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Bioorganic & Medicinal Chemistry 16 (2008) 822–829
Bioorganic & Medicinal Chemistry

A new synthesis of the ORL-1 antagonist 1-[(3*R*,4*R*)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidinyl]-3-ethyl-1,3-dihydro-2*H*-benzimidazol-2-one (J-113397) and activity in a calcium mobilization assay

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Received 19 July 2007; revised 21 September 2007; accepted 10 October 2007
Available online 13 October 2007

Abstract—A new chiral synthesis of the ORL-1 antagonist 1-[(3*R*,4*R*)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidinyl]-3-ethyl-1,3-dihydro-2*H*-benzimidazol-2-one (**2**, J-113397) was developed. J-113397 has a K_e = 0.85 nM in an ORL-1 calcium mobilization assay and is 89-, 887-, and 227-fold selective for the ORL-1 receptor relative to the μ, δ, and κ opioid receptors.

1. Introduction

The opioid-like receptor (ORL-1), also known as OP4 and recently named NOP (the fourth member of the opioid peptide receptor family) or nociceptin/orphanin FQ receptor, was identified in 1994 as an orphan opioid receptor.[1,2] Even though this G-protein coupled receptor displays a significant homology with the classical μ-, κ-, δ-opioid receptors (NOP, KOP, and DOP, respectively), it does not bind classical opioid ligands with high affinity. A 17-amino acid peptide was identified in 1995 as an endogenous ligand for ORL-1 and was named nociceptin (NC) or orphanin FQ (OFQ) (**1**).[3] It was found to be specific to ORL-1,[4] as it did not bind other opioid receptors. Nociceptin and ORL-1 receptors are widely distributed in the central nervous system and appear to play an important role in anxiety,[5] pain,[6] cognition,[7–10] and locomotion.[11] Importantly, antagonists for this system may be useful in pain management without having the undesirable side effects of opioids such as abuse, dependence, and withdrawal.[12]

The first selective small organic molecule antagonist, 1-[(3*R*,4*R*)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidinyl]-3-ethyl-1,3-dihydro-2*H*-benzimidazol-2-one (J-113397, **2**, Chart 1), was reported by Ozaki et al. in 1998.[13–15] The lead compound 1-(1-benzyl-4-piperidinyl)-1,3-dihydro-2*H*-benzimidazol-2-one (**3**, Chart 1) was identified from a chemical library and showed high affinity for ORL-1 but poor selectivity relative to μ- and κ-receptors.

In this study, we report a new synthesis of J-113397 and present its potency at the ORL-1, μ, δ, and κ opioid receptors using in vitro efficacy assays.

2. Chemistry

The synthesis used for **1** is shown in Scheme 1. This synthesis provides the desired optical isomer **2** without the use of a chiral column needed in the originally reported syntheses.[13–17]

Several groups have reported the reduction of piperidine β-ketoester **5** generated from **4** with Baker's yeast to be both diastereoselective and enantioselective.[17–19] We were pleased to find that only the cis diastereomer **6**

Keywords: J-113397; ORL-1 antagonist; Chiral synthesis; NOP.
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doi:10.1016/j.bmc.2007.10.023

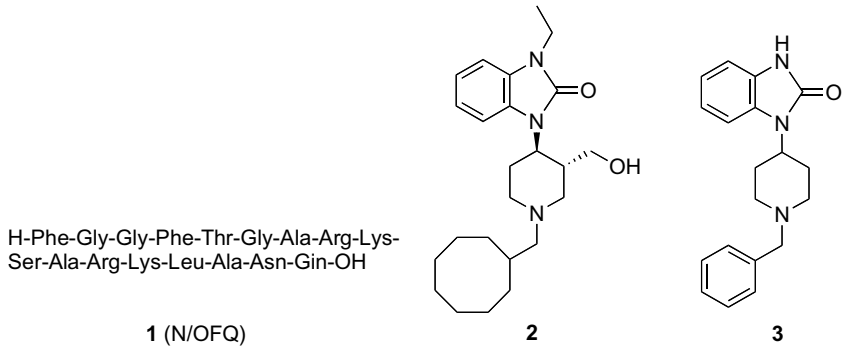
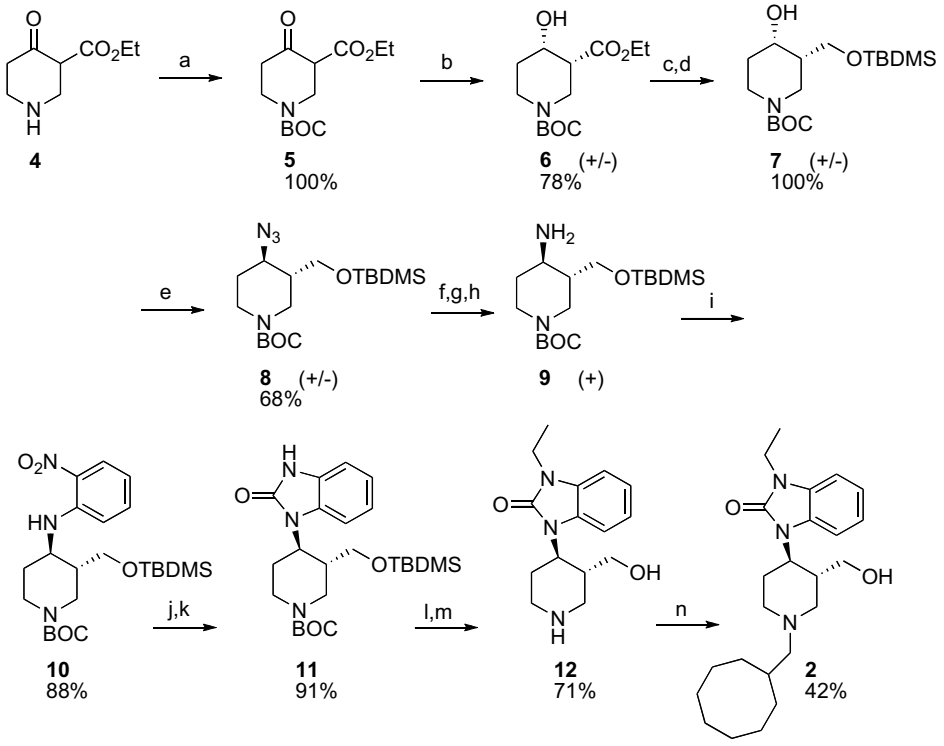


Chart 1.



Scheme 1. Reagents: (a) $(BOC)_2O$, Et_3N, CH_2Cl_2; (b) Baker's yeast, sucrose, tap water; (c) $LiBH_4$,THF; (d) TBDMSCI, DMAP Et_3N, CH_2Cl_2; (e) ZnN_6*2 Py, PPh_3, DIAD, toluene; (f) H_2, Pd/C, MeOH; (g) (*R*)-3-bromo-8-camphorsulfonic acid, ammonium salt; (h) $NaHCO_3$, $CHCl_3$; (i) 3-fluoronitrobenzene, *n*-BuOH, Na_2CO_3; (j) Raney Ni, hydrazine hydrate, EtOH; (k) *N*,*N*′-disuccimidyl carbonate, DMF; (l) NaH, Etl, DMF; (m) HCl, MeOH; (n) $NaBH(OAc)_3$, THF, cyclooctanecarboxaldehyde.

was isolated in good yield (78%), however, in our hands the enantioselectivity was quite low (ee around 30%) despite numerous changes to conditions. Other groups have reported this problem.[20] with the activity of the yeast at the heart of the problem. The optical rotation of the product **6** varies from batch to batch of yeast (from +5° to +15°). To overcome this problem, the single diastereomer was reduced with lithium borohydride and protected as the *tert*-butyldimethylsilyl ether **7**. The secondary alcohol was converted to the azide **8** using the Mitsunobu conditions with zinc azide/bis pyridine complex,[21] resulting in complete inversion of configuration at C4 of the piperidine ring. The primary amine, (±)-**9**, obtained by hydrogenation of the azide, was resolved to give (+)- and (−)-**9** using (+)- and (−)-3-bromo-8-camphorsulfonic acid ammonium salt. A single-crystal X-ray analysis of the (−)-3-bromo-8-camphorsulfonic acid salt showed that (−)-**9** possessed the (3*S*,4*S*)-stereochemistry and thus (+)-**9** was the (3*R*,4*R*)-isomer (Fig. 1). Thus, (+)-**9** was used to complete the synthesis of **2**. From this versatile intermediate, the synthesis of **2** proceeded by coupling the chiral 4-amino-piperidine (+)-**9** with 1-fluoro-2-nitrobenzene to give **10** in high yield (88%). Reduction of the nitro-group using Raney Nickel and hydrazine hydrate in ethanol followed by treatment with *N*,*N*′-disuccinimidyl carbonate afforded benzimidazolone **11** in 91% yield. Chiral-HPLC analysis of **11** showed a 99% ee. Alkylation of the benzimidazolone nitrogen with sodium hydride and iodoethane in DMF, followed by removal of the *tert*-butyloxycarbonyl and *tert*-butyldimethylsilanyl protecting groups with hydrogen chloride in methanol, afforded the deprotected piper-

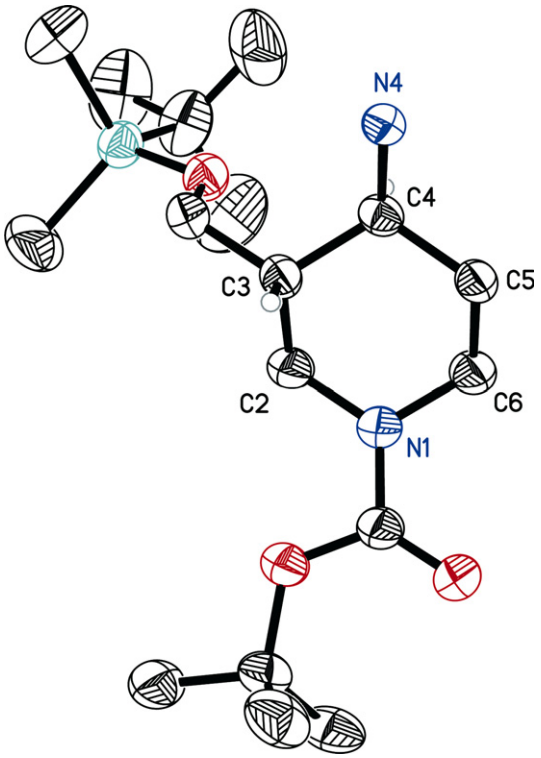


Figure 1. The conformation of (−)-**9** as determined by single-crystal X-ray diffraction. The 3S4S conformation was determined with respect to a reference molecule (3-bromocamphorsulfonic acid) which is not shown in the figure. All hydrogen atoms (except those on C3 and C4) and one of the positions for the disordered *tert*-butyl group off N1 have been omitted for clarity. Displacement ellipsoids are shown at the 30% level.

idine intermediate **12**. Reductive alkylation of **12** with cyclooctanecarboxaldehyde using sodium triacetoxyborohydride afforded **2** (J-113397) with an optical rotation almost identical to that previously described {observed $[\alpha]_D^{20}$ +6.25 (*c* 0.272, 0.1 M HCl), lit. $[\alpha]_D^{20}$ +6.4 (*c* 1, 0.1 M HCl)}.

3. Biology

3.1. Creation of an ORL-1 stable cell line

Human ORL-1 cDNA in the mammalian expression vector pcDNA3.1+ was purchased from the University of Missouri-Rolla cDNA Resource Center. CHO cells stably expressing the promiscuous G_q protein $G_{\alpha 16}$ (Molecular Devices, Sunnyvale, CA) were transfected with the ORL-1 vector using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). This was done in order to create a cell line where ORL-1 is also coupled to activation of phospholipase C and the mobilization of internal calcium in addition to G_i. The transfected cells were selected in 400 μg/mL geneticin. The clone used for this experiment was selected after screening the surviving cells for functional ORL-1 receptor expression using nociceptin and the Calcium 3 assay kit (Molecular Devices). These assays were run according to manufacturer's specifications using a FlexStationII384 plate reader (Molecular Devices) pre-warmed to 37 °C.

3.2. Functional assays and data analysis

Compound **2** was evaluated at 10 μM for intrinsic activity and for its ability to inhibit the mobilization of internal calcium and subsequent fluorescence elicited by nociceptin (EC_{80}; 1.5 nM Table 1). All test conditions were run in duplicate. For these assays, 96-well cell culture plates were seeded with 10,000 cells the day before the assay. On the day of assay, the cells were incubated at 37 °C with the Calcium 3 fluorescent dye (1/3 the standard concentration) for 1 h. For the antagonist assays, the test compound was added fifteen minutes before the end of the incubation period. The plate was then placed into the FlexStation (37 °C) and basal fluorescence recorded for 13 s followed by the addition of test compound or nociceptin (EC_{80}), and fluorescence recorded for an additional 47 s. The effect of **2** on the mobilization of internal calcium was determined using the MAX–MIN option in the recording software. Because **2** did not possess detectable intrinsic activity and it inhibited nociception-stimulated mobilization of internal calcium, it had its apparent affinity (K_e) determined. For these experiments, we assessed the ability of a single concentration of **2** to shift the nociceptin concentration response curve to the right using the same assay conditions described above. The EC_{50} values for nociceptin (A) and nociceptin + **2** (A′) were used to calculate the test compound K_e using the formula: $K_e = [L]/(DR - 1)$, where [L] equals the concentration of **2** in the assay and DR equals the dose ratio or A′/A. The concentration of test compound was selected to produce at least a twofold increase in the nociceptin EC_{50}, and eight different concentrations of nociceptin were used such that clear upper and lower asymptotes were obtained for both curves. Two different concentrations of **2** were used to determine its K_e. Compound **2** was then evaluated for its activity (agonist and antagonist) and selectivity for the human κ, μ, and δ opioid receptors using the [^{35}S]GTPγS functional binding assay and membrane homogenates prepared from CHO cells stably expressing one of these receptors. The [^{35}S]GTPγS binding assays were conducted as previously described.[22] A three-parameter logistic equation (shared bottom and top) was fitted to the concentration response data with Prism (v4 for Macintosh, GraphPad Software; San Diego, CA)

Table 1. Apparent affinities (K_e) for J-113397 (**2**) at cloned human ORL-1, μ, δ, and κ receptors[a]

Compound	K_e (nM)						
	ORL-1	μ	δ	κ	μ/ORL-1	δ/ORL-1	κ/ORL-1
2	0.85 ± .09	75.6 ± 15	754 ± 193	195 ± 34	89	887	227

[a] The apparent affinities (K_e) for **2** represent the mean ± SE from at least three independent experiments.

to calculate the EC_{50} values. These values are reported as means ± SE from at least three independent experiments.

4. Results and discussion

The original synthesis[13] of **2** as well as an improved synthesis described by Kawamoto et al.[17] and De Risi et al.[16] committed to a choice of N-substituent in the first step and involved the use of a chiral column. In a more recent report, Sulima et al. reported a new approach for the synthesis of J-113397 that did not require a chiral column.[23] In this study, we developed an alternate synthetic approach to **2** where the N-substituent is added in the last step (Scheme 1). Thus, in contrast to the Kawamoto type synthesis.[17] the present route could be used to prepare **2** as well as N-substituent analogs of **2**. The present synthesis involves 14 steps starting with **4** (one, an optical resolution; another, the neutralization of the resolved salt to give the freebase) with eight isolated intermediates. Note, however, intermediate **5** is commercially available. We chose to synthesize **5** since we had a large amount of **4** in hand. The overall yield was 6%. The Kawamoto synthesis involves nine steps (one being a separation of diastereoisomers and one a resolution with a chiral column) with six isolated intermediates. The overall yield was 8.3%.

In agreement with the originally reported data,[13] we found that **2** is a potent antagonist at the ORL-1 receptor (K_e = 0.85 nM). We found that **2** was 89-, 887-, and 227-fold selective for the ORL-1 receptor relative to the μ, δ, and κ opioid receptors, respectively. While these selectivities are good, they are much less than the 956-, >4347-, and 609-fold selectivity for the ORL-1 receptor relative to the μ, δ, and κ opioid receptors originally reported using competition binding assays using opioid receptors heterologously expressed in CHO cells.[13] These differences in selectivity between the assays could be due to our use of functional assays that assess the ability of **2** to disrupt receptor activation by an agonist as opposed to interfering with the binding of a radioligand. Nevertheless, the rank order potency of **2** at the various receptors is similar in both assays.

In summary, a new synthesis of the ORL-antagonist **2**, which proceeds without the need of a chiral column, was developed. Coupling the human ORL-1 to the mobilization of internal calcium via $G_{\alpha 16}$ identified J-113397 as a potent ORL-1 antagonist, and more importantly, it provides a rapid method to evaluate compounds at this receptor.

5. Experimental

Melting points were determined on a Thomas–Hoover capillary tube apparatus. All optical rotations were determined at the sodium D line using a Rudolph Research Autopol III polarimeter (1 dm cell). NMR spectra were recorded on a 300 MHz (Bruker AVANCE 300) spectrometer using tetramethylsilane as internal standard. Chromatography was performed using a CombiFlash Companion and RediSep columns by Isco, using the solvent system specified in the section below. Elemental analyses were carried out by Atlantic Microlab, Inc. Baker's yeast was purchased from ICN biomedicals.

5.1. Synthesis of 1-*tert*-butyl-3-ethyl-4-oxopiperidine-1,3-dicarboxylate (5)

A solution of di-*tert*-butyl dicarbonate (35.5 g, 0.17 mol) in CH_2Cl_2 (70 mL, anhydrous) was added dropwise to a stirred solution of ethyl 4-oxopiperidine-3-carboxylate hydrochloride (**4**, 30.4 g, 0.146 mol) and triethylamine (29 mL, 208 mmol) in CH_2Cl_2 (70 mL, anhydrous). After being stirred at rt for 16 h, the reaction mixture was washed with HCl (1 M, 2× 250 mL) and brine (2× 150 mL). The organic layer was dried over Na_2SO_4 and evaporated to dryness under reduced pressure to afford 40 g (100%) of **5** as a white solid. The material was used without further purification. ^{1}H NMR ($CDCl_3$) δ 12.06 (s, 1H), 4.25 (q, 2H, J = 6.9 Hz), 4.08 (br s, 2H), 3.58 (t, 2H, J = 5.85 Hz), 2.39 (t, 2H, J = 5.85 Hz), 1.49 (s, 9H), 1.32 (t, 3H, J = 6.9 Hz); ^{13}C NMR ($CDCl_3$) δ 170.8, 168.05, 154.6, 96.29, 80.1, 60.6, 40.7, 40.3, 28.7, 28.4, 14.2; MS (APCI) m/z 172.1 $(M+H-BOC)^+$.

5.2. 1-*tert*-Butyl-3-ethyl (3,4-*cis*)-4-hydroxypiperidine-1,3-dicarboxylate (6)

Baker's yeast (210 g, dry active), sucrose (210 g, pure cane sugar), and tap water (2100 mL) were mixed in a vat (~20 L). The mixture was stirred for 6 h at rt, after which no rising in the yeast was observed. To the yeast mixture was added 1-*tert*-butyl-3-ethyl-4-oxopiperidine-1,3-dicarboxylate (**5**) (26.1 g, 0.096 mol) in EtOH (100 mL). After being stirred at rt for 65 h, the reaction mixture was transferred to six 250 mL-centrifuge bottles and centrifuged at 5000 rpm for 20 min at 8 °C. The supernatant in each bottle was decanted and the solids were mixed with water (180 mL per bottle). The mixture was centrifuged again at 5000 rpm for 20 min at 5 °C. The supernatants were pooled and the washing process of the solids was repeated 2 more times. All the supernatants were combined and extracted with CH_2Cl_2 (5× 800 mL). The organic phases with a small amount of emulsion were combined and washed with brine (1 L), dried over Na_2SO_4, and filtered through a membrane filter with Celite on top. The solvent was evaporated and the residue was dried in vacuo to give 18 g (69%) of **6** as a white solid. $[\alpha]_D^{20}$ +10.5 (c 1.34, CH_2Cl_2).

The remaining solids were mixed with MeOH (150 mL per bottle). The mixture was filtered through a Celite pad. The filter cake was washed with MeOH (2× 20 mL). All the filtrates were combined, concentrated to about 250 mL, and extracted with $CHCl_3$ (4× 50 mL). The extracts were combined, washed with brine (3× 50 mL), and dried over Na_2SO_4. Evaporation of the solvent under reduced pressure afforded an additional 2.11 g (8%) of **6** as an orange oil (total yield: 77%). ^{1}H NMR ($CDCl_3$) δ 4.29 (br s, 1H), 4.19 (q, 2H, J = 7.2 Hz), 4.17 (br s, 1H), 3.73 (dt, 1H, J = 3.6 and 13.2 Hz), 3.40 (br s, 1H), 3.34–3.25 (m, 1H), 2.66–2.61

(m, 1H), 1.88–1.83 (m, 1H), 1.69–1.66 (br s, 2H), 1.48 (s, 9H), 1.30 (t, 3H, $J = 7.2$ Hz); ^{13}C NMR ($CDCl_3$) δ 173.4, 155.1, 104.5, 80.2, 65.4, 61.4, 46.3, 41.0, 39.1, 31.8, 29.0, 28.8, 14.5; MS (ESI) m/z 274.6 $(M+H)^+$.

5.3. 3-(*tert*-Butyldimethylsilanyloxymethyl)-piperidine-1-carboxylic acid *tert*-butyl ester (7)

To a solution of 1-*tert*-butyl-3-ethyl (3,4-*cis*)-4-hydroxypiperidine-1,3-dicarboxylate (**6**) (8.78 g, 0.032 mol) in anhydrous THF (1000 mL) was added dropwise a solution of $LiBH_4$ in THF (16.1 mL, 32.2 mmol). The reaction mixture was then heated at reflux for 10 min and allowed to cool to rt over 20 min. The mixture was cooled to 0 °C and an aqueous saturated solution of $KHSO_4$ (1000 mL) was slowly added. The two layers were separated and the aqueous phase was extracted with EtOAc (2× 500 mL). The combined organic layers (including the THF layer from the first separation) were washed with brine (600 mL), dried over $MgSO_4$, and evaporated to dryness to give 7.43 g (99.5%) of (3,4-*cis*)-4-hydroxy-3-hydroxymethyl-piperidine-1-carboxylic acid *tert*-butyl ester as a clear oil. ^{1}H NMR ($CDCl_3$) δ 4.23–4.19 (m, 1H), 4.21 (t, 2H, $J = 3$ Hz), 3.56 (dd, 2H, $J = 3.9$ and 13.2 Hz), 3.43–3.36 (m, 2H), 3.29 (br s, 1H), 1.87–1.68 (m, 4H), 1.47 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 211.2, 80.1, 69.9, 63.4, 54.2, 42.0, 32.5, 32.1, 29.6, 28.8, 25.2; MS (APCI) m/z 132.2 $(M+H-BOC)^+$.

To a solution of (3,4-*cis*)-4-hydroxy-3-hydroxymethyl-piperidine-1-carboxylic acid *tert*-butyl ester (7.39 g, 0.032 mol) in anhydrous CH_2Cl_2 (65 mL) were added 4-dimethylaminopyridine (0.43 g, 0.035 mol), triethylamine (5.8 mL, 41.54 mmol), and finally *tert*-butyldimethylsilyl chloride (5.8 g, 0.039 mol). The mixture was stirred at rt for 16 h, during that time the reaction turned cloudy with some solid at the bottom and on the walls of the flask. The reaction mixture was diluted with 500 mL of CH_2Cl_2, washed with water (2× 50 mL) and brine (50 mL). The organic layer was dried over Na_2SO_4, evaporated to dryness, and dried under vacuum to give 11 g (99.7%) of **7** as a clear oil. ^{1}H NMR ($CDCl_3$) δ 4.15 (br s, 1H), 3.73 (br s, 1H), 3.51 (dd, 2H, $J = 3.9$ and 13.2 Hz), 3.38–3.29 (m, 3H), 1.82–1.64 (m, 3H), 1.43 (s, 9H), 0.89 (s, 9H), 0.06 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 148.9, 107.0, 80.2, 68.4, 42.0, 39.4, 32.4, 28.8, 26.1, 18.4, −3.2; MS (APCI) m/z 246.5 $(M+H-BOC)^+$.

5.4. 4-Azido-3-(*tert*-butyldimethylsilanyloxymethyl)-piperidine-1-carboxylic acid *tert*-butyl ester (8)

To a solution of 3-(*tert*-butyldimethylsilanyloxymethyl)-piperidine-1-carboxylic acid *tert*-butyl ester (**7**) (1.23 g, 0.0036 mol) and triphenylphosphine (1.88 g, 0.007 mol) in anhydrous toluene (18 mL) was added zinc azide bis pyridine complex (830 mg, 2.68 mmol). The mixture was stirred at rt for 5 min and then cooled to 0 °C. Diisopropylazodicarboxylate (1.38 mL, 7.14 mmol) was then slowly added to the mixture. The ice bath was removed and the reaction mixture was stirred at rt for 4 h. The reaction mixture was then filtered through a pad of Celite (3 cm). The filter cake was washed with 20% EtOAc–hexanes (2× 25 mL). All the filtrates were combined and concentrated to 3.4 g. The crude product was then purified by using a RediSep column (SiO_2, 40 g) eluted with 0–10% B (0–5 min) (A = hexanes, B = EtOAc), 10% B (5–13 min), 10–20% B (13–17 min), 20% B (17–20 min), and 20–40% B (20–25 min), to give 0.882 g (68%) of **8** as a clear oil. ^{1}H NMR ($CDCl_3$) δ 4.07 (dd, 2H, $J = 3.2$ and 13.1 Hz), 3.67 (br s, 2H), 3.37 (br s, 2H), 2.81–2.73 (m, 2H), 2.03–1.98 (m, 1H), 1.60–1.55 (m, 3H), 1.46 (s, 9H), 0.90 (s, 9H), 0.07 (s, 3H), 0.06 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 155.1, 132.6, 132.5, 129.0, 128.8, 80.2, 62.1, 59.6, 43.7, 42.5, 30.5, 28.9, 28.8, 26.4, 26.3, 22.1, −5.2.

5.5. 4-Amino-3-(*tert*-butyldimethylsilanyloxymethyl)-piperidine-1-carboxylic acid *tert*-butyl ester (9)

Anhydrous methanol (100 mL) was added to a mixture of 4-azido-3-(*tert*-butyldimethylsilanyloxymethyl)-piperidine-1-carboxylic acid *tert*-butyl ester (**8**) (5.74 g, 0.015 mol) and palladium on active carbon (10% wt, 0.6 g). The reaction flask was connected to a hydrogenation system and the mixture was degassed and then stirred under H_2 for 16 h. The reaction mixture was then filtered through a pad of Celite and the filter cake was washed with methanol (2× 50 mL). The filtrates were combined and then evaporated to dryness to give 5.24 g of product. Purification of the product by flash chromatography (SiO_2, 40% EtOAc/hexanes) $CHCl_3$–CH_3OH–NH_4OH (80/18/2) then afforded 3.56 g (67%) of **9** as a clear oil. ^{1}H NMR ($CDCl_3$) δ 4.07–4.01 (m, 2H), 3.66 (br s, 2H), 2.66 (t, 2H, $J = 12$ Hz), 2.52–2.44 (m, 1H), 1.76–1.70 (m, 1H), 1.40 (s, 9H), 1.35–1.22 (m, 4H), 0.84 (s, 9H), 0.06 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 155.2, 79.9, 63.4, 50.8, 46.8, 43.0, 35.6, 28.8, 28.5, 26.3, 18.6, −5.1; MS (APCI) m/z 345.5 $(M+H)^+$.

5.6. Resolution of racemic amine 9 using (+)-3-bromo-8-camphorsulfonic acid ammonium salt

4-Amino-3-(*tert*-butyldimethylsilanyloxymethyl)-piperidine-1-carboxylic acid *tert*-butyl ester (**9**) (805 mg, 2.34 mmol) was dissolved in methanol (8 mL). To the well-stirred solution was added [(1*R*)-(endo, anti)]-(+)-3-bromocamphor-8-sulfonic acid ammonium salt (390 mg, 1.17 mmol). The mixture was stirred until a clear mixture was observed (about 20 min). The solvent was slowly evaporated under a stream of N_2 (3.5 h). An additional volume of methanol (2 mL) was added and evaporated under N_2 to give a thick oil that was placed under vacuum yielding a white foam. That foam was dissolved in *tert*-butyl methyl ether (TBME) (2 mL). The solvent was slowly evaporated under a stream of N_2 (0.5 h). Toluene (6 mL) was added, followed by hexanes (2 mL), and the solution was left at rt for 16 h. The resulting crystals were washed with 50% toluene–hexanes (5 mL), then with hexanes (5 mL). The white crystals were dried under vacuum to give 357 mg of resolved complex. Part of the crystals (22 mg) was dissolved in $CHCl_3$ (1 mL), and $NaHCO_3$ (saturated, 1 mL) was added. The mixture was stirred at rt for 30 min. The phases were separated and the aqueous layer was extracted with $CHCl_3$ (2× 1 mL). The combined organic

layers were washed with brine (1 mL), dried over Na_2SO_4, and evaporated to dryness to give 14 mg of free amine. $[\alpha]_D^{20}$ +11.53 (*c* 0.23, CH_3OH).

The mother liquor was dissolved in $CHCl_3$ (10 mL) and treated with $NaHCO_3$ (saturated, 10 mL). The mixture was stirred at rt for 30 min. The phases were separated and the aqueous layer was extracted with $CHCl_3$ (2× 10 mL). The combined organic layer was washed with brine (10 mL), dried over Na_2SO_4, and evaporated to dryness to give 560 mg of free amine. $[\alpha]_D^{20}$ −5.5, (*c* 1.09, CH_3OH).

The rest of the resolved complex was dissolved in $CHCl_3$ (20 mL) and treated with $NaHCO_3$ (saturated, 20 mL). The mixture was stirred at rt for 30 min. The phases were separated and the aqueous layer was extracted with $CHCl_3$ (2× 10 mL). The combined organic layer was washed with brine (10 mL), dried over Na_2SO_4, and evaporated to dryness to give 0.177 g of resolved free amine.

5.7. Resolution of 9 using (−)-3-bromo-8-camphorsulfonic acid ammonium salt

From 343 mg of **9** (1 mmol) and [(1*S*)-endo, anti]-(−)-3-bromocamphor-8-sulfonic acid ammonium salt (333 mg, 1.0 mmol) in methanol as described above the respective (−)-sulfonic acid salt was prepared. The resulting glassy solid was dissolved in 2 mL of TBME at ambient temperature and allowed to crystallize undisturbed for 15 h. The solution was very slowly concentrated to half the volume over a 4-h period. The crystalline salt was carefully separated by filtration, washed with three 0.4 mL portions of TBME. The combined filtrate and washings were allowed to slowly evaporate to half the volume, which furnished additional crystalline salt. After filtration this salt was washed with toluene–30% TBME and combined with the first fraction to yield 217 mg of white crystalline salt. This material was dissolved in 1.5 mL of ethyl acetate, 3 volumes of toluene were added, and the solution was concentrated to an oil. A solution of the material in 2 mL of toluene was left to crystallize undisturbed for 30 h. White clusters of crystals were separated and washed twice with 0.5 mL toluene and air dried for 2 h. An ^1H NMR spectrum ($CDCl_3$) showed characteristic signals at d 7.89 (br s, 3H), 4.46 (d, 1H, *J* = 3Hz), 1.32 (s, 9H), 1.08 (s, 3H), 0.83 (s, 3H), 0.79 (s, 9H), and 0.14 (s, 6H). A sample of 20 mg was used for X-ray diffraction analysis.

5.8. 3-(*tert*-Butyldimethylsilanyloxymethyl)-4-(2-nitrophenylamino)-piperidine-1-carboxylic acid *tert*-butyl ester (10)

A mixture of resolved amine (+)-**9** (172 mg, 0.5 mmol), sodium carbonate (64 mg, 0.6 mmol), *n*-butanol (5 mL), and 1-fluoro-2-nitrobenzene (0.16 mL, 0.75 mmol) was refluxed for 4 h. The reaction mixture was cooled to rt, filtered through a pad of Celite. The flask was washed with ether (3× 10 mL) and the washing was filtered through the pad. The filtrates were combined, washed with water (2× 5 mL) and brine (5 mL), and dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography (SiO_2, 10% EtOAc/hexanes) to afford 192 mg (88%) of pure product **10**. ^1H NMR ($CDCl_3$) δ 8.18 (d, 1H, *J* = 8.67 Hz), 8.09 (d, 1H, *J* = 8.29 Hz), 7.40 (t, 1H, *J* = 7.54 Hz), 7.02 (br s, 1H), 6.64 (t, 1H, *J* = 7.8 Hz), 4.28–3.95 (m, 2H), 3.71–3.65 (m, 3H), 3.20–2.76 (m, 2H), 2.09 (br d, 1H, *J* = 12 Hz), 1.75–1.74 (m, 1H), 1.49 (s, 9H), 0.87 (s, 9H), 0.01 (s, 3H), −0.07 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 162.5, 154.9, 145.3, 136.4, 127.3, 115.6, 114.4, 80.1, 61.7, 50.1, 44.3, 28.6, 28.0, 18.7, −5.5; MS (APCI) *m*/*z* 366.9 $(M+H-BOC)^+$.

5.9. 3-(*tert*-Butyldimethylsilanyloxymethyl)-4-(2-oxo-2,3-dihydrobenzimidazol-1-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (11)

To a solution of pure nitro compound **10** (587 mg, 1.26 mmol) in EtOH (20 mL) were added Raney Nickel (2.2 mL) and hydrazine hydrate (0.74 mL, 15 mmol). The mixture was stirred at rt for 2 h and then filtered through a pad of Celite. The flask was washed with EtOH (50 mL) and the washings were filtered through the pad. The filtrates were combined and concentrated. The residue was dissolved in Et_2O (10 mL), washed with brine (10 mL), and dried over Na_2SO_4. The solvent was evaporated under reduced pressure and the residue was dried in vacuo to give 510 mg (91%) of 3-(*tert*-butyldimethylsilanyloxymethyl)-4-(2-aminophenylamino)-piperidine-1-carboxylic acid *tert*-butyl ester as orange foam. ^1H NMR ($CDCl_3$) δ 6.77–6.68 (m, 4H), 4.14 (dd, 1H, *J* = 4 and 13.5 Hz), 3.75–3.72 (m, 2H), 3.38 (br s, 5H), 2.92–2.79 (m, 2H), 2.11 (dd, 1H, *J* = 3.15 and 13 Hz), 1.68–1.65 (m, 1H), 1.47 (s, 9H), 0.90 (s, 9H), 0.04 (s, 3H), 0.02 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 155.0, 136.4, 126.0, 120.6, 119.3, 117.1, 79.7, 62.9, 51.5, 28.6, 26.1, 18.4, −5.3; MS (APCI) *m*/*z* 436.7 $(M+H)^+$.

3-(*tert*-Butyldimethylsilanyloxymethyl)-4-(2-aminophenylamino)-piperidine-1-carboxylic acid *tert*-butyl ester (510 mg, 1.17 mmol) and *N*,*N*′-disuccinimidyl carbonate (600 mg, 2.34 mmol) were dissolved in DMF (20 mL). The reaction mixture was stirred at rt for 2 days. It was then treated with brine (5 mL) and water (5 mL). The aqueous phase was extracted with Et_2O (4× 10 mL). The combined organic phase was washed with brine (2× 5 mL) and dried over Na_2SO_4. Evaporation of the solvent under reduced pressure afforded 600 mg (100%) of **11** as an orange oil. The chiral purity of benzimidazolone **11** was determined by chiral-HPLC: ChromTech Chiral-AGP column 4 × 150 mm, 5 μm, solvent A: [10 mM $K_2HPO_4 + KH_2PO_4$, pH 7.0], solvent B: CH_3OH, 5% B, flow rate: 1 mL/min, UV: 235 nm, retention time: 16.8 min. ^1H NMR ($CDCl_3$) δ 10.74 (s, 1H), 7.11–7.00 (m, 4H), 4.36–4.32 (m, 2H), 3.44–3.37 (m, 2H), 2.80–2.38 (m, 5H), 1.80–1.77 (m, 1H), 1.48 (s, 9H), 0.78 (s, 9H), −0.17 (s, 3H), −0.19 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 155.6, 154.9, 128.4, 121.4, 121.0, 109.9, 79.9, 61.8, 51.9, 36.6, 28.6, 25.9, 18.2, −5.6; MS (ESI) *m*/*z* 460.9 $(M-H)^-$.

5.10. 1-Ethyl-3-(3-hydroxymethyl-4-piperidinyl)-1,3-dihydrobenzimidazol-2-one (12)

To a solution of benzimidazolone **11** (600 mg, 1.17 mmol) in DMF (25 mL) cooled at 0 °C was added NaH (60% suspension in mineral oil, 170 mg, 4.2 mmol). The reaction mixture was slowly warmed to rt over 1 h. Iodoethane (0.59 mL, 7.3 mmol) was added and the reaction mixture was stirred at rt for 90 min. It was then cooled to 0 °C and treated with water (30 mL). The aqueous phase was extracted with EtOAc (4× 15 mL). The combined organic layer was washed with brine (15 mL) and dried over Na_2SO_4. Evaporation of the solvent under reduced pressure afforded 580 mg (100%) of 3-(*tert*-butyldimethylsilanyloxymethyl)-4-(3-ethyl-2-oxo-2,3-dihydrobenzimidazol-1-yl)-piperidine-1-carboxylic acid *tert*-butyl ester. ^{1}H NMR ($CDCl_3$) δ 7.13–6.99 (m, 4H), 4.39–4.27 (m, 3H), 3.93 (q, 2H, J = 7.3 Hz), 3.43–3.33 (m, 2H), 2.96–2.37 (m, 4H), 1.79 (d, 2H, J = 11.1 Hz), 1.50 (s, 9H), 1.33 (t, H, J = 7.1 Hz), 0.81 (s, 9H), −0.13 (s, 3H), −0.15 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 154.8, 153.5, 129.2, 121.0, 120.9, 109.1, 107.6, 79.8, 62.0, 52.3, 35.8, 29.8, 29.4, 28.9, 28.5, 25.9, 18.2, 13.6, −5.7; MS (ESI) *m*/*z* 490.8 $(M+H)^+$.

3-(*tert*-Butyldimethylsilanyloxymethyl)-4-(3-ethyl-2-oxo-2,3-dihydrobenzimidazol-1-yl)-piperidine-1-carboxylic acid *tert*-butyl ester (130 mg, 0.27 mmol) was dissolved in HCl/MeOH (10% vv, 4.5 mL). The solution was stirred at rt for 3 h, then concentrated to about 1 mL. The residue was mixed with aqueous HCl (0.5 M, 5 mL). The aqueous solution was washed with Et_2O (3× 3 mL), basified to pH 12 with NaOH (2 M), and extracted with $CHCl_3$ (4× 7 mL). The extracts were combined, washed with H_2O (2× 5 mL), dried over Na_2SO_4, evaporated in vacuo to give 56 mg (76%) of **12** as a clear oil. ^{1}H NMR ($CDCl_3$) δ 7.32 (d, 1H, J = 7.5 Hz), 7.14–6.89 (m, 3H), 4.47 (dt, 1H, J = 3.7 and 12.2 Hz), 4.02–3.89 (m, 2H), 3.34–3.21 (m, 4H), 2.90–2.45 (m, 5H), 2.26 (br s, 1H), 1.92–1.91 (m, 1H), 1.34 (t, 3H, J = 7.35 Hz); ^{13}C NMR ($CDCl_3$) δ 154.6, 129.4, 128.3, 121.4, 121.3, 110.2, 108.1, 61.7, 51.9, 49.2, 46.6, 36.2, 30.1, 29.9, 13.7; MS (APCI) *m*/*z* 276.3 $(M+H)^+$.

5.11. 1-[(3*R*,4*R*)-1-Cyclooctylmethyl-3-hydroxymethyl-4-piperidinyl]-3-ethyl-1,3-dihydro-2*H*-benzimidazol-2-one (2) hydrochloride

To a stirred solution of 1-ethyl-3-(3-hydroxymethyl-4-piperidinyl)-1,3-dihydrobenzimidazol-2-one (**12**) (24.1 mg, 0.085 mmol) in THF (1 mL) were added cyclooctanecarboxaldehyde (0.01 mL, 0.1 mmol) and sodium triacetoxyborohydride (28 mg, 0.13 mmol). After 2.5 d at rt, the mixture was mixed with EtOAc (1 mL) and H_2O (1 mL). The organic layer was separated, washed with $NaHCO_3$ (satd, 1 mL), brine (1 mL), dried over Na_2SO_4, and placed under vacuum. Purification of the crude product by prep TLC (Silica gel, 1000 μm) with 70% EtOAc/hexanes afforded 14.4 mg (42%) of **2**. ^{1}H NMR ($CDCl_3$) δ 7.33–7.27 (m, 1H), 7.20–7.02 (m, 3H), 4.37 (dq, 1H, J = 3.8 Hz), 3.98–3.92 (m, 2H), 3.33 (br s, 2H), 3.00 (d, 3H, J = 9.42 Hz), 2.60 (dq, 1H, J = 3.7 and 12.3 Hz), 2.31–2.03 (m, 5H), 1.72–1.46 (m, 14H), 1.34 (t, 3H, J = 7.1 Hz), 1.28–1.22 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 155.0, 129.7, 128.5, 122.2, 121.5, 121.5, 110.6, 108.3, 66.5, 62.3, 56.9, 54.0, 52.2, 41.4, 36.5, 35.3, 31.3, 31.3, 29.8, 29.2, 27.6, 27.6, 26.8, 26.0, 26.0, 25.4, 13.9; MS (ESI) *m*/*z* 400.6 $(M+H)^+$.

1-[(3*R*,4*R*)-1-Cyclooctylmethyl-3-hydroxymethyl-4-piperidinyl-3-ethyl-1,3-dihydro-2*H*-benzimidazol-2-one (14.4 mg) was dissolved in 10% MeOH/HCl (1 mL). The solvent was removed under reduced pressure and the oily residue was then dissolved in Et_2O (1 mL). Some white solids precipitated on the flask walls. The solvent was removed under reduced pressure and the residue was placed under vacuum to give 13.6 mg of the hydrochloride salt as a white solid. $[\alpha]_D^{20}$ +6.25 (*c* 0.272, 0.1 M HCl) ($C_{24}H_{38}ClN_3O_2 \cdot H_2O$) C, H, N.

5.12. X-ray crystal structure of (−)-3-bromo-8-camphorsulfonic acid salt of (−)-9

Single-crystal X-ray diffraction data were collected at 295 K using Cu Kα radiation and a Bruker SMART 6000 CCD area detector. A $0.42 \times 0.10 \times 0.07$ mm^3 crystal was mounted on a glass rod and transferred to the diffractometer. The crystal was orthorhombic in space group $P2_12_12_1$ with unit cell dimensions a = 6.9722 (2) Å, b = 20.3743(6) Å, c = 24.2633(7) Å. Corrections were applied for Lorentz, polarization, and absorption effects. Data were 98.1% complete to 67.16° θ (approximately 0.83 Å) with an average redundancy of 5.0. The structure was solved by direct methods and refined by full-matrix least squares on F^2 values using the programs found in the SHELXTL suite (Bruker, SHELXTL v6.12, 2006, Bruker AXS Inc., Madison, WI). Parameters refined included atomic coordinates and anisotropic thermal parameters for all non-hydrogen atoms. Hydrogen atoms on carbons were included using a riding model [coordinate shifts of C applied to H atoms] with C–H distance set at 0.96 Å. The absolute configuration was set based on the reference molecule (3-bromocamphorsulfonic acid) of known configuration. The *tert*-butyl group off N1 was refined with a disorder over two positions with an occupancy ratio of 52:48. The *tert*-butyl group off the silica atom showed a similar disorder, but the occupancy of the minor position was less than 25% and this disorder was omitted from the final cycles of refinement.

Acknowledgments

This research was supported by the National Institute on Drug Abuse, Grants DA05477 and DA09045.

Supplementary data

Crystal data, structural refinement analysis, atomic coordinates, bond lengths, bond angles, anisotropic displacement parameters, hydrogen coordination, isotropic displacement parameters of (−)-**9**-(3-bromo-8-camphorsulfonic acid) salt, and results from elemental analysis.

This material is available free of charge via the Internet at http://pubs.acs.org. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.10.023.

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