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Probes for narcotic receptor mediated phenomena 49. N-Substituted *rac-cis*-4a-arylalkyl-1,2,3,4,4a,9a-hexahydrobenzofuro[2,3-*c*]pyridin-6-ols

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Abstract

Racemic N-substituted -1,2,3,4,4a,9a-hexahydrobenzofuro[2,3-*c*]pyridin-6-ols containing *cis*-4a-arylalkyl groups were explored as probes for opioid receptors. Specifically *cis*-4a-phenylpropyl, -phenylbutyl, and -phenylpentyl groups coupled with widely varied substituents on the nitrogen atom were synthesized and their pharmacological profiles at opioid receptors examined. The study yielded compounds with good affinity and moderate to potent antagonist activity at the μ - and δ -opioid receptors, and agonist activity at the κ -opioid receptor. An *N*-allyl substituent in the C4a phenylpropyl series induced 6-fold higher affinity at δ - than μ -receptors, while an *N*-CPM substituent in the C4a (CH₂)₃Ph series led to a compound with high δ -affinity and potent δ -antagonist activity.

Keywords

Synthesis; *cis*-4a-arylalkyl-1,2,3,4,4a,9a-hexahydrobenzofuro[2,3-*c*]pyridin-6-ols; opioid receptors; [³⁵S]GTP- γ -S efficacy assays; μ - and δ -receptor antagonists

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Appendix A. Supplementary data

1. Introduction

From our work[1-3] and that of Hutchison et al.[4] it has been shown that *cis*-1,2,3,4,4a,9a-hexahydrobenzofuro[2,3-*c*]pyridin-6-ols (**1**, Fig. 1), when suitably substituted on the tertiary nitrogen and the C4a-position, have high or moderate affinity for either μ -, δ - and κ -opioid receptors, and sometimes at a combination of these receptors [4]. In principle, then, substituent modifications at these positions could lead to compounds that interact with a desirable combination of $\tilde{\mu}$ and δ -opioid receptor affinity. It has been found that compounds that combine both μ -agonist and δ -antagonist behavior can minimize at least some of the undesired side-effects that are frequently observed with the use of opioid analgesics[5-7]. It has also been noted that a δ -agonist can counteract the respiratory depression induced by μ -agonists without interfering with antinociceptive activity [8]. We have prepared derivatives of the *cis*-1,2,3,4,4a,9a-hexahydrobenzofuro[2,3-*c*]pyridin-6-ols to see if we could find new, interesting δ -ligands, or compounds that interact with both μ & δ receptors.

Benzofuro[2,3-*c*]pyridinols can be viewed as truncated *para*-d oxide-bridged phenylmorphans (**2**, Fig., 1, *rac*-(3*R*,6*aS*,11*aR*)-2-methyl-1,3,4,5,6,11a-hexahydro-2*H*-3,6a-methanobenzofuro[2,3-*c*]azocin-8-ol). In the *para*-d series, neither the *N*-methyl nor the *N*-phenethyl-substituted isomer appreciably interacted with opioid receptors [9-11]. The C4a group in its abridged form, however, gives the benzofuropyridinols a partial flexibility, hopefully enabling the interaction of these C4a-arylalkyl substituted benzofuro[2,3-*c*]pyridinols with suitable amino acids in the opioid receptors that could not be reached with the inflexible, rigid, *para*-d oxide-bridged phenylmorphans. In other hexahydrobenzofuro[2,3-*c*]pyridin-6-ols this flexibility was shown to allow a conformational overlap that resembles morphine more than the oxide-bridged phenylmorphans [2]. For these reasons we concentrated on the synthesis and pharmacological evaluation of the C4a-phenylpropyl, -phenylbutyl and -phenylpentyl substituents combined with a variety of N-substituents. These compounds were obtained using our previous discovery of a simpler route for the synthesis of C4a-substituted hexahydrobenzofuro[2,3-*c*]pyridin-6-ols that has enabled us to study compounds with a variety of substituents in the C4a-position as well as on the piperidine nitrogen atom [1, 3, 12].

2. Results and discussion

2.1 Chemistry

We synthesized compounds **7a-7c** with a C4a-phenylpropyl, phenylbutyl and phenylpentyl group as the arylalkyl groups from 4-(2,5-dimethoxyphenyl)-1-methyl-1,2,3,6-tetrahydropyridine **3** (Scheme 1), using established procedures [12]. The N-position was subjected to different variations in these compounds. Further modification was achieved first by initial acetate protection of the *N*-methyl phenolic compounds **7a-7c** to give **8a-8c** [12]. Sequential treatment of **8a-8c** with Troc-Cl, deprotection of the acetate, and Zn-dust mediated removal of the Troc group gave the *N*-nor compounds **9a-9c**. Compounds **9a-9c** was N-alkylated to give compounds **10-12** (Scheme 1) that included the *N*-cyclopropylmethyl, *N*-allyl, *N*-phenalkyl, and fluoro- and trifluoromethyl-substituted *N*-phenethyl substituents.

2.2 Opioid receptor affinity

Among the compounds with the shortest aralkyl chain at C4a, in the C4a-phenylpropyl series (Table 1, **10a-10i**), three compounds with the least bulky moieties on nitrogen had the highest affinity for the μ -receptor, the phenethyl (**10a**), *o*-fluorophenylethyl (**10c**) and cyclopropylmethyl (**10g**) (K_i = 14, 17 and 13 nM, respectively). Remarkably, seven of the 9 compounds in this C4a-substituted series had good to excellent affinity for the δ -receptor. All except **10h** and **10i** were found to have K_i < 20 nM, and four of them had single-digit nanomolar affinity (**10a**, **10d**, **10f**, and **10g**, K_i = 6.9, 7.1, 9.1, and 5.5 nM, respectively). Several 4a-substituted 1,2,3,4,4a,9a-hexahydrobenzofuro[2,3-*c*]pyridin-6-ols have been found that interact with δ -receptors (Table 1). The combination of the moderate bulk at C4a combined with N-aralkyl (**10a** and **10d**), *N-p*- and *o*-fluorophenethyl-substituted (**10b**, **10c** and **10e** (the *p*-trifluoromethylphenethyl compound)), or N-allyl (**10f**) moieties, and, especially, with *N*-cyclopropylmethyl-substitution, appeared to have enabled at least some interaction with suitable amino acids in the δ -receptor. With the moderately bulky group, the phenylpropyl moiety, at both C4a and on the nitrogen atom (**10d**), the δ -selectivity was greatest ($\mu/\delta > 10$, $\mu/\kappa > 12$). Thus, 2,4a-bis(3-phenylpropyl)-1,2,3,4,4a,9a-hexahydrobenzofuro[2,3-*c*]pyridin-6-ol (**10d**) was the most selective ligand for the δ -receptor among all of the new compounds. It was found to be a δ -antagonist in the functional [35 S]GTP- γ -S assay and had no μ - or κ -agonist activity, but was not very efficacious (K_e = 103 nM).

Five of the compounds synthesized with a C4a-phenylbutyl substituent (Table 1, **11a-11i**), those combined with a phenethyl (**11a**), a *p*- or *o*-fluorophenethyl (**11b**, **11c**), the *p*-trifluoromethylphenethyl (**11e**), and cyclopropylmethyl (**11f**) substituent on nitrogen were found to have good affinity for the μ -receptor, with K_i 's ranging between 4 and 14 nM. Four of those compounds were found to interact well with the δ -receptor, with K_i 's between 7 and 21 nM (**11a**, **11b**, **11e** and **11f**), and one had high affinity and moderate selectivity for the κ -receptor (**11f**, K_i = 1.6 nM). Most notably, 2-phenethyl-4a-(4-phenylbutyl)-1,2,3,4,4a,9a-hexahydrobenzofuro[2,3-*c*]pyridin-6-ol (**11a**) showed good and equal affinity for both μ - and δ -receptors (K_i = 7 nM), coupled with only a little affinity ($K_i > 100$ nM) for the κ -receptor (μ or $\delta/\kappa > 15$).

Compounds in the C4a-phenylpentyl series (Table 1, **12a-12f**) were found to have the highest affinity for the μ -receptor. Five of the 6 compounds, all but **12f**, had single-digit K_i 's, between 3 and 6 nM (compounds with an N-phenethyl (**12a**), a *p*- or *o*-fluorophenethyl (**12b**, **12c**), the *p*-trifluoromethylphenethyl (**12d**), and cyclopropylmethyl (**12e**). These were the same N-substituents found to have good affinity in the C4a-phenylbutyl series – but in the C4a-phenylpentyl series they had even higher affinity for the μ -receptor. However, none of the C4a-phenylpentyl compounds had appreciable δ -receptor affinity, all K_i 's were found to be > 20 nM. The N-cyclopropylmethyl (**12e**) compound also had high κ -receptor affinity (K_i = 3.7 nM).

2.3 Efficacy of selected compounds ([35 S]GTP- γ -S assay)

Among the C4a-phenylpropyl compounds, only two showed moderate to good efficacy as μ - and δ -receptor antagonists (Table 2), the *N*-allyl compound, **10f**, and the *N*-

cyclopropylmethyl compound **10g**. The N-allyl substituted compound **10f** was more efficacious at the δ - than the μ -receptor ($K_e = 14$ and 47 nM, respectively), and had perhaps 3-fold μ/κ selectivity. The compound had a small amount of undesired agonist activity at the κ -receptor (Table 2). κ -Receptor agonist activity has been known to induce dysphoric effects in humans.[13] The *N*-cyclopropylmethyl compound **10g** had considerably greater activity as μ - and δ -receptor antagonists than **10f** (K_e at μ - and $\delta = 9$ and 4 nM, respectively). Although the μ/κ selectivity was similar to that in **10f**, it showed an $ED_{50} = 32$ nM ($E_{max} = 91$) as a κ -agonist.

Of those examined in the C4a-phenylbutyl series only the *N*-cyclopropylmethyl compound **11f** had moderate efficacy as a μ -antagonist ($K_e = 23$ nM), less efficacy as a δ -antagonist ($K_e = 64$ nM), and was about equally efficacious at κ , but as a κ -agonist ($ED_{50} = 35$ nM ($E_{max} = 83\%$)). The *N*-cyclopropyl compound **12e** had similar activity to **11f**. It's efficacy was moderate ($K_e = 21$ and 35 nM) as an antagonist at μ - and δ , and showed the highest activity at κ as an agonist ($ED_{50} = 13$ nM ($E_{max} = 70\%$)). Regardless of the substituent at C4a, the *N*-cyclopropylmethyl compounds were all κ -agonists, no κ -antagonist was found.

2.4. Conclusion

We evaluated the effect of different C4a substituents and varied the substituent at the *N*-position of the benzofuropyridin-6-ol series of compounds. We obtained δ -antagonists with high or moderate efficacy (**10f**, **10g**, and **12e**), and these also had μ -antagonist activity. Efforts are underway to further modify the promising compounds through optical resolution of these racemic compounds, attempting to remove κ -agonist activity that exists in the efficacious compounds by modifying the *N*-substituent, and examining as yet untried moieties at the C4a position. The results from that study will be reported in due course.

3. Experimental

3.1 Chemistry

Melting points were determined on a Buchi B-545 instrument and are uncorrected. Proton and carbon nuclear magnetic resonance (1H and ^{13}C NMR) spectra were recorded in $CDCl_3$ with tetramethylsilane (TMS) as the internal standard on a Varian Gemini-300 spectrometer and a Bruker DMX500 wide-bore spectrometer ((proton frequency 500.13 MHz) running XWINNMR v3.1, carbon 125.757) in $CDCl_3$ (unless otherwise noted) with the values given in ppm (TMS as internal standard) and J (Hz) assignments of 1H resonance coupling. Mass spectra (HRMS) were recorded on a VG 7070E spectrometer or a JEOL SX102a mass spectrometer. Thin layer chromatography (TLC) analyses were carried out on Analtech silica gel GHLF 0.25 mm plates using various gradients of $CHCl_3$ / MeOH containing 1% NH_4OH or gradients of EtOAc : *n*-hexane. Visualization was accomplished under UV light or by staining in an iodine chamber. Flash column chromatography was performed with Flukasilica gel 60 (mesh 220-400). Atlantic Microlabs, Inc., Norcross, GA, or Micro-Analysis, Inc., Wilmington, DE, performed elemental analyses, and the results were within $\pm 0.4\%$ of the theoretical values.

3.1.1 Method A: General procedure[12] to synthesize acetate 8a-8c—Compound **7** (1.0 equiv) was treated with acetic anhydride (5.0 equiv) and heated to reflux. After completion of the reaction the acetic anhydride was evaporated off to give a sticky solid. This solid was applied to a silica-gel column and the desired product was eluted using 75% EtOAc in hexanes to give a sticky brown solid **8a-8c**

3.1.1.1 2-Methyl-4a-(4-phenylpropyl)-1,2,3,4,4a,9a-hexahydrobenzofuro[2,3-c]pyridin-6-yl acetate (8a): Using Method A, **7a**[12] (7.5 g, 22 mmol) gave **8a** (7.4 g, 87%) as a sticky solid. ¹H NMR (500 MHz, CDCl₃) δ 7.26 (dd, *J* = 9.1, 5.6 Hz, 2H), 7.18 (d, *J* = 7.2 Hz, 1H), 7.12 (d, *J* = 7.3 Hz, 2H), 6.83 (d, *J* = 2.3 Hz, 1H), 6.77 (d, *J* = 8.5 Hz, 1H), 6.72 (d, *J* = 2.2 Hz, 1H), 4.48 (t, *J* = 6.0 Hz, 1H), 2.75 (dd, *J* = 12.2, 5.2 Hz, 1H), 2.54 (q, *J* = 8.1 Hz, 2H), 2.38 (dq, *J* = 17.2, 5.9 Hz, 2H), 2.28 (s, 3H), 2.25 (s, 3H), 2.16-2.11 (m, 1H), 2.00 (td, *J* = 7.0, 3.6 Hz, 1H), 1.79-1.70 (m, 2H), 1.58-1.55 (m, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 169.9, 156.4, 144.6, 142.0, 135.0, 128.5, 128.4, 125.9, 121.0, 116.7, 110.8, 85.4, 56.4, 51.8, 46.3, 46.2, 45.6, 38.8, 36.3, 33.0, 25.8, 21.32, 21.22. HRMS (TOF MS ES⁺) calcd for C₂₃H₂₈NO₃ (M + H)⁺ 366.2069, found: 366.2061.

3.1.1.2 2-Methyl-4a-(4-phenylbutyl)-1,2,3,4,4a,9a-hexahydrobenzofuro[2,3-c]pyridin-6-yl acetate (8b): Using Method A, 2.2g of **7b**[12] gave 2.2 g **8b** (89%). ¹H NMR (500 MHz, CDCl₃): δ 7.26-7.24 (m, 2H), 7.14 (dd, *J* = 23.1, 7.1 Hz, 2H), 6.84 (dd, *J* = 8.5, 2.3 Hz, 2H), 6.78-6.76 (m, 2H), 4.47 (t, *J* = 6.0 Hz, 1H), 2.74 (dd, *J* = 12.2, 5.2 Hz, 1H), 2.56 (t, *J* = 7.7 Hz, 2H), 2.37 (ddd, *J* = 18.1, 12.0, 6.3 Hz, 2H), 2.28 (s, 3H), 2.25 (s, 3H), 2.16-2.12 (m, 2H), 2.00 (ddd, *J* = 14.2, 6.1, 3.5 Hz, 2H), 1.82-1.77 (m, 1H), 1.69 (dt, *J* = 10.7, 5.3 Hz, 2H), 1.57 (dt, *J* = 15.7, 7.9 Hz, 2H), 1.29 (dd, *J* = 18.4, 11.3 Hz, 4H). ¹³C NMR (126 MHz, CDCl₃): δ 170.0, 156.4, 144.6, 142.6, 135.2, 128.5, 128.4, 128.4, 125.8, 120.9, 116.6, 110.8, 85.4, 56.5, 51.9, 46.4, 45.7, 39.0, 35.8, 33.1, 32.1, 23.7, 21.3. HRMS (TOF MS ES⁺) calcd for C₂₄H₃₀NO₃(M + H)⁺ : 380.2226; found: 380.2237.

3.1.1.3 2-Methyl-4a-(5-phenylpentyl)-1,2,3,4,4a,9a-hexahydrobenzofuro[2,3-c]pyridin-6-yl acetate (8c): Using Method A, 5.0g of **7c**[12] gave 5.18 g of **8c** (91%). ¹H NMR (500 MHz, CDCl₃): δ 7.28 (s, 1H), 7.25 (s, 1H), 7.18-7.13 (m, 3H), 6.83 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.78-6.75 (m, 2H), 4.47 (t, *J* = 5.8 Hz, 1H), 2.72 (dd, *J* = 12.2, 5.0 Hz, 1H), 2.54 (t, *J* = 7.6 Hz, 2H), 2.38 (t, *J* = 6.1 Hz, 2H), 2.28 (s, 3H), 2.25 (s, 3H), 2.17-2.13 (m, 2H), 1.99 (ddd, *J* = 13.9, 6.0, 3.1 Hz, 1H), 1.85 (t, *J* = 3.1 Hz, 3H), 1.77 (td, *J* = 9.1, 4.6 Hz, 2H), 1.64 (dd, *J* = 7.6, 4.8 Hz, 1H), 1.58-1.53 (m, 1H), 1.53-1.48 (m, 1H), 1.25 (d, *J* = 3.5 Hz, 2H). ¹³C NMR (126 MHz, CDCl₃): δ 170.0, 155.1, 145.3, 142.4, 128.4, 128.23, 125.6, 122.5, 117.2, 117.1, 112.9, 111.4, 82.0, 51.5, 50.1, 44.4, 39.5, 35.8, 31.3, 29.3, 29.0, 23.4, 23.3, 21.2. HRMS (TOF MS ES⁺) calcd for C₂₅H₃₂NO₃ (M + H) : 394.2382; found: 394.2375.

3.1.2 Method B: General procedure for synthesis of compounds 9a-9c—To compound **8** (1.0 equiv) in DCE was added Troc-Cl (1.2 equiv) and proton-sponge (1.2 equiv) and stirred overnight for 12 h. The reaction mixture was then washed with 1N HCl and the mixture was extracted with ethyl acetate. The aqueous layer was washed twice with ethyl acetate and the combined organic extracts were evaporated to give a brown oil. The

crude oil was triturated with hexanes and treated with K_2CO_3 in methanol and stirred for 4h. After completion of the reaction, as indicated by TLC, the mixture was extracted with chloroform, dried over sodium sulfate and used without purification in the next step. The crude oil was taken up in AcOH (7.0 mL) and Zn powder (1.2 eq) was added to it slowly with vigorous stirring. After the reaction was complete (as determined by TLC), the reaction mixture was filtered through a plug of cotton and the cotton was washed with CH_2Cl_2 . Ammonium hydroxide (10.0 mL) was slowly added to the organic solution followed by extraction of the mixture with CH_2Cl_2 . Evaporation of the organic layer followed by trituration of the crude oil with 2-propanol gave **9a-9c** as a sticky brown solid

3.1.2.1 4a-(3-Phenylpropyl)-1,2,3,4,4a,9a-hexahydrobenzofuro[2,3-c]pyridin-6-ol (9a):

Using Method B, 2.2g of **8a** gave 1.7g **9a** (96%). 1H NMR (500 MHz, $CDCl_3$): δ 7.21 (t, J = 7.2 Hz, 2H), 7.11 (t, J = 9.8 Hz, 3H), 6.56 (dd, J = 20.1, 13.3 Hz, 3H), 4.24 (t, J = 4.7 Hz, 1H), 2.98 (dd, J = 13.6, 4.2 Hz, 1H), 2.73 (dd, J = 13.3, 5.8 Hz, 2H), 2.52 (d, J = 14.0 Hz, 3H), 1.83 (d, J = 14.1 Hz, 1H), 1.70-1.51 (m, 5H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 161.9, 154.5, 152.6, 143.4, 133.1, 129.44, 129.36, 126.8, 115.9, 111.6, 86.5, 48.0, 41.3, 40.9, 37.1, 32.9, 26.9. HRMS (TOF MS ES^+) calcd for $C_{20}H_{24}NO_2$ (M + H) $^+$: 310.1807; found: 310.1806.

3.1.2.2 4a-(4-Phenylbutyl)-1,2,3,4,4a,9a-hexahydrobenzofuro[2,3-c]pyridin-6-ol (9b):

Using Method B, 2.2 g of **8b** gave 1.7g **9b** (91%). 1H NMR (500 MHz, $CDCl_3$): δ 7.15 (t, J = 7.5 Hz, 2H), 7.05 (d, J = 7.3 Hz, 3H), 6.55 (dd, J = 19.2, 8.1 Hz, 3H), 4.38 (t, J = 3.7 Hz, 1H), 3.15 (dd, J = 13.9, 3.4 Hz, 1H), 3.06 (t, J = 7.0 Hz, 1H), 2.93-2.90 (m, 1H), 2.63 (dd, J = 9.2, 4.0 Hz, 1H), 2.50 (t, J = 7.5 Hz, 2H), 1.78 (d, J = 4.7 Hz, 2H), 1.66 (ddd, J = 23.9, 12.4, 4.1 Hz, 2H), 1.52 (t, J = 7.5 Hz, 2H), 1.29 (t, J = 6.1 Hz, 1H), 1.18 (t, J = 6.1 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 153.2, 152.7, 143.7, 136.1, 129.4, 115.2, 111.3, 111.1, 84.5, 47.5, 46.5, 42.3, 39.7, 36.7, 34.0, 33.4, 25.2, 24.6. HRMS (TOF MS ES^+) calcd for $C_{21}H_{26}NO_2$ (M + H) $^+$: 324.1964; found: 324.1975.

3.1.2.3 4a-(5-Phenylpentyl)-1,2,3,4,4a,9a-hexahydrobenzofuro[2,3-c]pyridin-6-ol (9c):

Using Method B, 5.0g of **8c** gave 2.5g **9c** (58%). 1H NMR (500 MHz, $CDCl_3$): δ 7.26 (t, J = 7.3 Hz, 3H), 7.15 (dd, J = 16.7, 7.4 Hz, 2H), 6.63 (d, J = 8.2 Hz, 1H), 6.54-6.52 (m, 2H), 4.28 (t, J = 3.8 Hz, 1H), 3.02 (qd, J = 14.6, 3.9 Hz, 2H), 2.77 (d, J = 5.9 Hz, 1H), 2.66 (d, J = 5.0 Hz, 1H), 2.56 (t, J = 7.6 Hz, 2H), 1.69 (q, J = 11.5 Hz, 3H), 1.57 (d, J = 6.9 Hz, 3H), 1.28 (t, J = 6.2 Hz, 4H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 152.6, 150.8, 142.7, 135.8, 128.5, 128.3, 125.8, 114.5, 110.7, 110.3, 83.9, 50.9, 46.3, 46.0, 41.5, 38.4, 36.0, 33.9, 31.4, 30.0, 24.1. HRMS (TOF MS ES^+) calcd for $C_{22}H_{28}NO_2$ (M + H) $^+$: 338.2120; found: 338.2130.

3.1.3 Method C: General procedure for synthesis of compounds 10-12—A

mixture of the amine **9**, alkyl bromide (1.2 equiv) and $NaHCO_3$ (2 equiv) in DMF (20 mL) was heated at 50 °C for 20 h. After cooling to room temperature, the reaction mixture was poured into ice- H_2O , and the product was extracted with Et_2O . The ethereal extracts were washed with saturated NH_4Cl solution, dried over Na_2SO_4 , and the solvent was removed *in vacuo* to afford an oil. The crude oil was subjected to flash chromatography on silica gel using 75% $EtOAc$ in hexanes as the eluent to give the **10-12** as solids.

3.1.3.1 4a-(3-Phenylpropyl)-2-(phenethyl)-1,2,3,4,4a,9a-hexahydrobenzofuro[2,3-c]pyridin-6-ol (10a): Using Method C, 500mg of **9a** gave 220mg compound **10a** (33%). Mp 179-180 °C; ¹H NMR (500 MHz, CDCl₃): δ 7.27-7.24 (m, 5H), 7.15 (td, *J* = 16.6, 7.3 Hz, 5H), 6.66 (d, *J* = 8.4 Hz, 1H), 6.58 (dd, *J* = 8.4, 2.5 Hz, 1H), 6.51 (d, *J* = 2.4 Hz, 1H), 4.47 (t, *J* = 6.2 Hz, 1H), 2.95-2.92 (m, 1H), 2.78 (t, *J* = 8.1 Hz, 2H), 2.55 (dt, *J* = 13.8, 6.7 Hz, 4H), 2.37 (dd, *J* = 11.6, 7.2 Hz, 1H), 2.21 (t, *J* = 9.3 Hz, 1H), 2.03 (dt, *J* = 14.4, 2.7 Hz, 1H), 1.82-1.80 (m, 1H), 1.72 (dd, *J* = 13.1, 9.9 Hz, 2H), 1.57 (q, *J* = 10.6 Hz, 2H). ¹³C NMR (126 MHz, CDCl₃): δ 152.9, 150.1, 142.2, 140.3, 134.9, 128.5, 128.4, 126.2, 126.0, 114.6, 113.6, 113.1, 110.9, 110.8, 85.0, 60.6, 54.6, 49.9, 46.4, 39.6, 36.4, 33.6, 32.7, 25.9. HRMS (TOF MS ES⁺) calcd for C₂₈H₃₂NO₂ (M + H)⁺: 414.2433; found: 414.2432. Anal. calcd for (C₂₈H₃₁NO₂): C, 81.32; H, 7.56; N, 3.39; found: C, 81.17; H, 7.61; N, 3.35.

3.1.3.2 2-(4-Fluorophenethyl)-4a-(3-phenylpropyl)-1,2,3,4,4a,9a-hexahydrobenzofuro[2,3-c]pyridin-6-ol (10b): Using Method C, 800mg of **9a** gave 600mg **10b** (54%). Mp 143-146 °C; ¹H NMR (500 MHz, CDCl₃): δ 7.26 (t, *J* = 5.2 Hz, 3H), 7.18 (d, *J* = 7.2 Hz, 1H), 7.10 (dd, *J* = 12.0, 6.3 Hz, 3H), 6.95 (t, *J* = 8.6 Hz, 2H), 6.66 (d, *J* = 8.4 Hz, 1H), 6.58 (dd, *J* = 8.3, 2.3 Hz, 1H), 6.51 (d, *J* = 2.2 Hz, 1H), 4.47-4.44 (m, 1H), 2.92-2.90 (m, 1H), 2.74 (t, *J* = 7.7 Hz, 2H), 2.53 (dd, *J* = 12.9, 6.2 Hz, 4H), 2.38 (dd, *J* = 7.5, 2.6 Hz, 1H), 2.20 (t, *J* = 10.0 Hz, 1H), 2.04-2.01 (m, 1H), 1.80-1.78 (m, 1H), 1.71 (d, *J* = 12.4 Hz, 2H), 1.57 (d, *J* = 10.4 Hz, 2H). ¹³C NMR (126 MHz, CDCl₃): δ 162.5, 160.5, 152.9, 150.0, 142.1, 135.8, 135.81, 134.9, 130.1, 130.0, 128.5, 128.4, 125.9, 115.3, 115.1, 114.5, 113.0, 110.8, 110.7, 84.9, 60.5, 60.4, 54.5, 49.8, 46.4, 39.4, 36.4, 32.6, 25.8. HRMS (TOF MS ES⁺) calcd for C₂₈H₃₁NFO₂ (M + H)⁺: 432.2339; found: 432.2334. Anal. calcd for (C₂₈H₃₀NFO₂): C, 77.93; H, 7.01; N, 3.25; found: C, 77.56; H, 7.01; N, 3.21.

3.1.3.3 (2-(2-Fluorophenethyl)-4a-(3-phenylpropyl)-1,2,3,4,4a,9a-hexahydrobenzofuro[2,3-c]pyridin-6-ol (10c): Using Method C, 800mg of **9a** gave 530mg **10c** (47%). Mp 196-199 °C; ¹H NMR (500 MHz, CDCl₃): δ 7.25 (d, *J* = 10.3 Hz, 3H), 7.14 (dt, *J* = 24.0, 8.0 Hz, 3H), 7.02 (dd, *J* = 23.8, 8.5 Hz, 3H), 6.65 (d, *J* = 8.4 Hz, 1H), 6.59 (d, *J* = 2.2 Hz, 1H), 6.51 (s, 1H), 4.46 (s, 1H), 2.93 (s, 1H), 2.81 (s, 3H), 2.57-2.52 (m, 3H), 2.38 (s, 1H), 2.24-2.22 (m, 1H), 2.04-2.01 (m, 1H), 1.80-1.79 (m, 2H), 1.73-1.68 (m, 2H), 1.62-1.53 (m, 2H). ¹³C NMR (126 MHz, CDCl₃): δ 162.2, 160.3, 153.0, 150.0, 142.2, 131.1, 131.0, 128.5, 128.4, 128.0, 127.9, 127.8, 125.9, 124.2, 124.1, 115.4, 115.28, 114.5, 110.8, 85.3, 58.7, 54.4, 49.7, 46.3, 39.5, 36.3, 32.5, 26.7. HRMS (TOF MS ES⁺) calcd for C₂₈H₃₁NFO₂ (M + H)⁺: 432.2339; found: 432.2334. Anal. calcd for (C₂₈H₃₀NFO₂·0.25 H₂O): C, 77.12; H, 7.05; N, 3.21; found: C, 77.37; H, 7.05; N, 3.21.

3.1.3.4 2,4a-Bis(3-phenylpropyl)-1,2,3,4,4a,9a-hexahydrobenzofuro[2,3-c]pyridin-6-ol (10d): Using Method C, 550mg of **9a** gave 425 mg **10d** (56%). Mp 152-155 °C; ¹H NMR (500 MHz, CDCl₃): δ 7.25 (d, *J* = 5.2 Hz, 5H), 7.14 (td, *J* = 16.5, 7.1 Hz, 5H), 6.63 (d, *J* = 8.4 Hz, 1H), 6.56 (dd, *J* = 8.4, 2.6 Hz, 1H), 6.49 (d, *J* = 2.5 Hz, 1H), 4.41 (t, *J* = 6.3 Hz, 1H), 2.84-2.81 (m, 1H), 2.58 (t, *J* = 7.7 Hz, 3H), 2.51 (dd, *J* = 13.3, 7.6 Hz, 4H), 2.32 (d, *J* = 6.6 Hz, 2H), 2.24 (t, *J* = 6.0 Hz, 1H), 2.10-2.05 (m, 1H), 2.01-1.97 (m, 1H), 1.80-1.67 (m, 4H), 1.59-1.52 (m, 8H). ¹³C NMR (126 MHz, CDCl₃): δ 150.7, 142.1, 141.8, 134.4, 128.5, 128.4, 128.3, 125.9, 125.8, 114.7, 111.0, 110.6, 84.5, 57.9, 49.7, 46.3, 39.7, 36.3, 33.7, 32.2, 32.0,

28.1, 25.7. HRMS (TOF MS ES⁺) calcd for C₂₉H₃₄NO₂ (M + H)⁺: 428.2590; found: 428.2573. Anal. calcd for (C₂₉H₃₃NO₂.0.25 H₂O): C, 80.61; H, 7.81; N, 3.24; found: C, 80.65; H, 7.68; N, 3.26.

3.1.3.5 4a-(3-Phenylpropyl)-2-(4-(trifluoromethyl)phenethyl)-1,2,3,4,4a,

9ahexahydrobenzofuro[2,3-c]pyridin-6-ol (10e): Using Method C, 500 mg of **9a** gave 250 mg **10e** (32%). Mp 139-142 °C; ¹H NMR (500 MHz, CDCl₃): δ 7.52 (d, *J* = 7.7 Hz, 2H), 7.26 (d, *J* = 7.9 Hz, 3H), 7.18 (d, *J* = 7.0 Hz, 2H), 7.11 (d, *J* = 7.2 Hz, 2H), 6.66 (d, *J* = 8.3 Hz, 1H), 6.59-6.58 (m, 1H), 6.52 (s, 1H), 4.45 (t, *J* = 5.8 Hz, 1H), 2.91 (t, *J* = 5.7 Hz, 1H), 2.82 (t, *J* = 7.7 Hz, 2H), 2.55 (dt, *J* = 11.3, 6.2 Hz, 4H), 2.39 (dd, *J* = 11.6, 7.2 Hz, 1H), 2.21 (t, *J* = 13.9 Hz, 1H), 2.04-2.01 (m, 1H), 1.80-1.69 (m, 3H), 1.57 (q, *J* = 10.6 Hz, 2H). ¹³C NMR (126 MHz, CDCl₃): δ 152.9, 150.0, 144.4, 142.1, 134.9, 129.1, 128.51, 128.4, 125.9, 125.44, 125.41, 123.3, 114.5, 113.0, 110.8, 110.7, 84.9, 59.9, 54.5, 49.8, 46.4, 39.4, 36.4, 33.4, 32.6, 25.8. HRMS (TOF MS ES⁺) calcd for C₂₉H₃₁NF₃O₂ (M + H)⁺: 482.2307; found: 482.2295. Anal. calcd for (C₂₉H₃₀NO₂F₃.0.25 H₂O): C, 71.66; H, 6.32; N, 2.88; found: C, 71.58; H, 6.38; N, 2.91.

3.1.3.6 2-Allyl-4a-phenethyl-1,2,3,4,4a,9a-hexahydrobenzofuro[2,3-c]pyridin-6-ol (10f):

Using Method C, 500mg of **9a** gave 125mg **10f** (25%). Mp 143-148 °C; ¹H NMR (500 MHz, CDCl₃): δ 7.19-7.17 (m, 2H), 7.10 (d, *J* = 7.0 Hz, 2H), 7.04 (d, *J* = 7.2 Hz, 1H), 6.57 (d, *J* = 8.4 Hz, 1H), 6.52-6.50 (m, 1H), 6.43 (d, *J* = 2.2 Hz, 1H), 5.79-5.74 (m, 1H), 5.08-5.05 (m, 2H), 4.36 (t, *J* = 6.2 Hz, 1H), 2.88 (t, *J* = 6.9 Hz, 2H), 2.77-2.74 (m, 1H), 2.46-2.41 (m, 3H), 2.19 (dd, *J* = 12.2, 7.2 Hz, 1H), 2.05-2.01 (m, 1H), 1.94-1.91 (m, 1H), 1.68-1.60 (m, 2H), 1.52-1.46 (m, 2H). ¹³C NMR (126 MHz, CDCl₃): δ 152.9, 149.9, 142.2, 135.0, 134.8, 128.5, 128.4, 125.9, 118.4, 114.4, 113.0, 110.8, 85.0, 61.8, 54.4, 49.7, 46.3, 39.4, 36.4, 32.7, 25.8. HRMS (TOF MS ES⁺) calcd for C₂₃H₂₈NO₂ (M + H)⁺: 350.2120; found: 350.2117. Anal. calcd for (C₂₃H₂₇NO₂.0.25 H₂O): C, 78.04; H, 7.83; N, 3.96; found: C, 78.34; H, 7.83; N, 3.96.

3.1.3.7 2-(Cyclopropylmethyl)-4a-phenylpropyl-1,2,3,4,4a,9a-

hexahydrobenzofuro[2,3-c]pyridin-6-ol (10g): Using Method C, 500mg of **9a** gave 200mg **10g** (34%). Mp 166-169 °C; ¹H NMR (500 MHz, CDCl₃): δ 7.24 (s, 2H), 7.17 (d, *J* = 6.8 Hz, 1H), 7.11 (d, *J* = 6.9 Hz, 2H), 6.65 (d, *J* = 8.1 Hz, 1H), 6.58 (d, *J* = 6.9 Hz, 1H), 6.51 (s, 1H), 4.46 (s, 1H), 2.98-2.96 (m, 1H), 2.64-2.60 (m, 1H), 2.52 (d, *J* = 5.3 Hz, 3H), 2.33-2.16 (m, 5H), 2.02 (dd, *J* = 12.5, 0.6 Hz, 1H), 1.79 (s, 1H), 1.71-1.52 (m, 3H), 0.85-0.83 (m, 1H), 0.50 (d, *J* = 7.0 Hz, 2H), 0.07 (s, 2H). ¹³C NMR (126 MHz, CDCl₃): δ 153.0, 149.9, 142.2, 135.0, 135.0, 128.5, 128.4, 125.9, 114.4, 113.0, 110.8, 110.7, 85.1, 63.7, 54.2, 50.0, 48.7, 46.4, 39.5, 36.4, 32.5, 25.8, 8.2, 4.3. HRMS (TOF MS ES⁺) calcd for C₂₄H₃₀NO₂ (M + H)⁺: 364.2277; found: 364.2292. Anal. calcd for (C₂₄H₂₉NO₂): C, 79.30; H, 8.04; N, 3.85; found: C, 79.09; H, 7.93; N, 3.79.

3.1.3.8 4a-(3-Phenylpropyl)-2-(2-(trifluoromethyl)phenethyl)-1,2,3,4,4a,

9ahexahydrobenzofuro[2,3-c]pyridin-6-ol (10h): Using Method C, 350mg of **9a** gave 122 mg **10h** (22%). Mp 140-143 °C; ¹H NMR (500 MHz, CDCl₃): δ 7.60 (d, *J* = 7.8 Hz, 1H), 7.43 (d, *J* = 7.6 Hz, 2H), 7.30-7.24 (m, 3H), 7.17 (s, 1H), 7.11 (d, *J* = 7.4 Hz, 2H), 6.65 (d, *J*

= 8.3 Hz, 1H), 6.59-6.57 (m, 1H), 6.52 (s, 1H), 4.47 (s, 1H), 2.94-2.93 (m, 3H), 2.58-2.52 (m, 6H), 2.41 (dd, $J = 1.9, 0.6$ Hz, 1H), 2.24 (t, $J = 9.7$ Hz, 1H), 2.02 (d, $J = 14.0$ Hz, 1H), 1.81-1.78 (m, 1H), 1.72-1.68 (m, 1H), 1.57 (d, $J = 11.2$ Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3): δ 152.9, 150.1, 142.2, 138.8, 134.8, 131.8, 128.5, 128.4, 126.3, 126.11, 125.9, 123.6, 114.6, 110.8, 110.8, 84.9, 63.4, 60.2, 49.7, 46.4, 39.5, 36.4, 32.6, 30.0, 25.8. HRMS (TOF MS ES^+) calcd for $\text{C}_{29}\text{H}_{31}\text{NF}_3\text{O}_2$ ($\text{M} + \text{H}$) $^+$: 482.2307; found: 482.2283. Anal. calcd for ($\text{C}_{29}\text{H}_{30}\text{NF}_3\text{O}_2$): C, 72.33; H, 6.28; N, 2.91; found: C, 72.04; H, 6.17; N, 2.84.

3.1.3.9 4a-(3-Phenylpropyl)-2-(3-(trifluoromethyl)phenethyl)-1,2,3,4,4a,9a-

hexahydrobenzofuro[2,3-c]pyridin-6-ol (10i): Using Method C, 400 mg of **9a** gave 170mg **10i** (27%). Mp 149-152 °C; ^1H NMR (500 MHz, CDCl_3): δ 7.45-7.41 (m, 2H), 7.35 (d, $J = 18.0$ Hz, 2H), 7.25 (d, $J = 10.5$ Hz, 2H), 7.16 (dd, $J = 14.4, 7.1$ Hz, 2H), 7.11 (d, $J = 7.2$ Hz, 1H), 6.65 (d, $J = 8.4$ Hz, 1H), 6.59 (s, 1H), 6.51 (d, $J = 2.1$ Hz, 1H), 4.45 (d, $J = 5.6$ Hz, 1H), 2.90 (dt, $J = 1.9, 0.9$ Hz, 2H), 2.82 (t, $J = 7.5$ Hz, 2H), 2.55 (dt, $J = 14.3, 6.8$ Hz, 3H), 2.41-2.39 (m, 1H), 2.22 (t, $J = 9.2$ Hz, 1H), 2.01 (d, $J = 13.5$ Hz, 1H), 1.79-1.78 (m, 2H), 1.71-1.66 (m, 2H), 1.57 (t, $J = 10.0$ Hz, 2H). ^{13}C NMR (126 MHz, CDCl_3): δ 153.1, 150.4, 142.4, 132.5, 129.2, 128.8, 128.7, 126.2, 125.8, 125.7, 123.4, 123.4, 114.8, 111.1, 111.0, 85.2, 60.3, 54.7, 50.1, 46.7, 39.7, 36.7, 33.6, 32.9, 26.1. HRMS (TOF MS ES^+) calcd for $\text{C}_{29}\text{H}_{31}\text{NF}_3\text{O}_2$ ($\text{M} + \text{H}$) $^+$: 482.2307; found: 482.2283. Anal. calcd for ($\text{C}_{29}\text{H}_{30}\text{NF}_3\text{O}_2$): C, 72.33; H, 6.28; N, 2.91; found: C, 72.18; H, 6.29; N, 2.83.

3.1.3.10 2-Phenethyl-4a-(4-phenylbutyl)-1,2,3,4,4a,9a-hexahydrobenzofuro[2,3-

c]pyridin-6-ol (11a): Using Method C, 200mg of **9b** gave 170mg of **11a** (64%). Mp 179-182 °C; ^1H NMR (500 MHz, CDCl_3): δ 7.29-7.25 (m, 4H), 7.21-7.15 (m, 3H), 7.12 (d, $J = 7.1$ Hz, 3H), 6.67 (d, $J = 8.4$ Hz, 1H), 6.60 (dd, $J = 8.3, 2.5$ Hz, 1H), 6.56 (d, $J = 2.4$ Hz, 1H), 4.48 (t, $J = 6.3$ Hz, 1H), 2.95 (dd, $J = 12.0, 5.6$ Hz, 1H), 2.80 (t, $J = 8.2$ Hz, 2H), 2.57 (td, $J = 13.9, 5.9$ Hz, 3H), 2.39 (dd, $J = 12.0, 7.3$ Hz, 1H), 2.23 (t, $J = 9.2$ Hz, 1H), 2.05-2.02 (m, 2H), 1.83 (td, $J = 9.3, 4.5$ Hz, 1H), 1.69 (dt, $J = 12.1, 6.1$ Hz, 2H), 1.55 (t, $J = 6.9$ Hz, 3H), 1.29 (dt, $J = 12.0, 5.7$ Hz, 2H). ^{13}C NMR (126 MHz, CDCl_3): δ 152.9, 150.1, 142.6, 140.2, 135.1, 128.8, 128.6, 128.5, 128.4, 128.3, 126.2, 125.8, 114.5, 110.9, 110.8, 85.0, 60.6, 54.6, 49.9, 46.5, 39.7, 35.8, 33.5, 32.5, 32.1, 23.7. HRMS (TOF MS ES^+) calcd for $\text{C}_{29}\text{H}_{34}\text{NO}_2$ ($\text{M} + \text{H}$) $^+$: 428.2590; found: 428.2592. Anal. calcd for ($\text{C}_{29}\text{H}_{33}\text{NO}_2$): C, 81.46; H, 7.78; N, 3.28; found: C, 81.06; H, 7.57; N, 3.14.

3.1.3.11 2-(4-Fluorophenethyl)-4a-(4-phenylbutyl)-1,2,3,4,4a,9a-

hexahydrobenzofuro[2,3-c]pyridin-6-ol (11b): Using Method C, 300mg of **9b** gave 170mg **11b** (41%). Mp 147-149 °C; ^1H NMR (500 MHz, CD_3OD): δ 7.22-7.20 (m, 3H), 7.13 (d, $J = 7.2$ Hz, 2H), 7.06 (t, $J = 7.9$ Hz, 2H), 6.90 (t, $J = 8.6$ Hz, 2H), 6.62 (d, $J = 8.4$ Hz, 1H), 6.55 (dd, $J = 8.4, 2.4$ Hz, 1H), 6.50 (d, $J = 2.2$ Hz, 1H), 4.41 (t, $J = 6.1$ Hz, 1H), 2.87 (dd, $J = 11.7, 5.2$ Hz, 1H), 2.71 (t, $J = 8.1$ Hz, 2H), 2.50 (t, $J = 7.8$ Hz, 3H), 2.33 (dd, $J = 11.9, 7.2$ Hz, 1H), 2.19-2.15 (m, 1H), 2.00-1.97 (m, 1H), 1.77 (dt, $J = 10.9, 5.9$ Hz, 1H), 1.64 (dt, $J = 12.2, 6.2$ Hz, 2H), 1.50 (t, $J = 7.8$ Hz, 2H), 1.25 (dt, $J = 11.8, 6.2$ Hz, 2H), 1.17 (d, $J = 6.1$ Hz, 2H). ^{13}C NMR (126 MHz; CDCl_3): δ 162.5, 160.6, 152.9, 150.0, 142.6, 135.9, 135.1, 130.2, 130.1, 128.5, 128.4, 125.8, 115.4, 115.2, 114.5, 110.9, 110.7, 85.0, 64.7, 60.6, 54.6, 49.9, 46.5, 35.7, 32.7, 32.5, 32.1, 32.0, 23.6. HRMS (TOF MS ES^+) calcd

for $C_{29}H_{33}NFO_2$ ($M + H$)⁺: 446.2495; found: 446.2491. Anal. calcd for ($C_{29}H_{32}NFO_2$): C, 78.17; H, 7.24; N, 3.14; found: C, 77.90; H, 6.88; N, 3.08.

3.1.3.12 2-(2-Fluorophenethyl)-4a-(4-phenylbutyl)-1,2,3,4,4a,9a-

hexahydrobenzofuro[2,3-c]pyridin-6-ol (11c): Using Method C, 300mg of **9b** gave 175mg **11c** (42%). Mp 195-198 °C; ¹H NMR (500 MHz, CDCl₃): δ 7.25 (s, 3H), 7.18-7.12 (m, 4H), 7.06-6.98 (m, 2H), 6.67 (d, *J* = 8.4 Hz, 1H), 6.59 (dd, *J* = 8.4, 2.5 Hz, 1H), 6.55 (d, *J* = 2.4 Hz, 1H), 4.46 (t, *J* = 6.3 Hz, 1H), 2.96-2.92 (m, 1H), 2.82 (t, *J* = 8.0 Hz, 2H), 2.57 (q, *J* = 7.9 Hz, 4H), 2.39 (dd, *J* = 12.0, 7.3 Hz, 1H), 2.25-2.21 (m, 1H), 2.03 (dt, *J* = 11.3, 2.3 Hz, 1H), 1.83-1.79 (m, 1H), 1.70 (d, *J* = 4.3 Hz, 2H), 1.56 (dd, *J* = 15.9, 6.7 Hz, 4H), 1.33-1.26 (m, 2H). ¹³C NMR (126 MHz; CDCl₃): δ 162.2, 160.2, 152.9, 149.8, 142.6, 135.1, 131.1, 131.0, 128.4, 127.9, 125.7, 124.1, 115.4, 115.2, 114.4, 110.8, 110.7, 85.0, 58.8, 54.4, 49.7, 46.4, 39.6, 35.8, 32.5, 32.1, 26.7, 23.6. HRMS (TOF MS ES⁺) calcd for $C_{29}H_{33}NFO_2$ ($M + H$)⁺: 446.2495; found: 446.2491. Anal. calcd for ($C_{29}H_{32}NFO_2$): C, 78.17; H, 7.24; N, 3.14; found: C, 78.01; H, 7.22; N, 3.03.

3.1.3.13 4a-(4-Phenylbutyl)-2-(3-phenylpropyl)-1,2,3,4,4a,9a-hexahydrobenzofuro[2,3-c]pyridin-6-ol (11d): Using Method C, 200mg of **9b** gave 90mg of **11d** (33%). Mp 137-140 °C; ¹H NMR (500 MHz, CDCl₃): δ 7.33 (q, *J* = 7.5 Hz, 3H), 7.27 (dd, *J* = 10.0, 7.9 Hz, 3H), 7.22 (t, *J* = 6.7 Hz, 2H), 7.17 (d, *J* = 7.3 Hz, 2H), 6.72 (d, *J* = 8.1 Hz, 1H), 6.65-6.63 (m, 2H), 4.54 (t, *J* = 6.5 Hz, 1H), 2.98 (dd, *J* = 11.9, 5.4 Hz, 1H), 2.79 (t, *J* = 7.7 Hz, 1H), 2.62 (dt, *J* = 31.8, 7.7 Hz, 4H), 2.43 (dd, *J* = 8.4, 4.5 Hz, 2H), 2.33 (dd, *J* = 12.0, 7.6 Hz, 1H), 2.18 (t, *J* = 9.5 Hz, 1H), 2.09-2.06 (m, 2H), 1.98 (t, *J* = 7.6 Hz, 1H), 1.90 (t, *J* = 7.6 Hz, 2H), 1.72 (dt, *J* = 12.4, 6.2 Hz, 1H), 1.58 (t, *J* = 7.7 Hz, 2H), 1.35 (d, *J* = 5.5 Hz, 2H), 1.30 (d, *J* = 6.2 Hz, 1H). ¹³C NMR (126 MHz, CDCl₃): δ 152.3, 150.6, 142.5, 141.8, 134.6, 128.5, 128.4, 128.3, 125.92, 125.8, 125.7, 114.6, 111.1, 110.6, 84.6, 62.2, 57.9, 54.4, 49.8, 46.4, 39.9, 35.8, 34.2, 33.7, 32.1, 32.0, 31.8, 28.1, 23.5. HRMS (TOF MS ES⁺) calcd for $C_{30}H_{36}NO_2$ ($M + H$)⁺: 442.2746; found: 442.2758. Anal. calcd for ($C_{30}H_{35}NO_2 \cdot 0.25 H_2O$): C, 80.77; H, 8.02; N, 3.14; found: C, 81.03; H, 7.68; N, 3.12.

3.1.3.14 4a-(4-Phenylbutyl)-2-(4-(trifluoromethyl)phenethyl)-1,2,3,4,4a,9a-

hexahydrobenzofuro[2,3-c]pyridin-6-ol (11e): Using Method C, 300mg of **9b** gave 200mg **11e** (44%). Mp 157-160 °C; ¹H NMR (500 MHz, CDCl₃): δ 7.52 (d, *J* = 7.9 Hz, 2H), 7.26 (t, *J* = 7.9 Hz, 4H), 7.17 (t, *J* = 7.3 Hz, 1H), 7.13 (t, *J* = 10.7 Hz, 2H), 6.67 (d, *J* = 8.4 Hz, 1H), 6.59 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.54 (d, *J* = 2.0 Hz, 1H), 4.45 (t, *J* = 6.1 Hz, 1H), 2.90 (dd, *J* = 12.0, 5.1 Hz, 1H), 2.83 (t, *J* = 8.0 Hz, 2H), 2.56 (dd, *J* = 13.6, 7.7 Hz, 4H), 2.40 (dd, *J* = 12.0, 7.1 Hz, 1H), 2.25-2.18 (m, 1H), 2.03 (dd, *J* = 10.0, 4.5 Hz, 1H), 1.82-1.77 (m, 1H), 1.69 (dt, *J* = 12.3, 6.3 Hz, 1H), 1.56 (td, *J* = 16.5, 9.3 Hz, 5H), 1.32-1.22 (m, 2H), 1.22 (d, *J* = 6.1 Hz, 1H). ¹³C NMR (126 MHz; CD₃OD): δ 152.9, 149.9, 144.5, 142.6, 135.1, 129.1, 128.5, 128.4, 125.8, 125.5, 125.4, 114.4, 110.9, 110.8, 85.0, 60.0, 54.5, 49.9, 46.4, 39.5, 35.8, 33.4, 32.6, 32.1, 23.7. HRMS (TOF MS ES⁺) calcd for $C_{30}H_{33}NO_2F_3$ ($M + H$)⁺: 496.2463; found: 496.2480. Anal. calcd for ($C_{30}H_{32}NO_2F_3 \cdot 0.25 H_2O$): C, 72.05; H, 6.55; N, 2.80; found: C, 72.25; H, 6.58; N, 2.77.

3.1.3.15 2-(Cyclopropylmethyl)-4a-(4-phenylbutyl)-1,2,3,4,4a,9a-

hexahydrobenzofuro[2,3-c]pyridin-6-ol (11f): Using Method C, 250mg **9b** gave 150mg **11f** (51 %). Mp 162-165 °C; ¹H NMR (500 MHz, CD₃OD): δ 7.25 (d, *J* = 6.4 Hz, 2H), 7.16 (t, *J* = 7.3 Hz, 1H), 7.11 (d, *J* = 7.2 Hz, 2H), 6.65 (d, *J* = 8.4 Hz, 1H), 6.59-6.57 (m, 1H), 6.53 (d, *J* = 2.5 Hz, 1H), 4.47 (t, *J* = 6.1 Hz, 1H), 3.00 (d, *J* = 6.3 Hz, 1H), 2.65 (dd, *J* = 2.5, 1.2 Hz, 1H), 2.54 (t, *J* = 7.8 Hz, 3H), 2.34 (d, *J* = 3.4 Hz, 1H), 2.27-2.18 (m, 2H), 2.02 (ddd, *J* = 14.0, 4.9, 3.3 Hz, 1H), 1.83 (d, *J* = 9.4 Hz, 2H), 1.67 (t, *J* = 6.0 Hz, 2H), 1.54 (dd, *J* = 15.5, 7.7 Hz, 2H), 1.31-1.26 (m, 2H), 0.86 (t, *J* = 4.6 Hz, 1H), 0.50 (d, *J* = 7.7 Hz, 2H), 0.07 (s, 2H). ¹³C NMR (126 MHz; CDCl₃): δ 152.8, 150.1, 142.6, 135.1, 128.4, 128.3, 125.8, 114.4, 110.9, 110.7, 84.9, 63.7, 54.4, 49.9, 46.5, 39.7, 35.8, 32.3, 32.1, 23.6, 8.1, 4.2, 4.1. HRMS (TOF MS ES⁺) calcd for C₂₅H₃₂NO₂ (M + H)⁺: 378.2433; found: 378.2433. Anal. calcd for (C₂₅H₃₁NO₂): C, 79.54; H, 8.28; N, 3.71; found: C, 81.03; H, 7.68; N, 3.12.

3.1.3.16 4a-(4-Phenylbutyl)-2-(2-(trifluoromethyl)phenethyl)-1,2,3,4,4a,9a-

hexahydrobenzofuro[2,3-c]pyridin-6-ol (11g): Using Method C, 200mg of **9b** gave 105mg **11g** (34%). Mp 161-164 °C; ¹H NMR (500 MHz, CD₃OD): δ 7.57 (d, *J* = 7.7 Hz, 1H), 7.41 (t, *J* = 7.4 Hz, 1H), 7.27-7.21 (m, 4H), 7.11 (dd, *J* = 27.4, 7.2 Hz, 3H), 6.64 (d, *J* = 8.4 Hz, 1H), 6.57-6.55 (m, 1H), 6.52 (d, *J* = 2.3 Hz, 1H), 4.44 (t, *J* = 6.2 Hz, 1H), 2.92 (dd, *J* = 9.5, 6.2 Hz, 3H), 2.54 (td, *J* = 13.4, 6.9 Hz, 4H), 2.39 (dd, *J* = 11.9, 7.1 Hz, 1H), 2.22 (t, *J* = 9.1 Hz, 1H), 2.02-1.99 (m, 1H), 1.79 (dt, *J* = 10.8, 5.8 Hz, 1H), 1.67 (td, *J* = 12.5, 4.8 Hz, 2H), 1.51 (t, *J* = 6.8 Hz, 4H), 1.31-1.23 (m, 2H). ¹³C NMR (126 MHz; CD₃OD): δ 152.9, 150.0, 142.6, 138.9, 135.1, 131.9, 131.8, 128.9, 128.7, 128.5, 128.4, 126.3, 126.2, 126.1, 125.8, 123.6, 114.5, 110.9, 110.8, 85.0, 60.3, 54.4, 49.6, 46.4, 39.7, 35.8, 32.7, 32.1, 30.0, 23.7. HRMS (TOF MS ES⁺) calcd for C₃₀H₃₃NO₂F₃ (M + H)⁺: 496.2463; found: 496.2480. Anal. calcd for (C₃₀H₃₂NO₂F₃): C, 72.71; H, 6.51; N, 2.83; found: C, 72.42; H, 6.46; N, 2.79.

3.1.3.17 2,4a-Bis(4-phenylbutyl)-1,2,3,4,4a,9a-hexahydrobenzofuro[2,3-c]pyridin-6-ol

(11h): Using Method C, 200mg of **9b** gave 125mg **11h** (44%). Mp 168-172 °C; ¹H NMR (500 MHz, CDCl₃): δ 7.26-7.24 (m, 5H), 7.16 (d, *J* = 7.0 Hz, 3H), 7.11 (d, *J* = 7.4 Hz, 2H), 6.65 (d, *J* = 8.3 Hz, 1H), 6.59-6.56 (m, 1H), 6.52 (d, *J* = 1.8 Hz, 1H), 4.43-4.41 (m, 1H), 2.84-2.81 (m, 1H), 2.57 (dt, *J* = 28.9, 7.5 Hz, 3H), 2.48 (d, *J* = 9.0 Hz, 1H), 2.33-2.29 (m, 2H), 2.25-2.21 (m, 2H), 2.09-2.05 (m, 2H), 2.00-1.97 (m, 2H), 1.78-1.75 (m, 1H), 1.68-1.64 (m, 2H), 1.55-1.50 (m, 4H), 1.30-1.26 (m, 2H). ¹³C NMR (126 MHz, CDCl₃): δ 153.3, 150.1, 142.93, 142.83, 135.5, 128.83, 128.75, 128.72, 128.69, 128.64, 126.1, 114.6, 111.18, 111.01, 106.1, 85.5, 58.9, 55.1, 50.3, 46.8, 40.1, 36.22, 36.12, 32.8, 32.4, 29.8, 26.9, 23.9. HRMS (TOF MS ES⁺) calcd for C₃₁H₃₈NO₂ (M + H)⁺: 456.2903; found: 456.2920. Anal. calcd for (C₃₁H₃₇NO₂): C, 81.72; H, 8.19; N, 3.07; found: C, 81.57; H, 8.01; N, 2.93.

3.1.3.18 4a-(4-Phenylbutyl)-2-(3-(trifluoromethyl)phenethyl)-1,2,3,4,4a,9a-

hexahydrobenzofuro[2,3-c]pyridin-6-ol (11i): Using Method C, 350mg of **9b** gave 260mg of **11i** (37%). Mp 128-130 °C; ¹H NMR (500 MHz, CD₃OD): δ 8.21 (tdd, *J* = 24.8, 13.7, 9.9 Hz, 3H), 8.08 (t, *J* = 5.2 Hz, 3H), 8.00 (d, *J* = 7.0 Hz, 1H), 7.94 (d, *J* = 7.4 Hz, 2H), 7.50 (d, *J* = 8.4 Hz, 1H), 7.42 (d, *J* = 8.5 Hz, 1H), 7.38 (d, *J* = 1.0 Hz, 1H), 5.29 (t, *J* = 6.1 Hz, 1H), 3.75 (dd, *J* = 11.9, 5.2 Hz, 1H), 3.65 (q, *J* = 8.4 Hz, 3H), 3.39 (td, *J* = 14.2, 6.0 Hz, 4H), 3.23 (dd, *J* = 11.9, 7.2 Hz, 1H), 3.06 (t, *J* = 9.4 Hz, 1H), 2.85 (d, *J* = 14.2 Hz, 1H), 2.65 (dd,

$J = 10.1, 3.2$ Hz, 1H), 2.52 (td, $J = 12.5, 4.8$ Hz, 2H), 2.37 (dt, $J = 14.6, 7.4$ Hz, 3H), 2.13 (t, $J = 6.6$ Hz, 2H). ^{13}C NMR (126 MHz; CD_3OD): δ 152.8, 150.1, 142.6, 141.2, 135.1, 132.2, 130.9, 130.7, 128.9, 128.5, 125.8, 125.6, 125.5, 123.2, 123.1, 114.5, 110.9, 110.7, 88.2, 84.9, 60.0, 54.5, 54.4, 49.9, 46.4, 39.6, 35.8, 33.3, 32.5, 23.7. HRMS (TOF MS ES^+) calcd for $\text{C}_{30}\text{H}_{33}\text{NO}_2\text{F}_3$ ($\text{M} + \text{H}$): 496.2463; found: 496.2480. Anal. calcd for ($\text{C}_{30}\text{H}_{32}\text{NO}_2\text{F}_3$): C, 72.71; H, 6.51; N, 2.83; found: C, 72.40; H, 6.45; N, 2.90.

3.1.3.19 2-Phenethyl-4a-(5-phenylpentyl)-1,2,3,4,4a,9a-hexahydrobenzofuro[2,3-

c]pyridin-6-ol (12a): Using Method C, 200mg of **9c** gave 150mg **12a** (57%). Mp 153-155 °C; ^1H NMR (500 MHz, CDCl_3): δ 7.26 (d, $J = 1.5$ Hz, 5H), 7.16 (q, $J = 10.2$ Hz, 5H), 6.66 (dd, $J = 8.0, 0.8$ Hz, 1H), 6.59-6.56 (m, 2H), 4.98 (s, 1H), 4.47 (t, $J = 5.3$ Hz, 1H), 2.91 (dd, $J = 11.7, 4.6$ Hz, 1H), 2.79 (t, $J = 8.0$ Hz, 2H), 2.55 (dd, $J = 14.3, 7.4$ Hz, 5H), 2.42-2.39 (m, 1H), 2.25-2.21 (m, 1H), 2.03 (d, $J = 14.1$ Hz, 1H), 1.81-1.77 (m, 1H), 1.66 (s, 3H), 1.55-1.49 (m, 2H), 1.24 (s, 2H). ^{13}C NMR (126 MHz, CDCl_3): δ 152.8, 152.7, 149.9, 149.8, 142.8, 142.7, 140.2, 140.1, 135.2, 128.8, 128.3, 126.2, 126.1, 125.7, 114.3, 110.8, 110.7, 84.9, 60.6, 54.5, 49.9, 46.4, 36.0, 35.9, 33.5, 32.7, 31.5, 29.9, 23.8. HRMS (TOF MS AP^+) calcd for $\text{C}_{30}\text{H}_{36}\text{NO}_2$ ($\text{M} + \text{H}$) $^+$: 442.2740; found: 442.2746. Anal. calcd for ($\text{C}_{30}\text{H}_{35}\text{NO}_2$): C, 81.59; H, 7.99; N, 3.17; found: C, 81.45; H, 8.24; N, 3.00.

3.1.3.20 2-(4-Fluorophenethyl)-4a-(5-phenylpentyl)-1,2,3,4,4a,9a-

hexahydrobenzofuro[2,3-c]pyridin-6-ol (12b): Using Method C, 300mg of **9c** gave 205mg **12b** (50%). Mp 132-135 °C; ^1H NMR (500 MHz, CDCl_3): δ 7.28-7.25 (m, 3H), 7.16-7.09 (m, 4H), 6.96-6.93 (m, 2H), 6.66-6.65 (m, 1H), 6.59-6.55 (m, 2H), 4.51 (s, 1H), 4.45-4.43 (m, 1H), 2.90-2.87 (m, 1H), 2.76-2.73 (m, 2H), 2.57-2.52 (m, 4H), 2.39 (dd, $J = 12.1, 7.5$ Hz, 1H), 2.24-2.17 (m, 1H), 2.01 (d, $J = 14.3$ Hz, 1H), 1.81-1.76 (m, 1H), 1.64 (t, $J = 7.7$ Hz, 1H), 1.56 (s, 4H), 1.50 (t, $J = 8.5$ Hz, 1H), 1.26-1.21 (m, 2H). ^{13}C NMR (126 MHz, CDCl_3): δ 162.5, 153.0, 149.9, 142.8, 136.0, 135.9, 135.3, 130.2, 130.1, 128.5, 128.3, 125.8, 115.3, 115.2, 114.4, 110.8, 110.7, 85.1, 60.6, 54.6, 49.9, 46.4, 39.7, 36.0, 32.9, 32.8, 31.4, 29.9, 23.9. HRMS (TOF MS ES^+) calcd for $\text{C}_{30}\text{H}_{35}\text{NFO}_2$ ($\text{M} + \text{H}$) $^+$: 460.2652; found: 460.2639. Anal. calcd for ($\text{C}_{30}\text{H}_{34}\text{NFO}_2 \cdot 0.25 \text{H}_2\text{O}$): C, 77.64; H, 7.49; N, 3.02; found: C, 77.85; H, 7.27; N, 2.74.

3.1.3.21 2-(2-Fluorophenethyl)-4a-(5-phenylpentyl)-1,2,3,4,4a,9a-

hexahydrobenzofuro[2,3-c]pyridin-6-ol (12c): Using Method C, 250mg of **9c** gave 120mg of **12c** (35%). Mp 180-183 °C; ^1H NMR (500 MHz, CDCl_3): δ 7.26 (d, $J = 5.1$ Hz, 4H), 7.15 (dd, $J = 14.1, 5.7$ Hz, 4H), 7.06-6.98 (m, 2H), 6.66 (d, $J = 8.2$ Hz, 1H), 6.59-6.56 (m, 1H), 4.46 (t, $J = 6.0$ Hz, 1H), 2.94-2.90 (m, 1H), 2.82 (t, $J = 7.8$ Hz, 2H), 2.55 (t, $J = 7.6$ Hz, 4H), 2.40 (dd, $J = 11.9, 7.0$ Hz, 1H), 2.23-2.22 (m, 1H), 2.18 (d, $J = 5.3$ Hz, 1H), 2.03-2.01 (m, 1H), 1.79-1.77 (m, 1H), 1.63 (s, 3H), 1.56-1.49 (m, 2H), 1.24 (s, 2H). ^{13}C NMR (126 MHz; CDCl_3): δ 168.0, 162.2, 160.3, 152.4, 150.8, 142.7, 134.8, 132.6, 131.1, 128.5, 126.9, 125.7, 124.2, 115.4, 114.6, 111.0, 110.7, 84.7, 68.3, 58.7, 54.2, 49.7, 36.0, 32.3, 31.4, 30.5, 29.1, 26.6, 23.8. HRMS (TOF MS ES^+) calcd for $\text{C}_{30}\text{H}_{35}\text{NFO}_2$ ($\text{M} + \text{H}$): 460.2652; found: 460.2639. Anal. calcd for ($\text{C}_{30}\text{H}_{34}\text{NFO}_2 \cdot 0.25 \text{H}_2\text{O}$): C, 77.64; H, 7.49; N, 3.02; found: C, 77.75; H, 7.39; N, 2.98.

3.1.3.22 4a-(5-Phenylpentyl)-2-(4-(trifluoromethyl)phenethyl)-1,2,3,4,4a,

9ahexahydrobenzofuro[2,3-c]pyridin-6-ol (12d): Using Method C, 300mg of **9c** gave 115mg of **12d** (25%). Mp 124-127 °C; ¹H NMR (500 MHz, CDCl₃): δ 7.51 (d, *J* = 7.8 Hz, 2H), 7.26 (t, *J* = 8.8 Hz, 4H), 7.14 (t, *J* = 11.4 Hz, 3H), 6.66 (d, *J* = 8.2 Hz, 1H), 6.57 (d, *J* = 10.7 Hz, 2H), 4.46 (t, *J* = 5.6 Hz, 1H), 2.87 (dt, *J* = 33.8, 6.6 Hz, 3H), 2.55 (q, *J* = 8.1 Hz, 6H), 2.43 (dt, *J* = 8.7, 4.5 Hz, 1H), 2.24 (t, *J* = 9.0 Hz, 1H), 2.03-2.00 (m, 1H), 1.79 (t, *J* = 9.6 Hz, 1H), 1.63 (d, *J* = 9.4 Hz, 2H), 1.55-1.49 (m, 2H), 1.24 (d, *J* = 2.1 Hz, 2H). ¹³C NMR (126 MHz, CDCl₃): δ 152.6, 150.0, 144.2, 142.7, 135.1, 129.1, 128.5, 128.3, 125.7, 125.4, 125.4, 123.3, 114.4, 110.8, 110.7, 84.7, 69.1, 60.1, 54.3, 49.8, 46.2, 39.3, 36.0, 33.3, 32.6, 31.5, 29.9, 23.9. HRMS (TOF MS ES⁺) calcd for C₃₁H₃₅NO₂F₃ (M + H)⁺: 510.2620; found: 510.2618. Anal. calcd for (C₃₁H₃₄NO₂F₃): C, 73.06; H, 6.72; N, 2.75; found: C, 72.68; H, 6.67; N, 2.63.

3.1.3.23 2-(Cyclopropylmethyl)-4a-(5-phenylpentyl)-1,2,3,4,4a,9a-

hexahydrobenzofuro[2,3-c]pyridin-6-ol (12e): Using Method C, 150mg of **9c** gave 72mg of **12e** (41%). Mp 141-144 °C; ¹H NMR (500 MHz, CDCl₃): δ 7.26 (t, *J* = 7.3 Hz, 3H), 7.15 (dd, *J* = 18.8, 7.6 Hz, 2H), 6.64 (d, *J* = 8.2 Hz, 1H), 6.56 (dd, *J* = 11.2, 2.9 Hz, 2H), 4.45 (t, *J* = 6.3 Hz, 1H), 2.96 (dd, *J* = 12.0, 5.4 Hz, 1H), 2.62 (d, *J* = 6.1 Hz, 1H), 2.54 (t, *J* = 7.6 Hz, 3H), 2.34 (dd, *J* = 12.1, 7.3 Hz, 2H), 2.25-2.18 (m, 3H), 2.03-1.99 (m, 1H), 1.82-1.77 (m, 1H), 1.61-1.55 (m, 4H), 1.50-1.48 (m, 1H), 1.25 (s, 3H), 0.86-0.83 (m, 1H), 0.50 (d, *J* = 8.0 Hz, 1H), 0.06 (d, *J* = 3.2 Hz, 1H). ¹³C NMR (126 MHz, CDCl₃): δ 153.0, 149.9, 142.8, 135.3, 128.5, 128.3, 125.7, 114.3, 110.8, 110.7, 85.1, 63.8, 54.5, 50.0, 46.4, 39.8, 36.0, 32.6, 31.4, 29.9, 23.9, 8.2, 4.19, 4.07. HRMS (MS AP⁺) calcd for C₂₆H₃₄NO₂(M + H)⁺: 392.2590; found: 392.2586. Anal. calcd for (C₂₆H₃₃NO₂): C, 79.76; H, 8.50; N, 3.58; found: C, 79.40; H, 8.49; N, 3.45.

3.1.3.24 4a-(5-Phenylpentyl)-2-(2-(trifluoromethyl)phenethyl)-1,2,3,4,4a,

9ahexahydrobenzofuro[2,3-c]pyridin-6-ol (12f): Using Method C, 200mg of **9c** gave 105mg of **12f** (35%). Mp 138-141 °C; ¹H NMR (500 MHz, CDCl₃): δ 7.60 (d, *J* = 7.6 Hz, 1H), 7.44 (t, *J* = 7.4 Hz, 1H), 7.27 (q, *J* = 9.7 Hz, 4H), 7.15 (dd, *J* = 18.7, 7.3 Hz, 3H), 6.65 (d, *J* = 8.4 Hz, 1H), 6.59 (dd, *J* = 11.9, 3.4 Hz, 2H), 4.47 (t, *J* = 5.8 Hz, 1H), 2.95 (d, *J* = 6.1 Hz, 3H), 2.60-2.53 (m, 5H), 2.46-2.43 (m, 1H), 2.26 (t, *J* = 7.8 Hz, 1H), 2.04-2.01 (m, 1H), 1.81 (s, 1H), 1.62-1.49 (m, 4H), 1.26 (s, 4H), 0.89-0.88 (m, 1H). ¹³C NMR (126 MHz, CDCl₃): δ 152.6, 152.4, 150.3, 150.2, 142.8, 142.7, 138.7, 135.0, 131.9, 128.8, 128.6, 126.4, 126.3, 126.1, 126.0, 125.7, 125.6, 123.5, 114.5, 110.8, 110.7, 84.8, 60.3, 54.2, 49.6, 46.3, 39.7, 39.5, 36.0, 32.4, 31.5, 29.8, 23.8. HRMS (TOF MS ES⁺) calcd for C₃₁H₃₅NO₂F₃ (M + H)⁺: 510.2620; found: 510.2618. Anal. calcd for (C₃₁H₃₄NO₂F₃.0.25 H₂O): C, 72.42; H, 6.76; N, 2.72; found: C, 72.14; H, 6.69; N, 2.59.

3.1.4 Opioid binding assays—As previously described,[14, 15] the recombinant CHO cells (hMOR-CHO, hDOR-CHO and hKORCHO) were produced by stable transfection with the respective human opioid receptor cDNA, and provided by Dr. Larry Toll (SRI International, CA). The cells were grown in plastic flasks in DMEM (90%) (hDOR-CHO and hKOR-CHO) or DMEM/ F-12 (45%/ 45%) medium (hMOR-CHO) containing 5% FBS (Invitrogen), 5% FetalClone II (HyClone), and Geneticin (G-418: 0.10-0.2 mg/ml)

(Invitrogen) under 95% air/5% CO₂ at 37° C. Cell monolayers were harvested and frozen in - 80 °C. The hKOR-CHO, hMOR-CHO and hDOR-CHO cells are used for opioid binding experiments. For the [³⁵S]-GTP-γ-S binding experiments, we use hKOR-CHO and hMOR-CHO cells for assaying KOR and MOR receptor function. Currently, we use the NG108-15 neuroblastoma×glioma cell for the DOR [³⁵S]-GTP-γ-S binding assay, and obtain an excellent signal-to-noise ratio. In summary, we use the hDOR-CHO cells for DOR binding assays, and the NG108-15 cells for the DOR [³⁵S]-GTP-γ-S binding assay.

As noted formerly,[15, 16] [³H][D-Ala2-MePhe4,Gly-ol5]enkephalin ([³H]DAMGO, SA=44-48 Ci/mmol) was used to label MOR, [³H][D-Ala2,D-Leu5]enkephalin ([³H]DADLE, SA=40-50 Ci/mmol) to label DOR and [³H](−)-U69,593 (SA=50 Ci/mmol) to label KOR binding sites. On the day of the assay, cell pellets were thawed on ice for 15 min then homogenized with a polytron in 10 mL/pellet of ice-cold 10 mM Tris-HCl, pH 7.4. Membranes were then centrifuged at 30,000 × g for 10 min, resuspended in 10 ml/pellet ice-cold 10mM Tris-HCl, pH 7.4 and again centrifuged 30,000 × g for 10 min. Membranes were then resuspended in 25 °C 50 mM Tris-HCl, pH 7.4 (~100 mL/pellet hMOR-CHO, 50 mL/pellet hDOR-CHO and 120 mL/pellet hKOR-CHO). All assays took place in 50 mM Tris-HCl, pH 7.4, with a protease inhibitor cocktail [bacitracin (100 µg/mL), bestatin (10 µg/mL), leupeptin (4 µg/mL) and chymostatin (2 µg/mL)], in a final assay volume of 1.0 mL. All drug dilution curves were made up with buffer containing 1 mg/mL BSA. Nonspecific binding was determined using 20 µM levallorphan ([³H]DAMGO and [³H]DADLE) and 1 µM (−)-U69,593 (for [³H]U69,593 binding). [³H]Radioligands were used at ~ 2 nM concentrations. Triplicate samples were filtered with Brandel Cell Harvesters (Biomedical Research & Development Inc., Gaithersburg, MD), over Whatman GF/B filters, after a 2 h incubation at 25 °C. The filters were punched into 24-well plates to which was added 0.6 mL of LSC-cocktail (Cytoscint). Samples were counted, after an overnight extraction, in a Trilux liquid scintillation counter at 44% efficiency. Opioid binding assays had ~30 µg protein per assay tube. Inhibition curves were generated by displacing a single concentration of radioligand by 10 concentrations of drug. The pooled data of three experiments (typically 30 data points) were fit to the two-parameter logistic equation for the best-fit estimates of the IC₅₀ and N values: $Y=100/(1+([INHIBITOR]/IC_{50})^N)$, where “Y” is the percent of control value. K_i values for test drugs are calculated according to the standard equation: $K_i = IC_{50}/(1+[radioligand]/K_d)$. For the [³H]radioligands, the following K_d values (nM ± SD, n = 3) were used in the K_i calculation: [³H]DAMGO (0.93 ± 0.04), [³H]DADLE (1.9 ± 0.3) and [³H](−)-U69,593 (11 ± 0.6).

3.1.5. [³⁵S]GTP-γ-S binding assays—As previously described,[15, 16] the assays were conducted with minor modifications of published methods[17]. In this description, buffer “A” is 50 mM Tris-HCl, pH 7.4, containing 100 mM NaCl, 10 mM MgCl₂, 1 mM EDTA and buffer “B” is buffer A plus 1.67 mM DTT and 0.15% BSA. 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 bacitracin. The homogenate was centrifuged at 30,000 × g for 10 min at 4 °C, and the supernatant discarded. The membrane pellets were resuspended in buffer B and used for [³⁵S]GTP-γ-S binding assays. Test tubes received the following additions: 50 µL buffer A

plus 0.1% BSA, 50 μ L GDP in buffer A/0.1% BSA (final concentration = 40 μ M), 50 μ L drug in buffer A/0.1% BSA, 50 μ L [35 S]-GTP- γ -S in buffer A/0.1% BSA (final concentration = 50 pM), and 300 μ L of cell membranes (50 μ g of protein) in buffer B. The final concentrations of reagents in the [35 S]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, 40 μ M GDP and 0.1% BSA. Incubations proceeded for 3 h at 25 °C. Nonspecific binding was determined using GTP- γ -S (40 μ M). Bound and free [35 S]GTP- γ -S was separated by vacuum filtration (Brandel) through GF/B filters. The filters were punched into 24-well plates to which was added 0.6 mL LSC-cocktail (Cytoscint). Samples were counted, after an overnight extraction, in a Trilux liquid scintillation counter at 27% efficiency.

For the [35 S]GTP- γ -S binding experiments,[15, 16] K_e values were determined using the “shift” experimental design, agonist (DAMGO) dose-response curves were generated, using hMORCHO cells, in the absence and presence (ten points/curve) of a test compound. The data of several experiments, 3 or more, were pooled, and the K_e values were calculated according to the equation: [Test Drug]/($EC_{50-2}/EC_{50-1} - 1$), where EC_{50-2} is the EC_{50} value in the presence of the test drug and EC_{50-1} is the value in the absence of the test drug.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CPM	cyclopropylmethyl
Ph	phenyl
BSA	bovine serum albumin
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid

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Highlights

- Synthesis of *cis*-4a-arylalkyl 1,2,3,4,4a,9a-hexahydrobenzofuro[2,3-*c*]pyridin-6-ols.
- Binding studies determined opioid receptor affinity; [³⁵S]GTP-γ-S assays evaluated efficacy.
- Compounds were synthesized with μ- and δ-antagonist activity, and κ-agonist activity.
- An *N*-allyl substituent in the C4a (CH₂)₃Ph series gave a μ/δ ratio of 6.
- An *N*-CPM, C4a (CH₂)₃Ph substituted compound showed potent δ-antagonist activity.

Scheme 1.

Reagents and conditions:[12] (a) *n*-BuLi, Br(CH₂)_n-Ph (where n = 3, 4, or 5), -78 °C to rt; (b) (i) NBS, -78 °C to rt, (ii) NaBH₃CN, 0 °C; (c) HBr, reflux; (d) Et₃N, sealed tube, 100 °C; (e) Ac₂O, reflux; (f) (i) proton sponge, Troc-Cl, (ii) K₂CO₃, MeOH, (iii) Zn, AcOH; (g) (i) R₂Br, DMF, NaHCO₃, 50 °C, (ii) K₂CO₃, MeOH.

Figure 1.
General structure of benzofuro[2,3-c]pyridinol and *para*-d oxide bridged phenylmorphans

Table 1

Opioid receptor binding affinities of compounds in the C4a-phenylpropyl, -phenylbutyl, and -phenypentyl series

Cmpd		$K_i \pm SD$ (nM)		
$R_1 = (CH_2)_3Ph$	$R_2 =$	μ	δ	κ
10a	CH_2CH_2Ph	14 ± 1	6.9 ± 0.4	110 ± 6
10b	$p\text{-}F\text{-}CH_2CH_2Ph$	47 ± 2	15 ± 2	110 ± 9
10c	$o\text{-}F\text{-}CH_2CH_2Ph$	17 ± 2	13 ± 0.7	160 ± 10
10d	$CH_2CH_2CH_2Ph$	73 ± 5	7.1 ± 0.9	88 ± 6
10e	$p\text{-}CF_3CH_2CH_2Ph$	27 ± 0.2	18 ± 2	139 ± 12
10f	$CH_2CH=CH_2$	53 ± 3	9.1 ± 1	5.1 ± 0.2
10g	CPM	13 ± 1	5.5 ± 1	1.4 ± 0.1
10h	$o\text{-}CF_3CH_2CH_2Ph$	351 ± 26	229 ± 22	795 ± 43
10i	$m\text{-}CF_3CH_2CH_2Ph$	93 ± 8	$116 \pm$	923 ± 58
$R_1 = (CH_2)_4Ph$	$R_2 =$			
11a	CH_2CH_2Ph	7.7 ± 0.5	7.3 ± 0.5	117 ± 13
11b	$p\text{-}F\text{-}CH_2CH_2Ph$	12 ± 1	21 ± 3	147 ± 19
11c	$o\text{-}F\text{-}CH_2CH_2Ph$	10.1 ± 1	26 ± 4	230 ± 30
11d	$CH_2CH_2CH_2Ph$	80 ± 3	57 ± 4	424 ± 41
11e	$p\text{-}CF_3CH_2CH_2Ph$	3.8 ± 0.6	21 ± 4	163 ± 16
11f	CPM	14 ± 0.5	16 ± 3	1.6 ± 0.1
11g	$o\text{-}CF_3CH_2CH_2Ph$	294 ± 21	589 ± 61	1769 ± 129
11h	$(CH_2)_4Ph$	pi^d	68 ± 6	410 ± 36
11i	$m\text{-}CF_3CH_2CH_2Ph$	38 ± 3	148 ± 19	3184 ± 482
$R_1 = (CH_2)_5Ph$	$R_2 =$			
12a	CH_2CH_2Ph	5.6 ± 0.8	25 ± 4	82 ± 4
12b	$p\text{-}F\text{-}CH_2CH_2Ph$	4.2 ± 0.5	44 ± 5	130 ± 7
12c	$o\text{-}F\text{-}CH_2CH_2Ph$	5.8 ± 0.6	54 ± 3	177 ± 11
12d	$p\text{-}CF_3CH_2CH_2Ph$	3.1 ± 0.4	127 ± 12	297 ± 12
12e	CPM	3.9 ± 0.8	24 ± 2	3.7 ± 0.2
12f	$o\text{-}CF_3CH_2CH_2Ph$	159 ± 16	719 ± 36	1119 ± 63
Morphine		2.6 ± 0.01	605 ± 36	299 ± 49
DAMGO		1.2 ± 0.1	1500 ± 170	-

Cmpd	$R_1 = (CH_2)_3Ph$	$R_2 =$	$K_i \pm SD$ (nM)		
			μ	δ	κ
U69,593			>2 μM	>12 μM	3.5 ± 0.3

Opiate receptor binding assays were performed using CHO hMOR, CHO hDOR and CHO hKOR cells. The data of three experiments were pooled (N=30 data points) and fit to the two-parameter logistic equation for the best-fit estimates ($\pm SD$) of the IC_{50} , the value of which was then used to calculate the K_i value, as described in section 3.1.4; Ph = phenyl; CPM = cyclopropylmethyl

a [3H]-DAMGO

b [3H]-DADLE

c [3H]-U69,593

All data are n equal or greater than 3.

^d Partial inhibitor (compound **11h** appeared to show some inverse agonist activity).

Table 2

Antagonist ($Ke \pm SD$, nM) and agonist ($ED_{50} \pm SD$, nM, and $E_{max} \pm SD$) activities from [^{35}S]GTP- γ -S functional assays.

Compd		Antagonist activity		Agonist Activity	
$R_1 = (CH_2)_3Ph$	R_2	MOR Ke (nM)	DOR Ke (nM)	MOR ED_{50} (E_{max}) ^a	KOR ED_{50} (E_{max}) ^a
10a	CH_2CH_2Ph	-	192 ± 18	560 ± 120 (73 ± 4)	I
10b	<i>p</i> -F- CH_2CH_2Ph	518 ± 83	176 ± 19	I	I
10c	<i>o</i> -F- CH_2CH_2Ph	-	492 ± 53	854 ± 164 (79 ± 4)	I
10d	$CH_2CH_2CH_2Ph$	1301 ± 257	103 ± 14	I	I
10e	<i>p</i> -CF ₃ CH_2CH_2Ph	359 ± 82	437 ± 43	I	I
10f	$CH_2CH=CH_2$	47 ± 6	14 ± 3	I	137 ± 26 (83 ± 3)
10g	CPM	9 ± 0.82	4 ± 0.64	I	32 ± 5 (91 ± 2)
$R_1 = (CH_2)_4Ph$	$R_2 =$				
11a	CH_2CH_2Ph	-	2747 ± 229	542 ± 125 (77 ± 4)	I
11b	<i>p</i> -F- CH_2CH_2Ph	-	675 ± 95	1166 ± 278 (52 ± 3)	I
11c	<i>o</i> -F- CH_2CH_2Ph	-	2779 ± 388	1008 ± 180 (84 ± 4)	I
11f	CPM	23 ± 2	64 ± 6	I	35 ± 4 (83 ± 2)
11i	<i>m</i> -CF ₃ CH_2CH_2Ph	410 ± 45	595 ± 90	I	I
$R_1 = (CH_2)_5Ph$	$R_2 =$				
12a	CH_2CH_2Ph	-	836 ± 94	1077 ± 156 103 ± 4	I
12b	<i>p</i> -F- CH_2CH_2Ph	-	1208 ± 185	929 ± 125 109 ± 4	I
12c	<i>o</i> -F- CH_2CH_2Ph	-	I	1032 ± 134 107 ± 4	I
12e	CPM	21 ± 2	35 ± 7	I	13 ± 3 (70 ± 3)
Naloxone		2.3 ± 0.3			-
(-)-U50,488		-			27 ± 3 (99 ± 2)

None of the compounds tested had KOR antagonist activity or DOR agonist activity; Ph = phenyl, CPM = cyclopropylmethyl; I = inactive ($ED_{50} > 1500$); - = not tested

^a E_{max} = the percent the compound stimulated GTP- γ -S binding as compared to the stimulation produced by 1000 nM DAMGO, 500 nM SNC80 or 500 nM (-)-U50,488 (when the % stimulation is considerably less than 100%, the compound is said to be a partial agonist).