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Synthesis and biological characterization of (3*R*,4*R*)-4-(2-(benzhydryloxy)ethyl)-1-((*R*)-2-hydroxy-2-phenylethyl)-piperidin-3-ol and its stereoisomers for monoamine transporters

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Abstract

In this report we describe synthesis and biological evaluation of a series of asymmetric 4-(2-(benzhydryloxy)ethyl)-1-((*R*)-2-hydroxy-2-phenylethyl)-piperidin-3-ol based dihydroxy compounds where the hydroxy groups are located both on the piperidine ring and also on the N-phenylethyl side chain exo-cyclically. In vitro uptake inhibition data indicates high affinity of these molecules for the dopamine transporter (DAT) in addition to their moderate to high affinity for the norepinephrine transporter (NET). Interestingly, compounds **9b** and **9d** exhibited affinities for all three monoamine transporters with highest potency at DAT and NET and moderate potency at the serotonin transporter (SERT) (K_i 2.29, 78.4 and 155 nM for **9b** and 1.55, 14.1 and 259 nM for **9d**, respectively). Selected compounds, **9a**, **9d** and **9d'** were tested for their locomotor activity effects in mice, and for their ability to occasion the cocaine discriminative stimulus in rats. These test compounds generally exhibited a much longer duration of action than cocaine for elevating locomotor activity, and dose-dependently completely generalized the cocaine discriminative stimulus.

Keywords

Cocaine; dopamine; structure-activity relationships; monoamine transporters

Introduction

Cocaine is a powerful drug of abuse and is well known for its strong reinforcing activity. Addiction to cocaine has greatly impacted the nation in terms of its economy and securing law and order.^[1] Furthermore, this addiction has significantly contributed to the spreading of HIV infection and hepatitis as needle sharing is a pervasive problem among drug abusers. Currently, no effective medication is available for the treatment of cocaine addiction and there is a great need for the development of an effective medication.^[2,3]

Cocaine binds to the dopamine (DAT), serotonin (SERT) and norepinephrine (NET) monoamine transporter proteins in the brain.^[4,5] Multiple evidences indicate that the potent

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reinforcing property of cocaine can mostly be accounted for from its binding to the DAT. The dopamine hypothesis of cocaine addiction received additional support from in vivo experiments and molecular biological studies involving DAT knockout mice.^[6] Thus, binding potencies of dopamine receptor agonists and dopamine transporter specific compounds correlate very well with their relative reinforcing effects in animal drug discrimination and self-administration experiments.^[7,8] Finally, in recent Positron Emission Tomography (PET) studies it was demonstrated that the subjective effect of cocaine in humans directly correlates with the extent to which it occupies the dopamine transporter, and substantial amount of DAT occupancy is required by cocaine analogues to decrease cocaine self-administration in primates.^[9,10] Furthermore, it was reported that binding to DAT is mainly responsible for cocaine's reinforcing effect using a mouse knock-in model.^[11]

In spite of a strong correlation between dopaminergic activity and cocaine reinforcing effects, the serotonin (5-HT) system has also been implicated in some important effects of cocaine. Dopaminergic compounds with increased activity for serotonin system reduce the reinforcing potential of these drugs. Monoamine releaser compounds with both potent dopamine and serotonin releasing activities exhibit reduced reinforcing activity.^[12] In addition it has also been demonstrated that SERT blockers can reduce cocaine self-administration.^[13] Considering cocaine's affinity for all three monoamine transporters in the brain, possibility of compounds with multiple monoamine transporter activities for modulating cocaine's effect will be high.

Many of the advances made in developing drugs for the treatment of cocaine addiction are focused on targeting the DAT.^[14] Molecules with diverse structures have been developed for the DAT. Detailed structure activity relationship (SAR) studies of these different categories of molecules have been described in recent review papers. In our ongoing effort to design novel molecules targeting monoamine transporters for the development of pharmacotherapies for cocaine addiction, we have developed a large number of flexible piperidine analogs of GBR 12909 exhibiting potent affinity at the DAT.^[15-18] In one of our piperidine analogs of GBR 12909, the presence of a hydroxyl group on the piperidine ring enhanced the potency and selectivity for DAT compared to the parent compound **I** (see Figure 1).^[19] DAT binding affinity was 20-fold higher in the most active enantiomer, compound **II** (D-84, Figure 1) of the molecule compared to the parent non-hydroxylated molecule, which was attributed to the formation of an H-bond between the hydroxyl moiety in the compound and the DAT. Our site-directed mutagenesis study pointed to a role for the Asp68 residue in the first trans-membrane domain of DAT^[20], but more recent structural information from the bacterial homolog of DAT, the LeuT places this residue at the cytoplasmic end of the transmembrane helix.^[21] At the present time, it is not known whether DAT inhibitors can bind so deep down in the protein structure, although GBR-like compounds have sufficient lipophilicity to penetrate the membrane. Introduction of a hydroxyl group into compound **I** (Figure 1) also increased its in vivo potency significantly compared to the non-hydroxylated counterpart, suggesting an additional influence of the hydroxyl group controlling penetration of the blood-brain barrier.

In a further attempt to expand our SAR studies, a second hydroxyl group was introduced on the phenyl alkyl side chain of these molecules resulting in the design of the current set of compounds. In GBR 12909-like molecules, such introductions of a hydroxyl group on phenylalkyl side chains increases activity.^[22] In our current design, we wanted to explore the influence of introducing two stereochemically different hydroxyl groups, one located on the piperidine ring and the other one located as an exocyclic hydroxyl group on the N-alkyl-phenyl side chain, on the affinity and selectivity for DAT, SERT and NET and their ability

to produce cocaine-like activity. In this report, we describe the design, synthesis and biological characterization of dihydroxy analogs of I (Figure 1).

Chemistry

Schemes 1-3 outline the synthesis of our target compounds. In Scheme 1, 4-vinylpyridine (**1**) was reacted in presence of sodium methoxide with benzhydrol (**2**) in a Michael-type reaction to yield intermediate **3**. This reaction did not go to completion despite using 4 equivalents of **2**. Dibenzhydryl ether, self condensation product of benzhydrol, was found to be the major side product. Changing the equivalents of benzhydrol from 4 to 2 has little effect on the final yield of the product. Compound **3** was converted to 1,2,3,6-tetrahydropyridine **4** by a well-known reaction; initially converting pyridine to its pyridinium salt by reaction with benzyl bromide and reducing the salt with sodium borohydride.^[23] The change of solvent for preparation of the quaternary salt from MeOH to AcCN resulted in moderate improvement in the yield of the tetrahydropyridine **4**. N-debenzylation of **4** was carried out by its initial reaction with methyl chloroformate followed by hydrolysis of the resulting carbamate by Claisen's alkali gave amine **5** in good yield.^[17]

Opening of the optically active epoxides **6a-d** with **5** in refluxing ethanol gave secondary alcohols (represented as O-acylated products **7a-d**) in good yield (Scheme 2). Major side product of this reaction was the constitutional isomer, i.e., primary alcohol. The alcohols were not different on TLC whereas their O-acyl derivatives were found to exhibit slightly different R_f values. Careful and rapid separation of the acylated compounds using column chromatography gave the desired acetates. Hydroboration of the olefinic bond of **7a-d** by borane reagent (generated in situ by reaction of boron trifluoride with sodium borohydride) and subsequent oxidative cleavage by $H_2O_2/NaOH$ yielded diastereomeric pairs *trans*-(**8a**, **8a'**), *trans*-(**8b**, **8b'**), *trans*-(**8c**, **8c'**) and *trans*-(**8d**, **8d'**) in good yields. The *trans* structure and the absolute stereochemistry of the lead molecule II (D-84^[19], Figure 1) has been confirmed by NMR and X-ray analysis as reported previously. Separation of the diastereomeric pair was carried out by semipreparative HPLC, and the deprotection of the exocyclic secondary alcohols was done by reaction with potassium carbonate in methanol to yield final compounds **9a-d** and **9a'-9d'** (Scheme 3).

The assignment of the absolute stereochemistry at the exocyclic chiral center was based on the absolute stereochemistry of the epoxides **6a-6d**. Racemic epoxides were resolved using hydrolytic kinetic resolution and their optical purity was checked by analytical HPLC using chiral column.^[24]

Acetates derived from S-epoxides **6b** and **6d**, i.e., **7b** and **7d**, respectively, exhibited dextrorotatory behavior whereas their enantiomers **7a** and **7c**, derived from R-epoxides, **6a** and **6c**, were levorotary. After hydroboration reaction, diastereomeric pair was obtained from each secondary acetate. In case of pair (**8a**, **8a'**) which was derived from (–)-**7a**, compound **8a** showed optical rotation of -12.72° whereas **8a'** showed optical rotation of -48.76° . Our previous work on **II** and **III** (Figure 1) established the absolute stereochemistry at the cyclic chiral center to be (3*S*,4*S*) for the (–)-enantiomer and (3*R*,4*R*) for the (+)-isomer.^[19] Compound **8a** exhibited higher optical rotation compared to its precursor (–)-**7a** (-20.22° vs -12.72°) whereas **8a'** showed decreased rotation (-48.76° vs -12.72°). Thus, we assigned the absolute stereochemistry at the cyclic chiral centers as (3*R*,4*R*) for **8a** and (3*S*,4*S*) for **8a'**. Similar assignment of absolute stereochemistry was done for the remaining molecules.

Results and Discussion

As described earlier, the design of our molecules included in this manuscript was based upon structural extension of **II** (Figure 1) which exhibited high affinity and selectivity for DAT.^[19-20] We were particularly interested to uncover any additional influence of the exocyclic second hydroxyl group incorporated in the molecular template of **II** on potency of uptake inhibition of these compounds. Specifically, we were interested to determine how the activity from such a structural modification would compare with **II** which contains a stereochemically fixed *R*-hydroxyl group on the piperidine ring. From the structural point of view, by introducing an exocyclic hydroxyl group in the racemic version of **II** led to formation of two diastereomeric molecules each containing three asymmetric centers. Thus, these new derivatives were structurally more complex and could, thereby, provide unique information about their interaction with all three monoamine transporters.

All synthesized derivatives were tested for their affinities for the dopamine transporter (DAT), serotonin transporter (SERT), and norepinephrine transporter (NET) in the brain by measuring their potency in inhibiting the uptake of [³H]DA, [³H]5-HT, and [³H]NE, respectively. Compounds were also tested for their binding potency at the DAT by their ability to inhibit binding of [³H]WIN 35, 428.

Compounds **9a** and **9a'** represent the first set of two diastereomers synthesized from the intermediate **7a**. In this intermediate the stereochemistry of the exocyclic hydroxyl group was fixed in *R*-configuration. The hydroxylation of the double bond in the piperidine ring in intermediate **7a** produced hydroxyl group with *trans* stereochemistry with the production of two diastereomers **9a** (3*R*,4*R*,2' *R*) and **9a'** (3*S*,4*S*,2' *R*). The hydroxyl group on the piperidine ring in compound **9a** represents identical *R*-absolute stereochemistry as in **II** (3*R*,4*R*) whereas the same hydroxyl group in **9a'** represents the stereochemistry of compound **III** (D-83, Figure 1) which is the enantiomer of **II** (Figure 1). Uptake inhibition activity indicated that **9a** potently inhibited both dopamine and norepinephrine uptake with low nano molar potency (*K_i* 6.23 and 7.56 nM for DAT and NET, respectively). The activity for SERT was moderate (*K_i* 456 nM). The diastereomer **9a'** exhibited similar inhibition profile although the affinities for DAT and NET was three fold less (*K_i* 18.4 and 27.5 nM, respectively). Thus, in these two stereomers, the modest difference in activity is mainly originating from the difference in stereochemical configuration of the piperidynyl 3-hydroxyl group as the exocyclic hydroxyl configuration remains the same in both compounds.

For compounds **9b** and **9b'** the intermediate was **7b** which contains an exocyclic hydroxyl group with the *S*-absolute configuration. Compound **9b** with (3*R*,4*R*)-absolute configuration was highly potent in inhibiting uptake of DAT (*K_i* 4.69 nM) and was moderately potent in inhibiting uptake of SERT and NET (*K_i* 155 and 78.4 nM for SERT and NET, respectively). Thus, in comparison to **9a**, **9b** exhibited similar potency for DAT, however, unlike **9a** it exhibited 3-fold higher potency for SERT and 10-fold lower potency for NET (*K_i* 155 and 78 nM, respectively). This demonstrates the differential influence of stereochemically different exocyclic hydroxyl groups on uptake inhibition profile. Interestingly, compound **9b'** showed higher potency for DAT compared to **9b** (*K_i* 1.06 vs. 4.69 nM) which indicated that the dominant structural feature which influences interaction of these compounds with DAT is the nature of stereochemistry of the exocyclic hydroxyl group as the presence of *S*-hydroxyl group enhanced interaction with DAT. Compound **9b'** was relatively weaker at both SERT and NET compared to **9b**.

We observed a similar trend of activity in the fluoro derivatives **9c** and **9c'**. Between these two diastereomers, the slightly higher affinity for DAT uptake inhibition was exhibited by

9c' (K_i 2.44 vs. 4.04 nM for **9c'** and **9c**, respectively). Both these compounds exhibited similar high potency for NET (K_i 24.8 and 21.9 nM for **9c'** and **9c**, respectively). However, compound **9c** was less potent at SERT compared to **9c'** (K_i 1055 vs. 402 nM). Similar results were exhibited by the other two diastereomeric compounds **9d** and **9d'**. The activity for DAT and NET exhibited by these two compounds were very similar when **9d** was slightly more potent compared to **9d'** (K_i 1.55 and 14.1 nM vs. 2.97 and 20.6 nM, respectively for **9d** and **9d'**). Interestingly, compound **9d** with (3R,4R,2'S)-absolute configuration exhibited greater affinity for the SERT. Similarly, compound **9b** with (3R,4R,2'S)-absolute configuration displayed higher activity for SERT compared to **9b'**. Following in vitro uptake inhibition, compounds **9a**, **9d** and **9d'** were selected for in vivo locomotor and drug discrimination studies. Among these three compounds, **9a** and **9d** were non-selective, exhibiting high affinity for both DAT and NET, and moderate affinity for SERT. In this regard, these compounds were more potent in inhibiting dopamine uptake compared to cocaine (Table 1).^[27] As multiple monoamine transporters might be involved in cocaine's reinforcing effect, these two compounds are thought to be good candidates for further in vivo evaluation in behavioral studies. On the other hand, compound **9d'** exhibited very weak affinity for SERT and high affinity for DAT and NET. Thus, this compound was a good candidate for in vivo evaluation in order to assess whether the absence of SERT activity will produce any difference in behavioral outcome compared to the previous two compounds.

Effects of **9a**, **9d** and **9d'** on locomotor activity

All doses of all three drugs significantly ($P < 0.05$) increased distance traveled relative to their respective vehicle condition at least during one of the 24 10-min recording periods except at 1 mg/kg for **9d** and **9d'**. Doses and drugs which did significantly increase distance traveled generally had their peak effects occur within the first 20 min of the test session, except for **9a** at 1 mg/kg in which cumulative distance traveled (cm) was slightly greater between 20-30 min than between 10-20 min of the test session, and at 100 mg/kg for **9d'** which didn't significantly increase locomotor activity until 100 min into the test session.

Figure 2 shows the effects of test compounds on mean total distance traveled (cm) during each hour of the 4-h test session. The greatest increases in locomotor activity accumulated within any one hour of the test session occurred at 30 mg/kg for each test compound. Relative to each compound's vehicle condition, all compounds were elevating locomotor activity at 3 h into the test session; for **9a** these effects persisted even at 4 h into the test session especially at 30 and 100 mg/kg.

Effects of cocaine and **9a**, **9d** and **9d'** in cocaine discrimination tests

As shown in the top panel of Figure 3, increasing the dose of cocaine increased the percentage of cocaine-lever responding until at 10 and 30 mg/kg complete (~80%) cocaine-lever selection was occasioned. The ED_{50} (95% CI) for cocaine was 3.11 (1.70-5.70) mg/kg. Tests with saline occasioned near-zero levels of cocaine-lever pressing. These data indicate that cocaine had been effectively established as a discriminative stimulus prior to tests with the other test compounds. Increasing the doses of each test compound also increased the percentage of cocaine-lever selection and completely occasioned the cocaine discriminative stimulus. **9a** was about equipotent as cocaine for doing so, followed by **9d** and **9d'**, respectively. The ED_{50} s (95% CI) for occasioning the cocaine discriminative stimulus for **9a**, **9d** and **9d'** were 3.10 (1.05-9.09), 9.10 (4.10-20.19), and 17.12 (6.41-45.75) mg/kg, respectively.

Figure 3 (lower panel) shows that increasing the dose of all drugs progressively decreased response rates. Cocaine was more potent for suppressing lever pressing than the other drugs, which appeared to be about equipotent for doing so. The ED_{50} s (95% CI) for cocaine and **9a**

were 17.09 (6.32-46.24) and 58.32 (11.51-295.60) mg/kg, respectively. The ED₅₀s for suppressing response rates for **9d** and **9d'** could not be estimated because their 95% CIs.

Conclusion

In this report, we describe design and development of a series of asymmetric dihydroxy molecules which are an extension of our earlier work based on compounds II and III. The goal was to assess the effect of stereochemistry of different hydroxyl groups on affinity and selectivity for monoamine transporters. Our results indicate that selectivity and potency were moderately influenced by variation of stereochemistry of the two hydroxyl groups. One interesting difference between these series of molecules and the parent II is that the current molecules in general exhibited less selectivity for the DAT as they exhibited moderate to high uptake inhibition activity for the NET and the SERT. In fact, compounds **9b** and **9b'** while exhibiting high affinity for DAT, were also quite potent at NET and SERT. Locomotor activity tests with three selected compounds **9a**, **9d** and **9d'**, indicated that these derivatives exhibit a long duration of action with **9a** exhibiting the longest duration of action. All three compounds completely occluded the cocaine discriminative stimulus and did so with ED₅₀s ranging between 3.09 mg/kg (**9a**) to 17.12 mg/kg (**9d'**).

Experimental

Reagents and solvents were obtained from commercial suppliers and used as received unless otherwise indicated. Dry solvent was obtained according to the standard procedure. All reactions were performed under inert atmosphere (N₂) unless otherwise noted. Analytical silica gel 60 F₂₅₄-coated TLC plates were purchased from EMD Chemicals, Inc. and were visualized with UV light or by treatment with phosphomolybdic acid (PMA), Dragendorff's reagent or ninhydrin. Flash column chromatography was carried out on Whatman Purasil® 60A silica gel 230-400 mesh. ¹H NMR spectra were routinely obtained at Varian 400 MHz FT NMR. TMS was used as an internal standard. Normal phase HPLC analyses were performed using Waters® high-performance liquid chromatograph (Waters, Millipore, MA) consisting of Waters Delta 600 pump and Waters 2487 dual λ absorption detector. Analytical HPLC analyses were carried out using Nova-Pak Silica 60 Å 4 μm (150 mm×3.9 mm; Waters) column. For semi-preparative analyses, Nova-Pak Silica 6 μm (300 mm×19 mm; Waters) column was used. Optical purity of chiral epoxides was determined using (*R,R*)-Whelk-O1 5 μm, 100 Å column (25 cm×4.6 mm; Regis Technologies, Morton Grove, IL). Optical rotations were recorded on Perkin-Elmer 241 polarimeter. Melting points were recorded using MEL-TEMP II (Laboratory Devices Inc., USA) capillary melting point apparatus and were uncorrected. Elemental analyses were performed by Atlantic Microlab, Inc. and were within ± 0.4% of the theoretical value.

Synthesis of 4-(2-(Benzhydryloxy)ethyl)pyridine (3)—To a stirred solution of 4-vinylpyridine (**1**) (10 g, 95.1 mmol) and benzhydrol (**2**) (35 g, 190 mmol), was added NaOMe (1.54 g, 28.5 mmol). The reaction mixture was heated at 130-140 °C for 20 hrs, cooled to RT and acidified with 1 N HCl (150 mL) followed by extraction with EtOAc (3×30 mL) to remove unreacted **2** and the side product dibenzhydryl ether. The aqueous layer was further basified with 10 % NaOH (pH 10) and extracted with CH₂Cl₂ (3×30 mL). Combined organic layers were washed with water, dried (Na₂SO₄) and the solvent evaporated in vacuo. Unreacted **1** was removed by vacuum distillation to afford pure **3** as pale brown oil (8.7 g, 52%); *R*_f = 0.27 (Hex:EtOAc 5:1); ¹H-NMR (400 MHz, CDCl₃): δ=2.90–2.93 (t, *J*=6.4 Hz, 2H), 3.66–3.67 (t, *J*=6.8 Hz, 2H), 5.32 (s, 1H), 7.13–7.14 (d, *J*=5.6 Hz, 2H), 7.17–7.33 (m, 10H), 8.47–8.48 (d, *J*=6.0 Hz, 2H) ppm

Synthesis of 4-(2-(Benzhydryloxy)ethyl)-1-benzyl-1,2,3,6-tetrahydropyridine (4)
—A mixture of **3** (4 g, 13.8 mmol) and benzyl bromide (2.86 g, 16.7 mmol) in dry AcCN

(20 mL) was refluxed for 6 hrs. The solvent was removed in vacuo. After drying for 2 hrs in vacuo, the residue was dissolved in dry MeOH (30 mL) and cooled in an ice-bath. NaBH₄ (0.76 g, 20 mmol) was then added slowly portionwise over a period of 1.5 hrs, and the solution was gradually brought to RT. The reaction was quenched with water after stirring for 4 hrs, and MeOH was removed in vacuo. The residual product was dissolved in EtOAc, washed with water, dried (Na₂SO₄), and concentrated. The crude product was purified by column chromatography (Hex:EtOAc 5:1) to produce **4** as a colorless oil (5 g, 94%): *R*_f = 0.32 (Hex:EtOAc 5:1); ¹H-NMR (400 MHz, CDCl₃): δ = 2.09 (brs, 2H), 2.32–2.35 (t, *J* = 6.8 Hz, 2H), 2.52–2.54 (t, *J* = 5.6 Hz, 2H), 2.94–2.94 (brm, 2H), 3.52–3.55 (m, 4H), 5.34 (s, 1H), 5.41–5.42 (brs, 1H), 7.12–7.37 (m, 15H) ppm.

Synthesis of 4-(2-(Benzhydryloxy)ethyl)-1,2,3,6-tetrahydropyridine (**5**)—A

solution of **4** (12.44 g, 32.4 mmol) and methyl chloroformate (5.0 mL, 64.8 mmol) in benzene (40 mL) was refluxed for 6 hrs. After completion of reaction (monitored by TLC), the solvent was removed in vacuo to yield methyl 4-(2-(benzhydryloxy)ethyl)-5,6-dihydropyridine-1(2*H*)-carboxylate in the form of viscous oil. It was purified by column chromatography (Hex:EtOAc 9:1) to get pure carbamate (6.7 g, 59%).

To a stirred solution of above carbamate (6.5 g, 18.5 mmol) in EtOH (25 mL) was added Claisen's alkali (6.46 g KOH dissolved in 5 mL water and 20 mL MeOH). The solution was refluxed overnight, concentrated in vacuo, diluted with water (40 mL) and extracted with ether (3X30 mL). The combined ethereal extracts were dried (Na₂SO₄) and evaporated to afford viscous liquid. The product was purified by column chromatography (EtOAc:MeOH:TEA 20:10:2.5) to obtain compound **5** as yellow viscous liquid (4.8 g, 88%): *R*_f = 0.4 (Hex:EtOAc 9:1); ¹H-NMR (400 MHz, CDCl₃): δ = 2.03 (brm, 2H), 2.29–2.32 (t, *J* = 6.4 Hz, 2H), 2.92–2.95 (t, *J* = 5.6 Hz, 2H), 3.31 (brs, 2H), 3.49–3.53 (t, 2H), 5.32 (s, 1H), 5.41 (brs, 1H), 5.76 (brs, 1H), 7.17–7.33 (m, 10H) ppm. ¹³C NMR (75 MHz, CDCl₃) 28.1, 38.0, 42.2, 44.1, 67.8, 83.9, 120.0, 127.3, 127.6, 128.4, 134.2, 142.9 ppm.

General Procedure I—A solution of **5** (1 equiv.) and styrene oxide **6** (1 equiv.) in EtOH was refluxed overnight. The reaction mixture was cooled to RT and the solvent was evaporated in vacuo. The brownish oily residue was purified by column chromatography over silica gel (Hex:EtOAc 1:1) in order to remove any unreacted starting materials, if any, to yield a colorless oil which was found to be a mixture of regioisomers [in case of compound **7a**, (*R*)-2-(4-(2-(benzhydryloxy)ethyl)-5,6-dihydropyridin-1(2*H*)-yl)-1-phenylethanol, the desired product, and (*R*)-2-(4-(2-(benzhydryloxy)ethyl)-5,6-dihydropyridin-1(2*H*)-yl)-2-phenylethanol] which was then subjected to acetylation using pyridine and acetic anhydride. The reaction mixture was kept at RT with occasional shaking. After 4 hrs, the solvent was removed in vacuo and the residue was taken up in CH₂Cl₂ (15 mL), washed with water (10 mL), dried over Na₂SO₄ and the solvent removed in vacuo. Column chromatography of the crude product over silica gel (Hex:EtOAc 8:2) yielded the acetylated product.

(*R*)-2-(4-(2-(Benzhydryloxy)ethyl)-5,6-dihydropyridin-1(2*H*)-yl)-1-phenylethyl acetate (7a**)**—General procedure I was followed using **5** (0.5 g, 1.7 mmol), (*R*)-styrene oxide **6a** (0.21 g, 1.7 mmol) and EtOH (20 mL). Yield: 0.7 g. For acetylation, the mixture of regioisomers (0.7 g, 1.693 mmol), pyridine (10 mL) and Ac₂O (0.21 mL, 2.2 mmol, 1.3 equiv) were used to afford **7a** as colorless oil (0.26 g, 34%): *R*_f = 0.56 (Hex:EtOAc 1:1); ¹H-NMR (400 MHz, CDCl₃): δ = 2.03–2.07 (brs, 5H), 2.31–2.34 (t, *J* = 6.4 Hz, 2H), 2.54–2.71 (m, 3H), 2.91–2.97 (dd, *J* = 8.8 Hz, 1H), 3.04 (brs, 2H), 3.51–3.54 (t, 2H), 5.34 (s, 1H), 5.39 (brs, 1H), 5.95–5.98 (dd, 1H), 7.03–7.34 (m, 15H) ppm; [α]_D²⁵ = –20.22° (c = 1.05 in CHCl₃). ¹³C NMR (75 MHz, CDCl₃) 21.8, 29.9, 37.4, 50.1, 52.8, 63.7, 68.0, 73.8, 84.0,

120.5, 126.7, 127.2, 127.6, 128.0, 128.5, 134.0, 139.8, 142.7, 170.6 ppm; $[\alpha]^{25}_D = -20.22^\circ$ ($c = 1.05$ in CHCl_3).

(S)-2-(4-(2-Benzhydryloxy)ethyl)-5,6-dihydropyridin-1(2H)-yl)-1-phenylethyl acetate (7b)—General procedure I was followed using **5** (0.5 g, 1.7 mmol), (*S*)-styrene oxide **6b** (0.21 g, 1.7 mmol) and EtOH (20 mL). Yield: 0.65 g. For acetylation, the mixture of regioisomers (0.5 g, 1.2 mmol), pyridine (10 mL) and Ac_2O (0.15 mL, 1.6 mmol, 1.3 equiv) were used to afford **7b** as colorless oil (0.2 g, 36%): $R_f = 0.52$ (Hex:EtOAc 1:1); $^1\text{H-NMR}$ (400 MHz, CDCl_3): $\delta = 2.03\text{--}2.07$ (brs, 5H), 2.31–2.34 (t, $J = 6.4$ Hz, 2H), 2.59–2.74 (m, 3H), 2.95–2.30 (dd, $J = 9.2$ Hz, 1H), 3.04 (brs, 2H), 3.51–3.54 (t, 2H), 5.33 (s, 1H), 5.39 (brs, 1H), 5.96–6.0 (dd, 1H), 7.21–7.37 (m, 15H) ppm; $[\delta]^{25}_D = +21.17^\circ$ ($c = 1.02$ in CHCl_3).

(R)-2-(4-(2-(Benzhydryloxy)ethyl)-5,6-dihydropyridin-1(2H)-yl)-1-(4-fluorophenyl)ethyl acetate (7c)—General procedure I was followed using **5** (0.5 g, 1.7 mmol), (*R*)-4-fluorostyrene oxide **6c** (0.232 g, 1.7 mmol) and EtOH (20 mL). Yield: 0.38 g. For acetylation, the mixture of regioisomers (0.38 g, 0.9 mmol), pyridine (10 mL) and Ac_2O (0.11 mL, 1.15 mmol, 1.3 equiv) were used to afford **7c** as pale yellow oil (0.18 g, 43%): $R_f = 0.56$ (Hex:EtOAc 1:1); $^1\text{H-NMR}$ (400 MHz, CDCl_3): $\delta = 2.02\text{--}2.06$ (brs, 5H), 2.30–2.38 (t, 2H, $J = 6.4$ Hz, 2H), 2.52–2.69 (m, 3H), 2.91–2.96 (dd, $J = 8.8$ Hz, 1H), 3.04 (brs, 2H), 3.51–3.55 (t, 2H), 5.37 (s, 1H), 5.39 (brs, 1H), 5.95–5.98 (dd, 1H), 6.99–7.04 (t, 2H), 7.21–7.33 (m, 12H) ppm; $[\alpha]^{25}_D = -24.24^\circ$ ($c = 0.77$ in CHCl_3).

(S)-2-(4-(2-(benzhydryloxy)ethyl)-5,6-dihydropyridin-1(2H)-yl)-1-(4-fluorophenyl)-ethyl acetate (7d)—General procedure I was followed using **5** (0.48 g, 1.6 mmol), (*S*)-4-fluorostyrene oxide **6d** (0.23 g, 1.6 mmol) and EtOH (20 mL). Yield: 0.42 g. For acetylation, the mixture of regioisomers (0.42 g, 1 mmol), pyridine (10 mL) and acetic anhydride (0.12 mL, 1.3 mmol, 1.3 equiv) were used to afford **7d** as a pale yellow oil (0.25 g, 54%): $R_f = 0.51$ (Hex:EtOAc 1:1); $^1\text{H-NMR}$ (400 MHz, CDCl_3): $\delta = 2.03\text{--}2.06$ (brs, 5H), 2.31–2.34 (t, $J = 6.4$ Hz, 2H), 2.60–2.73 (m, 3H), 2.95–3.0 (dd, $J = 8.8$ Hz, 1H), 3.04 (brs, 2H), 3.51–3.54 (t, 2H), 5.33 (s, 1H), 5.39 (brs, 1H), 5.96–5.99 (dd, 1H), 6.99–7.03 (t, 2H), 7.23–7.33 (m, 12H) ppm; $[\alpha]^{25}_D = +24.89^\circ$ ($c = 0.83$ in CHCl_3).

General Procedure II. Synthesis of (8a, 8a'), (8b, 8b'), (8c, 8c') and (8d, 8d')—

Into a stirred solution of NaBH_4 in dry THF at 0°C was added dropwise 48 % w/w BF_3 -ether complex. The cooling bath was removed, and the solution was allowed to stir for 1 hr at RT. The mixture was then cooled in an ice-bath. Into the cooled solution was added dropwise a solution of **8** in THF (10 mL). The solution was brought back to RT and stirred for an additional 2 hrs. The solution was again cooled to 0°C and water, EtOH and 3N aq. NaOH solution were added followed by dropwise addition of 30 % H_2O_2 . The reaction mixture was stirred at 55°C overnight, cooled to RT and the solvent evaporated in vacuo. The product was partitioned between water and EtOAc. The aqueous layer was extracted with EtOAc and the combined organic layers were dried (Na_2SO_4) and concentrated to give crude diastereomeric mixture which was chromatographed to give pale yellow viscous oil.

Separation of diastereomers—Diastereomeric mixtures (**8a, 8a'**), (**8b, 8b'**), (**8c, 8c'**) and (**8d, 8d'**) were separated by semipreparative HPLC using a normal-phase column (Nova-Pack Silica 6 μM). The mobile phase used was either 4 or 5 % 2-propanol in hexanes with a flow rate of 15 mL/min. Final purity of the separated diastereomers was checked by an analytical normal phase column (Nova-Pack Silica 60 Å 4 μM) using the same mobile phase with a flow rate of 1 mL/min.

(1*R*)-2-(4-(2-(Benzhydryloxy)ethyl)-3-hydroxypiperidin-1-yl)-1-phenylethyl acetate (8a and 8a')—General procedure II was used. The quantities of the chemicals in order of addition were : NaBH₄ (0.12 g, 3.1 mmol), THF (20 mL), BF₃-ether complex (0.4 mL, 3.3 mmol), **7a** (0.7 g, 1.5 mmol), water (2 mL), EtOH (2 mL), 3N NaOH (1.1 mL) and 30 % H₂O₂ (0.8 mL, 7.7 mmol).

Separation of diastereomers 8a and 8a'—In semipreparative HPLC run, with 4% 2-propanol in hexanes as the mobile phase, the retention times of **8a** and **8a'** were observed to be 14.31 and 17.96 min., respectively. In case of analytical HPLC, the respective retention times for **8a** and **8a'** were 4.71 and 5.52 min.

(*R*)-2-((3*R*,4*R*)-4-(2-(Benzhydryloxy)ethyl)-3-hydroxypiperidin-1-yl)-1-phenylethyl acetate (8a)—The HPLC separation yielded **8a** as colorless oil (0.18 g, 25%): *R*_F = 0.26 (Hex:EtOAc 2:1); ¹H-NMR (400 MHz, CDCl₃): δ = 1.34–1.39 (m, 2H), 1.55–1.66 (m, 2H), 1.85–1.91 (m, 1H), 1.98–2.03 (t, *J* = 9.6 Hz, 1H), 2.08 (s, 3H), 2.13–2.19 (t, *J* = 10.8 Hz, 1H), 2.56–2.61 (dd, *J* = 13.6, 4.4 Hz, 1H), 2.71–2.73 (brd, *J* = 11.2 Hz, 1H), 2.82–2.88 (m, 1H), 3.05–3.08 (dd, *J* = 10.4, 3.2 Hz, 1H), 3.33–3.38 (m, 1H), 3.48–3.54 (m, 1H), 3.55–3.60 (m, 1H), 5.36 (s, 1H), 5.91–5.95 (dd, *J* = 8.8, 4.4 Hz, 1H), 7.22–7.35 (m, 15H) ppm; ¹³C NMR (75 MHz, CDCl₃) 21.9, 30.4, 34.0, 54.1, 60.0, 63.8, 68.2, 71.9, 72.8, 84.4, 127.2, 127.7, 128.0, 128.2, 129.1, 140.0, 142.0, 142.2, 170.1 ppm; [α]_D²⁵ = –12.72° (c = 0.99 in MeOH).

(*R*)-2-((3*S*,4*S*)-4-(2-(Benzhydryloxy)ethyl)-3-hydroxypiperidin-1-yl)-1-phenylethyl acetate (8a')—The HPLC separation yielded **8a'** as colorless oil (0.12 g, 17%): *R*_F = 0.23 (Hex:EtOAc 2:1); ¹H-NMR (400 MHz, CDCl₃): δ = 1.34–1.40 (m, 2H), 1.54–1.66 (m, 2H), 1.82–1.91 (m, 1H), 2.02–2.13 (m, 5H), 2.53–2.58 (dd, *J* = 13.6, 1H), 2.80–2.87 (m, 2H), 2.97–3.01 (dd, *J* = 10.8, 3.6 Hz, 1H), 3.37–3.42 (m, 1H), 3.48–3.53 (m, 1H), 3.56–3.60 (m, 1H), 5.37 (s, 1H), 5.93–5.96 (dd, *J* = 8.8, 4 Hz, 1H), 7.22–7.35 (m, 15H) ppm. [α]_D²⁵ = –48.76° (c = 1.05 in MeOH).

(1*S*)-2-(4-(2-(Benzhydryloxy)ethyl)-3-hydroxypiperidin-1-yl)-1-phenylethyl acetate (8b and 8b')—General procedure II was used. The quantities of the chemicals in order of addition were: NaBH₄ (0.02 g, 0.5 mmol), THF (10 mL), BF₃-ether complex (0.07 mL, 0.5 mmol), **7b** (0.12 g, 0.25 mmol), water (0.3 mL), EtOH (0.5 mL), 3N NaOH (0.2 mL) and 30 % H₂O₂ (0.2 mL, 1.26 mmol).

Separation of diastereomers 8b and 8b'—In semipreparative HPLC run, with 5 % 2-propanol in hexanes as mobile phase, the retention times of **8b'** and **8b** were observed to be 13.57 and 18.15 min., respectively. In case of analytical HPLC, the respective retention times for **8b'** and **8b** were 3.71 and 4.37 min.

(*S*)-2-((3*S*,4*S*)-4-(2-(benzhydryloxy)ethyl)-3-hydroxypiperidin-1-yl)-1-phenylethyl acetate (8b')—The HPLC separation yielded **8b'**, as colorless oil (30 mg): *R*_F = 0.24 (Hex:EtOAc 2:1); ¹H-NMR (400 MHz, CDCl₃): δ = 1.30–1.38 (m, 2H), 1.54–1.66 (m, 2H), 1.84–1.91 (m, 1H), 1.98–2.03 (t, *J* = 9.6 Hz, 1H), 2.08 (s, 3H), 2.13–2.19 (t, *J* = 10.8 Hz, 1H), 2.56–2.61 (dd, *J* = 13.2, 4 Hz, 1H), 2.71–2.74 (brd, *J* = 11.6 Hz, 1H), 2.82–2.88 (m, 1H), 3.05–3.08 (dd, *J* = 10.4, 2.8 Hz, 1H), 3.33–3.38 (m, 1H), 3.48–3.53 (m, 1H), 3.55–3.60 (m, 1H), 5.36 (s, 1H), 5.91–5.95 (dd, *J* = 8.8, 4.4 Hz, 1H), 7.22–7.35 (m, 15H) ppm; [α]_D²⁵ = +12.98° (c = 1.47 in CHCl₃).

(*S*)-2-((3*R*,4*R*)-4-(2-(benzhydryloxy)ethyl)-3-hydroxypiperidin-1-yl)-1-phenylethyl acetate (8b)—The HPLC separation yielded **8b**, as colorless oil (30 mg):

$R_f = 0.21$ (Hex:EtOAc 2:1); $^1\text{H-NMR}$ (400 MHz, CDCl_3): $\delta = 1.30\text{--}1.39$ (m, 2H, *H-4*, *H-5ax*), 1.55–1.66 (m, 2H), 1.82–1.92 (m, 1H), 2.02–2.13 (m, 5H), 2.54–2.58 (dd, $J = 13.6$, 4 Hz, 1H), 2.80–2.87 (m, 2H), 2.97–3.01 (dd, $J = 10.8$, 3.2 Hz, 1H), 3.37–3.42 (m, 1H), 3.48–3.53 (m, 1H), 3.56–3.61 (m, 1H), 5.37 (s, 1H), 5.93–5.96 (dd, $J = 8.8$, 4 Hz, 1H), 7.22–7.35 (m, 15H) ppm; $[\alpha]_D^{25} = -48$. 76° ($c = 1.05$ in MeOH).

(1*R*)-2-(4-(2-(Benzhydryloxy)ethyl)-3-hydroxypiperidin-1-yl)-1-(4-fluorophenyl)ethyl acetate (8c and 8c')—General procedure II was used. The

quantities of the chemicals in order of addition were : NaBH_4 (0.03 g, 0.8 mmol), THF (10 mL), BF_3 -ether complex (0.1 mL, 0.8 mmol), **7c** (0.18 g, 0.4 mmol), water (0.3 mL), EtOH (0.4 mL), 3N NaOH (0.3 mL) and 30 % H_2O_2 (0.2 mL, 1.9 mmol).

Separation of diastereomers 8c and 8c'—In semipreparative HPLC run, with 5 % 2-propanol in hexanes as mobile phase, the retention times of **8c** and **8c'** were observed to be 13.35 and 18.49 min., respectively. In case of analytical HPLC, the respective retention times for **8c** and **8c'** were 3.92 and 4.41 min.

(*R*)-2-((3*R*,4*R*)-4-(2-(Benzhydryloxy)ethyl)-3-hydroxypiperidin-1-yl)-1-(4-fluorophenyl)ethyl acetate (8c)—The HPLC separation yielded **8c**, as colorless oil (26

mg): $R_f = 0.27$ (Hex:EtOAc 2:1); $^1\text{H-NMR}$ (400 MHz, CDCl_3): $\delta = 1.28\text{--}1.38$ (m, 2H), 1.54–1.65 (m, 2H), 1.83–1.92 (m, 1H), 1.97–2.0 (t, $J = 10$ Hz, 1H), 2.07 (s, 3H), 2.12–2.17 (t, $J = 9.2$ Hz, 1H), 2.54–2.58 (dd, $J = 13.6$, 4.4 Hz, 1H), 2.71–2.73 (brd, $J = 11.6$ Hz, 1H), 2.80–2.85 (m, 1H), 3.01–3.05 (dd, $J = 10.4$, 3.6 Hz, 1H), 3.31–3.37 (m, 1H), 3.48–3.54 (m, 1H), 3.57–3.61 (m, 1H), 5.37 (s, 1H), 5.87–5.90 (dd, 1H, $J = 8.4$, 4.8 Hz, 1H), 7.0–7.04 (t, 2H), 7.23–7.33 (m, 12H) ppm; $[\alpha]_D^{25} = -12$. 95° ($c = 1.0$ in CHCl_3).

(*R*)-2-((3*S*,4*S*)-4-(2-(Benzhydryloxy)ethyl)-3-hydroxypiperidin-1-yl)-1-(4-fluorophenyl)ethyl acetate (8c')—The HPLC separation yielded **8c'**, as colorless oil (23

mg): $R_f = 0.24$ (Hex:EtOAc 2:1); $^1\text{H-NMR}$ (400 MHz, CDCl_3): $\delta = 1.31\text{--}1.40$ (m, 2H), 1.55–1.65 (m, 2H), 1.82–1.91 (m, 1H), 2.01–2.14 (m, 5H), 2.51–2.56 (dd, $J = 13.6$, 3.6 Hz, 1H), 2.76–2.84 (m, 2H), 2.98–3.01 (dd, $J = 10.8$, 3.2 Hz, 1H), 3.36–3.41 (m, 1H), 3.48–3.53 (m, 1H), 3.56–3.61 (m, 1H), 5.37 (s, 1H), 5.89–5.92 (dd, $J = 8.8$, 4.4 Hz, 1H), 6.97–7.05 (t, 2H), 7.23–7.33 (m, 12H) ppm; $[\alpha]_D^{25} = -36.33^\circ$ ($c = 0.9$ in CHCl_3).

(1*S*)-2-(4-(2-(benzhydryloxy)ethyl)-3-hydroxypiperidin-1-yl)-1-(4-fluorophenyl)ethyl acetate (8d and 8d')—General procedure II was used. The

quantities of the chemicals in order of addition were: NaBH_4 (0.04 g, 1.1 mmol), THF (10 ml), BF_3 -ether complex (0.14 mL, 1.1 mmol), **7d** (0.25 g, 0.5 mmol), water (0.4 mL), EtOH (0.6 mL), 3 N NaOH (0.4 mL) and 30 % H_2O_2 (0.4 mL, 4.1 mmol).

Separation of diastereomers 8d and 8d'—In semipreparative HPLC run, with 5 % 2-propanol in hexanes as mobile phase, the retention times of **8d'** and **8d** were observed to be 13.4 and 17.69 min., respectively. In case of analytical HPLC, the respective retention times for **8d'** and **8d** were 3.41 and 4.23 min.

(*S*)-2-((3*S*,4*S*)-4-(2-(benzhydryloxy)ethyl)-3-hydroxypiperidin-1-yl)-1-(4-fluorophenyl)ethyl acetate (8d')—The HPLC separation yielded **8d'**, as colorless oil

(20 mg): $R_f = 0.28$ (Hex:EtOAc 2:1); $^1\text{H-NMR}$ (400 MHz, CDCl_3): $\delta = 1.28\text{--}1.38$ (m, 2H), 1.54–1.65 (m, 2H), 1.83–1.92 (m, 1H), 1.97–1.99 (t, $J = 13.2$ Hz, 1H), 2.07 (s, 3H), 2.11–2.14 (t, $J = 8.8$ Hz, 1H), 2.54–2.58 (dd, $J = 13.6$, 4.8 Hz, 1H), 2.70–2.73 (brd, $J = 11.2$ Hz, 1H), 2.79–2.85 (m, 1H), 3.01–3.05 (dd, $J = 10.4$, 2.8 Hz, 1H), 3.31–3.36 (m, 1H, *H-3ax*),

3.48–3.53 (m, 1H), 3.55–3.60 (m, 1H, CH₂CHO), 5.36 (s, 1H), 5.87–5.90 (dd, $J=8.4$, 4.8 Hz, 1H), 6.99–7.04 (t, 2H), 7.22–7.33 (m, 12H) ppm; $[\alpha]^{25}_D = +13.30^\circ$ ($c = 1.0$ in CHCl₃).

(S)-2-((3R,4R)-4-(2-(Benzhydryloxy)ethyl)-3-hydroxypiperidin-1-yl)-1-(4-fluorophenyl)ethyl acetate (8d)—The HPLC separation yielded **8d**, as colorless oil (20 mg): $R_f=0.24$ (Hex:EtOAc 2:1); $^1\text{H-NMR}$ (400 MHz, CDCl₃): $\delta = 1.31$ – 1.40 (m, 2H), 1.54 – 1.64 (m, 2H), 1.82 – 1.91 (m, 1H), 2.01 – 2.12 (m, 5H), 2.51 – 2.56 (dd, $J=13.2$, 4.4 Hz, 1H), 2.76 – 2.84 (m, 2H), 2.97 – 3.01 (dd, $J=10.8$, 3.2 Hz, 1H), 3.35 – 3.40 (m, 1H), 3.48 – 3.53 (m, 1H), 3.56 – 3.61 (m, 1H), 5.37 (s, 1H), 5.89 – 5.92 (dd, $J=8.8$, 4.4 Hz, 1H), 6.99 – 7.03 (t, 2H), 7.22 – 7.33 (m, 12H) ppm; $[\alpha]^{25}_D = +36.5^\circ$ ($c = 1.0$ in CHCl₃).

General Procedure III. Synthesis of 9a-9d and 9a'-9d'—Fractions I and II obtained from HPLC separation were individually deacetylated with anhyd. K₂CO₃ (0.56 equiv) in MeOH (15 mL) at RT for 4 hrs. MeOH was removed in vacuo, and the residue was partitioned between CH₂Cl₂ and water. The organic layer was separated and aqueous layer was further extracted with CH₂Cl₂ (2X20 ml). Combined organic layers were dried (Na₂CO₃) and concentrated. The crude product was purified by column chromatography (EtOAc, EtOAc:MeOH 9:1, and MeOH).

(3R,4R)-4-(2-(Benzhydryloxy)ethyl)-1-((R)-2-hydroxy-2-phenylethyl)-piperidin-3-ol (9a)—Compound **8a** (0.18 g, 0.4 mmol) from HPLC separation of diastereomeric mixture **8a** and **8a'** was stirred with anhyd. K₂CO₃ (30 mg, 0.2 mmol) in MeOH to produce **9a** as a white solid (0.15 g, 93%): $R_f = 0.16$ (Hex:EtOAc 1:1); mp: 154 – 156°C (oxalate salt); $[\alpha]^{25}_D = -12.48^\circ$ ($c = 1.0$ in CHCl₃); $^1\text{H-NMR}$ (400 MHz, CDCl₃): $\delta = 1.39$ – 1.46 (m, 2H), 1.59 – 1.66 (m, 2H), 1.87 – 1.94 (m, 2H), 2.22 – 2.31 (t, $J=10.8$ Hz, 1H), 2.44 – 2.53 (m, 2H), 2.70 – 2.73 (brd, 1H, $J=11.2$ Hz), 3.30 – 3.34 (dd, $J=10.8$, 3.2 Hz, 1H), 3.40 – 3.46 (m, 1H), 3.51 – 3.57 (m, 1H), 3.60 – 3.65 (m, 1H), 4.72 – 4.76 (dd, $J=10$, 4 Hz, 1H), 5.39 (s, 1H), 7.24 – 7.35 (m, 15H) ppm; $^{13}\text{C NMR}$ (100 MHz, CDCl₃) 32.2 , 34.4 , 42.8 , 55.0 , 58.6 , 66.2 , 68.2 , 69.1 , 71.9 , 84.8 , 126.1 , 127.0 , 127.2 , 127.9 , 128.6 , 128.8 , 141.8 , 142.0 , 142.4 ppm; % Purity (HPLC): >98 , $t_R=5.63$ min (Hex:IPA 95:5); Anal calcd for C₂₈H₃₃NO₃·(COOH)₂·H₂O: C 66.77, H 6.91, N 2.60, found: C 67.05, H 6.53, N 2.60.

(3S,4S)-4-(2-(Benzhydryloxy)ethyl)-1-((R)-2-hydroxy-2-phenylethyl)piperidin-3-ol (9a')—Compound **8a'** (0.12 g, 0.26 mmol) from HPLC separation was stirred with anhyd. K₂CO₃ (20 mg, 0.14 mmol) in MeOH to produce **9a'** as a white solid (0.08 g, 80 %): $R_f = 0.13$ (Hex:EtOAc 1:1); mp: 84 – 86°C (oxalate salt); $[\alpha]^{25}_D = -50.73^\circ$ ($c = 0.95$ in CHCl₃); $^1\text{H-NMR}$ (400 MHz, CDCl₃): $\delta = 1.32$ – 1.43 (m, 2H), 1.60 – 1.71 (m, 2H), 1.88 – 2.01 (m, 2H), 2.12 – 2.18 (t, $J=10.4$ Hz, 1H), 2.43 – 2.55 (m, 2H), 2.98 – 3.01 (dd, $J=11.2$, 3.2 Hz, 1H), 3.04 – 3.07 (brd, $J=10.8$ Hz, 1H), 3.43 – 3.48 (m, 1H), 3.52 – 3.57 (m, 1H), 3.60 – 3.65 (m, 1H), 4.70 – 4.73 (dd, 1H, $J=10.4$, 3.6 Hz), 5.39 (s, 1H), 7.24 – 7.36 (m, 15H) ppm; $^{13}\text{C NMR}$ (100 MHz, CDCl₃) 31.2 , 34.2 , 42.8 , 51.6 , 62.1 , 66.4 , 68.2 , 68.9 , 72.1 , 84.6 , 126.1 , 127.1 , 127.2 , 127.8 , 127.9 , 128.5 , 128.8 , 141.9 , 142.2 , 142.4 ppm; % Purity (HPLC): >98 , $t_R=7.0$ min (Hex:IPA 95:5); Anal calcd for C₂₈H₃₃NO₃·(COOH)₂·0.5H₂O: C 67.91, H 6.84, N 2.64, found: C 68.14, H 6.82, N 2.81.

(3S,4S)-4-(2-(Benzhydryloxy)ethyl)-1-((S)-2-hydroxy-2-phenylethyl)piperidin-3-ol (9b')—Compound **8b'** (30 mg, 0.06 mmol) was stirred with anhyd. K₂CO₃ (5 mg, 0.04 mmol) in MeOH to produce **9b'** as a white solid (25 mg, 93 %): $R_f = 0.18$ (Hex:EtOAc 1:1); mp: 152 – 154°C (oxalate salt); $[\alpha]^{25}_D = +13.33^\circ$ ($c = 0.96$ in CHCl₃); $^1\text{H-NMR}$ (400 MHz, CDCl₃): $\delta = 1.38$ – 1.44 (m, 2H), 1.60 – 1.67 (m, 2H), 1.87 – 1.94 (m, 2H), 2.22 – 2.27 (dt, 1H, $J=11.2$ Hz, H6-ax), 2.43 – 2.53 (m, 2H), 2.69 – 2.72 (brd, $J=10.8$ Hz, 1H), 3.30 – 3.34 (dd, $J=10.8$, 3.2 Hz, 1H), 3.40 – 3.46 (m, 1H), 3.51 – 3.57 (m, 1H), 3.60 – 3.64 (m, 1H), 4.72 – 4.75

(dd, $J=9.6$, 4 Hz, 1H), 5.39 (s, 1H), 7.24–7.34 (m, 15H) ppm; ^{13}C NMR (100 MHz, CDCl_3) 31.3, 34.5, 42.9, 55.0, 58.6, 66.2, 68.3, 69.2, 72.1, 84.8, 126.1, 127.0, 127.1, 127.9, 128.6, 128.8, 141.7, 141.8, 142.2 ppm; % Purity (HPLC): >98, $t_R=5.33$ min (Hex:IPA 95:5); Anal calcd for $\text{C}_{28}\text{H}_{33}\text{NO}_3\cdot(\text{COOH})_2$: C 69.08, H 6.76, N 2.69, found: C 68.84, H 6.78, N 2.67.

(3R,4R)-4-(2-(Benzhydryloxy)ethyl)-1-((S)-2-hydroxy-2-phenylethyl)piperidin-3-ol (9b)—Compound **8b** (30 mg, 0.06 mmol) was stirred with anhyd. K_2CO_3 (5 mg, 0.04 mmol) in MeOH to produce **9b** as a white solid (25 mg, 93 %): $R_f=0.14$ (Hex:EtOAc 1:1); mp: 82–84 °C (oxalate salt); $[\alpha]_D^{25} = +50.64^\circ$ ($c=0.95$ in CHCl_3); ^1H -NMR (400 MHz, CDCl_3): $\delta=1.32$ – 1.43 (m, 2H), 1.60 – 1.71 (m, 2H), 1.87 – 2.01 (m, 2H), 2.12 – 2.17 (t, $J=10.4$ Hz, 1H), 2.43 – 2.54 (m, 2H), 2.98 – 3.01 (dd, $J=10.8$, 3.2 Hz, 1H), 3.04 – 3.07 (brd, $J=10.4$ Hz, 1H), 3.43 – 3.48 (m, 1H), 3.51 – 3.57 (m, 1H), 3.60 – 3.64 (m, 1H), 4.70 – 4.73 (dd, $J=10.4$, 3.6 Hz, 1H), 5.39 (s, 1H), 7.23–7.37 (m, 15H) ppm; ^{13}C NMR (100 MHz, CDCl_3) 31.2, 34.3, 42.9, 51.6, 62.0, 66.3, 68.3, 69.1, 72.2, 84.7, 126.1, 127.1, 127.2, 127.8, 127.9, 128.6, 128.8, 141.8, 142.0, 142.3 ppm; % Purity (HPLC): >98, $t_R=6.06$ min (Hex:IPA 95:5); Anal calcd for $\text{C}_{28}\text{H}_{33}\text{NO}_3\cdot(\text{COOH})_2$: C 69.08, H 6.76, N 2.69, found: C 69.09, H 6.76, N 2.70.

(3R,4R)-4-(2-(Benzhydryloxy)ethyl)-1-((R)-2-(4-fluorophenyl)-2-hydroxyethyl)piperidin-3-ol (9c)—Compound **8c** (30 mg, 0.05 mmol) was treated with anhyd. K_2CO_3 (4.1 mg, 0.03 mmol) in MeOH to produce **9a** as a white solid (20 mg, 74%): $R_f=0.19$ (Hex:EtOAc 1:1); mp: 160–162 °C (oxalate salt); $[\alpha]_D^{25} = -13.84^\circ$ ($c=0.65$ in CHCl_3); ^1H -NMR (400 MHz, CDCl_3): $\delta=1.39$ – 1.42 (m, 2H), 1.61 – 1.67 (m, 2H), 1.87 – 1.95 (m, 2H), 2.23 – 2.28 (t, $J=11.2$ Hz, 1H), 2.40 – 2.50 (m, 2H), 2.70 – 2.73 (brd, $J=11.2$ Hz, 1H), 3.29 – 3.33 (dd, $J=10.4$, 3.2 Hz, 1H), 3.40 – 3.46 (m, 1H), 3.52 – 3.57 (m, 1H), 3.60 – 3.65 (m, 1H), 4.70 – 4.73 (dd, $J=10.4$, 3.6 Hz, 1H), 5.39 (s, 1H), 7.00–7.04 (t, 2H), 7.23–7.34 (m, 12H) ppm; ^{13}C NMR (100 MHz, CDCl_3) 31.3, 34.5, 43.0, 54.8, 58.6, 66.2, 68.2, 68.6, 72.0, 84.0, 115.3, 115.5, 127.0, 127.1, 127.7, 127.9, 128.0, 128.7, 138.0, 141.7, 141.9 ppm; % Purity (HPLC): >98, $t_R=6.25$ min (Hex:IPA 95:5); Anal calcd for $\text{C}_{28}\text{H}_{32}\text{FNO}_3\cdot(\text{COOH})_2$: C 66.78, H 6.35, N 2.60, found: C 66.51, H 6.45, N 2.68.

(3S,4S)-4-(2-(benzhydryloxy)ethyl)-1-((R)-2-(4-fluorophenyl)-2-hydroxyethyl)piperidin-3-ol (9c')—Compound **8c'** (23 mg, 0.05 mmol) was stirred with anhyd. K_2CO_3 (3.6 mg, 0.03 mmol) in MeOH to produce **9c'** as a white solid (20 mg, 95 %): $R_f=0.14$ (Hex:EtOAc 1:1); mp: 82–84 °C (oxalate salt); $[\alpha]_D^{25} = -45.78^\circ$ ($c=0.95$ in CHCl_3); ^1H -NMR (400 MHz, CDCl_3): $\delta=1.34$ – 1.41 (m, 2H), 1.61 – 1.71 (m, 2H), 1.88 – 2.00 (m, 2H), 2.13 – 2.18 (t, $J=10$ Hz, 1H), 2.38 – 2.52 (m, 2H), 2.97 – 3.01 (dd, 1H, $J=12$, 3.6 Hz, H_{2eq}), 3.02 – 3.05 (brd, $J=10.8$ Hz, 1H), 3.43 – 3.49 (m, 1H), 3.52 – 3.57 (m, 1H), 3.60 – 3.65 (m, 1H), 4.67 – 4.71 (dd, $J=10.4$, 3.2 Hz, 1H), 5.39 (s, 1H), 7.00–7.04 (t, 2H), 7.24–7.36 (m, 12H); ^{13}C NMR (100 MHz, CDCl_3) 31.0, 34.1, 42.7, 51.4, 61.7, 66.1, 68.0, 68.4, 71.7, 84.6, 115.1, 115.3, 126.8, 127.0, 127.5, 127.7, 128.5, 128.6, 138.0, 141.5, 141.7 ppm; % Purity (HPLC): >98, $t_R=7.76$ min (Hex:IPA 95:5); Anal calcd for $\text{C}_{28}\text{H}_{32}\text{FNO}_3\cdot(\text{COOH})_2\cdot 0.5\text{H}_2\text{O}$: C 65.68, H 6.43, N 2.55, found: C 65.36, H 6.42, N 2.57.

(3S,4S)-4-(2-(Benzhydryloxy)ethyl)-1-((S)-2-(4-fluorophenyl)-2-hydroxyethyl)piperidin-3-ol (9d')—Compound **8d'** (20 mg, 0.04 mmol) was stirred with anhyd. K_2CO_3 (3.1 mg, 0.02 mmol) in MeOH to produce **9d'** as a white solid (17 mg, 93%): $R_f=0.21$ (Hex:EtOAc 1:1); mp: 164–166 °C (oxalate salt); $[\alpha]_D^{25} = +14.3^\circ$ ($c=0.65$ in CHCl_3); ^1H -NMR (400 MHz, CDCl_3): $\delta=1.40$ – 1.51 (m, 2H), 1.60 – 1.70 (m, 2H), 1.87 – 1.98 (m, 2H), 2.26 – 2.31 (t, $J=11.2$ Hz, 1H), 2.46 – 2.51 (m, 2H), 2.77 – 2.80 (brd, $J=12$ Hz, 1H), 3.33 – 3.37 (dd, $J=10.4$, 3.2 Hz, 1H), 3.44 – 3.50 (m, 1H), 3.52 – 3.57 (m, 1H), 3.61 – 3.65 (m, 1H), 4.74 – 4.78 (t, $J=6.8$ Hz, 1H), 5.39 (s, 1H), 7.00–7.04 (t, 2H), 7.24–7.35 (m, 12H, ArH) ppm; ^{13}C NMR (100 MHz, CDCl_3) 31.4, 34.5, 43.0, 55.1, 58.6, 66.2, 68.3, 68.6, 72.1,

84.8, 115.3, 115.5, 127.0, 127.1, 127.7, 127.9, 128.0, 128.7, 138.0, 141.7, 142.0 ppm; % Purity (HPLC): >98, t_R =5.96 min (Hex:IPA 95:5); Anal calcd for $C_{28}H_{32}FNO_3 \cdot (COOH)_2$: C 66.78, H 6.35, N 2.60, found: C 66.49, H 6.54, N 2.58.

(3*R*,4*R*)-4-(2-(Benzhydryloxy)ethyl)-1-((*S*)-2-(4-fluorophenyl)-2-hydroxyethyl)piperidin-3-ol (9d)—Compound **8d** (20 mg, 0.04 mmol) was stirred with anhyd K_2CO_3 (3.1 mg, 0.02 mmol) in MeOH to produce **9c'** as a white solid (17 mg, 93%): R_f = 0.15 (Hex:EtOAc 1:1); mp: 84–86 °C (oxalate salt); $[\alpha]_D^{25} = +43.13^\circ$ ($c = 0.8$ in $CHCl_3$); 1H -NMR (400 MHz, $CDCl_3$): δ = 1.33–1.39 (m, 2H), 1.61–1.71 (m, 2H), 1.88–2.0 (m, 2H), 2.12–2.17 (t, J =10.4 Hz, 1H), 2.38–2.51 (m, 2H), 2.97–3.0 (dd, J = 11.2, 4 Hz, 1H), 3.02–3.05 (brd, J =11.2 Hz, 1H), 3.42–3.48 (m, 1H), 3.52–3.57 (m, 1H), 3.60–3.65 (m, 1H), 4.67–4.70 (dd, J =10.4, 3.2 Hz, 1H), 5.39 (s, 1H), 7.00–7.04 (t, 2H), 7.23–7.34 (m, 12H, *ArH*) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) 31.2, 34.3, 42.9, 51.6, 62.0, 66.3, 68.3, 68.6, 72.2, 84.7, 115.3, 115.5, 127.0, 127.2, 127.7, 127.8, 127.9, 128.7, 128.0, 128.8, 138.0, 141.8, 142.0 ppm; % Purity (HPLC): >98, t_R =7.39 min (Hex:IPA 95:5); Anal calcd for $C_{28}H_{32}FNO_3 \cdot (COOH)_2 \cdot 0.5H_2O$: C 65.68, H 6.43, N 2.55, found: C 65.30, H 6.44, N 2.65.

Behavioral Pharmacology Methods and Materials

Subject—Male, Long-Evans hooded rats (Harlan Sprague Dawley Inc) were used for discrimination studies. Subjects were individually housed in an AALAC accredited animal facility and given *ad libitum* food and water. Free feeding weights were obtained and food (Harlan Teklad, Madison, Wisconsin) was subsequently restricted to 15 g a day until rats achieved 85% of their *ad libitum* weights. The rats were maintained at their target level throughout the rest of the study by adjustments in post-session feedings. Experimental sessions were conducted Monday-Friday during the light phase of a 12h/12h light/dark cycle.

Adult, male Swiss-Webster mice weighing 25–35 g were used in the locomotor activity studies. Mice were allowed to acclimate to the vivarium environment one week prior to the start of testing and were housed five per cage, with continuous access to food (Harlan Teklad, Madison, Wisconsin) and water.

Apparatus—Rat discrimination studies were conducted in two-lever operant conditioning chambers (Med-Associates Inc., St. Albans, VT) equipped with a house light and food dispenser that delivered 45 mg food pellets (Research Diets, Noyes Precision Pellets, New Brunswick, NJ). Scheduling of pellet deliveries and collection of data were accomplished by a microcomputer and associated interface (Med-Associates Inc., St. Albans, VT, MED-PC® IV).

Locomotor activity tests were conducted in automated activity monitoring devices, enclosed in sound and light attenuating chambers (AccuScan Instruments, Columbus, OH). The interior of each device was divided into two separate $20 \times 20 \times 30$ cm arena's permitting the independent and simultaneous measurement of two mice. Sixteen photobeam sensors were spaced 2.5 cm apart along the walls of the chamber and were used to detect movement.

Drug Discrimination Procedure in Rats—Drug discrimination training occurred during daily (Mon-Fri) 15-min experimental sessions. The rats were initially trained to press one of two levers under a fixed-ratio 1 (FR 1) schedule of reinforcement. The response requirement was gradually increased to FR 10. During the next few sessions the rats were reinforced only for pressing the alternate lever until they pressed reliably under FR 10 scheduling conditions, after which drug discrimination training commenced. Rats were injected with 10 mg/kg cocaine or saline vehicle i.p., 10 min prior to the start of the session.

For each rat, one lever was designated correct following drug administration and the other as correct following saline administration. The lever upon which the rats initially acquired the lever press response was designated as the saline-appropriate lever. All responses on the inappropriate lever were recorded, but had no programmed consequences. The lever on which the rats were initially trained and on which they acquired the lever-press response was designated as the vehicle-appropriate lever. Alternation of cocaine and saline injections proceeded according to a two-monthly cycle (Month #1: CSSCS, SCCSC, SCSCS, CSCSC; Month #2: SCCSS, CSCSC, CSSCC, SCSCS; in which C=cocaine S=saline). Lever pressing produced pellet delivery only on the injection-appropriate lever for that day. Incorrect presses reset the response requirement on the correct lever.

Substitution tests began once a rat met the following criteria: 1) the first completed fixed-ratio (FFR) occurred on the lever designated correct on at least eight of ten consecutive sessions; and 2) at least 80% of the total responses were emitted on the correct lever during those eight sessions. After these initial training criteria were met, testing could occur twice a week, on Tuesdays and Fridays, provided that the rats completed the FFR on the correct lever during the most recent training drug and saline sessions; otherwise, a training day was administered. Test sessions were identical to training sessions except completion of the FR10 contingencies on either lever resulted in pellet delivery. Dose-response curves were collected first with cocaine (1-30 mg/kg) before substitution tests with test compounds began. Tests with **9a** (1, 10, and 30 mg/kg), **9d** (1, 3, 10, 17 and 30 mg/kg) and **9d'** (1, 10, 17, 30 and 56 mg/kg) were subsequently conducted.

Locomotor Activity Procedure—On the test day mice were brought to the laboratory where they were allowed to acclimate in their home cages for approximately 30 min. Mice were injected i.p with vehicle or drug and immediately placed in the test chambers. Their activity was recorded for 240 minutes. The total distance traveled (cm) during the experimental session was recorded for each mouse. Doses of 1, 30, 100 mg/kg were tested for each drug.

Drugs—Cocaine HCl was obtained from the National Institute on Drug Abuse and was dissolved in 0.05 % saline and administered 10 min prior to start of discrimination sessions. **9a**, **9d** and **9d'** were synthesized as described above. Vehicle for all test compounds except cocaine was 20% w/v β -cyclodextrin (Cavitron 82003, Cargill Food and Pharma specialists, Cedar Rapids, IA) in sterile water. All test compounds (except cocaine) were given i.p 20 min before the start of discrimination test sessions. This pre-treatment time was based on data obtained from locomotor studies indicating that the peak effect of these drugs for elevating locomotor activity generally occurred between 10-20 min of their administration. All drugs were administered in a volume of 1 and 10 ml/kg for rat and mouse tests, respectively.

Data Analysis—For drug discrimination studies, the percentage of cocaine-lever responding (%CLR) was calculated for each subject by dividing the number of lever presses emitted upon the cocaine lever by the total number of presses emitted upon both levers and multiplying this quotient by 100. Individual values of %CLR were then averaged ($\pm$ SEM). Complete generalization to the drug lever was inferred when %CLR was 80%. Mean response rates for each test condition were calculated by dividing the total number of lever presses emitted upon both levers by the session duration (900 s) for each subject, and then these rates were averaged ($\pm$ SEM). If a rat failed to make at least ten lever presses during a test at a lower dose, it was not tested at higher doses, and its data were excluded from calculations of %CLR but were included for mean response rate determinations. This exclusion was made to prevent near-zero rates of responding from disproportionately influencing percent cocaine lever responding. ED₅₀ values and their confidence intervals

(CI) were calculated for %CLR and for reducing response rates using nonlinear regression analysis (Prism 5, GraphPad software, San Diego, CA).

For locomotor activity tests, total distance traveled (cm) during each 10 min of the 240 min test session was recorded. Data were analyzed using a repeated measures ANOVA (Prism 5, GraphPad software, San Diego, CA). Post tests were performed using the Bonferroni test comparing dose to vehicle at each of the 24, 10-min time points. Differences were inferred when $P < 0.05$.

The research was conducted under National Institutes of Health Guidelines for the Care and Use of Laboratory Animals in an Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC)-approved laboratory. The protocol was approved by Virginia Commonwealth University's Institutional Animal Care and Use Committee.

Biochemical Pharmacology Methods and Materials—The affinity of test compounds for rat monoamine transporters was monitored exactly as described by us previously.^[25] Briefly, rat striatum was used for measuring binding of [³H]WIN 35,428 to DAT and uptake of [³H]dopamine by DAT. Rat cerebral cortex was used for assessing uptake of [³H]serotonin by SERT and hippocampus for uptake of [³H]norepinephrine by NET. At least five triplicate concentrations of each test compound were studied, spaced evenly around the IC₅₀ value. The latter was estimated by nonlinear computer curve-fitting procedures and converted to K_i with the Cheng-Prusoff equation as described previously.^[26]

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References

1. Musto DF. Sci. Am. 1991; 265:40–47. [PubMed: 1882226]
2. Carroll FI, Howell LL, Kuhar MJ. J. Med. Chem. 1999; 42:2721–2736. [PubMed: 10425082]
3. Dutta AK, Zhang S, Kolhatkar R, Reith MEA. Eur. J. Pharmacol. 2003; 479:93–106. [PubMed: 14612141]
4. Ritz MC, Cone EJ, Kuhar MJ. Life Sci. 1990; 46:635–645. [PubMed: 2308472]
5. Ritz MC, Lamb RJ, Goldberg SR, Kuhar MJ. Science. 1987; 237:1219–1223. [PubMed: 2820058]
6. Giros B, Jaber M, Jones SR, Wightman RM, Caron MG. Nature. 1996; 379:606–612. [PubMed: 8628395]
7. Witkin JM, Nichols DE, Terry P, Katz JL. J. Pharmacol. Exp. Ther. 1991; 257:706–713. [PubMed: 1674533]
8. Bergman J, Madras BK, Johnson SE, Spealman RD. J. Pharmacol. Exp. Ther. 1989; 251:150–155. [PubMed: 2529365]
9. Volkow ND, Wang GJ, Fischman MW, Foltin RW, Fowler JS, Abumrad NN, Vitkun S, Logan J, Gatley SJ, Pappas N, Hitzemann R, Shea CE. Nature. 1997; 386:827–830. [PubMed: 9126740]
10. Wilcox KM, Lindsey KP, Votaw JR, Goodman MM, Martarello L, Carroll FI, Howell LL. Synapse. 2002; 43:78–85. [PubMed: 11746736]
11. Tilley MR, Cagniard B, Zhuang X, Han DD, Tiao N, Gu HH. BMC Neurosci. 2007; 8:42. [PubMed: 17584943]
12. Rothman RB, Blough BE, Woolverton WL, Anderson KG, Negus SS, Mello NK, Roth BL, Baumann MH. J. Pharmacol. Exp. Ther. 2005; 313:1361–1369. [PubMed: 15761112]
13. Kleven MS, Woolverton WL. Drug Alcohol. Depend. 1993; 31:149–158. [PubMed: 8436060]
14. Singh S. Chem. Rev. 2000; 100:925–1024. [PubMed: 11749256]
15. Dutta AK, Xu C, Reith ME. J. Med. Chem. 1996; 39:749–756. [PubMed: 8576918]

16. Dutta AK, Coffey LL, Reith ME. J. Med. Chem. 1997; 40:35–43. [PubMed: 9016326]
17. Dutta AK, Fei XS, Beardsley PM, Newman JL, Reith ME. J. Med. Chem. 2001; 44:937–948. [PubMed: 11300876]
18. Dutta AK, Xu C, Reith ME. J. Med. Chem. 1998; 41:3293–3297. [PubMed: 9703474]
19. Ghorai SK, Cook C, Davis M, Venkataraman SK, George C, Beardsley PM, Reith ME, Dutta AK. J. Med. Chem. 2003; 46:1220–1228. [PubMed: 12646032]
20. Zhen J, Maiti S, Chen N, Dutta AK, Reith ME. Eur. J. Pharmacol. 2004; 506:17–26. [PubMed: 15588620]
21. Yamashita A, Singh SK, Kawate T, Jin Y, Gouaux E. Nature. 2005; 437:215–223. [PubMed: 16041361]
22. a Hsin L-W, Dersch CM, Baumann MH, Stafford D, Glowa JR, Rothman RB, Jaconson AE, Rice KC. J. Med. Chem. 2002; 45:1321–1329. [PubMed: 11882001] b Lewis DB, Matecka D, Zhang Y, Hsin L-W, Dersch CM, Stafford D, Glowa JR, Rothman RB, Rice KC. J. Med. Chem. 1999; 42:5029–5042. [PubMed: 10585212]
23. Dutta AK, Reith ME, Madras BK. Synapse. 2001; 39:175–181. [PubMed: 11180505]
24. Tokunaga M, Larrow JF, Kakiuchi F, Jacobsen EN. Science. 1997; 277:936–938. [PubMed: 9252321]
25. Dutta AK, Ghosh B, Biswas S, Reith ME. Eur. J. Pharmacol. 2008; 589:73–79. [PubMed: 18561912]
26. Cheng Y, Prusoff WH. Biochem. Pharmacol. 2001; 22:3099–3108. [PubMed: 4202581]
27. Zhen J, Reith MEA, Carr KD. Brain Research. 2006; 1082:98–101. [PubMed: 16516172]

Figure 1.
Molecular structure of DAT blockers.

Figure 2.

Results from tests with **9a** (■), **9d** (▲) **9d'** (▼) and from each drug's respective vehicle test (open symbols corresponding to each drug) on mean total distance traveled (cm) during each hour of the four hour test session. N=8 at each dose and condition. Results of statistical comparisons are provided in the text.

Figure 3.

Effects of cocaine, **9a**, **9d** and **9d'** on the percentage of cocaine lever responding (top panel) and on rate of responding (bottom panel) in rats trained to discriminate 10 mg/kg cocaine from saline. The data points above "S" and "V" indicate the mean percentage of cocaine lever responding and rate of responding following administration of saline and 20% w/v b-cyclodextrin, the vehicles for cocaine and the "D" drugs, respectively. If a rat failed to emit

10 total lever presses during a test session at a lower dose it was not tested at higher doses, and its data for that dose were not included in the analysis of % cocaine-lever presses (although they were included in the response rate analysis; see text). % Cocaine Lever Presses: Cocaine (N=7 except N=6 at 30 mg/kg); **9a** (N=7 except N=2 at 30 mg/kg); **9d** (N=7 except N=6 at 3, and N=4 at 17 and 30 mg/kg); **9d'** (N=6 except N=5 at 17, N=3 at 30, and N=2 at 56 mg/kg). Response Rate: Cocaine (N=7); **9a** (N=7 except N=5 at 30 mg/kg); **9d** (N=7 except N=6 at 3, and N=4 at 17 and 30 mg/kg); **9d'** (N=6 except N=5 at 17 and N=2 at 56 mg/kg).

Scheme 1.

Synthesis of intermediate **5**, Reagents and Conditions: a) NaOMe, 130-140 °C; b) BzBr, AcCN, reflux; c) NaBH₄, MeOH; d) ClCOOMe, reflux; e) Claisen's alkali, reflux.

Scheme 2.

Synthesis of **8a-d** and **8a'-d'**, Reagents and Conditions: a : EtOH, reflux; b : Ac₂O, Pyridine;
c : BF₃-ether, NaBH₄; d : NaOH, H₂O₂

Scheme 3.

Synthesis of **9a-d** and **9a'-d'**, Reagents and Conditions: a = Anhydrous K₂CO₃/MeOH

Table 1

Uptake inhibition affinity of hydroxypiperidine derivatives at the DAT, SERT and NET in rat brain

Compound	DAT binding, Ki, nM, [³ H]WIN 35, 428 ^[a]	DAT uptake, Ki, nM, [³ H]DA ^[b]	SERT uptake, Ki, nM, [³ H]5-HT ^[c]	NET uptake, Ki, nM [³ H]NE ^[d]
GBR 12909	44.6 ± 6.3	10.6 ± 2.2	91.1 ± 12.8	102 ± 32
cocaine	233 ± 32	135 ± 68 ^[e]	NT ^[f]	135 ^[g]
I	4.14 ± 0.77 ^[h]	3.22 ± 1.0 ^[h]	NT ^[f]	NT
II (D-84)	0.473 ± 0.085	3.01 ± 0.54	1,083 ± 196	199 ± 18
III (D-83)	33.6 ± 3.6	28.3 ± 4.5	1,263 ± 180	184 ± 10
9a (D-225)	6.76 ± 1.75	6.23 ± 2.38	456 ± 102	7.56 ± 3.05
9a' (D-226)	40.1 ± 19.2	18.4 ± 9.5	420 (5) ± 62	27.5 ± 13.5
9b (D-276)	2.29 ± 0.85	4.69 ± 2.06	155 ± 26	78.4 ± 18.1
9b' (D-275)	0.486 ± 0.065	1.06 ± 0.42	231 ± 49	115 ± 14
9c (D-231)	2.17 ± 0.67	4.04 ± 1.22	1,055 ± 130	21.9 ± 4.3
9c' (D-230)	NT ^c	2.44 ± 0.37	402 ± 67	24.8 ± 2.8
9d (D-232)	2.65 ± 0.99	1.55 ± 0.27	259 ± 36	14.1 ± 0.3
9d' (D-233)	2.59 ± 0.66	2.97 ± 0.72	1,790 ± 312	20.6 ± 3.9

Results are average ± SEM of three to eight independent experiments assayed in triplicate.

^[a]For binding assays, the DAT was labeled with [³H]WIN 35, 428.^[b]For uptake by the DAT, the accumulation of [³H]DA was measured^[c]ibid for SERT, [³H]5-HT^[d]ibid for NET, [³H]NE^[e]data from our previous work [27]^[f]not tested^[g]tested once^[h]data from our previous work [19].

Table 2

Selectivity of hydroxypiperidine derivatives at the DAT, SERT and NET

Compound	Selectivity ratio		
	SERT uptake/DAT uptake	NET uptake/DAT uptake	DAT uptake/DAT binding
9a (D-225)	73	1.2	0.92
9a' (D-226)	23	1.5	0.46
9b (D-276)	33	17	2.0
9b' (D-275)	220	110	2.2
9c (D-231)	260	5.4	2.0
9c' (D-230)	160	10	ND
9d (D-232)	170	9.1	0.59
9d' (D-233)	600	7.0	1.2

Table 3

Elemental Analysis

Compound	Elemental Analysis					
	Calculated			Found		
	C	H	N	C	H	N
9a. (COOH) ₂ .H ₂ O	67.0	6.90	2.60	67.05	6.53	2.63
9a'. (COOH) ₂ .0.5 H ₂ O	68.14	6.82	2.65	68.14	6.82	2.81
9b. (COOH) ₂	67.45	6.87	2.62	67.50	6.72	2.69
9b'. (COOH) ₂	69.08	6.76	2.69	68.84	6.78	2.67
9c. (COOH) ₂	66.55	6.37	2.59	66.51	6.45	2.68
9c'. (COOH) ₂ .0.5 H ₂ O	65.25	6.46	2.54	65.36	6.42	2.57
9d. (COOH) ₂ .0.5 H ₂ O	65.25	6.46	2.54	65.30	6.44	2.65
9d'. (COOH) ₂	66.55	6.37	2.59	66.49	6.54	2.58