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3-Aza-6,8-dioxabicyclo[3.2.1]octanes as new enantiopure heteroatom-rich tropane-like ligands of human dopamine transporter

Nicoletta Cini,[a] Elisa Danieli,[b] Gloria Menchi,[b] Andrea Trabocchi,[b] Anna Bottoncetti,[a] Silvia Raspanti,[a] Alberto Pupi[a,†] and Antonio Guarna[b,*,†]

[a]*Dipartimento di Fisiopatologia Clinica, Unità di Medicina Nucleare, Università degli Studi di Firenze, Viale Pieraccini 6, I-50134 Firenze, Italy*
[b]*Dipartimento di Chimica Organica "Ugo Schiff", Università degli Studi di Firenze, Polo Scientifico di Sesto e Agraria, Via della Lastruccia 13, I-50019 Sesto Fiorentino, Italy*

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Abstract—CNS diseases such as Parkinson, schizophrenia, and attention deficit hyperactivity disorder (ADHD) are characterized by a significant alteration of dopamine transporter (DAT) density. Thus, the development of compounds that are able to selectively interact with DAT is of great interest. Herein we describe the design and synthesis of a new set of 3-aza-6,8-dioxabicyclo[3.2.1]octanes having a tropane-like structure with additional heteroatoms at positions 3 and 6. The compounds were evaluated for their in vitro receptor binding properties toward human dopamine (hDAT) and serotonin (hSERT) transporters using [^{3}H]WIN35,428 and [^{3}H]citalopram as specific radioligands, respectively. Biological assays revealed that some compounds having the N-3 atom substituted with aryl groups possess significant affinity and selectivity for monoamine transporters, and in particular, compound **5d** displayed an IC_{50} of 21 nM toward DAT, and a good selectivity toward SERT (IC_{50} = 1042 nM). These results suggest that 3-aryl-3-aza-6,8-dioxabicyclo[3.2.1]octanes may represent a new class of DAT ligands.

1. Introduction

Abnormalities in the dopamine neurotransmitter system are implicated in several psychomotor disorders such as Parkinson's disease, schizophrenia, attention deficit hyperactivity disorder (ADHD), and depression.[1–4] In particular, in vitro studies of human Parkinsonian patients showed that dopamine transporter (DAT) striatal expression is considerably depleted, and the extent of depletion is correlated with the progression of the disease.[5,6] In contrast, ADHD has been demonstrated to be accompanied by a significant increase in the dopamine transporter density.[7]

The investigation of dopaminergic agents and, in particular, selective dopamine transporter ligands, has received considerable attention, especially for the development of new imaging agents which could be biochemical probes for the measurement of the density of presynaptic dopamine transporter sites by emission tomography. This could be a potentially valuable technique for the diagnosis, management of treatment, and study of the pathogenesis of addictive and psychomotor disorders in humans.

Moreover, as it has been recognized that DAT plays a key role in the modulation of postsynaptic dopamine levels, and as cocaine is able to bind to DAT and to inhibit the dopamine reuptake,[8–11] much interest has been oriented toward the generation of molecules, respectively, capable of inhibiting or favoring dopamine reuptake in presynaptic neurons by selective interaction with DAT, as they may contribute to the generation of new therapeutics against CNS disorders. Although studies of structure–activity relationships (SAR) did not provide a comprehensive picture of the binding interaction to DAT at molecular level yet, studies on cocaine and its tropane analogues (see Fig. 1)[8,12–26] showed that two

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* Corresponding author. Tel.: +39 055 4573481; fax: +39 055 4573569; e-mail: antonio.guarna@unifi.it
† These authors contributed equally to this work.

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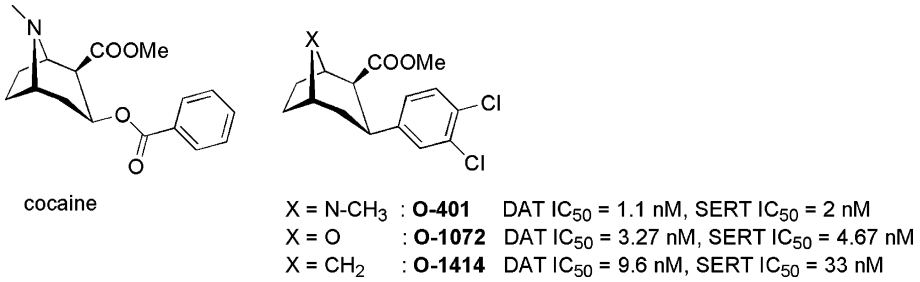


Figure 1. Structures of cocaine and 3-phenyltropane-based compounds as DAT inhibitors.[27,32]

elements play important roles in the binding and reuptake activities: (1) the presence of an aromatic ring connected directly or indirectly to the 3 position of the tropane ring, and the nature of the aromatic substituents, strongly influences the dopamine transporter ligand recognition. Specifically, an increase in the electronic density on this part of the molecule is associated with high DAT affinity; (2) the relative position of the aromatic group at position 3 and of the substituent at position 2.[26] In fact, it has been shown that the orientation of the 3-aryl moiety in either α-(*endo*) or β-(*exo*) configuration modulates the selectivity of these compounds toward DAT with respect to other monoamine transporters.[27] Moreover, the orientation of the aromatic ring in a more planar relationship with the tropane skeleton due to the presence of a double bond at C-2 and C-3 greatly enhances biological selectivity.[24] Still, 2α-configuration of the substituent in the tropane ring leads to much less active DAT inhibitors.[28–30] Moreover, it was described in literature that the presence of a nitrogen atom at 8-position of tropanes is not an absolute requisite for binding to DAT, and different 3-phenyltropanes bearing either an oxygen or a carbon atom at 8-position (Fig. 1) have been reported to maintain a good affinity.[27,31,32]

Among all DAT inhibitors based on tropane-like scaffolds reported to date, only few modifications of the core structure have been investigated. Recently, a nice example of a new scaffold having the nitrogen atom shifted from position 8 to 6 has been reported, which showed interesting inhibiting properties compared to cocaine.[33] We reasoned that, since there are no examples in literature describing the introduction of more than one heteroatom in the scaffold, especially in the key position 3 bearing an aromatic substituent, it would have been noteworthy to use a new molecule having a tropane-like structure to test the stereoelectronic features correlated to the binding potency toward DAT, thus providing new chemical insights into the design of DAT inhibitors. During the last five years we have been developing a new class of enantiopure 3-aza-6,8-dioxa-bicyclo[3.2.1]octane scaffolds as γ/δ bicyclic amino acids named BTAa,[34,35] which are obtained from the combination of tartaric acid and α-amino carbonyl derivatives. More recently, a new set of proline-like bicyclic α-amino acids, named BG(g)S(s), has been reported, which derive from the combination of glyceraldehyde and serinol derivatives.[36] Since they present the carboxyl moiety of BTAa formally shifted from position 7 to 4, we envisioned the possibility of using the Bgs stereoisomer, obtained from L-glyceraldehyde and D-serine derivatives, as tropane-mimetics containing three heteroatoms in the core structure, and possessing the correct absolute configuration with respect to cocaine. Indeed, these compounds show structural similarity with the bicyclic structure of tropane, and possess the correct configuration at C-4 carbon atom having the carbomethoxy group oriented in 4-*exo* configuration (Fig. 2).

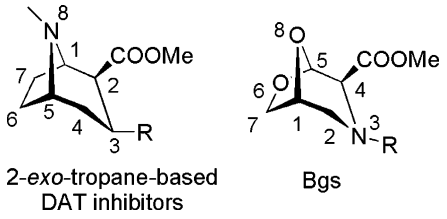


Figure 2. 2-*exo*-Tropane-based DAT inhibitors (left) and Bgs-derived compounds (right).

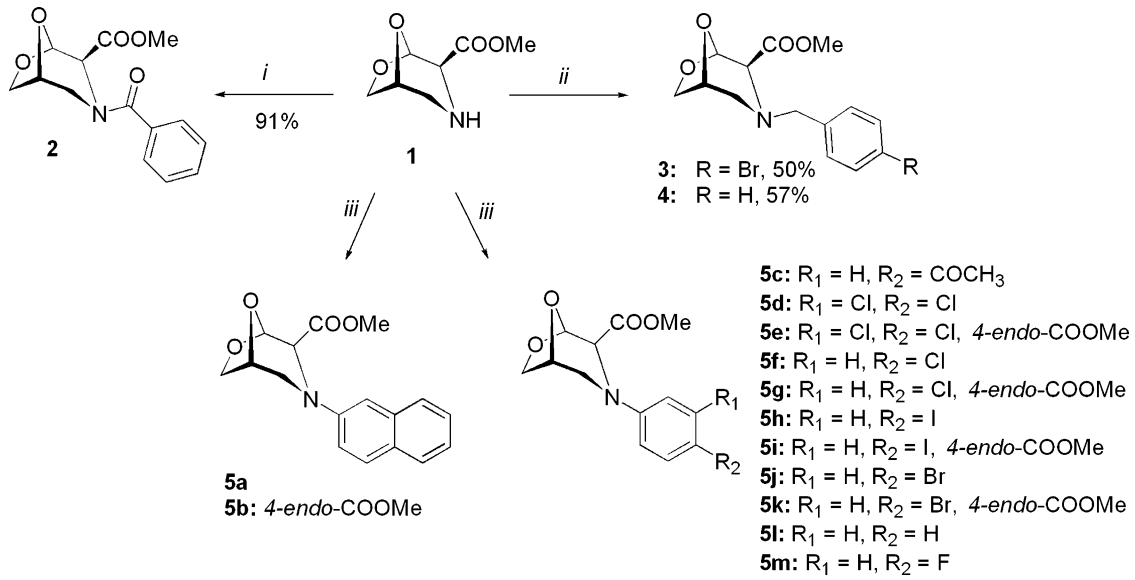
Moreover, the presence of heteroatoms might contribute to decreasing the non-specific binding of these molecules in the brain, which is a limiting factor for their possible use as radiolabeled imaging agents. More generally, such molecules were used to test the effect on binding potency and selectivity of the presence of two additional heteroatoms at positions 3 and 6 of the scaffold with respect to the tropane structure. Therefore, a collection of suitably functionalized Bgs molecules having different substituents at the nitrogen atom was prepared in order to establish how such heteroatom might influence the topology of the aromatic ring in terms of binding affinity.

Finally, the stereoelectronic properties were computed, and analyzed together with the affinity results in order to draw a rationale connected with the structural features of the new proposed scaffolds.

2. Results and discussion

2.1. Chemistry

Fmoc-Bgs amino acid was obtained in 24% yield according to the previously reported method.[36] After treatment with diazomethane, the corresponding *N*-Fmoc-amino



Scheme 1. Reagents and conditions: (i) PhCOCl, Et_3N, dry THF, rt, overnight; (ii) benzaldehyde or *p*-Br-benzaldehyde, $NaBH(OAc)_3$, DCE, rt, overnight; (iii) $ArB(OH)_2$, base, dry CH_2Cl_2, $Cu(OAc)_2$, 4 Å molecular sieves, O_2, 25–50 °C, 24–72 h.

ester was obtained in 91% yield,[37] and further deprotected using diethylamine in dry acetonitrile, to afford H-Bgs-OMe **1** in quantitative yield.

Treatment of **1** (Scheme 1) with benzoyl chloride in the presence of triethylamine gave the corresponding *N*-benzoyl-Bgs compound **2** (Scheme 1, path i), whereas *p*-bromobenzyl and benzyl groups were introduced by reductive amination of the corresponding functionalized benzaldehyde using $NaBH(OAc)_3$ in 1,2-dichloroethane (Scheme 1, path ii), thus giving **3** and **4**, respectively.[38] Low yields of reductive aminations of **1** (50% and 57%) were probably due to steric hindrance and to the poor nucleophilicity of the N-3 associated with the presence of the ester group.

The insertion of an aromatic ring directly bound to the nitrogen atom proved to be more challenging (Scheme 1, path iii). The effect of steric hindrance in the arylation of secondary amines having substituents adjacent to the amine function has been reported in the literature, and low yields were accounted for the reaction.[39–42] Initially, arylation of the amine group using aryl bromides in refluxing toluene for 20 h and in the presence of BINAP/$Pd(OAc)_2$ as catalyst failed,[39] and only the unreacted starting material was recovered. Attempts at increasing the conversion using 1,4-dioxane as solvent did not work.

The choice of different catalytic systems, depending on the aryl bromide used (i.e., $Pd_2(dba)_3$/BINAP, $PdCl_2(dppf)$), in higher amounts, as reported in the literature,[43] was likewise unsuccessful. In contrast, arylation of **1** using arylboronic acids and $Cu(OAc)_2$ under an oxygen atmosphere in dry CH_2Cl_2, and in the presence of a base gave the desired products, though in low yields (5–30%, see Table 1). The use of a base such as triethylamine or pyridine was found to be crucial, as the reaction failed when a base was absent.

As reported in the literature, the presence of molecular oxygen in copper-promoted N-arylation reactions has been hypothesized to facilitate the oxidation of the intermediate Cu(II)- to the Cu(III)-complex, which can undergo more facile reductive-elimination than the Cu(II)-complex to form the desired products.[41] Therefore, the effect of raising oxygen pressure on the reaction was evaluated, but only a modest increase in the final yield was observed, and in some cases epimerization at C-4 stereocenter occurred. The use of pyridine in place of triethylamine, in refluxing CH_2Cl_2, gave the desired products in modest yield, and epimerization at C-4

Table 1. N-Arylation reaction of **1** with selected arylboronic acids using cupric acetate

Compound	Boronic acid	*T* (°C)	PO_2 (atm)	Time (h)	Base	Yield (%)	*exo*:*endo*[a]
1	4-Acetyl-phenyl	25	1	24	Et_3N	5	*exo* only
2	4-Bromo-phenyl	25	5	24	Et_3N	27	1:1
3	4-Iodo-phenyl	25	17	24	Et_3N	25	1:1
4	4-Chloro-phenyl	50	17	24	Et_3N	26	2:3
5	3,4-Dichloro-phenyl	50	1	72	Py	18	1:2
6	2-Naphthyl	50	1	72	Py	15	1:2
7	Phenyl	30	17	40	Py	14	*exo* only
8	4-Fluorophenyl	30	17	40	Py	30	*exo* only

[a] Based on the yield of isolated products.

carbon atom of the scaffold was observed in variable extent, even at one atmosphere of oxygen. Finally, when both temperature and reaction time were lowered, 4-*endo* products were not obtained using pyridine, even at high pressures of oxygen. Thus, it was found that epimerization at C-4 could be due to different combinations of reaction conditions, and that a marked improvement of the yield was never observed. All the obtained diastereomeric compounds having the carbomethoxy group in 4-*endo* configuration were easily separated from the corresponding 4-*exo* isomers by chromatography. Both isomers were then subjected to binding assay to gain further insight on the influence of the orientation of the carbomethoxy group within the 3-aza-6,8-dioxa[3.2.1]bicyclic scaffold on binding affinity and selectivity for the DAT.

Structural assignment of the configuration at C-4 stereocenter for *N*-aryl compounds **5a**–**m** was achieved by comparison of the chemical shift data and the *J* values of H-4 and H-5 protons, as NOE experiments were not diagnostic for such structural determination. Specifically, it was found a typical NMR pattern of the H-4 proton for 4-*exo*- and 4-*endo*-structures with respect to H-5, and the corresponding *J* value proved to be dependent upon the relative positions of the two protons (Fig. 3 and Table 2). All the 4-*exo*-structures displayed a chemical shift of H-4 between 4.59 ppm and 4.34 ppm, and a *J* value between 0 and 2 Hz, whereas 4-*endo* stereoisomers, as well as showing similar chemical shift values for H-5, displayed an upfield shift of H-4 between 4.21 and 4.03 ppm, and a greater *J* value between 2.8 and 3.4 Hz, indicating a lower dihedral angle between H-4 and H-5.

2.2. Biology

The assays were performed on crude membrane receptor preparations originated from recombinant human dopamine transporter (hDAT) and from recombinant human serotonin transporter (hSERT) transfected into CHO and HEK293 cells, respectively.[44] The IC_{50} values of Bgs scaffolds toward DAT and SERT are reported in Table 3, and were assayed by using a competitive displacement of a specific radioligand for each transporter ([^{3}H]WIN35,428 8 nM for DAT and [^{3}H]citalopram 1 nM for SERT).[45,46] Competition studies were carried out using a fixed concentration of the radioligand and a range of concentrations between 100 μM and 1 nM of test compounds. All active Bgs molecules inhibited [^{3}H]WIN35,428 and [^{3}H]citalopram binding in a concentration-dependent manner.

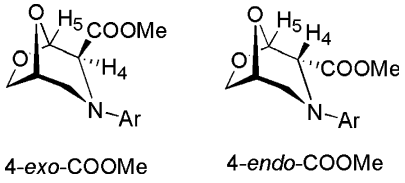


Figure 3. 4-*exo*- and 4-*endo*-Bgs structures.

Table 2. NMR data for 4-*exo*- and 4-*endo*-aryl-Bgs compounds

Structure	δ H-5 (ppm)	δ H-4 (ppm)	$^3J_{4,5}$ (Hz)
4-*exo*-COOMe	5.88–5.82	4.59–4.34	2.0–0
4-*endo*-COOMe	5.84–5.80	4.21–4.03	3.4–2.8

2.3. Discussion

In the present study, we report on the effect of replacing the tropane ring with a 3-aza-6,8-dioxa[3.2.1]bicyclic scaffold on binding affinity and selectivity for the dopamine transporter. Selected functional groups were introduced at position 3 of the scaffold. Specifically benzoyl, Fmoc, substituted benzyl, and aryl derivatives were introduced on Bgs in order to evaluate their effect on the receptor binding affinity. The lack of binding affinity of Fmoc-, benzoyl- (**2**), and benzyl- (**3** and **4**) derivatives (Table 3) could be probably due to the wrong orientation of the phenyl ring with respect to the correct geometry for the interaction with the hydrophobic pocket of DAT and SERT receptors. Moreover, the inactivity of **2**, **3**, and **4** indicated a moderate interaction of N-3 atom with DAT, as a marked difference in nitrogen nucleophilicity (benzoyl vs benzyl) did not evidence variations in the inhibitory potency. Indeed, only some of *N*-aryl-functionalized compounds **5a**–**m** proved to display affinity toward DAT, as showed in Table 2. Compound **5d**, having 3,4-dichloro-phenyl substituent at position 3, displayed low nanomolar activity toward DAT (IC_{50} = 21 nM), being about 8-fold more potent than the other bioactive *N*-aryl-Bgs. The higher SERT binding affinity of *N*-(2-naphthyl)-Bgs-OMe (**5a**) with respect to DAT was striking, and could be due to a higher steric hindrance associated with the hydrophobic pocket of DAT receptor compared to the SERT binding site, which is larger enough to accommodate a naphthyl group. More generally, the 4-*endo* scaffolds were totally inactive toward SERT and showed poor affinity toward DAT with respect to the corresponding 4-*exo* isomers, in accordance with the reported evidence that the corresponding stereochemistry change in the tropane ring leads to a strong affinity decrease.[28–30]

The presence of functional groups on the aromatic ring proved to influence the affinity (see **5d**, **5f**, **5h**, and **5j** compared to **5c**, **5l**, and **5m**). In particular, for both 4-*endo* and 4-*exo* isomers, different functionalization of the phenyl ring with halogens resulted in the same trend of DAT affinity, being 3,4-Cl_2 > 4-Cl > 4-Br > 4-I > 4-F, 4-H. In general, Bgs compounds were less potent compared to the corresponding 8-oxatropanes,[27] however a remarkable selectivity toward SERT was observed in variable extent. As reported by Meltzer, the heteroatom at position 8 directs the ligand orientation in the dopamine transporter site, as the length of the hydrogen bond between such heteroatom and the amino acid in the receptor binding site influences the position of the 3-aryl ring in the hydrophobic pocket, and the deeper placement of the 3-aryl substituent into the acceptor site of DAT, the higher potency results. Given that Bgs compounds show the same heteroatom at position 8 compared to 8-oxatropanes, the different potency could be

Table 3. Inhibition of [^{3}H]WIN35,428 binding to hDAT and [^{3}H]citalopram binding to hSERT by Bgs scaffolds

Compound	R	C-4 substituent	IC_{50}[a] (nM)		Selectivity[b]
			DAT	SERT	
2	Benzoyl	COOMe	>10,000	>10,000	—
—	Fmoc	COOMe	>10,000	>10,000	—
4	Benzyl	COOMe	>10,000	>10,000	—
3	4′-Br-Benzyl	COOMe	>10,000	>10,000	—
5c	4′-CH_3CO–Ph	COOMe	>10,000	>10,000	—
5d	3′,4′-Cl_2–Ph	COOMe	21	1042	50
5e	3′,4′-Cl_2–Ph	4-*endo*-COOMe	267	>10,000	>37
5a	2′-Naphthyl	COOMe	5456	598	0.11
5b	2′-Naphthyl	4-*endo*-COOMe	>10,000	>10,000	—
5f	4′-Cl–Ph	COOMe	163	>10,000	>61
5g	4′-Cl–Ph	4-*endo*-COOMe	1107	>10,000	>9
5h	4′-I–Ph	COOMe	219	3159	14.4
5i	4′-I–Ph	4-*endo*-COOMe	>10,000	>10,000	—
5j	4′-Br–Ph	COOMe	188	2218	12
5k	4′-Br–Ph	4-*endo*-COOMe	5320	>10,000	>1.8
5l	Ph	COOMe	>10,000	>10,000	—
5m	4′-F–Ph	COOMe	>10,000	>10,000	—

[a] IC_{50} values are expressed as a means of three experiments performed in triplicate.
[b] Selectivity results from the ratio of SERT to DAT IC_{50} values.

due to an altered fitting within the receptor, as a consequence of the presence of O-6 and N-3 heteroatoms in the bicyclic structure. Moreover, the flipping capability of the nitrogen atom could emphasize both the flexibility and the direction of the aromatic group, and either a share of O-6 in the interaction of O-8 with the transporter, or an additional interaction of O-6 within the receptor site could impart a twist with respect to the typical orientation of the tropane skeleton determining an uncorrected position of the aryl moiety. An electronic component could exert a role to the selectivity and the binding affinity toward DAT, too, due to the presence of the nitrogen atom at position 3 and also of oxygen atom at position 6, thus altering the overall charge distribution of the scaffold, as outlined in Section 3.4.

2.4. Computational analysis

In an attempt to form a clearer basis to the potential mode of binding of the new compounds, we looked at the electronic and spatial properties of these compounds in the molecular modeling package Spartan.[47] Specifically, lead compound **5d** was modeled in comparison to the known 8-oxatropane **O-1072**, to test the stereoelectronic differences of the two molecules.

Conformational searches of **O-1072** and **5d** were carried out using Monte Carlo method within MMFF94 force field,[48] and the AM1[49] semiempirical method was used to optimize the global minimum conformer. Electrostatic potential maps and atomic electrostatic charge densities (Table 4) for the minimum energy conformers of **O-1072** and **5d** were computed using ab initio quantum chemical procedures at the 3-21G* level[50] to gain insights on the basis for the stereoelectronic features leading to a different binding interaction to the DAT. The superimposition of the two global minimum structures of **5d** and **O-1072** gave a RMS value of 0.162 Å, and confirmed that the best orientation of the aromatic ring for Bgs scaffolds was found in *N*-aryl-Bgs compounds (Fig. 4). Moreover, structural data of the two compounds showed strict resemblance with respect to the bicyclic structure, and the orientation of the aromatic ring turned out to be similar.

Table 4. Atomic electrostatic charge densities for **O-1072** and **5d** calculated at the 3-21G* level

Atom[a]	**O-1072**	**5d**
C-5 (**O-1072**)/C-1 (**5d**)	0.38	0.25
C-4 (**O-1072**)/C-2 (**5d**)	−0.43	−0.17
C-3 (**O-1072**)/N-3 (**5d**)	0.54	−0.12
C-2 (**O-1072**)/C-4 (**5d**)	−0.64	−0.53
C-1 (**O-1072**)/C-5 (**5d**)	0.36	0.67
C-7 (**O-1072**)/O-6 (**5d**)	−0.17	−0.53
C-6 (**O-1072**)/C-7 (**5d**)	−0.16	0.15
O-8	−0.57	−0.56
C=O	1.19	1.16
C=*O*	−0.67	−0.65
C-1′	−0.12	0.25
C-2′	−0.27	−0.23
C-3′	0.15	0.08
C-4′	−0.03	0
C-5′	−0.08	−0.06
C-6′	−0.21	−0.40
m-Cl	−0.07	−0.08
p-Cl	−0.10	−0.07

[a] See Figure 2 for the numeration of atoms of **O-1072** and **5d**.

With regard to the electronic properties of *N*-aryl-Bgs compounds, the presence of a heteroatom-rich structure modified the electrostatic potential of the scaffold, whereas the electron density at the aromatic ring remained substantially unchanged, as shown in Figure 5.

In particular, the presence of oxygen and nitrogen atoms at positions 6 and 3 of **5d**, respectively, contributed to increase the electron density in such regions of the molecule, thus allowing additional interactions or variations of the molecular recognition about the receptor, which

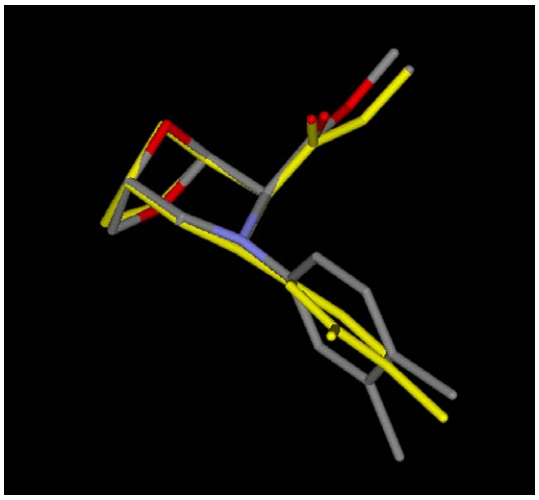


Figure 4. Superimposition of **5d** with **O-1072** (yellow).

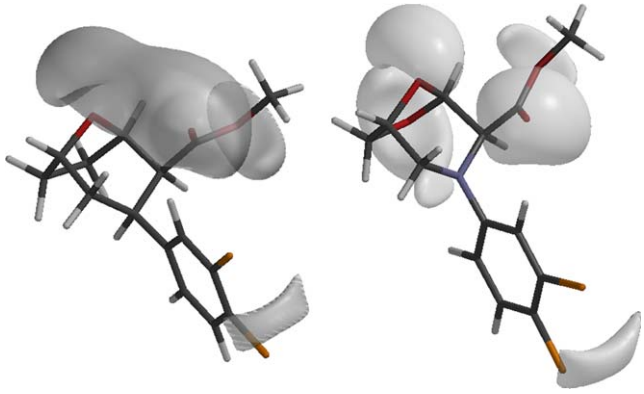


Figure 5. Three-dimensional isopotential contours of molecular electrostatic potential at −20 kcal/mol for **O-1072** (left) and compound **5d** (right).

in turn could affect affinity and selectivity toward DAT (Table 4).

Moreover, the dipolar moment of the **5d** compared to **O-1072** turned out to be different both in the absolute value and in the orientation (**5d**: $\mu = 2.82$ D, **O-1072**: $\mu = 3.41$ D; **5d**: $x = 0.489$, $y = 2.279$, $z = -1.588$; **O-1072**: $x = 1.459$, $y = -0.981$, $z = -2.922$). The electronic distribution was different with respect to the atoms of the bicyclic structure, except for C-4, C-1, and O-8 for **5d**, whereas only the C ipso of the aromatic ring (C-1′) seemed to experience different electrostatic charge densities, due to the nature of the vicinal atom at position 3 (Table 4). Thus, modeling calculations supported the hypothesis that a different electrostatic charge distribution within the bicyclic structure of **5d** compared to **O-1072**, as a consequence of the presence of two additional heteroatoms, could contribute to modulate the affinity and selectivity of **5d** toward DAT. Ultimately, the differences in the molecular electrostatic potential of **5d** and **O-1072** contributed to assume the existence of additional electrostatic interactions of O-6 with DAT, in order to explain the biological outcome of this new class of compounds.

2.5. Conclusion

The synthesis of a collection of 3-aza-6,8-dioxabicyclo[3.2.1]octane scaffolds, which possessed affinity and selectivity toward the dopamine transporter, was developed. In particular, the investigation of different substituents at nitrogen atom suggested the strict relationship between the topology of the aromatic group and the binding affinity and selectivity toward DAT and SERT monoamine transporters. Only *N*-aryl-Bgs-OMe molecules showed binding properties, in analogy with 3-aryl-tropane molecules, and specifically, compound **5d** possessing a 3,4-dichloro-phenyl group and **5a** having a naphthyl group were the most potent ligands for DAT and SERT, respectively. All the 4-*endo* isomers showed poor activity toward DAT with respect to the corresponding 4-*exo* molecules. In general, *N*-aryl-Bgs compounds showed low potency toward DAT, but nice selectivity was observed, suggesting a possible role of the two heteroatoms in exerting stereoelectronic effects responsible for a different arrangement of the scaffold within the active site of the transporters. Molecular modeling calculations on **5d** and on the reference compound **O-1072** revealed that the two compounds showed close similarity with respect to the bicyclic structure and to the orientation of the aromatic ring. The heteroatom-rich structure of *N*-aryl-Bgs **5d** modified the electrostatic potential of the bicyclic tropane scaffold, whereas the electron density at the aromatic ring remained substantially unchanged, indicating that the electrostatic charge distribution within the bicyclic structure of **5d** may modulate the affinity toward DAT, and the SERT to DAT selectivity as a consequence of a less favorable interaction at the SERT. Hence, Bgs scaffolds proved to be promising compounds for the development of new DAT ligands, as the synthesis is simple and occurs with complete stereochemical control.

3. Experimental

3.1. General

Melting points are uncorrected. Chromatographic separations were performed on silica gel using flash-column techniques; R_f values refer to TLC carried out on 25 mm silica gel 60 F_{254} plates with the same eluant indicated for column chromatography. ^1H and ^{13}C spectra were recorded with a Varian Gemini 200 MHz or Varian Mercury 400 MHz instrument using $CDCl_3$ solutions unless otherwise stated. EI-mass spectra were carried out at 70 eV ionizing voltage using a Shimadzu GCMS-QP5050 spectrometer. IR spectra were recorded with a Perkin-Elmer FT-IR-1600 spectrophotometer. Elemental analyses were obtained with a Perkin-Elmer 2400/2 C analyzer. Radioactivity was measured with a Perkin-Elmer TopCount NXT microplate scintillation counter. THF was distilled from Na/benzophenone. CH_2Cl_2 was distilled from CaH_2. All reactions requiring anhydrous conditions were performed in oven-dried glassware.

[^{3}H]WIN35,428 in ethanol (1 mCi/mL, specific activity 87 Ci/mmol, chemical purity 97%) and [^{3}H]citalopram in ethanol (1 mCi/mL, specific activity 84.2 Ci/mmol, chemical purity 97%) were purchased from Perkin-Elmer Life Sciences (Boston, MA). Crude membrane receptor preparations from cells that express recombinant human dopamine transporter (hDAT) and recombinant human serotonin transporter (hSERT) were obtained from Perkin-Elmer Life Science (Boston, MA). The receptor concentration (B_{max}) ranged from 4.2 to 1.7 and from 6.8 and 4.8 pmol/mg protein for hDAT and hSERT, respectively.

3.2. Chemistry

3.2.1. (1*R*,4*R*,5*S*)-6,8-Dioxa-3-aza-bicyclo[3.2.1]octane-4-*exo*-carboxylic acid methyl ester (1). To a solution of Fmoc-Bgs-OH (1.94 g, 5.09 mmol), prepared as reported,[36] in MeOH was dropwise added at 0 °C a freshly prepared ethereal solution of CH_2N_2 until a permanent yellow color persisted. After solvent evaporation, the corresponding methyl ester (1.83 g, 91%) was obtained as an oil. 2:3 mixture of rotamers ^{1}H NMR δ 7.75 (d, J = 7.4 Hz, 2H), 7.54 (m, 2H), 7.43–7.26 (m, 4H), 5.80 and 5.76 (s, 1H), 4.60–4.40 (m, 4H), 4.28 (m, 1H), 3.99–3.80 (m, 3H), 3.76 and 3.73 (s, 3H), 3.57–3.47 (m, 1H); ^{13}C NMR δ 168.1 (s), 156.2 and 155.9 (s), 143.5 (s, 2C), 141.2 (s, 2C), 127.7 (d, 2C), 127.1 (d, 2C), 124.9 and 124.8 (d, 2C), 120.0 (d, 2C), 98.7 and 98.3 (d), 71.8 and 71.3 (d), 67.8 (t) and 67.7 (t), 67.1 (t) and 67.0 (t), 60.1 and 59.7 (d), 52.5 and 52.4 (q), 47.2 and 47.1 (d), 46.4 and 45.9 (t); MS *m*/*z* 395 (M^+, 3), 179 (100), 172 (19), 113 (8), 59 (38). Successively, to a solution of Fmoc-Bgs-OMe (1.68 g, 4.24 mmol) in dry acetonitrile (50 mL) was added Et_2NH (1.76 mL, 17.0 mmol), then the solution was stirred at room temperature for 3.5 h. After evaporation of the solvent, the residue was dissolved in MeOH and stirred for 30 min. The white solid was filtered off and the solvent was removed, then the crude product was purified by flash chromatography (EtOAc/pet. ether, 2:1 then MeOH) to give pure **1** (720 mg, 98%) as an oil. $[\alpha]_D^{27}$ +34.9 (*c* 0.54, $CHCl_3$); ^{1}H NMR δ 5.72 (s, 1H), 4.45 (m, 1H), 4.18 (d, J = 7.0 Hz, 1H), 3.80 (m, 1H), 3.77 (s, 3H), 3.45 (s, 1H), 3.42 (d, J = 11.4 Hz, 1H), 2.64 (d, J = 11.4 Hz, 1H), 1.98 (br, 1H); ^{13}C NMR δ 170.5 (s), 99.9 (d), 73.5 (d), 67.0 (t), 60.0 (d), 52.0 (q), 46.0 (t); MS *m*/*z* 173 (M^+, 15), 128 (100), 114 (47), 86 (48), 59 (15). Anal. Calcd for $C_{13}H_{17}NO_3$: C, 66.36; H, 7.28; N, 5.95. Found: C, 66.31; H, 7.22; N, 5.86.

3.2.2. (1*R*,4*S*,5*S*)-*N*-(Benzoyl)-6,8-dioxa-3-aza-bicyclo[3.2.1]octane-4-*exo*-carboxylic acid methyl ester (2). To a solution of **1** (50 mg, 0.289 mmol) in dry THF were added dropwise at 0 °C Et_3N (77.2 μL, 0.462 mmol) and benzoyl chloride (41 μL, 0.289 mmol). The mixture was stirred at room temperature overnight, then brine was added and the two phases were separated. The aqueous layer was extracted with CH_2Cl_2 twice, and then the organic phases were dried (Na_2SO_4) and evaporated to give pure **2** (73 mg, 91%) as an oil. $[\alpha]_D^{23}$ −14.51 (*c* 0.84, $CHCl_3$); 1:1 mixture of rotamers ^{1}H NMR δ 7.37 (m, 5H), 5.89 (d, J = 1.8 Hz, 1H) and 5.72 (d, J = 2.2 Hz, 1H), 5.14 (s, 1H), 4.65 (m, 1H), 4.47–4.24 (m, 2H), 4.10 (d, J = 7.4 Hz, 1H), 3.95–3.87 (m, 2H), 3.84–3.71 (m, 4H), 3.82 (s, 3H) and 3.75 (s, 3H), 3.43 (m, 1H); ^{13}C NMR δ 172.2 (s), 167.8 (s), 134.6 (s), 130.1 (d), 130.0 (d), 128.7 (d), 128.6 (d), 126.7 (d), 126.6 (d), 99.0 (d), 98.4 (d), 72.3 (d), 71.6 (d), 67.3 (t), 66.8 (t), 52.7 (q), 49.9 (d), 44.1 (t); MS *m*/*z* 277 (M^+, 27), 219 (4), 105 (100), 77 (71); IR ($CHCl_3$) 1746, 1710, 1654, 1417 cm^{-1}. Anal. Calcd for $C_{14}H_{15}NO_5$: C, 60.64; H, 5.45; N, 5.05. Found: C, 60.79; H, 5.38; N, 4.98.

3.2.3. (1*R*,4*S*,5*S*)-*N*-(4-Bromo-benzyl)-6,8-dioxa-3-aza-bicyclo[3.2.1]octane-4-*exo*-carboxylic acid methyl ester (3). Compound **1** (70 mg, 0.404 mmol) and 4-bromobenzaldehyde (75.2 mg, 0.404 mmol) were mixed in 1,2-dichloroethane (2.0 mL) and then treated with sodium triacetoxyborohydride (120 mg, 0.566 mmol). The mixture was stirred under a nitrogen atmosphere overnight. The reaction mixture was quenched by adding aqueous saturated $NaHCO_3$ and the product was extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4) and the solvent was evaporated to give the crude free base. Purification by flash chromatography (EtOAc/pet. ether, 1:3, R_f = 0.47) gave **3** (69 mg, 50%) as an oil. $[\alpha]_D^{24}$ −11.34 (*c* 0.42, $CHCl_3$); ^{1}H NMR (400 MHz) δ 7.43 (d, J = 8.4 Hz, 2H), 7.21 (d, J = 8.4 Hz, 2H), 5.67 (d, J = 2.0 Hz, 1H), 4.51 (d, J = 5.2 Hz, 1H), 4.10 (d, J = 6.0 Hz, 1H), 3.87 (d, J = 14.0 Hz, 1H), 3.76 (m, 2H), 3.75 (s, 3H), 3.51 (d, J = 2.0 Hz, 1H), 3.35 (d, J = 11.2 Hz, 1H), 2.44 (d, J = 11.2 Hz, 1H); ^{13}C NMR (100 MHz) δ 169.9 (s), 137.5 (s), 131.5 (d), 130.2 (d), 121.0 (s), 99.7 (d), 73.2 (d), 67.6 (t), 65.2 (d), 57.3 (t), 51.5 (q), 50.6 (t); MS *m*/*z* 344 (M^+ + 2, 0.8), 342 (M^+, 1), 298 (7), 296 (7), 284 (15), 282 (17), 171 (77), 169 (78), 84 (100); IR ($CHCl_3$) 1740, 1601, 1486 cm^{-1}. Anal. Calcd for $C_{14}H_{16}BrNO_4$: C, 49.14; H, 4.71; N, 4.09. Found: C, 49.18; H, 4.69; N, 4.01.

3.2.4. (1*R*,4*S*,5*S*)-*N*-(Benzyl)-6,8-dioxa-3-aza-bicyclo[3.2.1]octane-4-*exo*-carboxylic acid methyl ester (4). Compound **4** was prepared as described for **3**. Purification by flash chromatography (EtOAc/pet. ether, 1:3, R_f = 0.44) gave pure **4** (172 mg, 57%) as an oil. $[\alpha]_D^{23}$ −5.17 (*c* 0.69, $CHCl_3$); ^{1}H NMR δ 7.28 (m, 5H), 5.67 (d, J = 2 Hz, 1H), 4.51 (m, 1H), 4.13 (d, J = 6.2 Hz, 1H), 3.84 (m, 3H), 3.75 (s, 3H), 3.53 (d, J = 2 Hz, 1H), 3.36 (d, J = 11.4 Hz, 1H), 2.46 (d, J = 11.4 Hz, 1H); ^{13}C NMR δ 170.1 (s), 138.5 (s), 128.5 (d), 128.4 (d), 127.2 (d), 99.8 (d), 73.3 (d), 67.6 (t), 65.3 (d), 57.9 (t), 51.4 (q), 50.6 (t); MS *m*/*z* 263 (M^+, 6), 218 (5), 204 (11), 158 (2), 105 (20), 91 (100); IR ($CHCl_3$) 1740, 1602, 1494, 1451, 1250 cm^{-1}. Anal. Calcd for $C_{14}H_{17}NO_4$: C, 63.87; H, 6.51; N, 5.32. Found: C, 64.02; H, 6.42; N, 5.24.

3.2.5. General procedure for N-arylation. A 150 mL stainless steel autoclave was charged with H-Bgs-OMe **1** (50 mg, 0.289 mmol, 1 equiv) dissolved in dry CH_2Cl_2 (2 mL), the appropriate boronic acid (0.867 mmol, 3 equiv), anhydrous cupric acetate (0.578 mmol, 2 equiv), base (0.867 mmol, 3 equiv), 4 Å molecular

sieves, and then oxygen up to the desired atmosphere (see Table 1). The vessel was stirred and heated. After filtration over Celite of the reaction mixture, the solvent was evaporated and the crude oil was purified by flash chromatography.

3.2.6. (1*R*,4*S*,5*S*)-*N*-Naphthalen-2-yl-6,8-dioxa-3-aza-bicyclo[3.2.1]octane-4-*exo*-carboxylic acid methyl ester (5a) and (1*R*,4*R*,5*S*)-*N*-naphthalen-2-yl-6,8-dioxa-3-aza-bicyclo[3.2.1]octane-4-*endo*-carboxylic acid methyl ester (5b). Compounds **5a** and **5b** were obtained as orange oils in 5% and 10% yield, respectively. Compound **5a**: $R_f = 0.6$ (EtOAc/pet. ether, 1:1); ^{1}H NMR δ 7.84–7.64 (m, 3H), 7.44–7.28 (m, 2H), 7.08–7.00 (m, 2H), 5.88 (s, 1H), 4.78 (m, 1H), 4.59 (s, 1H), 4.10 (d, $J = 6.6$ Hz, 1H), 3.91–3.74 (m, 2H), 3.66 (s, 3H), 3.5–3.43 (m, 1H); MS *m*/*z* 299 (M^+, 11), 58 (100). Anal. Calcd for $C_{17}H_{17}NO_4$: C, 68.21; H, 5.72; N, 4.68. Found: C, 67.97; H, 5.62; N, 4.63.

Compound **5b**: $R_f = 0.4$ (EtOAc/pet. ether, 1:1); ^{1}H NMR δ 7.78–7.63 (m, 3H), 7.40–7.29 (m, 2H), 7.12–6.94 (m, 2H), 5.83 (d, $J = 3.2$ Hz, 1H), 4.79 (m, 1H), 4.46 (d, $J = 8.0$ Hz, 1H) 4.21 (d, $J = 3.2$ Hz, 1H), 3.95 (m, 1H), 3.72 (s, 3H), 3.55 (m, 1H), 3.41(m, 1H); MS *m*/*z* 299 (M^+, 6), 178 (100). Anal. Calcd for $C_{17}H_{17}NO_4$: C, 68.21; H, 5.72; N, 4.68. Found: C, 68.16; H, 5.68; N, 4.61.

3.2.7. (1*R*,4*S*,5*S*)-*N*-(4-Acetyl-phenyl)-6,8-dioxa-3-aza-bicyclo[3.2.1]octane-4-*exo*-carboxylic acid methyl ester (5c). A yellow oil was obtained in 5% yield. $R_f = 0.14$ (Et_2O/pet. ether, 2:1). ^{1}H NMR δ 7.89 (d, $J = 9.1$ Hz, 2H), 6.72 (d, $J = 9.1$ Hz, 2H), 5.88 (s, 1H), 4.77 (m, 1H), 4.49 (s, 1H), 4.03 (d, $J = 7.0$ Hz, 1H), 3.88 (m, 1H), 3.71 (s, 3H), 3.50–3.43 (m, 2H), 2.52 (s, 3H); MS *m*/*z* 291 (M^+, 19.1), 146 (100). Anal. Calcd for $C_{15}H_{17}NO_5$: C, 61.85; H, 5.88; N, 4.81. Found: C, 61.94; H, 5.92; N, 5.02.

3.2.8. (1*R*,4*S*,5*S*)-*N*-(3,4-Dichloro-phenyl)-6,8-dioxa-3-aza-bicyclo[3.2.1]octane-4-*exo*-carboxylic acid methyl ester (5d) and (1*R*,4*R*,5*S*)-*N*-(3,4-dichloro-phenyl)-6,8-dioxa-3-aza-bicyclo[3.2.1]octane-4-*endo*-carboxylic acid methyl ester (5e). Compounds **5d** and **5e** were obtained as colorless oils in 6% and 12% yield, respectively. Compound **5d**: $R_f = 0.49$ (EtOAc/pet. ether, 1:1); ^{1}H NMR δ 7.26 (d, $J = 9.1$ Hz, 1H), 6.80 (d, $J = 2.9$ Hz, 1H), 6.54 (dd, $J = 9.1$, 2.9 Hz, 1H), 5.84 (d, $J = 1.8$ Hz, 1H), 4.73 (m, 1H), 4.34 (d, $J = 1.8$ Hz, 1H), 4.02 (d, $J = 6.6$ Hz, 1H), 3.86 (m, 1H), 3.71 (s, 3H), 3.65 (d, $J = 12.1$ Hz, 1H), 3.26 (d, $J = 12.1$ Hz, 1H); ^{13}C NMR δ 168.9 (s), 148.5 (s), 133.2 (s), 130.6 (d), 122.0 (s), 114.6 (d), 112.3 (d), 99.0 (d), 71.8 (d), 67.4 (t), 62.2 (d), 52.3 (q), 48.2 (t); MS *m*/*z* 319 (M^+, 11), 317 (M^+, 17), 260 (17), 258 (23), 176 (14), 174 (94) 172 (100). Anal. Calcd for $C_{13}H_{13}Cl_2NO_4$: C, 49.08; H, 4.12; N, 4.40. Found: C, 48.87; H, 4.08; N, 4.46.

Compound **5e**: $R_f = 0.42$ (EtOAc/pet. ether, 1:1); ^{1}H NMR δ 7.27 (d, $J = 8.8$ Hz, 1H), 6.76 (d, $J = 2.9$ Hz, 1H), 6.45 (dd, $J = 8.8$, 2.9 Hz, 1H), 5.81 (d, $J = 2.9$ Hz, 1H), 4.82 (m, 1H), 4.12 (d, $J = 7.0$ Hz, 1H), 4.05 (d, $J = 2.9$ Hz, 1H), 3.93 (m, 1H), 3.76 (s, 3H), 3.56 (d, $J = 11$ Hz, 1H), 3.39 (dd, $J = 11$, 3.7 Hz, 1H); ^{13}C NMR δ 168.6 (s), 147.9 (s), 132.6 (s), 130.1 (d), 122.5 (s), 115.8 (d), 133.3 (d), 98.0 (d), 70.9 (d), 67.0 (t), 62.5 (d), 52.4 (q), 51.5 (t); MS *m*/*z* 319 (M^+, 9), 317 (M^+, 12), 260 (5), 258 (8), 176 (10), 174 (73), 172 (100). Anal. Calcd for $C_{13}H_{13}Cl_2NO_4$: C, 49.08; H, 4.12; N, 4.40. Found: C, 48.85; H, 4.07; N, 4.37.

3.2.9. (1*R*,4*S*,5*S*)-*N*-(4-Chloro-phenyl)-6,8-dioxa-3-aza-bicyclo[3.2.1]octane-4-*exo*-carboxylic acid methyl ester (5f) and (1*R*,4*R*,5*S*)-*N*-(4-chloro-phenyl)-6,8-dioxa-3-aza-bicyclo[3.2.1]octane-4-*endo*-carboxylic acid methyl ester (5g). Compounds **5f** and **5g** were obtained as oils in 10% and 16% yield, respectively. Compound **5f**: $R_f = 0.6$ (EtOAc/pet. ether, 1:1); ^{1}H NMR δ 7.18 (d, $J = 9.2$ Hz, 2H), 6.64 (d, $J = 9.2$ Hz, 2H), 5.83 (s, 1H), 4.75 (m, 1H), 4.37 (s, 1H), 4.03 (d, $J = 6.6$ Hz, 1H), 3.85 (t, $J = 6.6$ Hz, 1H), 3.68 (s, 3H), 3.65 (m, 1H), 3.27 (d, $J = 11.6$ Hz, 1H); ^{13}C NMR δ 169.2 (s), 148.1 (s), 129.1 (d), 124.9 (s), 114.0 (d), 99.0 (d), 72.1 (d), 67.4 (t), 62.4 (d), 52.2 (q), 48.2 (t); MS *m*/*z* 285 ($M^+ + 2$, 9), 283 (M^+, 29), 226 (9), 224 (29), 199 (17), 197 (52), 140 (42), 138 (100), 113 (17), 111 (48), 85 (32), 59 (10), 57 (92). Anal. Calcd for $C_{13}H_{14}ClNO_4$: C, 55.04; H, 4.97; N, 4.94. Found: C, 54.97; H, 4.86; N, 4.85.

Compound **5g**: $R_f = 0.51$ (EtOAc/pet. ether, 1:1); ^{1}H NMR δ 7.20 (d, $J = 9.0$ Hz, 2H), 6.61 (d, $J = 9.0$ Hz, 2H), 5.79 (d, $J = 2.8$ Hz, 1H), 4.79 (m, 1H), 4.16 (d, $J = 6.2$ Hz, 1H), 4.03 (d, $J = 2.8$ Hz, 1H), 3.92 (t, $J = 6.2$ Hz, 1H), 3.74 (s, 3H), 3.59 (d, $J = 11.4$ Hz, 1H), 3.34 (dd, $J = 11.4$, 3.2 Hz, 1H); ^{13}C NMR δ 169.0 (s), 147.3 (s), 128.7 (d), 124.9 (s), 115.8 (d), 98.4 (d), 71.4 (d), 67.1 (t), 63.0 (d), 52.4 (q), 52.2 (t); MS *m*/*z* 285 ($M^+ + 2$, 5), 283 (M^+, 15), 226 (4), 224 (12), 199 (11), 197 (30), 140 (37), 138 (100), 113 (14), 111 (45), 85 (16), 59 (11), 57 (50). Anal. Calcd for $C_{13}H_{14}ClNO_4$: C, 55.04; H, 4.97; N, 4.94. Found: C, 54.91; H, 4.87; N, 4.88.

3.2.10. (1*R*,4*S*,5*S*)-*N*-(4-Iodo-phenyl)-6,8-dioxa-3-aza-bicyclo[3.2.1]octane-4-*exo*-carboxylic acid methyl ester (5h) and (1*R*,4*R*,5*S*)-*N*-(4-iodo-phenyl)-6,8-dioxa-3-aza-bicyclo[3.2.1]octane-4-*endo*-carboxylic acid methyl ester (5i). Compounds **5h** and **5i** were obtained as oils in 12% and 13% yield, respectively. Compound **5h**: $R_f = 0.6$ (EtOAc/pet. ether, 1:1); ^{1}H NMR (400 MHz) δ 7.49 (d, $J = 8.8$ Hz, 2H), 6.49 (d, $J = 8.8$ Hz, 2H), 5.83 (d, $J = 1.6$ Hz, 1H), 4.74 (m, 1H), 4.36 (d, $J = 1.6$ Hz, 1H), 4.02 (d, $J = 6.8$ Hz, 1H), 3.85 (t, $J = 6.8$ Hz, 1H), 3.68 (s, 3H), 3.64 (d, $J = 10$ Hz, 1H), 3.28 (d, $J = 11.6$ Hz, 1H); ^{13}C NMR δ 169.2 (s), 148.9 (s), 137.9 (d), 115.0 (d), 99.0 (d), 80.7 (s), 72.0 (d), 67.4 (t), 62.1 (d), 52.2 (q), 47.9 (t); MS *m*/*z* 375 (M^+, 45), 316 (18), 289 (60), 230 (85), 203 (25), 76 (86), 57 (100). Anal. Calcd for $C_{13}H_{14}INO_4$: C, 41.62; H, 3.76; N, 3.73. Found: C, 41.57; H, 3.66; N, 3.64.

Compound **5i**: $R_f = 0.51$ (EtOAc/pet. ether, 1:1); ^{1}H NMR δ 7.50 (d, $J = 9.2$ Hz, 2H), 6.44 (d, $J = 9.2$ Hz, 2H), 5.80 (d, $J = 3.4$ Hz, 1H), 4.80 (m, 1H), 4.13 (d,

J = 6.6 Hz, 1H), 4.03 (d, J = 3.4 Hz, 1H), 3.92 (t, J = 6.6 Hz, 1H), 3.74 (s, 3H), 3.58 (d, J = 11.4 Hz, 1H), 3.35 (dd, J = 11.4, 3.8 Hz, 1H); ^{13}C NMR δ 169.1 (s), 148.7 (s), 137.6 (d), 116.5 (d), 98.4 (d), 81.9 (s), 71.4 (d), 67.3 (t), 62.9 (d), 52.6 (q), 51.9 (t); MS *m*/*z* 375 (M^+, 38), 316 (11), 289 (54), 230 (87), 203 (25), 76 (100), 59 (46), 57 (87). Anal. Calcd for $C_{13}H_{14}INO_4$: C, 41.62; H, 3.76; N, 3.73. Found: C, 41.51; H, 3.70; N, 3.68.

3.2.11. (1*R*,4*S*,5*S*)-*N*-(4-Bromo-phenyl)-6,8-dioxa-3-aza-bicyclo[3.2.1]octane-4-*exo*-carboxylic acid methyl ester (5j) and (1*R*,4*R*,5*S*)-*N*-(4-bromo-phenyl)-6,8-dioxa-3-aza-bicyclo[3.2.1]octane-4-*endo*-carboxylic acid methyl ester (5k). Compounds **5j** and **5k** were obtained as oils in 13% and 14% yield, respectively. Compound **5j**: R_f = 0.66 (EtOAc/pet. ether, 1:1); ^{1}H NMR δ 7.32 (d, J = 8.8 Hz, 2H), 6.60 (d, J = 8.8 Hz, 2H), 5.83 (d, J = 1.8 Hz, 1H), 4.73 (m, 1H), 4.37 (d, J = 1.8 Hz, 1H), 4.03 (d, J = 6.6 Hz, 1H), 3.85 (m, 1H), 3.68 (s, 3H), 3.65 (m, 1H), 3.27 (m, 1H); ^{13}C NMR δ 169.2 (s), 147.8 (s), 132.0 (d), 114.5 (d), 111.2 (s), 99.0 (d), 72.0 (d), 67.4 (t), 62.3 (d), 52.2 (q), 48.1 (t); MS *m*/*z* 329 (M^+ + 2, 42), 327 (M^+, 43), 270 (45), 268 (45), 243 (98), 241 (100), 184 (4.3), 182 (4.8), 172 (2), 157 (5), 155 (5), 117 (6.5), 91 (34), 85 (9), 59 (24), 57 (63). Anal. Calcd for $C_{13}H_{14}BrNO_4$: C, 47.58; H, 4.30; N, 4.27. Found: C, 47.67; H, 4.25; N, 4.16.

Compound **5k**: R_f = 0.58 (EtOAc/pet. ether, 1:1); ^{1}H NMR δ 7.33 (d, J = 9.0 Hz, 2H), 6.55 (d, J = 9.0 Hz, 2H), 5.8 (d, J = 3.2 Hz, 1H), 4.80 (m, 1H), 4.15 (d, J = 6.8 Hz, 1H), 4.03 (d, J = 3.2 Hz, 1H), 3.92 (t, J = 6.8 Hz 1H), 3.74 (s, 3H), 3.58 (d, J = 11.0 Hz, 1H), 3.34 (dd, J = 11.0, 3.0 Hz, 1H); ^{13}C NMR δ 169.1 (s), 147.6 (s), 131.8 (d), 116.2 (d), 112. 3 (s), 98.5 (d), 71.5 (d), 67.3 (t), 63.1 (d), 52.6 (q), 52.2 (t); MS *m*/*z* 329 (M^+ + 2, 9), 327 (M^+, 9), 270 (7.2), 268 (7.4), 243 (25.4), 241 (27), 184 (58), 182 (61), 157 (16), 155 (20), 57 (100). Anal. Calcd for $C_{13}H_{14}BrNO_4$: C, 47.58; H, 4.30; N, 4.27. Found: C, 47.45; H, 4.21; N, 4.19.

3.2.12. (1*R*,4*S*,5*S*)-*N*-(Phenyl)-6,8-dioxa-3-aza-bicyclo[3.2.1]octane-4-*exo*-carboxylic acid methyl ester (5l). A yellowish oil was obtained (14%). R_f = 0.47 (EtOAc/pet. ether, 1:2); ^{1}H NMR δ 7.24 (m, 2H), 6.82 (t, J = 7.3 Hz, 1H), 6.72 (d, J = 8.8 Hz, 2H), 5.83 (d, J = 1.4 Hz, 1H), 4.75 (m, 1H), 4.43 (d, J = 1.4 Hz, 1H), 4.04 (d, J = 6.3 Hz, 1H), 3.85 (t, J = 6.3 Hz, 1H), 3.70 (m, 1H), 3.68 (s, 3H), 3.34 (d, J = 11.8 Hz, 1H); ^{13}C NMR δ 169.6 (s), 148.7 (s), 129.3 (d), 118.9 (d), 112.8 (d), 99.2 (d), 72.2 (d), 67.4 (t), 62.3 (d), 52.1 (q), 48.0 (t); MS *m*/*z* 249 (M^+, 30), 190 (21), 163 (25), 117(8), 104 (100), 91 (13), 77 (88). Anal. Calcd for $C_{13}H_{15}NO_4$: C, 62.64; H, 6.07; N, 5.62. Found: C, 62.57; H, 5.98; N, 5.59.

3.2.13. (1*R*,4*S*,5*S*)-*N*-(4-Fluoro-phenyl)-6,8-dioxa-3-aza-bicyclo[3.2.1]octane-4-*exo*-carboxylic acid methyl ester (5m). A brown oil was obtained (30%). R_f = 0.48 (EtOAc/pet. ether, 1:2; 1% Et_3N); ^{1}H NMR δ 6.95 (m, 2H), 6.67 (dd, J = 9.0, 4.4 Hz, 2H), 5.82 (d, J = 2.0 Hz, 1H), 4.72 (m, 1H), 4.36 (d, J = 2.0 Hz, 1H), 4.05 (d, J = 6.6 Hz, 1H), 3.85 (t, J = 6.6 Hz, 1H), 3.70 (m, 1H), 3.67 (s, 3H), 3.23 (dd, J = 9.8, 1.8 Hz, 1H); ^{13}C NMR δ 169.3 (s), 156.4 (s, J_F = 236 Hz), 145.1 (s), 115.6 (d, J_F = 22 Hz), 114.2 (d, J_F = 7.5 Hz), 99.1 (d), 72.2 (d), 67.4 (t), 62.9 (d), 52.1 (q), 48.5 (t); MS *m*/*z* 267 (M^+, 29), 208 (33), 181 (51), 122 (100), 109 (10), 75 (26), 59 (9), 57 (12). Anal. Calcd for $C_{13}H_{14}FNO_4$: C, 58.42; H, 5.28; N, 5.24. Found: C, 58.38; H, 5.14; N, 5.17.

3.3. Biology

3.3.1. Receptor preparation. The membranes suspended in 50 mM Tris–HCl, pH 7.4, 10% sucrose for dopamine transporter and 50 mM Tris–HCl, pH 7.4, 5 mM KCl, 10% sucrose for serotonin transporter were thawed, diluted with binding buffer (50 mM Tris–HCl, 100 mM NaCl, pH 7.4, for hDAT and 50 mM Tris–HCl, 120 mM NaCl, 5 mM KCl, pH 7.4, for hSERT), and processed with a Polytron homogenizer (Ultraturrax T8, IKA, Wilmington, NC).

3.3.2. Dopamine and serotonin transporters' binding affinity assays. Competition binding studies were performed with a fixed concentration of radioligand ([^{3}H]WIN35,428 8 nM to label DAT and [^{3}H]citalopram 1 nM to label SERT) and concentrations ranging from 100 μM to 1 nM of the tested molecules. Stock solutions of 1 mM were prepared by dissolving the tested compounds in an EtOH/H_2O mixture where the EtOH final concentration was not superior to 2%. A 0.5% polyethylenimine solution (0.05 mL to each well) was added to UniFilter-96 GF/C glass fiber filter plates (Perkin-Elmer Life Sciences, Boston, MA) and the plates were allowed to incubate at 4 ° C for 2 h. All assays were performed in triplicate on 96-well microplates in a final volume of 0.2 mL, each containing the following species: 0.04 mL of radioligand, 0.04 mL of tested compound, 0.02 mL of diluted membrane suspension, and 0.10 mL of incubation buffer. Non-specific binding was defined as radioligand bound in the presence of an excess (10 μM final) of GBR12909 or Citalopram in DAT and SERT assays, respectively. After incubation for 2 h, binding was terminated by rapid filtration over a FilterMate Harvester system (Perkin-Elmer Life Sciences, Boston, MA). Filters were washed nine times with the cold buffer 50 mM Tris–HCl, 0.9% NaCl, pH 7.4, for DAT and 0.9% NaCl for SERT, dried, and counted in the TopCount NXT microplate scintillation counter (Perkin-Elmer Life Sciences, Boston, MA) using 0.25 mL of MicroScint-40 liquid scintillation (Perkin-Elmer Life Sciences, Boston, MA).

3.3.3. Data analysis. Relative affinity values (IC_{50}) were determined by fitting binding inhibition values by non-linear regression using GraphPad curve fitting software (PRISM, San Diego, California).

3.4. Molecular modeling

Calculations were performed using SPARTAN version 5.1[47] running on a SGI IRIX 6.5 workstation. Conformational searches of **O-1072** and **5d** were carried out using Monte Carlo method within

MMFF94 force field,[48] and the AM1 semiempirical method[49] was used to optimize the global minimum conformer. The geometry of the most abundant minimum energy conformer was successively subjected to ab initio single point calculation of the electronic properties at the 3-21G*/HF level[50] of quantum chemical theory. The electrostatic potentials were sampled over the entire accessible surface of the molecules (corresponding roughly to a van der Waals contact surface).

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References and notes

1. Hornykiewicz, O. *Pharmacol. Rev.* **1966**, *18*, 925–961.
2. Rowley, M.; Bristow, L. J.; Hutson, P. H. *J. Med. Chem.* **2001**, *44*, 477–501.
3. Nieoullon, A. *Prog. Neurobiol.* **2002**, *67*, 53–83.
4. Castellanos, F. X.; Tannock, R. *Nat. Rev. Neurosci.* **2002**, *3*, 617–628.
5. Kaufman, M. J.; Madras, B. K. *Synapse* **1991**, *9*, 43–49.
6. Morgan, G. F.; Nowotnik, D. P. *Drug News Perspect.* **1999**, *12*, 137.
7. Dougherty, D. D.; Bonab, A. A.; Spencer, T. J.; Rauch, S. L.; Madras, D. K.; Fischman, A. J. *Lancet* **1999**, *354*, 2132–2133.
8. Carroll, F. I.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J. *J. Med. Chem.* **1992**, *35*, 969–981.
9. Fowler, J. S.; Volkow, N. D.; Wang, G. J.; Gatley, S. J.; Logan, J. *Nucl. Med. Biol.* **2001**, *28*, 561–572.
10. Volkow, N. D.; Gatley, S. J.; Fowler, J. S.; Logan, J.; Fischman, M.; Gifford, A. N.; Pappas, N.; King, P.; Vitkun, S.; Ding, Y. S.; Wang, G. J. *Synapse* **1998**, *24*, 399–402.
11. Kuhar, M. J.; Ritz, M. C.; Boja, J. W. *Trends Neurosci.* **1991**, *14*, 299–302.
12. Carroll, F. I.; Gao, Y.; Rahman, M. A.; Abraham, P.; Parham, K.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J. *J. Med. Chem.* **1991**, *34*, 2719–2725.
13. Carroll, F. I. *J. Med. Chem.* **2003**, *46*, 1775–1794.
14. Carroll, F. I.; Kotian, P.; Dehghani, A.; Gray, J. L.; Kuzemko, M. A.; Parham, K. A.; Abraham, P.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J. *J. Med. Chem.* **1995**, *38*, 379–388.
15. Carroll, F. I.; Gray, J. L.; Abraham, P.; Kuzemko, M. A.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J. *J. Med. Chem.* **1993**, *36*, 2886–2890.
16. Holmquist, C. R.; Keverline-Frantz, K. I.; Abraham, P.; Boja, J. W.; Kumar, M. J.; Carroll, F. I. *J. Med. Chem.* **1996**, *39*, 4139–4141.
17. Meltzer, P. C.; Liang, A. Y.; Madras, B. K. *J. Med. Chem.* **1994**, *37*, 2001–2010.
18. Meltzer, P. C.; Liang, A. Y.; Brownell, A.-L.; Elmaleh, D. R.; Madras, B. K. *J. Med. Chem.* **1993**, *36*, 855–862.
19. Kozikowski, A. P.; Saiah, M. K. E.; Johnson, K. M.; Bergmann, J. S. *J. Med. Chem.* **1995**, *38*, 3086–3093.
20. Meltzer, P. C.; Liang, A. Y.; Madras, B. K. *J. Med. Chem.* **1996**, *39*, 371–379.
21. Lewin, A. H.; Gao, Y.; Abraham, P.; Boja, J. W.; Kuhar, M. J.; Carroll, F. I. *J. Med. Chem.* **1992**, *35*, 135–140.
22. Abraham, P.; Pitner, J. B.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J.; Carroll, F. I. *J. Med. Chem.* **1992**, *35*, 141–144.
23. Carroll, F. I.; Mascarella, S. W.; Kuzemko, M. A.; Gao, Y.; Abraham, P.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J. *J. Med. Chem.* **1994**, *37*, 2865–2873.
24. Meltzer, P. C.; Blundell, P.; Huang, H.; Liu, S.; Yong, Y. F.; Madras, B. K. *Bioorg. Med. Chem.* **2000**, *8*, 581–590.
25. Milius, R. A.; Saha, J. K.; Madras, B. K.; Neumeyer, J. L. *J. Med. Chem.* **1991**, *34*, 1728–1731.
26. Singh, S. *Chem. Rev.* **2000**, *100*, 925–1024.
27. Meltzer, P. C.; Liang, A. Y.; Blundell, P.; Gonzalez, M. D.; Chen, Z.; Clifford, G.; Madras, B. K. *J. Med. Chem.* **1997**, *40*, 2661–2673.
28. Ritz, M. C.; Cone, E. J.; Kuhar, M. J. *Life Sci.* **1990**, *46*, 635–645.
29. Reith, M. E. A.; Meisler, B. E.; Sershen, H.; Lajtha, A. *Biochem. Pharmacol.* **1986**, *35*, 1123–1129.
30. Clarke, R. L.; Daum, S. J.; Gambino, A. J.; Aceto, M. D.; Pearl, J.; Levitt, M.; Cumiskey, W. R.; Bogado, E. F. *J. Med. Chem.* **1973**, *16*, 1260–1267.
31. Madras, B. K.; Pristupa, Z. B.; Niznik, H. B.; Liang, A. Y.; Blundell, P.; Gonzales, M. D.; Meltzer, P. C. *Synapse* **1996**, *24*, 340–348.
32. Meltzer, P. C.; Blundell, P.; Yong, Y. F.; Zhengming, C.; George, C.; Gonzalez, M. D.; Madras, B. K. *J. Med. Chem.* **2000**, *43*, 2982–2991.
33. Quirante, J.; Vila, X.; Bonjoch, J.; Kozikowski, A. P.; Johnson, K. M. *Bioorg. Med. Chem.* **2004**, *12*, 1383–1391.
34. Guarna, A.; Guidi, A.; Machetti, F.; Menchi, G.; Occhiato, E. G.; Scarpi, D.; Sisi, S.; Trabocchi, A. *J. Org. Chem.* **1999**, *64*, 7347–7364.
35. Trabocchi, A.; Menchi, G.; Guarna, F.; Machetti, F.; Scarpi, D.; Guarna, A. *Synlett* **2006**, 331–353.
36. Bicyclic compounds from Glyceraldehyde and Serine derivatives (BG(g)S(s)). Upper cases refer to the stereoisomer derived from D-glyceraldehyde and L-serine derivatives, whereas lower cases refer to compounds derived from their enantiomers. See: Trabocchi, A.; Cini, N.; Menchi, G.; Guarna, A. *Tetrahedron Lett.* **2003**, *44*, 3489–3492.
37. Diazomethane proved to be necessary for ester generation, as degradation was observed when common esterification methods, such as Fischer reaction with $SOCl_2$, were attempted.
38. Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. *J. Org. Chem.* **1996**, *61*, 3849–3862.
39. Wolfe, J. P.; Wagaw, S.; Marcoux, J. F.; Buchwald, S. L. *Acc. Chem. Res.* **1998**, *31*, 805–818.
40. Quach, T. D.; Batey, R. A. *Org. Lett.* **2003**, *5*, 4397–4400.
41. Lam, P. Y. S.; Bonne, D.; Vincent, G.; Clark, C. G.; Combs, A. P. *Tetrahedron Lett.* **2003**, *44*, 1691–1694.
42. Antilla, J. C.; Buchwald, S. L. *Org. Lett.* **2001**, *3*, 2077–2079.
43. Wolfe, J. P.; Buchwald, S. L. *Tetrahedron Lett.* **1997**, *38*, 6359–6362.
44. In the literature is reported the affinity of new compounds toward DAT and SERT receptors using different biological systems, such as monkey caudate putamen, rat striatum, and HEK cells transfected with hDAT. See, for example: Ref. 27; Ref. 14 (a) Meltzer, P. C.; Liu, S.; Blanchette, H. S.; Blundell, P.; Madras, B. K. *Bioorg.*

Med. Chem. **2002**, *10*, 3583–3591; This fact does not facilitate the exact comparison of the affinity of different classes of compounds. However in some cases, when the same class is tested in two different systems, a common general trend of variation of the affinity was observed. See: (b) Dar, D. E.; Thiruvazhi, M.; Kitayama, S.; Kopajtic, T. A.; Gamliel, A.; Slusher, B. S.; Carroll, F. I.; Uhl, G. R. *Eur. J. Med. Chem.* **2005**, *40*, 1013–1021.

45. Pristupa, Z. B.; Wilson, J. M.; Hoffman, B. J.; Kish, S. J.; Niznik, H. B. *Mol. Pharmacol.* **1994**, *45*, 125–135.
46. Goulet, M.; Miller, G. M.; Bendor, J.; Liu, S.; Meltzer, P. C.; Madras, B. K. *Synapse* **2001**, *42*, 129–140.
47. Wavefunction, Inc., Irvine, CA.
48. (a) Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 490–519; (b) Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 520–552; (c) Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 553–586; (d) Halgren, T. A.; Nachbar, R. B. *J. Comput. Chem.* **1996**, *17*, 587–615; (e) Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 616–641.
49. Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902–3909.
50. Hehre, W. J.; Radam, L.; Schleyer, P. R.; Pople, J. A. *Ab initio Molecular Orbital Theory*; Wiley: New York, 1986.