEM/F12 media containing 10% fetal bovine serum and antibiotics as has been described previously.[14] Compounds were added to the apical side at a concentration of 10 μM in a transport buffer comprising of 1X Hank's balanced salt solution, 25 mM D-glucose and buffered with HEPES to pH 7.4. Samples were incubated for 1 h at 37 °C and carefully collected from both the apical and basal side of the filters. Compounds selected for MDCK-mdr1 cell assays were infused on an Applied Biosystems API-4000 mass spectrometer to optimize for analysis using multiple reaction monitoring (MRM). Flow injection analysis was also conducted to optimize for mass spectrometer parameters. Samples from the apical and basolateral side of the MDCK cell assay were dried under nitrogen on a Turbovap LV. The chromatography was conducted with an Agilent 1100 binary pump with a flow rate of 0.5 mL/min. Mobile phase solvents were A, 0.1% formic acid in water, and B, 0.1% formic acid in methanol. The initial solvent conditions were 10% B for 1 minute, then a gradient was used by increasing to 95% B over 5 minutes, then returning to initial conditions. Data reported are average values from 2–3 measurements.

In vitro stability testing

Stability of compounds to plasma and S9 fraction was performed as previously described.[15] *In vitro* testing for metabolic stability was conducted in mixed gender pooled hepatic S9 fraction supplied by Xenotech, LLC, Lenexa, KS. Identity of the donors was unknown.

For the hepatic S9 metabolism studies, all samples were tested at 10μM final concentration in a 1 mL volume containing 1 mg/mL S9. Samples were incubated in a buffer containing 50 mM potassium phosphate, pH 7.4 with 3 mM $MgCl_2$ and a NADPH regeneration system comprising of NADP (1 mM), glucose-6-phosphate (5 mM) and glucose-6-phosphate

dehydrogenase (1 unit/mL). Triplicate samples were incubated for 60 min. Reactions were terminated by addition of 3 volumes of acetonitrile and processed as described for the MDCK-mdr1 assays, but standard curves were prepared in blank matrix for each compound for quantitative assessment. Data reported are average values from 3 measurements.

PAMPA

PAMPA was conducted using a 96-well plate based kit from BD Biosciences (RTP, NC) and manufacturer's instructions were followed closely. Briefly, all samples were tested at 10μM final concentration by adding them to the apical (top) compartment in PBS at pH 5.5 or 7.4. Samples were incubated at room temperature for 4 hours. Apical to basal transport was evaluated by sampling from the top and bottom wells of the plate and measuring concentrations of compounds in each compartment using LC-MS. Processing and methods for analytical evaluation were similar to those described for MDCK-mdr1 transport assays. Data reported are average values from 3 measurements.

hERG Assay

Preparations of membranes overexpressing human hERG were purchased from Perkin Elmer. The binding assays were performed for 60 min using 4 μg hERG expressing membranes, ~3 nM [^{3}H]Astemizole, and various concentrations of the test agent in a binding buffer (10 mM HEPES, pH 7.4, 130 mM NaCl, 5 mM KCl, 0.8 mM $MgCl_2$, 1 mM NaEDTA, 10 mM glucose, 0.1% BSA). Binding was terminated by rapid filtration onto GF/B fiber filtermats, presoaked in 0.3% polyethyleneimine, followed by rapid washing 6 times (2 mL) with ice-cold solution containing 25 mM Tris-HCl, pH 7.4, 130 mM NaCl, 5 mM KCl, 0.8 mM $MgCl_2$, 0.05 mM $CaCl_2$, and 0.1% BSA using a Brandel harvester. Filters were dried and counted after addition of a scintillant. Data were analyzed using non-linear regression (GraphPad Prism) and K_i values were determined as described before.[16] All experiments were performed at least twice in duplicate and data reported are mean values.

Solubility Determination

For these experiments, 10 mM DMSO stocks of compounds were directly diluted into 10 mM phosphate buffer at pH 7.4 or 3 and shaken for 90 min at room temperature. The final concentration of DMSO was 1%. After the incubation, samples were filtered through a 0.4 micron filterplate (Millipore). Filtrates were carefully collected. Analysis of compounds was performed by LC/MS using previously available methods and concentrations determined. Data are reported as mean values from three determinations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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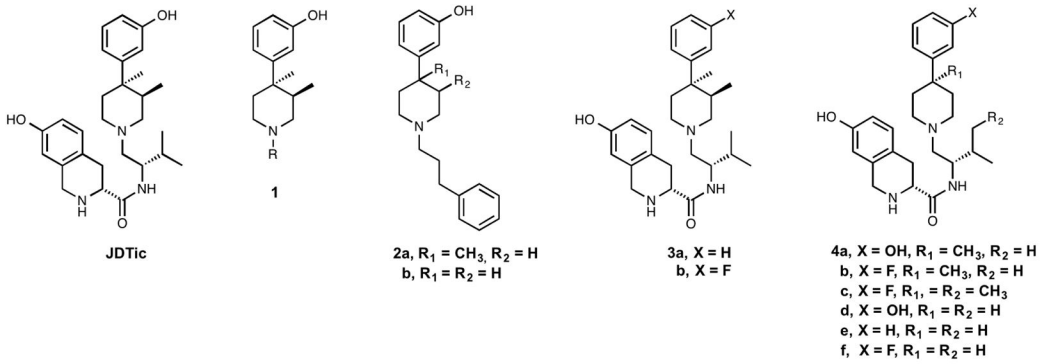
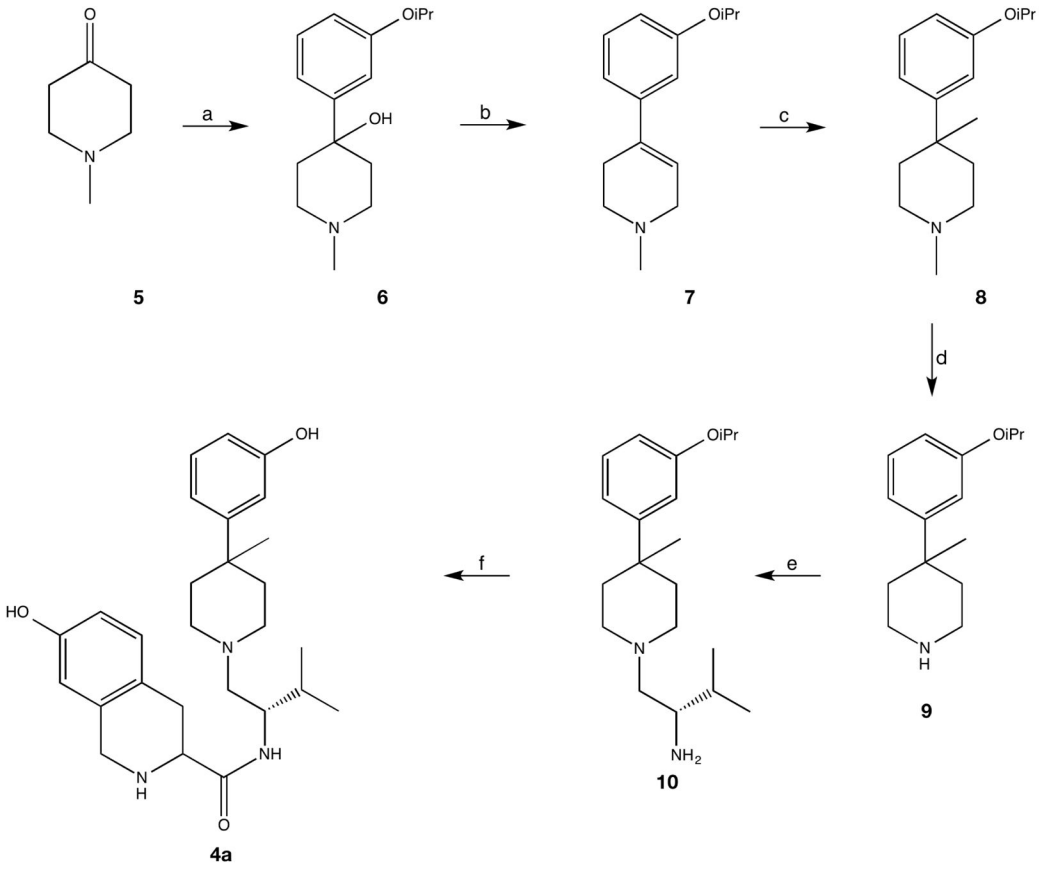
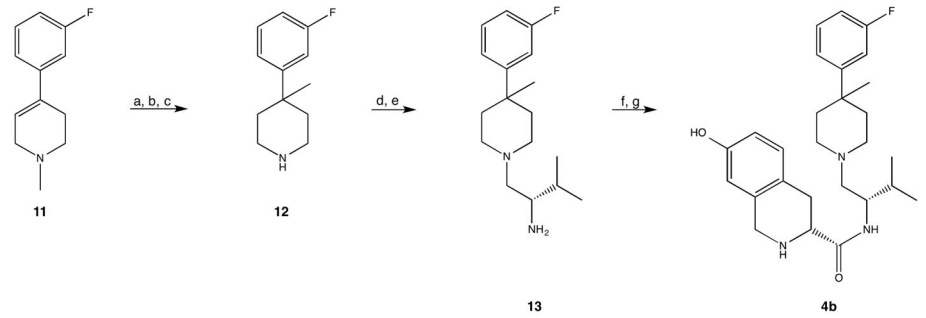


Figure 1.
Structures of JDTic, **2a–b, 3a–b,** and **4a–f**.



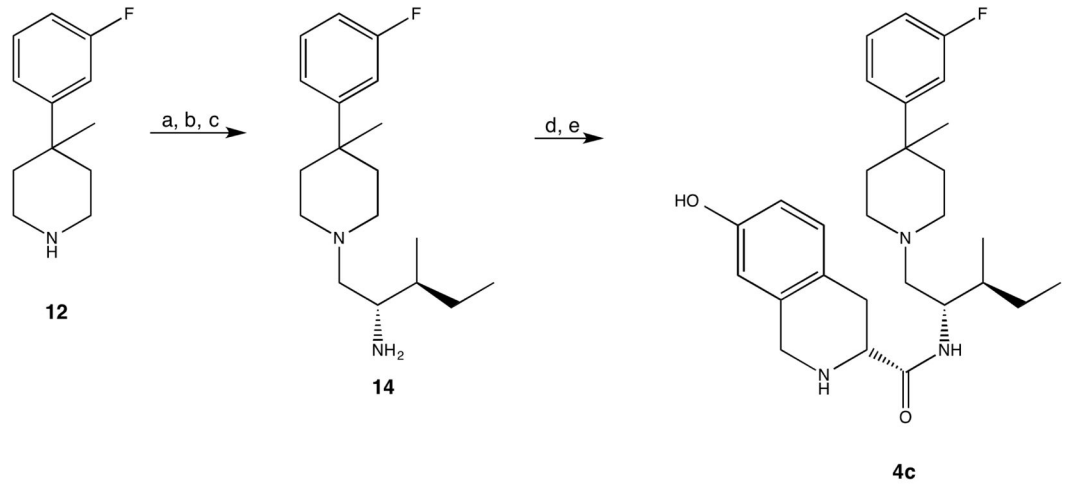
Scheme 1.

Reagents and Conditions: a) 3-isopropoxyphenyl lithium, THF; b) TsOH, toluene; c) 1. *n*-BuLi, THF, −15 °C; 2. Me_2SO_4, −50 °C; 3. $NaBH_4$, CH_3OH; d) 1. 1-chloroethyl chloroformate, DCE; 2) CH_3OH, reflux; e) 1. Boc-L-valinal, $NaBH_3CN$; 2. TFA, DCM; f) 1. Boc-7-hydroxy-D-Tic-OH, HBTU, NEt_3; 2. HBr, AcOH.



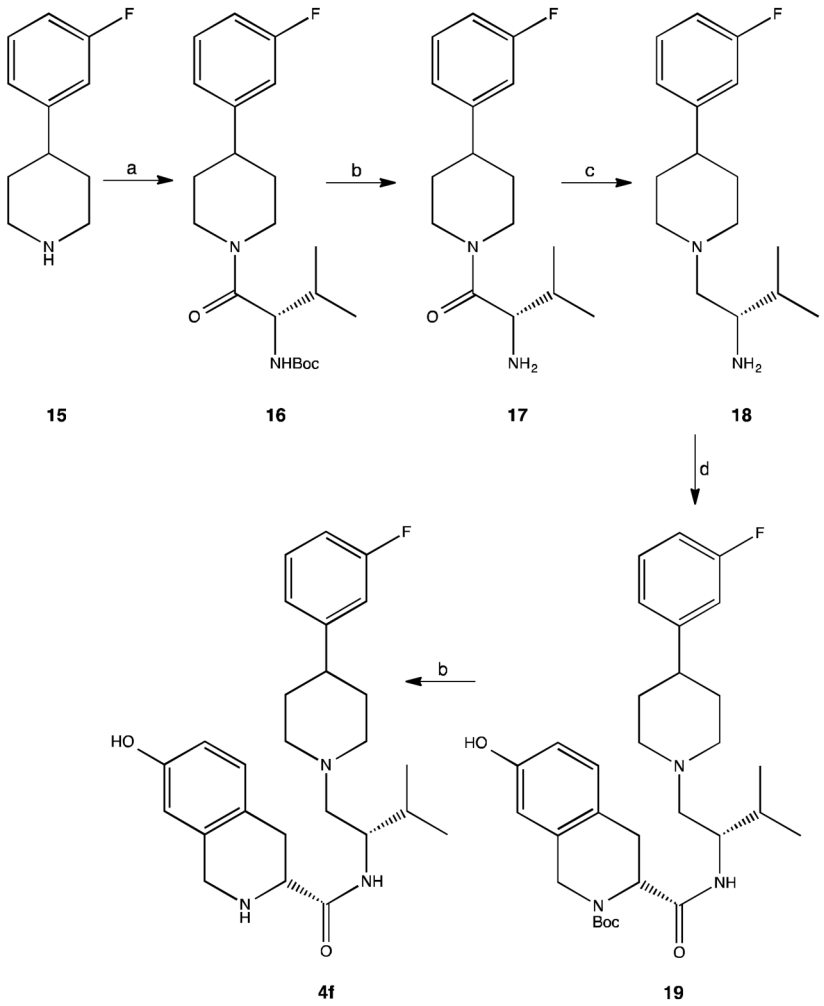
Scheme 2.

Reagents and conditions: a) 1. *n*-BuLi, THF, −78 °C; 2. Me_2SO_4; b) $NaBH_4$, CH_3OH; c) 1. ACE-Cl, DCE; 2. CH_3OH, reflux; d) HBTU, NEt_3, Boc-L-valine, CH_3CN; e) 1. HCl; 2. BH_3 • SMe_2; f) Boc-7-hydroxy-D-Tic-OH, EDC • HCl, HOBt, DIPEA, THF; g) HCl, CH_3CN, dioxane.



Scheme 3.

Reagents and conditions: a) Boc-L-isoleucine, HOBt, EDC, DIPEA, CH_3CN; b) HCl, CH_3OH; c) $BH_3 \cdot S(CH_3)_2$; d) Boc-7-hydroxy-D-Tic-OH, EDC • HCl, DIPEA, HOBt, THF; e) HCl, CH_3CN, dioxane.



Scheme 4.

Reagents: a) N-Boc-L-valine, HBTU, CH_3CN, TEA; b) HCl, MeOH; c) B_2H_6, THF; d) EDC, HOBt, TEA, Boc-7-hydroxy-D-Tic-OH, DCM.

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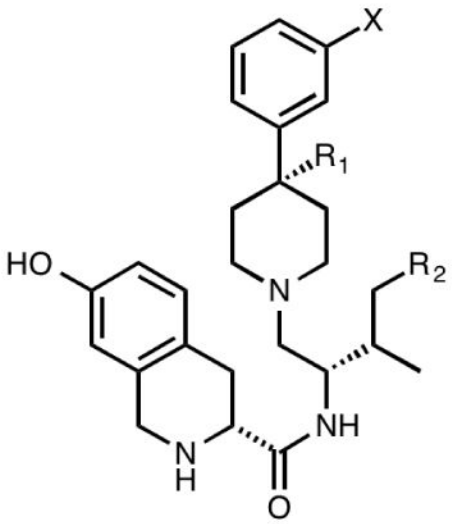
Table 1

Inhibition of Agonist-Stimulated [35S]GTPγS Binding by Compounds in Cloned Human μ, δ, and κ Opioid Receptors



Compd				K_e (nM)[a]				
	X	R_1	R_2	μ, DAMGO	δ, DPDPE	κ, U69,593	μ/κ	δ/κ
nor-BNI	—	—	—	26	29	0.05	520	580
JDTic	—	—	—	25 ± 4	74 ± 2	0.02	1255	3800
3a	—	—	—	8.9	442	0.024	370	18,400
3b	—	—	—	14.8	249	0.01	1480	24,900
4a	OH	CH_3	H	67.5 ± 10.4	927 ± 350	0.69 ± 0.21	98	1344
4b	F	CH_3	H	38.0 ± 6.4	1480 ± 350	0.18 ± 0.04	211	8211
4c	F	CH_3	CH_3	11.3 ± 4.3	903 ± 200	0.033 ± 0.01	342	27,363
4d	OH	H	H	12.3 ± 1.0	240 ± 73	0.10 ± 0.03	123	2400
4e	H	H	H	3.96 ± 1.1	281 ± 44	0.051 ± 0.01	77	5500
4f	F	H	H	3.55 ± 1.1	128 ± 36	0.023 ± 0.01	154	5600

[a] K_e values are the mean ±SEM of at least three independent experiments performed in duplicate.

Table 2

In vitro ADME data for **3a, 3b,** and **4 a–f**

Compd	hERG (K_i, μM)	MDCK-mdr1 (% Transported, A to B)	Solubility (μM)		Plasma Stability (% of Parent)	S9 Stability (% of Parent)	PAMPA (% Transported)		TPSA	cLogP	LogBB
			pH 7.4	pH 3			pH 7.4	pH 5.5			
JDTic	8.820	11	11	34	97	76	26.8	57.9	84.83	3.60	−0.57
3a	7.048	27	11	42	67.0	82.0	91.4	67.3	64.60	3.89	−0.23
3b	6.251	6	10	47	49.6	77.5	19.6	3	64.60	4.15	−0.19
4a	>10	<1	44	101	62.5	62.5	1	2.6	84.83	3.43	−0.59
4b	4.237	1.3							64.60	3.97	−0.21
4c	1.666								64.60	4.38	−0.15
4d	1.732								84.83	3.23	−0.63
4e	0.777								64.6	3.53	−0.28
4f	0.436								64.60	3.75	−0.25