

Bioisosteric Heterocyclic Versions of 7-[[2-(4-Phenyl-piperazin-1-yl)ethyl]propylamino]-5,6,7,8-tetrahydronaphthalen-2-ol: Identification of Highly Potent and Selective Agonists for Dopamine D3 Receptor with Potent in Vivo Activity

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In the current report, we extend the SAR study on our hybrid structure 7-[[2-(4-phenyl-piperazin-1-yl)ethyl]propylamino]-5,6,7,8-tetrahydronaphthalen-2-ol further to include heterocyclic bioisosteric analogues. Binding assays were carried out with HEK-293 cells expressing either D2 or D3 receptors with tritiated spiperone to evaluate inhibition constants (K_i). Functional activity of selected compounds in stimulating GTP γ S binding was assessed with CHO cells expressing human D2 receptors and AtT-20 cells expressing human D3 receptors. The highest binding affinity and selectivity for D3 receptors were exhibited by (–)-**34** ($K_i = 0.92$ nM and D2/D3 = 253). In the functional GTP γ S binding assay, (–)-**34** exhibited full agonist activity with picomolar affinity for D3 receptor with high selectivity ($EC_{50} = 0.08$ nM and D2/D3 = 248). In the in vivo rotational study, (–)-**34** exhibited potent rotational activity in 6-OH-DA unilaterally lesioned rats with long duration of action, which indicates its potential application in neuroprotective treatment of Parkinson's disease.

Introduction

¹Parkinson's disease (PD) is a chronic progressive neurodegeneration disorder that is characterized by a gradual loss of dopaminergic neurons in the pars compacta of the substantia nigra.¹ It is estimated that PD affects approximately 1–2% of people older than 65 years of age. It is primarily a sporadic disorder, although a rare subset of population (<10%) acquires this disease because of several genetic defects.^{2,3} Some of the symptoms associated with PD involve rigidity, bradykinesia, resting tremor, and postural instability along with cognitive and psychiatric complications.^{1,4,5} Several therapies are being used in the clinic for symptomatic treatment of PD.⁶ The dopamine precursor L-dopa has been used as a gold standard treatment agent for PD since its discovery almost 40 years ago and is still considered a main therapy.⁷ However, long-term use of L-dopa gives rise to motor fluctuations with dyskinesias and the decrease in duration of response to a given L-dopa dose.⁸ Prolong use of L-dopa also gives rise to "on" and "off" episodes resulting in additional complications. It has also been speculated that long-term exposure to L-dopa may be toxic to dopamine neurons, hence accelerating the dopamine neurodegeneration process.^{9,10}

Dopamine receptors, which belong to the G-protein-coupled receptor (GPCR) class, in the brain have been studied extensively over a period of several decades. Dopamine receptors are classified into two main classes based on their pharmaco-

logical properties. They are known as D1-type and D2-type. D1-type consists of D1 and D5 subtypes, whereas D2, D3, and D4 receptors belong to D2-type.^{11–17} This classification was based on distinct physiological and pharmacological properties of these two receptors as stimulation of the D1-type receptor leads to activation of adenylate cyclase, which promotes synthesis of cAMP, whereas D2-type receptor activation leads to inhibition of adenylate cyclase activity. In the CNS, D1-type receptors are located postsynaptically, whereas D2-type receptors are located both pre- and postsynaptically and have a high affinity for dopamine.¹⁸

Since the discovery of the D3 receptor from molecular cloning in early 1990s, there has been a great deal of interest in understanding the functional properties of the D3 receptor to delineate its role in different disease processes in the CNS.¹⁹ Neuroanatomical location of the D3 receptor is different from D2 because it concentrates in the limbic regions, which have been implicated in different psychiatric diseases.²⁰ In human brain, the highest expression of D3 receptor was found in the area of the ventral striatum and associated striatum.²¹ The D3 receptor has been implicated in neuroprotection, as D3 preferring agonists, e.g., pramipexole and ropinirole, could protect dopamine neurons against MPTP and 6-OH-DA^a neurotoxicity more robustly than less selective D3 agonists.^{22–24} In another study, it was demonstrated that clinically relevant low doses of pramipexole effectively protected dopamine fibers from MPTP neurotoxicity.²⁵ A role of D3 receptor has also been implicated in production of neurotrophic factors such as BDNF. In a recent study, D3-preferring agonists have been demonstrated to induce production of neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and glial cell line-derived factor (GDNF) in cultured mesencephalic dopamine neurons and also in differentiated SH-SY5Y cells.^{26–28} D3 agonist has also been shown to play a role as a neurorestorative agent in some of these experiments.²⁹ Evidence from some of these early studies

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^a Abbreviations: GTP γ S, guanosine 5'-[γ -thio]triphosphate; 7-OH-DPAT, 7-hydroxy-2-(dipropylamino)tetralin; 6-OH-DA, 6-hydroxydopamine; CHO, Chinese hamster ovary; HEK, human embryonic kidney.

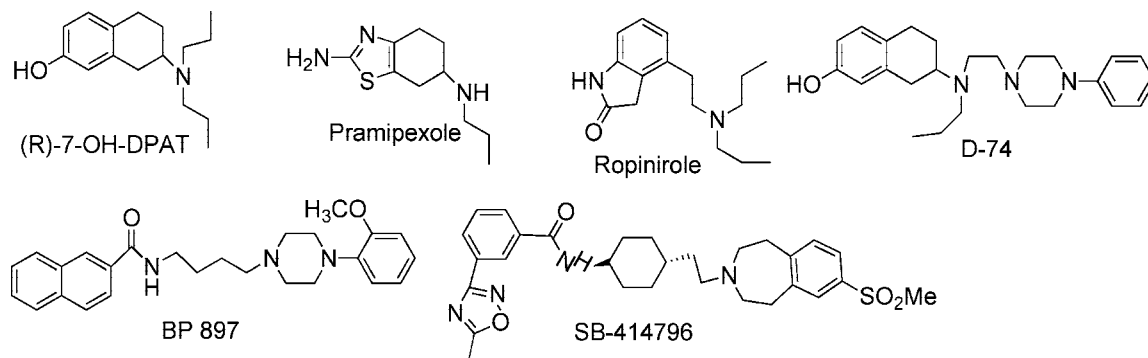


Figure 1. Molecular structures of dopamine D3 receptor selective agonists and antagonists.

indicates that selective D3-preferring agonists not only will have potential to alleviate motor dysfunction in PD patients but also will have a potential to provide neuroprotective effect in restoration of lost dopaminergic function. Furthermore, it has been increasingly evident from primate and rodent experiments that treatment with L-dopa leads to overexpression of D3 receptor in the basal ganglia and the development of dyskinesia due to treatment with L-dopa can be reduced significantly by treatment with D3 preferring agonist.^{30,31} Consequently, high affinity agonist for D3 receptor with high selectivity will be able to delineate more effectively roles of functional properties of D3 receptor and its importance in various neurodisorders.

Since the discovery of the D3 receptor, an intensive effort has been directed toward development of selective ligands for this receptor. A large number of molecules have been developed with various selectivities for the D3 receptor.^{32,33} The task of developing selective ligands has been particularly challenging considering the fact that D2 and D3 receptors share considerable homology in their molecular structures. In the primary structure sequence the extent of homology is greater than 50% which when extended to the transmembrane domains, the homology increases to greater than 90%, and both receptors share very similar active binding sites.³⁴ Therefore, it is challenging to develop drugs, either agonist or antagonist, selective for D3 compared to D2, which becomes especially difficult when development of a selective agonist is considered. This is due to the fact that both receptors share nearly identical active binding sites for agonist interaction. Numerous ligands as antagonists have been developed so far with a number of lead compounds showing high selectivity for the D3 receptor. Most of these compounds contain a piperazine ring connected to a suitable benzamide-type moiety via variable linker size.^{32,33,35} Some of the most selective molecules derived from this template are shown in Figure 1. On the other hand, selectivities of agonists developed so far have not been very high for the reason described above. Some of the agonists with the highest D3 selectivity are pramipexole, ropinirole, and 7-OH-DPAT. Most of the agonists developed so far are based on the aminotetralin structure.

Our hybrid drug development approach, combining aminotetralin and piperazine moieties together via a linker, resulted in a number of potent agonists exhibiting high affinities for D2/D3 receptors with preferential affinity for the D3 receptor. Some of these molecules also exhibited good selectivity for the D3 receptor.^{36,37} So far, structurally, we have focused on compounds with an aminotetralin moiety containing phenolic or equivalent moiety. However, in this report, we wanted to explore the replacement of the catechol ring by several bioisosteric moieties to observe the effect of such replacement on both affinity and selectivity for the D3 receptor. A catechol or phenolic moiety

in aminotetralins is known to possess poor in vivo stability due to formation of metabolites via conjugation with glucuronic acid, also resulting in poor oral bioavailability.³⁸ In our current studies, we have included a number of heterocyclic moieties including 2-aminothiazole, pyrazole, isoxazole, and thia- and selenodiazole rings.

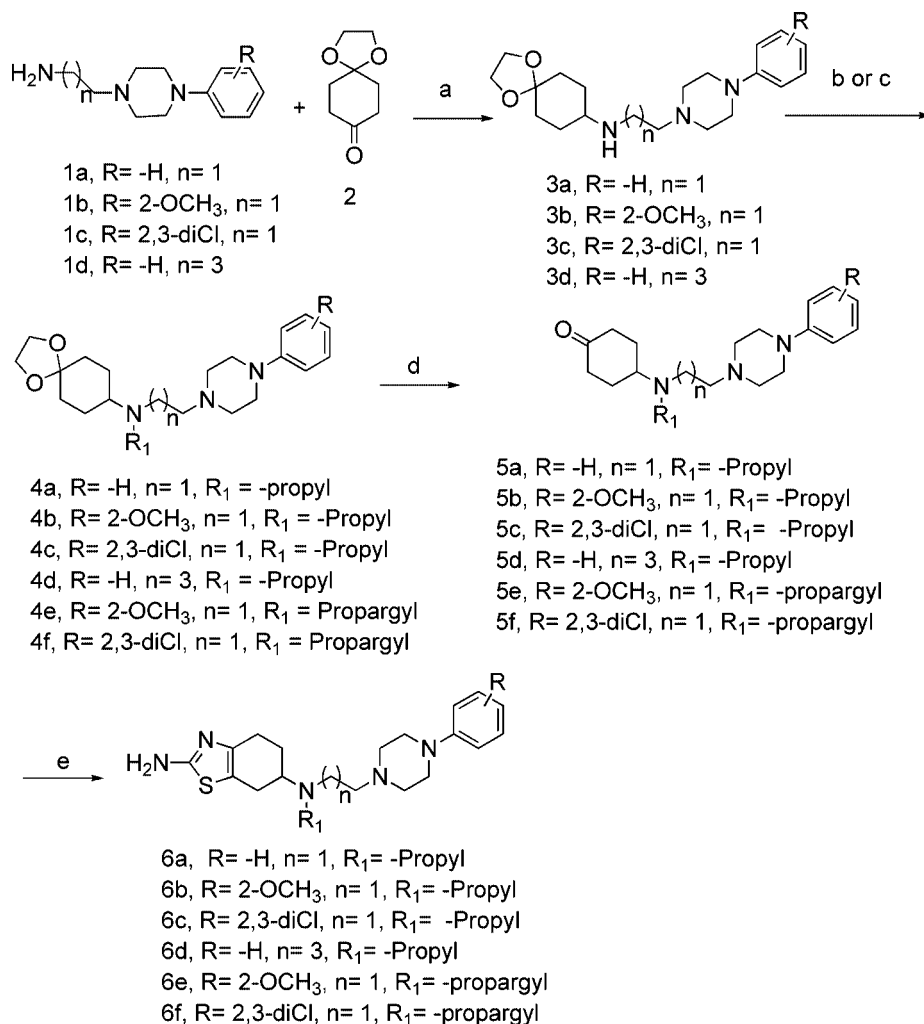
Chemistry

Syntheses of the target molecules are shown in Schemes 1–5. Scheme 1 describes the synthesis of **6a–f**. Appropriately substituted amines **1a–d** were prepared by following our previously described procedure.³⁹ Reductive amination of these amines with 1,4-cyclohexanedione monoethylene ketal in the presence of sodium triacetoxyborohydride produced compounds **3a–d**. N-Alkylation of amine with propyl bromide in the presence of a base produced **4a–d** in good yield. Similarly, N-alkylation of **3b,c** with propargyl bromide in the presence of sodium hydride produced **4e–f**. Next, hydrolysis of ketal in the presence of 2 N HCl produced keto derivatives **5a–f**. The final target 2-aminothiazol derivatives **6a–f** were produced by first reacting **5a–f** with pyrrolidine to produce the enamine intermediate followed by reaction with sulfur and cyanamide at room temperature overnight.⁴⁰

Scheme 2 describes the synthesis of number of heterocyclic target derivatives. Ketone **5a** was reacted with ethyl formate in the presence of sodium ethoxide to produce hydroxymethylene derivative **7**, which on treatment with semicarbazide followed by treatment with concentrated H₂SO₄ produced pyrazole analogue **8**.⁴¹ The isoxazole derivative **9b** was synthesized by treating **7** with hydroxylamine hydrochloride in acetic acid, and the isomeric isoxazole **9a** was produced by treatment of **7** with hydroxylamine in pyridine. In the next reaction, compound **5a** was converted into semicarbazone **10** by reacting with semicarbazide hydrochloride in the presence of sodium acetate in methanol. Semicarbazone **10** was next treated with thionyl chloride to produce target **11**.⁴¹ Similarly, selenium derivative **12** was produced by treating compound **10** with selenium dioxide.

Scheme 3 describes synthesis of pyrazole derivative target **19**. The starting 3-ethoxy-2-cyclohexen-1-one was first converted into 6-hydroxymethylene derivative followed by its conversion into pyrazole derivative **14** on treatment with hydrazine.⁴² Protection of pyrazole N-atom with mesitylene-sulfonyl chloride followed by acid catalyzed hydrolysis of enol ether yielded compound **16**.⁴³ Reductive amination with amine **1a** produced compound **17** in good yield. Intermediate **17** on reaction with propionyl chloride followed by reduction with LAH produced the final compound **19**.

Schemes 4 and 5 describe synthesis of various enantiomeric analogues of 2-aminothiazole derivatives. A new synthesis of

Scheme 1^a

^a (a) 1,4-Cyclohexanedione monoethylene ketal, Na(OAc)₃BH, AcOH, dichloroethane, room temp, overnight; (b) bromopropane, K₂CO₃, DMF, reflux, 3 h; (c) propargyl bromide, NaH, THF/DMF = 5:1, room temp to 60 °C, 3 h; (d) 2 N HCl, THF, reflux, 4 h; (e) (i) pyrrolidine, TsOH, benzene, Dean–Stark reflux, 1.5 h; (ii) S₈, MeOH, NH₂CN, room temp, overnight.

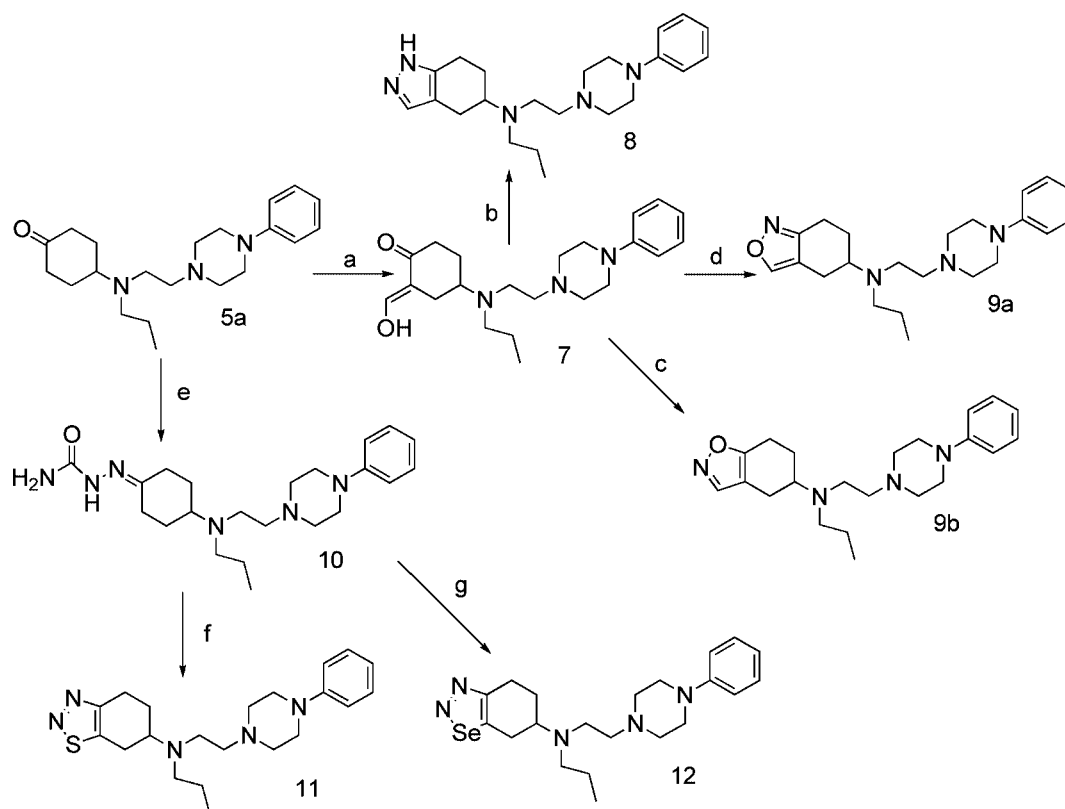
pramipexole was devised by us, which involved protection of amino group in intermediate **20** by mesitylenesulfonyl chloride followed by hydrolysis of ketal and conversion into 2-aminothiazole derivative **23**.⁴⁴ Deprotection of protected group yielded racemic pramipexole in good yield. Racemic pramipexole was next resolved by following a patent procedure with some modification.⁴⁵ Separated enantiomers (+)-**24** and (–)-**24** were then used in the synthesis of various optically active 2-aminothiazole derivatives as described in Scheme 5.

Various substituted phenyl piperazine derivatives **25a–d** were treated with chloroacetyl chloride to provide substituted piperazine derivatives **26a–d**. N-Alkylation of enantiomeric pramipexole with various chloropiperazine derivatives in the presence of a base and potassium iodide yielded enantiomeric substituted amides **27–30**. Reduction of amide with borane produced the final optically active targets (+)-**31**, (–)-**31**, (+)-**32**, (–)-**32**, (+)-**33**, (–)-**33**, and (–)-**34**.

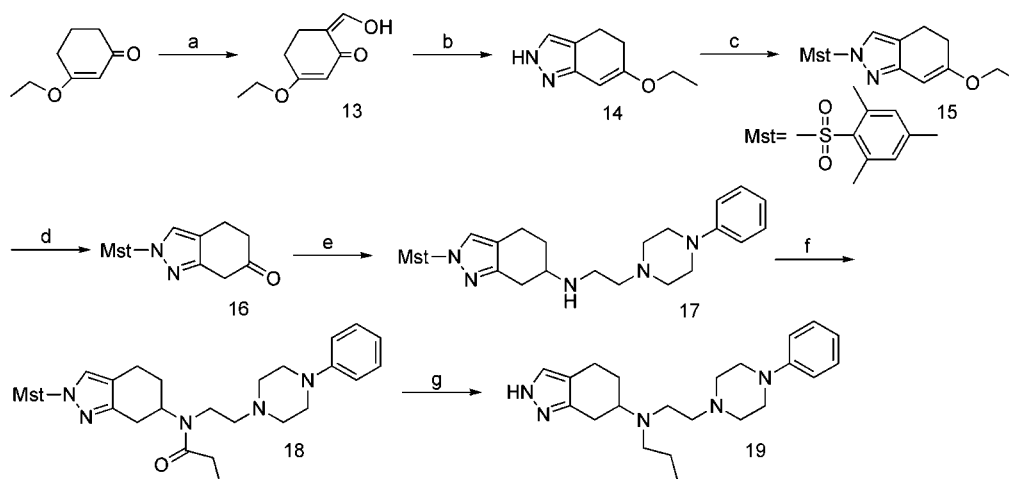
Results and Discussion

One of our main goals in this structure–activity relationship (SAR) study is to delineate an optimum pharmacophoric structure with bioisosteric heterocyclic moieties in the hybrid structure backbone for high D3 selectivity. Several structurally different heterocyclic derivatives have been synthesized and

characterized. In this regard, we first wanted to optimize 2-aminothiazole ring-based derivatives as one of our target molecules. To this end, a series of racemic derivatives **6a–f** were synthesized and characterized. In an earlier report, we described the development of compound **6a**.³⁶ In this report, we resynthesized the compound because we wanted to recharacterize it with our new cell lines. In the **6a–f** series of compounds, N-aromatic and N-alkyl substitutions were explored. The two principle aromatic substitutions were 2-methoxy- and 2,3-dichlorophenyl groups. The N-alkyl substitutions mainly consisted of propyl and propargyl groups. The dichloro derivative **6c** was the most potent with moderate selectivity for the D3 receptor ($K_i = 1.82$ nM for D3; D2/D3 K_i ratio = 37.6, Table 1). The 2-methoxy substituted compound **6b** was somewhat less potent compared to **6c**; however, the selectivity for D3 receptor was similar to that of **6c** (D2/D3: 32.6 and 37.6 for **6b** and **6c**, respectively, Table 1). In accordance to our previous results with compounds containing four methylene linker, compound **6d** produced lower potency at both D2 and D3 receptors compared to **6a**.^{36,39} This result indicates that the mode of interaction of our hybrid derivatives may not be identical with the other class of molecules interacting with D2/D3 receptors. Next, in compounds **6e** and **6f** the N-propyl group was replaced by an N-propargyl group, which for the most part

Scheme 2^a

^a (a) NaOEt, ethyl formate, benzene, room temp, overnight; (b) (i) semicarbazide HCl, sodium acetate, EtOH, 0 °C, 1 h; (ii) concentrated H₂SO₄/H₂O (1:3), reflux, 15 min; (c) NH₂OH·HCl, AcOH, 80 °C, 1 h; (d) NH₂OH·HCl, pyridine, reflux, 6 h; (e) semicarbazide HCl, sodium acetate, MeOH, reflux, 4 h; (f) thionyl chloride, -10 °C to room temp, 30 min; (g) SeO₂, AcOH, room temp, 30 min.

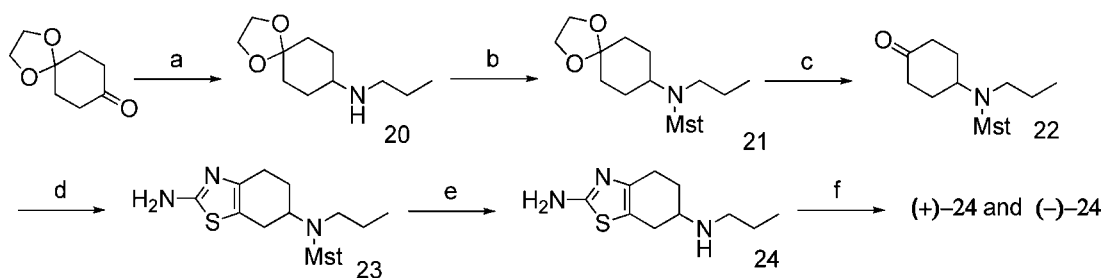
Scheme 3^a

^a (a) NaOEt, ethyl formate, benzene, room temp, overnight; (b) NH₂NH₂, ethanol, reflux, 6 h; (c) 2-mesitylenesulfonyl chloride, NaOH, dichloromethane, reflux, overnight; (d) 3 N HCl, THF, room temp, 16 h; (e) **1a**, NaCNBH₃, AcOH, dichloroethane, room temp, overnight; (f) propionyl chloride, Et₃N, dichloromethane, 0 °C to room temp, 4 h; (g) LiAlH₄, THF, reflux, 4 h.

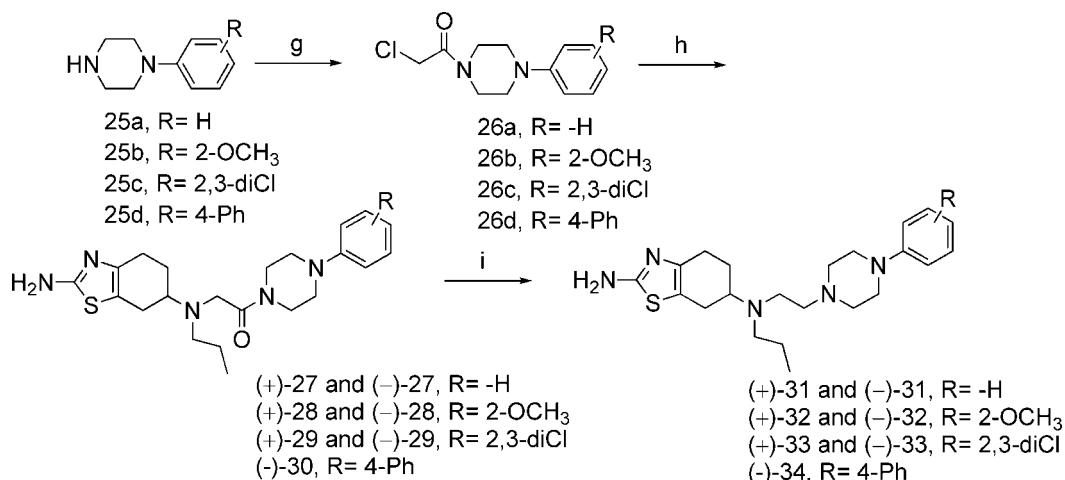
maintained the potency with less selectivity for the D₃ receptor. Interestingly, *N*-propargyl substituted compounds **6e** and **6f** exhibited higher affinity for the D₂ receptor compared to their corresponding *N*-propyl substituted compounds (81.2 and 34.9 nM for **6e** and **6f** vs 166 and 68.4 nM for **6b** and **6c**).

In our next SAR exploration, several bioisosteric heterocyclic derivatives were designed and synthesized as described in Schemes 2 and 3. A suitably substituted pyrazole ring is a well-known bioisosteric equivalent of the catechol moiety for agonist interaction with dopamine receptors; thus, quinpirole and other

related pyrazole derivatives have been developed as agonist for D₂/D₃ receptors.⁴² In the design of heterocyclic analogues, we wanted to introduce pyrazole, isoxazoles, and thia- and selenodiazole heterocyclic ring systems into our hybrid template. As expected from our previous results on bioisosteric derivatives, pyrazole derivative **8** exhibited good potency for the D₃ receptor but was weak at D₂ with overall good selectivity for the D₃ receptor ($K_i = 46.7$ nM, D₂/D₃ = 39.3, Table 1). On the other hand, neither isoxazole **9b** nor its isomer **9a** was very active at the D₂/D₃ receptor, indicating nonsuitability of these

Scheme 4^a

^a (a) *n*-Propylamine, NaCNBH₃, AcOH, dichloroethane, room temp, overnight; (b) 2-mesitylenesulfonyl chloride, NaOH, dichloromethane, reflux, overnight; (c) 2 N HCl, THF, reflux, 6 h; (d) pyrrolidine, TsOH, benzene, Dean–Stark reflux, 1.5 h; (ii) S₈, MeOH, NH₂CN, room temp, overnight; (e) 33% HBr in AcOH, phenol, ethyl acetate, 0 °C to room temp, 10 h; (f) HCl, methanol, L-(+)- or D-(-)-tartaric acid, MeOH, recrystallized from MeOH.

Scheme 5^a

^a (g) Chloroacetyl chloride, Et₃N, dichloromethane, 0 °C to room temp, 2 h; (h) (+)-24 or (-)-24, K₂CO₃, KI, acetonitrile, 60 °C, 4 h; (i) BH₃ in THF, THF, room temp, 36 h.

hetero rings as bioisosteres for dopamine D2/D3 receptors. Similarly, thiadiazolo derivative **11** and the selenium analogue **12** were weak at the D2/D3 receptors. Isomeric pyrazole derivative **19** likewise was also weak at both dopamine receptors. Thus, the heterocyclic rings other than pyrazole moiety were not suitable for interaction with D2/D3 receptors.

In Schemes 4 and 5 we describe synthesis of enantiomers of the racemic compounds shown in Scheme 1. In general the (-)-isomer was more active than the (+)-isomer. The two enantiomers of **6a**, (+)-**31** and (-)-**31**, exhibited differential activity with higher potency, and selectivity for the D3 receptor resided in (-)-**31** ($K_i = 4.15$ nM for D3 and D2/D3 = 58.6). It is important to point out that the racemic mixture **6a** exhibited somewhat greater selectivity for D3 receptor than (-)-**31** (D2/D3 = 107 vs 58.6 for **6a** and (-)-**31**, respectively). This could be due to the presence of complex interaction with the D2 receptor. However, such anomaly was not observed with the enantiomers of **6b** and **6c**. Among these enantiomers, (-)-**33** exhibited the most potent affinity for the D3 receptor ($K_i = 1.80$ nM for D3).

A recent publication has demonstrated that *N*-substituted fused aromatic rings substituted piperazine moiety is well tolerated by the D3 receptor, indicating the possible existence of a hydrophobic binding pocket.⁴⁶ These results further indicate that the bicyclic system is tolerated well on either side of those molecules. In our further exploration to evaluate the effect of an additional hydrophobic interaction in developing molecules with higher selectivity for the D3 receptor, we decided to replace the *N*-phenyl moiety by a linearly fused biphenyl moiety in (-)-**34**. In this regard such a linearly fused biphenyl moiety has

been shown to introduce higher potency and selectivity for D3 receptors in other molecular structures.³² The biphenyl derivative (-)-**34** exhibited subnanomolar potency and high selectivity for the D3 receptor ($K_i = 0.92$ nM for D3 and D2/D3 = 248). Compound (-)-**34** was most potent and selective for D3 in the current series of molecules.

Next, the two compounds (-)-**33** and (-)-**34** were selected based on the binding results for evaluation in the GTPγS binding functional assay for D2 and D3 receptors. In this assay, stimulation of the binding of nonhydrolyzable [³⁵S]GTPγS by agonist was measured and compared with the full agonist DA. The assays were carried out with the cloned human D2 and D3 receptors expressed in CHO and AtT cells.⁴⁷ The half-maximal stimulation (EC₅₀) of (-)-**33** and (-)-**34** along with percent of maximal stimulation compared with a maximal efficacious ($E_{max} = 100\%$) concentration of dopamine indicated full agonist activity of both compounds at D2 and D3 receptors. Both compounds exhibited extremely high affinity in the picomolar range for stimulating the D3 receptor and are two of the most potent agonists for the D3 receptor known to date (EC₅₀ = 0.089 and 0.060 nM for (-)-**34** and (-)-**33**). They also exhibited high selectivity toward preferential stimulation of D3 compared to D2 receptor (D2/D3 EC₅₀ = 248 and 223 for (-)-**34** and (-)-**33**, respectively). In this regard, compound (-)-**34** exhibited greater functional selectivity than 7-OH-DPAT in GTPγS binding assay (D2/D3 = 248 vs 74.95 for (-)-**34** and 7-OH-DPAT (Table 2), respectively).

In Vivo Pharmacology with 6-OH-DA Lesioned Rats. Compounds (-)-**33** and (-)-**34** were chosen for in vivo evaluation in rats carrying unilateral lesion in the medial

Table 1. Affinity for Cloned D2L and D3 Receptors Expressed in HEK Cells Measured by Inhibition of [³H]Spiperone Binding^a

compd	[³ H]spiperone		D2L/D3 (<i>K_i</i> ratio)
	D2L <i>K_i</i> (nM)	D3 <i>K_i</i> (nM)	
7-OH-DPAT	202 ± 34	2.35 ± 0.29	86
D-74	142 ± 23	1.56 ± 0.36	91
6a	639 ± 98	5.96 ± 1.60	107
6b (D-210)	166 ± 19	5.09 ± 0.51	32.6
6c (D-219)	68.4 ± 25.9	1.82 ± 0.43	37.6
6d (D-203)	769 ± 173	12.4 ± 2.2	62.0
6e (D-218)	81.2 ± 11.1	34.8 ± 6.4	2.33
6f (D-220)	34.9 ± 1.3	7.10 ± 0.63	4.92
8 (D-189)	1835(5) ± 445	46.7 ± 12.6	39.3
9b (D-193)	915 ± 165	155 ± 47	5.90
9a (D-192)	813 ± 182	254 ± 99	3.20
11 (D-191)	746 ± 173	160 ± 15	4.66
12 (D-190)	189 ± 55	40.7 ± 17.5	4.64
19 (D-201)	2,570 ± 620	188 ± 23	13.7
(+)- 31 (D-255)	1979 ± 567	44.0 ± 10.6	45.0
(-)- 31 (D-258)	243 ± 65	4.15 ± 0.76	58.6
(+)- 32 (D-257)	243 ± 47	101 ± 41	2.40
(-)- 32 (D-259)	288 ± 86	7.01 ± 1.16	41.1
(+)- 33 (D-256)	44.2 ± 6.9	12.0 ± 2.9	3.68
(-)- 33 (D-260)	56.8 ± 15.4	1.80 ± 0.32	31.6
(-)- 34 (D-264)	264 ± 40	0.92 ± 0.23	253

^a Results are the mean ± SEM for three to seven experiments each performed in triplicate.

forebrain bundle induced by application of the neurotoxin 6-hydroxydopamine (6-OH-DA). This results in destruction of dopamine neurons and development of high supersensitivity of dopamine receptors on the lesioned side. Such surgically modified rats when challenged with direct acting dopamine agonists produce contralateral rotations away from the lesioned side. This rat model is considered to be one of the standard models for preclinical screening of drugs for possible antiparkinsonian property.⁴⁸ Two different doses (2.5 and 5 μmol/kg) of each compound were tested to observe their effect in producing contralateral rotations. At the 5 μmol/kg dose, both (-)-**33** (2.96 mg/kg) and (-)-**34** (3.58 mg/kg) produced potent rotations that lasted for more than 10 h (Figure 3). Compound (-)-**34** was more potent in producing maximum rotations compared to (-)-**33** (3866 vs 2766 for (-)-**34** and (-)-**33**, respectively). Peak effects of both compounds were reached at around 7.4 h. This indicates long duration of action of both compounds in producing contralateral rotations. When tested at a lower dose (2.5 μmol/kg), these two compounds, (-)-**33** (1.48 mg/kg) and (-)-**34** (1.79 mg/kg), produced lower number of rotations, indicating dose dependency for the effect. The total number of rotations produced by these two compounds at the lower dose (2.5 μmol/kg) was comparable (2092 vs 2281 for (-)-**34** and (-)-**33**, respectively). The rotations in this case lasted for more than 8 h (Figure 2).

Conclusion

Novel heterocyclic analogues based on our earlier lead molecule 7-[[2-(4-phenylpiperazin-1-yl)ethyl]propylamino]-

Table 2. Stimulation of [³⁵S]GTPγS Binding to the Cloned hD2 Receptor Expressed in CHO Cells and Cloned hD3 Receptor Expressed in AtT-20 Cells^a

compd	CHO-D2		AtT-D3		D2/D3
	EC ₅₀ [³⁵ S]GTPγS (nM)	<i>E</i> _{max} (%)	EC ₅₀ [³⁵ S]GTPγS (nM)	<i>E</i> _{max} (%)	
dopamine	209(4) ± 29	100 (definition)	8.53 ± 0.62	100 (definition)	24.50
7-OH-DPAT	39.8 ± 19.4	61.4 ± 8.6	0.531 ± 0.042	45.3 ± 2.3	74.95
(-)- 33	13.4 ± 2.4	78.8 ± 0.8	0.06 ± 0.015	95.7 ± 10.7	223
(-)- 34	19.9 ± 0.9	119 ± 6	0.085 ± 0.016	102 ± 19	248

^a EC₅₀ is the concentration producing half-maximal stimulation. For each compound, maximal stimulation (*E*_{max}) is expressed as percent of the *E*_{max} observed with 1 mM (D2) or 100 μM (D3) of the full agonist DA (% *E*_{max}). Results are the mean ± SEM for three to four experiments each performed in triplicate.

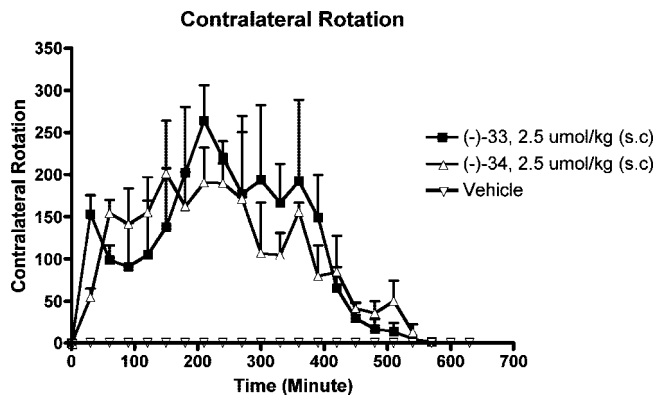


Figure 2. Effect on turning behavior of (-)-**33** and (-)-**34** and vehicle in 6-OH-DA unilaterally lesioned rats studied over 12 h. Each point is the mean ± SEM for four rats. All drugs were administered sc. One way ANOVA analysis demonstrates a significant effect among treatments: $F(3,95) = 29.48$ ($P < 0.0001$). Dunnett's analysis shows that the effect of (-)-**33** and (-)-**34** on rotations at a dose of 2.5 μmol/kg is statistically significantly different compared to vehicle ($P < 0.01$).

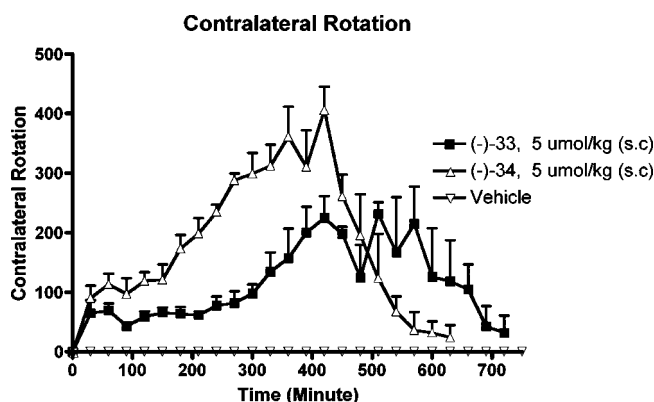


Figure 3. Effect on turning behavior of (-)-**33** and (-)-**34** and vehicle in 6-OH-DA unilaterally lesioned rats studied over 12 h. Each point is the mean ± SEM for four rats. All drugs were administered sc. One way ANOVA analysis demonstrates significant effect among treatments: $F(3,95) = 33.58$ ($P < 0.0001$). Dunnett's analysis shows that the effect of (-)-**33** and (-)-**34** on rotations at a dose of 5 μmol/kg is statistically significantly different compared to vehicle ($P < 0.01$).

5,6,7,8-tetrahydronaphthalen-2-ol have been developed. Among various heterocyclic derivatives, only pyrazole and 2-aminothiazole compounds exhibited potent affinity and selectivity for the D3 receptor. Several enantiomers of 2-aminothiazole compounds were synthesized and characterized. Higher affinity and selectivity mainly resided in the (-)-isomer. Compound (-)-**34** turned out to be the most potent and D3 selective compound in the binding assay. In the GTPγS functional assay, both (-)-**33** and (-)-**34** exhibited high selectivity for the D3 receptor with

picomolar affinity in stimulating the binding of nonhydrolyzable [^{35}S]GTP γ S and did so as full agonists. Thus, (–)-**33** and (–)-**34** are two of the most potent agonists for D3 receptor known to date.⁴⁹ A PD animal model, based on measuring in vivo rotational activity in 6-OH-DA induced unilaterally lesioned rats, indicated potent activity of these two compounds with long duration of action. **In this rotational study, both compounds exhibited comparable activity at a dose of 2.5 $\mu\text{Mol/kg}$ but (–)-**34** was somewhat more active at a higher 5 $\mu\text{Mol/kg}$ dose. Detailed pharmacological evaluation to evaluate relative contribution of D2 and D3 receptors in rotation by (–)-**33** and (–)-**34** will be done at some point of time in future.

Experimental Section

Analytical silica gel-coated TLC plates (silica gel 60 F₂₅₄) were purchased from EM Science and were visualized with UV light or by treatment with either phosphomolybdic acid (PMA) or ninhydrin. Flash chromatography was carried out on Baker silica gel 40 mM. ¹H NMR spectra were routinely obtained on GE-300 MHz and Varian 400 MHz FT NMR spectrometers. The NMR solvent used was either CDCl₃ or CD₃OD as indicated. TMS was used as an internal standard. Elemental analyses were performed by Atlantic Microlab, Inc. and were within $\pm 0.4\%$ of the theoretical value. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter. [^3H]spiperone (15.0 Ci/mmol) and [^{35}S]GTPS (1250 Ci/mmol) were from Perkin-Elmer (Boston, MA). 7-OH-DPAT and (+)-butaclamol were from Sigma-Aldrich (St. Louis, MO).

Procedure A. Synthesis of *N*-(2-(4-Phenylpiperazin-1-yl)ethyl)-1,4-dioxaspiro[4.5]decan-8-amine (3a). Amine **1a** (1.0 g, 4.87 mmol), 1,4-cyclohexanedione monoethylene ketal **2** (0.84 g, 5.36 mmol), and a few drops of acetic acid (0.3 mL) in dichloroethane (30 mL) were stirred under nitrogen atmosphere at 0 °C for 30 min before the addition of sodium triacetoxyborohydride (1.55 g, 7.31 mmol). The reaction mixture was stirred for 8 h at room temperature under nitrogen atmosphere. The reaction mixture was diluted with EtOAc (100 mL), quenched by addition of 10% aqueous HCl (10 mL), followed by addition of saturated NaHCO₃ solution (20 mL). The organic layer was washed with brine (25 mL), dried over Na₂SO₄, evaporated, and the crude product was purified by flash chromatography by using solvent system ethyl acetate/methanol/triethylamine (95:4:1) to yield 1.5 g of compound **3a** (90%). ¹H NMR (400 MHz, CDCl₃): δ 1.42–1.49 (m, 3H), 1.53–1.60 (m, 3H), 1.69 (bs, 2H), 1.77 (d, 2H, $J = 12.0$ Hz), 1.87–1.91 (m, 2H), 2.55 (t, 4H, $J = 4.0$ Hz), 2.60 (t, 4H, $J = 4.4$ Hz), 2.76 (t, 1H, $J = 8.0$ Hz), 3.19 (t, 4H, $J = 4.0$ Hz, Ph-NCH₂), 6.84 (t, 1H, $J = 8.0$ Hz), 6.92 (d, 2H, $J = 8.0$ Hz), 7.24–7.28 (m, 2H).

Synthesis of *N*-(2-(4-(2-Methoxyphenyl)piperazin-1-yl)ethyl)-1,4-dioxaspiro[4.5]decan-8-amine (3b). Amine **1b** (1.25 g, 5.31 mmol) was reacted with 1,4-cyclohexanedione monoethylene ketal **2** (0.83 g, 5.31 mmol), NaCNBH₃ (1.0 g, 15.93 mmol), and HOAc (0.3 mL) in 1,2-dichloroethane (20 mL) to yield 1.09 g of compound **3b** (55%) (procedure A). ¹H NMR (400 MHz, CDCl₃): δ 1.52–1.63 (m, 3H), 1.78–1.81 (m, 2H), 1.94–1.97 (m, 1H), 2.63–2.69 (m, 9H), 2.86 (t, 2H, $J = 6.4$ Hz), 3.10 (bs, 4H), 3.86 (s, 4H), 3.94 (s, 3H), 6.85–7.03 (m, 4H).

Synthesis of *N*-(2-(4-(2,3-Dichlorophenyl)piperazin-1-yl)ethyl)-1,4-dioxaspiro[4.5]decan-8-amine (3c). Amine **1c** (0.65 g, 2.37 mmol) was reacted with 1,4-cyclohexanedione monoethylene ketal (0.37 g, 2.37 mmol), NaCNBH₃ (0.52 g, 8.30 mmol), and HOAc (0.2 mL) in 1,2-dichloroethane (10 mL) to yield 1.09 g of compound **3c** (55%) (procedure A). ¹H NMR (400 MHz, CDCl₃): δ 1.42–1.61 (m, 4H), 1.77–1.80 (m, 2H), 1.89–1.93 (m, 2H), 2.54–2.65 (m, 7H), 2.77–2.80 (t, $J = 6.0$ Hz), 3.06 (bs, 4H), 3.94 (s, 4H), 6.95–6.97 (m, 1H), 7.14–7.16 (m, 2H).

Synthesis of *N*-(4-(4-Phenylpiperazin-1-yl)butyl)-1,4-dioxaspiro[4.5]decan-8-amine (3d). Amine **1d** (0.57 g, 2.44 mmol) was reacted with 1,4-cyclohexanedione monoethylene ketal (0.42 g, 2.69 mmol), NaCNBH₃ (0.34 g, 5.47 mmol), and HOAc (0.15 mL) in

1,2-dichloroethane (10 mL) (procedure A). Purification by column chromatography was done over silica gel by using solvent system dichloromethane/methanol (100:2) to afford 0.75 g of product **3d** (82%). ¹H NMR (400 MHz, CDCl₃): δ 1.53–1.61 (m, 2H), 1.74–1.96 (m, 8H), 2.09–2.15 (m, 2H), 2.52–2.58 (m, 2H), 2.71–2.73 (t, 2H, $J = 4.8$ Hz), 2.77–2.79 (t, 2H, $J = 4.8$ Hz), 2.93–2.96 (t, 2H, $J = 6.0$ Hz), 3.20–3.22 (t, 1H, $J = 5.2$ Hz), 3.25–3.27 (t, 4H, $J = 4.4$ Hz), 3.74 (s, 4H), 6.88–6.96 (m, 3H), 7.25–7.30 (m, 2H).

Procedure B. Synthesis of *N*-(2-(4-Phenylpiperazin-1-yl)ethyl)-*N*-propyl-1,4-dioxaspiro[4.5]decan-8-amine (4a). Compound **3a** (2.05 g, 5.93 mmol), 1-bromopropane (**3**) (2.93 g, 23.83 mmol), and K₂CO₃ (17.87 mmol) in dry DMF (20 mL) were stirred at 60 °C for 10 h, and the mixture was poured into water (60 mL) and extracted with Et₂O (3 \times 100 mL). The combined organic layer was washed with brine, dried over MgSO₄, evaporated, and the residue was purified by flash chromatography using solvent system ethyl acetate/methanol/triethylamine (95:4:1) to yield 2.12 g of pure compound **4a** (92%). ¹H NMR (400 MHz, CDCl₃): δ 0.86 (t, 3H, $J = 4.0$ Hz, N-CH₂CH₂C H₃), 1.43 (m, 2H, -CH₂CH₃), 1.54–1.61 (m, 4H), 1.71–1.80 (m, 5H), 2.4–2.67 (m, 10H), 3.19 (t, 4H, $J = 4.5$ Hz, Ph-N(CH₂)₂), 3.94 (s, 4H, -O(CH₂)₂O-), 6.84 (t, 1H, $J = 8.0$ Hz), 6.92 (d, 2H, $J = 8.0$ Hz), 7.24–7.28 (m, 2H).

Synthesis of *N*-(2-(4-(2-Methoxyphenyl)piperazin-1-yl)ethyl)-*N*-propyl-1,4-dioxaspiro[4.5]decan-8-amine (4b). Compound **3b** (1.09 g, 2.90 mmol) was reacted with 1-bromopropane (**3**) (0.8 mL, 8.80 mmol) and K₂CO₃ (1.22 g, 8.8 mmol) in dry DMF (20 mL) by following procedure B. The residue was purified by flash chromatography using solvent system ethyl acetate/methanol/triethylamine (90:9:1) to yield 0.70 g of pure compound **4b** (58%). ¹H NMR (400 MHz, CDCl₃): δ 1.03 (t, 3H, $J = 7.2$ Hz), 1.65–2.03 (m, 8H), 2.31–2.33 (d, 2H, $J = 10.4$ Hz), 3.04–3.1 (m, 2H), 3.43 (bs, 8H), 3.64–3.94 (m, 12H), 6.87–6.91 (m, 3H), 7.04–7.08 (m, 1H).

Synthesis of *N*-(2-(4-(2,3-Dichlorophenyl)piperazin-1-yl)ethyl)-*N*-propyl-1,4-dioxaspiro[4.5]decan-8-amine (4c). Compound **3c** (1.20 g, 2.90 mmol) was reacted with 1-bromopropane (**3**) (0.8 mL, 8.80 mmol) and K₂CO₃ (1.22 g, 8.8 mmol) in dry DMF (20 mL) by following procedure B. The residue was purified by flash chromatography by using solvent system ethyl acetate/methanol/triethylamine (90:9:1) to yield 0.60 g of pure compound **4c** (45%). ¹H NMR (400 MHz, CDCl₃): δ 0.87 (t, 3H, $J = 7.2$ Hz), 1.39–1.61 (m, 6H), 1.691.80 (m, 4H), 2.42–2.67 (m, 10 H), 3.06 (bs, 4H), 3.19 (t, 1H, $J = 5.2$ Hz), 3.93 (s, 4H), 6.94–6.98 (m, 1H), 7.13–7.15 (m, 2H).

Synthesis of *N*-(4-(4-Phenylpiperazin-1-yl)butyl)-*N*-propyl-1,4-dioxaspiro[4.5]decan-8-amine (4d). Compound **3d** (0.91 g, 2.44 mmol) was reacted with 1-bromopropane (**3**) (0.67 mL, 7.32 mmol) and K₂CO₃ (1.01 g, 7.32 mmol) in dry DMF (20 mL) by following procedure B. The residue was purified by flash chromatography using solvent system ethyl acetate/methanol/triethylamine (90:9:1) to yield 0.61 g of pure compound **4d** (60%). ¹H NMR (400 MHz, CDCl₃): δ 0.86 (t, 3H, $J = 7.2$ Hz), 1.39–1.56 (m, 10H), 1.68–1.80 (m, 4H), 2.37–2.48 (m, 6H), 2.55–2.61 (m, 5H), 3.21 (t, 4H, $J = 5.2$ Hz), 3.93 (s, 4H), 6.83–6.87 (m, 1H), 6.93–6.95 (d, 2H, $J = 8.0$ Hz), 7.24–7.28 (t, 2H, $J = 8.8$ Hz).

Procedure C. Synthesis of *N*-(2-(4-(2-Methoxyphenyl)piperazin-1-yl)ethyl)-*N*-(prop-2-ynyl)-1,4-dioxaspiro[4.5]decan-8-amine (4e). Into a suspension of NaH (60% dispersion in mineral oil) (1.4 g, 34.89 mmol) in 10 mL of anhydrous THF/DMF mixture (5:1) at 0 °C was added compound **3b** (1.31 g, 3.49 mmol) dissolved in 5 mL of the same solvent under nitrogen atmosphere. Reaction mixture was stirred for 30 min at 0 °C before propargyl bromide (80 wt % dispersion in toluene) (3.11 g, 20.93 mmol) was added. The reaction mixture was stirred at 60 °C for 3 h. The reaction mixture was cooled to room temperature. Drop by drop water was added to quench the reaction mixture, and the solvent was removed in vacuo. Reaction mixture was washed with 20 mL of water. The aqueous layer was extracted with Et₂O (3 \times 50 mL). The combined organic layer was washed with brine, dried over MgSO₄, evaporated,

and the residue was purified by flash chromatography using solvent system ethyl acetate/methanol (95:5) to yield 0.44 g of pure compound **4e** (31%). ¹H NMR (400 MHz, CDCl₃): δ 1.51–1.66 (m, 4H), 1.78–1.89 (m, 4H), 2.18 (t, 1H, *J* = 2.4 Hz), 2.53–2.70 (m, 7H), 2.80 (t, 2H, *J* = 8.0 Hz), 3.10 (bs, 4H), 3.50–3.51 (d, 2H, *J* = 2.0 Hz), 3.86 (s, 3H, –OCH₃), 3.93 (s, 4H), 6.85–7.01 (m, 4H).

Synthesis of *N*-(2-(4-(2,3-Dichlorophenyl)piperazin-1-yl)ethyl)-*N*-(prop-2-ynyl)-1,4-dioxaspiro[4.5]decan-8-amine (4f). Compound **3c** (0.73 g, 1.76 mmol) was reacted with propargyl bromide (0.79 mL, 7.05 mmol) in the presence of NaH (0.42 g, 10.57 mmol) in THF/DMF mixture (20 mL) by following procedure C. The residue was purified by flash chromatography by using solvent system hexane/ethyl acetate (50:50) to yield 0.31 g of pure compound **4f** (39%). ¹H NMR (400 MHz, CDCl₃): δ 1.51–1.63 (m, 4H), 1.79–1.89 (m, 4H), 2.19 (t, 1H, *J* = 2.4 Hz), 2.54–2.68 (m, 7H), 2.79 (t, 2H, *J* = 7.6 Hz), 3.07 (s, 4H), 3.51–3.52 (d, 2H, *J* = 2.0 Hz), 3.94 (s, 4H), 6.94–6.97 (dd, 1H, *J* = 6.4 Hz, *J* = 3.2 Hz), 7.11–7.15 (m, 2H).

Procedure D. Synthesis of 4-((2-(4-Phenylpiperazin-1-yl)ethyl)(propyl)amino)cyclohexanone (5a). Into a solution of compound **4a** (2.05 g, 5.97 mmol) in 20 mL of THF was added 2 N HCl (50 mL), and the reaction mixture was stirred at 80 °C under nitrogen atmosphere for 6 h. THF was removed under reduced pressure. Reaction mixture was made alkaline by saturated NaHCO₃ solution. The aqueous layer was extracted with EtOAc (3 × 100 mL) and the combined organic layer was washed with brine, dried over Na₂SO₄, and evaporated to yield crude product which was purified by flash chromatography on silica gel using solvent system ethyl acetate/methanol/triethyl amine (95:4:1) to yield 0.96 g of pure **5a** (52%). ¹H NMR (400 MHz, CDCl₃): δ 0.89 (t, 3H, *J* = 7.6 Hz), 1.42–1.51 (m, 2H), 1.69–1.79 (qd, 2H, *J*₁ = 11.4 Hz, *J*₂ = 4.8 Hz), 2.03–2.07 (m, 2H), 2.30–2.38 (m, 2H), 2.42–2.51 (m, 6H), 2.63–2.69 (m, 6H), 2.95–3.02 (tt, 1H, *J*₁ = 11.2, *J*₂ = 3.2 Hz, –CH–N), 3.20 (t, 4H, *J* = 5.2 Hz, Ph–NCH₂), 6.83–6.87 (t, 1H, *J* = 7.2 Hz), 6.91–6.94 (d, 2H, *J* = 8.4 Hz), 7.24–7.28 (m, 2H).

Synthesis of 4-((2-(4-Methoxyphenyl)piperazin-1-yl)ethyl)(propyl)amino)cyclohexanone (5b). Compound **4b** (0.4 g, 0.96 mmol) dissolved in 5 mL of THF was hydrolyzed in the presence of 2 N HCl (25 mL) by following procedure D. The residue was purified by flash chromatography using solvent system ethyl acetate/methanol (90:10) to yield 0.30 g of pure compound **5b** (83%). ¹H NMR (400 MHz, CDCl₃): δ 0.89 (t, 3H, *J* = 7.2 Hz), 1.42–1.52 (m, 2H), 1.67–1.79 (m, 2H), 2.03–2.07 (m, 2H), 2.30–2.53 (m, 8H), 2.63–2.70 (m, 6H), 2.95–3.02 (tt, 1H, *J*₁ = 3.2 Hz, *J*₂ = 10.8 Hz), 3.10 (bs, 4H), 3.86 (s, 3H, –OC H₃), 6.85–7.02 (m, 4H).

Synthesis of 4-((2-(4-(2,3-Dichlorophenyl)piperazin-1-yl)ethyl)(propyl)amino)cyclohexanone (5c). Compound **4c** (0.59 g, 1.29 mmol) dissolved in 5 mL of THF was hydrolyzed in the presence of 2 N HCl (25 mL) by following procedure D. The residue was purified by flash chromatography using solvent system ethyl acetate/methanol (95:5) to yield 0.31 g of pure compound **5c** (58%). ¹H NMR (400 MHz, CDCl₃): δ 0.89 (t, 3H, *J* = 7.6 Hz), 1.45–1.50 (m, 2H), 1.76–1.80 (m, 2H), 2.03–2.30 (m, 2H), 2.32–2.69 (m, 13 H), 2.95–3.07 (m, 5H), 3.67 (t, 1H, *J* = 5.6 Hz), 6.94–6.97 (dd, 1H, *J*₁ = 6.6 Hz, *J*₂ = 3.2 Hz), 7.13–7.15 (m, 2H).

Synthesis of 4-((4-(4-Phenylpiperazin-1-yl)butyl)(propyl)amino)cyclohexanone (5d). Compound **4d** (0.50 g, 1.2 mmol) dissolved in 5 mL of THF was hydrolyzed in the presence of 2 N HCl (25 mL) by following procedure D. The residue was purified by flash chromatography using solvent system ethyl acetate/methanol/triethylamine (95:4:0.5) to yield 0.36 g of pure compound **5d** (80%). ¹H NMR (400 MHz, CDCl₃): δ 0.88 (t, 3H, *J* = 7.2 Hz), 1.43–1.76 (m, 11H), 1.85–2.05 (m, 5H), 2.33–2.50 (m, 5H), 2.61 (t, 4H, *J* = 4.4 Hz), 3.21 (t, 4H, *J* = 5.2 Hz), 6.84–6.87 (t, 1H, *J* = 7.2 Hz), 6.93–6.95 (d, 2H, *J* = 8.0 Hz), 7.27 (t, 2H, *J* = 8.8 Hz).

Synthesis of 4-((2-(4-(2-Methoxyphenyl)piperazin-1-yl)ethyl)(prop-2-ynyl)amino)cyclohexanone (5e). Compound **4e** (0.44 g, 1.06 mmol) dissolved in 5 mL of THF was hydrolyzed in the presence of 2 N HCl (25 mL) by following procedure D. The residue was purified by flash chromatography using solvent system ethyl acetate/methanol/triethylamine (95:4:0.5) to yield 0.30 g of pure compound **5e** (76%). ¹H NMR (400 MHz, CDCl₃): δ 1.75–1.84 (m, 2H), 2.02–2.04 (m, 2H), 2.16 (bs, 1H), 2.20–2.28 (m, 2H), 2.36–2.42 (m, 2H), 2.48–2.52 (t, 2H, *J* = 6.8 Hz), 2.63 (bs, 4H), 2.74–2.78 (t, 2H, *J* = 7.2 Hz), 2.91–2.96 (m, 1H), 3.02 (bs, 4H), 3.49 (bs, 2H), 3.76 (s, 3H), 6.77–6.93 (m, 4H).

Synthesis of 4-((2-(4-(2,3-Dichlorophenyl)piperazin-1-yl)ethyl)(prop-2-ynyl)amino)cyclohexanone (5f). Compound **4f** (0.31 g, 0.69 mmol) dissolved in 5 mL of THF was hydrolyzed in the presence of 2 N HCl (25 mL) by following procedure D. The residue was purified by flash chromatography by using solvent system ethyl acetate/methanol/triethylamine (95:4:0.5) to yield 0.20 g of pure compound **5f** (73%). ¹H NMR (400 MHz, CDCl₃): δ 1.84–1.93 (m, 2H), 2.10–2.14 (m, 2H), 2.22 (t, 1H, *J* = 2.4 Hz, –NCH₂CC H), 2.29–2.37 (m, 2H), 2.46–2.52 (m, 2H), 2.58 (t, 2H, *J* = 7.2 Hz), 2.69 (bs, 4H), 2.83 (t, 2H, *J* = 7.2 Hz), 2.99–3.07 (m, 5H), 3.58 (d, 2H, *J* = 2.0 Hz), 6.95–6.97 (dd, 1H, *J*₁ = 6.8 Hz, *J*₂ = 2.4 Hz), 7.14–7.17 (m, 2H).

Procedure E. Synthesis of *N*⁶-(2-(4-Phenylpiperazin-1-yl)ethyl)-*N*⁶-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine (6a). Compound **5a** (0.53 g, 1.54 mmol) and pyrrolidine (0.15 mL, 1.85 mmol) in the presence of catalytic amount of *p*-toluenesulfonic acid (0.005 mol ratio) in anhydrous benzene were heated to reflux in a Dean–Stark apparatus for 1.5 h. Reaction mixture was then cooled to room temperature and concentrated under vacuum. The residue was then dissolved in anhydrous methanol. Sulfur powder (S₈) (59 mg, 0.23 mmol) was added at room temperature, stirred for 20 min, and then cooled to 0 °C. Cyanamide (78 mg, 1.55 mmol) in methanol was added to it, and the reaction mixture was stirred at room temperature for 12 h. Methanol was evaporated in vacuo. The residue was dissolved in ethyl acetate, washed with water and brine, dried over MgSO₄, evaporated. The crude residue was purified by flash chromatography using solvent system ethyl acetate/methanol (90:10) to yield 0.26 g of pure compound **6a** (43%). ¹H NMR (400 MHz, CDCl₃): δ 0.87–0.92 (m, 3H), 1.40–1.52 (m, 2H), 1.70–1.77 (m, 1H), 1.98–2.03 (m, 1H), 2.44–2.61 (m, 6H), 2.64–2.75 (m, 8H), 3.02–3.10 (m, 1H), 3.19–3.21 (t, 4H, *J* = 4.8 Hz), 4.82 (bs, 2H), 6.83–6.88 (m, 1H), 6.92–6.95 (m, 2H), 7.24–7.28 (m, 2H). The product was converted into corresponding dioxalate salt, mp 160–163 °C. Anal. (C₂₂H₃₃N₅S·2C₂H₂O₄·2H₂O) C, H, N.

Synthesis of *N*⁶-(2-(4-(2-Methoxyphenyl)piperazin-1-yl)ethyl)-*N*⁶-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine (6b). Compound **5b** (0.36 g, 0.96 mmol) was treated with pyrrolidine (0.08 mL, 1.01 mmol) in the presence of catalytic amount of *p*-toluenesulfonic acid under reflux followed by addition of sulfur powder (31 mg, 0.12 mmol) and cyanamide (41 mg, 1.01 mmol) by following procedure E. The crude residue was purified by flash chromatography using solvent system ethyl acetate/methanol (90:10) to yield 0.20 g of pure compound **6b** (48%). ¹H NMR (400 MHz, CDCl₃): δ 0.89 (t, 3H, *J* = 7.6 Hz), 1.43–1.52 (m, 2H), 1.68–1.76 (m, 1H), 1.98–2.04 (m, 1H), 2.45–2.77 (m, 14H), 3.01–3.10 (m, 5H), 3.86 (s, 3H), 4.75 (bs, 2H, –NH₂), 6.85–7.02 (m, 4H). The product was converted into corresponding tetrahydrochloride salt, mp 105–108 °C. Anal. (C₂₃H₃₅N₅OS·4HCl·2H₂O) C, H, N.

Synthesis of *N*⁶-(2-(4-(2,3-Dichlorophenyl)piperazin-1-yl)ethyl)-*N*⁶-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine (6c). Compound **5c** (0.315 g, 0.76 mmol) was treated with pyrrolidine (57.4 mg, 0.07 mL, 0.81 mmol) in the presence of catalytic amount of *p*-toluenesulfonic acid under reflux followed by addition of sulfur powder (24.5 mg, 0.095 mmol) and cyanamide (32.1 mg, 0.76 mmol) by following procedure E. The crude residue was purified by flash chromatography using solvent system ethyl acetate/methanol (90:10) to yield 0.20 g of pure compound **6c** (56%). ¹H NMR (400 MHz, CDCl₃): δ 0.89 (t, 3H, *J* = 7.6 Hz),

1.44–1.50 (m, 2H), 1.66–1.77 (m, 1H), 1.97–2.00 (m, 1H), 2.45–2.73 (m, 14H), 3.01–3.06 (m, 4H), 3.19 (t, 1H, $J = 4.8$ Hz), 4.91 (bs, 2H, $-NH_2$), 6.94–6.96 (dd, 1H, $J_1 = 6.6$ Hz, $J_2 = 3.2$ Hz), 7.13–7.15 (m, 2H). The product was converted into corresponding dioxalate salt, mp 175–177 °C. Anal. ($C_{22}H_{31}Cl_2N_5S \cdot 2C_2H_2O_4 \cdot 0.9H_2O$) C, H, N.

Synthesis of N^6 -(4-(4-Phenylpiperazin-1-yl)butyl)- N^6 -propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine (6d). Compound **5d** (0.36 g, 0.96 mmol) was treated with pyrrolidine (0.08 mL, 1.01 mmol) in the presence of catalytic amount of *p*-toluenesulfonic acid under reflux followed by addition of sulfur powder (30.8 mg, 0.12 mmol) and cyanamide (40.4 mg, 0.96 mmol) by following procedure E. The crude residue was purified by flash chromatography using solvent system ethyl acetate/methanol (95:5) to yield 0.20 g of pure compound **6d** (49%). 1H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, 3H, $J = 7.2$ Hz), 1.35–1.57 (m, 7H), 1.66–1.75 (m, 1H), 1.96–1.99 (m, 1H), 2.38–2.72 (m, 13H), 2.99–3.10 (m, 1H), 3.21 (t, 4H, $J = 4.8$ Hz), 4.77 (bs, 2H, $-NH_2$), 6.85 (t, 1H, $J = 7.2$ Hz), 6.92–6.94 (d, 2H, $J = 8.0$ Hz), 7.26 (t, 2H, $J = 8.4$ Hz). The product was converted into corresponding tetrahydrochloride salt, mp 210–213 °C. Anal. ($C_{24}H_{37}N_5S \cdot 4HCl$) C, H, N.

Synthesis of N^6 -(2-(4-(2-Methoxyphenyl)piperazin-1-yl)ethyl)- N^6 -(prop-2-ynyl)-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine (6e). Compound **5e** (0.29 g, 0.79 mmol) was treated with pyrrolidine (0.07 mL, 0.83 mmol) in the presence of catalytic amount of *p*-toluenesulfonic acid under reflux followed by addition of sulfur powder (27 mg, 0.104 mmol) and cyanamide (33.2 mg, 0.79 mmol) by following procedure E. The crude residue was purified by flash chromatography using solvent system ethyl acetate/methanol (95:5) to yield 0.18 g of pure compound **6e** (52%). 1H NMR (400 MHz, $CDCl_3$): δ 1.69–1.79 (m, 1H), 2.08–2.14 (m, 1H), 2.22 (bs, 1H), 2.56–2.71 (m, 12H), 3.10 (bs, 5H), 3.57 (d, 2H, $J = 1.6$ Hz), 3.86 (s, 3H), 5.07 (bs, 2H), 6.85–7.01 (m, 4H). The product was converted into corresponding dioxalate salt, mp 150–154 °C. Anal. ($C_{22}H_{31}N_5OS \cdot 2C_2H_2O_4 \cdot 0.8H_2O$) C, H, N.

Synthesis of N^6 -(2-(4-(2,3-Dichlorophenyl)piperazin-1-yl)ethyl)- N^6 -(prop-2-ynyl)-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine (6f). Compound **5f** (0.205 g, 0.50 mmol) was treated with pyrrolidine (0.05 mL, 0.55 mmol) in the presence of catalytic amount of *p*-toluenesulfonic acid in benzene under reflux followed by addition of sulfur powder (16.1 mg, 0.063 mmol) and cyanamide (21.1 mg, 0.50 mmol) by following procedure E. The crude residue was purified by flash chromatography using solvent system ethyl acetate/methanol (95:5) to yield 0.10 g of pure compound **6f** (43%). 1H NMR (400 MHz, $CDCl_3$): δ 1.69–1.79 (m, 1H), 2.08–2.14 (m, 1H), 2.23–2.27 (m, 1H), 2.57–3.07 (m, 17H), 3.58 (s, 2H), 4.99 (bs, 2H, $-NH_2$), 6.94–6.97 (dd, 1H, $J_1 = 6.40$ Hz, $J_2 = 2.8$ Hz), 7.12–7.15 (m, 2H). The product was converted into corresponding dioxalate salt, mp 170–172 °C. Anal. ($C_{22}H_{27}Cl_2N_5S \cdot 2C_2H_2O_4 \cdot 0.2H_2O$) C, H, N.

Synthesis of 2-Hydroxymethylene-4-[[2-(4-phenylpiperazin-1-yl)ethyl]propylamino]cyclohexanone (7). Na metal (2.0 g) was stirred in excess EtOH (40 mL) under nitrogen atmosphere for half an hour until evolution of hydrogen gas ceases. The excess EtOH was removed under reduced pressure. This freshly prepared NaOEt was dried completely and used for the subsequent reaction. Compound **5a** (1.11 g, 3.22 mmol) in anhydrous benzene and excess NaOEt (3.5 g, 51.43 mmol) was stirred under nitrogen atmosphere at 0 °C for 10 min. Ethyl formate (2.1 mL, 2.58 mmol) was added dropwise, and the reaction mixture was stirred overnight at room temperature under nitrogen atmosphere.

Benzene layer was extracted with water (two to three times). The aqueous extract containing sodium salt of **7** was made slightly acidic with dilute HCl, then buffered with 2 N $KHCO_3$ and extracted with CH_2Cl_2 (3 \times 100 mL). The organic extract was washed with brine, dried over Na_2SO_4 , evaporated under reduced pressure to yield sufficiently pure 1.10 g of compound **7** (92%). 1H NMR (400 MHz, $CDCl_3$): δ 0.89 (t, 3H, $J = 7.2$ Hz, $N-CH_2CH_2C H_3$), 1.45 (m, 2H, $CH_2C H_2CH_3$), 1.55–1.65 (m, 1H), 1.89–1.92 (m, 1H), 2.24–2.33 (m, 2H), 2.44–2.53 (m, 6H), 2.64–2.71 (m, 6H),

2.80–2.86 (m, 1H, $N-CH$), 3.20 (t, 4H, $J = 4.8$ Hz, $Ph-N(CH_2)_2$), 6.86 (t, 1H, $J = 7.2$ Hz), 6.93 (d, 2H, $J = 8.4$ Hz), 7.26 (t, 2H, $J = 8.0$ Hz), 8.65 (s, 1H, $-C=CH-OH$).

Synthesis of N -(2-(4-Phenylpiperazin-1-yl)ethyl)- N -propyl-4,5,6,7-tetrahydro-1*H*-indazol-5-amine (8). Compound **7** (0.67 g, 1.80 mmol) was dissolved in 25 mL of ethanol, and a solution of semicarbazide HCl (0.4 g, 3.60 mmol) and sodium acetate (0.29 g, 3.60 mmol) in 10 mL of water was added dropwise. The mixture was stirred at 0 °C for 1 h until the TLC showed disappearance of the starting material. The solvent was evaporated in vacuo. The reaction mixture was heated under reflux in 15 mL of concentrated H_2SO_4 /water mixture 1:3 (20 mL) for 15 min. The mixture was made alkaline with NaOH solution and extracted with CH_2Cl_2 (3 \times 50 mL). The organic layer was washed with brine, dried over Na_2SO_4 , evaporated under reduced pressure, and purified by flash chromatography on silica gel using solvent system ethyl acetate/methanol/triethylamine (85:13:2) to yield 0.46 g of pure compound **8** (69%). 1H NMR (400 MHz, $CDCl_3$): δ 0.89 (t, 3H, $J = 7.6$ Hz, $N-CH_2CH_2CH_3$), 1.44–1.54 (m, 2H), 1.65–1.76 (m, 1H), 2.02–2.08 (m, 1H), 2.44–2.55 (m, 5H), 2.65–2.75 (m, 8H), 2.83–2.99 (m, 2H), 3.20 (t, 4H, $J = 4.8$ Hz, $Ph-N(CH_2)_2$), 6.85 (t, 1H, $J = 7.2$ Hz), 6.92 (d, 2H, $J = 8.0$ Hz), 7.24–7.28 (m, 3H, Ph (2H)) and $N=CH-C$). The product was converted into corresponding tetrahydrochloride salt, mp 162–164 °C. Anal. ($C_{22}H_{33}N_5 \cdot 4HCl \cdot 0.5H_2O$) C, H, N.

Synthesis of [2-(4-Phenylpiperazin-1-yl)ethyl]propyl(4,5,6,7-tetrahydrobenzo[d]isoxazol-5-yl)amine (9b). Into a solution of compound **7** (0.67 g, 1.80 mmol) in acetic acid (20 mL), $NH_2OH \cdot HCl$ (0.26 g, 3.74 mmol) was added, and the mixture was heated for 1 h at 80 °C. The reaction mixture was cooled and poured into ice/water (50 mL). The solution was made alkaline with 2 N NaOH, and the water layer was extracted with CH_2Cl_2 (3 \times 50 mL). The organic layer was washed with brine, dried over Na_2SO_4 , and evaporated under reduced pressure. The crude mass was purified by column chromatography on silica gel by using solvent system ethyl acetate/methanol/triethylamine (95:4:1) to yield 0.53 g of pure compound **9b** (80%). 1H NMR (400 MHz, $CDCl_3$): δ 0.98 (t, 3H, $J = 7.2$ Hz), 1.43–1.52 (m, 2H), 1.62–1.73 (m, 1H), 2.06–2.10 (m, 1H), 2.42–2.52 (m, 5H), 2.62–2.72 (m, 7H), 2.76–2.81 (m, 1H), 2.90–2.97 (m, 1H), 3.02–3.08 (m, 1H), 3.20 (t, 4H, $J = 4.8$ Hz), 6.86 (t, 1H, $J = 7.6$ Hz), 6.92 (d, 2H, $J = 8.0$ Hz), 7.24–7.28 (m, 2H), 8.09 (s, 1H, $-O-N=C H-C$). The product was converted into corresponding trihydrochloride salt, mp 145–148 °C. Anal. ($C_{22}H_{32}N_4O \cdot 3HCl \cdot 0.5H_2O$) C, H, N.

Synthesis of [2-(4-Phenylpiperazin-1-yl)ethyl]propyl(4,5,6,7-tetrahydrobenzo[c]isoxazol-5-yl)amine (9a). Into a solution of compound **7** (1.84 g, 4.95 mmol) in 30 mL of pyridine, $NH_2OH \cdot HCl$ (0.86 g, 1.24 mmol) in water (3 mL) was added dropwise. The mixture was then heated under reflux for 6 h, cooled, and diluted with water. The product was extracted with CH_2Cl_2 (3 \times 50 mL). The organic layer was washed with brine, dried over Na_2SO_4 , and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using solvent system ethyl acetate/methanol/triethylamine (95:5:1) to yield 0.90 g of pure compound **9a** (49.3%). 1H NMR (400 MHz, $CDCl_3$): δ 0.89 (t, 3H, $J = 7.60$ Hz), 1.42–1.51 (m, 2H), 1.61–1.72 (m, 1H), 2.05–2.09 (m, 1H), 2.41–2.51 (m, 5H), 2.61–2.78 (m, 8H), 2.89–2.97 (m, 1H), 3.01–3.02 (m, 1H), 3.19 (t, 4H, $J = 4.8$ Hz, $Ph-N(CH_2)_2$), 6.85 (t, 1H, $J = 7.6$ Hz), 6.92 (d, 2H, $J = 8.0$ Hz), 7.24–7.28 (m, 2H), 8.07 (s, 1H, $-N-O=CH-C$). The product was converted into corresponding trihydrochloride salt, mp 148–150 °C. Anal. ($C_{22}H_{32}N_4O \cdot 3HCl \cdot 0.2H_2O$) C, H, N.

Synthesis of 2-(4-((2-(4-Phenylpiperazin-1-yl)ethyl)(propylamino)cyclohexylidene)hydrazinecarboxamide (10). A mixture of semicarbazide HCl (0.65 g, 5.82 mmol) and sodium acetate (0.48 g, 5.82 mmol) was dissolved in methanol, and the residue formed was filtered off. Compound **5a** (1.0 g, 2.91 mmol) in methanol was added to the filtrate, and the reaction mixture was heated under reflux for 4 h. The reaction mixture was concentrated and diluted with cold water. The aqueous layer was extracted with CH_2Cl_2 (150 mL). The organic layer was washed with brine, dried over Na_2SO_4 ,

evaporated and the resulting crude mass was purified by column chromatography on silica gel column using solvent system ethyl acetate/methanol/triethylamine (80:15:5) to yield 0.69 g of pure compound **10** (70%). ¹H NMR (400 MHz, CDCl₃): δ 0.88 (t, 3H, *J* = 7.6), 1.40–1.57 (m, 4H), 1.68 (bs, 2H), 1.81–1.98 (m, 5H), 2.16–2.24 (m, 1H), 2.42–2.49 (m, 4H), 2.63–2.65 (m, 6H), 2.76–2.81 (m, 1H), 3.2 (t, 4H, *J* = 4.8 Hz, Ph–N(CH₂)₂), 6.84–6.87 (t, 1H, *J* = 7.6 Hz), 6.92–6.94 (d, 2H, *J* = 8.0 Hz), 7.24–7.84 (t, 2H, *J* = 8.0 Hz), 7.79 (s, 1H, –NNHCO).

Synthesis of [2-(4-Phenylpiperazin-1-yl)ethyl]propyl(4,5,6,7-tetrahydrobenzo[1,2,3]thiadiazol-6-yl)amine (11). Compound **10** (0.45 g, 2.49 mmol) was added to an excess of thionyl chloride (15 mL) in portionwise manner at –10 °C in stirred conditions under nitrogen atmosphere. The reaction mixture was allowed to attain room temperature. Then CH₂Cl₂ (100 mL) was added and the diluted reaction mixture was treated with saturated Na₂CO₃ solution. The organic layer was separated and washed thoroughly with brine, dried over Na₂SO₄, and evaporated. The crude reaction mixture was purified by column chromatography on silica gel column using solvent system ethyl acetate/methanol/triethylamine (95:4:1) to yield 0.30 g of pure compound **11** (69.3%). ¹H NMR (400 MHz, CDCl₃): δ 0.90 (t, 3H, *J* = 7.6 Hz), 1.44–1.53 (m, 2H), 1.74–1.85 (m, 1H), 2.16–2.20 (m, 1H), 2.47–2.85 (m, 11H), 3.00–3.21 (m, 7H), 3.43–3.48 (dd, 1H, *J*₁ = 16.4 Hz, *J*₂ = 4 Hz, –SCCH–), 6.85 (t, 1H, *J* = 7.6 Hz), 6.93 (d, 2H, *J* = 8.0 Hz), 7.24–7.28 (m, 2H). The product was converted into corresponding trihydrochloride salt, mp 160–162 °C. Anal. (C₂₁H₃₁N₅S·3HCl·0.6H₂O) C, H, N.

Synthesis of [2-(4-Phenylpiperazin-1-yl)ethyl]propyl(4,5,6,7-tetrahydrobenzo[1,2,3]selenadiazol-6-yl)amine (12). The semicarbazone **10** (0.46 g, 2.49 mmol) was dissolved in glacial acetic acid (20 mL) and warmed gently with stirring to provide a clear solution. Selenium dioxide (0.33 g, 2.99 mmol) was added in a portionwise manner into the solution. The reaction mixture was stirred for 30 min. The deposited selenium was removed by filtration. The filtrate was diluted with cold water (50 mL) and washed with saturated NaHCO₃ solution. The aqueous layer was extracted with CH₂Cl₂ (150 mL). The organic layer was washed with brine, dried over Na₂SO₄, evaporated under reduced pressure. The crude mass was purified by column chromatography on silica gel column using solvent system ethyl acetate/methanol/triethylamine (95:4:1) to yield 0.36 g of pure compound **11** (73%). ¹H NMR (400 MHz, CDCl₃): δ 0.89 (t, 3H, *J* = 7.6 Hz), 1.42–1.51 (m, 2H), 1.74–1.84 (m, 1H), 2.12–2.20 (m, 1H), 2.48–2.83 (m, 11H), 3.15–3.29 (m, 7H), 3.53–3.56 (dd, 1H, *J*₁ = 16.2 Hz, *J*₂ = 4 Hz, –SeCCH–), 6.85 (t, 1H, *J* = 7.6 Hz), 6.92 (d, 2H, *J* = 8.0 Hz), 7.24–7.28 (m, 2H). The product was converted into corresponding trihydrochloride salt, mp 210–212 °C. Anal. (C₂₁H₃₁N₅Se·3HCl·0.7H₂O) C, H, N.

Synthesis of 3-Ethoxy-6-hydroxymethylenecyclohex-2-enone (13). Into a stirred solution of 3-ethoxy-2 cyclohexen-1-one (3.50 g, 25 mmol) and freshly prepared sodium ethoxide (obtained from 7.0 g (30.0 mmol) of sodium metal) in benzene (100 mL) at 0 °C was added ethyl formate (7.4 g, 100 mmol) dropwise under nitrogen atmosphere. The mixture was stirred overnight at room temperature. Benzene layer was extracted with water (two to three times). The aqueous extract containing sodium salt of **13** was made slightly acidic with dilute HCl, then buffered with 2 N KHCO₃ and extracted with 3 × 100 mL of CH₂Cl₂. The combined organic layer was washed with brine, dried over Na₂SO₄, and evaporated under reduced pressure. The crude mass was purified by flash chromatography over a silica gel column using hexane/ethyl acetate (1:1) as eluant to yield 3.04 g of title compound **13** (72%). ¹H NMR (400 MHz, CDCl₃): δ 1.36 (t, 3H, *J* = 6.8 Hz, –OCH₂CH₃), 2.39–2.44 (m, 4H, –OCCH₂CH₂C), 3.89–3.95 (q, 2H, *J* = 7.2 Hz, –OCH₂CH₃), 5.31 (s, –OCCHCO), 7.17 (d, 1H, *J* = 9.2 Hz, –CCHO).

Synthesis of 6-Ethoxy-4,5-dihydro-2H-indazole (14). Into the solution of compound **13** (0.4 g, 2.37 mmol) in 20 mL of ethanol was added hydrazine (0.15 mL, 4.76 mmol) under nitrogen atmosphere. The reaction mixture was refluxed for 6 h. The solvent was evaporated in vacuo and the crude product was purified by

flash chromatography over a silica gel column using hexane/ethyl acetate (1:1) as eluant to afford 0.34 g compound **14** (87%). ¹H NMR (400 MHz, CDCl₃): δ 1.36 (t, 3H, *J* = 6.8 Hz, –OCH₂CH₃), 2.46 (t, 2H, *J* = 8.0 Hz, OCCH₂CH₂), 2.75 (t, 2H, *J* = 8.0 Hz, –OCCH₂CH₂–), 3.86–3.91 (q, 2H, *J* = 7.2 Hz, –OCH₂CH₃), 5.62 (s, 1H, –OCCHCN), 7.19 (s, 1H, –CCHNH–).

Synthesis of 6-Ethoxy-2-(2,4,6-trimethylbenzenesulfonyl)-4,5-dihydro-2H-indazole (15). Into a stirred mixture of (0.28 g, 1.7 mmol) of compound **14** and 0.38 mL of 20% sodium hydroxide solution (1.87 mmol) in 40 mL of CH₂Cl₂, 2-mesitylene sulfonyl chloride was added. The reaction mixture was kept in refluxing condition overnight. The reaction mixture was diluted with CH₂Cl₂ (50 mL) and washed with water. The water layer was extracted with CH₂Cl₂ (3 × 50 mL). The combined organic layer was washed with brine, dried over Na₂SO₄, and evaporated in vacuo. The crude mass was purified by flash chromatography over a silica gel column using hexane/ethyl acetate (9:1) as eluant to yield 0.51 g of title compound **15** (86.4%). ¹H NMR (400 MHz, CDCl₃): δ 1.33 (t, 3H, *J* = 6.8 Hz, –CH₂CH₃), 2.28 (s, 3H, CH₃(Mst–)), 2.43 (t, 2H, *J* = 7.6), 2.6 (s, 2H), 2.66 (s, 6H, C H₃(Mst–)), 2.74 (t, 2H, *J* = 7.6), 5.56 (s, 1H, –OCCHCN), 6.95 (s, 2H, Ar–H), 7.73 (s, 1H, –CCHN–).

Synthesis of 2-(2,4,6-Trimethylbenzenesulfonyl)-2,4,5,7-tetrahydroindazol-6-one (16). Into the stirred solution of compound **15** (1.69 g, 4.87 mmol) in 20 mL of THF at 0 °C was added 2.7 mL of 3 N HCl dropwise. After HCl addition, the reaction mixture was stirred in an ice bath for 30 min. Then it was stirred at room temperature under nitrogen atmosphere for 16 h. THF was removed under reduced pressure. Excess saturated NaHCO₃ solution was added. The water layer was extracted three to four times with EtOAc (3 × 100 mL). The combined organic layer was washed with brine, dried over Na₂SO₄, and evaporated in vacuo to yield sufficiently pure 1.5 g of compound **16** (96%). ¹H NMR (400 MHz, CDCl₃): δ 2.31–2.32 (m, 3H, –Mst–CH₃), 2.58–2.62 (m, 2H, –COCH₂CH₂), 2.66 (s, 6H, –Mst–CH₃), 2.91 (t, 2H, *J* = 6.8 Hz, –COCH₂CH₂), 3.55 (s, 2H, –COCH₂C–), 6.99 (s, 2H, Ar–H), 7.98 (s, 1H, –CCHN–).

Synthesis of [2-(4-Phenylpiperazin-1-yl)ethyl][2-(2,4,6-trimethylbenzenesulfonyl)-4,5,6,7-tetrahydro-2H-indazol-6-yl]amine (17). A mixture of amine **1a** (0.3976 g, 1.94 mmol), compound **16** (1.29 mmol), and acetic acid in dichloroethane (20 mL) was stirred at room temperature under nitrogen atmosphere for 15–20 min. NaCNBH₃ (0.33 g, 5.17 mmol) dissolved in 2 mL of methanol was added dropwise. The reaction mixture was stirred for 4 h at room temperature. Water was added followed by dilute HCl. Excess HCl was then neutralized with saturated NaHCO₃ solution. The water layer was extracted with CH₂Cl₂ (3 × 100 mL). The combined organic layer was washed with brine, dried over Na₂SO₄, and evaporated in vacuo. The crude mass was purified by flash chromatography over a silica gel column using ethyl acetate/methanol/triethylamine (95:4:1) as eluant to yield 0.47 g of title compound **17** (48%). ¹H NMR (400 MHz, CDCl₃): δ 1.56–1.66 (m, 1H, –NHCHCH₂–), 1.95–2.00 (m, 1H), 1.78 (broad s, 1H, –NH–), 2.29–2.30 (m, 3H, Mst–C H₃), 2.39–2.46 (m, 1H), 2.53–2.65 (m, 14H), 2.69–2.86 (m, 2H), 2.92–3.03 (m, 1H), 3.16–3.21 (m, 4H, N(CH₂)₂), 3.36–3.41 (m, 1H, –NHCH(CH₂)₂), 6.83–6.96 (m, 5H, Ar–H), 7.24–7.29 (m, 2H, Ar–H), 7.83 (s, 1H, –CCHN–).

Synthesis of N-[2-(4-Phenylpiperazin-1-yl)ethyl][2-(2,4,6-trimethylbenzenesulfonyl)-4,5,6,7-tetrahydro-2H-indazol-6-yl]propionamide (18). Into a stirred solution of compound **17** (0.4729 g, 0.93 mmol) and 1 mL of Et₃N in anhydrous CH₂Cl₂ (20 mL) under nitrogen atmosphere at 0 °C was added propionyl chloride (0.20 mL, 2.33 mmol). The reaction mixture was stirred for 2 h. The reaction mixture was diluted with CH₂Cl₂ (20 mL) and washed with water and brine. The organic layer was dried over Na₂SO₄ and evaporated in vacuo. The crude mass was purified by flash chromatography over a silica gel column using ethyl acetate/methanol/triethylamine (95:4:1) as eluant to yield 0.44 g of title compound **18** (84%). ¹H NMR (400 MHz, CDCl₃): δ 1.12–1.21 (m, 3H), 1.74 (s, 1H), 1.88–1.99 (m, 2H), 2.31–2.32 (m, 3H),

Mst-CH₃), 2.35–2.46 (m, 2H), 2.57–2.74 (m, 14H, Mst-CH₃), 2.81–2.85 (m, 1H), 3.16–3.20 (m, 4H, N(CH₂)₂), 3.29–3.36 (m, 1H), 3.38–3.49 (m, 2H), 6.83–6.99 (m, 5H, Ar-H), 7.24–7.29 (m, 2H, Ar-H), 7.83–7.89 (d, 1H, *J* = 23.6, 1H, -CCHN-).

Synthesis of [2-(4-Phenylpiperazin-1-yl)ethyl]propyl(4,5,6,7-tetrahydro-2H-indazol-6-yl)amine (19). Compound **18** (0.44 g, 0.78 mmol) in anhydrous THF was added dropwise into the suspension of LiAlH₄ (0.18 g, 4.68 mmol) in anhydrous THF at 0 °C under nitrogen atmosphere. The reaction mixture was refluxed for 8 h and cooled to room temperature. The reaction mixture was diluted with EtOAc, and saturated NaOH/H₂O (4 mL) was added dropwise. The mixture was stirred for 15–20 min. Then it was filtered, and the organic layer was dried over Na₂SO₄ and evaporated in vacuo. The crude mass was purified by flash chromatography over a silica gel column using ethyl acetate/methanol/triethylamine (95:4:1) as eluant to yield 0.22 g of title compound **19** (78%). The product was converted into corresponding tetrahydrochloride salt, mp 230–233 °C. Anal. (C₂₂H₃₃N₅·4HCl·0.5H₂O) C, H, N. ¹H NMR (400 MHz, CDCl₃): δ 0.89 (t, 3H, *J* = 7.2 Hz, CH₂C H₃), 1.44–1.53 (m, 2H, CH₂CH₃), 1.56–1.66 (m, 1H), 1.99–2.02 (m, 1H), 2.49–2.59 (m, 6H), 2.62–2.68 (m, 5H), 2.71–2.75 (m, 3H), 2.84–2.89 (dd, 2H, *J*₁ = 15.6, *J*₂ = 4.4, -NCHCH₂CN-), 2.99–3.06 (m, 1H), 3.19 (t, 4H, *J* = 8.0 Hz), 6.85 (t, 1H, *J* = 7.2 Hz, Ar-H), 6.90–6.93 (d, 2H, *J* = 8.4, Ar-H), 7.23–7.27 (m, 3H, 2Ar-H, -CCHNH-).

Synthesis of *N*-Propyl-1,4-dioxaspiro[4.5]decan-8-amine (20). 1,4-Cyclohexanedione monoethylene ketal (10 g, 64.03 mmol) was reacted with *n*-propylamine (10.5 mL, 128.06 mmol) in the presence of NaCNBH₃ (10.06 g, 160.07 mmol) and HOAc (7.6 mL) in 1,2-dichloroethane (100 mL) by following procedure A. The crude reaction mixture was purified by column chromatography on silica gel column using solvent system dichloromethane/methanol (100:1) to yield 12.04 g of pure compound **20** (94%). Compound **20** was also purified in some batches by dissolving the crude mixture in ethanol/ether and passing HCl gas through the solution for 15 min till the solution is acidic to litmus paper. The precipitated HCl salt was collected by filtration, which was used directly in next step without converting the salt back to free base. ¹H NMR (400 MHz, CDCl₃): δ 0.91 (t, 3H, *J* = 7.2 Hz), 1.36–1.59 (m, 6H), 1.74–1.79 (m, 2H), 1.84–1.89 (m, 2H), 2.49–2.60 (m, 3H), 3.94 (s, 4H, -O(CH₂)₂O-).

Synthesis of 2,4,6-Trimethyl-*N*-propyl-*N*-(1,4-dioxaspiro[4.5]decan-8-yl)benzenesulfonamide (21). Into a stirred mixture of compound **20** (2.17 g, 10.89 mmol) and 2.4 mL of 20% w/v NaOH solution (11.98 mmol) in 20 mL of dichloromethane was added 2-mesitylenesulfonyl chloride (2.62 g, 11.98 mmol). The reaction mixture was refluxed for 12 h. The reaction mixture was diluted with CH₂Cl₂ (25 mL) and washed with water. The water layer was extracted with CH₂Cl₂ (3 × 50 mL). The combined organic layer was washed with brine, dried over Na₂SO₄, and evaporated in vacuo. The crude mass was purified by flash chromatography over a silica gel column using hexane/ethyl acetate (9:1) as eluant to yield 2.90 g of title compound **21** (70%). ¹H NMR (400 MHz, CDCl₃): δ 0.75 (t, 3H, *J* = 7.6 Hz, -CH₂CH₂CH₃), 1.36–1.43 (m, 2H, -CH₂CH₂CH₃), 1.94–2.01 (m, 2H), 2.11–2.16 (m, 2H), 2.31 (s, 3H), 2.40–2.43 (q, 4H, *J* = 4.0 Hz), 2.63 (s, 6H), 3.05–3.10 (m, 2H, -NCH₂CH₂CH₃), 3.90 (s, 4H, -O(CH₂)₂O-), 4.06–4.12 (tt, 1H, *J*₁ = 12.0 Hz, *J*₂ = 3.2 Hz, -CH₂CHN-), 6.96 (bs, 2H, -Ar-H).

Synthesis of 2,4,6-Trimethyl-*N*-(4-oxocyclohexyl)-*N*-propylbenzenesulfonamide (22). Into a stirred solution of compound **21** (38.5 g, 0.10 mol) in 100 mL of THF was added 400 mL of 1 N HCl. The reaction mixture was stirred at 80 °C for 6 h. THF was evaporated. The reaction mixture was neutralized by using saturated NaHCO₃ solution. The aqueous layer was extracted using ethyl acetate (3 × 200 mL). The combined organic layer was washed with brine, dried over Na₂SO₄, and evaporated in vacuo. The crude mass was purified by flash chromatography over a silica gel column using hexane/ethyl acetate (8:2) as eluant to yield 31.0 g of title compound **22** (91%). ¹H NMR (400 MHz, CDCl₃): δ 0.74 (t, 3H, *J* = 7.2 Hz, -CH₂CH₂CH₃), 1.36–1.42 (m, 2H, -CH₂CH₂CH₃),

1.92–1.99 (m, 2H), 2.11–2.15 (m, 2H), 2.31 (s, 3H), 2.40–2.43 (q, 4H, *J* = 4.0 Hz), 2.63 (s, 6H), 3.05–3.09 (m, 2H, -NCH₂CH₂CH₃), 4.06–4.12 (tt, 1H, *J*₁ = 12.0 Hz, *J*₂ = 3.2 Hz, -CH₂CHN-), 6.96 (bs, 2H, -Ar-H).

Synthesis of *N*-(2-Amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)-2,4,6-trimethyl-*N*-propylbenzenesulfonamide (23). Compound **22** (1.21 g, 3.59 mmol) was treated with pyrrolidine (0.36 mL, 4.30 mmol) in the presence of catalytic amount of *p*-toluenesulfonic acid (3.4 mg, 0.0179 mmol) under reflux followed by the addition of sulfur powder (0.14 g, 0.54 mmol) and cyanamide (0.18 g, 4.30 mmol) by following procedure E. The crude residue was purified by flash chromatography using solvent system hexane/ethyl acetate (20:80) to yield 0.80 g of pure compound **23**. (58%). ¹H NMR (400 MHz, CDCl₃): δ 0.79 (t, 3H, *J* = 8.0 Hz, CH₂CH₃), 1.44–1.58 (m, 2H), 1.90–1.94 (m, 1H), 2.03–2.05 (m, 1H), 2.30 (s, 3H), 2.53–2.62 (m, 7H), 2.67–2.71 (m, 2H), 2.76–2.83 (m, 1H), 3.11–3.22 (m, 2H), 3.90–3.98 (m, 1H), 4.8 (bs, 2H, -NH₂), 6.94 (s, 2H).

Synthesis of (±)-*N*⁶-Propyl-4,5,6,7-tetrahydrobenzo[*d*]thiazole-2,6-diamine. ((±)-24). Into a suspension of compound **23** (16.73 g, 42.51 mmol) and phenol (40 g, 435.08 mmol) in 200 mL of ethyl acetate at 0 °C was added 200 mL of 33% HBr in acetic acid drop by drop under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 12 h. The reaction mixture was evaporated to dryness. The solid HBr salt of the compound was triturated with ethyl acetate, filtered, and crystallized using solvent system ethanol/methanol. The white crystals of the HBr salt were filtered and dried under vacuum. The salt was dissolved in water. The water layer was made saturated with powdered K₂CO₃. The aqueous layer was extracted with ethyl acetate (3 × 200 mL). The combined organic layer was dried over Na₂SO₄ and evaporated in vacuo to yield 6.88 g of pure title compound (±)-**24** (77%). ¹H NMR (400 MHz, CDCl₃): δ 0.93 (t, 3H, *J* = 7.6 Hz), 1.49–1.57 (m, 2H), 1.64–1.70 (m, 1H), 2.0–2.05 (m, 1H), 2.36–2.42 (m, 1H), 2.52–2.66 (m, 4H), 2.83–2.95 (dd, 1H, *J*₁ = 15.2 Hz, *J*₂ = 4.4 Hz), 2.96–3.00 (m, 1H), 5.13 (bs, 2H).

Resolution of (±)-Pramipexole (24) via Its Monohydrochloride Tartrate (U.S. Patent 6,727,367). Into a warm solution of (±)-**24** (10.57 g, 50 mmol) in CH₃OH (100 mL), concentrated HCl (4.13 mL, 50 mmol) was added dropwise, and the solid that formed was dissolved by further heating. L-(+)-tartaric acid (7.50 g, 50 mmol) was added and the solution mixed until white crystals precipitated. After cooling, the crystals were collected and washed with cold CH₃OH before recrystallizing from CH₃OH.

The free base was liberated using saturated K₂CO₃ and extracting with EtOAc (4×). This gave the (*S*)-enantiomer as a white solid with a maximum optical rotation of [α]_D -94° (*c* 0.5, CH₃OH). (If the maximum rotation had not been reached, the procedure was repeated for a second time.)

The different filtrates were concentrated and converted to the free base. The samples that had a positive optical rotation were further separated using D-(-)-tartaric acid to give the pure (*R*)-enantiomer.

Procedure F. Synthesis of 2-Chloro-1-(4-phenylpiperazin-1-yl)ethanone (26a). Into the solution of 1-phenylpiperazine (5.6 mL, 36.98 mmol) and triethylamine (10 mL) in anhydrous dichloromethane (50 mL) was added chloroacetyl chloride (3.2 mL, 40.68 mmol) at 0 °C under nitrogen atmosphere. Reaction mixture was stirred for half an hour and diluted with dichloromethane. The organic layer was washed with water, dried over Na₂SO₄, and evaporated in vacuo. The crude residue was purified by flash chromatography on silica gel column using solvent system hexane/ethyl acetate (75:25) to yield 6.05 g of pure compound **26a** (69%). ¹H NMR (400 MHz, CDCl₃): δ 3.19 (t, 2H, *J* = 4.8 Hz), 3.24 (t, 2H, *J* = 5.2 Hz), 3.69 (t, 2H, *J* = 5.2 Hz), 3.80 (t, 2H, *J* = 5.6 Hz), 4.12 (s, 2H), 6.91–6.95 (m, 3H), 7.26–7.32 (m, 2H).

Synthesis of 2-Chloro-1-(4-(2-methoxyphenyl)piperazin-1-yl)ethanone (26b). 1-(2-Methoxyphenyl)piperazine HCl (5.89 g, 25.0 mmol) was treated with chloroacetyl chloride (2.2 mL, 27.0 mmol) in the presence of triethylamine (8.7 mL) in 100 mL of anhydrous CH₂Cl₂ by following procedure F. Reaction mixture was

purified by flash chromatography using solvent system hexane/ethyl acetate (50:50) to yield 6.10 g of pure compound **26b** (91%). ¹H NMR (400 MHz, CDCl₃): δ 3.06 (t, 2H, *J* = 5.2 Hz), 3.12 (t, 2H, *J* = 5.2 Hz), 3.71 (t, 2H, *J* = 5.2 Hz), 3.82 (t, 2H, *J* = 5.2 Hz), 3.89 (s, 3H), 4.12 (s, 2H), 6.87–6.97 (m, 3H), 7.02–7.08 (m, 1H).

Synthesis of 2-Chloro-1-(4-(2,3-dichlorophenyl)piperazin-1-yl)ethanone (26c). 1-(2,3-Dichlorophenyl)piperazine HCl (6.0 g, 22.42 mmol) was treated with chloroacetyl chloride (1.96 mL, 24.66 mmol) in the presence of triethylamine (5 mL) in 75 mL of anhydrous CH₂Cl₂ by following procedure F. Reaction mixture was purified by flash chromatography using solvent system hexane/ethyl acetate (70:30) to yield 5.0 g of pure compound **26c** (62%). ¹H NMR (400 MHz, CDCl₃): δ 3.05 (t, 2H, *J* = 4.8 Hz), 3.10 (t, 2H, *J* = 4.4 Hz), 3.72 (t, 2H, *J* = 5.2 Hz), 3.82 (t, 2H, *J* = 4.8 Hz), 4.12 (s, 2H), 6.93–6.95 (dd, 1H, *J*₁ = 8.0 Hz, *J*₂ = 2.0 Hz), 7.15–7.23 (m, 2H).

Synthesis of 1-(4-Biphenyl)piperazine Trifluoroacetate (25d). Into the suspension of *tert*-butyl piperazine-1-carboxylate (0.44 g, 2.36 mmol), sodium *tert*-butoxide (0.413 g, 4.29 mmol), and the catalyst dichlorobis(tri-*o*-tolylphosphine)palladium(II) (50 mg, 0.0644 mmol, 3 mol %) in anhydrous toluene was added 4-bromobiphenyl (0.5 g, 2.15 mmol). Reaction mixture was refluxed for 3 h. The crude reaction mixture was filtered through Celite, washed with ethyl acetate, evaporated in vacuo, and purified by flash chromatography on silica gel column using solvent system hexane/ethyl acetate (90:10) to yield 0.60 g of pure compound *tert*-butyl 4-(biphenyl-4-yl)piperazine-1-carboxylate (80%). This compound was deprotected to afford 1-(4-biphenyl)piperazine. Deprotection of *tert*-butyl 4-(biphenyl-4-yl)piperazine-1-carboxylate was done as follows.

Into the solution of *tert*-butyl 4-(biphenyl-4-yl)piperazine-1-carboxylate (3.6 g, 10.64 mmol) in 25 mL of dry CH₂Cl₂ was added trifluoroacetic acid (25 mL) dropwise at room temperature. Reaction mixture was stirred at room temperature for 6 h. The reaction mixture was concentrated under reduced pressure. The salt of the product was recrystallized from solvent system dichloromethane/hexane to yield 2.81 g of the desired compound 1-(4-biphenyl)piperazine as trifluoroacetate salt form (75%) **25d**. ¹H NMR (400 MHz, CDCl₃): δ 1.63 (bs, 1H, –NH), 3.05 (t, 4H, *J* = 4.4 Hz), 3.20 (t, 4H, *J* = 5.2 Hz), 6.99–7.01 (d, 2H, *J* = 8.8 Hz), 7.26–7.30 (m, 1H), 7.40 (t, 2H, *J* = 7.6 Hz), 7.51–7.57 (m, 4H).

Synthesis of 1-(4-(Biphenyl-4-yl)piperazin-1-yl)-2-chloroethanone (26d). 1-(4-Biphenyl)piperazine trifluoroacetate **25d** (2.81 g, 7.98 mmol) was treated with chloroacetyl chloride (0.70 mL, 8.78 mmol) in the presence of triethylamine (5 mL) in anhydrous CH₂Cl₂ (50 mL) by following procedure F. Reaction mixture was purified by flash chromatography using solvent system ethyl acetate/methanol/triethylamine (90:10:1) to yield 2.0 g of pure compound **26d** (80%). ¹H NMR (400 MHz, CDCl₃): δ 3.23–3.30 (tt, 4H, *J*₁ = 19.6 Hz, *J*₂ = 4.8 Hz), 3.69–3.71 (t, 2H, *J* = 5.6 Hz), 3.80–3.82 (t, 2H, *J* = 5.2 Hz), 4.12 (s, 2H), 6.99–7.01 (d, 2H, *J* = 8.8 Hz), 7.28–7.32 (m, 1H), 7.40–7.43 (t, 2H, *J* = 8.0 Hz), 7.53–7.57 (t, 4H, *J* = 8.8 Hz).

Procedure G. Synthesis of (+)-2-((2-Amino-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)(propylamino)-1-(4-phenylpiperazin-1-yl)ethanone ((+)-27). Into a suspension of (+)-**24** (0.20 g, 0.95 mmol), K₂CO₃ (0.39 g, 2.84 mmol), and a catalytic amount of KI in acetonitrile (25 mL) was added **26a** (0.34 g, 1.42 mmol). Reaction mixture was refluxed for 3 h. The crude reaction mixture was filtered, washed with ethyl acetate, evaporated in vacuo, and purified by flash chromatography on silica gel column using solvent system ethyl acetate/methanol (95:5) to yield 0.15 g of pure compound (+)-**27** (38%). ¹H NMR (400 MHz, CDCl₃): δ 0.87–0.90 (m, 3H), 1.47–1.52 (m, 2H), 1.73–1.78 (m, 1H), 1.98–2.05 (m, 1H), 2.51–2.73 (m, 6H), 3.06–3.26 (m, 5H), 3.40–3.50 (q, 2H, *J* = 13.6 Hz), 3.74–3.86 (m, 4H), 4.69 (bs, 2H), 6.89–6.96 (m, 3H), 7.26–7.31 (m, 2H).

Synthesis of (–)-2-((2-Amino-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)(propylamino)-1-(4-phenylpiperazin-1-yl)ethanone ((–)-27). Compound (–)-**24** (0.21 g, 1.0 mmol) was treated with **26a** (0.36 g, 1.5 mmol) in the presence of base K₂CO₃ (0.28 g, 2.0

mmol) and a catalytic amount of KI by following procedure G. The reaction mixture was purified by flash chromatography on silica gel column using solvent system ethyl acetate/methanol (90:10) to yield 0.15 g of pure compound (–)-**27** (36%). ¹H NMR (400 MHz, CDCl₃): δ 0.89 (t, *J* = 7.2 Hz, 3H), 1.49 (h, *J* = 7.2 Hz, 2H), 1.81–1.68 (m, 1H), 2.06–1.96 (m, 1H), 2.65–2.45 (m, 4H), 2.76–2.65 (m, 2H), 3.26–3.04 (m, 5H), 3.50–3.38 (m, 2H), 3.90–3.66 (m, 4H), 4.94 (bs, 2H), 6.97–6.86 (m, 3H), 7.32–7.25 (m, 2H).

Synthesis of (+)-2-((2-Amino-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)(propylamino)-1-(4-(2-methoxyphenyl)piperazin-1-yl)ethanone ((+)-28). Compound (+)-**24** (0.3 g, 1.42 mmol) was treated with **26b** (0.57 g, 2.13 mmol) in the presence of base K₂CO₃ (0.39 g, 2.84 mmol) and a catalytic amount of KI by following procedure G. The reaction mixture was purified by flash chromatography on silica gel column using solvent system diethyl ether/methanol (92:8) to yield 0.24 g of pure compound (+)-**28** (38%). ¹H NMR (400 MHz, CDCl₃): δ 0.89 (t, 3H, *J* = 7.6 Hz), 1.43–1.52 (m, 2H), 1.71–1.80 (m, 1H), 2.00–2.04 (m, 1H), 2.47–2.63 (m, 4H), 2.69–2.73 (m, 2H), 2.97–3.13 (m, 5H), 3.40–3.50 (q, 2H, *J* = 13.6 Hz), 3.72–3.89 (m, 7H), 4.71 (bs, 2H), 6.88–6.94 (m, 3H), 7.02–7.06 (m, 1H).

Synthesis of (–)-2-((2-Amino-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)(propylamino)-1-(4-(2-methoxyphenyl)piperazin-1-yl)ethanone ((–)-28). Compound (–)-**24** (0.21 g, 1.0 mmol) was treated with **26b** (0.34 g, 1.25 mmol) in the presence of base K₂CO₃ (0.21 g, 1.5 mmol) and a catalytic amount of KI following procedure G. The reaction mixture was purified by flash chromatography on silica gel column using solvent system ethyl acetate/methanol (90:10) to yield 0.15 g of pure compound (–)-**28** (34%). ¹H NMR (400 MHz, CDCl₃): δ 0.89 (t, 3H, *J* = 7.6 Hz), 1.43–1.56 (m, 2H), 1.68–1.82 (m, 1H), 2.00–2.05 (m, 1H), 2.47–2.65 (m, 4H), 2.65–2.76 (m, 2H), 2.96–3.16 (m, 5H), 3.40–3.52 (q, 2H, *J* = 13.6 Hz), 3.70–3.92 (m, 7H), 4.88 (bs, 2H), 6.86–6.97 (m, 3H), 7.00–7.08 (m, 1H).

Synthesis of (+)-2-((2-Amino-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)(propylamino)-1-(4-(2,3-dichlorophenyl)piperazin-1-yl)ethanone ((+)-29). Compound (+)-**24** (0.3 g, 1.42 mmol) was treated with **26c** (0.65 g, 2.13 mmol) in the presence of base K₂CO₃ (0.39 g, 2.84 mmol) and catalytic amount of KI by following procedure G. The reaction mixture was purified by flash chromatography on silica gel column using solvent system ethyl acetate/methanol (95:5) to yield 0.31 g of pure compound (+)-**29** (45%). ¹H NMR (400 MHz, CDCl₃): δ 0.90 (t, 3H, *J* = 7.2 Hz), 1.43–1.52 (m, 2H), 1.71–1.81 (m, 1H), 2.00–2.05 (m, 1H), 2.48–2.63 (m, 4H), 2.70–2.75 (m, 2H), 2.98–3.13 (m, 5H), 3.40–3.50 (q, 2H, *J* = 13.6 Hz), 3.76–3.91 (m, 4H), 4.71 (bs, 2H), 6.91–6.94 (dd, 1H, *J*₁ = 7.6 Hz, *J*₂ = 1.6 Hz), 7.14–7.22 (m, 2H).

Synthesis of (–)-2-((2-Amino-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)(propylamino)-1-(4-(2,3-dichlorophenyl)piperazin-1-yl)ethanone ((–)-29). Compound (–)-**24** (0.21 g, 1.0 mmol) was treated with **26c** (0.34 g, 1.1 mmol) in the presence of base K₂CO₃ (0.17 g, 1.25 mmol) and a catalytic amount of KI following procedure G. The reaction mixture was purified by flash chromatography on silica gel column using solvent system ethyl acetate/methanol (90:10) to yield 0.21 g of pure compound (–)-**29** (44%). ¹H NMR (400 MHz, CDCl₃): δ 0.90 (t, 3H, *J* = 7.2 Hz), 1.43–1.55 (m, 2H), 1.69–1.82 (m, 1H), 1.97–2.06 (m, 1H), 2.46–2.65 (m, 4H), 2.65–2.75 (m, 2H), 2.95–3.15 (m, 5H), 3.40–3.50 (q, 2H, *J* = 13.6 Hz), 3.68–3.95 (m, 4H), 4.81 (bs, 2H), 6.91–6.94 (dd, 1H, *J*₁ = 7.6 Hz, *J*₂ = 1.6 Hz), 7.13–7.22 (m, 2H).

Synthesis of (–)-2-((2-Amino-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)(propylamino)-1-(4-(biphenyl-4-yl)piperazin-1-yl)ethanone ((–)-30). Compound (–)-**24** (0.3 g, 1.42 mmol) was treated with **26d** (0.67 g, 2.13 mmol) in the presence of base K₂CO₃ (0.39 g, 2.84 mmol) and a catalytic amount of KI following procedure G. The reaction mixture was purified by flash chromatography on silica gel column using solvent system ethyl acetate/methanol (95:5) to yield 0.25 g of pure compound (–)-**30** (36%). ¹H NMR (400 MHz, CDCl₃): δ 0.89–0.92 (t, 3H, *J* = 7.2 Hz),

1.44–1.53 (m, 2H), 1.75–1.79 (m, 1H), 2.02–2.05 (m, 1H), 2.52–2.75 (m, 6H), 3.06–3.26 (m, 5H), 3.42–3.51 (q, 2H, $J = 13.6$ Hz), 3.77–3.89 (m, 4H), 4.70 (bs, 2H), 7.00–7.02 (d, 2H, $J = 8.0$ Hz), 7.30–7.32 (m, 1H), 7.40–7.44 (t, 2H, $J = 8.0$ Hz), 7.53–7.58 (t, 4H, $J = 9.2$ Hz).

Procedure H. Synthesis of (+)-*N*⁶-(2-(4-Phenylpiperazin-1-yl)ethyl)-*N*⁶-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine ((+)-31). Into the solution of (+)-27 (0.26 g, 0.63 mmol) in dry THF (5 mL) at 0 °C was added (3.8 mL, 3.8 mmol) solution of borane–THF complex (1 M solution) with stirring under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 36 h and quenched with methanol. The solvent was evaporated. The white solid complex was suspended in 6 N HCl in methanol and stirred for 2 h at room temperature. Methanol was evaporated under vacuo. Reaction mixture was made alkaline using saturated Na₂CO₃/NaHCO₃ solution. The aqueous layer was extracted with ethyl acetate (3 × 100 mL), dried over Na₂SO₄, concentrated under vacuo, and purified by flash chromatography on silica gel column using solvent system ethyl acetate/methanol (90:10) to afford 0.14 g of compound (+)-31 (56%). [α]_D +40.6° (c 1, dichloromethane). ¹H NMR (400 MHz, CDCl₃): δ 0.87–0.92 (m, 3H), 1.43–1.52 (m, 2H), 1.68–1.77 (m, 1H), 1.98–2.01 (m, 1H), 2.44–2.60 (m, 6H), 2.64–2.75 (m, 8H), 3.02–3.08 (m, 1H), 3.19–3.21 (t, 4H, $J = 4.8$ Hz), 4.81 (bs, 2H), 6.83–6.87 (m, 1H), 6.92–6.94 (m, 2H), 7.24–7.28 (m, 2H). The product was converted into corresponding tetrahydrochloride salt, mp 230–233 °C. Anal. (C₂₂H₃₃N₅S·4HCl) C, H, N.

Synthesis of (–)-*N*⁶-(2-(4-Phenylpiperazin-1-yl)ethyl)-*N*⁶-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine ((–)-31). Compound (–)-27 (0.15 g, 0.36 mmol) in 25 mL of anhydrous THF was treated with borane–THF complex (1.8 mL) by following procedure H. Crude reaction mixture was purified by flash chromatography on silica gel column using solvent system ethyl acetate/methanol (80:20) to afford compound (–)-31. [α]_D –38.4° (c 1.0, dichloromethane). ¹H NMR (400 MHz, CDCl₃) δ 7.26 (t, $J = 8.0$ Hz, 2H), 6.93 (d, $J = 8.0$ Hz, 2H), 6.85 (t, $J = 7.2$ Hz, 1H), 4.83 (bs, 2H), 3.24–3.16 (m, 4H), 3.11–3.01 (m, 1H), 2.78–2.63 (m, 7H), 2.63–2.45 (m, 6H), 2.04–1.96 (m, 1H), 1.79–1.66 (m, 1H), 1.48 (h, $J = 7.2$ Hz, 2H), 0.89 (t, $J = 7.2$ Hz, 3H). The product was converted into corresponding tetrahydrochloride salt (0.09 g), mp ~210 °C (dec). Anal. (C₂₂H₃₃N₅S·4HCl·0.3Et₂O) C, H, N.

Synthesis of (+)-*N*⁶-(2-(4-(2-Methoxyphenyl)piperazin-1-yl)ethyl)-*N*⁶-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine ((+)-32). Compound (+)-28 (0.24 g, 0.54 mmol) in 5 mL of anhydrous THF was treated with borane–THF complex (3.3 mL) by following procedure H. Crude reaction mixture was purified by flash chromatography on silica gel column using solvent system ethyl acetate/methanol (90:10) to afford 0.15 g of compound (+)-32 (64%). [α]_D +36.2° (c 1, dichloromethane). ¹H NMR (400 MHz, CDCl₃): δ 0.87–0.92 (m, 3H), 1.42–1.51 (m, 2H), 1.70–1.74 (m, 1H), 1.98–2.02 (m, 1H), 2.46–2.60 (m, 6H), 2.65–2.74 (m, 8H), 3.01–3.09 (m, 5H), 3.86 (s, 3H, –OCH₃), 4.74 (bs, 2H, –NH₂), 6.84–7.01 (m, 4H). The product was converted into corresponding dioxalate salt, mp 170–173 °C. Anal. (C₂₃H₃₅N₅OS·2C₂H₂O₄·1.7H₂O) C, H, N.

Synthesis of (–)-*N*⁶-(2-(4-(2-Methoxyphenyl)piperazin-1-yl)ethyl)-*N*⁶-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine ((–)-32). Compound (–)-28 (0.15 g, 0.34 mmol) in 25 mL of anhydrous THF was treated with borane–THF complex (1.8 mL) by following procedure H. Crude reaction mixture was purified by flash chromatography on silica gel column using solvent system ethyl acetate/methanol (80:20) to afford compound (–)-32. [α]_D –40.2° (c 1.0, dichloromethane). ¹H NMR (400 MHz, CDCl₃) δ 7.03–6.82 (m, 4H), 4.84 (bs, 2H), 3.86 (s, 3H), 3.20–3.00 (m, 5H), 2.84–2.64 (m, 8H), 2.64–2.45 (m, 6H), 2.06–1.96 (m, 1H), 1.80–1.67 (m, 1H), 1.49 (h, $J = 7.2$ Hz, 2H), 0.89 (t, $J = 7.2$ Hz, 3H). The product was converted into corresponding tetrahydrochloride salt (0.10 g), mp ~210 °C (dec). Anal. (C₂₃H₃₅N₅OS·4HCl·0.6H₂O) C, H, N.

Synthesis of (+)-*N*⁶-(2-(4-(2,3-Dichlorophenyl)piperazin-1-yl)ethyl)-*N*⁶-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine ((+)-33). Compound (+)-29 (0.31 g, 0.64 mmol) in 5 mL of anhydrous THF was treated with borane–THF complex (3.9 mL) by following procedure H. Crude reaction mixture was purified by flash chromatography on silica gel column using solvent system ethyl acetate/methanol (90:10) to afford 0.14 g of compound (+)-33 (47%). [α]_D +36.4° (c 1, dichloromethane). ¹H NMR (400 MHz, CDCl₃): δ 0.89 (t, 3H, $J = 7.2$ Hz), 1.43–1.52 (m, 2H), 1.67–1.77 (m, 1H), 1.98–2.04 (m, 1H), 2.44–2.60 (m, 6H), 2.66–2.77 (m, 8H), 3.01–3.09 (m, 5H), 4.99 (bs, 2H, –NH₂), 6.94–6.98 (m, 1H), 7.12–7.17 (m, 2H). The product was converted into corresponding trihydrochloride salt, mp 175–178 °C. Anal. (C₂₂H₃₁Cl₂N₅S·3HCl·1.8H₂O) C, H, N.

Synthesis of (–)-*N*⁶-(2-(4-(2,3-Dichlorophenyl)piperazin-1-yl)ethyl)-*N*⁶-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine ((–)-33). Compound (–)-29 (0.21 g, 0.44 mmol) in 25 mL of anhydrous THF was treated with borane–THF complex (2.2 mL) following procedure H. Crude reaction mixture was purified by flash chromatography on silica gel column using solvent system ethyl acetate/methanol (80:20) to afford compound (–)-33. [α]_D –35.9° (c 1.0, dichloromethane). ¹H NMR (400 MHz, CDCl₃): δ 0.90 (t, 3H, $J = 7.2$ Hz), 1.45–1.55 (m, 2H), 1.66–1.77 (m, 1H), 1.97–2.05 (m, 1H), 2.46–2.64 (m, 6H), 2.64–2.82 (m, 8H), 3.00–3.14 (m, 5H), 5.28 (bs, 2H, –NH₂), 6.94–6.97 (m, 1H), 7.11–7.17 (m, 2H). The product was converted into corresponding trihydrochloride salt (0.12 g), mp ~210 °C (dec). Anal. (C₂₂H₃₁N₅S·3HCl·0.8H₂O) C, H, N.

Synthesis of (–)-*N*⁶-(2-(4-(Biphenyl-4-yl)piperazin-1-yl)ethyl)-*N*⁶-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine ((–)-34). Compound (+)-30 (0.23 g, 0.47 mmol) in 5 mL of anhydrous THF was treated with borane–THF complex (4.0 mL) by following procedure H. Crude reaction mixture was purified by flash chromatography on silica gel column using solvent system ethyl acetate/methanol (90:10) to afford 0.12 g of compound (–)-34 (54%). [α]_D –34.4° (c 1, dichloromethane). ¹H NMR (400 MHz, CDCl₃): δ 0.88–0.91 (t, 3H, $J = 7.2$ Hz), 1.45–1.51 (q, 2H, $J = 7.2$ Hz), 1.68–1.78 (m, 1H), 1.97–2.04 (m, 1H), 2.44–2.75 (m, 14H), 3.03–3.08 (m, 1H), 3.24–3.27 (m, 4H), 4.76 (bs, 2H), 6.98–7.01 (m, 2H), 7.28–7.31 (m, 1H), 7.38–7.43 (m, 2H), 7.50–7.59 (m, 4H). The product was converted into corresponding trioxalate salt., mp 198–200 °C. Anal. (C₂₈H₃₇N₅S·2C₂H₂O₄·H₂O) C, H, N.

Measurement of Binding Potencies at Dopamine D2 and D3 Receptors. [³H]Spiperone Binding. Binding affinities were assessed according to the general procedure described in our previous study (Table 1).³⁹ Human embryonic kidney (HEK) 293 cells, newly transfected with rat D2L and D3 receptors, were generously donated by Dr. Kim A. Neve (Oregon Health Sciences University). As described previously, membranes preparations from these cells were incubated with each test compound and [³H]spiperone (0.4 nM, 15 Ci/mmol, Perkin-Elmer) was incubated for 1 h at 30 °C in 50 mM Tris-HCl (pH 7.4), with 0.9% NaCl, and 0.025% ascorbic acid in the absence of GTP. (+)-Butaclamol (2 μ M) was used to define nonspecific binding. Assays were terminated by addition of ice-cold buffer and filtration in a MACH 3-96 Tomtec harvester (Wallac, Gaithersburg, MD). IC₅₀ values were estimated by nonlinear regression analysis with the logistic model in the least-squares fitting program ORIGIN and converted to inhibition constants (K_i) by the Cheng–Prusoff equation.⁵⁰ In this conversion, the K_d values for [³H]spiperone binding were 0.057 nM for D₂ receptors and 0.125 nM for D₃ receptors.

Measurement of Stimulation of Dopamine D2 and D3 receptors. [³⁵S]GTP γ S Binding. All procedures were as described in our recent work.³⁹ The general procedures used for measuring [³⁵S]GTP γ S binding are modified from protocols described for DA receptors⁵¹ and other G-protein-coupled receptors.^{52,53} Chinese hamster ovary (CHO) cells expressing human D2L receptors and ATt-20 cells expressing human D3 receptors served as the source for membrane fractions that were washed and resuspended with assay buffer containing Mg²⁺, Na⁺, EGTA, and bovine serum

albumin. The GTP γ S binding assays were performed in triplicate in a final volume of 1 mL containing test drug, DA (1 mM for D2 cells and 100 μ M for D3 cells) as indicator of binding plateau, [³⁵S]GTP γ S (1.72 nM, 1,250 Ci/mmol, Perkin-Elmer), and cell suspension (cells suspended in assay buffer and GDP for final concentration of 3 μ M in assay). After incubation at room temperature in a shaking water bath for 60 min, cells were harvested on Brandel GF/B filter mats with a 24-pin Brandel harvester (Biomedical Research & Development Laboratories, Inc., Gaithersburg, MD). A Beckman LS 6500 scintillation counter was used to determine ³⁵S radioactivity at 70% efficiency. Nonspecific binding of [³⁵S]GTP γ S measured in the presence of 10 μ M GTP γ S was a very small fraction (5% or less) of basal binding in the absence of drug and did not impact the EC₅₀ (concentration producing half-maximal stimulation) of the test drug estimated by nonlinear logarithmic fitting (logistics model) with OriginPro 7.0. The plateau binding (maximal binding stimulation) with test drug was expressed as percent of maximal binding observed with the full agonist DA (% E_{max}); each filter mat used for harvesting and scintillation counting contained varying concentrations of test drug (for EC₅₀ determination) and a fixed [DA] (see above, for maximally achievable binding with full agonist).

In Vivo Rotational Experiment with 6-OH-DA Lesioned Rats. The lesioned rats were purchased from Taconic Biotechnology (Rensselaer, NY), and their unilateral lesion was checked twice by apomorphine challenge following the surgery. Animals were housed in a temperature and humidity controlled room with a 12 h light/dark cycle. Food and water were accessible to animals freely throughout the duration of study. All testing occurred during the light component. All animal procedures were reviewed and approved by Wayne State University animal investigation committee consistent with AALAC guidelines.

The first 14 days postlesion challenge with apomorphine was done to observe a complete rotation session postadministration. In the second challenge with apomorphine (0.05 mg/kg) 21 days postlesion, contralateral rotations were recorded for 30 min; apomorphine produced rotations in all four rats (average rotation of >250), indicating successful unilateral lesion. In these rats, lesion was performed on the left side with the rotations produced upon agonist challenge occurring clockwise. In this study, apomorphine was also used as a reference compound. The test drugs were dissolved in 5% β -hydroxycyclodextrin solution and were administered. The number of rotations was measured over 10 h. For control, vehicle was administered alone. Rotations were measured in the Rotomax rotometry system (AccuScan Instruments, Inc., Columbus, OH) equipped with Rotomax analyzer, high resolution sensor, and animal chambers with harnesses. Data were analyzed with Rotomax Window software program. Test drugs (–)-**33** (2.5 and 5 μ mol/kg) and (–)-**34** (2.5 and 5 μ mol/kg) dissolved in 5% β -hydroxycyclodextrin solution were administered sc. The rotations were measured in a rotational chamber immediately after administration of drugs. The data were collected at every 30 min. Data were analyzed by the Graph Pad (version 4, San Diego, CA) program. Compounds (–)-**33** and (–)-**34** produced contralateral rotations in all lesioned rats which lasted over 8–10 h.

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Supporting Information Available: Elemental analysis data for all final targets. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

(1) Wooten, G. F. *Movement Disorders. Neurologic Principles and Practice*; McGraw-Hill: New York, 1997; pp 153–160.

- (2) Mouradian, M. M. Recent advances in the genetics and pathogenesis of Parkinson disease. *Neurology* **2002**, *58*, 179–85.
- (3) Moore, D. J.; West, A. B.; Dawson, V. L.; Dawson, T. M. Molecular pathophysiology of Parkinson's disease. *Annu. Rev. Neurosci.* **2005**, *28*, 57–87.
- (4) Paulus, W.; Jellinger, K. The neuropathologic basis of different clinical subgroups of Parkinson's disease. *J. Neuropathol. Exp. Neurol.* **1991**, *50*, 743–755.
- (5) Sherer, T. B.; Betarbet, R.; Greenamyre, J. T. Pathogenesis of Parkinson's disease. *Curr. Opin. Invest. Drugs* **2001**, *2*, 657–662.
- (6) Dutta, A. K.; L, W. Existing dopaminergic therapies for Parkinson's disease. *Expert Opin. Ther. Pat.* **2006**, *16*, 1613–1625.
- (7) Birkmayer, W.; Hornykiewicz, O. The effect of 1-3,4-dihydroxyphenylalanine (= DOPA) on akinesia in parkinsonism. 1961. *Wien. Klin. Wochenschr.* **2001**, *113*, 851–854.
- (8) Marsden, C. D.; Parkes, J. D. "On-off" effects in patients with Parkinson's disease on chronic levodopa therapy. *Lancet* **1976**, *1*, 292–296.
- (9) Fahn, S. Is levodopa toxic? *Neurology* **1996**, *47*, S184–S195.
- (10) Olanow, C. W. Oxidation reactions in Parkinson's disease. *Neurology* **1990**, *40* (Suppl. 32-7), 37–39.
- (11) Kebabian, J. W.; Calne, D. B. Multiple receptors for dopamine. *Nature* **1979**, *277*, 93–96.
- (12) Giros, B.; Martres, M. P.; Sokoloff, P.; Schwartz, J. C. Gene cloning of human dopaminergic D3 receptor and identification of its chromosome. *C. R. Acad. Sci., Ser. III* **1990**, *311*, 501–508.
- (13) Sokoloff, P.; Giros, B.; Martres, M. P.; Bouthenet, M. L.; Schwartz, J. C. Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. *Nature* **1990**, *347*, 146–151.
- (14) Van Tol, H. H.; Bunzow, J. R.; Guan, H. C.; Sunahara, R. K.; Seeman, P.; Niznik, H. B.; Civelli, O. Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine. *Nature* **1991**, *350*, 610–614.
- (15) Sunahara, R. K.; Guan, H. C.; O'Dowd, B. F.; Seeman, P.; Laurier, L. G.; Ng, G.; George, S. R.; Torchia, J.; Van Tol, H. H.; Niznik, H. B. Cloning of the gene for a human dopamine D5 receptor with higher affinity for dopamine than D1. *Nature* **1991**, *350*, 614–619.
- (16) Strange, P. G. New insights into dopamine receptors in the central nervous system. *Neurochem. Int.* **1993**, *22*, 223–236.
- (17) Civelli, O.; Bunzow, J. R.; Grandy, D. K. Molecular diversity of the dopamine receptors. *Annu. Rev. Pharmacol. Toxicol.* **1993**, *33*, 281–307.
- (18) Emilien, G.; Maloteaux, J. M.; Geurts, M.; Hoogenberg, K.; Cragg, S. Dopamine receptors—physiological understanding to therapeutic intervention potential. *Pharmacol. Ther.* **1999**, *84*, 133–156.
- (19) Joyce, J. N. Dopamine D3 receptor as a therapeutic target for antipsychotic and antiparkinsonian drugs. *Pharmacol. Ther.* **2001**, *90*, 231–259.
- (20) Sokoloff, P.; Giros, B.; Martres, M. P.; Andrieux, M.; Besancon, R.; Pilon, C.; Bouthenet, M. L.; Souil, E.; Schwartz, J. C. Localization and function of the D3 dopamine receptor. *Arzneim.-Forsch.* **1992**, *42*, 224–230.
- (21) Gurevich, E. V.; Joyce, J. N. Distribution of dopamine D3 receptor expressing neurons in the human forebrain: comparison with D2 receptor expressing neurons. *Neuropsychopharmacology* **1999**, *20*, 60–80.
- (22) Zou, L.; Xu, J.; Jankovic, J.; He, Y.; Appel, S. H.; Le, W. Pramipexole inhibits lipid peroxidation and reduces injury in the substantia nigra induced by the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in C57BL/6 mice. *Neurosci. Lett.* **2000**, *281*, 167–170.
- (23) Kitamura, Y.; Kohno, Y.; Nakazawa, M.; Nomura, Y. Inhibitory effects of talipexole and pramipexole on MPTP-induced dopamine reduction in the striatum of C57BL/6N mice. *Jpn. J. Pharmacol.* **1997**, *74*, 51–57.
- (24) Iida, M.; Miyazaki, I.; Tanaka, K.; Kabuto, H.; Iwata-Ichikawa, E.; Ogawa, N. Dopamine D2 receptor-mediated antioxidant and neuroprotective effects of ropinirole, a dopamine agonist. *Brain Res.* **1999**, *838*, 51–59.
- (25) Joyce, J. N.; Woolsey, C.; Ryoo, H.; Borwege, S.; Hagner, D. Low dose pramipexole is neuroprotective in the MPTP mouse model of Parkinson's disease, and downregulates the dopamine transporter via the D3 receptor. *BMC Biol.* **2004**, *2*, 22.
- (26) Du, F.; Li, R.; Huang, Y.; Li, X.; Le, W. Dopamine D3 receptor-preferring agonists induce neurotrophic effects on mesencephalic dopamine neurons. *Eur. J. Neurosci.* **2005**, *22*, 2422–2430.
- (27) Presgraves, S. P.; Borwege, S.; Millan, M. J.; Joyce, J. N. Involvement of dopamine D(2)/D(3) receptors and BDNF in the neuroprotective effects of S32504 and pramipexole against 1-methyl-4-phenylpyridinium in terminally differentiated SH-SY5Y cells. *Exp. Neurol.* **2004**, *190*, 157–170.
- (28) Joyce, J. N.; Millan, M. J. Dopamine D3 receptor agonists for protection and repair in Parkinson's disease. *Curr. Opin. Pharmacol.* **2007**, *7*, 100–105.

- (29) Van Kampen, J. M.; Eckman, C. B. Dopamine D3 receptor agonist delivery to a model of Parkinson's disease restores the nigrostriatal pathway and improves locomotor behavior. *J. Neurosci.* **2006**, *26*, 7272–7280.
- (30) Sanchez-Pernaute, R.; Jenkins, B. G.; Choi, J. K.; Iris Chen, Y. C.; Isacson, O. In vivo evidence of D3 dopamine receptor sensitization in parkinsonian primates and rodents with l-DOPA-induced dyskinesias. *Neurobiol. Dis.* **2007**, *27*, 220–227.
- (31) Sokoloff, P.; Diaz, J.; Le Foll, B.; Guillin, O.; Leriche, L.; Bezard, E.; Gross, C. The dopamine D3 receptor: a therapeutic target for the treatment of neuropsychiatric disorders. *CNS Neurol. Disord. Drug Targets* **2006**, *5*, 25–43.
- (32) Boeckler, F.; Gmeiner, P. The structural evolution of dopamine D3 receptor ligands: structure–activity relationships and selected neuropharmacological aspects. *Pharmacol. Ther.* **2006**, *112*, 281–333.
- (33) Luedtke, R. R.; Mach, R. H. Progress in developing D3 dopamine receptor ligands as potential therapeutic agents for neurological and neuropsychiatric disorders. *Curr. Pharm. Des.* **2003**, *9*, 643–671.
- (34) Park, B. H.; Fishburn, C. S.; Carmon, S.; Accili, D.; Fuchs, S. Structural organization of the murine D3 dopamine receptor gene. *J. Neurochem.* **1995**, *64*, 482–486.
- (35) Elsner, J.; Boeckler, F.; Heinemann, F. W.; Hubner, H.; Gmeiner, P. Pharmacophore-guided drug discovery investigations leading to bioactive 5-aminotetrahydropyrazolopyridines. Implications for the binding mode of heterocyclic dopamine D3 receptor agonists. *J. Med. Chem.* **2005**, *48*, 5771–5779.
- (36) Dutta, A. K.; Venkataraman, S. K.; Fei, X. S.; Kolhatkar, R.; Zhang, S.; Reith, M. E. Synthesis and biological characterization of novel hybrid 7-[[2-(4-phenyl-piperazin-1-yl)-ethyl]-propyl-amino]-5,6,7,8-tetrahydro-naphthalen-2-ol and their heterocyclic bioisosteric analogues for dopamine D2 and D3 receptors. *Bioorg. Med. Chem.* **2004**, *12*, 4361–4373.
- (37) Dutta, A. K.; Fei, X. S.; Reith, M. E. A novel series of hybrid compounds derived by combining 2-aminotetralin and piperazine fragments: binding activity at D2 and D3 receptors. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 619–622.
- (38) Swart, P. J.; Jansman, F. G.; Drenth, B. F.; de Zeeuw, R. A.; Dijkstra, D.; Horn, A. S. Impact of structural differences on the in vitro glucuronidation kinetics of potentially dopaminergic hydroxy-2-aminotetralins and naphthoxazines using rat and human liver microsomes. *Pharmacol. Toxicol.* **1991**, *68*, 215–219.
- (39) Biswas, S.; Zhang, S.; Fernandez, F.; Ghosh, B.; Zhen, J.; Kuzhikandathil, E.; Reith, M. E.; Dutta, A. K. Further structure activity relationships study of hybrid 7-[[2-(4-phenyl-piperazin-1-yl)-ethyl]-propyl-amino]-5,6,7,8-tetrahydro-naphthalen-2-ol analogues: identification of a high affinity D3-preferring agonist with potent in vivo activity with long duration of action. *J. Med. Chem.* **2008**, *51*, 101–117.
- (40) Kozikowski, A.; Tuckmantel, W.; Saxena, A.; Doctor, B. Synthesis of the thiazolone analogue of the acetylcholinesterase inhibitor, huperzine A. *Helv. Chim. Acta* **1994**, *77*, 1256–1266.
- (41) Berney, D.; Schuh, K. Structural analogues of apomorphine. *Helv. Chim. Acta* **1982**, *65*, 1304–1309.
- (42) Bach, N. J.; Kornfeld, E. C.; Jones, N. D.; Chaney, M. O.; Dorman, D. E.; Paschal, J. W.; Clemens, J. A.; Smalstig, E. B. Bicyclic and tricyclic ergoline partial structures. Rigid 3-(2-aminoethyl)pyrroles and 3- and 4-(2-aminoethyl)pyrazoles as dopamine agonists. *J. Med. Chem.* **1980**, *23*, 481–491.
- (43) Snider, B.; O'Neil, S. Formal synthesis of (±)-upial. Oxidative free-radical cyclization of unsaturated 1,3-cyclohexanediones. *Tetrahedron* **1995**, *51*, 12983–12994.
- (44) Frydman, B.; Blokhin, A. V.; Brummel, S.; Wilding, G.; Maxuitenko, Y.; Sarkar, A.; Bhattacharya, S.; Church, D.; Reddy, V. K.; Kink, J. A.; Marton, L. J.; Valasinas, A.; Basu, H. S. Cyclopropane-containing polyamine analogues are efficient growth inhibitors of a human prostate tumor xenograft in nude mice. *J. Med. Chem.* **2003**, *46*, 4586–4600.
- (45) Karel, P. Process for Resolution of 2-Amino-6-propylamino-4,5,6,7-tetrahydrobenzothiazole and Compounds Thereof. U.S. Patent 6,727,367 B2, 2004.
- (46) Leopoldo, M.; Lacivita, E.; De Giorgio, P.; Colabufo, N. A.; Niso, M.; Berardi, F.; Perrone, R. Design, synthesis, and binding affinities of potential positron emission tomography (PET) ligands for visualization of brain dopamine D3 receptors. *J. Med. Chem.* **2006**, *49*, 358–365.
- (47) Kuzhikandathil, E. V.; Bartoszyk, G. D. The novel antidyskinetic drug sarizotan elicits different functional responses at human D2-like dopamine receptors. *Neuropharmacology* **2006**, *51*, 873–884.
- (48) Ungerstedt, U. 6-Hydroxy-dopamine induced degeneration of central monoamine neurons. *Eur. J. Pharmacol.* **1968**, *5*, 107–110.
- (49) Newman-Tancredi, A.; Cussac, D.; Audinot, V.; Nicolas, J. P.; De Ceuninck, F.; Boutin, J. A.; Millan, M. J. Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. II. Agonist and antagonist properties at subtypes of dopamine D(2)-like receptor and alpha(1)/alpha(2)-adrenoceptor. *J. Pharmacol. Exp. Ther.* **2002**, *303*, 805–814.
- (50) Cheng, Y.; Prusoff, W. H. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (I₅₀) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.
- (51) Newman-Tancredi, A.; Cussac, D.; Audinot, V.; Millan, M. J. Actions of roxindole at recombinant human dopamine D2, D3 and D4 and serotonin 5-HT1A, 5-HT1B and 5-HT1D receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1999**, *359*, 447–453.
- (52) Lazareno, S. Measurement of agonist-stimulated [35S]GTP gamma S binding to cell membranes. *Methods Mol. Biol.* **1997**, *83*, 107–116.
- (53) Bidlack, J. M.; Parkhill, A. L. Assay of G protein-coupled receptor activation of G proteins in native cell membranes using [35S]GTP gamma S binding. *Methods Mol. Biol.* **2004**, *237*, 135–143.

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