Probes for Narcotic Receptor Mediated Phenomena. 39. Enantiomeric N-Substituted Benzofuro[2,3-c]pyridin-6-ols: Synthesis and Topological Relationship to Oxide-Bridged Phenylmorphans

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Enantiomers of N-substituted benzofuro[2,3-c]pyridin-6-ols have been synthesized, and the subnanomolar affinity and potent agonist activity of the known racemic N-phenethyl substituted benzofuro[2,3-c]pyridin-6-ol can now be ascribed to the 4aS,9aR enantiomer. The energy-minimized structures suggest that the active enantiomer bears a greater three-dimensional resemblance to morphine than to an ostensibly structurally similar oxide-bridged phenylmorphan. Structural features of the enantiomers of N-substituted benzofuro[2,3-c]pyridin-6-ols were compared to provide the rationale for their binding affinity.

Introduction

In 1989, Hutchison, et al.3 published the synthesis of racemic N-substituted benzofuro[2,3-c]pyridin-6-ols and noted that one of them, the N-phenethyl derivative of the cis-benzofuropyridin-6-ol had high μ-opioid affinity (Ki = 0.9 nM) and potent antinociceptive activity. These compounds appeared to have marked structural resemblance to the oxide-bridged phenylmorphans. We published our initial work on the synthesis of the 12 racemic topologically rigid N-methyl-substituted a-through-f-oxide-bridged phenylmorphans (Figure 1) in 19844-5 and have recently reported the preparation of the final pair of oxide-bridged phenylmorphans.4-13 Some of the N-phenethyl analogues have been synthesized and their pharmacological activity determined.12,13 A few of the enantiomers of the racemates have also been examined. The topology of the ortho-b, -d, and -f isomers has been reported,13 and comparisons have previously been made to a highly potent azabicyclo[3.3.1]nonane μ-opioid agonist.14 We believed that we could gain information about the three-dimensional shape of a molecule required for it to interact with a specific opioid receptor if any of the 12 oxide-bridged racemic phenylmorphans was found to have agonist or antagonist activity. There was no way then, or now, to determine the agonist or antagonist conformation of a ligand acting at an opioid receptor.

1Dedicated to the 100th anniversary of the Division of Medicinal Chemistry.
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3Abbreviations: para-d-oxide-bridged phenylmorphan, (3R*,6aS*,11aR*)-1,3,4,5,6,11a-hexahydro-2-fl-3,6a-methanobenzofuro[2,3-c]jazocin-8-ol; EI, electron impact; HRMS, high-resolution mass spectra; TLC, thin layer chromatography.

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unsatisfactory results. The use of anhydrous aluminum chloride in dry nitromethane at 60 °C gave, in a rapid reaction, the desired product 1 in 84% yield, after workup. At a lower temperature (10 °C) the reaction resulted in the formation of only the 2,5-dimethoxypropiophenone intermediate and little of the desired O-demethylated product 1.

The reaction sequence to the known racemic secondary amine 8 (Scheme 1) was similar to that used by Hutchison et al.3 but modified for the larger scale synthesis. The yield in the conversion of 1 to the propiophenone 2 was improved from 65%3 to 90% by running the reaction in acetone, monitoring its completion by TLC and NMR, and by crystallization using hexane and isopropanol. Borane reduction16 of the amide 7 gave the racemic cis-6-methoxyamine 8.

Compound 8 was treated with (S)-(+) mandelic acid to give the salt with the (+)-amine base 9 (Scheme 2). From the

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**Figure 1.** Twelve racemic N-methyl substituted oxide-bridged phenylmorphan isomers.

**Scheme 1**

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Reagents and conditions: (a) propionyl chloride, AlCl₃, CH₃NO₂, 60 °C; (b) BrCH₂CO₂Et, K₂CO₃, acetone, reflux, 30 h; (c) (EtO)₂POCH₂CN, NaH, THF, 0 °C, 2 h; (d) NaH, EtOH, reflux, 15 min; (e) H₂, 5% Pt/C, acetic acid, 50 psi, room temperature; (f) 60% NaH in mineral oil, EtOH, reflux, 8.5 h; (g) NaBH₄, (CH₃O)₂SO₂, THF, reflux 48 h, purified through oxalate salt (oxalic acid, acetone), followed by KOH (aqueous) and distillation of the free base at 183 °C, in vacuo (68%). The asterisk (*) indicates that the yield of (+)-7 after the combination steps of c and f was about 60%. 
mother liquors of the recrystallization of the salt, material enriched in the (−)-enantiomer 10 was obtained and converted to the base, and treatment with (R)-(−)-mandelic acid gave the desired salt with the (−)-amine base 10 (Scheme 2). The enantiomers (+)-9 and (−)-10 were obtained from their mandelate salts on basification. The melting points of these enantiomers were similar, and their optical rotations were about equal and opposite.

Derivatization of (+)-9 or (−)-10 with R-(+)-1-phenethyl isocyanate to form the diastereomeric ureas enabled the determination of the optical purity of these amines. The optical purity of 9 was established by the disappearance of the chemical shift at δ 1.453 and that of 10 by the disappearance of the chemical shift at δ 1.408. Their optical purity was estimated to be ~99% ee.

The chirality of (−)-10 was determined to be 4aS,9aR by X-ray crystallographic analysis of the salt (−)-10: R-(−)-mandelate (Figure 2). N-Alkylation of the secondary amine 4aR,9aS-9 or 4aS,9aR-10 (Scheme 3) gave the dextrorotary N-methyl (4aR,9aS-11) and N-phenylethyl analogue (4aR,9aS-12) and their levorotatory relatives (4aS,9aR-18 and 4aS,9aR-19). Cleavage of the aromatic methoxy group with 48% HBr gave the phenols, 4aR,9aS-14 and 4aR,9aS-15 and 4aS,9aR-21 and 4aS,9aR-22 (Scheme 3). The (+)-S- and R-2'-hydroxyphenethyl compounds 4aR,9aS-16 and 4aR,9aS-17 and the (−)-S- and R-2'-hydroxyphenethyl compounds 4aS,9aR-23 and 4aS,9aR-24 (Scheme 3) were obtained from the 4aR,9aS-aminophenol 13 and the 4aS,9aR-aminophenol 20, respectively. These phenols (4aR,9aS-13 and 4aS,9aR-20) were prepared by 48% HBr cleavage of the aromatic methoxy secondary amine compounds 4aR,9aS-9 and 4aS,9aR-10. The various chiral compounds were generally prepared in moderate to high yields.

Results and Discussion

Five compounds (20–24) were examined for their efficacy as µ-agonists in the [35S]GTP-γ-S functional assay (Table 1). In that assay, the secondary amine 20 was found to be a weak partial agonist (ED50 = 150 nM) and had weak antagonist activity (Ki = 95 nM), the N-methyl substituted pyridin-6-ol 21 had good affinity for the µ-receptor (Ki = 16 nM) but had little efficacy in the functional assay. 23 was noted to be a weak partial agonist, compound 24 was a weak agonist, and the 4aS,9aR-N-phenethyl substituted pyridin-6-ol (22) was, as expected, a potent agonist with very high affinity for the µ-receptor (Ki = 0.7 nM). As shown in Table 1, the 4aS,9aR compounds (all levorotatory) were found to have much higher affinity than the comparable 4aR,9aS compounds (all dextrorotatory). Clearly, the activity of the racemic mixtures

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Figure 2. X-ray crystallographic structure of the R-(−)-mandelic acid salt of 4aS,9aR-(−)-cis-4a-ethyl-6-methoxy-1,2,3,4,4a,9a-hexahydrobenzofuro[2,3-c]pyridine (−)-10. Displacement ellipsoids are shown at the 50% level.

Scheme 2

Scheme 3

Reagents and conditions: (a) (S)-(−)-mandelic acid, EtOAc/Et2O (recrystallized from acetonitrile/Et2O (1:1) (1×), EtOAc/Et2O (1:1) (1×), and acetonitrile/Et2O (1:1) (1×)), free-based with NaOH (2 N), extracted by CHCl3, solvent removed, high vacuum distilled at 130°C; (b) (R)-(−)-mandelic acid, EtOAc/Et2O (recrystallized from EtOAc/Et2O (1:1) (1×), acetonitrile/Et2O (1:1) (2×)), 68% recovery. Free-based with NaOH (2 N), extracted with CHCl3, solvent removed, high vacuum distilled at 125°C.
Table 1. \[^{1}H\]opioid Receptor Binding Data\(^a\) for cis-Benzofuro\([2,3-c]\)pyridin-6-ols and Functional Data (\[^{35}S\]GTP-\(\gamma\)-S)\(^b\) for Selected Compounds

<table>
<thead>
<tr>
<th>(K_i) (nM)</th>
<th>(\mu)</th>
<th>(\delta)</th>
<th>(\kappa)</th>
<th>(\mu)-agonism (E_{max}) (%)</th>
<th>(ED_{50}) (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4aS,9aR</td>
<td>48 ± 2.9</td>
<td>1350 ± 90</td>
<td>450 ± 34</td>
<td>97 ± 4</td>
<td>150 ± 25</td>
</tr>
<tr>
<td>20(^f)</td>
<td>15.9 ± 1</td>
<td>75 ± 8.8</td>
<td>88 ± 8</td>
<td>45 ± 2</td>
<td>241 ± 47</td>
</tr>
<tr>
<td>21</td>
<td>0.70 ± 0.06</td>
<td>443 ± 32</td>
<td>413 ± 44</td>
<td>100 ± 3</td>
<td>560 ± 60</td>
</tr>
<tr>
<td>22</td>
<td>9.3 ± 0.7</td>
<td>807 ± 45</td>
<td>1090 ± 60</td>
<td>36 ± 2</td>
<td>480 ± 90</td>
</tr>
<tr>
<td>23 (2'S)</td>
<td>200 ± 9</td>
<td>2490 ± 230</td>
<td>5870 ± 470</td>
<td>85 ± 3</td>
<td>650 ± 100</td>
</tr>
<tr>
<td>24 (2'R)</td>
<td>200 ± 9</td>
<td>2490 ± 230</td>
<td>5870 ± 470</td>
<td>85 ± 3</td>
<td>650 ± 100</td>
</tr>
</tbody>
</table>

\(^a\) Assays\(^2\) were conducted using CHO cells, which were stably transfected and express the \(\mu\)-, \(\delta\)-, or \(\kappa\)-opioid receptors, respectively. For all results, \(n = 3\). For comparison, the \(K_i\) of morphine at \(\mu = \pm 5.55 ± 0.01\) nM. \(^b\) \[^{35}S\]GTP-\(\gamma\)-S binding was performed using CHO hMOR cells, which express the human \(\mu\)-opioid receptor. For all values, \(n = 3\). \(^c\) \[^{3}H\]DAMGO binding. \(^d\) \[^{3}H\]JADLE binding. \(^e\) \[^{3}H\]U69,593 binding. \(^f\) The \(E_{max}\) is the extrapolated maximal stimulation where 100% is defined as the stimulation produced by 10 \(\mu\)M DAMGO (DAMGO \(ED_{50}\) at \(\mu = 42 ± 4\) nM). \(^g\) Secondary amine 20 is a \(\mu\)-antagonist, \(K_i = 95 ± 16\) nM (\[^{35}S\]GTP-\(\gamma\)-S assay with CHO hMOR cells, \(n = 5\)).

Examined by Hutchison et al.\(^3\) reflected the activity of the compounds with the 4aS,9aR stereochemistry. The 4aS,9aR-N-phenethyl compound 22 had subnanomolar affinity for the \(\mu\)-opioid receptor and slightly more than 100-fold less affinity at the \(\delta\)- and \(\kappa\)-receptors. Interestingly, introduction of a hydroxy substituent in the N-phenethyl side chain decreased affinity for the \(\mu\)-receptor 10-fold with the hydroxy in the \(\epsilon\) configuration (4aS,9aR,2'S-23) and over 100-fold when the hydroxy was in the \(\gamma\)-configuration (4aS,9aR,2'R-24).

Figure 3 illustrates the geometry optimized conformers of the high affinity N-phenethyl compound 22 in the gaseous phase in its protonated form; the active form of 22 is likely to be protonated at physiological pH. For the comparison of the energetics, the Gibbs free energy of each conformer was calculated at 298.15 K from its fully optimized geometry in CHCl\(_3\). The topology of 22, except for the equatorial \(N\)-phenethyl substituent, is identical to the X-ray structure of 10 as evidenced by the less than 0.04 Å root-mean-square deviation (rmsd) between the heavy atoms of both the dihydrofuran and the piperidine rings. Conformer B1 is epimeric to A and was obtained by nitrogen inversion; it is 2.6 kcal/mol less stable relative to the equatorial \(N\)-phenethyl moiety; conformer C is slightly (0.26 kcal/mol) more stable than A, and this stability may arise from the charge interaction between the dihydrofuran oxygen and the hydrogen on the nitrogen atom, as evidenced by their distance of 2.4 Å. Note that A can undergo the piperidine conformation change first, followed by the nitrogen inversion to conformer C. Both the reaction pathway and the energy barrier between the two conformers A and C are not known and are currently being investigated with the ab initio replica path method.\(^18\) Nonetheless, considering that both the nitrogen inversion energy barrier and the conformation energy barrier of the neutral \(N\)-methylpiperidine are known to be less than 11 kcal/mol,\(^19,20\) 22 may well undergo a rapid interconversion between A and C and thus is likely to exist as a conformer mixture at room temperature.

Energetics suggest that 22 exists in CHCl\(_3\) predominantly in the form of A and C (\(>95\%\)) at room temperature. To identify the conformer most likely to be recognized at the \(\mu\)-opioid receptor, the heavy atoms of the piperidine rings of A and C and the oxide-bridged para-d phenylmorphinan isomer (which was found to be structurally more similar to 22 than any of the other oxide-bridged phenylmorphinans) were fitted to morphine with the equatorial \(N\)-methyl as shown in Figure 4. Their respective rmsd value of the fitting is 0.13, 0.12, and 0.07 Å. The dihydrofuran ring of conformer C overlaps well with that of the para-d isomer that is known to bind poorly to the \(\mu\)-opioid receptor (\(K_i = 1220\) nM).\(^13\) This indicates that conformer C, although thermodynamically more stable than A, is not recognized by the receptor, and thus, the subnanomolar affinity of 22 is likely to arise from conformer A. The benzoferan ring in conformer A overlaps better with the benzofuran ring of morphine than that of the para-d isomer (Figure 4). The affinity of 22 for the \(\mu\)-receptor is much closer to that of morphine (\(K_i = 2.55 ± 0.1\) nM) than to the para-d isomer.

This work has enabled us to determine the enantiomer responsible for the \(\mu\)-affinity and activity of the racemic \(N\)-substituted benzofuro\([2,3-c]\)pyridin-6-ols.\(^3\) In addition, we have compared the structures of the energy-minimized conformers of the most active enantiomer 22 and the various oxide-bridged phenylmorphinans, determining that the activity and affinity of 22 were not likely to be due to conformer C, which is structurally similar to the para-d-oxide-bridged phenylmorphinan. Compound 22, however, was found to have a more stable conformer A, topologically similar to morphine, that could be responsible for its activity. Also, we synthesized new N-derivatives with a hydroxyl substituent on the phenylethyl side chain (23 and 24) and determined that a hydroxyl moiety on that side chain hindered binding and reduced efficacy.

**Experimental Section**

All reactions were performed in oven-dried glassware under an argon atmosphere. Some of the large scale mixtures were magnetically stirred. All melting points were determined on a
Thomas-Hoover capillary melting point apparatus and are uncorrected. The optical rotation data were obtained on a Perkin-Elmer polarimeter model 341 or on a Jasco DIP-370 digital polarimeter (589 nm).\(^1\) H NMR (in CDCl\(_3\) with TMS at \(\delta \) ppm) spectra were recorded on a Varian XL-300 spectrometer at 300 MHz and on a Varian AS-400 spectrometer at 400 MHz. Mass spectra (EI) and high-resolution mass spectra (HRMS) were obtained using a JEOL SX102 instrument. Thin layer chromatography (TLC) was performed on precoated plates of silica gel GF (0.25 mm, F254, Alltech) using various gradients of CHCl\(_3\)/MeOH containing 1% NH\(_4\)OH or gradients of ethyl acetate/n-hexanes. Visualization was accomplished under UV or by staining in an iodine chamber. Flash chromatography was conducted using silica gel (230−400 mesh, Merck). Elemental analyses were performed by Atlantic Microlabs Inc., Norcross, GA, to determine the purity of the compounds and were within ±0.4% of theoretical values, confirming ≥95% purity.

**2-Hydroxy-5-methoxypropiophenone (1).** In a 5 L three-necked flask fitted with a mechanical stirrer, thermometer, nitrogen purge and pressure equalizing funnel was placed 500 mL of dry nitromethane. The stirred solvent was cooled to −20 °C, and 277.82 g (2.08 mol, 1.15 equiv) of anhydrous AlCl\(_3\) was added slowly (exothermic) keeping the temperature at <0 °C with a dry ice−acetone bath. A 50 mL portion of nitromethane was used to wash in the AlCl\(_3\). Propionyl chloride (201.05 g, 2.17 mol, 1.20 equiv) was then slowly added keeping the temperature at <−20 °C. A mixture of 150 mL of nitromethane and 1,4-dimethoxybenzene (250.0 g, 1.81 mol) was heated to solution. This solution was added slowly to the reaction mixture beginning at −20 °C, and the temperature was allowed to rise to 10 °C. A 50 mL portion of nitromethane was used as wash-in. This is a very rapid reaction. TLC (petroleum ether/Et\(_2\)O, 3:1) of an aliquot quenched with H\(_2\)O gave essentially a single spot indicating the intermediate product 2,5-dimethoxypropiophenone, no starting material, and only a trace of \(\text{I}^1\). The stirred mixture was slowly and cautiously heated to reflux with the rapid evolution of HCl and methyl chloride. At 60 °C TLC showed ∼2:1 mixture of intermediate and the desired \(\text{I}\). After 1.5 h of reflux, TLC showed the absence of the intermediate product. The reaction mixture was cooled and slowly quenched with 500 mL of H\(_2\)O, and the nitromethane evaporated under water aspirator vacuum. The mixture was extracted with CHCl\(_3\) (600 mL, 2 × 200 mL) and the combined CHCl\(_3\) washed with 250 mL of H\(_2\)O and evaporated in vacuo. The dark residue was vacuum

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**Figure 3.** Geometry optimized conformers of the protonated 4aS,9aR-(−)-cis-4a-ethyl-2-(2-phenylethyl)-1,2,3,4,4a,9a-hexahydrobenzofuro-[2,3-c]pyridin-6-ol (22) in the gaseous phase. Topology of A is essentially identical with the X-ray structure of 10 in Figure 2 (without the N-phenylethyl moiety). Conformer B1 is epimeric to A with respect to the nitrogen. B2 is another representation of B1 via a 180° rotation, and C is formed by a conformational change of the piperidine ring of B2 or B1. \(\phi_1\) is the dihedral angle of O9−C9a−C1−N2, and \(\phi_2\) is the dihedral angle of C4a−C4−C3−N2.
distilled at 120–125 °C/1–2 mm to give 288.84 g of yellow oil. This was dissolved in CH₂OH (722 mL) and treated dropwise with H₂O (577 mL) (CH₃OH/H₂O, 2.5:2) to give crystalline material (cooling to a final temperature of 15 °C). This was filtered and washed with a mixture of CH₃OH (360 mL) and H₂O (289 mL) (CH₃OH/H₂O, 1.25:1) at 15 °C and dried on the filter to constant weight to give 273.9 g (84%) of rac-2-carbethoxy-3-(cyanoethyl)-3-ethyl-5-methoxy-2,3-dihydrobenzofuran (4). A mixture of compound 3 (130.7 g), EtOH (anhydrous, 18.5 mL), and sodium hydride (60% in mineral oil, 1.807 g) was refluxed for 15 min. The completion of the reaction was monitored by NMR. The yield of the 4 was improved by modifying the workup procedure of Hutchison et al. After the mixture was cooled to room temperature, EtOH was evaporated from the reaction mixture, which was then mixed with ice–water (100 mL) and extracted with CH₂Cl₂ (100 mL × 3). The crude product (130.69 g, 100%) was used to synthesize 5 (cis-(2R*,3S*)-ethyl 3-(2-aminoethyl)-3-ethyl-5-methoxy-2,3-dihydrobenzofuran-2-carboxylate) and 6 (trans-(2R*,3R*)-ethyl 3-(2-aminoethyl)-3-ethyl-5-methoxy-2,3-dihydrobenzofuran-2-carboxylate).

**Figure 4.** Overlay of the piperidine ring of conformers A and C of compound 4a,S,9a,R(−)-22, and the para-d-oxide-bridged phenyl-morphan, onto that of morphine, illustrating the topographical similarity of conformer A with morphine and conformer C with the para-d-oxide-bridged phenylmorphine that has little affinity for the μ-opioid receptor. Hydrogen atoms are not shown.
concentrated to afford the crude racemic amine (8, 56.21 g). Oxalic acid (21.69 g, 0.24 mol) and crude amine 8 (56.21 g) were dissolved in acetone (400 mL). The clear-yellow solution was stirred until the solid oxalate salt precipitated from the solution. After the mixture was cooled to 0 °C, the oxalate was filtered, washed with cold acetone (300 mL), and dried in vacuo to afford 8-oxalate (50.48 g). The oxalate was placed in a separatory funnel and dissolved in H₂O (200 mL), and a KOH aqueous solution (40.2 g in 160 mL H₂O) was added. The mixture was shaken to give 8 as the free base, and CHCl₃ (200 mL x 4) was added to extract the amine. The first two CHCl₃ extracts were combined, washed with H₂O (200 mL), then filtered through Celite. The third extract was washed with that H₂O (200 mL) and filtered through Celite, and the Celite filter-cake was washed with CHCl₃. This was repeated again with the fourth CHCl₃ extract. The organic solvent was removed in vacuo to give 8 (42.16 g, 81%). Compound 8 was distilled under water aspirator vacuum at 183 °C to give the pure racemic amine 8 (35.2 g, 68%) as a colorless viscous oil. MS m/z 234 [M + H]^+.

4aR,9aS- (+)-cis-4a-Ethyl-6-methoxy-1,2,3,4,4a,9a-hexahydrobenzofuro[2,3-c]pyridine (-) -9). Racemic amine 8 (33.58 g, 0.144 mol) and S- (+)-mandelic acid (22.34 g, 0.147 mol) were heated to solution in EtOAc (91 mL) in a 250 mL beaker. Ether (105 mL) was then slowly added to the hot solution. Scratching or a seed crystal was used to induce crystallization. Another portion of Et₂O (27 mL) was added to the boiling mixture. Scratching was continued until crystallization ended. The solution was cooled in a chilled water bath, and another portion of Et₂O (4.5 mL) was added. An oil appeared with this addition, and a small amount of EtOAc was added to resolubilize the oil. The solid was filtered and washed with a portion of a mixture of EtOAc (91 mL) and Et₂O (136.5 mL) and was briefly vacuum-dried (to avoid drying any oil on the crystals). Three additional washes used the remaining amount of the rinsing solvent mixture. Ether (25 mL) was used as a final rinse to dry the filter cake. The white solid (30.55 g) was recrystallized 3x from EtOAc or acetone to give pure (+)-9: S- (+)-mandelate (17.96 g, 64%), mp 144.5–145.5 °C. The mandelate salt was dissolved in CHCl₃ (40 mL), and NaOH (2 N, 45 mL) was used to convert it to the free base. The aqueous phase was extracted with CHCl₃ (3 x). The combined organic phase was washed with H₂O (30 mL) and the solvent removed in vacuo to give (+)-9 as the free base (11.23 g). High vacuum distillation at 120–125 °C gave a quantitative yield of (+)-9 (10.88 g) as a colorless viscous oil. The oil was slowly crystallized to a white solid, mp 50–51 °C, δ (6H, t, J = 7.4 Hz), 1.58–1.84 (4H, m), 2.65 (1H, dd, J = 4.2, 8.1, 12.6 Hz), 2.79 (1H, dd, J = 4.5, 6.6, 11.1 Hz), 2.94 (1H, dd, J = 5.2, 13.6 Hz), 3.09 (1H, dd, J = 4.4, 13.6 Hz), 3.77 (3H, s), 4.30 (1H, t, J = 7.2 Hz), 1.58 (1H, sextet, J = 7.3 Hz), 1.69 (1H, sextet, J = 7.2 Hz), 1.78 (1H, dd, J = 4.2, 9.3, 13.8 Hz), 2.02 (1H, dd, J = 3.4, 5.8, 13.8 Hz), 2.12 (1H, dd, J = 3.4, 9.4, 14.6 Hz), 2.25 (3H, s), 2.30 (1H, dd, J = 6.9, 12.3 Hz), 2.42 (1H, m), 2.75 (1H, dd, J = 1.4, 5.4, 12.3 Hz), 3.77 (3H, s), 4.45 (1H, dd, J = 5.3, 6.8 Hz), 6.65 (2H, m), 6.72 (1H, m), MS m/z 248 [M + H]^+. Anal. (C₁₇_H₂₃_NO₂) C, H, N.

4aR,9aS- (+)-cis-4a-Ethyl-6-methoxy-1,2,3,4,4a,9a-hexahydrobenzofuro[2,3-c]pyridine (+)-9). Formaldehyde solution (37% aqueous, 89 μL) was added to the argon replaced by hydrogen via a hydrogen balloon connected to the flask through a needle (16 gauge, 1/2″). The reaction was complete after 4 h at room temperature (monitored by TLC (CHCl₃/MeOH/28% NH₄OH, 90:9:1)). The mixture was then filtered through Celite, the filtered catalyst was rinsed with ethanol, and the solvent was removed in vacuo. Column chromatography (CHCl₃/MeOH/28% NH₄OH, 90:9:1) afforded (+)-9 (247 mg, 100%) as a colorless viscous oil that crystallized on standing, mp 50–51 °C. [α]D²⁰ +89.3 (c 0.750, CDCl₃). NMR (CDCl₃, 300 MHz): δ 0.84 (3H, J = 7.5 Hz), 5.18 (1H, sextet, J = 3.3, 11.4 Hz), 2.59 (1H, dd, J = 3.3, 5.4, 14.1 Hz), 2.21 (1H, dt, J = 3.3, 11.4 Hz), 2.36 (1H, dd, J = 7.2, 12.0 Hz), 2.59 (3H, m), 2.79 (2H, m), 2.94 (1H, dd, J = 1.5, 6.0, 12.0 Hz), 3.78 (3H, s), 4.48 (1H, dd, J = 5.7, 7.2 Hz), 6.62 (2H, m), 6.73 (1H, m), 7.15–7.29 (5H, m), MS m/z 338 [M + H]^+. Anal. (C₁₇_H₂₃_NO₂) C, H, N.
monitored by TLC (CHCl₃/MeOH, 3:1) purification afforded 4a, 9a-S-4-amino-2-methyl-1,2,3,4,4a,9a-hexahydrobenzofuro[2,3-c]pyridizine (16). Compound (12) (326 mg) was dissolved in a mixture of hydrobromic acid (2 mL, 48%), and acetic acid (16.5 mL). The mixture was refluxed for about 7 h with the completion of the reaction monitored by TLC (CHCl₃/MeOH: 3:1). The acidic solution was then concentrated under aspirator vacuum, redissolved in H₂O (15 mL), and the solvent was removed in vacuo to get crude (15). Column chromatography (CHCl₃/MeOH: 3:1) afforded (12) (304 mg, 88%) as a white solid, mp 171.5–173.5°C. [α]D₂₀ +115.8° (c 0.810, CDCl₃). NMR (CDCl₃, 300 MHz): δ 0.83 (3H, t, J = 7.5 Hz), 1.58 (1H, sextet, J = 7.2 Hz), 1.69 (1H, sextet, J = 7.2 Hz), 1.80 (1H, ddd, J = 4.2, 9.9, 14.1 Hz), 2.05 (1H, ddd, J = 3.4, 5.6, 9.0 Hz), 2.22 (1H, dt, J = 3.3, 10.5 Hz), 2.37 (1H, ddd, J = 7.4, 11.8 Hz), 2.59 (3H, m), 2.79 (2H, m), 2.94 (1H, ddd, J = 1.5, 6.0, 12.0 Hz), 4.48 (1H, dd, J = 6.0, 7.2 Hz), 4.58 (2H, m), 6.68 (2H, m), 7.15–7.29 (5H, m). MS m/z 324 [M + H]+. Anal. (C₁₉H₁₇NO₂) C, H, N.

4a, 9a-S-4-amino-2-methyl-1,2,3,4,4a,9a-hexahydrobenzofuro[2,3-c]pyridizine (16). Compound (12) (306 mg, 352 mg (352 mg) was dissolved in hydrobromic acid (2 mL, 48%), and the solution was removed in vacuo to get crude (17). Column chromatography (CHCl₃/MeOH: 3:1) afforded (17) (371 mg, 92%) as a white solid, mp 171.5–172.5°C. [α]D₂₀ +115.8° (c 0.810, CDCl₃). NMR (CDCl₃, 300 MHz): δ 0.83 (3H, t, J = 7.5 Hz), 1.58 (1H, sextet, J = 7.2 Hz), 1.69 (1H, sextet, J = 7.2 Hz), 1.80 (1H, ddd, J = 4.2, 9.9, 14.1 Hz), 2.05 (1H, ddd, J = 3.4, 5.6, 9.0 Hz), 2.22 (1H, dt, J = 3.3, 10.5 Hz), 2.37 (1H, ddd, J = 7.4, 11.8 Hz), 2.59 (3H, m), 2.79 (2H, m), 2.94 (1H, ddd, J = 1.5, 6.0, 12.0 Hz), 4.48 (1H, dd, J = 6.0, 7.2 Hz), 4.58 (2H, m), 6.68 (2H, m), 7.15–7.29 (5H, m). MS m/z 324 [M + H]+. Anal. (C₁₉H₁₇NO₂) C, H, N.
X-ray Crystal Structure of 4a,5a,9a-R(-)cis-4a-Ethyl-6-methoxy-2,3,4,9a-hexahydrobenzofuro[2,3-c]pyridin-6-ol (−)-(22). Compound (−)-(20) (283.5 mg), (R)(−)-stereoycline (326.2 mg), and toluene (anhydrous, 8 mL) were reacted and purified as shown for (−)-(16) to give (−)-(24) (255 mg, 58%) as a white solid, mp 165.0–168.0 °C. [α] D 25° = −177.0° (c 0.775, CDCl3). NMR (CDCl3, 400 MHz): δ 0.84 (3H, t, J = 7.3 Hz), 1.58 (1H, sextet, J = 7.2 Hz), 1.71 (1H, sextet, J = 7.2 Hz), 1.85 (1H, dd, J = 5.3, 9.2, 14.0 Hz), 2.04 (1H, dt, J = 4.3, 14.0 Hz), 2.37 (1H, dd, J = 6.8, 12.4 Hz), 2.48 (4H, m), 3.14 (1H, dd, J = 5.2, 12.4 Hz), 4.46 (1H, dd, J = 5.3, 6.9 Hz), 4.68 (1H, dd, J = 5.2, 8.8 Hz), 6.59 (2H, m), 6.67 (1H, m), 7.21–7.35 (5H, m). MS m/z = 340 [M + H]. Anal. (C23H23NO3) C, H, N.

X-ray Crystal Structure of 4a,5a,9a-R(-)cis-4a-Ethyl-6-methoxy-2,3,4,9a-hexahydrobenzofuro[2,3-c]pyridine (−)-(10) - R(−)-Mandelic Acid. Single-crystal X-ray diffraction data on the (R)(−)-mandelic acid salt of the enantiomer 10 were collected using Mo Kα radiation and a Bruker APEX 2 CCD area detector. 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, version 6.10, 2000, 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 adjusted to H atoms] with C-H distance set at 0.96 Å. A 0.504 mm × 0.112 mm × 0.037 mm crystal of (−)-(10) - R(−)-mandelic acid was prepared for data collection coating with high viscosity microscope oil (Paratone-N, Hampton Research). The oil-coated crystal was mounted on a MicroMesh mount (MiTeGen, Ithaca, NY) and transferred to the cold stream (113 K) on the diffractometer. The crystal was monoclinic in space group P21 with unit cell dimensions a = 11.1907(7) Å, b = 6.2958(4) Å, c = 13.8939(9) Å, and β = 96.147(1)°. Corrections were applied for Lorentz, polarization, and absorption effects. Data were 98.9% complete to 29.28° θ (approximately 0.73 Å) with an average redundancy of 3.76. The asymmetric unit contained a single molecule of (−)10 and a single molecule of (+)-mandelic acid.

Quantum Chemistry and Superposition Study. The geometry optimization for the conformers of compound 22, the para-dioxide-bridged phenylmorphan, and morphine, in their protonated forms, was done in the gaseous phase with the density functional theory at the level of B3LYP/6-31G*. The conformers of 22 were also fully optimized in CHCl3 with the polarized continuum model with the UKS parameters set to compare their energetics, which include the zero-point correction as well as the enthalpy and the entropy contribution at 298.15 K. For the superposition study, the optimized structures in the gaseous phase were overlaid onto morphine with the rigid fit of Quanta 2008 (Accelrys) using the heavy atoms of the piperidine ring as a common docking site.

Binding and Efficacy Assays, Cell Culture and Membrane Preparation. As noted previously, the recombinant CHO cells (hMOR-CHO, hDOR-CHO, and hKOR-CHO) were produced by stable transfection with the respective human opioid receptor cDNA and were provided by Dr. Larry Toll (SRI International, CA). The cells were grown on plastic flasks in DMEM (100%) (hDOR-CHO and hKOR-CHO) or DMEM/F-12 (50%/50%) medium (hMOR-CHO) containing 10% FBS, and G-41 (0.10–0.2 mg/mL) under 95% air/5% CO2 at 37 °C. Cell monolayers were harvested and frozen at −80 °C.

135S]GTP-γ-S Binding Assays. On the day of the assay, cells were thawed on ice for 15 min and homogenized using a Polytron in 50 mM Tris-HCl, pH 7.4, containing 4 μg/mL leupeptin, 2 μg/mL chymostatin, 10 μg/mL bestatin, and 100 μg/mL β-lactamase. The homogenate was centrifuged at 30000 g for 10 min at 4 °C and the supernatant discarded. The membrane pellets were resuspended in binding buffer and used for [35S]GTP-γ-S binding assays. [35S]GTP-γ-S binding was determined as described previously. Briefly, test tubes received the following additions: 50 μL of buffer A (50 mM Tris-HCl, pH 7.4, containing 100 mM NaCl, 10 mM MgCl2, 1 mM EDTA), 50 μL of GDP in buffer A/0.1% BSA (final concentration = 10 μM), 50 μL of drug in buffer A/0.1% BSA, 50 μL of [35S]GTP-γ-S in buffer A/0.1% BSA (final concentration = 50 nM), and 300 μL of cell membranes (50 μg of protein) in buffer B. The final concentrations of reagents in the [35S]GTP-γ-S binding assays were 50 mM Tris-HCl, pH 7.4, containing 100 mM NaCl, 10 mM MgCl2, 1 mM EDTA, 1 mM DTT, 10 μM GDP, and 0.1% BSA. Incubations proceeded for 3 h at 25 °C. Nonspecific binding was determined using GTP-γ-S (40 μM). Bound and free [35S]GTP-γ-S were separated by vacuum filtration through GF/B filters. The filters were punched into 24-well plates to which was added 0.6 mL of LSC cocktail (Cytosint). Samples were counted, after an overnight extraction, in a Trilux liquid scintillation counter at 27% efficiency.

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