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ORIGINAL RESEARCH ARTICLE

Further exploration of N-4 substituents on the piperazine ring of the hybrid template 5/ 7-{[2-(4-Aryl-piperazin-1-yl)-ethyl]-propyl-amino}-5,6,7,8-tetrahydro-naphthalen-2-ol and its analog: development of an exceptionally potent agonist for D_2 & D_3 receptors

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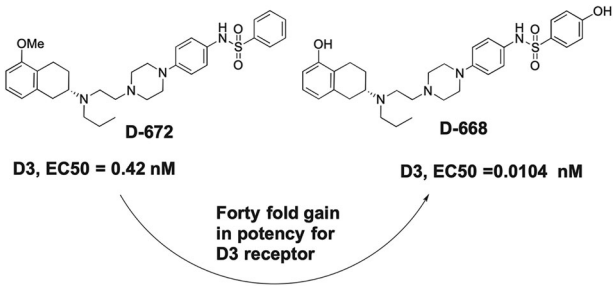
Abstract

In this manuscript we report a structure-activity relationship (SAR) study of analogs of 5/ 7-{[2-(4-Aryl-piperazin-1-yl)-ethyl]-propyl-amino}-5,6,7,8-tetrahydro-naphthalen-2-ol. Our study is focused on introduction of various bioisoteric and aromatic substitutions on the piperazine ring of the hybrid template to further probe into the accessory binding domains on dopamine D_2/D_3 receptors. Specifically, the goal behind this study is to delineate the nature of the binding pockets for such substitutions on the piperazine ring to determine their influence on binding affinity (Ki), as measured with tritiated spiperone and HEK-293 cells expressing either D_2 or D_3 receptors. Functional activity of selected compounds was assessed with the GTPγS binding assay. Our data indicates that various N-substitution with substituted and unsubstituted benzene sulfonyl group produced varied affinity and potency for D_2/D_3. Compound **D-660** produced highest selectivity for the D_3 receptor in the binding assay. In general, presence of hydroxyl group improved overall activity for both D_2/D_3 receptors. One such compound **D-668** produced exceptional potencies for both the receptors. Overall, our results suggest that binding to the sites removed from the orthosteric binding sites contribute significantly to enhance functional potencies of ligands.



Keywords Dopamine receptors · D_2 receptor · D_3 receptor · Agonist · Structure activity relationship study

Introduction

The dopamine (DA) receptors have been targeted for drug development for a number of Central Nervous System (CNS) disorders, including drug abuse, schizophrenia, and Parkinson's disease (PD) [1–7]. DA receptors are found throughout the CNS and periphery. Five subtypes of DA receptors have been identified and are classified as being either D_1-like or D_2-like [8]. These classifications are based on receptor pharmacology and function [9–12]. The D_1 and

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D_5 subtypes, known as D1-like, activate adenylate cyclase activity upon receptor activation. The D_2-like receptors, which include the D_2, D_3, and D_4 subtypes, inhibit adenylate cyclase activity. D_3 receptors were found to have a different distribution in the brain from that of D_2 receptors [13, 14]. Recent study on the brain distribution of D_3 receptors indicated highest density in the nucleus accumbens. In addition D_3 receptors are also expressed at a higher level compared to D_2 receptors in the extrastriatal regions and also in the thalamus [14]. The D_2 and D_3 receptor subtypes possess 50% overall structural homology, and 75–80% in the agonist binding domains [2, 15].

Many compounds have been developed with various selectivity for the D_3 versus D_2 receptor [16–23]. Due to high homology, development of selective agonists for D_3 receptor is rather difficult as both receptors share nearly identical orthosteric active binding sites for agonist interaction [21, 24–27]. Some of the well known D_3 selective agonists include ropinirole and pramipexole, and these agonists were shown to exhibit a 4- to 10-fold higher affinity for the D_3 than D_2 receptor [28]. In our own work, we have demonstrated development of some of the highly selective agonists for D_3 receptors e.g. D-264, D-443 etc. (Fig. 1) that have been reported to date [20–22]. In comparison, a number of highly selective D_3 antagonists have been developed. In the majority of these compounds there is a piperazine ring connected to a suitable benzamide-type moiety via a variable-size linker, such as in BP 897 (Fig. 1) [16, 17, 29–31].

In our previous structure activity relationship (SAR) study on our hybrid template developed earlier, we mapped out different aspects of structural alterations on affinity and selectivity for D_3 receptor [21–23, 32, 33]. Some of those studies involved the incorporation of hydrophobic moieties on the distal part of the hybrid molecular template which led to enhancement of affinity and selectivity for D_3 receptor in general [20, 34]. The compound D-264 (Fig. 1) is one such compound emerged from such studies. The compound D-264 also exhibited potent in vivo neuroprotection efficacy in the MPTP mouse model [4]. The part of the neuroprotection effect was attributed to its high affinity for the D_3 receptor [4]. In our current study, we wanted to explore the effect of bio-isosteric replacement of N-substituted

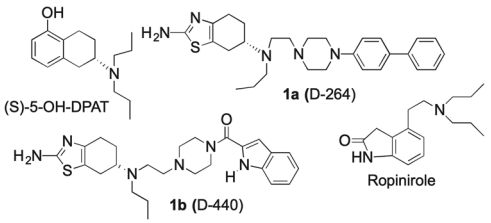


Fig. 1 Molecular structures of dopamine D_3 receptor preferring agonists

group on the piperazine ring in the highly D_3 selective compound D-443 (Fig. 1). Impact on binding due to strongly electron withdrawing phenyl sulfone group on basicity of the piperazine nitrogen atom was evaluated. Additionally, we wanted to evaluate effect of similar N-substitution on phenyl aniline moiety with introduction of additional aromatic hydrophobic moiety.

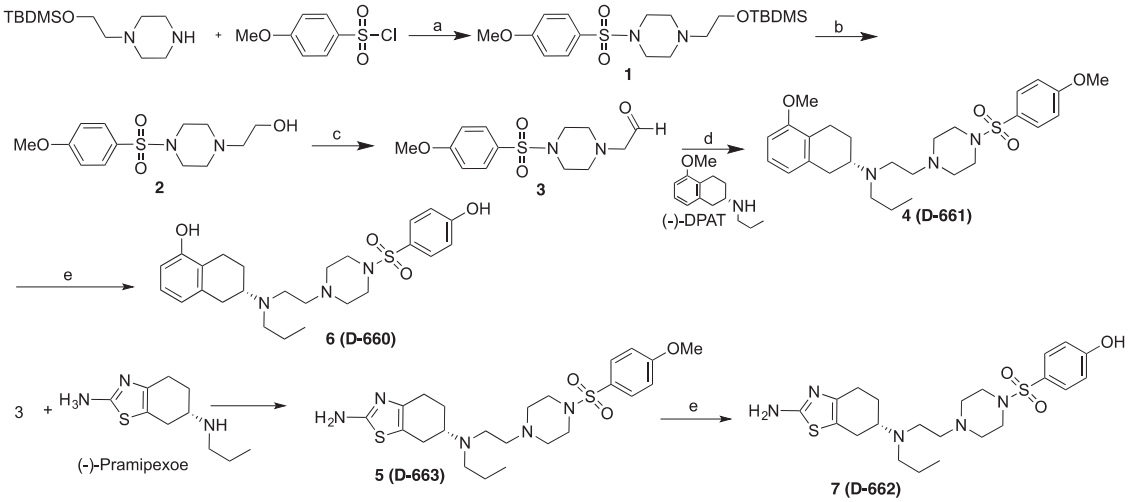
Results and discussion

Chemistry

Scheme 1 describes synthesis of four final target molecules. The starting compound 1-(2-((tert-butyldimethylsilyl)oxy)-ethyl)piperazine was synthesized by following a procedure published by us [35]. Derivatization of the starting compound with 4-methoxy-benzenesulfonyl chloride followed by deprotection of the TBDMS group produced the intermediate **2** which on oxidation produced the intermediate **3**. Reductive amination of **3** with either (*S*)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine ((-)-DPAT) or (*S*)-N^6-propyl-4,5,6,7-tetrahydrobenzo[*d*]thiazole-2,6-diamine ((-)-Pramipexole) in presence of NaBH(OAc)3 produced compounds **4** (**D-661**) and **5** (**D-663**). The optically active amine intermediates with known absolute stereochemistry were previously synthesized by us [20, 36]. Demethylation of the methoxy group in **4** and **5** in presence of boron tribromide produced compounds **6 (D-660)** and **7 (D-662)**.

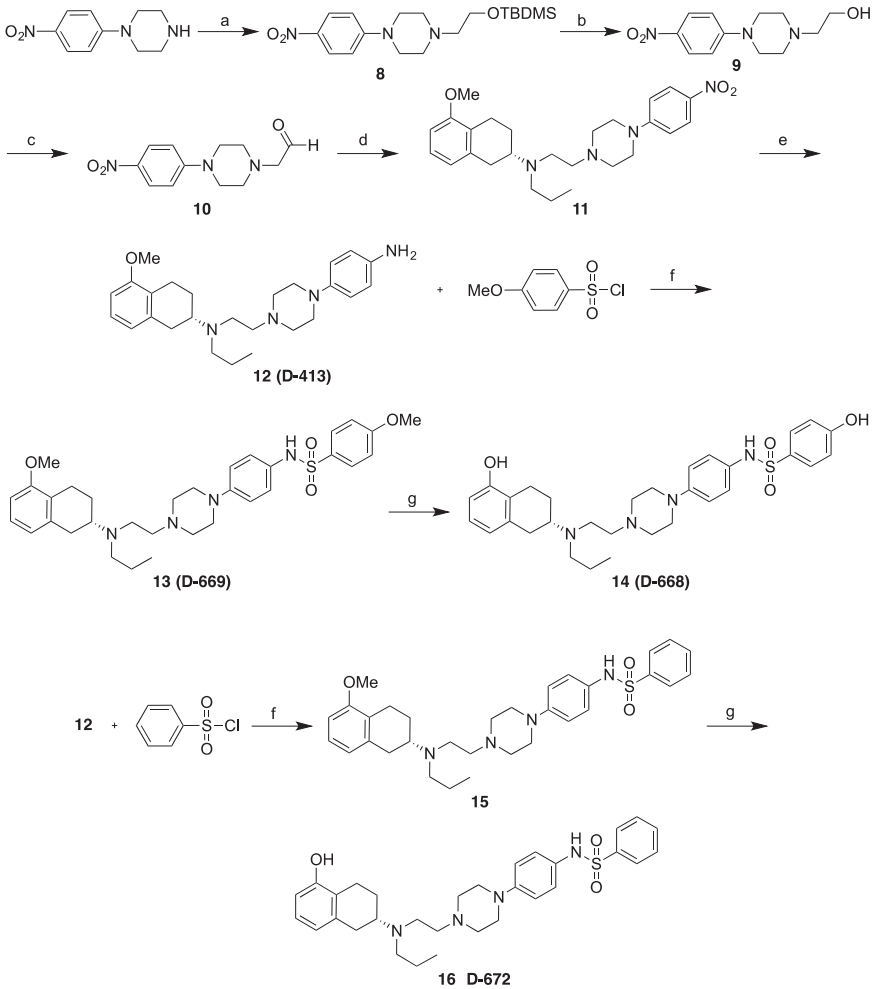
The Scheme 2 describes the synthesis of the final compounds **D-668, D-669** and **D-672**. The starting compound 1-(4-nitro-phenyl)-piperazine was N-alkylated with (2-bromoethoxy)(*tert*-butyl)dimethylsilane in presence of a base by following our earlier reported method to yield intermediate **8**. Deprotection of the TBDMS group followed by oxidation yielded **10** which underwent reductive amination followed by reduction of the nitro group to produce **12**. The compound **12** was transformed into **13** (**D-669**) by treatment with 4-methoxy-benzenesulfonyl chloride which on demethylation yielded **14** (**D-668**). The reaction of compound **12** with benzenesulfonyl chloride produced 15 which on demethylation yielded the final compound **16** (**D-672**).

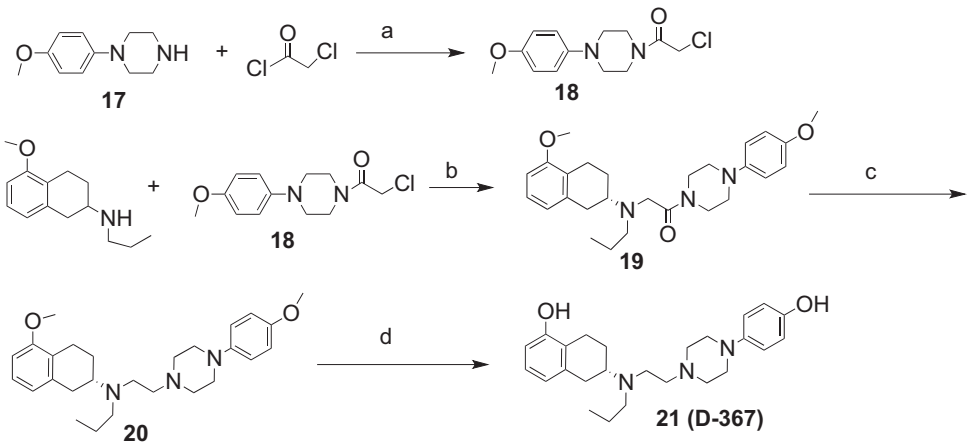
The Scheme 3 describes synthesis of hydroxy compound **21** (**D-367**). Reaction of Chloroacetylchloride with commercially available 4-methocy phenyl piperazine produced **18**. *N*-alkylation of 5-methoxy-*N*-propyl-1,2,3,4-tetrahydronaphthalen-2-amine with **18** followed by reduction produced the intermediate **20**. In the final step demethylation by refluxing with HBr yielded compound **21** (**D-367**).



Scheme 1 Reagents and conditions: **a** Et_3N, CH_2Cl_2, 0 °C, 1 h; **b** *n*-Bu_4NF, THF, 0 °C to rt, 2 h; **c** SO_3.py, CH_2Cl_2:DMSO (2:1), Et_3N, 0 °C to rt, 2 h; **d** (-)-DPAT or (-)-pramipexole, $NaBH(OAc)_3$, CH_2Cl_2, rt, 48 h; **e** BBr_3, CH_2Cl_2, −78 °C to rt, overnight

Scheme 2 Reagents and conditions: **a** (2-Bromo-ethoxy)-*tert*-butyl-dimethyl-silane, K_2CO_3, CH_3CN, 90 °C, overnight; **b** *n*-Bu_4NF, THF, 0 °C to rt, 2 h; **c** SO_3.py, CH_2Cl_2:DMSO (2:1), Et_3N, 0 °C to rt, 2 h; **d** (-)-DPAT, $NaBH(OAc)_3$, CH_2Cl_2, rt, 48 h; **e** H_2, Pd/C, 50 psi, MeOH/EtOAc, rt, overnight; **f** Et_3N, CH_2Cl_2, 0 °C to rt, 3 h; **g** BBr_3, CH_2Cl_2, −78 °C to rt, overnight





Scheme 3 Reagents and conditions: **a** Et_3N, CH_2Cl_2, rt; **b** K_2CO_3, CH_3CN, reflux; **c** $LiALH_4$, THF, reflux; **d** Aqueous HBr, reflux

Table 1 Ki values (nM) are for inhibition of [^{3}H] spiroperidol binding to HEK-D_2/D_3 cells and are given as the mean ± SEM for 3 to 6 independent experiments carried out in triplicate

Compound	Ki, (nM), D_2 [^{3}H] Spiperone	Ki, (nM), D_3 [^{3}H] Spiperone	D_2/D_3
(-)-5-OH-DPAT[a]	58.8 ± 11.0	1.36 ± 0.28	43.2
Ropinirole[a]	2674 ± 305	29.3 ± 4.2	91
D-264[a]	264 ± 40	0.92 ± 0.23	253
D-440[a]	1073 ± 92	1.84 ± 0.51	583
D-413	17.5 ± 2.8	1.46 ± 0.1	11.9
D-367	9.56 ± 1.48	0.35 ± 0.06	27.3
D-660	49.7 ± 8.6	0.50 ± 0.09	99
D-661	382 ± 7	7.75 ± 1.08	49.3
D-662	723 ± 80	6.86 ± 0.34	105
D-663	592 ± 62	4.59 ± 0.42	129
D-668	15.5 ± 1.5	0.91 ± 0.009	17
D-669	164 ± 17	16.73 ± 0.81	9.8
D-672	20.0 ± 2.9	0.70 ± 0.09	29

[a]See Gopishetty et al. [23]

Compound labels are presented in bold

Structure activity relationship study

Compounds **D-661, D-663, D-660** and **D-662** were designed as bio-isosteric mimics of compounds of highly selective D_3 agonist **D-440** where the amide moiety in **D-440** is replaced by a sulfone group. It is expected that sulfone group should further lower the basicity of the piperazine N-atom it is attached to. The binding data (Table 1) indicates the effect of such replacement led to somewhat different outcomes. Compounds **D-661** and **D-663** exhibited low nanomolar potency for D_3 while exhibiting moderate affinity for D_2 (K_i; $D_2 = 382$ & 592 nM and $D_3 = 7.75$ & 4.59 nM, respectively for **D-661** & **D-663**). Although the selectivity for D_3 receptor turned out to be much less compared to **D-264** and **D-440**. An interesting observation was made when demethylation of the methoxy group on the 4-methoxy-benzenesulfonyl moiety was carried out. The resultant compounds **D-660** and **D-662** exhibited very different profile from each other. The affinity of compound **D-660** for D_2/ D_3 receptors went up significantly compared to the parent **D-661** (K_i; $D_2 = 49.7$ nM and $D_3 = 0.50$ nM; $D_2/D_3 = 99$ for **D-660**). Thus, the compound **D-660** exhibited more than fifteen-fold increase in affinity for D_3 compared to **D-661** with concomitant increase in selectivity for D_3 receptor. However, no significant changes were observed for the corresponding **D-662**. The increase in binding affinity of **D-660** for both D_2/D_3 receptors correlates with potent functional activity in the [^{35}S]GTPγS binding assay which indicate sub-nanomolar potencies with full agonist activity (EC50; 0.7 and 0.36 nM for D_2/D_3 receptors for **D-660**, Table 2).

Our next series of compounds deals with further probing of electronic, hydrophobic and H-bonding on the distal part of the molecular template. To determine the effect of introduction of hydroxyl and amine functionalities on the aromatic ring, compounds **D-367** and **D-413** were designed and synthesized. Both phenolic and amine derivatives **D-367** and **D-413** exhibited high affinity for D_2/D_3 receptors (K_i; $D_2 = 17.5$ & 9.56 nM and $D_3 = 1.46$ & 0.35 nM, respectively for **D-413** & **D-367**). The data indicates possible role of H-bonding interaction originating from the hydroxyl and amine functionalities. We further wanted to probe the basicity of the nitrogen atom in the amine group as well as any hydrophobic effect from derivatization with aromatic sulfone moiety. Addition of unsubstituted phenyl sulfone yielded **D-672** which produced the comparable affinity for D_2/D_3 receptors as the parent **D-413** (K_i; $D_2 = 20$ nM and $D_3 = 0.7$ nM, for **D-672**). However, a considerable loss of affinity was observed when 4-methoxy phenyl sulfone was introduced as shown in **D-669** (K_i; $D_2 = 164$ nM and $D_3 = 16.73$ nM, for D-669). This could be due to unfavorable electronic effect of the methoxy group. Interestingly, high affinity was restored when

Table 2 EC_{50} values (nM) for stimulating [35 S]GTPγS binding

Compound	CHO-D_2		CHO-D_3		D2/D3
	EC_{50} (nM) [^{35}S]GTPγS	$\%E_{max}$	EC_{50} (nM) [^{35}S]GTPγS	$\%E_{max}$	
Dopamine	209 ± 29	100	4.76 ± 0.87	100	43.9
Ropinirole[a]	304 ± 11	83.9 ± 0.3	10.3 ± 1.5	66.6 ± 8.1	29.5
D-660	0.70 ± 0.11	93.7 ± 1.77	0.36 ± 0.05	92.7 ± 7.0	1.93
D-668	0.39 ± 0.09	106 ± 6	0.0104 ± 0.003	92.7 ± 6.9	37.5
D-672	0.93 ± 0.14	87.5 ± 4.9	0.42 ± 0.11	97.4 ± 8.6	2.21

Results are means ± SEM for 3–5 experiments each performed in triplicate.

[a]See Gopishetty et al. [23]

Compound labels are presented in bold

demethylation of the methoxy group in **D-669** was carried out to produce **D-668** (K_i; $D_2 = 15.5$ nM and $D_3 = 0.91$ nM, for **D-668**). This clearly indicates the role of the hydroxyl group in enhancing activity. Indeed, in the functional assay compound **D-668** produced exceptionally high potency for for both D_2 and D_3 receptors (EC50; 0.39 and 0.0104 nM for D_2/D_3 receptors for **D-668**, Table 2). A forty-fold increase of potency for D_3 receptor took place with **D-668** when compared to **D-672** which does not contain hydroxyl substitution on the aromatic ring of phenyl sulfone group (EC50; 0.0104 vs 0.42 nM).

Conclusion

In conclusion, our current SAR studies on hybrid template shed additional light on the influence of H-bonding, basicity of N-atoms and hydrophobic effect on the distal part of the molecule for interaction with D_2/D_3 receptors. In general, presence of the methoxy substituent on the aromatic ring lowered the affinity for D_2/D_3 receptors. However, the restoration of activity except for compound **D-662** upon replacement of methoxy by hydroxyl group might indicate possible involvement of H-bonding or favorable electronic effect. One of the lead compounds **D-660** produced high selectivity for the D_3 receptors. Selected compounds were found to have potent agonist activity in the functional assays. One of the compounds **D-668** produced exceptional potencies for both D_2 and D_3 receptors. Overall, our results suggest that binding to the sites removed from the orthosteric binding sites contribute to enhance functional activity of the ligands significantly.

Experimental description

Reagents and solvents were purchased from commercial suppliers and used as received unless otherwise noted. Dry solvent was obtained following the standard procedure. All reactions were performed under N_2 atmosphere unless otherwise indicated. Analytical silica gel 60 F_{254}-coated TLC plates were purchased from EMD Chemicals, Inc. and were visualized with UV light or by treatment with phosphomolybdic acid (PMA), Dragendorff's reagent, or ninhydrin. Whatman Purasil 60A silica gel 230–400 mesh was used for flash column chromatographic purifications. Proton nuclear magnetic resonance (^{1}H NMR) spectra were measured on Varian 400 and 600 MHz NMR spectrometer (Pao Alto, California, USA), using tetramethylsilane (TMS) as an internal standard. The NMR solvent used was either $CDCl_3$ or CD_3OD unless otherwise indicated. Optical rotations were recorded on Autopol III automatic polarimeter (Rudolph Research Analytical, Hackettstown, NJ, USA). Melting points were recorded using a MEL-TEMP II (Laboratory Devices Inc., California, U.S.) capillary melting point apparatus. Purity of the compounds was determined by elemental analysis and was within ±0.4% of the theoretical value (≥95% purity). Elemental analyses were performed by Atlantic Microlab, Inc, GA, USA. Selected compounds were furher analyzed by reverse phase HPLC (Waters 2489 Alliance Integrated System, Massachusetts, USA) to check for purity.

Procedure A. 1-[2-(*tert*-Butyl-dimethyl-silanyloxy)-ethyl]-4-(4-methoxy-benzene-sulfonyl)-piperazine (1)

To a stirring solution of 1-(2-((tert-butyldimethylsilyl)oxy)-ethyl)piperazine (1.0 g, 4.09 mmol) in CH_2Cl_2 (10 mL), Et_3N (2.57 mL, 18.41 mmol) and 4-methoxy-benzenesulfonyl chloride (1.01 g, 4.91 mmol) were added at 0 °C. The reaction mixture was stirred at the same temperature for 1 h after which it was quenched with saturated $NaHCO_3$ solution and the aqueous phase was extracted with CH_2C1_2 (3 × 30 mL). The organic portions were dried over Na_2SO_4 and rotary evaporated to dryness, which was purified by silica gel column chromatography (hexane:EtOAc = 7:3) to give compound **1** as white solid (1.4 g, 83%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.68 (dd, $J = 6.0$, 1.8 Hz, 2H), 6.99 (dd, $J = 6.0$, 1.8 Hz, 2H), 3.87 (s, 3H),

3.68 (t, $J = 6.0$ Hz, 2H), 3.00 (s, 4H), 2.61 (t, $J = 4.8$ Hz, 4H), 2.51 (t, $J = 6.0$ Hz, 2H), 0.86 (s, 9H), 0.02 (s, 6H).

2-[4-(4-Methoxy-benzenesulfonyl)-piperazin-1-yl]-ethanol (2)

Into a stirring solution of compound **1** (1.2 g, 2.89 mmol) in THF (12 mL) was added *n*-tetrabutylammonium fluoride (4.34 mL, 4.34 mmol, 1.0 M solution in THF) at 0 °C. The reaction mixture was then stirred at room temperature for 2 h. THF was evaporated in vacuo, and the residue was diluted with CH_2Cl_2 (25 mL) and washed with a saturated solution of $NaHCO_3$. The water layer was extracted with CH_2Cl_2 (3 × 40 mL). The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 19:1) to afford white solid **2** (0.71 g, 82%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.69 (dd, $J = 6.0$, 1.8 Hz, 2H), 7.01 (dd, $J = 6.0$, 1.8 Hz, 2H), 3.88 (s, 3H), 3.57–3.55 (m, 2H), 3.01 (s, 4H), 2.58 (s, 4H), 2.53–2.51 (m, 2H).

[4-(4-Methoxy-benzenesulfonyl)-piperazin-1-yl]-acetaldehyde (3)

Into a stirring solution of compound **2** (0.35 g, 1.17 mmol) in CH_2Cl_2 (8 mL) and DMSO (4 mL), was added Et_3N (1.14 mL, 8.16 mmol) at 0 °C. The reaction mixture was stirred for 5 min followed by addition of SO_3.py complex (0.927 g, 5.83 mmol) at 0 °C. Ice bath was removed and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was quenched by addition of water and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layer was dried using Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography with EtOAc as the eluent to give aldehyde **3** (0.32 g, 92%). The purified aldehyde was used immediately for next step. **^{1}H NMR** (600 MHz, $CDCl_3$): δ 9.60 (s, 1H), 7.70–7.67 (m, 2H), 7.02–6.99 (m, 2H), 3.88 (s, 3H), 3.23–3.22 (m, 2H), 3.06 (s, 4H), 2.61–2.60 (m, 4H).

Procedure B. (*S*)-{2-[4-(4-Methoxy-benzenesulfonyl)-piperazin-1-yl]-ethyl}-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (4) (D-661)

Into a stirring solution of aldehyde **3** (0.31 g, 1.04 mmol) in CH_2Cl_2 (15 mL) was added (*S*)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (0.23 g, 1.04 mmol). After the mixture was stirred for 1.5 h, $NaBH(OAc)_3$ (0.44 g, 2.08 mmol) was added portion wise and the mixture was stirred for 48 h at room temperature. The reaction mixture was quenched with a saturated solution of $NaHCO_3$ at 0 °C and extracted with CH_2Cl_2 (3 × 40 mL). The combined organic layer was dried over Na_2SO_4, and the solvent was removed under reduced pressure. Crude product was purified by column chromatography (hexane:EtOAc = 3:7) to afford compound **4** (0.45 g, 86%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.68 (dd, $J = 7.2$, 1.8 Hz, 2H), 7.07 (t, $J = 7.8$ Hz, 1H), 6.98 (dd, $J = 7.2$, 1.8 Hz, 2H), 6.67 (d, $J = 7.8$ Hz, 1H), 6.64 (d, $J = 7.8$ Hz, 1H), 3.86 (s, 3H), 3.79 (s, 3H), 3.06–2.94 (m, 5H), 2.89–2.84 (m, 1H), 2.80–2.77 (m, 1H), 2.71–2.66 (m, 1H), 2.62–2.43 (m, 11H), 2.00–1.97 (m, 1H), 1.50 (td, $J = 12$, 5.4 Hz, 1H), 1.42 (sx, $J = 7.2$ Hz, 2H), 0.85 (t, $J = 7.2$ Hz, 3H); $[\alpha]_D^{25} = -26.2$ ($c = 1.0$ in CH_2Cl_2); Anal. Calcd for $C_{27}H_{43}Cl_2N_3O_5S$: C, 54.72; H, 7.31; N, 7.09. Found: C, 54.43; H, 7.29; N, 6.92.

(*S*)-N^6-{2-[4-(4-Methoxy-benzenesulfonyl)-piperazin-1-yl]-ethyl}-N^6-propyl-4,5,6,7-tetrahydrobenzo[*d*]thiazole-2,6-diamine (5) (D-663)

Aldehyde **3** (0.265 g, 0.89 mmol) in CH_2Cl_2 (10 mL) was reacted with (*S*)-N^6-propyl-4,5,6,7-tetrahydrobenzo[*d*]thiazole-2,6-diamine (0.17 g, 0.80 mmol) and $NaBH(OAc)_3$ (0.376 g, 1.78 mmol) according to procedure B. Crude product was purified by column chromatography (EtOAc/MeOH 19:1) to afford compound **5** (0.275 g, 70%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.70–7.67 (m, 2H), 7.00–6.97 (m, 2H), 4.80 (bs, 2H), 3.87 (s, 3H), 3.02–2.93 (m, 5H), 2.68–2.60 (m, 3H), 2.58–2.52 (m, 6H), 2.49–2.39 (m, 5H), 1.94–1.92 (m, 1H), 1.66 (td, $J = 12$, 5.4 Hz, 1H), 1.41 (sx, $J = 7.2$ Hz, 2H), 0.85 (t, $J = 7.2$ Hz, 3H); $[\alpha]_D^{25} = -33.8$ ($c = 1.0$ in CH_2Cl_2); Anal. Calcd for $C_{23.8}H_{41}Cl_4N_5O_{3.2}S_2$: C, 43.69; H, 6.32; N, 10.70. Found: C, 43.82; H, 6.62; N, 10.66.

Procedure C. (*S*)-6-({2-[4-(4-Hydroxy-benzenesulfonyl)-piperazin-1-yl]-ethyl}-propyl-amino)-5,6,7,8-tetrahydro-naphthalen-1-ol (6) (D-660)

To a stirred solution of **4** (0.1 g, 0.2 mmol) in 8 mL of CH_2Cl_2 was added BBr_3 (1.2 mL, 1.2 mmol, 1.0 M solution in CH_2Cl_2) at −78 °C under N_2 atmosphere. The reaction mixture was stirred at −78 °C for 2 h and then at room temperature overnight. The reaction was quenched with a saturated solution of $NaHCO_3$ at 0 °C and the aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layer was dried over Na_2SO_4, and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 98:2) to yield compound **6** (0.055 g, 58%). **^{1}H NMR** (600 MHz, CD_3OD): δ 7.56–7.54 (m, 2H), 6.91–6.89 (m, 2H), 6.87 (d, $J = 7.8$ Hz, 1H), 6.53 (t, $J = 8.4$ Hz, 2H), 3.35 (s, 1H),

2.98–2.87 (m, 6H), 2.82–2.79 (m, 1H), 2.74–2.70 (m, 3H), 2.59–2.51 (m, 6H), 2.48 (t, $J = 7.2$ Hz, 4H), 2.05–2.02 (m, 1H), 1.55 (td, $J = 12$, 5.4 Hz, 1H), 1.49 (sx, $J = 7.2$ Hz, 2H), 0.88 (t, $J = 7.2$ Hz, 3H); $[\alpha]_D^{25} = -28.6$ ($c = 1.0$ in CH_3OH); Anal. Calcd for $C_{25}H_{39}Cl_2N_3O_5S$: C, 53.19; H, 6.96; N, 7.44. Found: C, 53.07; H, 6.94; N, 7.06.

(*S*)-4-(4-{2-[(2-Amino-4,5,6,7-tetrahydro-benzo[*d*] thiazol-6-yl)-propyl-amino]-ethyl}-piperazine-1-sulfonyl)-phenol (7) (D-662)

Compound **5** (0.15 g, 0.3 mmol) in 12 mL of CH_2Cl_2 was reacted with BBr_3 (0.9 mL, 0.9 mmol, 1.0 M solution in CH_2Cl_2) according to procedure C. The crude product was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 9:1) to afford **7** (0.09 g, 63%). **^{1}H NMR** (600 MHz, CD_3OD): δ 7.58–7.56 (m, 2H), 6.92–6.90 (m, 2H), 3.33 (s, 1H), 3.02–2.93 (m, 5H), 2.68–2.43 (m, 14H), 1.94–1.92 (m, 1H), 1.67 (td, $J = 12$, 5.4 Hz, 1H), 1.44 (sx, $J = 7.2$ Hz, 2H), 0.85 (t, $J = 7.2$ Hz, 3H); $[\alpha]_D^{25} = -40.6$ ($c = 1.0$ in CH_3OH); Anal. Calcd for $C_{24}H_{44}Cl_4N_5O_{4.5}S_2$: C, 42.35; H, 6.52; N, 10.29. Found: C, 42.11; H, 6.38; N, 10.49.

1-[2-(*tert*-Butyl-dimethyl-silanyloxy)-ethyl]-4-(4-nitro-phenyl)-piperazine (8)

A suspension of 1-(4-nitro-phenyl)-piperazine (2.0 g, 9.65 mmol), potassium carbonate (4.0 g, 28.95 mmol), and (2-bromoethoxy)(*tert*-butyl)dimethylsilane (2.54 g, 10.62 mmol) in acetonitrile (20 mL) was refluxed under N_2 for 15 h. The reaction mixture was filtered off and the filtrate was evaporated under reduced pressure. The residue was then diluted with EtOAc, washed with water, dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (hexane:EtOAc = 2:1) to afford compound **8** as yellow solid (2.75 g, 78%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.12 (dd, $J = 5.4$, 1.8 Hz, 2H), 6.82 (dd, $J = 5.4$, 1.8 Hz, 2H), 3.80 (t, $J = 6.0$ Hz, 2H), 3.43 (t, $J = 4.8$ Hz, 4H), 2.69 (t, $J = 4.8$ Hz, 4H), 2.60 (t, $J = 6.0$ Hz, 2H), 0.90 (s, 9H), 0.07 (s, 6H).

2-[4-(4-Nitro-phenyl)-piperazin-1-yl]-ethanol (9)

Into a stirring solution of compound **8** (1.4 g, 3.83 mmol) in THF (18 mL) was added *n*-tetrabutylammonium fluoride (5.75 mL, 5.75 mmol, 1.0 M solution in THF) at 0 °C. The reaction mixture was then stirred at room temperature for 2 h. THF was evaporated in vacuo, and the residue was diluted with EtOAc (25 mL) and washed with a saturated solution of $NaHCO_3$. The water layer was extracted with EtOAc (3 × 40 mL). The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 9:1) to give a yellow solid **2** (0.86 g, 89%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.12 (dd, $J = 5.4$, 1.8 Hz, 2H), 6.83 (dd, $J = 5.4$, 1.8 Hz, 2H), 3.69 (t, $J = 5.4$ Hz, 2H), 3.45 (t, $J = 4.8$ Hz, 4H), 2.68 (t, $J = 4.8$ Hz, 4H), 2.63 (t, $J = 5.4$ Hz, 2H).

[4-(4-Nitro-phenyl)-piperazin-1-yl]-acetaldehyde (10)

Into a stirring solution of compound **9** (0.4 g, 1.59 mmol) in CH_2Cl_2 (8 mL) and DMSO (4 mL), was added Et_3N (1.55 mL, 11.14 mmol) at 0 °C. The reaction mixture was stirred for 5 min followed by addition of SO_3.py complex (1.27 g, 7.96 mmol) at 0 °C. Ice bath was removed and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was quenched by addition of water and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layer was dried using Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography with EtOAc as the eluent to give aldehyde **10** (0.35 g, 88%). The purified aldehyde was used immediately for next step. **^{1}H NMR** (600 MHz, $CDCl_3$): δ 9.74 (s, 1H), 8.13–8.09 (m, 2H), 6.84–6.81 (m, 2H), 3.49 (t, $J = 4.8$ Hz, 4H), 3.29 (m, 2H), 2.70 (t, $J = 4.8$ Hz, 4H).

(*S*)-(5-Methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-{2-[4-(4-nitro-phenyl)-piperazin-1-yl]-ethyl}-propyl-amine (11)

Aldehyde **10** (0.34 g, 1.36 mmol) in CH_2Cl_2 (15 mL) was reacted with (*S*)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (0.27 g, 1.23 mmol) and $NaBH(OAc)_3$ (0.58 g, 2.73 mmol) according to procedure B. Crude product was purified by column chromatography with EtOAc as the eluent to afford compound **11** (0.5 g, 81%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.12 (dd, $J = 5.4$, 1.8 Hz, 2H), 7.09 (t, $J = 7.8$ Hz, 1H), 6.81 (dd, $J = 5.4$, 1.8 Hz, 2H), 6.71 (d, $J = 7.8$ Hz, 1H), 6.65 (d, $J = 7.8$ Hz, 1H), 3.81 (s, 3H), 3.42 (t, $J = 4.8$ Hz, 4H), 3.02–2.98 (m, 1H), 2.96–2.92 (m, 1H), 2.86–2.83 (m, 1H), 2.78–2.76 (m, 1H), 2.73 (t, $J = 7.2$ Hz, 2H), 2.66–2.62 (m, 4H), 2.55–2.49 (m, 5H), 2.07–2.05 (m, 1H), 1.57 (td, $J = 12$, 5.4 Hz, 1H), 1.48 (sx, $J = 7.2$ Hz, 2H), 0.90 (t, $J = 7.2$ Hz, 3H); $[\alpha]_D^{25} = -15.6$ ($c = 1.0$ in CH_2Cl_2).

(*S*)-{2-[4-(4-Amino-phenyl)-piperazin-1-yl]-ethyl}-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (12) D-413

To a suspension of Pd/C (0.005 g) in anhydrous MeOH (10 mL), compound **11** (0.475 g, 1.05 mmol), dissolved in

EtOAc/MeOH, was added under N_2 atmosphere. The reaction vessel was degassed by applying vacuum, replaced by H_2 gas (50 psi) and then stirred at room temperature overnight. The mixture was filtered through a pad of celite, washed with MeOH and the filtrate thus obtained was evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 9:1) to furnish compound **12 (D-413)** (0.385 g, 87%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.09 (t, $J = 7.8$ Hz, 1H), 6.81 (d, $J = 8.4$ Hz, 2H), 6.71 (d, $J = 7.2$ Hz, 1H), 6.66–6.64 (m, 3H), 3.81 (s, 3H), 3.06 (t, $J = 4.8$ Hz, 4H), 3.02–2.98 (m, 1H), 2.92–2.88 (m, 1H), 2.82–2.78 (m, 3H), 2.67 (s, 5H), 2.57–2.50 (m, 5H), 2.10 (bs, 1H), 1.64–1.49 (m, 3H), 0.90 (t, $J = 7.2$ Hz, 3H); $[\alpha]_D^{25} = -19.2$ ($c = 1.0$ in CH_3OH).

(*S*)-4-Methoxy-N-[4-(4-{2-[(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amino]-ethyl}-piperazin-1-yl)-phenyl]-benzenesulfonamide (13) (D-669)

Compound **12** (0.18 g, 0.43 mmol) was reacted with Et_3N (0.24 mL, 1.7 mmol) and 4-methoxy-benzenesulfonyl chloride (0.106 g, 0.51 mmol) in CH_2Cl_2 (5 mL) according to procedure A. The crude material thus obtained was purified by silica gel column chromatography (EtOAc:MeOH = 19:1) to give compound **13** (0.22 g, 87%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.61 (dd, $J = 5.4$, 1.8 Hz, 1H), 7.10–7.07 (m, 1H), 6.97 (dd, $J = 5.4$, 1.8 Hz, 1H), 6.93 (d, $J = 9.0$ Hz, 2H), 6.86–6.83 (m, 2H), 6.75 (dd, $J = 5.4$, 1.8 Hz, 2H), 6.70 (d, $J = 7.8$ Hz, 1H), 6.64 (d, $J = 7.8$ Hz, 1H), 3.81 (s, 3H), 3.80 (s, 3H), 3.24 (t, $J = 4.8$ Hz, 1H), 3.13 (t, $J = 4.8$ Hz, 3H), 3.01–2.97 (m, 1H), 2.93–2.83 (m, 2H), 2.77–2.71 (m, 3H), 2.64–2.57 (m, 4H), 2.53–2.49 (m, 5H), 2.06–2.04 (m, 1H), 1.56 (td, $J = 12$, 5.4 Hz, 1H), 1.48 (sx, $J = 7.2$ Hz, 2H), 0.89 (t, $J = 7.2$ Hz, 3H); $[\alpha]_D^{25} = -22.2$ ($c = 1.0$ in CH_2Cl_2); Anal. Calcd for $C_{37}H_{57}Cl_3N_4O_5S$: C, 57.25; H, 7.40; N, 7.22. Found: C, 57.64; H, 7.09; N, 7.39.

(*S*)-4-Hydroxy-N-[4-(4-{2-[(5-hydroxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amino]-ethyl}-piperazin-1-yl)-phenyl]-benzenesulfonamide (14) (D-668)

Compound **13** (0.14 g, 0.24 mmol) in 12 mL of CH_2Cl_2 was reacted with BBr_3 (1.42 mL, 1.42 mmol, 1.0 M solution in CH_2Cl_2) according to procedure C. The crude product was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 9:1) to afford **14** (0.075 g, 56%). **^{1}H NMR** (600 MHz, CD_3OD): δ 7.48 (d, $J = 8.4$ Hz, 2H), 6.94–6.91 (m, 3H), 6.78 (d, $J = 9.0$ Hz, 2H), 6.76 (d, $J = 9.0$ Hz, 2H), 6.60 (d, $J = 4.8$ Hz, 1H), 6.59 (d, $J = 4.8$ Hz, 1H), 3.53–3.49 (m, 1H), 3.24–3.16 (m, 2H), 3.09 (t, $J = 4.8$ Hz, 4H), 3.06–3.01 (m, 4H), 2.98–2.93 (m, 1H), 2.77–2.71 (m, 2H), 2.69 (t, $J = 4.8$ Hz, 4H), 2.62–2.57 (m, 1H), 2.25–2.22 (m, 1H), 1.79 (td, $J = 12$, 5.4 Hz, 1H), 1.72 (sx, $J = 7.2$ Hz, 2H), 0.99 (t, $J = 7.2$ Hz, 3H); $[\alpha]_D^{25} = -26.4$ ($c = 1.0$ in CH_3OH); Anal. Calcd for $C_{31}H_{45}Cl_3N_4O_5S$: C, 53.79; H, 6.55; N, 8.09. Found: C, 53.45; H, 6.63; N, 7.63.

(*S*)-*N*-[4-(4-{2-[(5-Methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amino]-ethyl}-piperazin-1-yl)-phenyl]-benzenesulfonamide (15)

Compound **12** (0.165 g, 0.39 mmol) was reacted with Et_3N (0.22 mL, 1.56 mmol) and benzenesulfonyl chloride (60 µL, 0.47 mmol) in CH_2Cl_2 (5 mL) according to procedure A. The crude material thus obtained was purified by silica gel column chromatography (EtOAc:MeOH = 19:1) to give compound **15** (0.19 g, 87%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.94 (dd, $J = 7.2$, 1.2 Hz, 1H), 7.69–7.63 (m, 2H), 7.53 (t, $J = 7.8$ Hz, 2H), 7.40 (t, $J = 7.8$ Hz, 1H), 6.92–6.91 (m, 1H), 6.86–6.84 (m, 1H), 6.79–6.78 (m, 1H), 6.75 (dd, $J = 4.8$, 1.8 Hz, 1H), 6.72–6.70 (m, 1H), 6.66–6.64 (m, 1H), 3.80 (s, 3H), 3.25 (t, $J = 5.4$ Hz, 2H), 3.13 (t, $J = 5.4$ Hz, 2H), 3.02–2.94 (m, 2H), 2.86–2.84 (m, 1H), 2.78–2.71 (m, 3H), 2.64–2.59 (m, 4H), 2.56–2.48 (m, 5H), 2.07–2.05 (m, 1H), 1.57 (td, $J = 12$, 5.4 Hz, 1H), 1.49 (sx, $J = 7.2$ Hz, 2H), 0.91–0.88 (m, 3H); $[\alpha]_D^{25} = -17.3$ ($c = 1.0$ in CH_2Cl_2).

(*S*)-*N*-[4-(4-{2-[(5-Hydroxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amino]-ethyl}-piperazin-1-yl)-phenyl]-benzenesulfonamide (16) (D-672)

Compound **15** (0.12 g, 0.21 mmol) in 10 mL of CH_2Cl_2 was reacted with BBr_3 (0.64 mL, 0.64 mmol, 1.0 M solution in CH_2Cl_2) according to procedure C. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 19:1) to afford **16** (0.075 g, 65%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.68 (d, $J = 7.8$ Hz, 2H), 7.54–7.48 (m, 1H), 7.41–7.37 (m, 2H), 6.95–6.91 (m, 3H), 6.75 (d, $J = 4.8$ Hz, 1H), 6.73 (d, $J = 4.8$ Hz, 1H), 6.54 (t, $J = 7.8$ Hz, 1H), 6.49–6.47 (m, 1H), 3.48–3.47 (m, 1H), 3.21–3.12 (m, 3H), 2.93–2.89 (m, 1H), 2.86–2.82 (m, 1H), 2.72–2.61 (m, 7H), 2.54–2.41 (m, 5H), 2.35–2.28 (m, 1H), 1.96 (bs, 1H), 1.48–1.36 (m, 3H), 0.87 (t, $J = 7.2$ Hz, 3H); $[\alpha]_D^{25} = -15.6$ ($c = 1.0$ in CH_2Cl_2); Anal. Calcd for $C_{31}H_{44}Cl_3N_4O_{3.5}S$: C, 55.81; H, 6.65; N, 8.40. Found: C, 55.73; H, 6.72; N, 7.98.

2-Chloro-1-[4-(4-methoxy-phenyl)-piperazin-1-yl]-ethanone (18)

Chloroacetylchloride (6.3 ml, 39.01 mmol) was added drop wise into a solution of 1-(4-Methoxy-phenyl)-piperazine (5 g, 26 mmol) and triethylamine (19.07 ml) in anhydrous methylene chloride at −40 °C under N_2 atmosphere and then stirred at room temperature for 30 min. The reaction

was diluted with CH_2Cl_2, washed with water, brine, and the organic layer was dried over Na_2SO_4, evaporated, purified by column chromatography Hexane/ EtOAc=50:50 to obtain pure product 18 (6.08 g, 89%).

^{1}H NMR (400 MHz, CDCl3) δ: 3.06 (t, $J = 5.2$ Hz, 2H); 3.12 (t, $J = 5.2$ Hz, 2H); 3.68 (t, $J = 4.8$ Hz, 2H); 3.74–3.84 (m, 5H); 4.11 (s, 2H); 6.86 (d, $J = 9.2$ Hz, 2H); 6.91 (d, $J = 9.2$ Hz, 2H).

1-[4-(4-Methoxy-phenyl)-piperazin-1-yl]-2-[(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amino]-ethanone (19)

Compound 5-methoxy-*N*-propyl-1,2,3,4-tetrahydronaphthalen-2-amine (2 g, 7.819 mmol), compound 18 (3.15 g, 11.73 mmol), K_2CO_3 (4.58 g, 23.46 mmol) were refluxed in CH_3CN (50 ml) for 2.5 hr. The solution was cooled, filtered, and concentrated. The crude material was then partitioned between EtOAc and H_2O, and the organic layer was separated, dried (Na_2SO_4), and concentrated. The crude mixture was purified by column chromatography EtOAc =100 to yield pure compound 19 (4.4 g, 98%).

^{1}H NMR (400 MHz, CDCl3) δ: 0.88 (t, $J = 7.2$ Hz, 3H); 1.42–1.70 (m, 3H); 1.97–2.12 (m, 1H); 2.46–2.64 (m, 3H); 2.74–3.12 (m, 8H); 3.40–3.52 (m, 2H); 3.61–3.92 (m, 4H); 3.77 (s, 3H); 3.79 (s, 3H); 6.64 (d, $J = 8$ Hz, 1H); 6.69 (d, $J = 7.6$ Hz, 1H); 6.84 (d, $J = 9.2$ Hz, 2H); 6.90 (d, $J = 9.2$ Hz, 2H); 7.08 (t, $J = 8$ Hz, 1H).

{2-[4-(4-Methoxy-phenyl)-piperazin-1-yl]-ethyl}-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (20)

To a suspension of $LiAlH_4$ (0.84 g, 4.42 mmol) in THF (50 ml) in an ice bath was added compound 19 (2 g, 2.21 mmol) in a solution of THF (25 ml). After addition, the mixture was refluxed for 2 h and cooled to 0 °C. 15% NaOH was added dropwise, and the mixture stirred for 20 min, and filtered. The solution was dried (Na_2SO_4), filtered, and concentrated to give 20 (1.09 g, 58%).

^{1}H NMR (400 MHz, CDCl3) δ: 0.90 (t, $J = 7.6$ Hz, 3H); 1.41–1.66 (m, 3H); 2.02–2.14 (m, 1H); 2.44–2.62 (m, 5H); 2.66 (t, $J = 4.8$ Hz, 4H); 2.70–3.06 (m, 6H); 3.10 (t, $J = 4.4$ Hz, 4H); 3.76 (s, 3H); 3.81 (s, 3H); 6.65 (d, $J = 8$ Hz, 1H); 6.71 (d, $J = 7.6$ Hz, 1H); 6.83 (d, $J = 9.2$ Hz, 2H); 6.90 (d, $J = 9.2$ Hz, 2H); 7.09 (t, $J = 8$ Hz, 1H).

6-({2-[4-(4-Hydroxy-phenyl)-piperazin-1-yl]-ethyl}-propyl-amino)-5,6,7,8-tetrahydro-naphthalen-1-ol (21) D-367

A mixture of 30 (1 g, 2.285 mmol) and 10 ml of 48% Aq. HBr was refluxed under N_2 atmosphere for 3 h. Reaction mixture was then cooled down, evaporated to dryness. Reaction mixture was then dissolved in sat. $NaHCO_3$ solution and extracted with ethyl acetate. The organic layer was dried (Na_2SO_4), filtered, and concentrated. Compound was purified over column chromatography using EtOAc/ MeOH=80:20 to yield pure compound **21** (**D-367**) (0.85 g, 91%).

^{1}H NMR (400 MHz, CDCl3) δ: 0.89 (t, $J = 7.6$ Hz, 3H); 1.32–1.58 (m, 3H); 1.94–2.06 (m, 1H); 2.38–2.60 (m, 6H); 2.60–2.81 (m, 7H); 2.82–3.02 (m, 2H); 3.04–3.22 (m, 4H); 6.54 (d, $J = 8$ Hz, 1H); 6.62 (d, $J = 7.2$ Hz, 1H); 6.70–6.78 (m, 2H); 6.78–6.88 (m, 2H); 6.98 (t, $J = 7.6$ Hz, 1H).

The free base of 23 was converted in to its hydrochloride salt. m.p. decomp. at 215–217 °C Anal. ($C_{25}H_{35}N_3O_2 \cdot 3HCl \cdot 0.5H_2O$) C, H, N.

DA D_2 and D_3 receptor assays

Binding potency was monitored by inhibition of [^{3}H]spiroperidol (16.2 Ci/mmole, Perkin-Elmer) binding to dopamine rD_2 and rD_3 receptors expressed in HEK-293 cells, in a buffer containing 0.9% NaCl under conditions corresponding to our 'high [radioligand] protocol' as described by us previously [37]. Observed IC_{50} values were converted to inhibition constants (K_i) by the Cheng–Prusoff equation (see Ghosh et al. [38]). Functional activity of test compounds in activating dopamine hD_2 and hD_3 receptors expressed in CHO cells was measured by stimulation of [^{35}S]GTPγS (1250 Ci/mmole, Perkin-Elmer) binding in comparison to stimulation by the full agonist dopamine as described by us previously.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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