Published in final edited form as:
Bioorg Med Chem. 2008 March 15; 16(6): 2769–2778.

Further structural optimization of *cis*-(6-benzhydryl-piperidin-3-yl)-benzylamine and 1,4-diazabicyclo[3.3.1]nonane derivatives by introducing an exocyclic hydroxyl group: Interaction with dopamine, serotonin and norepinephrine transporters

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Abstract

Our earlier effort to develop constrained analogues of flexible piperidine analogs for monoamine transporters led to the development of a series of 3,6-disubstituted piperidine derivatives, and a series of 4,8-disubstituted 1,4-diazabicyclo[3.3.1]nonane derivatives. In further structure-activity relationship (SAR) studies on these constrained derivatives, several novel analogues were developed where an exocyclic hydroxyl group was introduced on the N-alkyl-aryl side chain. All synthesized derivatives were tested for their affinities for the dopamine transporter (DAT), serotonin (5-HT) transporter (SERT), and norepinephrine transporter (NET) in the brain by measuring their potency in inhibiting the uptake of [^{3}H]DA, [^{3}H]5-HT, and [^{3}H]NE, respectively. Compounds were also tested for their binding potency at the DAT by their ability to inhibit binding of [^{3}H]WIN 35,428. The results indicated that position of the hydroxyl group on the N-alkyl side chain is important along with the length of the side chain. In general, hydroxyl derivatives derived from more constrained bicyclic diamines exhibited greater selectivity for interaction with DAT compared to the corresponding 3,6-disubstituted diamines. In the current series of molecules, compound **11b** with N-propyl side chain with the hydroxyl group attached in the benzylic position was the most potent and selective for DAT (Ki = 8.63 nM; SERT/DAT = 172 and NET/DAT = 48.4).

Introduction

Cocaine binds to several binding sites in the brain including those on monoamine transporter proteins. These proteins transport dopamine (DA), serotonin (5-HT) and norepinephrine (NE) (DAT, SERT, and NET, respectively). [1, 2] However, binding of cocaine to DAT is believed to be responsible for production of its powerful reinforcing effect. As no effective medication is currently available to treat cocaine dependence, the development of an effective pharmacotherapy for this disorder is urgently needed.

The dopamine hypothesis of cocaine addiction received further support from a series of in vivo experiments and also from molecular biological studies involving DAT knockout mice.[3, 4]

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Furthermore, in a recent experiment with knock-in mouse model it was demonstrated that binding to DAT is mainly responsible for its reinforcing effect.[5] This recent evidence further validates DAT as a target for drug development for cocaine addiction. DAT has been targeted for the development of pharmacotherapy for cocaine addiction for number of years. However, it is also important to mention that other studies have indicated the additional involvement of the serotonergic system in some of the subjective effects of cocaine.[6] The validity of DAT as a target for development of cocaine pharmacotherapy is evident from preclinical results in animal behavior studies which indicated that GBR 12909, a DAT blocker, could attenuate self-administration of cocaine without modulating food reinforcement in monkeys.[7] In a human clinical trial GBR 12909 was a non-stimulant.[8] However, the clinical trial of GBR 12909 was discontinued due to problems of QTc prolongation. In another ongoing study with a different DAT blocker, the phenyl tropane analogue RTI-336 is being evaluated preclinically as a pharmacotherapy for cocaine abuse.[9] Finally, a recent study on the mechanism of interaction of benztropine-like compounds with DAT suggests a link between conformational effects at DAT and their ability to serve in psychostimulant substitution therapy.[10, 11]

Structurally diverse molecules have been developed for DAT. These molecules are broadly categorized into four main classes depending on their chemical structure, known as the tropane, GBR, methylphenidate and mazindol class of derivatives. Detailed structure-activity relationship (SAR) studies of these different categories of molecules have been described in a recent review paper.[12]

In our earlier studies for development of novel molecules for DAT, we have developed a large number of flexible piperidine analogs of GBR 12909 exhibiting potent affinity at the DAT.[13–15] In order to address poor in vivo activity in these flexible molecules, we modified one of our lead flexible DAT-selective piperidine analogs, compound I in Figure 1, into a series of structurally constrained 3,6-disubstituted piperidine derivatives. The cis isomeric derivative from this novel series exhibited preferential affinity at the DAT over the trans derivative.[16] Further SAR exploration based on the novel *cis*-structure yielded more potent molecules for the DAT (compounds II and III in Figure 1), thus, confirming preferential affinity of this novel *cis*-template for the DAT.[17] In our efforts to further expand our SAR studies in searching for suitable pharmacotherapeutic agents for cocaine addiction, the design of more structurally constrained molecules of 3,6-disubstituted derivatives were undertaken. Thus, a further structural rigidification on this template was carried out by linking the two nitrogen atoms in the molecule by a bis-methylene-chain linker which yielded a novel series of 4,8-disubstituted 1,4-diazabicyclo[3,3,1]nonane derivatives where the piperazine and the piperidine rings were fused into each other. A brief SAR study was conducted which led to the discovery of lead molecules exhibiting high affinity and selectivity for the DAT, paralleling the results obtained from the corresponding lesser constrained 3,6-disubstituted versions.[17] However, the more rigid 1,4-diazabicyclo[3,3,1]nonane compounds exhibited greater selectivity for the DAT indicating enhanced effect of rigidity on selectivity (compound IV in Figure 1).[18] Furthermore, in vivo locomotor and drug discrimination experiments demonstrated more activity in these constrained derivatives compared to their flexible counterparts, indicating an effect of the constrained structure in efficient penetration of blood brain barrier. In this regard, more constrained 1,4-diazabicyclo[3,3,1]nonane seemed to exhibit higher in vivo potency.

In the present SAR study we examine the effect of introduction of an exocyclic hydroxyl group in the above constrained derivatives on affinity and selectivity for monoamine transporters. Specifically, we wanted to observe the effect of introduction of an exocyclic hydroxyl functionality in both optically pure (−)-*cis*-3,6-disubstituted monocyclic diamine and (−)-*cis*-bicyclic diamine structures to understand the influence of such a hydroxyl group in binding interaction. Introduction of a comparable hydroxyl group in the piperidine ring of our earlier flexible piperidine analogue produced highly potent and selective molecule for DAT.[19] In the

present study we also determine the influence of chirality of the newly introduced hydroxyl center on interaction with monoamine transporters.

Chemistry

Synthesis of target compounds are shown in Schemes 1, 2 and 3. Scheme 1 describes the synthesis of targets **4a** to **4d**. As described in our earlier publication,[17] optically active cis-amine was synthesized from racemic **1** by converting the amine into diastereoisomeric intermediates followed by separation and hydrolysis of the desired isomers to the corresponding amine *cis*-(−)-**3**. Treatment of (−)-**3** with optically active epoxides furnished the final hydroxy targets **4a–4d** in good yield. The optically active epoxides were synthesized by following the published procedure.[20]

Scheme 2 describes the synthesis of targets **7a–7d**. Here optically active amine (−)-**5** was synthesized by following our earlier procedure.[18] This amine was treated with racemic 2,3-epoxypropyl benzene, which produced two diastereomers **6a** and **6b**, which were separated by column chromatography. Amine *cis*-(−)-5 was further treated with enantiomeric (R ans S) 2-phenyloxirane and 2-(4-fluorophenyl)oxirane separately in ethanol to yield the target compounds **7a–7d** in reasonably good yield.

Scheme 3 describes the synthesis of targets **11a** and **11b**. N-alkylation of amine cis-(−)-**5** with 3-chloro-4′-fluoro propiophenone under basic condition produced **8** which was reduced by sodium borohydride to produce mixture of both R- and S-alcohols **9**. Alcohol **9** was next converted into diastereomeric caphanic esters, which were separated by semi-preparative HPLC process. The final targets, **11a** and **11b**, were produced after hydrolyzing the esters.

Results and Discussion

Our previous study with 3,6-disubstituted constrained piperidine derivatives produced high-affinity ligands for DAT.[17, 18] In the current study, we wanted to explore whether introduction of an exocyclic hydroxyl group can further increase affinity of these compounds for DAT or for other monoamine transporters. We had established earlier that (−)-enantiomeric (S,S) versions of disubstituted diamines exhibited the highest potency. Thus, in our design we selectively wanted to synthesize only the (−)-isomeric versions. Design of compounds **4a–b** and **4c–d** involved introduction of an exocyclic hydroxyl group to 3,6-disubstituted template. Between compounds **4a** and **4b**, compound **4b** has the hydroxyl group in a S-configuration as it was synthesized from S-epoxide. Compound **4b** was more potent in inhibiting uptake of radiolabeled DA and NE by DAT and NET, respectively, compared to **4a** (Ki (DAT) = 236 nM Vs. 152 nM for **4a** and **4b**, respectively and Ki (NET) = 1435 Vs. 306 nM for **4a** and **4b**, respectively). Thus, the difference in NE uptake inhibitory activity between the two compounds was much greater than the DA uptake inhibitory activity.

In designing the next two compounds, an additional methylene unit was introduced between the phenyl moiety and the hydroxyl center. This transformation made **4c–d** more potent DAT uptake inhibitors compared to **4a–b** (Table 1). Both **4c** and **4d** exhibited low nanomolar activity for inhibition of DA uptake activity (Ki (DAT) = 25 nM and 25.3 nM for **4c** and **4d**, respectively) and also the same relative potency was exhibited in the binding assay with the radiolabeled tropane ligand CFT. It is interesting to note that both compounds exhibited comparable inhibition activity, thus, not exhibiting any preference for chirality of the hydroxyl center. Thus, a minor change in molecular structure resulted in almost ten-fold increase of DAT inhibition potency in **4c–d** compared to **4a–b**.

In designing our next analogues, the bicyclic (−)-diamine template was chosen for introduction of exocyclic hydroxyl group. Thus in compounds **6a** and **6b**, which represent bicyclic versions

of **4c** and **4d**, a hydroxyl functionality was introduced with R- and S-stereocenters. These molecules exhibited differential potencies for inhibition of DA uptake with the S-hydroxyl stereo-center exhibiting greater potency than the R stereo-center (Ki (DAT) = 16.8 nM Vs. 82.9 nM for **6b** and **6a**, respectively). Thus, this result was somewhat different compared to their 3,6-disubstituted counterparts **4c–d**. Compound **6b** was 5 times more potent in inhibiting DA uptake than **6a** (Table 1). This might indicate an effect of a more constrained bicyclic structure on greater selectivity and affinity for DAT as we observed earlier.[18] The next series of compounds **7a–d**, which represents more constrained bicyclic versions of **4a–b**, yielded results from weak to strong potency for the DAT. However, these molecules, in contrary to previous derivatives, produced preferential interaction with DAT with an exocyclic hydroxyl group in the R-stereo center. The most potent compound identified in the 7-series was fluoro derivative **7c**, exhibiting the highest potency for DAT (Ki = 66.5 nM). In general, fluoro derivatives were more potent compared to unsubstituted versions. In general, compounds **7a–d** were much less potent than **6a–b**, indicating the importance of N-alkyl chain length in transporter interaction.

In the design of next two bicyclic amine analogues, **11a** and **11b**, the hydroxyl group was introduced in the benzyl position at the terminus of the propyl chain. Thus, in these two compounds the hydroxyl group was located furthest from the N-atom as compared to previous analogues. It is evident from uptake inhibition data that such location of the hydroxyl group produced maximal inhibition of DA uptake in one of the diastereomers, **11b** (Ki = 8.63 nM). Thus, compound **11b** exhibited maximum potency and selectivity for inhibition of dopamine uptake compared to inhibition of both serotonin and norepinephrine (SERT/DAT and NET/DAT; 172 and 48.4, Table 2). In fact, compound **11b** turned out to be the most potent and selective compound in this current series of molecules. The diastereomer **11a**, on the other hand, was less potent compared to **11b** even though its potency was comparable to the third best compound **4d** in the series (Ki (DAT) = 41.8 nM and 25.3 nM, respectively for **11a** and **4d**). It is evident from this result that the location of the hydroxyl group with respect to the aromatic ring and the N-atom played an important role in uptake inhibition activity.

Conclusion

The positional effect of the exo-cyclic hydroxyl group on N-alkyl side chain and the importance of the optimum length of N-alkyl side chain were evaluated for interaction with monoamine transporters. The results indicated that an N-propyl linker length was optimal for interaction with DAT. Position of an exo-cyclic hydroxyl group in the benzylic position of the *N*-propyl terminus produced the most active and selective DAT compound **11b** in the current series. In most cases a stereochemical preference was exhibited for the hydroxyl stereo center. In general, as expected from earlier findings, hydroxyl compound derived from more constrained bicyclic amine produced greater selectivity for DAT than 3,6-disubstituted amine derivatives.

Experimental detail

Analytical silica gel-coated TLC plates (Si 250F) were purchased from Baker, Inc and were visualized with UV light or by treatment with phosphomolybdic acid (PMA). Flash chromatography was carried out on Baker Silica Gel 40 mM. ^{1}H NMR spectra were routinely obtained at Varian 400 MHz FT NMR. The NMR solvent used was CDCl3 as indicated. TMS was used as an internal standard. Elemental analyses were performed by Atlantic Microlab, Inc and were within ± 0.4% of the theoretical value. Optical rotations were measured on Perkin-Elmer 241 polarimeter.

[^{3}H]WIN 35,428 (83.6 Ci/mmol), [^{3}H]dopamine (55.1 Ci/mmol), [3H]serotonin (30.0 Ci/mmol), and [^{3}H]norepinephrine (54.6 Ci/mmol) were obtained from Dupont-New England Nuclear (Boston, MA, U.S.A). WIN 35,428 napthalene sulfonate was purchased from Research

Biochemicals, Inc. (Natick, MA, U.S.A.). (−)-Cocaine HCl was obtained from the National Institute on Drug Abuse. GBR 12909 Dihydrochloride (1-[2-[bis(4-Fluorophenyl)methoxy] ethyl]-4-[3-phenylpropyl]piperazine) was purchased from SIGMA-ALDRICH (#D-052; St. Louis, MO).

Synthesis of *N*-(6- benzhydrylpiperidin-3-yl)-4,7,7-trimethyl-3-oxo-2-oxa-bicyclo [2.2.1]heptane-1-carboxamide (2a and 2b)—To a stirring solution of racemic *cis*-6-benzhydrylpiperidin-3-ylamine **1** (1.10 g, 4.12 mmol) in anhydrous CH_2Cl_2 (50 ml), triethylamine (2.08 g, 20.64 mmol) was added drop wise (1*S*)-(−)-camphanic chloride (1.07 g, 4.95 mmol) dissolved in 10 ml anhydrous CH_2Cl_2 under nitrogen. The reaction mixture was stirred at 0°C for 30 min and at room temperature for another 3 h under nitrogen atmosphere. The reaction mixture was then diluted with CH_2Cl_2 (50 ml) and washed with water (20 ml), dried over Na_2SO_4 and the solvent was evaporated in vacuo to afford a mixture of two diastereomers **2a** and **2b**. Each diastereoisomer was separated by flash column chromatography over silica gel using hexanes/diethyl ether (12:88) as a mobile phase.

Eluting first was **2a** (0.51 g, 55%) ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89 (3H, s, CH_3), 1.09 (3H, s, CH_3), 1.11 (3H, s, CH_3), 1.37–1.41 (1H, m, H-5), 1.48–1.56 (1H, m, H-5), 1.65–1.72 (2H, m, CCH_2C), 1.82–1.97 (3H, m, CCH_2C and H-4), 2.48–2.56 (1H, m, H-4), 2.78–2.84 (2H, m, H-2), 3.23 (1H, dt, *J* = 2.4 Hz, *J* = 10.4 Hz, H-6), 3.79 (1H, d, *J* = 10.0 Hz, $(Ph)_2CH$), 4.09–4.12 (1H, m, H-3), 7.13–7.37 (8H, m, ArH), 7.39–7.41 (2H, m, ArH). Eluting second was **2b** (0.45g, 49%) ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.82 (3H, s, CH_3), 1.02 (3H, s, CH_3), 1.05 (3H, s, CH_3), 1.32–1.35 (1H, m, H-5), 1.43–1.52 (1H, m, H-5), 1.57–1.64 (2H, m, CCH_2C), 1.71–1.90 (3H, m, CCH_2C and H-4), 2.41–2.50 (1H, m, H-4), 2.71–2.80 (2H, m, H-2), 3.16 (1H, dt, *J* = 2.0 Hz, *J* = 10.4 Hz, H-6), 3.71 (1H, d, *J* = 10.0 Hz, $(Ph)_2CH$), 4.01–4.07 (1H, m, H-3), 7.07–7.30 (8H, m, ArH), 7.33–7.35 (2H, m, ArH).

Synthesis of (−)-*cis*-6-Benzhydrylpiperidin-3-ylamine (3)—A solution of **2b** (0.55 g, 1.23 mmol) in conc. HCl/MeOH (50 ml, 1:4 ratio v/v) was refluxed for 72 h. Methanol was then evaporated under reduced pressure at 50° C and the remaining aqueous solution was neutralized by saturated $NaHCO_3$ solution. The solution was extracted with CH_2Cl_2 (3 × 50 ml). All organic layers were combined, washed with brine (50 ml) and dried over Na_2SO_4, concentrated and purified by flash column chromatography over silica gel using ethyl acetate/MeOH/Et_3N (80:15:5) to afford **3** as a white solid (0.26 g, 79%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.35–1.43 (2H, m, H-5), 1.59–1.64 (2H, m, H-4), 2.18 (2H, broad singlet, NH), 2.79–2.81 (2H, m, H-2), 2.98–3.04 (1H, m, H-3), 3.25 (1H, dt, *J* = 4 Hz, *J* = 10 Hz, H-6_{ax}), 3.80 (1H, d, *J* = 10.2 Hz, $(Ph)_2CH$), 7.12–7.40 (10H, m, ArH). $[\alpha]^{25}_D$ = (−) 41.9° (c 1, MeOH).

Procedure A. Synthesis of (*R*)-2-[(3*S*, 6*S*)-6-benzhydrylpiperidin-3-ylamino)-1-(4-fluorophenyl)ethanol (4a)—To a stirring solution of (−)-*cis*-6-benzhydrylpiperidin-3-ylamine **3** (0.058 g, 0.217 mmol) in dry ethanol (20 ml), was added *R*-(−)-4-fluoro styrene oxide (0.045 g, 0.326 mmol). The reaction mixture was refluxed overnight under nitrogen atmosphere. The solvent was evaporated and the product was purified by flash column chromatography over silica gel using diethyl ether/MeOH/Et_3N (92:8:0.2) to give **4a** (0.023 g, 26%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.30–1.38 (2H, m, H-5), 1.48–1.55 (1H, m, H-4_{ax}), 1.75–1.79 (1H, m, H-4_{eq}), 2.44–2.54 (1H, dd, *J* = 2.0 Hz, *J* = 10.0 Hz, $NHCH_2$), 2.71–2.78 (2H, m, H-2), 2.86–2.90 (1H, dd, *J* = 3.2 Hz, *J* = 12.4 Hz, $NHCH_2$), 2.97–3.00 (1H, m, H-3_{eq}), 3.25 (1H, dt, *J* = 3.2 Hz, J = 9.6 Hz, H-6_{ax}), 3.75 (1H, d, *J* = 10 Hz, $(Ph)_2CH$), 4.60–4.64 (1H, dd, *J* = 3.2 Hz, *J* = 9.6 Hz, CH-OH), 7.01 (2H, t, *J* = 8.4 Hz, ArH), 7.13–7.37 (12H, m, ArH). Free base converted into oxalate salt, m.p. 202–204 °C. $[\alpha]^{25}_D$ (oxalate salt) = (−) 21.5° (c 0.26, MeOH).

Analysis calculated for (**$C_{26}H_{29}FN_2O$.** $2(COOH)_2$, $0.5H_2O$) C, H, N.

Synthesis of (*S*)-2-[(3*S*, 6*S*)-6-benzhydrylpiperidin-3-ylamino)-1-(4-fluorophenyl) ethanol (4b)—Compound **3** (0.076 g, 0.285 mmol) was refluxed with *S*-(+)-4-fluoro styrene oxide (0.059 g, 0.427 mmol) (Procedure A) to yield **4b** (0.029 g, 25%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25–1.38 (2H, m, H-5), 1.47–1.53 (1H, m, H-4_{ax}), 1.79–1.83 (1H, m, H-4_{eq}), 2.43–2.53 (1H, dd, *J* = 2.8 Hz, *J* = 12.0 Hz, NHCH_2), 2.71–2.74 (2H, m, H-2), 2.87–2.97 (2H, m, NHCH_2 and H-3_{eq}), 3.25 (1H, dt, *J* = 3.2 Hz, *J* = 9.6 Hz, H-6_{ax}), 3.74 (1H, d, *J* = 10 Hz, $(Ph)_2$CH), 4.60–4.63 (1H, dd, *J* = 2.8 Hz, *J* = 8.8 Hz, C*H*-OH), 7.01 (2H, t, *J* = 8.8 Hz, ArH), 7.16–7.37 (12H, m, ArH). $[\alpha]^{25}_D$ (oxalate salt) = (+) 19.2° (*c* 0.38, MeOH). Free base was converted into oxalate salt 203–205 °C.

Analysis calculated for (**$C_{26}H_{29}FN_2O$.** 2$(COOH)_2$, 0.9H_2O) C, H, N.

Synthesis of (*R*)-1-[(3*S*, 6*S*)-6-benzhydrylpiperidin-3-ylamino)-3-phenylpropan-2-ol (4c)—Compound **3** (0.076 g, 0.285 mmol) was reacted with *R*-(+)-2,3-epoxypropyl benzene (0.057 g, 0.427 mmol) (Procedure A). The crude product was purified by flash column chromatography using diethyl ether/MeOH/Et_3N (90:10:0.2) to give **4c** (0.035 g, 30%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25–1.34 (2H, m, H-5), 1.44–1.52 (1H, m, H-4_{ax}), 1.72–1.75 (1H, m, H-4_{eq}), 2.32–2.38 (1H, dd, *J* = 2.8 Hz, *J* = 9.6 Hz, NHCH_2), 2.64–2.83 (5H, m, H-2, NHCH_2, Ph-CH_2), 2.90–2.93 (1H, m, H-3_{eq}), 3.23 (1H, dt, *J* = 3.2 Hz, *J* = 10.0 Hz, H-6_{ax}), 3.73–3.83 (2H, m, $(Ph)_2$CH and C*H*-OH), 7.12–7.31 (13H, m, ArH), 7.35–7.36 (2H, m, ArH). Optical rotation of free base, $[\alpha]^{25}_D$ = (−) 38.9 ° (*c* 0.57, MeOH). Free base was converted into oxalate salt 205–207 °C.

Analysis calculated for (**$C_{27}H_{32}N_2O$**. 2$(COOH)_2$, 0.5H_2O) C, H, N.

Synthesis of (*S*)-1-[(3*S*, 6*S*)-6-benzhydrylpiperidin-3-ylamino)-3-phenylpropan-2-ol (4d)—Compound **3** (0.075 g, 0.281 mmol) was reacted with *S*-(−)-2,3-epoxypropyl benzene (0.056 g, 0.422 mmol) (Procedure A) and was purified by flash column chromatography over silica gel using diethyl ether/MeOH/Et_3N (90:10:0.2) to yield **4d** (0.037 g, 33%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.23–1.35 (2H, m, H-5), 1.41–1.49 (1H, m, H-4_{ax}), 1.75–1.78 (1H, m, H-4_{eq}), 2.36–2.41 (1H, dd, *J* = 3.2 Hz, *J* = 8.8 Hz, NHCH_2), 2.66–2.84 (5H, m, H-2, NHCH_2, Ph-CH_2), 2.89–2.92 (1H, m, H-3_{eq}), 3.23 (1H, dt, *J* = 2.4 Hz, *J* = 10.0 Hz, H-6_{ax}), 3.75 (1H, d, *J* = 10.0 Hz, $(Ph)_2$CH) 3.78–3.85 (1H, m, and C*H*-OH), 7.12–7.31 (13H, m, ArH), 7.35–7.36 (2H, m, ArH). Optical rotation, $[\alpha]^{25}_D$ = (−) 49.3 ° (c 0.96, MeOH). Free base was converted into oxalate salt 206–209 °C

Analysis calculated for (**$C_{27}H_{32}N_2O$**. 2$(COOH)_2$, 0.3H_2O) C, H, N.

Synthesis of 1-((5*S*, 8*S*)-8-benzhydryl-1,4-diazabicyclo [3.3.1]nonane-2-yl)-3-phenylpropan-2-ol (6a and 6b)—To a stirring solution of *cis*-(−)-8-benzydryl-1,4-diazabicyclo[3.3.1]nonane **5** (0.100 g, 0.341 mmol) in dry ethanol was added 2,3-epoxypropyl benzene (0.068 g, 0.512 mmol). The reaction mixture was stirred overnight at 65°C (Procedure A). The diastereomers were separated by preparative TLC using acetone/diethyl ether (20:80) as mobile phase to afford **6a** and **6b**. Upper Fraction gave **6a** (0.026 g, 18%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.24–1.43 (2H, m, H-7), 1.54–1.66 (1H, m, H-6_{ax}), 2.03–2.06 (1H, m, H-6_{eq}), 2.28 (1H, t, *J* = 12.4 Hz, NCH_2CH), 2.39 (1H, bs, H-5), 2.57–2.87 (6H, m, NCH_2CH, NCH_2CH_2N, Ph-CH_2), 2.98–3.01 (1H, m, H-9_{ax}), 3.10 (1H, bd, *J* = 11.8 Hz, NCH_2CH_2N), 3.22–3.25 (1H, m, H-9_{eq}) 3.77 (1H, dt, *J* = 4.8 Hz, *J* = 11.2 Hz, H-8_{ax}), 3.84–3.91 (2H, m, C*H*-OH, $(Ph)_2$C*H*), 7.11–7.15 (2H, m, ArH), 7.19–7.31 (11H, m, ArH), 7.35–7.38 (2H, m, ArH). $[\alpha]^{25}_D$ (oxalate salt) = (−) 24.7 ° (*c* 0.42, MeOH).

Analysis calculated for (**$C_{29}H_{34}N_2O$.** 2$(COOH)_2$) C, H, N.

Lower fraction gave **6b** (0.024g, 16%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.22–1.29 (1H, m, H-7_{ax}), 1.34–1.42 (1H, m, H-7_{eq}), 1.47–1.56 (1H, m, H-6_{ax}), 2.02–2.07 (1H, m, H-6_{eq}), 2.20 (1H, dd, *J* = 10.8 Hz, *J* = 2.4 Hz, NC*H*$_2$CH), 2.48 (1H, bm, H-5), 2.67 (1H, dd, *J* = 8.0 Hz, *J* = 5.6 Hz, NC*H*$_2$CH), 2.75–3.10 (7H, m, NCH_2CH_2N, Ph-CH_2, H-9_{ax}), 3.17–3.20 (1H, m, H-9_{eq}) 3.75 (1H, dt, *J* = 4.8 Hz, *J* = 11.3 Hz, H-8_{ax}), 3.83–3.90 (2H, m, C*H*-OH, $(Ph)_2CH$), 7.12–7.15 (2H, m, ArH), 7.19–7.30 (11H, m, ArH), 7.33–7.38 (2H, m, ArH). $[\alpha]^{25}_D$ (oxalate salt)= (−) 31.2 ° (c 0.40, MeOH).

Analysis calculated for (**$C_{29}H_{34}N_2O$.** 2$(COOH)_2$) C, H, N.

Synthesis of (*R*)-2-((5*S*, 8*S*)-8-benzhydryl-1,4-diazabicyclo[3.3.1]nonane-2-yl)-1-phenylethanol (7a)—To a stirring solution of *cis*-(−)-8-benzydryl-1,4-diazabicyclo[3.3.1] nonane **5** (0.065 g, 0.222 mmol) in dry ethanol was added *R*-(+)-2-phenyloxirane (0.04 g, 0.333 mmol) (Procedure A). The compound was purified by flash column chromatography by using diethyl ether/MeOH (9:1) to afford **7a** (0.051 g, 56%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.26–1.33 (1H, m, H-7_{ax}), 1.38–1.47 (1H, m, H-7_{eq}), 1.54–1.63 (1H, m, H-6_{ax}), 2.00–2.03 (1H, m, H-6_{eq}), 2.38–2.47 (2H, m, NC*H*$_2$CH, H-5), 2.77 (1H, dd, *J* = 3.6 Hz, *J* = 12.4 Hz, NC*H*$_2$CH), 2.82–2.99 (3H, m, NCH_2CH_2N) 3.03–3.06 (1H, m, H-9_{ax}), 3.17–3.19 (1H, m, NCH_2CH_2N), 3.29–3.33 (1H, m, H-9_{eq}) 3.80 (1H, dt, *J* = 4.4 Hz, *J* = 10.8 Hz, H-8_{ax}), 3.91 (1H, d, *J* = 11.6 Hz, $(Ph)_2CH$), 4.69 (1H, dd, *J* = 3.2 Hz, *J* = 11.2 Hz, C*H*OH), 7.10–7.38 (15H, m, ArH). $[\alpha]^{25}_D$ = (−) 52.3 ° (*c* 0.52, MeOH). Free base was converted into oxalate salt 194–197 °C

Analysis calculated for (**$C_{28}H_{32}N_2O$.** 2$(COOH)_2$) C, H, N.

Synthesis of (*S*)-2-((5*S*, 8*S*)-8-benzhydryl-1,4-diazabicyclo[3.3.1]nonane-2-yl)-1-phenylethanol (7b)—Compound **5** (0.060 g, 0.205 mmol) was reacted with *S*-(−)-2-phenyloxirane (0.036 g, 0.307 mmol) (Procedure A) to yield **7b** (0.045 g, 53%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25–1.34 (1H, m, H-7_{ax}), 1.38–1.49 (1H, m, H-7_{eq}), 1.54–1.65 (1H, m, H-6_{ax}), 2.22–2.27 (1H, m, H-6_{eq}), 2.34 (1H, dd, *J* = 2.8 Hz, *J* = 10.4 Hz, NC*H*$_2$CH), 2.46–2.49 (1H, m, NCH_2CH_2N), 2.64 (1H, bs, H-5), 2.91–3.11 (6H, m, NCH_2CH_2N, NC*H*$_2$CH, H-9_{ax}), 3.24–3.27 (1H, m, H-9_{eq}) 3.72–3.83 (1H, m, H-8_{ax}), 3.88 (1H, d, *J* = 11.6 Hz, $(Ph)_2CH$), 4.67 (1H, dd, *J* = 3.6 Hz, *J* = 10.0 Hz, C*H*OH), 7.10–7.37 (15H, m, ArH). $[\alpha]^{25}_D$ = (−) 45.8 ° (*c* 0.52, MeOH). Free base was converted into oxalate salt 193–195 °C

Analysis calculated for (**$C_{28}H_{32}N_2O$.** 2$(COOH)_2$, 0.3H_2O) C, H, N.

Synthesis of (*R*)-2-((5*S*, 8*S*)-8-benzhydryl-1,4-diazabicyclo[3.3.1]nonane-2-yl)-1-4-fluorophenyl)ethanol (7c)—Compound **5** (0.060 g, 0.205 mmol) was reacted with *R*-(−)-2-(4-fluorophenyl)oxirane (0.042 g, 0.307 mmol) (Procedure A) to yield **7c** (0.034 g, 39%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25–1.32 (1H, m, H-7_{ax}), 1.35–1.47 (1H, m, H-7_{eq}), 1.54–1.64 (1H, m, H-6_{ax}), 2.00–2.03 (2H, m, H-6_{eq}), 2.32–2.38(1H, dd, *J* = 11.2 Hz, *J* = 0.8 Hz, NC*H*$_2$CH), 2.44 (1H, bs, H-5), 2.73 (1H, dd, *J* = 3.6 Hz, *J* = 12.8 Hz, NC*H*$_2$CH), 2.79–2.96 (3H, m, NCH_2CH_2N), 3.02–3.06 (1H, m, H-9_{ax}), 3.16–3.19 (1H, bm, NCH_2CH_2N), 3.27–3.31 (1H, m, H-9_{eq}), 3.75–3.86 (1H, m, H-8_{ax}), 3.90 (1H, d, *J* = 11.2 Hz, $(Ph)_2C$*H*), 4.66 (1H, dd, *J* = 3.2 Hz, *J* = 10.8 Hz, C*H*OH), 7.03 (2H, t, *J* = 8.4 Hz, ArH), 7.10–7.38 (12H, m, ArH). $[\alpha]^{25}_D$ (free base) = (−) 45.5 ° (*c* 0.57, MeOH). Free base was converted into oxalate salt 189–191 °C

Analysis calculated for (**$C_{28}H_{31}FN_2O$.** 2$(COOH)_2$, 0.2H_2O) C, H, N.

Synthesis of (*S*)-2-((5*S*, 8*S*)-8-benzhydryl-1,4-diazabicyclo[3.3.1]nonane-2-yl)-1-4-fluorophenyl)ethanol (7d)—Compound **5** (0.070 g, 0.239 mmol) was reacted with *S*-(+)-2-(4-fluorophenyl)oxirane (0.049 g, 0.358 mmol) (Procedure A) to yield **7d** (0.040 g,

39%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.27–1.34 (1H, m, H-7_{ax}), 1.37–1.49 (1H, m, H-7_{eq}), 1.59–1.68 (1H, m, H-6_{ax}), 2.20–2.32 (2H, m, H-6_{eq}, NC*H*$_2$CH), 2.45–2.49 (1H, m, NCH_2CH_2N), 2.62 (1H, bs, H-5), 2.91–3.11 (5H, m, NCH_2CH_2N, NC*H*$_2$CH, H-9_{ax}), 3.23–3.28 (1H, m, H-9_{eq}), 3.75–3.82 (1H, m, H-8_{ax}), 3.88 (1H, d, *J* = 11.2 Hz, $(Ph)_2C$*H*), 4.64 (1H, dd, *J* = 3.2 Hz, *J* = 10.0 Hz, C*H*OH), 6.99–7.04 (2H, m, ArH), 7.10–7.37 (12H, m, ArH). $[\alpha]^{25}_D$ (free base) = (−) 44.0 ° (*c* 0.56, MeOH). Free base was converted into oxalate salt 191–193 °C

Analysis calculated for (**$C_{28}H_{31}FN_2O$.** $2(COOH)_2$, $0.3H_2O$) C, H, N.

Synthesis of 3-((1*S*, 6*S*)-6-benzhydryl-2,5-diazabicyclo[3.3.1]nonane-2-yl)-1-(4-fluorophenyl)propan-1-one (8)—Into a stirred solution of *cis*-(−)-8-benzydryl-1,4-diazabicyclo[3.3.1] nonane **5** (0.250 g, 0.854 mmol) in dry acetonitrile was added K_2CO_3 (0.354 g, 2.56 mmol) followed by 3-chloro-4′-fluoro propiophenone (0.207 g, 1.11 mmol). Catalytic amount of potassium iodide was added and the reaction mixture was refluxed for 3 h under nitrogen atmosphere. After evaporation of solvent, the residue was dissolved in water and extracted with CH_2Cl_2 (2 × 100 ml), dried over Na_2SO_4 and evaporated. The compound was purified by flash column chromatography using diethyl ether/MeOH/Et_3N (93:7:0.2) to afford **8** (0.260 g, 68%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25–1.34 (1H, m, H-7_{ax}), 1.34–1.45 (1H, m, H-7_{eq}), 1.48–1.58 (1H, m, H-6_{ax}), 2.20–2.23 (1H, m, H-6_{eq}), 2.52 (1H, bs, H-5), 2.62–2.65 (1H, m, NC*H*$_2$CH$_2$), 2.80–3.16 (8H, m, NCH_2CH_2N, NC*H*$_2$CH$_2$, C*H*$_2$CO, H-9_{ax}), 3.20–3.23 (1H, m, H-9_{eq}) 3.77 (1H, dt, *J* = 4.4 Hz, *J* = 11.2 Hz, H-8_{ax}), 3.89 (1H, d, *J* = 11.8 Hz, $(Ph)_2CH$), 7.08–7.15 (2H, m, ArH), 7.20–7.28 (6H, m, ArH), 7.36 (2H, d, *J* = 7.2 Hz, ArH), 7.95–7.99 (2H, dt, *J* = 2.0 Hz, *J* = 5.2 Hz, ArH).

Synthesis of 3-((1*S*, 6*S*)-6-benzhydryl-2,5-diazabicyclo[3.3.1]nonane-2-yl)-1-(4-fluorophenyl)propan-1(R & S)-ol (9)—To a stirred solution of compound **8** (0.250 g, 0.564 mmol) dissolved in 25 ml of THF was added $NaBH_4$ (0.025 g, 0.677 mmol) followed by addition of 0.5 ml of water. The reaction mixture was stirred for 3 h under nitrogen atmosphere at RT. Water (5 ml) was added next into the reaction mixture. The solvent was evaporated and the residue was dissolved in water and extracted with CH_2Cl_2 (2 × 50 ml), dried over Na_2SO_4 and evaporated in vacuo. The crude product was purified by flash column chromatography using diethyl ether/MeOH/Et_3N (93:8:0.2) to afford **9** (0.195 g, 79%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25–1.41 (2H, m, H-7_{ax}, H-7_{eq}), 1.47–1.95 (3H, m, C*H*$_2$CHOH, H-6_{ax}), 2.09–2.18 (1H, m, H-6_{eq}), 2.59–2.96 (6H, m, H-5, NC*H*$_2$CH$_2$, NCH_2CH_2N), 3.04 (1H, d, *J* = 13.2 Hz, H-9_{ax}), 3.12–3.25 (2H, m, NCH_2CH_2N, H-9_{eq}), 3.73–3.82 (1H, m, H-8_{ax}), 3.88 (1H, dd, *J* = 1.6 Hz, *J* = 11.6 Hz, $(Ph)_2CH$), 4.84–4.95 (1H, m, C*H*OH), 7.00 (2H, dt, *J* = 1.6 Hz, *J* = 8.4 Hz, ArH), 7.10–7.16 (2H, m, ArH), 7.19–7.37 (10H, m, ArH).

Synthesis of 3-((1*S*, 6*S*)-6-benzhydryl-2,5-diazabicyclo[3.3.1]nonane-2-yl)-1-(4-fluorophenyl)propyl-4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]heptane-1-carboxylate (10a and 10b)—To a stirred solution of 3-((1*S*, 6*S*)-6-benzhydryl-2,5-diazabicyclo [3.3.1]nonane-2-yl)-1-(4-fluorophenyl)propan-1 (R & S)-ol **9** (0.195 g, 0.438 mmol), Et_3N (0.088 g, 0.876 mmol), and DMAP (10 mg) in 100 ml of dry CH_2Cl_2 under nitrogen atmosphere at 0°C was added (1S)-(−)-camphanic chloride (0.123 g, 0.569 mmol) dissolved in 10 ml dry CH_2Cl_2. The reaction mixture was stirred at 0°C for 30 min and at room temperature for 3 h under nitrogen. The reaction mixture was quenched with water (20 ml) and then diluted with CH_2Cl_2 (50 ml). Organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2 × 50 ml), dried over Na_2SO_4 and evaporated in vacuo. The crude product was purified by flash column chromatography over silica gel using diethyl ether/MeOH (95:5) to afford mixture of two diastereoisomers **10a** and **10b** (0.250 g, 91%). The diastereoisomers were separated by semipreparative HPLC using a normal phase column (Nova-pack silica 6μm). Hexanes/2-propanol/Et_3N (92:8:0.3) was used as a mobile phase with

a flow rate of 12 mL/min. The two fractions were eluted with retention time 2.83 min and 3.30 min for **10a** and **10b**, respectively.

Eluting first was **10a** (0.115 g, 42%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.79 (3H, s, CH_3), 0.97 (3H, s, CH_3), 1.07 (3H, s, CH_3), 1.24–1.49 (2H, m, H-7_{ax}, H-7_{eq}), 1.62–1.68 (1H, m, H-6_{ax}), 1.84–2.07 (3H, m, C*H*$_2$CHO, H-6_{eq}), 2.16–2.49 (5H, m, H-5, CC*H*$_2$C, NCH$_2$CH$_2$N), 2.59–2.63 (1H, m, NCH$_2$CH$_2$N), 2.68–2.75 (1H, m, NC*H*$_2$CH$_2$), 2.86–2.98 (2H, m, NC*H*$_2$CH$_2$, H-9_{ax}), 3.09–3.12 (1H, m, NCH$_2$CH$_2$N), 3.18–3.21 (1H, m, H-9_{eq}), 3.75 (1H, dt, *J* = 4.4 Hz, *J* = 11.2 Hz, H-8_{ax}), 3.87 (1H, d, *J* = 11.2 Hz, (Ph)$_2$C*H*), 5.94 (1H, t, *J* = 7.2 Hz, C*H*OCO), 6.97–7.03 (2H, m, ArH), 7.09–7.14 (2H, m, ArH), 7.19–7.27 (6H, m, ArH), 7.30–7.37 (4H, m, ArH).

Eluting second was **10b** (0.105 g, 38%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (3H, s, CH_3), 0.98 (3H, s, CH_3), 1.09 (3H, s, CH_3), 1.23–1.52 (2H, m, H-7_{ax}, H-7_{eq}), 1.62–1.68 (1H, m, H-6_{ax}), 1.84–1.99 (2H, m, C*H*$_2$CHO), 2.04–2.07 (1H, m, H-6_{eq}), 2.12–2.52 (5H, m, H-5, CC*H*$_2$C, NCH$_2$CH$_2$N), 2.68–2.75 (1H, m, NC*H*$_2$CH$_2$), 2.84–2.92 (1H, m, NCH$_2$CH$_2$), 2.95–2.99 (1H, m, H-9_{ax}), 3.08–3.11 (1H, m, NCH$_2$CH$_2$N), 3.18–3.22 (1H, m, H-9_{eq}), 3.76 (1H, dt, *J* = 4.8 Hz, *J* = 11.2 Hz, H-8_{ax}), 3.87 (1H, d, *J* = 11.6 Hz, (Ph)$_2$CH), 5.92 (1H, t, *J* = 6.8 Hz, C*H*OCO), 6.98–7.03 (2H, m, ArH), 7.09–7.15 (2H, m, ArH), 7.19–7.37 (10H, m, ArH).

Procedure B. Synthesis of (−)11a—The first eluting camphanic ester fraction **10a** (0.075 g, 0.120 mmol) was hydrolyzed with K_2CO_3 (20 mg) in methanol (20 ml) at room temperature for 12 h. Methanol was evaporated, water (20 ml) was added and the product was extracted with ethyl acetate (2 × 50 ml). Organic layer was dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by flash column chromatography over silica using diethyl ether/MeOH/Et$_3$N (93:8:0.2) to afford **11a** (0.048 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.25–1.44 (2H, m, H-7_{ax}, H-7_{eq}), 1.54–1.69 (2H, m, C*H*$_2$CHOH, H-6_{ax}), 1.80–1.90 (1H, m, C*H*$_2$CHOH), 2.15–2.18 (1H, m, H-6_{eq}), 2.63 (1H, bs, H-5), 2.67–2.974 (1H, m, NC*H*$_2$CH$_2$), 2.79–2.95 (4H, m, NC*H*$_2$CH$_2$, NCH$_2$CH$_2$N), 3.04 (1H, d, *J* = 13.2 Hz, H-9_{ax}), 3.13–3.18 (1H, m, NCH$_2$CH$_2$N), 3.22–3.25 (1H, m, H-9_{eq}), 3.79 (1H, dt, *J* = 4.8 Hz, *J* = 11.6 Hz, H-8_{ax}), 3.88 (1H, d, *J* = 11.6 Hz, (Ph)$_2$C*H*), 4.86 (1H, dd, *J* = 2.4 Hz, *J* = 9.6 Hz, C*H*OH), 7.00 (2H, t, *J* = 8.8 Hz, ArH), 7.11–7.16 (2H, m, ArH), 7.19–7.37 (10H, m, ArH). $[\alpha]^{25}_D$ = (−) 38.7 ° (*c* 1.08, MeOH). Free base was converted into oxalate salt 191–193 °C

Analysis calculated for (**$C_{29}H_{33}FN_2O$.** 2(COOH)$_2$, 1.5H$_2$O) C, H, N.

Synthesis of (−)11b—The second eluting fraction **10b** (0.105 g, 0.168 mmol) was hydrolyzed with K_2CO_3 (20 mg) in methanol (20 ml) to afford **11b** (0.071 g, 94%) (Procedure B). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.24–1.43 (2H, m, H-7_{ax}, H-7_{eq}), 1.48–1.54 (1H, m, H–6_{ax}), 1.71–1.79 (1H, m, C*H*$_2$CHOH), 1.88–1.95 (1H, m, C*H*$_2$CHOH), 2.09–2.12 (1H, m, H-6_{eq}), 2.60–2.66 (2H, m, H-5, NC*H*$_2$CH$_2$), 2.76–2.97 (4H, m, NC*H*$_2$CH$_2$, NCH$_2$CH$_2$N), 3.04 (1H, d, *J* = 13.2 Hz, H-9_{ax}), 3.12–3.15 (1H, m, NCH$_2$CH$_2$N), 3.21–3.24 (1H, m, H-9_{eq}), 3.78 (1H, dt, *J* = 4.8 Hz, *J* = 11.6 Hz, H-8_{ax}), 3.88 (1H, d, *J* = 11.6 Hz, (Ph)$_2$C*H*), 4.94 (1H, dd, *J* = 3.2 Hz, *J* = 7.6 Hz, C*H*OH), 7.00 (2H, t, *J* = 8.4 Hz, ArH), 7.10–7.16 (2H, m, ArH), 7.20–7.37 (10H, m, ArH). $[\alpha]^{25}_D$ = (−) 56.8 ° (*c* 1.0, MeOH). Free base was converted into oxalate salt 193–195 °C

Analysis calculated for (**$C_{29}H_{33}FN_2O$.** 2(COOH)$_2$, 0.5H$_2$O) C, H, N.

Transporter assays—The affinity of test compounds in binding to rat DAT and in inhibiting monoamine uptake was monitored as described by us previously.[21] Briefly, rat striatum was used for measuring binding of [^{3}H]WIN 35,428 by DAT and uptake of [^{3}H]DA by DAT. Rat cerebral cortex was used for assessing uptake of [^{3}H]serotonin by SERT and hippocampus for

uptake of [^{3}H]NE by NET. Nonspecific binding at DAT was defined with 100 uM cocaine; nonspecific uptake at DAT, SERT, and NET with 100 uM cocaine, 10 uM citalopram, and 10 uM desipramine, respectively. Test compounds were dissolved in dimethyl sulfoxide (DMSO), diluted out in 10% (v/v) DMSO, and added to assays resulting in a final DMSO concentration of 0.5% which by itself did not interfere with the assays. At lease 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[16].

Acknowledgements

This work was supported by the National Institute on Drug Abuse, Grant No. DA 12449 (AKD).

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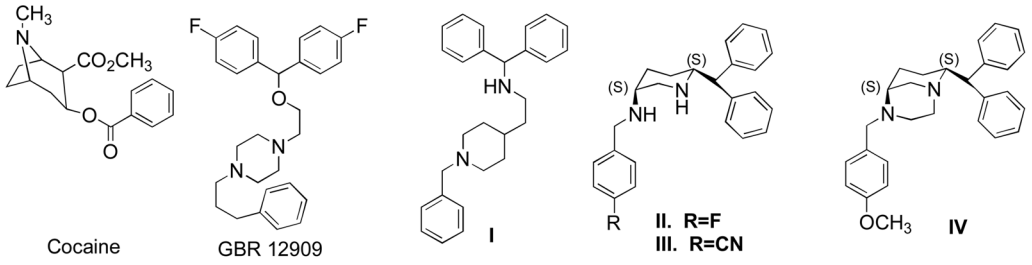
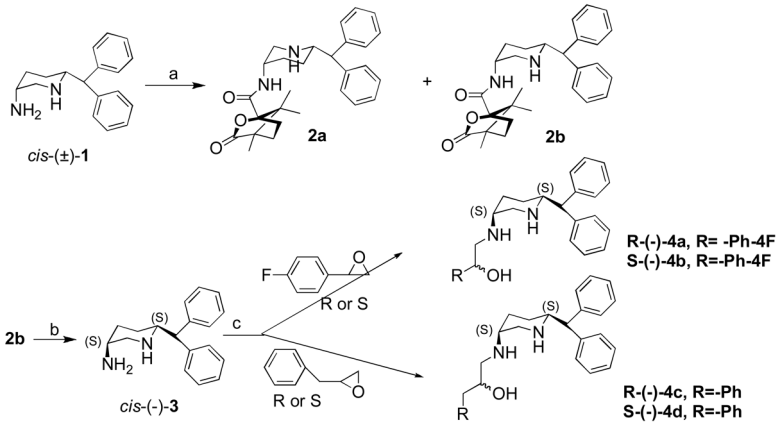
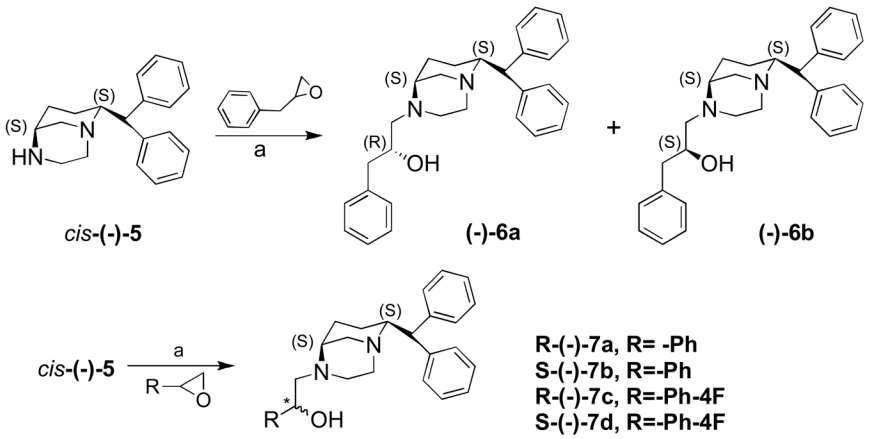


Figure 1.
Molecular structure of dopamine transporter blockers



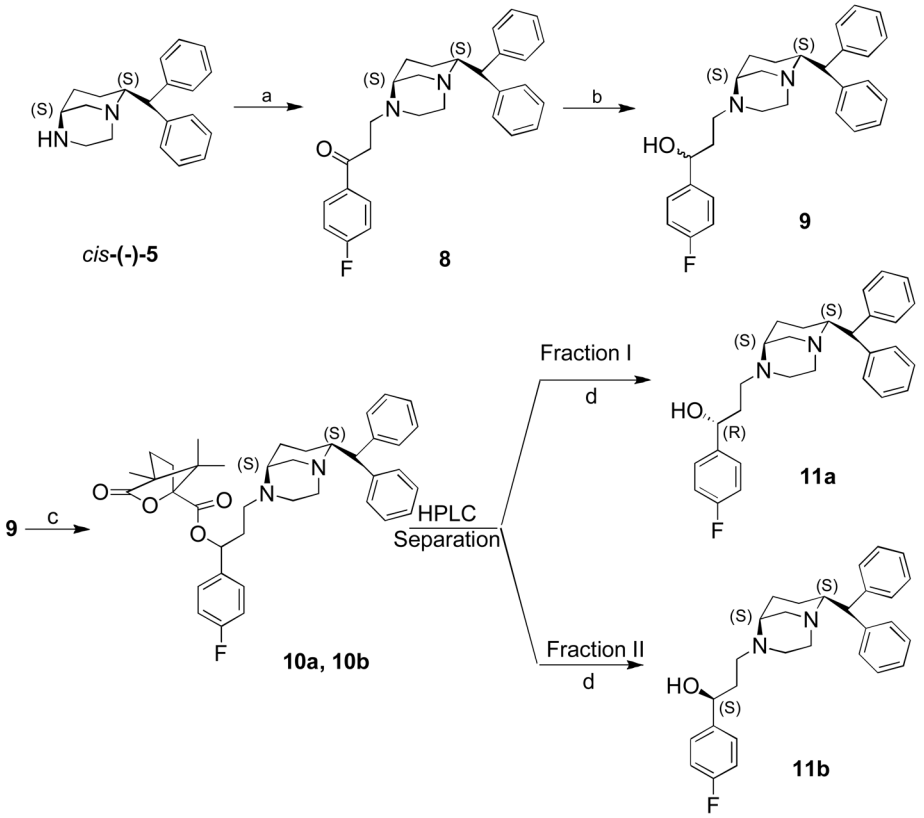
Reaction conditions: (a) 1*S*-(-)Camphanic chloride, Et_3N, CH_2Cl_2; (b). HCl/MeOH, reflux, 72 h; (c) R- or S-epoxide, EtOH, 65°C, overnight.

Scheme 1.



Reaction conditions: (a) epoxide, EtOH, 65°C, overnight.

Scheme 2.



Reaction conditions: (a) 3-chloro-4'-fluoro propiophenone, K_2CO_3, KI, MeCN, reflux, 3h; (b) $NaBH_4$, THF, H_2O, 3h, RT; (c) (1*S*)-(-)-camphanic chloride, Et_3N, DMAP, CH_2Cl_2, 3 h; (d) K_2CO_3, MeOH, 12 h, RT.

Scheme 3.

Table 1

Affinity of Drugs at DAT, SERT, and NET in Rat Brain

Compounds	DAT binding, Ki, nM, [^{3}H]WIN 35, 428[a]	DATuptake, Ki, nM, [^{3}H]DA[a]	SERT uptake, Ki, nM, [^{3}H]5-HT[a]	NET uptake, Ki, nM [^{3}H]NE[a]
GBR 12909[b]	10.6 ± 1.9	10.6 ± 2.2	91.1 ± 12.8	102 ± 32
III[b]	11.3 ± 0.9	9.10 ± 1.86		
IV[b]	22.5 ± 2.1	18.4 ± 0.9		
D-228, 4a (*S,S,R*)		236 ± 41	2,895 ± 755	1435 ± 495
D-227, 4b (*S,S,S*)		152 ± 46	3,117 ± 757	306 ± 89
D-254, 4c (*S,S,R*)	31.8 ± 6.0	25.1 ± 2.5	1,391 ± 298	170 ± 32
D-272, 4d (*S,S,S*)	28.9 ± 2.3	25.3 ± 6.9	2596 ± 718	231 ± 50
D-169, 6a (*S,S,R*)	148 ± 48	82.9 ± 7.2	11,216 ± 231	730 ± 79
D-170, 6b (*S,S,S*)	47.0 ± 5.6	16.8 ± 1.3	10,336 ± 539	259 ± 20
D-250, 7a (*S,S,R*)	228 ± 45	204 ± 34	12,904 ± 1440	954 ± 138
D-251, 7b (*S,S,S*)	1,039 ±179	266 ± 31	9,508 ±1,748	1,730 ± 387
D-252, 7c (*S,S,R*)	142 ± 22	66.5 ± 5.7	7,414 ± 978	319 ± 58
D-253, 7d (*S,S,S*)	649 ± 100	160 ± 12	7344 ± 1,437	728 ± 142
D-273 (11a)	19.9 ± 0.9	41.8 ± 6.9	11,884 ± 4136	388 ± 76
D-274 (11b)	13.5 ± 2.9	8.63 ± 1.36	1484 ± 366	418 ± 27

[a]For binding, the DAT was labeled with [^{3}H]WIN 35, 428. For uptake by DAT, SERT and NET, [3H]DA, [3H]5-HT and [3H]NE accumulation were measured. Results are average ± SEM of three to eight independent experiments assayed in triplicate.

[b]Results from Ref # 12 and 13.

Table 2

Selectivity ratio for uptake inhibition

Compound	SERT uptake/DAT uptake[a]	NET uptake/DAT uptake[a]	DAT uptake/DAT Binding[a]
GBR 12909	8.5	9.6	1.0
4a	12.2	6.0	
4b	20.5	2.0	
4c	55.4	6.7	0.78
4d	103	9.1	0.87
6a	135	8.8	0.56
6b	615	15.4	0.35
7a	63.2	4.6	0.89
7b	35.7	6.5	0.25
7c	111	4.7	0.46
7d	45.9	4.5	0.24
11a	284.3	9.2	2.1
11b	171.9	48.4	0.63

[a]Ratio of Ki values

Elemental Analysis Results of Final Products

Compounds	Calculated			Found		
	C	H	N	C	H	N
4a $2(COOH)_2\ 0.5H_2O$	60.70	5.77	4.72	60.78	5.72	4.69
4b $2(COOH)_2\ 0.9H_2O$	59.97	5.84	4.66	59.98	5.71	4.57
4c $2(COOH)_2\ 0.5H_2O$	63.15	6.32	4.75	63.15	6.31	4.74
4d $2(COOH)_2\ 0.3H_2O$	63.53	6.30	4.78	63.45	6.42	4.79
6a $2(COOH)_2$	65.33	6.31	4.62	65.17	6.32	4.58
6b $2(COOH)_2$ 0.2H2O	64.94	6.34	4.59	64.73	6.23	4.54
7a $2(COOH)_2$	64.85	6.12	4.73	64.75	6.20	4.71
7b $2(COOH)_2\ 0.3H_2O$	64.27	6.17	4.68	64.24	6.21	4.73
7c $2(COOH)_2\ 0.2H_2O$	62.57	5.81	4.56	62.48	5.87	4.60
7d $2(COOH)_2\ 0.3H_2O$	62.39	5.82	4.55	62.29	5.85	4.59
11a $2(COOH)_2\ 1.5H_2O$	60.82	6.19	4.30	60.43	5.95	4.23
11b $2(COOH)_2\ 0.5H_2O$	62.55	6.04	4.42	62.54	5.94	4.33