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Probes for narcotic receptor mediated phenomena. 44. Synthesis of an N-substituted 4-hydroxy-5-(3-hydroxyphenyl)morphan with high affinity and selective μ -antagonist activity

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Abstract

A simple three-step synthesis of 5-(3-hydroxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-4-ol (**3a**) was achieved using an osmium tetroxide mediated oxidation of the known intermediate **6**. A pyrrolidine-ring variant of **3a** (3-(7-(hydroxymethyl)-6-methyl-6-azabicyclo[3.2.1]octan-1-yl)phenol (**5**)) was isolated when other routes were used. The epimeric hydroxy analogue **4a** was synthesized by simple inversion of the stereochemistry at C-4. Both *N*-methyl (**3a** and **4a**) and *N*-phenethyl (**3b** and **4b**) derivatives were synthesized. The compounds were examined for their opioid receptor affinity and the *N*-phenethyl analogue **3b** was found to have relatively weak affinity for the μ -opioid receptor ($K_i = 74$ nM). However, the *N*-phenethyl analogue of the C-4 epimer, **4b**, had about 15 fold higher affinity than **3b** and was selective for the μ -opioid receptor ($K_i = 4.6$ nM). Compound **4b** was a moderately potent μ -opioid antagonist ($K_e = 12$ nM), as determined by [³⁵S]GTP- γ -S assays. Compounds **3b** and **4b** were energy minimized at the level of B3LYP/6-31G*, and then overlaid onto the 5-phenylmorphinan, the (1*R*,5*R*,9*S*)-(-)-enantiomer of **2b** (Fig. 1) with the α or β -OH group at the C-9 position. The spatial orientation of the hydroxyl moiety in **3b**, **4b**, **2a**, and **2b** is proposed to be the structural requirement for high μ -opioid receptor binding affinity and their agonist or antagonist activity. The modest change in spatial position of the hydroxyl moiety, and not the N-substituent, induced the change from potent agonist to an antagonist of moderate potency.

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Keywords

osmium tetroxide mediated oxidation; opioid receptor affinity; opioid receptor efficacy; 5-(3-hydroxyphenyl)-2-phenethyl-2-azabicyclo[3.3.1]nonan-4-ol synthesis; energy minimization for molecular overlay; X-ray crystallographic structure

1. Introduction

May and co-workers showed that racemic 5-(3-hydroxyphenyl)-2-methylmorphinan (**1**, Fig. 1) was as potent as morphine as an antinociceptive.[1] Over the past half-century since that discovery many interesting compounds based on **1** and its derivatives have been described. Recently, for example, Hiebel et al., [2] found that a 5-phenylmorphinan, the (1*R*,5*R*,9*S*)-(-)-enantiomer of **2b** (Fig. 1) with a β -hydroxy group at the C-9 position (axially oriented on the piperidine ring, *cis* to the equatorially oriented phenyl ring), had extremely high μ -opioid receptor affinity ($K_i = 0.19$ nM). It was at least 500 times more potent than morphine in vivo as an antinociceptive. Its C9 α -epimer (**2a**) had about 230 times less affinity. That work determined that the stereochemistry of the β -hydroxyl group at C-9 of the piperidine ring in compound **2** was important for its high binding affinity and its agonist activity as well. In order to explore the effect of the hydroxy group at a different position in the 5-phenylmorphinan bicyclic ring system, we decided to synthesize the C4-OH compounds **3** and **4** (Fig. 1). We realized that an *N*-methyl-substituted 5-phenylmorphinan (such as **3a**) usually[3] has less affinity and is less potent than the comparable *N*-phenethyl-substituted 5-phenylmorphinan (**3b**) and, as well, the agonist activity of a compound with an *N*-methyl substituent can sometimes be altered to opioid antagonist activity by conversion to the *N*-phenethyl analogue.[4] We therefore converted the *N*-methyl compounds **3a** and **4a** to their *N*-phenethyl relatives **3b** and **4b**. The geometries of **3b** and **4b** were then optimized with density functional theory at the level of B3LYP/6-31G*, and compared with those of C-9-hydroxy substituted **2a** and **2b** to define the structural requirements that give rise to both high affinity and agonist or antagonist activity for the 5-phenylmorphinan alcohols.

2. Chemistry

Many substituted 5-phenylmorphans have been obtained from the known 5-(3-methoxyphenyl)-2-methyl-2-azabicyclo[3.3.1] non-3-ene (**6**) (Scheme 1).[5] We hypothesized that the C4-OH substituted phenylmorphinan **3a** could be obtained by reduction of the double bond in **6**, following introduction of a bromine atom at C-4, to give **7**. The subsequent displacement of the bromine in **7** with potassium benzoate, followed by hydrolysis and O-demethylation might give the target compound **3a** (C4 α -OH, *cis* to the equatorially oriented phenyl ring, Scheme 1) that could be used to prepare its epimeric relative **4a** (C4 β -OH, *trans* to the equatorially oriented phenyl ring, Fig. 1).

Thus, treatment of intermediate **6** with NBS followed by reduction of the imine using sodium cyanoborohydride gave the known bromo compound **7**. [6] However, the bromine at C4 could not be displaced by potassium benzoate to give **8**; the C4-OH compounds (**3a**, **4a**) could not be obtained through this route. The product obtained from the attempted displacement of the bromine atom in **7** was determined by X-ray crystallography (Fig. 2) to be a five-membered ring compound (1-(3-methoxyphenyl)-6-methyl-6-azabicyclo[3.2.1]octan-7-yl)methyl benzoate (**10**, Scheme 1)). X-ray crystallographic analysis was also used to prove that alkaline hydrolysis of the benzoate **10** followed by cleavage of the phenolic ether with BBr₃ gave **5**, with the expected five-membered ring (3-(7-(hydroxymethyl)-6-methyl-6-azabicyclo[3.2.1]octan-1-yl)phenol, **5**, Fig. 2)). It is likely that nucleophilic displacement on the bromo compound **7** probably occurred via the

assistance of a neighboring nitrogen atom resulting in a putative aziridinium ion **9**. The formation of an aziridinium ion in a similar reaction was postulated by Burke et al.;[7] that 3-membered ring intermediate of Burke et al., was said to be opened by intramolecular attack by an oxygen on the adjacent aromatic ring and gave a pyran. In the case of **9**, attack by the benzoate anion from the reagent on the aziridinium ion gave the azabicyclo compound **10** (Scheme 1).

The synthesis of compounds **3a** and **4a** required an alternate route that would preclude aziridinium ion formation. Various reactions were unsuccessfully tried to convert **6** or **7** to the target compound **3** (Table 1). Finally, **6** was treated with osmium tetroxide (4% wt in water) in the presence of *N*-methylmorpholine *N*-oxide (NMO). This reaction gave a mixture of the hydroxy amide **11** and the ketoamide **12** (Scheme 2). When this mixture of **11** and **12** was subjected to LAH reduction under refluxing conditions a single product (**13**) was obtained. The epimeric alcohol **14** was not detected. O-Demethylation of **13** gave the desired C4 α -OH compound **3a**. The relative configuration of **3a** was determined to be 1*S**,4*R**,5*R** by X-ray crystallography (Fig. 3).

With compound **3a** in hand, the synthesis of **3b** was attempted via the transformation shown in Scheme 2. Stepwise treatment of **13** with Troc-Cl in the presence of proton sponge followed by removal of the Troc group with Zn/AcOH gave the secondary amine **15**. [8, 9] The *N*-phenethyl moiety was introduced by reacting **15** with 2-(bromoethyl)benzene and sodium bicarbonate in DMF at 50°C. Removal of the O-methyl protecting group with BBr₃ gave the *N*-phenethyl substituted compound **3b**.

After successfully obtaining the C4 α -OH compounds **3a** and **3b**, we then turned our attention to the synthesis of the C4 β -OH compound **4a**. Initial attempts involved oxidation of the C4 α -OH mixture in the methoxyphenyl compound **13** to a ketone, followed by selective reduction to the methoxyphenyl relative of **13** bearing the C4 β -OH group. This route however was unsuccessful and oxidation of **13** resulted mostly in recovered starting material and/or the over-oxidized ketoamide **12**.

Synthesis of **4** required the stereochemical inversion of the hydroxy group at C4 in compound **3**. However, that inversion necessitated circumvention of anchimeric assistance from the neighboring nitrogen atom that would result in five-membered ring formation. The problem was resolved by using the hydroxyamide **11** for further manipulations (Scheme 3). We anticipated that the amide would render the nitrogen atom ineffective for neighboring group participation, and that would obviate formation of the aziridinium ion. The alcohol in compound **11** was then converted to the mesylate using methanesulfonic anhydride. The mesylate **16** was treated with potassium benzoate and hydrolysed using methanolic KOH to give the hydroxy amide **17**, resulting in inversion of the stereochemistry at C4 to give the desired C4 β -OH compound. All that remained was the reduction of the amide and deprotection of the phenolic group. Although simple reduction of the amide **17** with LAH in THF resulted in decomposition, carrying out the LAH reduction in the presence of stoichiometric amounts of AlCl₃ provided a clean conversion to the hydroxy compound **14**. The TLC and ¹HNMR spectra of **14**, by comparison with the epimeric compound **13**, gave the initial indications that the stereochemical inversion of the α -mesylate moiety in **16** occurred to give the β -OH in compound **17** (Scheme 3). The NMR spectra of the epimers **13** and **14** were very different at around δ 3.5-4 and δ 6.6-6.8 (see Experimental Section). The relative configuration of the C4 β -OH epimer **14** (Scheme 3) was unequivocally confirmed by X-ray crystallographic analysis (Fig. 3) and was found to have a relative configuration of 1*R**,4*R**,5*S**. The desired phenolic compound **4a** was obtained by O-demethylation of **14** in the presence of BBr₃, and it was converted to the *N*-phenethyl compound **4b** as shown in

Scheme 3. Additionally, the keto amide **12** (Scheme 4) was used to synthesize the novel 5-phenylmorphinan ketal **20**.

3. Results and discussion

The affinity of compounds **3a**, **3b**, **4a**, **4b**, **5** and **20** for opioid receptors was tested in competitive binding studies (Table 2). Compound **4b** had good affinity for the μ -opioid receptor ($K_i \sim 5$ nM) and was more than 200 fold selective for the μ -opioid receptor over the δ -receptor, and more than 130 fold selective for the μ -opioid receptor over the κ -receptor. The novel ketal bearing compound **20** had moderate μ -receptor binding ($K_i = 22$ nM) and showed little κ -opioid receptor binding ($K_i = 140$ nM); it had even less affinity for δ -receptors. Compound **3b** had considerably less affinity ($K_i = 74$ nM) than **4b** for the μ -receptor. All the tested compounds had almost no affinity for the δ -opioid receptor (Table 2). Functional assays ($[^{35}\text{S}]\text{GTP-}\gamma\text{-S}$ assay, Table 3) showed that compound **4b** is a fairly potent antagonist at the μ -receptor ($K_e = 12$ nM), and compound **20** had moderate agonist activity at the μ -receptor ($\text{ED}_{50} = 89$ nM, Table 3). It is apparent that the potent agonist **2b**[2] has much higher affinity for μ - and δ -receptors than **4b** and that **4b**, unlike **2b**, acts as a moderately potent antagonist, not an agonist in the $[^{35}\text{S}]\text{GTP-}\gamma\text{-S}$ assay. This suggests that the position of the hydroxy substituent in the 5-phenylmorphinan is critical for optimal opioid receptor affinity as well as for their efficacy as opioid agonists or antagonists. The novel ketal bearing racemic 5-phenylmorphinan compound **20** had moderate affinity for μ -opioid receptors and appeared to have mostly μ -agonist activity, comparable to DAMGO (Table 3).

Fig. 4 shows an overlay of the geometry optimized compounds **3b** and **4b** on the previously reported **2a** and **2b**[2]; this superposition was obtained by the rigid fitting of the nine atoms of the morphan backbone of **3b**, **4b**, and **2a** to those of **2b**. The phenylethyl group overlaps well in space. However, due to the steric repulsion caused by the OH group on the piperidine ring, the phenolic rings do not overlap as well as the phenylethyl moiety, as shown by the dihedral angle of C15-C10-C5-C9 (ϕ). For **3b**, $\phi = 16.9^\circ$, **4b** $\phi = 12.3^\circ$, **2a** $\phi = 5.6^\circ$, and for **2b** $\phi = -19.2^\circ$. In a previous study, [2] we have shown that it requires very little energy (less than a 1 kcal/mol) to rotate the phenolic ring of **2b** in Fig. 4 to a more strained position ($\phi = -2.7^\circ$). This small energy penalty indicates that all the phenolic ring conformations shown in Fig. 4 are readily accessible, and thus are not likely to be responsible for the difference in the binding affinity and/or activity for **3b**, **4b**, **2a**, and **2b**. When the conformation of both the phenolic and the phenylethyl moieties are ruled out, the spatial orientation of the hydroxyl on the piperidine emerges as a primary cause for the observed pharmacological properties of these hydroxyl-substituted phenylmorphans. In terms of K_i , **4b** ($K_i = 4.6$ nM) and **2b** ($K_i = 0.19$ nM), both with an axial (β)-hydroxyl moiety (*cis* to the equatorially oriented C5-phenyl group), bind with much higher affinity to the μ -receptor than their respective epimers **3b** ($K_i = 74$ nM) and **2a** ($K_i = 54$ nM) that have an equatorially oriented (α)-hydroxyl (*trans* to the C5-phenyl group). These higher K_i values indicate that the β -hydroxyl of the piperidine ring either at C4 or C9 is better positioned than the corresponding α -hydroxyl to interact with the putative residue(s) at the binding site of the μ -opioid receptor, likely via H-bonding. Of the two β -OH's, it can be also inferred that the C9 β -OH of **2b** forms an even stronger H-bonding interaction at the binding site. Interestingly, when the geometry optimized 14-hydroxymorphine was overlaid onto **2b** (Fig. 5), it can be seen that the C14 β -OH essentially occupies the same space spanned by the C9 β -OH of **2b**. While the agonist potency of 14-hydroxymorphine is comparable to that of morphine, 14-hydroxydihydromorphine is twice as potent as dihydromorphine; all are agonists.[10] Coupling the fact that a C14 β -OH substituent on a morphine-like molecule does not generally change the compounds' action as an agonist with the likelihood that both the C-14 β -OH in, for example, 14-hydroxymorphine and the C9 β -OH in **2b** form the same H-bonding in the binding site, we propose that the putative H-bonding between the C9 β -OH

of **2b** and a polar residue or a water molecule at the binding site of the μ -receptor is stereospecific, and also that it plays a more prominent role in **2b** because of the greater structural flexibility of 5-phenylmorphinan. This stereospecific H-bonding interaction might also be responsible for the agonist activity for the hydroxyl-substituted phenylmorphinan **2b**, possibly by enhancing a charge interaction between the positively charged tertiary nitrogen of the 5-phenylmorphinan and an Asp residue at the binding site, as depicted in Fig. 5. Recent X-ray crystallographic structures of a GPCR, the β_1 -adrenergic receptor, [11] have shown the charge interaction between Asp-121 and the secondary nitrogen of its agonist ligands, suggesting that a similar charge interaction is also plausible at the μ -opioid receptor. The validity of the proposed stereospecific interactions (Fig. 5) can be tested when the X-ray crystallographic structure of the μ -opioid receptor becomes available. Nonetheless, in the absence of such an X-ray derived structure, the present study does provide essential structural motifs as to how the hydroxyl group of our ligands (**3b**, **4b**, **2a**, and **2b**) would interact with the opioid receptor.

3. Conclusions

Initial attempts to synthesize 4-hydroxy-substituted 5-phenylmorphinans resulted in a five-membered ring phenylmorphinan, presumably through an aziridinium ion intermediate. A key osmium tetroxide mediated oxidation of the known enamine eliminated aziridinium ion formation and resulted in the synthesis of the desired 4-hydroxy-substituted 5-phenylmorphinan. Both the racemic *N*-methyl and *N*-phenethyl substituted C4 α OH (1*S**,4*R**,5*R**) and C4 β OH (1*R**,4*R**,5*S**) 5-phenylmorphinan epimers were synthesized. Opioid receptor binding studies indicated that the racemic C4 β hydroxy-*N*-phenethyl compound **4b** had good affinity at μ -opioid receptors, and the [³⁵S]GTP- γ -S functional assay indicated that it was a moderately potent μ -antagonist. By comparing the receptor binding studies and the agonist/antagonist activity with the respective position of the OH on the piperidine ring of **3b**, **4b**, **2a**, and **2b**, the spatial position of the C9 β -OH in the 5-phenylmorphinan is proposed to be essential for a stereospecific interaction with amino acids in the receptor binding pocket, and this interaction is responsible for not only an enhancement of binding affinity but also for imparting agonist activity. The C4 β -OH in **4b** is at a comparable distance from the piperidine nitrogen atom as a C9 β -OH in **2b** but it is spatially oriented a little differently than the C9 β -OH in **2b**. Because of its spatial orientation, the C4 β -OH in **4b** is likely to interact with a different set of amino acids in the binding pocket and that interaction is likely to be responsible for both its high μ -opioid receptor affinity (albeit much lower affinity than found for **2b**), [2] and its opioid antagonist activity (as determined by the [³⁵S]GTP- γ -S functional binding assay, Table 3).

5. Experimental section

5.1. Materials and methods

Melting points were determined on a Buchi B-545 instrument and are uncorrected. Proton and carbon nuclear magnetic resonance (¹H- and ¹³C-NMR) spectra were recorded in CDCl₃ 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 CDCl₃ (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 CHCl₂/MeOH containing 1% NH₄OH 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 Fluka silica 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.

5.2. Preparation of compounds

5.2.1. 5-(3-Hydroxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-4-ol (3a)—The alcohol **13** (200 mg, 0.76 mmol) was dissolved in CHCl_3 (10 mL), BBr_3 (5 eq) was added, the reaction mixture was stirred at room temperature for 1 h, and the excess BBr_3 was quenched with cold MeOH (3 mL). Ammonium hydroxide (5 mL) was added and the organic layer was extracted into CHCl_3 . Drying over Na_2SO_4 , followed by evaporation of the solvent gave a crude solid. Recrystallization of the crude product from propan-2-ol gave pale brown crystalline material (115 mg, 61%). A small batch (50 mg) was dissolved in $\text{HBr}:\text{MeOH}$ (1:1, 5 mL) to give **3a**•HBr as pale white crystalline solid. Mp 191–193°C; IR (CHCl_3) 3053, 2938 cm^{-1} ; ^1H NMR (500 MHz; CD_3OD): δ 7.09 (t, $J = 8.2$ Hz, 1H), 6.93 (m, 2H), 6.60 (dd, $J = 8.5, 1.5$ Hz, 1H), 3.92 (dd, $J = 10.0, 7.5$ Hz, 1H), 2.95 (dd, $J = 11.8, 7.2$ Hz, 2H), 2.85 (t, $J = 11.2$ Hz, 1H), 2.54 (dd, $J = 13.2, 5.3$ Hz, 1H), 2.44 (s, 3H), 2.24 (dd, $J = 14.0, 3.5$ Hz, 1H), 2.16 (d, $J = 12.5$ Hz, 1H), 1.87 (dt, $J = 13.6, 6.7$ Hz, 1H), 1.74 (m, 2H), 1.59 (dt, $J = 13.1, 6.6$ Hz, 1H), 1.44 (m, 1H); ^{13}C NMR (126 MHz, CD_3OD): δ 158.07, 151.46, 129.83, 118.47, 114.57, 113.83, 74.51, 59.17, 55.02, 42.42, 40.42, 39.83, 30.42, 24.70, 23.12; HRMS (TOF MS ES^+) calcd for $\text{C}_{15}\text{H}_{22}\text{NO}_2$ ($M + \text{H}$) $^+$: 248.1651; found: 248.1653. Anal. calcd for $\text{C}_{15}\text{H}_{21}\text{NO}_2$: C, 72.84; H, 8.56; N, 5.66; found: C, 72.57; H, 8.66; N, 5.57.

5.2.2. 5-(3-Hydroxyphenyl)-2-phenethyl-2-azabicyclo[3.3.1]nonan-4-ol (3b)—Compound **15** (0.6 g, 2.42 mmol) was dissolved in DMF and treated with 2-(bromoethyl)benzene (0.36 mL, 2.66 mmol) and NaHCO_3 (407 mg, 4.84 mmol). The reaction was stirred at 50 °C for 5 h. After cooling the reaction to room temperature, water was added and the mixture was extracted into EtOAc. The organic solvent was removed in vacuo to give a brown oil. The crude oil was dissolved in CHCl_3 (20 mL) and BBr_3 (5 eq) was added and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was cooled to room temperature and the excess BBr_3 was quenched with cold MeOH (5 mL). Ammonium hydroxide (5 mL) was added and the organic layer was extracted into CHCl_3 . Drying over Na_2SO_4 , followed by evaporation of the solvent gave a crude solid. Purification by flash chromatography using CHCl_3 : MeOH : NH_4OH (90 : 9 : 1) gave **3b** as a pale brown solid (300 mg, 37% over two steps). Mp 120–123 °C; ^1H NMR (500 MHz, CD_3OD): δ 7.31 (t, $J = 7.4$ Hz, 2H), 7.27 (d, $J = 6.9$ Hz, 2H), 7.21 (t, $J = 7.1$ Hz, 1H), 7.15 (t, $J = 8.2$ Hz, 1H), 6.99 (m, 2H), 6.65 (dd, $J = 7.9, 1.4$ Hz, 1H), 4.00 (dd, $J = 9.9, 7.5$ Hz, 1H), 3.19 (m, 2H), 2.92–2.84 (m, 5H), 2.62 (m, 1H), 2.26 (m, 2H), 1.93 (td, $J = 13.4, 6.4$ Hz, 1H), 1.81–1.76 (m, 2H), 1.65 (dd, $J = 13.1, 7.3$ Hz, 1H), 1.52–1.47 (m, 1H); ^{13}C NMR (126 MHz, CD_3OD): δ 158.04, 151.56, 141.32, 129.80, 129.67 (2C), 129.48 (2C), 127.19, 118.44, 114.53, 113.78, 74.60, 57.99, 57.91, 52.91, 40.87, 39.72, 34.86, 30.48, 25.08, 23.23; HRMS (TOF MS AP^+) calcd for $\text{C}_{22}\text{H}_{28}\text{NO}_2$ ($M + \text{H}$) $^+$: 338.2120; found: 338.2112. Anal. calcd for ($\text{C}_{22}\text{H}_{27}\text{NO}_2 \bullet 0.5\text{H}_2\text{O}$): C, 76.26; H, 8.14; N, 4.04; found: C, 76.30; H, 8.19; N, 4.12.

5.2.3. 5-(3-Hydroxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-4-ol (4a)—Compound **4a** (50 mg, 66% yield) was from obtained from **14** (80 mg, 3.62 mmol) and BBr_3 (5 eq) as shown in the procedure for **3a**. Mp 159–161 °C; ^1H NMR (500 MHz, CD_3OD): δ 7.26 (t, $J = 7.8$ Hz, 1H), 6.91 (d, $J = 8.0$ Hz, 1H), 6.87 (s, 1H), 6.77 (dd, $J = 8.0, 2.0$ Hz, 1H), 4.35 (s, 1H), 3.88 (d, $J = 13.8$ Hz, 1H), 3.78 (s, 1H), 3.51 (d, $J = 14.0$ Hz, 1H), 2.98 (s, 3H), 2.80 (d, $J = 13.4$ Hz, 1H), 2.36 (dd, $J = 16.5, 5.5$ Hz, 1H), 2.18 (dd, $J = 14.5, 5.2$ Hz, 1H), 2.09 (d, $J = 13.5$ Hz, 1H), 2.01 (m, 1H), 1.86 (td, $J = 16.7, 6.0$ Hz, 2H), 1.68 (dt, $J = 13.4, 6.6$ Hz, 2H); ^{13}C NMR (126 MHz, CD_3OD): δ 158.42, 149.68, 130.59, 117.50, 114.29,

113.37, 70.48, 58.91, 58.49, 41.54, 39.79, 35.67, 30.32, 22.37, 20.83. HRMS (TOF MS ES⁺) calcd for C₁₅H₂₂NO₂ (M + H)⁺: 248.1651; found: 248.1653. Anal. calcd for (C₁₅H₂₁NO₂•0.75H₂O): C, 69.07; H, 8.69; N, 5.37; found: C, 69.22; H, 8.32; N, 5.29.

5.2.4. 5-(3-Hydroxyphenyl)-2-phenethyl-2-azabicyclo[3.3.1]nonan-4-ol (4b)—

Compound **4b** (75 mg, 28 % over two steps) was from obtained from **19** (200 mg, 0.81 mmol) as shown in the procedure for **3b**. Mp 146–148 °C; ¹H NMR (500 MHz, CD₃OD): δ 7.36 (m, 4H), 7.30 (m, 1H), 7.19 (t, *J* = 7.9 Hz, 1H), 6.84 (d, *J* = 7.8 Hz, 1H), 6.80 (s, 1H), 6.69 (d, *J* = 7.8 Hz, 1H), 4.29 (s, 1H), 3.87 (s, 1H), 3.82 (d, *J* = 13.5 Hz, 1H), 3.59 (d, *J* = 13.8 Hz, 1H), 3.33 (s, 2H), 3.13–3.05 (m, 2H), 2.82 (dd, *J* = 13.5, 1.0 Hz, 1H), 2.28 (m, 1H), 2.16 (dd, *J* = 15.0, 5.5 Hz, 1H), 2.05 (d, *J* = 13.5 Hz, 1H), 2.02 (m, 1H), 1.84 (m, 2H), 1.71 (m, 1H); ¹³C NMR (126 MHz; CD₃OD): δ 158.77, 149.68, 137.79, 130.64, 129.98 (2C), 129.87 (1C), 128.29, 117.40, 114.36, 113.46, 110.36, 70.69, 68.86, 66.92, 57.41, 57.39, 40.42, 35.81, 31.79, 26.50, 21.04; HRMS (TOF MS AP⁺) calcd for C₂₂H₂₈NO₂ (M + H)⁺: 338.2120; found: 338.2112. Anal. calcd for (C₂₂H₂₇NO₂•HBr•0.25H₂O): C, 62.49; H, 6.79; N, 3.31; found: C, 62.62; H, 6.41; N, 3.46.

5.2.5. 3-(7-(Hydroxymethyl)-6-methyl-6-azabicyclo[3.2.1]octan-1-yl)phenol (5)

—A 10% solution of LiOH in a 1:1 mixture of MeOH and H₂O (50 mL) was slowly added to a vigorously stirred solution of the ester **10** (1.0 g, 2.73 mmol) in MeOH (50 mL). The reaction mixture was stirred for 1 h at room temperature under a nitrogen atmosphere, then diluted with H₂O (30 mL), and extracted with CHCl₃ (3 x 30 mL). The organic layer was washed with H₂O, dried over Na₂SO₄ and evaporated to dryness to give the alcohol as a crude reddish solid. The alcohol was dissolved in CHCl₃ (20 mL) and BBr₃ (5 eq) was added and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was cooled to room temperature and the excess BBr₃ was quenched with cold MeOH (5 mL). Ammonium hydroxide (5 mL) was added and the organic layer was extracted into CHCl₃. Drying over Na₂SO₄, followed by evaporation of the solvent gave a crude solid. Crystallization of this solid from EtOH gave pale yellow needles of compound **5** (500 mg, 74%). Mp 190–193 °C; ¹H NMR (CDCl₃, 500 MHz) δ 7.10 (t, 1H, *J* = 7.5 Hz), 6.87 (d, 1H, *J* = 8.0 Hz), 6.83 (s, 1H), 6.61 (d, 1H, *J* = 7.0 Hz), 3.89 (t, 1H, *J* = 10.5 Hz), 3.75 (dd, 1H, *J* = 2.5 and 11.5 Hz), 3.09 (t, 1H, *J* = 4.0 Hz), 2.81 (d, 1H, *J* = 7.0 Hz), 2.65 (s, 3H), 2.42 (m, 1H), 1.93 (m, 2H), 1.70 (m, 3H), 1.53 (d, 1H, *J* = 11.5 Hz), 1.36 (dt, 1H, *J* = 5.5 and 12.0 Hz); ¹³C NMR (CDCl₃, 126 MHz) δ 158.58, 148.14, 130.19, 117.89, 114.10, 114.07, 80.01, 65.43, 62.37, 50.89, 47.89, 43.81, 32.65, 31.83, 20.90; HRMS (TOF MS ES⁺) calcd for C₁₅H₂₂NO₂ (M + H)⁺: 248.1651; found: 248.1653. Anal. calcd for (C₁₅H₂₁NO₂): C, 72.84; H, 8.56; N, 5.66; found: C, 72.72; H, 8.58; N, 5.58.

5.2.6. (1-(3-Methoxyphenyl)-6-methyl-6-azabicyclo[3.2.1]octan-7-yl)methyl benzoate (10)—

A mixture of bromo compound **7** [6] (4.1 g, 12.64 mmol) and potassium benzoate (10.1 g, 63.22 mmol) was heated in DMF at 130 °C for 5 h. After cooling to room temperature, the reaction mixture was diluted with water and the organic layer was extracted into ether dried over Na₂SO₄ and solvent was evaporated. The crude material was purified by flash chromatography using 30% ether in hexanes as an eluent. Compound **10** was obtained as an orange solid (3.0 g, 67%). A small batch was treated with HBr in methanol to give a white crystalline **10**.HBr. Mp 251–253 °C; ¹H NMR (CDCl₃, 500 MHz) δ 7.76 (d, 2H, *J* = 8.0 Hz), 7.49 (t, 1H, *J* = 7.0 Hz), 7.33 (t, 2H, *J* = 7.5 Hz), 7.22 (t, 1H, *J* = 8.0 Hz), 7.03 (d, 1H, *J* = 8.0 Hz), 6.98 (s, 1H), 6.72 (d, 1H, *J* = 8.0 Hz), 4.54 (m, 2H), 3.17 (t, 1H, *J* = 6.0 Hz), 3.13 (t, 1H, *J* = 5.0 Hz), 2.69 (s, 3H), 2.45 (t, 1H, *J* = 7.0 Hz), 2.17 (m, 1H), 2.04 (m, 1H), 1.79 (m, 3H), 1.56 (d, 1H, *J* = 11.5 Hz), 1.36 (dt, 1H, *J* = 5.5 and 12.5 Hz); ¹³C NMR (CDCl₃, 126 MHz) δ 166.69, 159.70, 147.07, 132.82, 130.19, 129.66 (2C), 129.33, 128.23 (2C), 118.40, 112.84, 110.59, 75.32, 65.12, 63.78, 55.31, 49.93, 46.34, 43.37, 31.29,

30.25, 19.97; HRMS (TOF MS ES⁺) calcd for C₂₃H₂₈NO₃ (M + H)⁺: 366.2069; found: 366.2071. Anal. C₂₃H₂₇NO₃•HBr•0.5 H₂O (C, H, N).

5.2.7. 4-Hydroxy-5-(3-methoxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-3-one (11)—To a solution of **6** (3.6 g, 14.8 mmol) in acetone (50 mL) OsO₄ (3 mL of 4% solution) was added to it followed by NMO (2.08 g, 17.8 mmol). The dark reaction mix was stirred for 12 h and quenched with satd. Na₂S₂O₃ solution. The dark crude mixture was applied to a column and eluted with 90% ethyl acetate in hexanes to give the hydroxy amide **11** (1.0 g, 25%). Further elution with 100% ethyl acetate gave the ketoamide **12** (1.8 g, 45%) as a pale brown solid. Mp 114–116 °C; ¹H NMR (CDCl₃, 500 MHz): δ 7.27 (t, *J* = 8.0 Hz, 1H), 7.10–7.08 (m, 2H), 6.80–6.78 (dd, *J* = 9.0, 2.0 Hz, 1H), 4.08 (s, 1H), 3.82 (s, 3H), 3.67 (s, 1H), 2.96 (s, 3H), 2.55 (d, *J* = 11.3 Hz, 1H), 2.37 (dd, *J* = 13.3, 1.3 Hz, 1H), 2.06 (dd, *J* = 13.2, 2.7 Hz, 1H), 1.94 (d, *J* = 12.1 Hz, 1H), 1.67 (d, *J* = 12.6 Hz, 1H), 1.46 (t, *J* = 13.4 Hz, 3H); ¹³C NMR (CDCl₃, 126 MHz): δ 174.07, 159.63, 149.00, 129.28, 118.00, 112.48, 111.34, 73.65, 55.74, 55.29, 41.26, 36.19, 33.59, 31.87, 27.55, 17.66; HRMS (TOF MS ES⁺) calcd for C₁₆H₂₂NO₃ (M + H)⁺: 276.1600; found: 276.1584. Anal. C₁₆H₂₁NO₃ (C, H, N).

5.2.8. 5-(3-Methoxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonane-3,4-dione (12)—Mp 114–117 °C; ¹H NMR (CDCl₃, 500 MHz): δ 7.17 (t, *J* = 8.0 Hz, 1H), 6.77 (d, *J* = 7.5 Hz, 2H), 6.73 (d, *J* = 9.0 Hz, 1H), 3.70 (s, 3H), 3.05 (s, 3H), 2.78 (d, *J* = 13.5 Hz, 1H), 2.24 (d, *J* = 12.9 Hz, 1H), 2.00 (dd, *J* = 14.0, 3.0 Hz, 1H), 1.92 (d, *J* = 14.0 Hz, 1H), 1.83 (m, *J* = 9.5 Hz, 2H), 1.60–1.47 (m, 2H); ¹³C NMR (CDCl₃, 126 MHz): δ 195.96, 159.30, 158.83, 143.81, 129.11, 118.70, 112.91, 112.01, 55.11, 54.85, 51.07, 38.35, 35.32, 34.01, 25.76, 17.48; HRMS (TOF MS AP⁺) calcd for C₁₆H₂₀NO₃ (M + H)⁺: 274.1443; found: 274.1451. Anal. C₁₆H₁₉NO₃ (C, H, N).

5.2.9. 5-(3-Methoxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-4-ol (13)—To a solution of the amide **11** (400 mg, 1.45 mmol) in THF (15 mL) was added solid LAH (165 mg, 3 eq.) and the solution was refluxed for 5 h. After the completion of reaction, the excess LAH was quenched drop-wise by the addition of EtOAc, followed by a dropwise addition of 10% NaOH solution (5 mL). The organic layer was washed with water and was then extracted in to CH₂Cl₂ (3 x 50 ml). Evaporation of the solvents gave a brown oil, which was purified by flash chromatography using 90:10:1 (CHCl₃ : MeOH : NH₄OH) to give a pale yellow oil in 76% yield. ¹H NMR (CDCl₃, 500 MHz): δ 7.17 (t, *J* = 8.2 Hz, 1H), 6.95 (m, 2H), 6.68 (dd, *J* = 8.1, 1.6 Hz, 1H), 3.82 (dd, *J* = 9.7, 7.5 Hz, 1H), 3.71 (s, 3H), 2.89 (dd, *J* = 11.5, 7.1 Hz, 1H), 2.83 (m, 1H), 2.76 (t, *J* = 11.0 Hz, 2H), 2.52 (dd, *J* = 13.4, 5.7 Hz, 1H), 2.32 (s, 3H), 2.13 (d, *J* = 12.1 Hz, 2H), 1.62 (dd, *J* = 12.8, 2.8 Hz, 2H), 1.51 (m, 1H), 1.27 (m, 1H); ¹³C NMR (CDCl₃, 126 MHz): δ 159.56, 149.97, 129.19, 118.44, 112.66, 111.12, 74.34, 67.99, 57.76, 55.19, 53.46, 42.17, 39.84, 38.82, 28.79, 24.03, 22.15; HRMS (TOF MS ES⁺) calcd for C₁₆H₂₄NO₂ (M + H)⁺: 262.1807; found: 262.1819. Anal. C₁₆H₂₃NO₂•0.75H₂O (C, H, N).

5.2.10. 5-(3-Methoxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-4-ol (14)—To a solution of LAH (192 mg, 5.08 mmol) and AlCl₃ (169 mg, 1.27 mmol) in THF was added amide **18** (700 mg, 2.54 mmol) in THF (20 mL) and the solution was refluxed for 5 h. After the completion of reaction, the excess LAH was quenched drop-wise by the addition of ethyl acetate, followed by a dropwise addition of 10% NaOH solution. The organic layer was washed with water and was then extracted in to CH₂Cl₂ (3 x 50 ml). Evaporation of the solvents gave a brown oil, which was purified into using flash chromatography using 90:10:1 Chloroform: methanol: ammonia to give a pale yellow solid (450 mg, 68%). Mp 118–120 °C; ¹H NMR (CDCl₃, 500 MHz): δ 7.29 (t, *J* = 7.9 Hz, 1H), 6.95 (d, *J* = 7.5 Hz,

1H), 6.89 (s, 2H), 6.78 (dd, $J = 8.0, 2.1$ Hz, 1H), 4.04 (s, 1H), 3.81 (s, 3H), 3.23 (dd, $J = 13.0, 3.0$ Hz, 1H), 3.04 (s, 1H), 3.02 (d, $J = 13.0$ Hz, 1H), 2.56 (dd, $J = 12.5, 1.5$ Hz, 1H), 2.42 (s, 3H), 2.21 (dd, $J = 13.0, 4.0$ Hz, 1H), 1.99 (dd, $J = 13.9, 6.0$ Hz, 1H), 1.71 (m, Hz, 3H), 1.61 (d, $J = 13.0$ Hz, 1H), 1.35 (m, 1H); ^{13}C NMR (CDCl_3 , 126 MHz): δ 160.30, 150.21, 130.07, 118.25, 112.50, 111.45, 73.03, 57.61, 55.56, 54.13, 43.05, 40.66, 36.29, 32.42, 23.81, 22.67; HRMS (TOF MS ES^+) calcd for $\text{C}_{16}\text{H}_{24}\text{NO}_2$ ($\text{M} + \text{H}$) $^+$: 262.1807; found: 262.1819. Anal. $\text{C}_{16}\text{H}_{23}\text{NO}_2 \bullet 0.75\text{H}_2\text{O}$ (C, H, N).

5.2.11. 5-(3-Methoxyphenyl)-2-azabicyclo[3.3.1]nonan-4-ol (15)—To compound **13** (1.0 g, 4.0 mmol) in DCE was added Troc-C (0.85 mL, 6.0 mmol) and proton-sponge (1.28 g, 6.0 mmol) and stirred overnight for 12 h. The reaction mixture was then washed with 1 N HCl and water and the organic layer was extracted in to ethyl acetate. The aqueous layer was washed with twice with ethyl acetate and the combined extracts were evaporated to give a brown oil. The crude oil was then taken up in AcOH (7 mL) and Zn powder (785 mg, 12 mmol) was added to it slowly with vigorous stirring. After the reaction is complete, the reaction mixture was filtered through a plug of cotton and washed with a CH_2Cl_2 . Ammonium hydroxide (10 mL) was slowly added to the organic layer followed by extraction of the organic layer into CH_2Cl_2 . Evaporation of the organic layer followed by trituration of the crude oil with 2-propanol gave **15** as a sticky solid (0.6 g, 63% over two steps). ^1H NMR (CD_3OD , 500 MHz): δ 7.25 (t, $J = 8.3$ Hz, 1H), 7.06 (m, 2H), 6.80-6.79 (d, $J = 9.0, 2.0$ Hz, 1H), 3.95 (dd, $J = 10.1, 8.0$ Hz, 1H), 3.80 (s, 3H), 3.49 (s, 1H), 3.27 (d, $J = 11.0$ Hz, 1H), 3.23 (d, $J = 7.5$ Hz, 1H), 2.68 (dd, $J = 14.0, 4.5$ Hz, 1H), 2.23 (d, $J = 13.9$ Hz, 1H), 2.05 (dt, $J = 12.3, 6.2$ Hz, 2H), 1.87 (m, 4H), 1.68 (dt, $J = 12.9, 6.5$ Hz, 1H); ^{13}C NMR (CD_3OD , 126 MHz): δ 161.09, 150.86, 130.22, 119.54, 114.09, 112.28, 73.43, 55.73, 40.87, 38.04, 30.34, 29.34, 22.40, (2C overlapped by CD_3OD signal between 50-48 ppm); HRMS (TOF MS ES^+) calcd for $\text{C}_{15}\text{H}_{22}\text{NO}_2$ ($\text{M} + \text{H}$) $^+$: 248.1651; found: 248.1653. Anal. $\text{C}_{15}\text{H}_{21}\text{NO}_2 \bullet 0.5\text{CH}_2\text{Cl}_2$ (C, H, N).

5.2.13. 5-(3-Methoxyphenyl)-2-methyl-3-oxo-2-azabicyclo[3.3.1]nonan-4-yl methanesulfonate (16)—Methanesulfonyl anhydride (2.53 g, 14.5 mmol) was added to a solution of **11** (2.0 g, 7.27 mmol) in CH_2Cl_2 at -20°C (20 mL) followed by triethylamine (2.53 mL, 18.1 mmol). The brown solution was slowly warmed up to room temperature and stirred under a nitrogen atmosphere at room temperature for 18 h. The reaction was quenched with a saturated solution of NaHCO_3 . The phases were separated and the aqueous layer was extracted with CH_2Cl_2 . The combined organic material was washed with H_2O , dried over Na_2SO_4 and evaporated to dryness to give the oily ester. The ester was purified by flash chromatography using 90% ethyl acetate in hexanes to yield **17** as a sticky solid (1.5 g, 58% yield). ^1H NMR (CDCl_3 , 500 MHz): δ 7.28 (dd, $J = 8.5$ Hz, 1H), 6.97 (d, $J = 6.4$ Hz, 2H), 6.83 (dd, $J = 7.6, 1.5$ Hz, 1H), 5.11 (s, 1H), 3.81 (s, 3H), 3.71 (s, 1H), 3.47 (s, 1H), 3.17 (s, 3H), 2.97 (s, 3H), 2.63 (t, $J = 14.3$ Hz, 2H), 2.05 (m, 2H), 1.94 (d, $J = 8.3$ Hz, 1H), 1.79 (d, $J = 3.5$ Hz, 1H), 1.58-1.48 (m, 2H); ^{13}C NMR (CDCl_3 , 126 MHz): δ 167.26, 159.90, 145.47, 129.78, 117.28, 112.97, 111.64, 85.16, 70.20, 55.39, 42.33, 41.73, 36.43, 33.80, 31.17, 27.02, 17.28; HRMS (TOF MS AP^+) calcd for $\text{C}_{15}\text{H}_{22}\text{NO}_2$ ($\text{M} + \text{H}$) $^+$: 354.1414; found: 354.1391. Anal. $\text{C}_{17}\text{H}_{23}\text{NO}_5\text{S} \bullet 0.75 \text{CH}_2\text{Cl}_2 \bullet 0.5 \text{H}_2\text{O}$ (C, H, N).

5.2.14. 4-Hydroxy-5-(3-methoxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-3-one (17)—A mixture of the mesylate **17** (1.0 g, 2.83 mmol) and potassium benzoate (2.3 g, 14.1 mmol) was heated in DMF at 120°C for 12 h. After cooling to room temperature, the reaction mixture was diluted with water and the organic layer was extracted into ether dried over Na_2SO_4 and solvent was evaporated. A 10% solution of KOH in a 1:1 mixture of MeOH and H_2O (20 mL) was slowly added to a vigorously stirred solution of the crude benzoate ester in MeOH (20 mL). The reaction mixture was stirred for 5 h at room

temperature under a nitrogen atmosphere, then diluted with H₂O (30 mL), and extracted with CHCl₃ (3 x 30 mL). The organic layer was washed with H₂O, dried over Na₂SO₄ and evaporated to dryness to give the alcohol as a crude brown solid. Purification of the crude solid by flash chromatography using ethyl acetate as the eluent gave pale brown solid **18** (0.4 g, 51% over two steps). Mp 164–167 °C; ¹H NMR (500 MHz, CDCl₃): δ 7.28 (d, *J* = 8.0 Hz, 1H), 6.87 (d, *J* = 7.7 Hz, 1H), 6.81 (s, 1H), 6.77 (d, *J* = 8.0 Hz, 1H), 4.22 (s, 1H), 3.80 (s, 3H), 3.73 (s, 1H), 2.96 (s, 4H), 2.59 (d, *J* = 11.6 Hz, 1H), 2.04 (d, *J* = 12.1 Hz, 2H), 1.94 (d, *J* = 12.5 Hz, 1H), 1.89 (d, *J* = 8.5 Hz, 1H), 1.43 (m, 3H); ¹³C NMR (126 MHz, CDCl₃): δ 171.91, 159.79, 147.49, 129.56, 117.80, 112.10, 111.17, 74.36, 55.40, 53.58, 42.05, 38.02, 33.69, 30.80, 26.88, 17.66; HRMS (TOF MS ES⁺) calcd for C₁₆H₂₂NO₃ (M + H)⁺: 276.1600; found: 276.1584. Anal. C₁₆H₂₁NO₃ (C, H, N).

5.2.15. 5-(3-Methoxyphenyl)-2-azabicyclo[3.3.1]nonan-4-ol (18)—Compound **19** (200 mg, 70 % over two steps) was from obtained from **14** (300 mg, 1.1 mmol) as shown in the procedure for compound **15**. ¹H NMR (500 MHz, CD₃OD): δ 7.27 (t, *J* = 7.5 Hz, 1H), 6.95 (d, *J* = 7.6 Hz, 1H), 6.90 (s, 1H), 6.79 (dd, *J* = 8.0, 1.4 Hz, 1H), 4.01 (s, 1H), 3.80 (s, 3H), 3.64 (dd, *J* = 14.8, 2.4 Hz, 1H), 2.98 (d, *J* = 14.8 Hz, 1H), 2.48-2.45 (m, 1H), 2.10 (t, *J* = 8.9 Hz, 2H), 2.09 (m, 1H), 1.77 (m, 3H), 1.61-1.55 (m, 1H); ¹³C NMR (126 MHz, CD₃OD): δ 161.3, 152.1, 130.3, 118.7, 112.8, 111.9, 72.5, 55.6, 50.4, 41.4, 37.1, 32.1, 30.4, 22.9 (1C overlapped by CD₃OD signal between 50-48 ppm). HRMS (TOF MS ES⁺) calcd for C₁₅H₂₂NO₂ (M + H)⁺: 248.1651; found: 248.1653. Anal. C₁₅H₂₁NO₂•0.5CH₂Cl₂ (C, H, N).

5.2.16. 5-(3-Methoxyphenyl)-2-methyl-2-azaspiro[bicyclo[3.3.1]nonane-4,2'-[1,3]dioxolan]-3-one (19)—To a solution of the keto-amide **12** (1.3 g, 4.76 mmol) in toluene, Ethylene glycol (10 mL) and PTSA (1.8 g, 9.52 mmol) were added and the mixture was refluxed for 8 h. The reaction mixture was then cooled to room temperature and toluene evaporation. Extraction of the organic layer into CHCl₃ followed by drying and evaporation of the solvent gave a brown sticky solid. The solid was purified by flash chromatography using 90% ethyl acetate as the eluent to give a pale yellow solid (1.0 g, 66%). Mp 191–193 °C; ¹H NMR (500 MHz, CDCl₃): δ 7.07 (t, *J* = 7.9 Hz, 1H), 6.87 (m, 2H), 6.63 (d, *J* = 7.9 Hz, 1H), 4.31 (q, *J* = 7.0 Hz, 1H), 3.81 (q, *J* = 5.2 Hz, 1H), 3.72 (q, *J* = 4.9 Hz, 1H), 3.65 (s, 3H), 3.55 (s, 1H), 2.79 (s, 4H), 2.73 (d, *J* = 12.7 Hz, 1H), 2.54 (d, *J* = 13.9 Hz, 1H), 1.78 (d, *J* = 13.0 Hz, 2H), 1.53 (d, *J* = 14.5 Hz, 1H), 1.43 (d, *J* = 13.6 Hz, 1H), 1.26 (dd, *J* = 13.3, 4.3 Hz, 2H); ¹³C NMR (126 MHz, CDCl₃): δ 171.02, 158.80, 145.53, 128.15, 119.22, 113.39, 112.80, 110.98, 105.45, 68.16, 65.05, 54.96, 54.55, 44.07, 34.22, 33.15, 26.67, 17.39; HRMS (TOF MS AP⁺) calcd for C₁₈H₂₄NO₄ (M + H)⁺: 318.1705; found: 318.1712. Anal. C₁₈H₂₃NO₄•0.5H₂O (C, H, N).

5.2.17. 3-(2-Methyl-2-azaspiro[bicyclo[3.3.1]nonane-4,2'-[1,3]dioxolane]-5-yl)phenol (20)—To a solution of the amide **19** (500 mg, 1.57 mmol) in THF was added solid LAH (179 mg, 4.73 mmol) and the solution was refluxed for 5 h. After the completion of reaction, the excess LAH was quenched drop-wise by the addition of ethyl acetate, followed by a dropwise addition of 10% NaOH solution. The organic layer was washed with water and was then extracted in to CH₂Cl₂ (3 x 50 mL). Evaporation of the solvents gave a brown oil, which was purified into using flash chromatography using 90:10:1 Chloroform: methanol: ammonia to give a pale yellow oil in yield. The crude oil was dissolved in CHCl₃ (20 mL) and BBr₃ (5 eq) was added and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was cooled to room temperature and the excess BBr₃ was quenched with cold methanol (5 mL). Ammonium hydroxide (5 mL) was added and the organic layer was extracted into CHCl₃. Drying over Na₂SO₄, followed by evaporation of the solvent gave a crude solid. Crystallization of the crude solid from 2-

propanol gave compound **20** as a HBr salt (200 mg, 35 % over two steps). Mp 243 °C; ¹H NMR (500 MHz, CD₃OD): δ 7.09 (t, *J* = 7.8 Hz, 1H), 6.90 (d, *J* = 8.4 Hz, 2H), 6.66 (d, *J* = 8.0 Hz, 1H), 3.85 (q, *J* = 6.5 Hz, 1H), 3.78 (q, *J* = 6.7 Hz, 1H), 3.66 (s, 1H), 3.54 (q, *J* = 6.5 Hz, 1H), 3.47 (d, *J* = 13.5 Hz, 1H), 3.35 (d, *J* = 13.8 Hz, 1H), 3.02 (q, *J* = 6.7 Hz, 1H), 2.92 (s, 3H), 2.85 (d, *J* = 13.8 Hz, 1H), 2.63 (dd, *J* = 14.2, 5.4 Hz, 1H), 2.28 (d, *J* = 14.3 Hz, 1H), 1.96 (d, *J* = 13.6 Hz, 2H), 1.80 (dd, *J* = 9.7, 8.4 Hz, 2H), 1.61 (dt, *J* = 13.4, 6.6 Hz, 1H); ¹³C NMR (126 MHz, CD₃OD): δ 157.78, 146.15, 129.46, 119.83, 115.98, 114.54, 108.71, 67.48, 66.21, 58.94, 57.82, 42.49, 41.69, 34.09, 32.41, 23.21, 20.71; HRMS (TOF MS AP⁺) calcd for C₁₇H₂₃NO₃ (M + H)⁺: 290.1756; found: 290.1742. Anal. C₁₇H₂₂NO₃•HBr (C, H, N).

5.3. Pharmacology

5.3.1 Cell culture and membrane preparation for binding and efficacy assays

—As noted previously, [12] 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-418 (0.10-0.2 mg/mL) under 95% air/5% CO₂ at 37 °C. Cell monolayers were harvested and frozen at –80 °C.

5.3.2 Opioid binding assays—We used [³H][D-Ala²-MePhe⁴, Gly-ol⁵]enkephalin ([³H]DAMGO, SA=44–48 Ci/mmol) 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 x g for 10 minutes, resuspended in 10 mL/pellet ice-cold 10mM Tris-HCl, pH 7.4 and again centrifuged 30,000 x 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) are 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). The corresponding Bmax values were (fmol/mg protein ± SD, n=3): [³H]DAMGO (1912±68), [³H]DADLE (3655±391) and [³H](–)-U69,593 (3320±364).

5.3.3. [³⁵S]GTP-γ-S binding assays—The assays were conducted with minor modifications of published methods.[13] 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 x 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 µL GDP in buffer A/0.1% BSA (final concentration = 40 µM), 50 µL drug in buffer A/0.1% BSA, 50 µL [³⁵S]-GTP-γ-S in buffer A/0.1% BSA (final concentration = 50 pM), 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, 40 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 (Brandel) through GF/B filters. The filters were punched into 24-well plates to which was added 0.6 mL LSC-cocktail (Cytosint). Samples were counted, after an overnight extraction, in a Trilux liquid scintillation counter at 27% efficiency.

For the [³⁵S]GTP-γ-S binding experiments, the percent stimulation of [³⁵S]GTP-γ-S binding was calculated according to the following formula: $(S - B)/B \times 100$, where B is the basal level of [³⁵S]GTP-γ-S binding and S is the stimulated level of [³⁵S]GTP-γ-S binding. Agonist dose-response curves (ten points/curve) are generated, and the data of several experiments, 3 or more, are pooled. The EC₅₀ values (the concentration that produces fifty percent maximal stimulation of [³⁵S]GTP-γ-S binding) and E_{max} are determined using either the program MLAB-PC (Civilized Software, Bethesda, MD), KaleidaGraph (Version 3.6.4, Synergy Software, Reading, PA) or Prism 4.0 (GraphPad Software, Inc, San Diego, CA). In most cases, the percent stimulation of the test compound is reported as a percent of the maximal stimulation of 1000 nM DAMGO, 500 nM SNC80 or 500 nM (–)-U50,488 in the appropriate cell type. For determination of K_e values using the “shift” experimental design, agonist (DAMGO, (–)-U50,488 or SNC80) dose-response curves are generated, using the appropriate cell type, in the absence and presence (ten points/curve) of a test compound. The data of several experiments, 3 or more, are pooled, and the K_e values are calculated according to the equation: $[\text{Test Drug}]/(\text{EC}_{50-2}/\text{EC}_{50-1} - 1)$, where EC₅₀₋₂ is the EC₅₀ value in the presence of the test drug and EC₅₀₋₁ is the value in the absence of the test drug.

5.4. X-ray crystal structure methods

Single-crystal X-ray diffraction data on compounds **3a**, **5**, **10**, and **14** were collected using MoKα radiation ($\lambda = 0.71073 \text{ \AA}$) and a Bruker APEX 2 CCD area detector. The structures were 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 v6.10, 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 applied to H atoms] with C-H distance set at 0.96 Å. Atomic coordinates for these compounds have been deposited with the Cambridge Crystallographic Data Centre (deposition numbers for **3a**, **5**, **10** and **14**, respectively: 826966, 826968, 826969 and 826967). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

5.4.1. Compound 3a—A 0.103 x 0.086 x 0.078 mm³ crystal of **3a** was prepared for data collection coating with high viscosity microscope oil (Paratone-N, Hampton Research). The

oil-coated crystal was placed on a MicroMesh mount (MiTeGen, Ithaca, NY) and transferred immediately to the cold stream on the diffractometer. The crystal was monoclinic in space group $P2_1/c$ with unit cell dimensions $a = 10.2454(10)$ Å, $b = 11.6443(12)$ Å, $c = 12.1069(12)$ Å, and $\beta = 106.926(3)^\circ$. Corrections were applied for Lorentz, polarization, and absorption effects. Data were 99.3% complete to $29.23^\circ \theta$ (approximately 0.73 Å) with an average redundancy of 4.14.

5.4.2. Compound 5—A $0.578 \times 0.413 \times 0.097$ mm³ crystal of **5** was prepared for data collection coating with high viscosity microscope oil (Paratone-N, Hampton Research). The oil-coated crystal was placed on a MicroMesh mount (MiTeGen, Ithaca, NY) and transferred immediately to the cold stream on the diffractometer. The crystal was monoclinic in space group $P1$ with unit cell dimensions $a = 8.3955(4)$ Å, $b = 9.8351(4)$ Å, $c = 10.8184(4)$ Å, $\alpha = 73.265(2)^\circ$, $\beta = 72.228(1)^\circ$, and $\gamma = 66.195(1)^\circ$. Corrections were applied for Lorentz, polarization, and absorption effects. Data were 85.8% complete to $25.00^\circ \theta$ (approximately 0.83 Å) with an average redundancy of 1.02.

5.4.3. Compound 10—A $0.237 \times 0.198 \times 0.068$ mm³ crystal of **10** was prepared for data collection coating with high viscosity microscope oil (Paratone-N, Hampton Research). The oil-coated crystal was placed on a MicroMesh mount (MiTeGen, Ithaca, NY) and transferred immediately to the cold stream on the diffractometer. The crystal was monoclinic in space group $P2_1/n$ with unit cell dimensions $a = 6.6975(4)$ Å, $b = 29.4278(14)$ Å, $c = 10.8008(4)$ Å, and $\beta = 90.533(2)^\circ$. Corrections were applied for Lorentz, polarization, and absorption effects. Data were 99.4% complete to $28.37^\circ \theta$ (approximately 0.75 Å) with an average redundancy of 4.41.

5.4.4. Compound 14—A $0.691 \times 0.565 \times 0.043$ mm³ crystal of **4b** was prepared for data collection coating with high viscosity microscope oil (Paratone-N, Hampton Research). The oil-coated crystal was placed on a MicroMesh mount (MiTeGen, Ithaca, NY) and transferred immediately to the cold stream on the diffractometer. The crystal was monoclinic in space group $P2_1/n$ with unit cell dimensions $a = 10.6423(11)$ Å, $b = 6.6202(7)$ Å, $c = 20.749(2)$ Å, and $\beta = 94.253(4)^\circ$. Corrections were applied for Lorentz, polarization, and absorption effects. Data were 99.4% complete to $29.23^\circ \theta$ (approximately 0.73 Å) with an average redundancy of 4.03.

5.5. Quantum Chemistry and Superposition Study

Geometry optimization for compounds **2a**, **2b**, **3b**, **4b** and 14 β -hydroxymorphine, in their protonated form, was conducted in the gaseous phase with the density functional theory at the level of B3LYP/6-31G*[14]. For the superposition study, the optimized structures in the gaseous phase were overlaid onto compound **2b** with the rigid fit of Quanta 2008 (Accelrys) using the nine heavy atoms of the phenylmorphane as a common docking site. The root mean square differences of such fit for each compound were all less than 0.13 Å.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

GPCR	G-protein coupled receptor
Asp	aspartic acid
^1H NMR	proton nuclear magnetic resonance
^{13}C NMR	carbon nuclear magnetic resonance
TLC	thin-layer chromatography
ESI	high resolution electrospray ionization
TOF MS ES⁺	time-of-flight mass spectra, HRMS

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Appendix. Supplementary material

Supplementary Material attached in Article

Highlights

- Simplified synthesis of 5-(3-hydroxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-4-ol.
- Stereochemical inversion for epimeric analogue.
- Opioid receptor affinity and efficacy.
- Energy minimization at B3LYP/6-31G* level for molecular overlay.

Fig. 1.
5-Phenylmorphans structures

Fig. 2.
X-ray crystallographic structure of (1-(3-methoxyphenyl)-6-methyl-6-azabicyclo[3.2.1]octan-7-yl)methyl benzoate. HBr (**10.HBr**, top), and 3-(7-(hydroxymethyl)-6-methyl-6-azabicyclo[3.2.1]octan-1-yl)phenol (**5.HBr**. H₂O bottom)

Fig. 3.
X-ray crystallographic structure of the epimeric alcohols **3a**. HBr (top, $1S^*,4R^*,5R^*$) and **14** (bottom, $1R^*,4R^*,5S$)

Fig. 4.

Two views of the overlapped spatial positions of the C4α and C4β of **4b** vs the C9α and C9β-hydroxyl groups in the 5-phenylmorphinan **2b**.^[2] Atoms represented by colors as follows: white, hydrogen; green, carbon; blue, nitrogen; red, oxygen.

Fig. 5. Overlay of the C14-OH group in 14-hydroxymorphine on the C9β-OH of the 5-phenylmorphinan **2b**.^[2] The dotted line indicates the putative charge interaction between the tertiary nitrogen and an Asp residue at the binding site of the μ-opioid receptor.

Scheme 1.

Formation of 3-(7-(hydroxymethyl)-6-methyl-6-azabicyclo[3.2.1]octan-1-yl)phenol (**5**).

Reagents and conditions: a) NBS; b) NaBH_3CN ; c) $\text{K-CO}_2\text{C}_6\text{H}_5$, DMF, 130 °C, 65%; d)

K_2CO_3 , MeOH; e) BBr_3 , CHCl_3 , 74% (over 2 steps).

Scheme 2.

Synthesis of *N*-methyl and *N*-phenethyl substituted (1*S**,4*R**,5*R**)-5-(3-hydroxyphenyl)-2-azabicyclo[3.3.1]nonan-4-ol (**3a** and **3b**). Reagents and conditions: a) OsO₄, NMO, acetone; b) LAH, THF, reflux; c) BBr₃, CHCl₃, 61% (over 2 steps); d) Troc-Cl, proton sponge; e) Zn, AcOH, 67% (over 2 steps); f) 2-(bromoethyl)benzene, NaHCO₃; g) BBr₃, CHCl₃, 37% (over 2 steps).

Scheme 3.

Synthesis of *N*-methyl and *N*-phenethyl substituted (1*R**,4*R**,5*S**)-5-(3-hydroxyphenyl)-2-azabicyclo[3.3.1]nonan-4-ol (**4a** and **4b**). Reagents and conditions: a) Mesyl anhydride, Et₃N, 58%; b) K-CO₂C₆H₅, DMF, 130 °C; c) K₂CO₃, MeOH-H₂O, 51% (over 2 steps); d) LAH, AlCl₃, 68%; e) BBr₃, CHCl₃, 66%; f) Troc-Cl; g) Zn/AcOH, 70% (over 2 steps); h) 2-(bromoethyl)benzene, NaHCO₃; i) BBr₃, CHCl₃, 37% (over 2 steps).

Scheme 4.

Synthesis of 3-(2-methyl-2-azaspiro[bicyclo[3.3.1]nonane-4,2'-[1,3]dioxolan]-5-yl)phenol (**20**). Reagents and conditions: a) Ethylene glycol, PTSA, toluene, reflux, 66%; b) LAH; c) BBr₃, CHCl₃, 35% (over 2 steps).

Table 1

Reactions attempted to avoid azirindium ion formation.

Comp.	Reagents/Conditions	Results
6	Borane-THF, H ₂ O ₂	decomposition
6	Borane-DMS, H ₂ O ₂	enamine reduction
6	Thexyl-Borane, H ₂ O ₂	enamine reduction
6	Oxirane, NaBH ₄	decomposition
7	DMF, KOH	decomposition
7	CuI, H ₂ O, DMF, heat	decomposition

Table 2

Binding affinity of test compounds at opioid receptors

Comp.	<i>K_i</i> (nM)		
	μ^a	δ^b	κ^c
2a[2]	45 ± 4	720 ± 40	690 ± 30
2b[2]	0.19 ± 0.01	13 ± 1	184 ± 9
3a	138 ± 8	7200 ± 481	3800 ± 150
3b	74 ± 6	5800 ± 424	751 ± 60
4a	346 ± 33	> 15,000	8300 ± 662
4b	4.6 ± 0.42	1000 ± 113	604 ± 28
5	315 ± 20	> 10,000	483 ± 24
20	22 ± 2.4	> 7800	140 ± 7
Morphine	2.6 ± 0.01	605 ± 36	299 ± 49
DAMGO	1.2 ± 0.1	1500 ± 170	
U69,593	2370 ± 94	12540 ± 1075	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 IC₅₀, the value of which was then used to calculate the *K_i* value, as described in section 5.3.2.

^a[³H]-DAMGO

^b[³H]-DADLE

^c[³H]-U69,593

Table 3Antagonist K_e values for the MOR receptor determined with the [35 S]GTP- γ -S functional binding assay^a

Comp.	K_e (nM) $\pm$ SD
20	154 $\pm$ 0.09
4b	12 $\pm$ 2
Naloxone	2.3 $\pm$ 0.3

^aCompounds **20** and **4b** demonstrated no agonist activity in the μ -receptor [35 S]GTP- γ -S binding assay. To determine the K_e value of these compounds as antagonists, “shift” experiments were conducted as described in Methods.

Briefly, DAMGO dose-response curves were generated in the absence and presence of a fixed concentration of **20** (100 nM) and **4b** (25 nM). The pooled data of three experiments were fit to the dose-response equation for the best-fit estimates of the ED50 and EMAX values. The K_e was calculated based on the increased value of the ED50 value observed in the presence of the test compound.