Further Structurally Constrained Analogues of *cis*-(6-Benzhydrylpiperidin-3-yl)benzylamine with Elucidation of Bioactive Conformation: Discovery of 1,4-Diazabicyclo[3.3.1]nonane Derivatives and Evaluation of Their Biological Properties for the Monoamine Transporters

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Our structure−activity relationship (SAR) study on piperidine analogues for monoamine transporters led to the development of a series of 3,6-disubstituted piperidine derivatives, structurally constrained versions of flexible piperidine analogues, with preferential affinity for the dopamine transporter (DAT). In our attempt to further rigidify this structure to study influence of rigidity on binding and in vivo activity, we have developed a series of 4,8-disubstituted 1,4-diazabicyclo[3.3.1]nonane derivatives. All synthesized derivatives were tested for their affinity at the DAT, serotonin transporter (SERT), and norepinephrine transporter (NET) in the brain by measuring their potency in competing for the binding of [3H]WIN 35, 428, [3H]citalopram, and [3H]nisoxetine, respectively. Selected compounds were also tested for their ability to inhibit uptake of [3H]DA. The SAR study led to the discovery of a potent lead compound (−)-*S,S*-**10c** which exhibited high affinity and selectivity for the DAT (IC$_{50}$ = 22.5 nM; SERT/DAT = 384 and NET/DAT > 444). It is interesting to note that both (−)-**10c** and the lead compound from the 3,6-disubstituted series (−)-**2** exhibited highest activity in their (*S,S*) isomer indicating similar requirement of regiospecificity for maximum interaction. Overall, our current SAR results corresponded well with the results from less constrained 3,6-disubstituted versions of these molecules albeit the former class exhibited more stringent requirement in molecular structure for activity. However, the potent compounds in the current series exhibited greater selectivity for the DAT compared to their corresponding lesser constrained 3,6-disubstituted versions indicating an effect of rigidity in selective interaction with the transporter proteins. In an effort to elucidate the bioactive conformational structure of the lead molecules in the current and the 3,6-disubstituted series, a preliminary molecular modeling study was carried out where the most rigid derivative (−)-**10c** was used as a template structure. Compounds (−)-**2** and (−)-**10c** exhibited stimulant activity in locomotor tests in mice in which (−)-**2** exhibited a slower onset and longer duration of action compared to (−)-**10c**. Both compounds occasioned complete cocaine-like responding in mice trained to discriminate 10 mg/kg ip cocaine from vehicle.

Introduction

In recent years a significant advancement has been made in understanding the mechanism of action of cocaine. Cocaine binds to several binding sites in the brain located primarily on transporter proteins for the monoamines dopamine (DAT for DA), serotonin (SERT for SER), and norepinephrine (NET for NE).[1] There is much evidence that the strong reinforcing effects of cocaine originate from its binding to the DAT.[2−7] However, the effect of serotonergic system has also been implicated in the production of some of the cocaine effects.[8]

Many of the advances made in developing drugs for the treatment of cocaine addiction are focused on targeting the DAT.[9] Molecules with diverse structures have been developed for the DAT.[10,11] These molecules are broadly categorized into four main classes depending on their chemical structure and are known as tropane, GBR, methyl phenidate, and mazindol class of derivatives. Detailed structure−activity relationship (SAR) studies of these different categories of molecules have been described in the recent review papers.[10−12] A selective DAT blocker that is devoid of much of the commonly associated NET activity will be of interest for further study. Such compounds are also expected to exhibit less cardio toxicity.

In our ongoing effort to design novel molecules targeting monoamine transporters in the development of pharmacotherapies for cocaine addiction, we have developed a large number of flexible piperidine analogues of GBR 12935 (Figure 1) exhibiting potent affinity at

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(-)-S,S-**2** ; R=CN
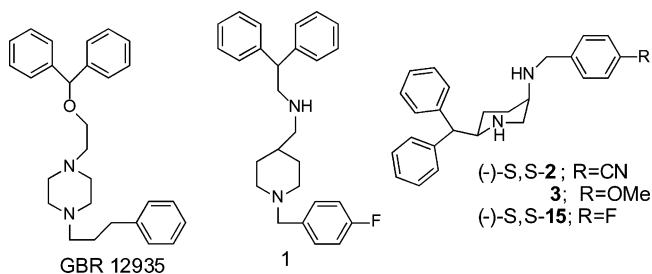**3**; R=OMe
(-)-S,S-**15**; R=F

GBR 12935

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Figure 1. Molecular structures of dopamine transporter blockers.

the DAT.[13-15] Recently, we modified one of our flexible DAT-selective piperidine analogues leading to a series of structurally constrained 3,6-disubstituted piperidine derivatives.[16,17] Between two isomeric derivatives characterized in this series, the cis derivative exhibited preferential affinity at the DAT over the trans derivative.[16] Further structure–activity relationship (SAR) exploration based on the novel cis-structure yielded more potent molecules for the DAT, thus confirming preferential affinity of this novel template for the DAT.[17] In this regard, central piperazine ring-constrained GBR 12909 derivatives and modified GBR structures based on the tropane moiety have also been developed recently.[18-20]

One of our research goals is to explore possible bioactive conformations of our molecules interacting with the DAT. In this effort, and to expand our SAR studies further in the search for suitable pharmacotherapeutic agents for cocaine addiction, the design of more structurally constrained molecules of 3,6-disubstituted derivative was undertaken. Thus, we have carried out a further structural rigidification on this template by linking the two nitrogen atoms in the molecule by a bis-methylene-chain linker (Figure 2) which yielded a novel series of 4,8-disubstituted 1,4-diazabicyclo[3.3.1]nonane derivatives. We hypothesized that a highly conformationally constrained molecule shaped to the complementary binding sites of a target will exhibit high affinity due to the less entropic penalty encountered (compared to flexible molecule) resulting from binding to the transporter.[21] To elucidate the structure of a bioactive conformer, we planned to conduct a preliminary molecular modeling studies with the lead constrained bicyclic and 3,6-disubstituted derivatives. We have already shown that the substitutions of 3a,6e in the *S,S*-absolute configuration with respect to the chair form of the piperidine ring, as depicted in compound *S,S*-(−)-**2**, is a pharmacophoric requirement in the 3,6-disubstituted series. A further conformational restriction on this template with maintenance of appreciable activity at the target receptor site will qualify to act as a template for determination of

the bioactive conformation.[22,23] Thus, the current series of 4,8-disubstituted 1,4-diazabicyclo[3.3.1]nonane derivatives possesses a constrained semicaged structure, which should act as a template for the determination of the bioactive conformation for 3,6-disubstituted derivatives.

In addition, we wanted to study the effect of structural rigidity on in vivo activity, as a structurally constrained molecule could exhibit favorable pharmacokinetic properties.[24] In addition to assessing the effect of changes in lipophilicity (ClogP), it is of interest to examine whether the change in molecular shape associated with the present constrained molecules will lead to enhanced blood–brain barrier-penetrating activity. Recent studies have demonstrated eloquently that besides lipophilicity, there are other factors which can influence oral bioavailability and crossing of the blood–brain barrier. These factors include polar molecular surface area, number of H-bonds (donor + acceptor), and number of rotatable bonds in drug molecules.[25-28] It is likely that a combination of molecular shape and change of overall polarity and lipophilicity might contribute to an overall change in pharmacokinetic and pharmacodynamic properties including a more efficient crossing of the blood–brain barrier.

In the present work, the cis 3,6-disubstituted piperidine molecule was further converted into a series of more structurally constrained 4,8-disubstituted 1,4-diazabicyclo[3.3.1]nonane derivatives where piperazine and piperidine rings were fused into each other. This new structural motif represents a higher degree of structural organization and can potentially assume a lesser number of structural variability in its lowest energy state compared to the parent compound. Various structural variations on the exocyclic N-atom were explored for preferential binding affinity to the DAT. Binding affinity of the enantiomers was also evaluated to determine any stereoselective preference in binding interaction. Selected molecules with highest potencies for the DAT, both from the 3,6-disubstituted and the 1,4-diazabicyclo[3.3.1]nonane series, were evaluated in animal experiments to evaluate their in vivo activity.

Chemistry

Compound **4**, which provided a structural backbone for construction of a fused bicyclic structure, was synthesized by a six-step process as reported by us before.[17] Thus, the secondary amine in the piperidine ring in compound **4** was alkylated by reacting with ethyl bromoacetate in the presence of a base to give compound **5** in good yield (see Scheme 1). Deacetylation of the exocyclic primary amine under acidic conditions followed by cyclization in the presence of sodium bicarbonate
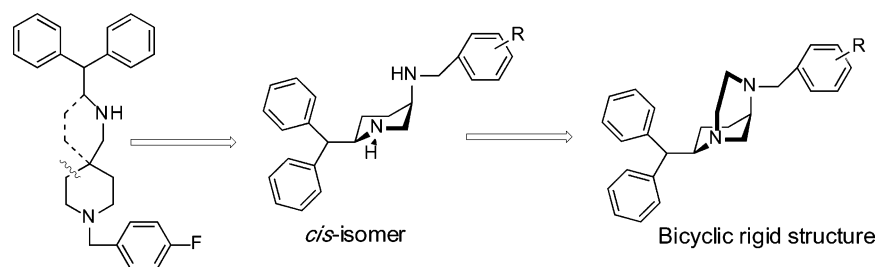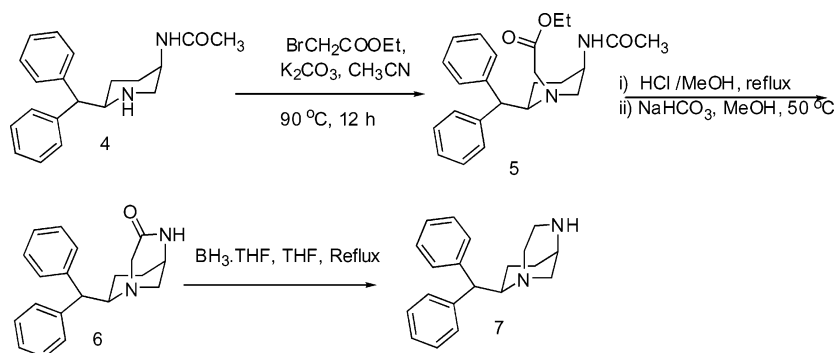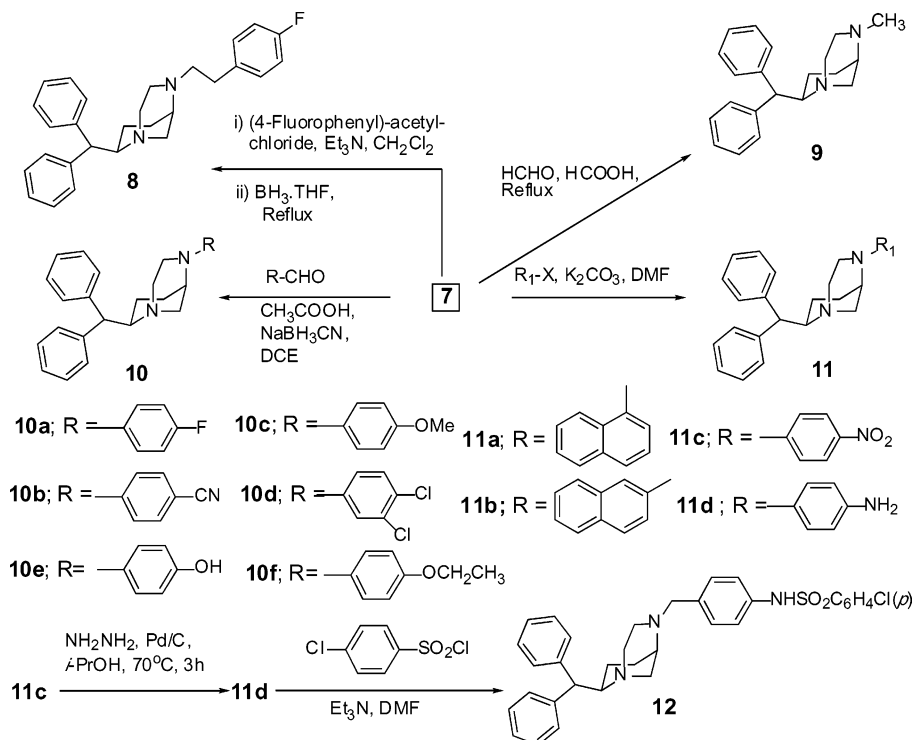

cis-isomer

Bicyclic rigid structure

Figure 2. Evolution of different generation of conformationally constrained structure from flexible piperidine derivative.

Scheme 1

Scheme 2

produced cyclic amide **6**. Reduction of **6** by borane yielded racemic bicyclic diamine **7** which served as a starting precursor for the synthesis of most of our targets.

Thus, the diamine **7** (Scheme 2) was reacted with the appropriate aldehyde under reductive amination conditions to synthesize compounds **10a**–**f**. The intermediate **7** was also reacted with the respective alkyl halide to obtain compounds **11a**–**c**. The aromatic nitro group in **11c** was reduced to obtain amino derivative **11d**, which was next functionalized by reacting with aryl sulfonyl chloride in the presence of a base to obtain **12**. Compound **8** was prepared by reacting **7** with 4-fluorophenylacetyl chloride followed by reduction of intermediate amide by BH_3, while the secondary nitrogen atom in **7** was methylated by reacting it with formaldedye and formic acid to obtain **9**.[29]

Optically active isomers of the most potent methoxy derivative **10c** were synthesized from optically active bicyclic diamine **7** (Scheme 3). Our initial attempt to isolate optically active isomers via preparation of diastereomers with optically active methoxy mandelic acid did not produce good results, as separation of diaster-

eomers turned out to be difficult. Thus, racemic bicyclic diamine **7** was resolved by forming a diastereomeric mixture (**13a** and **13b**) with 1*S*-(−) camphanic chloride followed by hydrolysis of the individual diastereomer to give optically pure bicyclic diamine, (+)-**14a** and (−)-**14b**. Each of these optically pure bicyclic amines was reacted with *p*-anisaldehyde to give (+)-**10c** and (−)-**10c** in good yield. To determine absolute configuration
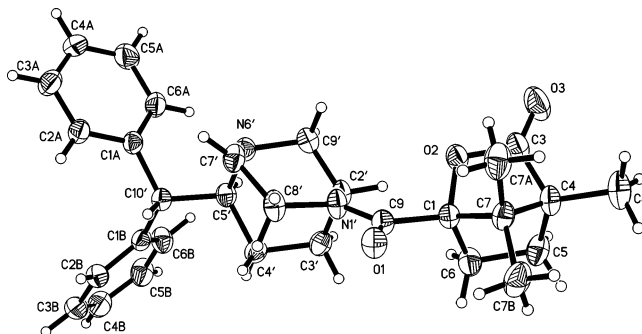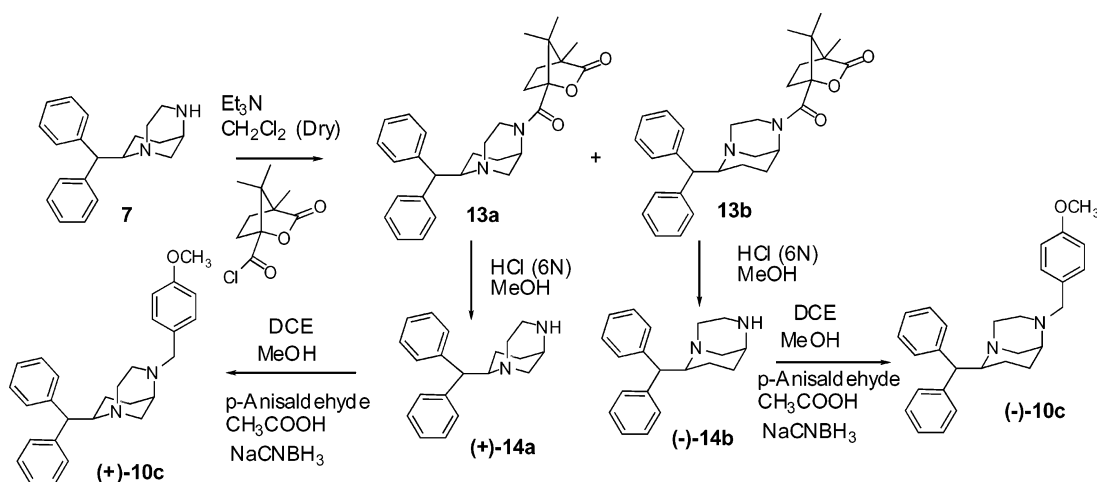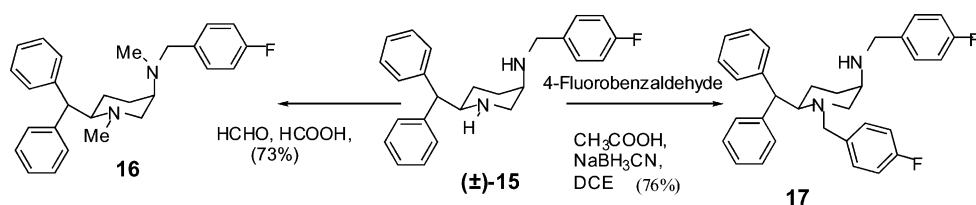

Figure 3. The molecular structure and numbering scheme for compound (−)-**13b** with displacement ellipsoids drawn at the 30% probability level.

Scheme 3

Scheme 4

of the optically pure compounds, compound (−)-**13b** was crystallized for X-ray structure evaluation. The absolute configuration assignment from this X-ray structure determination corresponded to a designation of *S,S* at the 5 and 8 asymmetric carbon centers (see Figure 3). Hence, the compound (−)-**10c** also possesses *S,S*-configuration. Thus, the enantiomer (+)-**10c** must have the *R,R*-configuration. It is evident from the X-ray analysis that the benzhydryl group in compound **13b** is in an equatorial position. The equatorial orientation of the diphenyl group was also confirmed by ^{1}H NMR data; also see Supporting Information.

Syntheses of compounds **16** and **17** were carried out using different routes starting with the compound **15** (Scheme 4), which was synthesized by following our earlier synthetic procedure. Compound **15** underwent methylation on the both N-atoms to produce **16** by treatment with formic acid and formaldehyde in very good yield. Compound **17** was synthesized by reductive amination of **15** with 4-fluorobenzaldehyde in good yield.

Results and Discussion

Our earlier effort to derive structurally constrained derivatives of flexible piperidine analogues of GBR 12909 led us to develop a series of 3,6-disubstituted piperidine derivatives. Our SAR studies were able to demonstrate certain differences in molecular interactions of these molecules with the DAT compared with their flexible counterparts.[17] In this report, we have introduced an additional element of rigidity in the 3,6-disubstituted structures by connecting the two N-atoms by a bis-methylene linker tether. The resulting 1,4-diazabicyclo[3.3.1]nonane derivatives derived from this transformation have a semi-cage-type configuration in which piperidine and piperazine moieties are embedded into each other. In this new bicyclic template, the 4,8-disubstituted 1,4-diazabicyclo[3.3.1]nonane derivatives

have both nitrogen atoms in a tertiary configuration as opposed to the secondary nature of the two N-atoms in 3,6-disubstituted derivatives.

In our initial attempt to decipher possible changes in biological properties as a result of such changes, we focused on modification of substitutions on the secondary nitrogen atom in compound **7**. Derivatization of the basic bicyclic structure **7** with a substituted-benzyl group produced compounds **10a**−**f**, **11d** and **12**. All of these derivatives have either an electron-withdrawing or electron-donating substituents at the 4-position of the aromatic ring with compound **10d** having one additional substituent at the 3-position of the aromatic ring. Derivatives with electron-withdrawing substitutions (F, CN, 3,4 di-Cl) as in the racemic compounds **10a**, **10b**, and **10d** exhibited good binding affinity (81.2, 110, and 210 nM, see Table 1) while derivatives with electron-donating groups showed moderate affinity with the exception of the methoxy derivative, racemic compound **10c** (68.9 nM), which was found to be the most potent racemic derivative in the current series. These results concur with our previous finding with the 3,6-disubstituted derivatives where we observed that either electron-withdrawing or electron releasing group at the 4-position of the exocyclic aromatic ring increases the activity as compared to the unsubstituted aromatic ring. A change in rank order of potency was observed between the current series and the 3,6-disubstituted series of molecules. In the current series of compounds the rank order potency for different aromatic substitutions was found to be 4-OCH$_3$, 4-F > 4-CN > 3,4-di-Cl, 4-OCH$_2$-CH$_3$ whereas in the 3,6-disubstituted compounds the most potent compound was 4-CN substituted derivative.[17] All of the current derivatives were found to have less binding affinity as compared to their more flexible 3,6-disubstituted counterpart with the exception of the methoxy and fluoro derivatives. On the other hand, all

Table 1. Affinity of Drugs at the Dopamine, Serotonin, and Norepinephrine Transporters in Rat Brain Tissue

compd	DAT binding, IC$_{50}$, nM, [^{3}H]WIN 35, 428[a]	SERT binding, IC$_{50}$, nM, [^{3}H]citalopram[a]	NET binding, IC$_{50}$, nM, [^{3}H]nisoxetine[a]	DAT uptake, IC$_{50}$ nM, [^{3}H]DA [a]	ClogP[c]
cocaine	266 ± 37[b]	737 ± 160	3530 ± 550		2.57
GBR 12909	10.6 ± 1.9[b]	132 ± 0	496 ± 22	6.63 ± 0.43	5.37
1	19.7 ± 1.4[b]	137 ± 46	1110 ± 120	49.6 ± 7.2	5.62
(−)-*S,S*-**2**[b]	11.3 ± 0.9	434 ± 27	1670 ± 90	9.10 ± 1.86	4.87
3[b]	47.5 ± 6.2	1040 ± 110	1110 ± 60	40.5 ± 21.8	
8	565 ± 22	2550 ± 700	>10000		
9	4710 ± 830	157000 ± 5000	60100 ± 19800		
10a	81.2 ± 11.9	8860 ± 730	56400 ±5,000	56.9 ± 14.7	
10b	110 ± 14	3500 ± 1000	>10000		
10c	68.9 ± 7.3	5830 ± 170	>10000	57.2 ± 16.4	
(+)-*R,R*-**10c**	329 ± 30	6630 ± 1130	>10000	269 ± 4	
(−)-*S,S*-**10c**	22.5 ± 2.1	8650 ± 830	>10000	18.4 ± 0.9	6.12
10d	210 ± 39	2340 ± 270	8030 ± 1540		
10e	581 ± 46	8320 ± 1120	>10000	357 ± 35	
10f	215 ± 16	12200 ± 200	>10000	138 ± 20	
11a	958 ± 46	1890 ± 710	1080 ± 660		
11b	308 ± 13	672 ± 92	5790 ± 1210		
11d	1400 ± 60	>10000	>10000	809 ± 35	
12	371 ± 1	4130 ± 490	3480 ± 660		
(−)-*S,S*-**15**[b]	33.8 ± 5	1330 ± 120	1420 ± 560	53.8 ± 7.4	
16	225 ± 10	2360 ± 200	7000 ± 1,320		
17	1240 ± 60	18900 ± 900	12600 ± 2230		

[a] For binding, the DAT was labeled with [^{3}H]WIN35,428, the SERT with [^{3}H]citalopram and the NET with [^{3}H]nisoxetine. For uptake by DAT, [^{3}H]DA accumulation was measured. Results are average ± SEM of three to eight independent experiments assayed in triplicate. [b] See ref 17. [c] Value calculated based on a program developed by BioByte Corp. CA.

Table 2. Selectivity of Various Ligands for Their Activity at Monoamine Transporter

compound	SERT binding/ DAT binding	NET binding/ DAT binding	[^{3}H]DA uptake/ DAT binding
GBR 12909	12	47	0.62
2	38	148	0.80
3	22	23	0.85
8	4	>18	
9	33	13	
10a	109	695	0.7
10b	32	>91	
10c	85	>145	0.8
(+)-**10c**	20	>30	0.8
(−)-**10c**	384	>444	0.8
10d	11	38	
10e	14	>17	0.6
11a	2	1	
11b	2	19	
11d	>7	>7	0.6
12	11	9	
16	10	31	
17	10	12	

of the current more constrained derivatives exhibited more selectivity compared to 3,6-disubstituted derivatives for binding to the DAT. Thus, the racemic methoxy derivative **10c** exhibited higher selectivity for the DAT compared to its more flexible 3,6-disubstituted counterpart compound **3** (SERT/DAT = 85 and NET/DAT> 145 vs SERT/DAT = 22 and NET/DAT = 23, see Table 2).[17] It is evident that this increase in selectivity is due to the introduction of an additional rigidity in the bicyclic template which selectively reduced affinity for the SERT and NET while maintaining its activity for the DAT. Furthermore, due to introduction of rigidity, this new template is less flexible in accommodating different substitutions at the exocyclic N-benzylic ring for generation of activity indicating selective discrimination at the receptor recognition sites. Thus, for compounds with different substitutions at the 4-position of the phenyl group, the binding affinity in the flexible 3,6-disubstituted piperidine derivatives ranged from 32

to 114 nM, while for 4,8-disubstituted 1,4-diazabicyclo-[3.3.1]nonane derivatives the binding affinity ranged from 22 to 1400 nM. Replacement of the methoxy group by a hydroxy functionality in compound **10e** reduced the affinity for DAT by almost 8-fold and profoundly decreased the affinity for NET (IC$_{50}$ >10 000 nM). Interestingly, this observation was in contrast to our previous results with the corresponding 3,6-disubstituted hydroxy derivative where we observed that the such transformation from a methoxy to hydroxy group increased affinity for the NET (IC$_{50}$ went from 1110 to 201 nM).[23] Compound **11d** (IC$_{50}$ = 1400 nM) with the electron-releasing amino group attached at the 4-position was almost inactive at all three transporters. However, when the amino group was transformed into an electron-withdrawing sulfone moiety as depicted in compound **12**, moderate potency was restored.

Replacement of the benzylic group by naphthalene moieties reduced the affinity for the DAT. However, the two isomeric naphthalene derivatives showed differential activity with 2-naphthyl derivative **11b** being more potent for the DAT than the 1-naphthyl derivative **11a** (308 vs 958 nM). Interestingly, the 2-naphthyl derivative exhibited reduced selectivity for the DAT as it possessed substantial affinity for the serotonin transporter (IC$_{50}$ = 672 nM). In fact, this compound had the highest affinity for the SERT in the current series of derivatives. In this regard, previous SAR study in the tropane- and piperidine-based cocaine analogues demonstrated much more profound effect of introduction of a naphthyl group in SERT affinity.[30,31] A drop in affinity for the DAT by almost 7-fold was observed in compound **8** where 4-fluorobenzyl group was replaced by a 4-fluorophenethyl group (565 vs 81.2 nM). This observation is in agreement with our previous studies with the flexible 3,6-disubstituted derivatives. Compound **9** was found to be inactive at all the transporters indicating the importance of the presence of hydrophobic aromatic moiety in binding interaction.
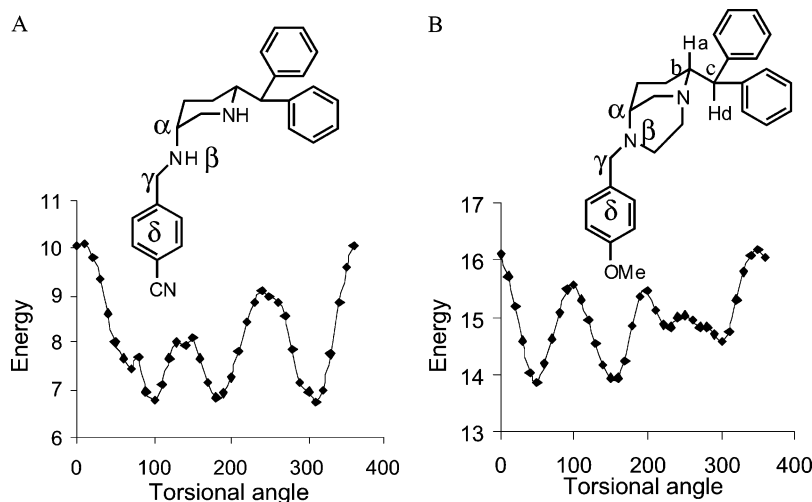
Figure 4. A. Energy Profile for every 10° change in torsional angle ($\alpha-\beta-\gamma-\delta$) for 3,6-disubstituted piperidine derivative (−)-**2**. B. Energy profile for every 10° change in torsional angle ($\alpha-\beta-\gamma-\delta$) for 4,8-disubstituted 1,4-di-azabicyclco[3.3.1]nonane derivative (−)-**10c**.

Compounds **16** and **17** were synthesized to observe the influence of the tertiary N-atoms in the absence of an additional cyclic piperazine ring system fused into the piperidine moiety as in the bicyclic derivatives. Compound **16** can be considered as an acyclic version of **10a**. Binding results (see Table 1) indicated weak activity in both **16** and **17** indicating the importance of structural rigidity in the interactions with DAT.

The most potent methoxy analogue (±)-**10c** was resolved into enantiomers and one of the enantiomers (−)-*S,S*-**10c** was found to be 15-fold more potent at DAT compared to (+)-*R,R*-**10c** (22.5 vs 329 nM, see Table 1). Thus, the current 1,4-diazabicyclo[3.3.1]nonane template exhibited the same stereoselectivity in terms of potency at DAT as its 3,6-disubstituted counterparts. However, the most potent enantiomer (−)-*S,S*-**10c** was much more selective for the DAT compared to the most potent and selective enantiomer of the 3,6-disubstituted version (−)-**2** (SERT/DAT= 384 vs 38 and NET/DAT = >444 vs 148, see Table 2). Moreover, (−)-**10c** exhibited comparable potency to GBR 12909 but was much more selective for the DAT than GBR 12909. Thus, by introducing an additional element of rigidity we have obtained derivatives which are much more selective in binding to the DAT while maintaining the affinity for DAT.

Molecular Modeling. Bioactive conformational structures of the present constrained derivatives were explored in a brief molecular modeling study. Since the three-dimensional structure of DAT is not known, it is currently impossible to elucidate the bioactive conformation of a ligand bound to the DAT. One theoretical solution to this problem was to develop sufficiently structurally constrained active molecules so as to "freeze" conformational flexibility to a minimum degree which can then be used as a template for determination of bioactive conformation for lesser constrained version of molecules belonging to the same subset of structures. Our current series of rigid bicyclic molecules were used as such a template for this purpose. Molecular minimization was performed (see details in the Experimental Section) by using SYBYL molecular modeling program (version 6.9, 2002, Tripos Associates, Inc., St. Louis, MO). X-ray structure and NMR data demonstrated that
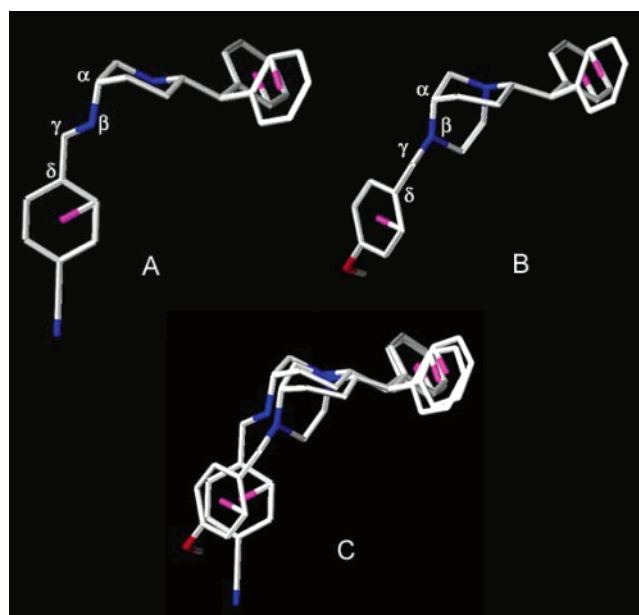

Figure 5. A. Conformational structures of 3,6-disubstituted piperidine compound (−)-**2** where torsional angle $\alpha-\beta-\gamma-\delta$ is 180°. B. Conformational structure of 4,8-disubstituted 1,4-diazabicylco[3.3.1]nonane series where torsional angle $\alpha-\beta-\gamma-\delta$ is 150°. C. Overlap of A and B with RMS value of 0.59. Hydrogen atoms are eliminated from the figure for simplicity.

the diphenyl benzyl group in (−)-**10c** is in an equatorial position with the torsional angle for Ha−Cb−Cc−Hd close to 180° as shown in the Figure 4B. Both the compounds (−)-**10c** and (−)-**2** were initially minimized by using SYBYL. The minimized structure of (−)-**10c** was further subjected to the grid scan conformational search procedure with the torsional angle corresponding to Cα−Nβ−Cγ−Cδ (Figure 5B) which resulted in generation of conformations within a narrow band of energy range (13.86 to 16.18 kcal/mol, Figure 4B) indicating fewer degree of conformational freedom. The corresponding plot of energy vs torsional angle produced two almost equivalent local energy minima (13.86 and 13.93 kcal/mol, Figure 4B); either one of them may potentially represent a structure of a probable bioactive conformer. Similarly, compound (−)-**2** was subjected to the identical minimization procedure by varying the same torsional

angle $\varphi1$ ($\alpha-\beta-\gamma-\delta$) which produced three local minima
with energy levels very closed to each other (Figure 4A).
In the next step, we examined each one of the two local
minima conformers of (−)-**10c** with the corresponding
local minima conformations of (−)-**2** by a multifit
alignment operational procedure option in the SYBYL
program. The goal was to find one of the local minima
conformers of (−)-**10c** producing the lowest rms value,
indicating a good fit, with one of the three structures of
(−)-**2**. It turned out that the conformer of (−)-**10c** with
a corresponding torsional angle of 150° produced the
best fit with the 3,6-disubstituted conformer having the
corresponding torsional angle 180° (Figure 5c). The
corresponding conformers of these two structures are
shown as A and B in Figure 5. The lowest rms value in
multifit operation indicated the best spatial fit occurring
between these two conformers. Thus, we propose that
the 3,6-disubstituted conformer B shown in Figure 5 is
a possible bioactive conformer based on the above
analysis involving a rigid template analogue. It is
important to mention here that we have carried out
similar studies with (−)-*S,S*-**15** which yielded an identi-
cal bioactive conformational structure for (−)-**15** as
discussed above for (−)-**2**. However, the energy profile
with the change of torsional angle for (−)-**15** produced
two local minima instead of three as was the case for
(−)-**2**. The results for (−)-**15** are detailed in the Sup-
porting Information section.

In Vivo Studies. Following characterization of in
vitro transporter activities, we subjected compounds (−)-
S,S-**2** and (−)-*S,S*-**10c** to in vivo locomotor and drug
discrimination studies.

**Effects of Cocaine, (−)-S,S-2, and (−)-S,S-10c on
Locomotor Activity.** Figure 6 shows the time-course
effects of cocaine, (−)-**2**, and (−)-**10c** during the first 60
min of testing as well as the summed effects across the
remaining 3 h. The cocaine data have been presented
previously[32] and are included for comparison purposes
(Figure 6A). Doses of 10 and 30 mg/kg cocaine produced
peak effects on activity during the first 10 min following
administration with the levels of activity declining by
60 min and remaining low for the remaining 3 h of
testing. Doses of 10 and 30 mg/kg (−)-**2** produced
sustained levels of increased activity beginning 30 min
postadministration and significantly increased total
distance traveled across the remaining 3 h ($F(5,42) =$
11.43, $P \leq 0.05$) (Figure 6B). Doses of 30 and 56 mg/kg
(−)-**10c** produced peak effects 10 min postadministra-
tion, whereas only a dose of 56 mg/kg significantly
increased activity across the remaining 3 h of the
experimental session ($F(3,28) = 8.23$, $P \leq 0.05$) (Figure
6C).

Figure 7 shows the effects of cocaine, (−)-**2** and (−)-
10c at each dose when averaged over the entire 4-hour
test session. The cocaine data have been presented
previously and are included for comparison purposes.[32]
Briefly, cocaine dose-dependently increased activity with
a dose 30 mg/kg producing a significant increase relative
to vehicle levels of activity. (−)-**2** produced a biphasic
effect on activity. There was a main effect of dose for
(−)-**2** ($F(5,42) = 10.31$, $P \leq 0.05$) such that doses of 10
and 30 mg/kg increased total distance traveled relative
to vehicle control, and a dose of 56 mg/kg produced
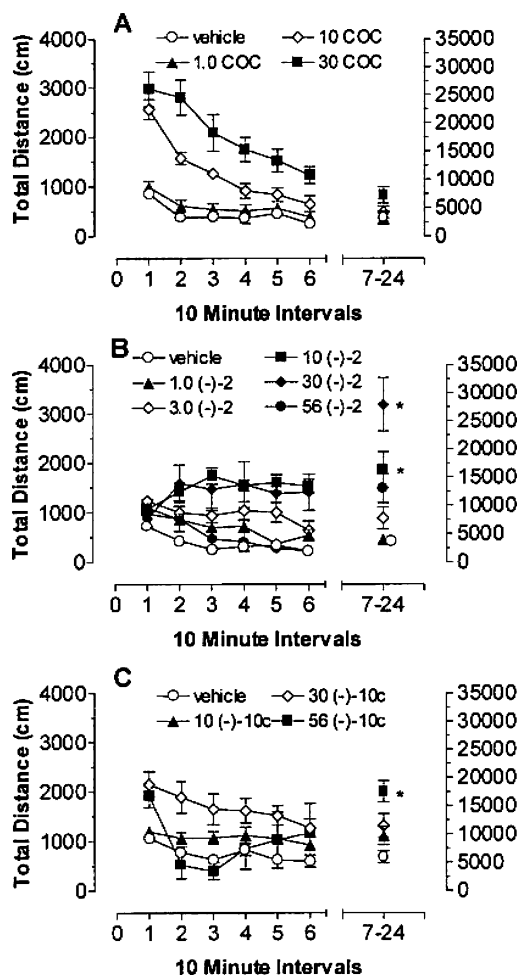activity levels similar to that of vehicle. There was a

Figure 6. Effects of cocaine, (−)-**2**, and (−)-**10c** on total
distance traveled (cm) across the first 60 min of the test session
as well as their effects when the data were summed across
the remaining 3 h of the test session (intervals 7−24). The
cocaine data are from ref 29 and are shown for comparison
purposes. * Indicates significant differences compared to
vehicle control based on Dunnett's posthoc tests.

Figure 7. Effects of cocaine, (−)-**2**, and (−)-**10c** on total
distance traveled (cm) when the data were summed across the
entire 4 h test session. The cocaine curve is from ref 29 and is
shown for comparison purposes. * Indicates significant differ-
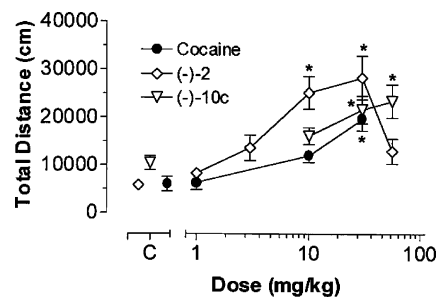ences compared to vehicle control based on Dunnett's posthoc
tests.

main effect of dose for (−)-**10c** ($F(3,28) = 5.31$, $P \leq 0.05$)
such that doses of 30 and 56 mg/kg increased activity
relative to vehicle control levels.

**Effects of Cocaine, (−)-2, and (−)-10C in Cocaine
Discrimination Tests.** As shown in Figure 8, cocaine
produced dose-dependent increases in cocaine lever
responding. The ED_{50} value (mg/kg) of cocaine for the
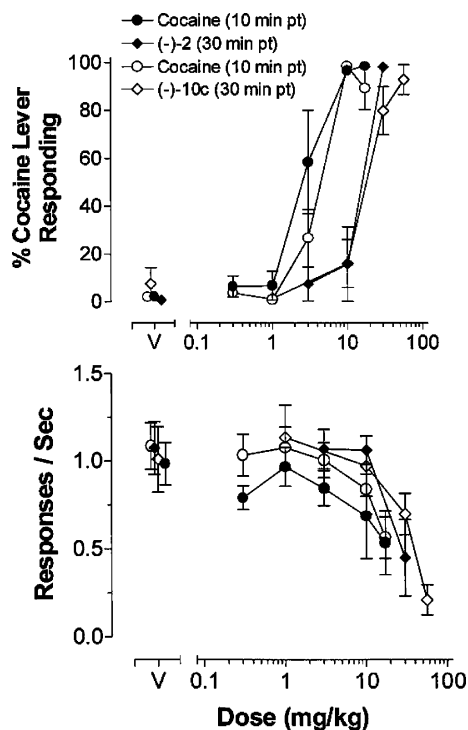mice tested with (−)-**2** was 3.00 (95% CL: 2.30−3.93),

Figure 8. Effects of cocaine (10 min pretreatment interval (PT), (−)-**2** (30 min PT), and (−)-**10c** (30 min PT) on the percentage of cocaine-lever responding (top panel) and rate of responding (bottom panel) in rats trained to discriminate 10 mg/kg cocaine from saline. Cocaine was tested in five and eleven mice in the (−)-**2** and (−)-**10c** groups, respectively. (−)-**2** and (−)-**10c** were tested in six and eleven mice, respectively. Filled and empty circles for cocaine tests correspond to tests in (−)-**2** and (−)-**10c** tested mice, respectively. The data points above "V" indicate the mean percentage of cocaine-lever responding and rate of responding following the administration of the respective vehicles.

and the ED_{50} value of cocaine for the mice tested with (−)-**10c** was 3.99 (3.03−5.26). In both groups of mice a dose of 17 mg/kg cocaine decreased rates of responding by 50% relative to saline levels. (−)-**2** produced dose-dependent increases in cocaine-lever responding with an ED_{50} value of 13.90 (6.64−29.12). At a dose of 30 mg/ kg (−)-**2** responding was eliminated in one of six mice tested. (−)-**10c** produced dose-dependent increases in cocaine-lever responding with an ED_{50} value of 18.23 (13.99−23.75). At a dose of 56 mg/kg (−)-**10c** responding was eliminated in five of ten mice tested.

It is important to note that as we moved from flexible molecule to constrained structures, we observed a gradual increase of in vivo activity. The parent flexible piperidine analogue **1** (Figure 1) and structurally similar compounds showed hardly any activity in locomotor and drug discrimination studies which may be due to poor blood−brain barrier crossing ability.[15] In this regard, the poor blood−brain barrier crossing ability of GBR and their related piperidine derivatives was attributed to their high lipophilicity (high ClogP). Interestingly, the present results indicate that rigidification of the parent structure **1** (Figure 1) into compounds (−)-**2** and (−)-**10c** preserves in vitro affinity for the DAT (Table 1) but markedly increases central activity; it is tempting to speculate that the more rigid compounds (−)-**2** and (−)-**10c** could be crossing the blood−brain barrier more efficiently. This could be explained, in part, by lipophi-

licity (ClogP), as the structurally constrained 3,6-disubstituted derivative (−)-**2** exhibits lower lipophilicity compared to the parent **1** (see Table 1). However, the ClogP value alone cannot explain the fast onset of action, almost comparable to cocaine, of (−)-**10c** which has higher ClogP value compared to both parent **1** and the 3,6-disubstituted version (−)-**2**. As mentioned earlier, there are other factors besides lipophilicity that control overall bioavailability and blood−brain penetration of a drug molecule.[25−28] Therefore, the pharmacokinetic and pharmacodynamic properties of the current compounds might be due to the combination of change of polarity, molecular shape, and other properties associated with these constrained molecules. Future studies with a larger set of training compounds will help to establish such relationships.

Conclusion

In this report, we describe a novel series of structurally constrained 1,4-diazabicyclo[3.3.1]nonane derivatives. 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. However, the current more rigid compounds exhibit greater selectivity for the DAT indicating enhanced effect of rigidity on selectivity. The bioactive conformation of the constrained piperidine derivative was explored by using compound (−)-**10c** as a template. Systematic molecular minimization with this template and with a corresponding lead 3,6-disubstituted version (−)-**2** followed by multifit alignment procedure enabled us to elucidate potential bioactive conformations of (−)-**2**. In vivo locomotor activity study was carried out with (−)-**2** and (−)-**10c** with both the compounds exhibiting locomotor stimulatory effects. In this study, compound (−)-**2** was slower acting with a longer duration of action compared to (−)-**10c**. In this regard, (−)-**10c** displayed fast onset of action as does cocaine even though it was less potent than cocaine in its initial action. However, (−)-**2** and (−)-**10c** were significantly longer acting than cocaine. In the cocaine discrimination test, both compounds within the dose range tested occasioned complete (≥80%) cocaine-like responding in mice trained to discriminate 10 mg/kg ip cocaine from vehicle.

Experimental Section

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. [1H] NMR spectra were routinely obtained on a Varian 400 MHz FT NMR. The NMR solvent used was $CDCl_3$ 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.

[^{3}H]WIN 35,428 (86.0 Ci/mmol), [^{3}H]nisoxetine (80.0 Ci/ mmol), and [^{3}H]dopamine (48.2 Ci/mmol) were obtained from Dupont-New England Nuclear (Boston, MA). [^{3}H]Citalopram (85.0 Ci/mmol) was from Amersham Pharmacia Biotech Inc. (Piscataway, NJ). Cocaine hydrochloride was purchased from Mallinckrodt Chemical Corp. (St. Louis, MO). WIN 35,428 naphthalene sulfonate was purchased from Research Bio-chemicals, Inc. (Natick, MA). (−)-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).

Molecular Modeling. Molecular modeling investigation was performed by using the SYBYL molecular modeling package (version 6.9, 2002, Tripos Associates, Inc., St. Louis, MO). The most active enantiomer (*SS*) was used for the molecular modeling studies. Molecular mechanics minimizations were performed using the Tripos force field and the Powell minimizer, with 1000 iterations or until convergence, defined as energy gradient of 0.005 kcal/mol or less. The Gastiger−Huckel method was used to calculate the partial charge distribution of the molecules. Electrostatic interactions were taken into consideration by using a distance dependent dielectric function. Grid scan conformational analysis was performed with minimized structure of (−)-**2** by varying torsional angle $\varphi1$ ($\alpha-\beta-\gamma-\delta$) by 10° (see Figure 4A). These conformations generated have energy ranging from 6.75 to 10.10 kcal/mol. A graph of energy vs torsional angle showed three minimum energy conformations where one of them has torsional angle $\varphi1$ 180° while the other two have a torsional angles $\varphi1$ of 100° and 310°. Any one of these three local minimum conformations can potentially represent a bioactive conformation since the energy differences among these three conformations are small. A similar grid scan conformational analysis was performed with (−) *SS* isomer of compound **10c** to generate conformations where energy ranged from (13.86−16.18 kcal/mol, see Figure 4B). A graph of energy vs torsional angle showed two minimum energy conformations when one of them has a torsional angle $\varphi1$ of 50° (13.86 kcal/mol), while the other one has a torsional angle $\varphi1$ of 150° (13.93 kcal/mol), Figure 4B. These two conformations were superimposed one at a time with the three local minimum energy conformations of (−)-**2**. Three points based on centroids of the respective three phenyl rings and the fourth point as the exocyclic nitrogen atom were selected to perform the fit atom program in SYBYL and to obtain RMS value as a measure of extent of overlap between the two structures. The best RMS value (0.59) was obtained for the overlap in which the torsional angle $\varphi1$ was 180° for compound (−)-**2** (Figure 4 A) while torsional angle $\varphi1$ was 150° for compound (−)-**10c** (Figure 4B).

Synthesis of (5-Acetylamino-2-benzhydrylpiperidin-1-yl)acetic Acid Ethyl Ester (5). A solution of *N*-(6-benzhydrylpiperidin-3-yl)acetamide **4** (0.50 g, 1.62 mmol), bromoethyl acetate (0.32 g, 1.94 mmol), and K_2CO_3 (0.51 g, 4.86 mmol) in dry acetonitrile (25 mL) was refluxed for overnight under nitrogen atmosphere. The solvent was removed under reduced pressure. The residue was partitioned between water and ethyl acetate. The organic layer was collected, and the water layer was extracted with additional ethyl acetate. The combined organic phase was dried over Na_2SO_4 and evaporated to give a crude product, which was purified by column chromatography (EtOAc/hexane = 4/1) to give **5**, as colorless oil: 0.51 g (80% yield). ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.18 (3H, t, $J = 7.4$ Hz, CH$_2$C*H$_3$*), 1.31−1.58 (3H, m, H-5, H-4$_{ax}$), 1.69−1.81 (1H, m, H-4$_{eq}$), 1.99 (3H, s, CH$_3$CO), 2.67 (1H, dd, $J = 4.8$ Hz, $J = 12$ Hz, H-2$_{ax}$), 2.93 (1H, dd, $J = 2.4$ Hz, $J = 12.4$ Hz, H-2$_{eq}$), 3.23−3.33 (2H, m, CH$_2$CO), 3.53 (1H, dt, $J = 3.2$ Hz, $J = 8$ Hz, C*H*CH(Ph)$_2$), 3.96−4.07 (3H, m, H-3, OC*H$_2$*CH$_3$), 4.23 (1H, d, $J = 8.4$ Hz, CH(Ph)$_2$), 6.26 (1H, bd, $J = 7.2$ Hz, NHCO), 7.14−7.19 (2H, m, ArH), 7.22−7.30 (8H, m, ArH).

Synthesis of 8-Benzhydryl-1,4-diaza-bicyclo[3.3.1]nonan-3-one (6). (5-Acetylamino-2-benzhydrylpiperidin-1-yl)-acetic acid ethyl ester **5** (0.3 g, 0.76 mmol) was dissolved in 2 N HCl/MeOH (15 mL), and the solution was refluxed for overnight. Methanol was then evaporated and the residue was neutralized using saturated NaHCO$_3$ solution. It was then extracted with dichloromethane (3×). The organic extracts were pooled, washed with brine, and dried over Na$_2$SO$_4$. The solvent was removed under vacuo to give crude product. Into the solution of crude in dry MeOH was added solid NaHCO$_3$ (0.1 g), and it was stirred for overnight at 50 °C under nitrogen atmosphere. Methanol was removed under reduced pressure. The residue was partitioned between water and ethyl acetate. Organic layer was collected, and the water layer was extracted

with additional ethyl acetate. The combined organic phase was dried (Na$_2$SO$_4$) and evaporated to give a crude product which was purified by column chromatography (EtOAc/MeOH = 8/1) to give **6**, as a while solid, mp: 294−295 °C: 0.18 g (77% yield). ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.31−1.40 (1H, m, H-7$_{ax}$), 1.39−1.55 (1H, m, H-7$_{eq}$), 1.70−1.84 (2H, m, H-6), 3.03 (1H, dd, $J = 1.6$ Hz, $J = 13.6$ Hz, H-9$_{ax}$), 3.22−3.30 (2H, m, H-9$_{eq}$, CH$_2$-CO), 3.41−3.51(2H, m, H-5, CH$_2$CO), 3.60 (1H, dt, $J = 3.2$ Hz, $J = 11.2$ Hz, C*H*CH(Ph)$_2$), 3.86 (1H, d, $J = 11.6$ Hz, CH(Ph)$_2$), 6.11 (1H, bs, NH), 7.11−7.18 (2H, m), 7.20−7.34 (8H, m, ArH).

Synthesis of 8-Benzhydryl-1,4-diazabicyclo[3.3.1]nonane (7). Into a stirred solution of 8-benzhydryl-1,4-diazabicyclo[3.3.1]nonan-3-one **6** (0.18 g, 0.58 mmol) in 5 mL of dry THF was added 1 M BH$_3$/THF (1.1 mL, 1.1 mmol). The reaction mixture was refluxed for 6 h. After the solution was cooled to room temperature, methanol (2 mL) was added slowly. The solvent was removed under reduced pressure, 10% HCl/MeOH (5 mL) was added into the residue, and the solution was next refluxed for 1 h. Solid NaHCO$_3$ was added, and methanol was removed in vacuo. The residue was partitioned between water and ethyl acetate. The organic layer was collected, and the water layer was extracted with additional ethyl acetate (3×). The combined organic phase was dried over Na$_2$SO$_4$ and evaporated to give crude product, which was purified by flash chromatography (EtOAc/MeOH/Et$_3$N = 7/7/1) to give **7** as a colorless oil: 0.12 g (70% yield). ^{1}H NMR (CDCl$_3$/D$_2$O, 300 MHz) δ 1.33−1.44 (1H, m, H-7$_{ax}$), 1.60−1.77 (1H, m, H-7$_{eq}$), 1.82−2.04 (2H, m, H-6), 2.62−2.73 (2H, m, H-5, NCH$_2$CH$_2$N), 2.82−2.97 (2H, m, H-9ax, NCH$_2$CH$_2$N), 3.02−3.19 (1H, m, −NCH$_2$CH$_2$N-), 3.21−3.30 (1H, m, H-9$_{eq}$), 3.41 (1H, dt, $J = 4.8$ Hz, $J = 12.3$ Hz, NCH$_2$CH$_2$N), 3.80 (1H, dt, $J = 4.8$ Hz, $J = 11.2$ Hz C*H*CH(Ph)$_2$), 3.90 (1H, d, $J = 11.2$ Hz, CH(Ph)$_2$), 7.11−7.18 (2H, m, ArH), 7.20−7.30 (6H, m, ArH), 7.33−7.40 (2H, m, ArH).

Synthesis of 8-Benzhydryl-4-[2-(4-fluorophenyl)ethyl]-1,4-diazabicyclo[3.3.1]nonane (8). Into a stirred solution of 8-benzhydryl-1,4-diazabicyclo[3.3.1]nonane **7** (0.06 g, 0.22 mmol) and Et$_3$N (0.04 g, 0.44 mmol) in 10 mL of dry CH$_2$Cl$_2$ under nitrogen at 0 °C was added a solution of 4-fluorophenyl)-acetyl chloride (0.05 g, 0.03 mmol) dissolved in CH$_2$Cl$_2$. The temperature was allowed to rise to RT, and the mixture was stirred for 3 h and then washed with water. The aqueous layer was extracted with dichloromethane (3×). The combined organic phase was washed with brine and dried over Na$_2$SO$_4$. Solvent was then evaporated under vacuo to give a crude product which was reacted with 1 M BH$_3$/THF (1.1 mL, 1.1 mmol) in dry THF to give a crude product which was purified by flash chromatography (ethyl acetate/hexane = 5/1) to give **8** as a colorless oil, 0.052 g (53% yield). ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.23−1.32 (1H, m, H-7$_{ax}$), 1.33−1.45 (1H, m, H-7$_{eq}$), 1.46−1.60 (1H, m, H-6$_{ax}$), 2.14−2.24 (1H, m, H-6$_{eq}$), 2.57 (1H, bm, H-5), 2.63−2.89 (6H, m, NCH$_2$CH$_2$N, CH$_2$CH$_2$Ar), 2.90−3.05 (2H, m, NCH$_2$CH$_2$N, H-9$_{ax}$), 3.15 (1H, bd, $J = 14.4$ Hz, NCH$_2$CH$_2$N), 3.21−3.30 (1H, m, H-9$_{eq}$), 3.78 (1H, dt, $J = 4$, 11.6 Hz, C*H*CH(Ph)$_2$), 3.90 (1H, d, $J = 11.6$ Hz, CH(Ph)$_2$), 6.92−6.99 (2H, m, ArH), 7.10−7.19 (4H, m, ArH), 7.20−7.31 (6H, m, ArH), 7.38 (2H, d, $J = 7.2$ Hz, ArH). Free base was converted into its hydrochloride salt, mp 275−279 °C. Anal. [C$_{28}$H$_{31}$FN$_2$·2HCl·1.5H$_2$O] C, H, N.

Synthesis of 8-Benzhydryl-4-methyl-1,4-diazabicyclo-[3.3.1]nonane (9). 8-Benzhydryl-1,4-diazabicyclo[3.3.1]nonane **7** (0.05 g, 0.19 mmol) was refluxed with formaldehyde (1.0 g, 37%/H$_2$O) and formic acid (2.0 g, 88%/H$_2$O) for 3 h, and the excess reagent was removed under reduced pressure. The residue was partitioned between saturated solution of NaHCO$_3$ and ethyl acetate. The organic layer was collected, and the water layer was extracted with ethyl acetate (3×). The crude product was purified by flash chromatography (EtOAc/MeOH/Et$_3$N = 7/7/0.5) to give **9**, as a colorless oil, 0.05 g (87% yield). ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.23−1.40 (2H, m, H-7), 1.45−1.62 (1H, m, H-6$_{ax}$), 2.18−2.28 (1H, m, H-6$_{eq}$), 2.41 (3H, s, CH$_3$), 2.45−2.52 (1H, bm, H-5), 2.60 (1H, m, NCH$_2$CH$_2$N), 2.77−2.88 (1H, m, NCH$_2$CH$_2$N), 2.90−3.01 (1H, m, NCH$_2$CH$_2$N), 3.03 (1H, bd, $J = 12.8$ Hz, H-9$_{ax}$), 3.08−3.17 (1H, m, NCH$_2$CH$_2$N),

3.29 (1H, bd, J = 13.6 Hz, H-9$_{eq}$), 3,78 (1H, dt, J = 4.8 Hz, J = 11.2 Hz, CHCH(Ph)$_2$), 3.88 (1H, d, J = 11.2 Hz, CH(Ph)$_2$), 7.11−7.17 (2H, m, ArH), 7.19−7.30 (6H, m, ArH), 7.36 (2H, d, J = 8 Hz, ArH). Free base was converted into its hydrochloride salt, mp 258−262 °C. Anal. [C$_{21}$H$_{26}$N$_2$·2HCl·1.3H$_2$O] C, H, N.

Procedure A. Synthesis of 8-Benzhydryl-4-(4-fluorobenzyl)-1,4-diazabicyclo[3.3.1]nonane (10a). 8-Benzhydryl-1,4-diazabicyclo[3.3.1]nonane **7** (0.05 g, 0.16 mmol) was dissolved in 1,2 dichloroethane (10 mL), and into it was added *p*-fluorobenzaldehyde (0.03 g, 0.22 mmol) followed by acetic acid (0.01 g, 0.22 mmol). After 15−20 min of stirring under N$_2$, sodium cyanoborohydride (0.02 g, 0.28 mmol) was added followed by 1 mL of methanol. The mixture was stirred for 3−4 h. Into the solution was added 3−4 mL of water followed by few drops of HCl. Excess HCl was neutralized with NaHCO$_3$, and the mixture was extracted with CH$_2$Cl$_2$ (3×). All extracts were pooled, washed with brine, and dried over Na$_2$SO$_4$, and the solvent was evaporated under vacuo to give crude product which was purified by flash chromatography over a silica gel column using hexane:EtOAc (1/3) to yield **10a**, 0.03 g, (45% yield) as a colorless oil. ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.28−1.33 (1H, m, H-7$_{ax}$), 1.39−1.52 (2H, m, H-6$_{ax}$, H-7$_{eq}$), 2.20−2.28 (1H, m, H-6$_{eq}$), 2.41−2.53 (2H, m, H-5, NCH$_2$CH$_2$N), 2.73−2.94 (2H, m, NCH$_2$CH$_2$N), 2.97 (1H, bd, J = 12.8 Hz, H-9), 3.06−3.14 (1H, m, NCH$_2$CH$_2$N), 3.20−3.27 (1H, m, H-9$_{eq}$), 3.53−3.68 (2H, m, CH$_2$Ar), 3.78 (1H, dt, J = 4.8 Hz, J = 11.2 Hz, CHCH(Ph)$_2$), 3.90 (1H, d, J = 11.2 Hz, CH(Ph)$_2$), 6.97 (2H, t, J = 8.4 Hz, ArH *ortho* to F), 7.11−7.17 (2H, m, ArH) 7.21−7.31 (8H, m, ArH), 7.35−7.39 (2H, m, ArH). Free base was converted into its hydrochloride salt, mp 240−242 °C. Anal. [C$_{27}$H$_{29}$FN$_2$·2HCl·0.7H$_2$O] C, H, N.

Synthesis of 4-(8-Benzhydryl-1,4-diazabicyclo[3.3.1]non-4-ylmethyl)-benzonitrile (10b). 8-Benzhydryl-1,4-diazabicyclo[3.3.1]nonane **7** (0.05 g, 0.17 mmol) was reacted with 4-formylbenzonitrile (0.03 g, 0.25 mmol), acetic acid (0.02 g, 0.25 mmol), and NaBH$_3$CN (0.02 g, 0.30 mmol) in CH$_2$ClCH$_2$-Cl to give a crude product which was purified by flash chromatography (EtOAc/hexane = 3/1) to produce **10b**, 0.04 g (57% yield) as colorless oil (Procedure A). ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.32−1.41 (1H, m, H-7$_{ax}$), 1.42−1.58 (2H, m, H-6$_{ax}$, H-7$_{eq}$), 2.14−2.26 (1H, m, H-6$_{eq}$), 2.42−2.50 (2H, m, H-5, NCH$_2$-CH$_2$N), 2.80−2.96 (2H, m, NCH$_2$CH$_2$N), 3.0 (1H, d, J = 13.2 Hz, H-9$_{ax}$), 3.11 (1H, bd, J = 11.2 Hz, NCH$_2$CH$_2$N), 3.22−3.30 (1H, m, H-9$_{eq}$), 3.60−3.78 (2H, m, CH$_2$Ar), 3.80 (1H, dt, J = 4.8 Hz, J = 11.2 Hz, CHCH(Ph)$_2$), 3.90 (1H, d, J = 11.2 Hz, CH(Ph)$_2$), 7.14 (2H, t, J = 7.6 Hz, ArH), 7.15−7.31 (6H, m, ArH), 7.36 (2H, d, J = 7.2 Hz, ArH), 7.44 (2H, d, J = 8.0 Hz, ArH), 7.58 (2H, d, J = 8.4 Hz, ArH). Free base was converted into its oxalate salt, mp 195−198 °C. Anal. [C$_{28}$H$_{29}$N$_3$·(COOH)$_2$·1.15H$_2$O] C, H, N.

Synthesis of 8-Benzhydryl-4-(4-methoxy-benzyl)-1,4-diazabicyclo[3.3.1]nonane (10c). 8-Benzhydryl-1,4-diazabicyclo[3.3.1]nonane **7** (0.05 g, 0.17 mmol) was reacted with 4-methoxybenzaldehyde (0.03 g, 0.25 mmol), acetic acid (0.02 g, 0.25 mmol), and NaBH$_3$CN (0.02 g, 0.30 mmol) in CH$_2$ClCH$_2$-Cl to give a crude product which was purified by flash chromatography (EtOAc/hexane = 3/2) to produce **10c**, 0.03 g, (48% yield) as a colorless oil (Procedure A). ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.26−1.38 (1H, m, H-7$_{ax}$), 1.40−1.53 (2H, m, H-6$_{ax}$, H-7$_{eq}$), 2.20−2.30 (1H, m, H-6$_{eq}$), 2.42−2.56 (2H, m, H-5, NCH$_2$-CH$_2$N), 2.77−3.01 (3H, m, NCH$_2$CH$_2$N, H-9$_{ax}$), 3.10 (1H, bd, J = 14 Hz, NCH$_2$CH$_2$N), 3.20−3.27 (1H, m, H-9$_{eq}$), 3.51−3.60 (2H, m, CH$_2$Ar), 3.74−3.84 (4H, m, CHCH(Ph)$_2$, OCH$_3$), 3.91 (1H, d, J = 11.2 Hz, CH(Ph)$_2$), 6.81−6.87 (2H, m, ArH), 7.11−7.17 (2H, m, ArH), 7.21−7.30 (8H, m, ArH), 7.37 (2H, d, J = 6.4 Hz, ArH). Free base was converted into its hydrochloride salt, mp 273−275 °C. Anal. [C$_{28}$H$_{32}$N$_2$O·2HCl·1.5H$_2$O] C, H, N.

Synthesis of 8-Benzhydryl-4-(3,4-dichloro-benzyl)-1,4-diazabicyclo[3.3.1]nonane (10d). 8-Benzhydryl-1,4-diazabicyclo[3.3.1]nonane **7** (0.04 g, 0.14 mmol) was reacted with 3,4-dichlorobenzaldehyde (0.04 g, 0.21 mmol), acetic acid (0.01 g, 0.21 mmol), and NaBH$_3$CN (0.02 g, 0.25 mmol) in CH$_2$ClCH$_2$-Cl to give a crude product which was purified by flash

chromatography (EtOAc/hexane = 3/2) to produce **10d**, 0.04 g, (59% yield) as a colorless oil (Procedure A). ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.28−1.34 (1H, m, H-7$_{ax}$), 1.40−1.58 (2H, m, H-6$_{ax}$, H-7$_{eq}$), 2.14−2.24 (1H, m, H-6$_{eq}$), 2.38−2.51 (2H, m, H-5, NCH$_2$CH$_2$N), 2.77−2.95 (2H, m, NCH$_2$CH$_2$N), 2.99 (1H, bd, J = 11.2 Hz, H-9$_{ax}$), 3.10 (1H, bd, J = 13.2 Hz, NCH$_2$CH$_2$N), 3.20−3.28 (1H, m, H-9$_{eq}$), 3.50−3.67 (2H, m, CH$_2$Ar), 3.79 (1H, dt, J = 3.2 Hz, J = 11.2 Hz, CHCH(Ph)$_2$), 3.90 (1H, d, J = 11.2 Hz, CH(Ph)$_2$), 7.11−7.19 (3H, m, ArH), 7.23−7.30 (6H, m, ArH), 7.34−7.39 (3H, m, ArH), 7.42−7.44 (1H, m, ArH). Free base was converted into its mono oxalate salt, mp 189−190 °C. Anal. [C$_{27}$H$_{28}$Cl$_2$N$_2$·(COOH)$_2$] C, H, N.

Synthesis of 4-(8-Benzhydryl-1,4-diazabicyclo[3.3.1]-non-4-ylmethyl)phenol (10e). 8-Benzhydryl-1,4-diazabicyclo-[3.3.1]nonane **7** (0.05 g, 0.14 mmol) was reacted with 4-hydroxy benzaldehyde (0.03 g, 0.25 mmol), acetic acid (0.02 g, 0.25 mmol), and NaBH$_3$CN (0.02 g, 0.30 mmol) in CH$_2$ClCH$_2$Cl to give a crude product which was purified by flash chromatography (EtOAc) to produce **10e**, 0.05 g (66% yield) as a colorless oil (Procedure A). ^{1}H NMR (CDCl$_3$, 300 MHz) δ 1.32−1.41 (1H, m, H-7$_{ax}$), 1.42−1.64 (2H, m, H-6$_{ax}$, H-7$_{eq}$), 2.23−2.35 (1H, m, H-6$_{eq}$), 2.46−2.60 (2H, m, H-5, NCH$_2$CH$_2$N), 2.78−3.0 (3H, m, NCH$_2$CH$_2$N, H-9$_{ax}$), 3.12 (1H, bd, J = 11.7 Hz, NCH$_2$CH$_2$N), 3.20−3.26 (1H, m, H-9$_{eq}$), 3.48−3.64 (2H, m, CH$_2$Ar), 3.79 (1H, dt, J = 4.8 Hz, J = 10.4 Hz, CHCH(Ph)$_2$), 3.90 (1H, d, J = 11.2 Hz, CH(Ph)$_2$), 6.60 (2H, d, J = 8.8 Hz, ArH), 7.08−7.16 (4H, m, ArH), 7.20−7.28 (6H, m, ArH), 7.36 (2H, bd, J = 7.6 Hz, ArH). Free base was converted into its hydrochloride salt, mp 308−310 °C. Anal. [C$_{27}$H$_{30}$N$_2$O·2HCl·1.7H$_2$O] C, H, N.

Synthesis of 8-Benzhydryl-4-(4-ethoxy-benzyl)-1,4-diazabicyclo[3.3.1]nonane(10f). 8-Benzhydryl-1,4-diazabicyclo[3.3.1]nonane **7** (0.05 g, 0.17 mmol) was reacted with 4-ethoxybenzaldehyde (0.04 g, 0.25 mmol), acetic acid (0.02 g, 0.26 mmol), and NaBH$_3$CN (0.02 g, 0.26 mmol) in CH$_2$ClCH$_2$-Cl to give a crude product which was purified by flash chromatography (EtOAc/MeOH/hexane = 40/10/50) to produce **10f**, 0.03 g, (46% yield) as a colorless oil (Procedure A). ^{1}H NMR (CD$_3$OD, 400 MHz) δ 1.35 (3H, t, J = 7.2 Hz, CH$_3$), 1.38−1.45 (1H, m, H-7$_{ax}$), 1.53−1.65 (2H, m, H-6$_{ax}$, H-7$_{eq}$), 2.27−2.35 (1H, m, H-6$_{eq}$), 2.60−2.67(2H, m, NCH$_2$CH$_2$N, H-5), 2.80−2.92 (1H, m, NCH$_2$CH$_2$N), 2.96−3.12 (2H, m, H-9$_{ax}$, NCH$_2$CH$_2$N), 3.19 (1H, td, J = 3.6 Hz, J = 14.4 Hz, H-9$_{eq}$), 3.30−3.38 (1H, m, NCH$_2$CH$_2$N), 3.62−3.73 (2H, m, CH$_2$Ar), 3.99 (2H, q, J = 7.2 Hz, OCH$_2$), 4.03−4.09 (2H, m, CH(Ph)$_2$, CHCH(Ph)$_2$), 6.84 (2H, d, J = 8.8 Hz, ArH), 7.11−7.18 (2H, m, ArH), 7.21−7.29 (6H, m, ArH), 7.35 (2H, d, J = 7.2 Hz, ArH), 7.44 (2H, d, J = 7.6 Hz, ArH). Free base was converted into its hydrochloride salt. Anal. [C$_{29}$H$_{34}$N$_2$O·2HCl·2.5H$_2$O] C, H, N.

Synthesis of 8-Benzhydryl-4-naphthalen-1-ylmethyl-1,4-diazabicyclo[3.3.1]nonane (11a). A mixture of 8-benzhydryl-1,4-diazabicyclo[3.3.1]nonane **7** (0.06 g, 0.20 mmol), 1-chloromethyl-naphthalene (0.05 g, 0.30 mmol), and K$_2$CO$_3$ (0.28 g) in dry DMF (6 mL) was stirred at 85 °C for overnight. The reaction mixture was diluted with 20 mL of water and extracted with Et$_2$O (3×). The combined organic phase was dried over Na$_2$SO$_4$ and evaporated under vacuo to give crude product, which was purified by flash chromatography (EtOAc/hexane = 3/1), to give **11a** as a colorless oil, 0.05 g (56% yield). ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.30−1.44 (1H, m, H-7$_{ax}$), 1.50−1.66 (2H, m, H-6$_{ax}$, H-7$_{eq}$), 2.35−2.48 (1H, m, H-6$_{eq}$), 2.50−2.64 (2H, m, H-5, NCH$_2$CH$_2$N), 2.84−3.04 (3H, m, NCH$_2$CH$_2$N, H-9$_{ax}$), 3.14 (1H, bd, J = 12.0 Hz, NCH$_2$CH$_2$N), 3.20−3.30 (1H, m, H-9$_{eq}$), 3.78−3.89 (1H, m, CHCH(Ph)$_2$), 3.93−4.09 (2H, m, CH$_2$Ar), 4.15 (1H, d, J = 13.2 Hz, CH(Ph)$_2$), 7.13−7.12(2H, m, ArH), 7.24−7.36 (6H, m, ArH). 7.38−7.55 (6H, m, ArH), 7.77 (1H, d, J = 8.0 Hz, ArH), 7.83−7.90 (1H,m, ArH), 8.29−8.36 (1H,m, ArH). Free base was converted into its hydrochloride salt, mp 238−240 °C. Anal. [C$_{31}$H$_{32}$N$_2$·2HCl·1.5H$_2$O] C, H, N.

Synthesis of 8-Benzhydryl-4-naphthalen-2-ylmethyl-1,4-diazabicyclo[3.3.1]nonane (11b). A mixture of 8-Benzhydryl-1,4-diazabicyclo[3.3.1]nonane **7** (0.04 g, 0.13 mmol), 2-bromomethyl-naphthalene (0.04 g, 0.16 mmol), and K$_2$CO$_3$ (0.28 g) in dry DMF (5 mL) was stirred at 85 °C for overnight. The reaction mixture was then diluted with water and

extracted with Et_2O (3×). Combined organic phase was dried over Na_2SO_4 and evaporated under vacuo to give crude product, which was purified by flash chromatography (EtOAc/hexane = 3/1) to give **11b** as a colorless oil, 0.04 g (70% yield). ^{1}H NMR (CDCl_3, 400 MHz) δ 1.29−1.38 (1H, m, H-7$_{ax}$), 1.46−1.56 (2H, m, H-6$_{ax}$, H-7$_{eq}$), 2.28−2.35 (1H, m, H-6$_{eq}$), 2.48−2.60 (3H, m, H-5, NCH$_2$CH$_2$N), 2.85−3.01 (3H, m, NCH$_2$CH$_2$N, H-9$_{ax}$), 3.11 (1H, bd, J = 10.4 Hz, NCH$_2$CH$_2$N), 3.24−3.32 (1H, m, H-9$_{eq}$), 3.72−3.89 (3H, m, CH$_2$Ar, C*H*CH(Ph)$_2$), 3.92 (1H, d, J = 11.6 Hz, CH(Ph)$_2$), 7.10−7.17 (2H, m, ArH), 7.20−7.30 (6H, m, ArH), 7.34−7.54 (5H, m, ArH), 7.73 (1H, s, ArH), 7.75−7.84 (3H, m, ArH). Free base was converted into its oxalate salt. Anal. [C$_{31}$H$_{32}$N$_2$·(COOH)$_2$·0.2H$_2$O] C, H, N.

Synthesis of 8-Benzhydryl-4-(4-nitro-benzyl)-1,4-diazabicyclo[3.3.1]nonane (11c). A mixture of 8-benzhydryl-1,4-diazabicyclo[3.3.1]nonane **7** (0.08 g, 0.27 mmol), 1-bromomethyl-4-nitro-benzene (0.07 g, 0.32 mmol), and K$_2$CO$_3$ (0.38 g) in dry DMF (10 mL) was stirred at 85 °C for overnight. The reaction mixture was diluted with 20 mL of water and extracted with Et$_2$O. The combined organic phase was dried over Na$_2$SO$_4$ and evaporated under vacuo to give crude product, which was purified by flash chromatography (EtOAc/hexane= 2/1) to give **11c** as a colorless oil: 0.07 g (65% yield). ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.29−1.36 (1H, m, H-7$_{ax}$), 1.45−1.59 (2H, m, H-6$_{ax}$, H-7$_{eq}$), 2.17−2.26 (1H, m, H-6$_{eq}$), 2.41−2.50 (2H, m, H-5, NCH$_2$CH$_2$N), 2.81−2.95 (2H, m, NCH$_2$CH$_2$N), 2.96−3.03 (1H, m, H-9$_{ax}$), 3.11 (1H, bd, J = 10.4 Hz, NCH$_2$CH$_2$N), 3.22−3.29 (1H, m, H-9$_{eq}$), 3.64−3.85 (3H, m, CH$_2$-Ar, C*H*CH(Ph)$_2$), 3.90 (1H, d, J = 11.2 Hz, CH(Ph)$_2$), 7.10−7.18 (2H, m, ArH), 7.20−7.30 (6H, m, ArH), 7.31−7.39 (2H, m, ArH), 7.47−7.53 (2H, m, ArH), 8.12−8.18 (2H, m, ArH).

Synthesis of 4-(8-Benzhydryl-1,4-diazabicyclo[3.3.1]-non-4-ylmethyl)phenylamine (11d). A mixture of 8-benzhydryl-4-(4-nitrobenzyl)-1,4-diazabicyclo[3.3.1]nonane **11c** (0.05 g, 0.11 mmol), Pd/C (cat. 10%, 0.02 g), and hydrazine (0.1 mL, 3.1 mmol) in isopropyl alcohol (5 mL) was heated at 70 °C for 3 h. After cooling, the solution was decanted, concentrated, and purified by flash chromatography (EtOAc/MeOH = 5/1) to give **11d** as a colorless oil: 0.04 g (87% yield). ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.26−1.34 (1H, m, H-7$_{ax}$), 1.44−1.56 (2H, m, H-6$_{ax}$, H-7$_{eq}$), 2.19−2.31 (1H, m, H-6$_{eq}$), 2.48−2.65 (2H, m, H-5, NCH$_2$CH$_2$N), 2.77−3.02 (3H, m, NCH$_2$CH$_2$N, H-9$_{ax}$), 3.09 (1H, bd, J = 13.2 Hz, NCH$_2$CH$_2$N), 3.25−3.35 (1H, m, H-9$_{eq}$), 3.60 (2H, bs, CH$_2$Ar), 3.71−3.83 (1H, m, C*H*CH(Ph)$_2$), 3.90 (1H, d, J = 11.2 Hz, CH(Ph)$_2$), 6.63 (2H, d, J = 8.0 Hz, ArH), 7.09−7.19 (4H, m, ArH), 7.20−7.30 (6H, m, ArH), 7.36 (2H, bd, J = 7.6 Hz, ArH). Free base was converted into its hydrochloride salt. Anal. [C$_{27}$H$_{31}$N$_3$·3HCl·1.75H$_2$O] C, H, N.

Synthesis of *N*-[4-(8-Benzhydryl-1,4-diazabicyclo[3.3.1]-non-4-ylmethyl)phenyl]-4-chlorobenzenesulfonamide (12). Into a stirred solution of 4-(8-benzhydryl-1,4-diazabicyclo[3.3.1]-non-4-ylmethyl)-phenylamine **11d** (0.04 g, 0.10 mmol) in dry DMF (6 mL) were added 4-chlorobenzenesulfonyl chloride (0.03 g, 0.13 mmol) and Et$_3$N (0.05 g, 0.50 mmol). The solution was stirred at 85 °C for overnight. It was then diluted with water and extracted with Et$_2$O (3×). Combined organic phase was dried over Na$_2$SO$_4$ and evaporated under vacuo to give crude product, which was purified by flash chromatography (EtOAc/hexane/MeOH = 40/40/3) to give **12** as a colorless oil, 0.03 g (64% yield). ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.24−1.32 (1H, m, H-7$_{ax}$), 1.40−1.54 (2H, m, H-6$_{ax}$, H-7$_{eq}$), 2.15−2.24 (1H, m, H-6$_{eq}$), 2.36−2.47 (2H, m, H-5, NCH$_2$CH$_2$N), 2.74−2.92 (2H, m, NCH$_2$CH$_2$N), 2.97 (1H, bd, J = 12.8 Hz, H-9$_{ax}$), 3.08 (1H, bd, J = 13.2 Hz, NCH$_2$CH$_2$N), 3.18−3.26 (1H, m, H-9$_{eq}$), 3.49−3.56 (2H, m, CH$_2$Ar), 3.73−3.83 (1H, m, C*H*CH(Ph)$_2$), 3.89 (1H, d, J = 11.2 Hz, CH(Ph)$_2$), 6.93−6.95 (2H, m, ArH), 7.10−7.16 (2H, m, ArH), 7.19−7.29 (8H, m, ArH), 7.33−7.41 (4H, m, ArH), 7.63−7.69 (2H, m, ArH). Free base was converted into its oxalate salt. mp 200−205 °C Anal. [C$_{33}$H$_{34}$ClN$_3$O$_2$S·(COOH)$_2$·1.7H$_2$O] C, H, N.

Synthesis of (6-Benzhydryl-1-methylpiperidin-3-yl)(4-fluorobenzyl)methylamine (16). A solution of *cis*-(6-benzhydryl-1-methylpiperidin-3-yl)(4-fluorobenzyl)methylamine **15** (0.06 g, 0.16 mmol), formaldehyde (0.5 g, 37%/H$_2$O), and formic

acid (1.0 g, 88%/H$_2$O) was refluxed for 3 h. The solution was then cooled to room temperature, and solvent was removed in vacuo. The residue was dissolved in EtOAc and washed by saturated NaHCO$_3$ solution and brine and then dried over Na$_2$SO$_4$. Solvent was then evaporated to give crude product, which was purified by flash chromatography (EtOAc/hexane = 3/2) to give **16** as colorless oil: 0.05 g (73% yield). ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.43−1.47 (1H, m, H-5$_{ax}$), 1.64−1.79 (3H, m, H-5$_{eq}$, H-4), 2.21 (3H, s, NCH$_3$), 2.39 (3H, s, NCH$_3$), 2.65 (2H, H-2$_{ax}$, H-3$_{eq}$), 2.99 (1H, m, H-2$_{eq}$), 3.43−3.47 (1H, m, H-6$_{ax}$), 3.53−3.61 (2H, m, CH$_2$C$_6$H$_4$F), 4.40 (1H, d, J = 11.6 Hz, CH(Ph)$_2$), 6.98−7.03 (1H, m, ArH *ortho* F), 7.13−7.16 (1H, m, ArH, *ortho* F), 7.2−7.36 (12H, m, ArH). Free base was converted into its hydrochloride salt, mp 200−205 °C. Anal. [C$_{27}$H$_{31}$FN$_2$·2HCl·1.4H$_2$O] C, H, N.

Synthesis of [6-Benzhydryl-1-(4-fluorobenzyl)piperidin-3-yl](4-fluorobenzyl)amine (17). *cis*-(6-Benzhydryl-1-methylpiperidin-3-yl)(4-fluorobenzyl)methylamine **(15)** (0.07 g, 0.18 mmol) were reacted with 4-fluorobenzaldehyde (0.022 g, 0.17 mmol), acetic acid (0.01 g, 0.18 mmol), and NaBH$_3$CN (0.01 g, 0.22 mmol) in CH$_2$ClCH$_2$Cl to give a crude product which was purified by flash chromatography (EtOAc/hexane = 3/2) to produce **17**, 0.07 g, (76% yield) as a white solid, mp: 138−139 °C. ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.46 (1H, d, J = 12.8 Hz, H-5$_{ax}$), 1.56−1.62(1H, m, H-5$_{eq}$), 1.75−1,83 (2H, m, H-4), 2.60 (1H, d, J = 11.2 Hz, H-2$_{ax}$), 2.72−2.83 (2H, m, H-3$_{eq}$, H-2$_{eq}$), 3.50 (1H, dd, J = 11.2 Hz, J = 2.4 Hz, H-6$_{ax}$), 3.72−3.84 (4H, m, CH$_2$C$_6$H$_4$F), 4.49 (1H, d, J = 11.2 Hz, CH(Ph)$_2$), 6.80−6.88(2H, m, ArH, *ortho* F), 6.99(2H, t, J = 8.5 Hz, ArH, *ortho* F), 7.13−7.29 (14H, m, ArH). Free base was converted into its hydrochloride salt, mp: 177−180 °C. Anal. [C$_{32}$H$_{32}$FN$_2$·2HCl·2.6H$_2$O] C, H, N.

Synthesis of 1-(8-Benzhydryl-1,4-diazabicyclo[3.3.1]-nonane-4-carbonyl)-4,7,7-trimethyl-2-oxabicyclo[2.2.1]-heptan-3-one (13a and 13b). Racemic 8-benzhydryl-1,4-diazabicyclo[3.3.1]nonane (±)-**7** (0.43 g, 1.49 mmol) was dissolved in dry CH$_2$Cl$_2$, and into it was added (1*S*)-(−)-camphanic chloride at 0 °C followed by Et$_3$N (0.75 g, 7.4 mmol). The solution was allowed to come to room temperature and stirred for another 3 h under N$_2$ atmosphere. It was then diluted with CH$_2$Cl$_2$ and washed with water (2 times) and brine. The organic layer was dried over Na$_2$SO$_4$ and solvent evaporated in vacuo to give a crude mixture of two diastereomers which was separated by flash chromatography over a silica gel column using hexane/ethyl acetate (1:1). Eluting first **13a** (0.25 g, 35%) ^{1}H NMR (CDCl$_3$, 400 MHz): δ 1.00 (3H, d, J = 7.2 Hz, CH$_3$), 1.09 (3H, d, J = 7.2 Hz, CH$_3$), 1.16 (3H, d, J = 21.6 Hz, CH$_3$), 1.63−2.14 (8H, m, CCH$_2$C), 2.31−2.45 (1H, m, NCH$_2$C), 2.78−2.92 (1H, m, NCH$_2$C), 2.94−3.36 (3H, m, H-5, NCH$_2$C), 3.68−3.82 (1H, m, NCH$_2$C), 3.82−3.96 (1H, m, CHCH(Ph)$_2$), 4.00−4.24 (2H, m, CH(Ph)$_2$, NCH$_2$C), 7.12−7.20 (2H, m, ArH), 7.20−7.32 (6H, m, ArH), 7.32−7.40 (2H, m ArH). Eluting second **13b** (0.21 g, 30%) ^{1}H NMR (CDCl$_3$, 400 MHz): δ 1.00 (3H, d, J = 8.8 Hz, CH$_3$), 1.10 (3H, d, J = 4.8 Hz, CH$_3$), 1.17 (3H, d, J = 9.6 Hz, CH$_3$), 1.30−2.05 (8H, m, CCH$_2$C), 2.32−2.42 (1H, m, NCH$_2$C), 2.94−3.02 (1H, m, NCH$_2$C), 3.12−3.30 (2H, m, H-5, NCH$_2$), 3.63−4.08 (m, 4H, NCH$_2$C, CHCH-(Ph)$_2$), 4.28−4.38 (1H, d, J = 14 Hz, CH(Ph)$_2$), 7.11−7.20 (2H, m, ArH), 7.22−7.32 (6H, m, ArH), 7.32−7.40 (2H, m, ArH).

Synthesis of (+)-8-Benzhydryl-1,4-diazabicyclo[3.3.1]-nonane ((+)-14a). A solution of **13a** (0.35 g, 0.74 mmol) in 6 N HCl/MeOH (20 mL) was refluxed for 48 h. Methanol was then evaporated in vacuo, and the aqueous solution was neutralized using saturated NaHCO$_3$ solution. It was then extracted using EtOAc (3×). All organic extracts were combined, washed with brine, and dried over Na$_2$SO$_4$. The solvent was then evaporated in vacuo to give a crude mixture, which was purified by flash chromatography over a silica gel column using MeOH/EtOAc/Et$_3$N (7:7:1) to yield (+) **14a** (0.2 g, yield 93%). Optical rotation of the free base was measured in Perkin-Elmer 241 polarimeter, $[\alpha]^{25}_D$ = +49 ° (*c* 1, MeOH).

Synthesis of (−)-8-Benzhydryl-1,4-diazabicyclo[3.3.1]-nonane ((−)-14b). 13b was treated in a similar way as shown above for **13a** to get (−)-8-benzhydryl-1,4-diazabicyclo[3.3.1]-

nonane **14b**. Optical rotation of the free base was measured in Perkin-Elmer 241 polarimeter, $[\alpha]^{25}_{D} = -46°$ (c 1, MeOH).

Synthesis of (+)-8-Benzhydryl-4-(4-methoxy-benzyl)-1,4-diazabicyclo[3.3.1]nonane ((+)-10c). The same procedure as followed in the synthesis of racemic (±)-**10c** was followed here. The optical rotation of the free base was measured in Perkin-Elmer 241 polarimeter, $[\alpha]^{25}_{D} = +52°$ (c 1, MeOH). The free base was converted into hydrochloride salt. Anal. [$C_{28}H_{32}O\cdot2HCl\cdot1.4H_2O$] C, H, N.

Synthesis of (−)-8-Benzhydryl-4-(4-methoxy-benzyl)-1,4-diazabicyclo[3.3.1]nonane ((−)-10c). Procedure for preparation of racemic compound **10c** was followed. Optical rotation of the free base was measured in Perkin-Elmer 241 polarimeter, $[\alpha]^{25}_{D} = -55°$ (c 1, MeOH). The free base was converted into hydrochloride salt. Anal. [$C_{28}H_{32}O\cdot2HCl\cdot H_2O$] C, H, N.

Biology. The affinity of test compounds in binding to rat DAT, SERT, and NET was assessed by measuring inhibition of binding of [³H]WIN 35,428, [³H]citalopram, and [³H]nisoxetine, respectively, exactly as described by us previously.[17,32] Briefly, rat striatum was the source for DAT, and cerebral cortex for SERT and NET. Final [Na⁺] was 30 mM for DAT and SERT assays. All binding assays were conducted at 0−4 °C, for a period of 2 h for [³H]WIN 35,428 and [³H]citalopram binding, and 3 h for [³H]nisoxetine binding. Nonspecific binding of [³H]WIN 35,428 and [³H]citalopram binding was defined with 100 μM cocaine, and that of [³H]nisoxetine binding with 1 μM desipramine. Test compounds were dissolved in dimethyl sulfoxide (DMSO) and diluted out in 10% (v/v) DMSO. Additions from the latter stocks resulted in a final concentration of DMSO of 0.5%, which by itself did not interfere with radioligand binding. At lease five triplicate concentrations of each test compound were studied, spaced evenly around the IC_{50} value. For DAT uptake assays, uptake of [³H]DA into rat striatal synaptosomes was measured exactly as described by us previously.[17,32] Briefly, rat striatal P_2 membrane fractions were incubated with test compounds for 8min followed by the additional presence of [³H]DA for 4 min at 25 °C. Nonspecific uptake was defined with 100 μM cocaine. Construction of inhibition curves and dissolvement of test compounds were as described above.

Locomotor Activity, Subjects. Adult male Swiss Webster mice (Harlan Sprague Dawley, Inc., Indianapolis, IN) weighing 30−35 g were used. Mice were housed five per cage with continuous access to food and water and were allowed to acclimate to the vivarium environment one week prior to the start of any experiment. The mice were housed in an AALAC-accredited animal facility with a controlled temperature (22−24 °C) on a 12 h light−dark cycle. All testing occurred during the light component.

Apparatus Procedure and Analyses. The locomotor activity chambers and procedure have been described in detail elsewhere.[15] Briefly, four commercially obtained, automated activity-monitoring devices each enclosed in sound- and light-attenuating chambers were used (AccuScan Instruments, Inc., Columbus, OH). The interior of each device was divided into two separate 20 × 20 × 30-cm arenas permitting the independent and simultaneous measurement of two mice. Sixteen photobeam sensors were spaced 2.5 cm apart along the walls of the chamber. Nonhabituated mice were administered a dose of a test compound or vehicle and immediately placed into automated activity chambers where distance traveled (cm) was recorded for 24 10-min periods. The summed total distance traveled for each drug during the 10 min intervals for the last 3 h (intervals 7−24) were analyzed using a one-factor (dose) ANOVA followed by Dunnett's posthoc tests when the overall ANOVA was significant. The alpha level for all comparisons was set at 0.05.

Drug Discrimination, Subjects. Adult male Swiss Webster mice (Harlan Sprague Dawley, Inc., Indianapolis, IN) were used. Mice were individually housed and maintained on a 12 h light−dark cycle with continuous access to water. Training and testing occurred during the light component. Mice were maintained at 35 ± 5 g by supplemental postsession feedings of laboratory chow (Harlan Tekled, Madison, WI).

Apparatus and Procedure. The drug discrimination chambers and procedure have been described in detail elsewhere.[15] Briefly, eight standard, light- and sound-attenuated mouse operant conditioning chambers were used (Med Associates, Inc., St. Albans, VT; model ENV-307A). Each chamber was equipped with two response levers separated by a trough into which a 0.01 mL dipper cup could be presented. A house light was centered at the top of the front panel and three cue lights were located above each lever. Control of lights, dipper presentations, and recording of lever presses were accomplished by a microcomputer system (Med-PC software, Med Associates, Inc., St. Albans, VT).

Mice were initially trained to press one of the two levers at a fixed-ratio 1 (FR1) schedule of reinforcement in which each lever press resulted in a 0.01 mL delivery of sweetened condensed milk. The response requirement was gradually increased to FR20. Subsequently, the mice were reinforced for pressing the opposite lever until reliable responding was obtained under FR20 conditions. Discrimination training occurred during daily (Mon-Fri) 15 min experimental sessions. Mice were injected with cocaine (10 mg/kg) or saline ip 10 min prior to the start of the session start. The cocaine- and saline-associated levers were counterbalanced across the mice. Responses on the injection-appropriate lever resulted in delivery of the sweetened milk solution. Responses on the injection-inappropriate lever resulted in resetting the response requirement on the correct lever. A schedule was used to determine which injection was administered, with the restriction that the same injection was not given on more than two consecutive sessions and over 30 training sessions the number of saline and cocaine injections were approximately equal.

Testing commenced when (1) a mouse completed the first fixed-ratio (FFR) on the correct lever on at least 8 or 10 consecutive days; and (2) at least 80% of the total responses were made on the correct lever during those eight sessions. Tests were conducted on Tuesdays and Fridays provided that the mouse completed the FFR on the correct lever during the most recent cocaine and saline training sessions, otherwise, a training session was administered. During test days, responding on either lever was reinforced with milk.

Analyses. The percentage of responses on the cocaine-lever was calculated for each mouse during training and test sessions by dividing the number of lever presses emitted upon the cocaine-lever by the total number of lever presses emitted on both levers, and then this quotient was multiplied by 100. Additionally, rate of responding was calculated for each mouse by dividing the total number of responses emitted on both levers by 900 s. Individual cocaine-lever responding percentages and responses per sec were then averaged (±SEM). If a mouse failed to complete a FFR then its data were excluded from calculations of mean cocaine-lever responding, but were included for mean response rate calculations. ED_{50} values (95% CL) (mg/kg) were calculated for percent cocaine-lever responding using a sigmoidal dose−response (variable slope) curve fitting procedure (GraphPad Prism; San Diego, CA). A log transformation on dose was used.

Drugs. Cocaine (U.S. National Institute Drug Abuse) was dissolved in 0.09% sterile saline. (−)-**2** was dissolved in 40% w/v hydroxypropyl-β-cyclodextrin (Cavitron 82003, Cerestar USA, Inc., Hammond, IN) and (−)-**10c** was dissolved in sterile water. All drugs were administered by the ip route in a volume of 10 mL/kg. Cocaine was administered 10 min prior to the session start and (−)-**2** and (−)-**10c** were administered 30 min prior to the session start.

Single-Crystal X-ray Diffraction Analysis of (−)-13b. $C_{30}H_{35}N_2O_3$, FW = 471.60, monoclinic space group $P2_1$, $a = 7.0137(2)$, $b = 13.7876(5)$, $c = 13.5686(4)$ Å, $b = 92.201(2)°$, $V = 1311.14(7)$ Å³, $Z = 2$, density (calcd) = 1.195 mg mm⁻³, λ (Cu Kα) = 1.54178 Å, $\mu = 0.607$ mm⁻¹, $F(000) = 506$, $T = 293$ K. A clear colorless $0.38 × 0.13 × 0.02$ mm crystal was used for data collection with a Bruker SMART 6000 CCD detector on a Platform goniometer using SMART.[33a] The Rigaku rotating Cu anode source was equipped with an incident beam Göbel mirrors. Lattice parameters were determined using

SAINT[33b] from 3762 reflections within $6.41 < 2q < 132.83$. Data were collected to $2q = 132.83°$. A set of 6693 reflections was collected in the w scan mode. There were 3748 unique reflections. Corrections were applied for Lorentz, polarization, and absorption effects. The structure was solved with SHELX-TL [33c] and refined with the aid of the SHELX system of programs. The full-matrix least-squares refinement on F^2 used one restraint and varied 317 parameters: atom coordinates and anisotropic thermal parameters for all non-H atoms, and the extinction coefficient. H atoms were included using a riding model [coordinate shifts of C applied to attached H atoms, C−H distances set to 0.96 to 0.93 Å, H angles idealized, U_{iso}-(H) were set to 1.2 to 1.5 U_{eq}(C)]. Final residuals were $R1 = 0.056$ for the 2996 observed data with $F_o > 4\sigma(F_o)$ and 0.068 for all data. Final difference Fourier excursions were 0.26 and −0.21 eÅ-3. The asymmetric unit contains one molecule of the title compound. Tables of coordinates, bond distances and bond angles, and anisotropic thermal parameters have been deposited with the Crystallographic Data Centre, Cambridge, CB2, and 1EW, England.

Supporting Information Available: Crystal structure, additional ^{1}H NMR, and molecular modeling data are available free of charge via the Internet at http://pubs.acs.org.

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