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Structural Modifications of Neuroprotective Anti-Parkinsonian (−)-*N*6-(2-(4-(Biphenyl-4-yl)piperazin-1-yl)-ethyl)-*N*6-propyl-4,5,6,7-tetrahydrobenzo[*d*]thiazole-2,6-diamine (D-264): An Effort toward the Improvement of in Vivo Efficacy of the Parent Molecule

Gyan Modi,[†] Tamara Antonio,[‡] Maarten Reith,[‡,§] and Aloke Dutta*[,†]

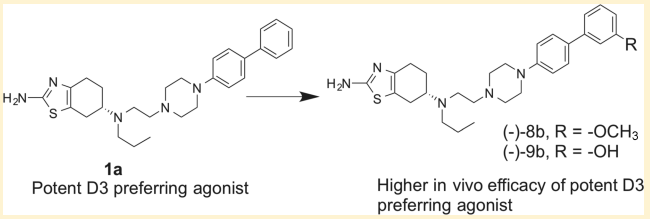
[†]Department of Pharmaceutical Sciences, Wayne State University, Detroit, Michigan 48202, United States

[‡]Department of Psychiatry, New York University, New York, New York 10016, United States

[§]Department of Biochemistry and Molecular Pharmacology, New York University, New York, New York 10016, United States

Supporting Information

ABSTRACT: In our overall goal to develop multifunctional dopamine D_2/D_3 agonist drugs for the treatment of Parkinson's disease (PD), we previously synthesized potent D_3 preferring agonist D-264 (**1a**), which exhibited neuroprotective properties in two animal models of PD. To enhance the in vivo efficacy of **1a**, a structure−activity relationship study was carried out. Competitive binding and [^{35}S]GTPγS functional assays identified compound (−)-**9b** as one of the lead molecules with preferential D_3 agonist activity (EC_{50}(GTPγS); D_3 = 0.10 nM; D_2/D_3 (EC_{50}): 159). Compounds (−)-**9b** and (−)-**8b** exhibited high in vivo activity in two PD animal models, reserpinized and 6-hydroxydopamine (OHDA)-induced unilateral lesioned rats. On the other hand, **1a** failed to show any in vivo activity in these models unless the compound was dissolved in 5−10% beta-hydroxy propyl cyclodextrin solution. Lead compounds exhibited appreciable radical scavenging activity. In vitro experiments with dopaminergic MN9D cells indicated neuroprotection by both **1a** and (−)-**9b** from toxicity of MPP+.



INTRODUCTION

Parkinson's disease (PD) is a progressive age-related neurodegenerative disorder of the central nervous system that is characterized by gradual loss of dopaminergic neurons in the substantia nigra region of the brain.[1] It is estimated that PD affects 1−2% of the people older than 65 years of age. According to a statistical analysis published by the Parkinson's Disease foundation, approximately 60 000 Americans are diagnosed with PD each year, and an estimated 7−10 million people worldwide are living with PD. Common symptoms associated with PD include rigidity, bradykinesia, resting tremors, postural instability, and cognitive psychiatric complications.[2−4] The etiology of PD is not clear yet, but it has been shown that both mitochondrial dysfunction and oxidative stress are interdependent, which is thought to play a central role in the pathogenesis of the disease process.[5,6] Oxidative stress and excessive amounts of metals especially iron can lead to the formation of reactive oxygen species (ROS). These mitochondria-derived ROS inhibit mitochondrial respiration and promote the aggregation of alpha synuclein protein (αSN), which ultimately forms Lewy bodies (LBs) and Lewy neuritis (LN).[7] LBs and LN are neuropathological hallmarks of PD and toxic toward dopaminergic neurons. Levodopa (L-DOPA) became available in 1960 for the treatment of PD and is still being considered a main stream therapy.[8] However, prolonged use of L-DOPA gives rise to "on" and "off" episodes along with motor fluctuations, and eventual oxidation of dopamine (DA) derived from L-DOPA further facilitates neurodegeneration.[9] One of the current strategies of PD therapy is to delay the initiation of L-DOPA therapy, by using various combinations of other therapeutic agents including, but not limited to, DA agonists, inhibitors of DA metabolism.[10] However, none of these strategies address the limitations of L-DOPA. Therefore, the need for therapeutic agents with disease-modifying effects is of paramount importance.

The DA receptors, belonging to a class of G-protein-coupled receptor (GPCR) family, are mainly found in the central nervous system (CNS) (controlling neuronal signaling thereby modulating many important behaviors) and in the periphery (to affect cardiovascular and renal functions).[10] The D_1-like receptors (D_1 and D_5 subtypes) and the D_2-like receptors (D_2, D_3, and D_4 subtypes) transduce signals via adenylate cyclase, an effectors molecule. Upon receptor activation, D_1-like receptors activate adenylate cyclase, whereas D_2-like receptors inhibit it. Interestingly, the DA D_3 receptor has a different distribution in the brain compared to the D_2 receptor.[11,12] The D_3 receptor is found to be densest in the limbic region of the brain, whereas the highest level of D_2 expression is in the striatum of the midbrain.[13] It is important to mention that D_2 and D_3 receptor subtypes exhibit

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50% homology in their amino acid sequence, which increases to 75−80% in the helical transmembrane spanning domains, where agonist binding sites are believed to be located.[14,15] This makes the task of developing D_3-selective ligands challenging. Interestingly, DA D_3 preferring agonists were shown to provide an additional neuroprotective effect compared to the DA D_2 receptor agonist, probably via the production of neurotrophic factor.[16,17] An enormous amount of work has been done to develop D_3 selective agonists and to identify key pharmacophoric features responsible for selectivity for D_3 receptor over D_2.[18−27] It is important to mention that D_3 receptor bound to an antagonist was recently crystallized to provide a detailed molecular structure.[28]

The research from the past two decades in the PD area has provided more insights into the basic pathogenetic factors of PD such as roles of oxidative stress, aggregation of αSN proteins in the form of soluble toxic aggregates and fibrils, and increased concentration of iron in the PD brain.[29−31] αSN is a component of Lewy bodies, a pathological hallmark of PD. αSN along with oxidized DA (DA-quinone) could have a synergistic effect in terms of disease susceptibility and progression.[32−35]

It is increasingly evident that drugs aimed at a single target may be inadequate for the treatment of complex diseases such as PD, which is multifactorial in nature. Thus, it is hypothesized that multifunctional drugs exhibiting multiple pharmacological activities addressing underlying pathogenic factors of PD will be effective as disease modifying agents.[36] With this in mind, we initiated our drug discovery approach aimed at identifying novel multifunctional agents possessing D_2/D_3 agonist or D_3 preferring agonist activity along with antioxidant, iron chelating, and modulation of αSN aggregation activities. In this regard, we have designed and explored a novel, hybrid molecular template by combining known D_2/D_3 agonists with D_2/D_3 antagonist fragments, which led to the development of a number of potent D_3 preferring and D_2/D_3 agonists and lead molecules exhibiting potent in vivo activity in PD animal models.[37−44] One such lead compound, D-264 (**1a**, Figure 1), exhibited potent in vivo activity in PD animal models and also exhibited neuroprotective properties in two different PD animal neuroprotection models.[45]

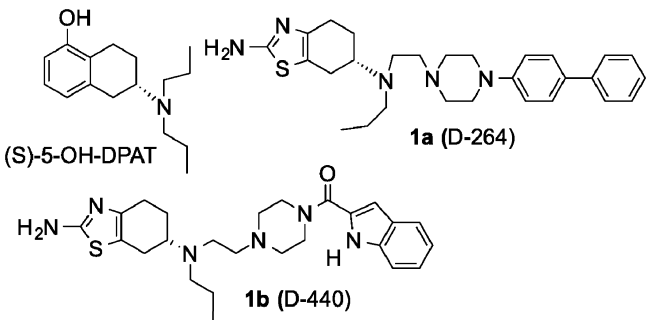

Figure 1. Molecular structures of D_2/D_3 agonists.

Although its neuroprotective action is an important feature, **1a** suffers from poor in vivo efficacy probably due to lack of sufficient brain penetration, although additionally high plasma protein binding and possibly binding to adipose tissue can also potentially contribute to less efficacy. In vivo activity of **1a** was enhanced significantly when **1a** was solubilized in 5−10% β-hydroxy-propyl-cyclodextrin (BHPC) solution presumably by encapsulating the molecule leading to enhanced blood brain barrier penetration of **1a**. The present structure−activity study with **1a**-related compounds has been designed to enhance the in vivo efficacy without compromising their multifunctional agonist and neuroprotective properties. Introduction of different polar hydroxyl group(s) will contribute toward reducing the lipophilicity of the parent **1a**, which will help bring the compounds, for example, (−)-**9b**, more in compliance with Lipinski's rule of five compared to the parent **1a**.[46] Consequently, this should contribute toward a higher in vivo efficacy.

CHEMISTRY

Scheme 1 describes the synthesis of final compounds (±)-**8a**, (±)-**8b**, (±)-**8c**, (±)-**9a**, (±)-**9b**, (±)-**9c**, (±)-**9d**, (−)-**11** and their enantiomers. Iodination of phenyl piperazine was done following the literature procedure. The 1-(4-iodophenyl) piperazine was treated with *t*-Boc-anhydride to synthesize the *t*-Boc protected intermediate (**1**). The *t*-Boc protected intermediate was then subjected to Suzuki coupling reaction[47,48] with various commercially available substituted benzene boronic acids. The amine protecting *t*-Boc group was removed by using trifluoroacetic acid. The free amines (**4a−d**) were subjected to N-alkylation reaction with TBDMS protected bromoalcohol to get intermediates (**5a−e**) which further underwent TBDMS elimination using tetrabutyl ammonium fluoride (TBAF) solution to get the alcohol intermediate (**6a−e**). These alcohol intermediates (**6a−e**) were oxidized under Swern oxidation conditions to get the arylpiperazine aldehydes (**7a−e**), which were further condensed with (±)-, *S*-(−), or *R*-(+)-pramipexole under reductive amination conditions to give four final compounds (±)-**8a**, (±)-**8b**, (±)-**8c**, (−)-**8b** and the four carbon linker intermediate (±)-**8d**. The demethylation of these intermediates with either boron tribromide or with freshly distilled aqueous hydrobromic acid (48%) yielded the four final compounds (**9a−d**) and their enantiomers. One of the intermediates described in Scheme 1, the arylpiperazine aldehyde, **7b**, was subjected under reductive amination conditions to react with (*S*)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine to get corresponding mehtoxy intermediates (−)-**10** and subsequently was treated with aqueous hydrobromic acid (48%) to furnish the final compound (−)-**11**.

Scheme 2 depicts the synthesis of final target compounds (±)-**22**. 2-Methoxyaniline (**12**) was subjected to cyclization by following the literature procedure[49] to produce the intermediate **13**. Further, bromination of the intermediate, **13**, yielded bromo derivative, **14**. This amine intermediate, **14**, was converted into *t*-Boc protected compound, **15**, followed by their Suzuki coupling reaction with commercially available benzene boronic acids, and subsequently *t*-Boc group was removed by using TFA to yield **17**. The free amine intermediate **17** was N-alkylated with (2-bromo ethoxy)-*tert*-butyldimethylsilane to get compound **18**, which on TBDMS elimination yielded alcohol, **19**. Compound **19** was converted into aldehyde derivatives **20** under Swern oxidation conditions followed by condensation with (±)-pramipexole under reductive amination conditions and subsequently treated with aqueous hydrobromic acid (48%) to yield the final compound **22**.

In Scheme 3, we describe the synthesis of bioisosteric analogues of 2-aminothiazole agonist pharmacophoic headgroup using the quinazoline moiety. The quanazoline derivatives were synthesized as reported in our earlier publication. Briefly, 1,4-cyclohexanedionemonoethyleneketal, on treatment with *n*-propylamine under reductive amination conditions, yielded intermediate **23**. This intermediate **23** was coupled with

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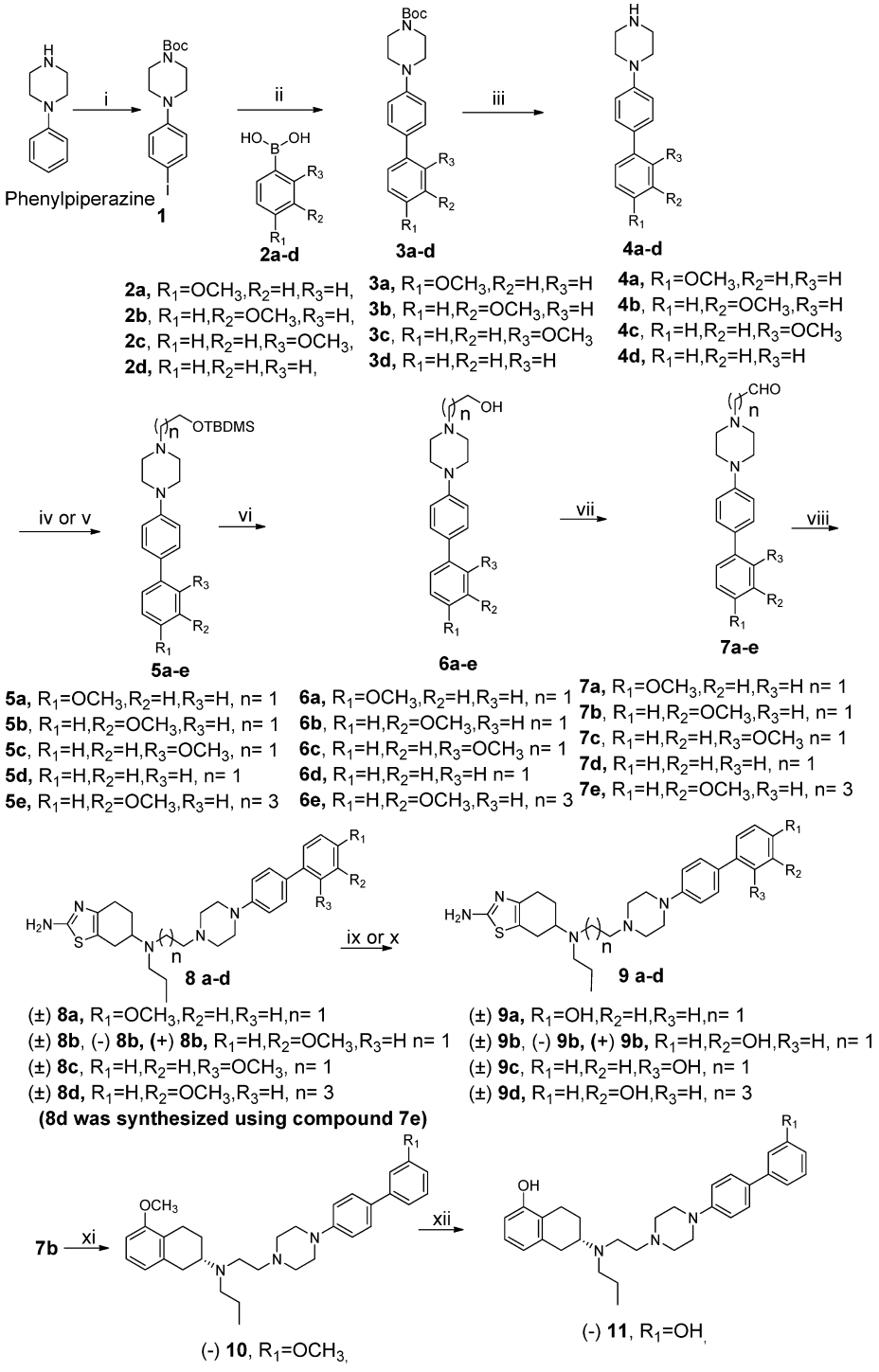
Scheme 1[a]



[a]Reagents and conditions: (i) (a) ICI, acetic acid, water, 55 °C, 1 h, 74%; (b) $(Boc)_20$, Et_3N, dichloromethane, rt, 12 h, 92%; (ii) $ArB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, dimethoxy ethane, ethanol, 95 °C, 1 h, 65−70%; (iii) TFA, dichloromethane, rt, 4 h, 90−95%; (iv) (2-bromoethoxy)-(*tert*-butyl)dimethylsilane, K_2CO_3, acetonitrile, reflux, 14 h, 80−85% (for compound **5a**−**d**); (v) (4-bromobutoxy)(*tert*-butyl)dimethylsilane, K_2CO_3, acetonitrile, reflux, 14 h (for compound **5e**), 80% (vi) *n*-Bu_4NF, THF, rt, 1.5 h, 90−95%; (vii) oxalyl chloride, DMSO, TEA, CH_2CI_2, −78 °C, 2 h, 65−70%; (viii) (±)-pramipexole, (−)-pramipexole, (+)-pramipexole $NaBH(OAc)_3$, CH_2CI_2, rt, 48 h, 60−65%; (ix) BBr_3,CH_2CI_2, −78 °C,48 h; (for compound (±)-**8a**, (±)-**8b**, and (±)-**8c**), 60−65%,48 h; (x) 48% aq. HBr, reflux, 6 h, 75−80% (for compound (−)-**8b**, (+)-**8b**, and for **8d**). (xi) (*S*)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine, $NaBH(OAc)_3$, CH_2CI_2, rt, 48 h, 60%; (xii) 48% aq. HBr, reflux, 6 h, 70%.

aldehyde **7d** and **7b** under reductive amination conditions to afford **24a** and **24b**. Removal of the ketal group by dilute HCl in THF followed by ring formation in a two-step synthesis afforded the final compound **26a** and the intermediate **26b**. Final target **27** was produced by demethylation of methoxy group of **26b**, using 48% aqueous HBr.

The synthesis of the final compound **35** is shown in Scheme 4. Mono-*t*-Boc protected amine **28** was reacted with commercially

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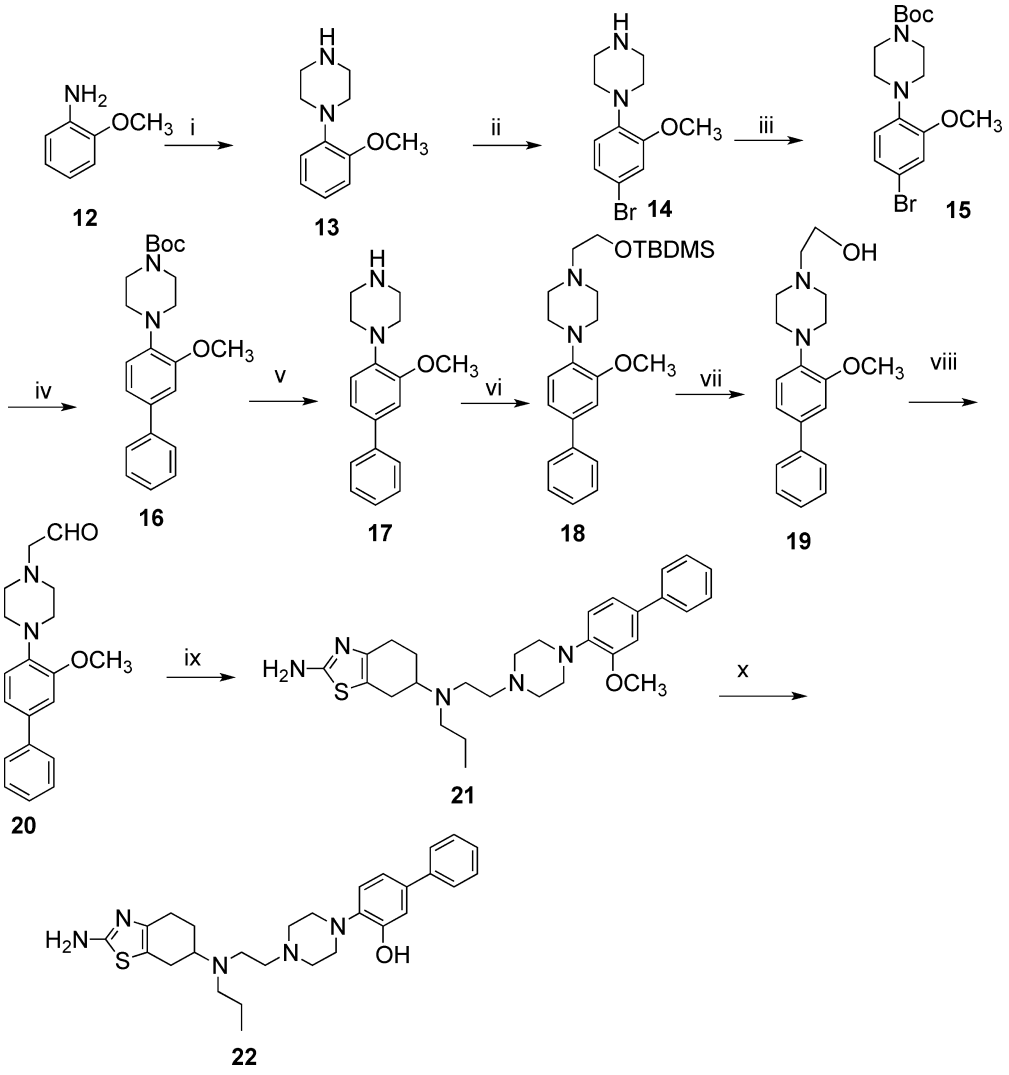
Scheme 2[a]



[a]Reagents and conditions: (i) Bis(2-chloroethyl)amine, diethylene glycol monomethyl ether, 150 °C, 7 h; 65−70% (ii) Br_2,CH_2Cl_2, 0 °C, 2 h, NaOH, 80%; (iii) $(Boc)_2O$, Et_3N, dichloromethane, rt, 12 h, 75−80%; (iv) phenyl boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, dimethoxy ethane, ethanol, 95 °C, 2 h, 76−80%; (v) TFA, dichloromethane, rt, 4 h, 90−95%; (vi) (2-bromo ethoxy)-*tert*-butyl-dimethyl-silane, K_2CO_3, CH_3CN, reflux, 12 h, 80−85%; (vii) *n*-Bu_4NF, THF, rt, 2 h, 70−75%; (viii) oxalyl chloride, DMSO, TEA, CH_2Cl_2, −78 °C, 2 h; 65−70% (ix) (±)-pramipexole, $NaBH(OAc)_3$, CH_2Cl_2, rt, 48 h; (x) 48% aq. HBr, reflux, 6 h.

available biphenyl carbonyl chloride **29** at room temperature in THF in the presence of diisopropylethylamine as base to provide **30**. The *t*-Boc group was removed using TFA followed by N-alkylation with TBDMS protected bromoethanol, and subsequently the TBDMS group was eliminated using TBAF to yield the corresponding alcohol **33**. Alcohol **33** was converted, under Swern oxidation conditions, into its aldehyde derivative **34** followed by reductive amination with (±)-pramipexole to afford the final compound **35**.

RESULTS AND DISCUSSION

Potency and Agonism at DA D_2 and D_3 Receptors. Our first-generation hybrid compound **1a**, a potent and D_3 preferring agonist with multifunctional properties for potential PD treatment, was the starting point for designing compounds with enhanced in vivo efficacy without compromising agonist potency. The structural modifications are mainly centered on the introduction of methoxy and hydroxyl groups at various positions on the biphenyl moiety of this hybrid molecule. Methoxy and hydroxyl substitutions also should help us to examine the possible contribution of any hydrogen-bonding interaction originating from this region of the molecule with D_2 and D_3 receptors. Apart from these modifications, other molecular alterations involving bioisosteric replacement of the thiazolidium moiety by aminotetraline or quanazoline rings, change of ethylene linker length, and incorporation of amide bond at the piperazine nitrogen atom distal to the agonist headgroup have also been incorporated.

First, the influence of methoxy and hydroxyl substitutions on the biphenyl ring of **1a** was tested in binding assays with rat DA D_2 and D_3 (rD_2 and rD_3) receptors expressed in HEK-293 cells. To this end, racemic derivatives **8a−c**, **9a−c**, and **22** were synthesized and characterized. It is evident from Table 1 that most of these compounds displayed high affinity for D_3 and moderate affinity for D_2 receptors. Among this series of analogues, compound **9b** with monohydroxyl substitution on the meta position of the phenyl ring distal to the piperazine was found to be the most potent and selective for D_3 tested at this

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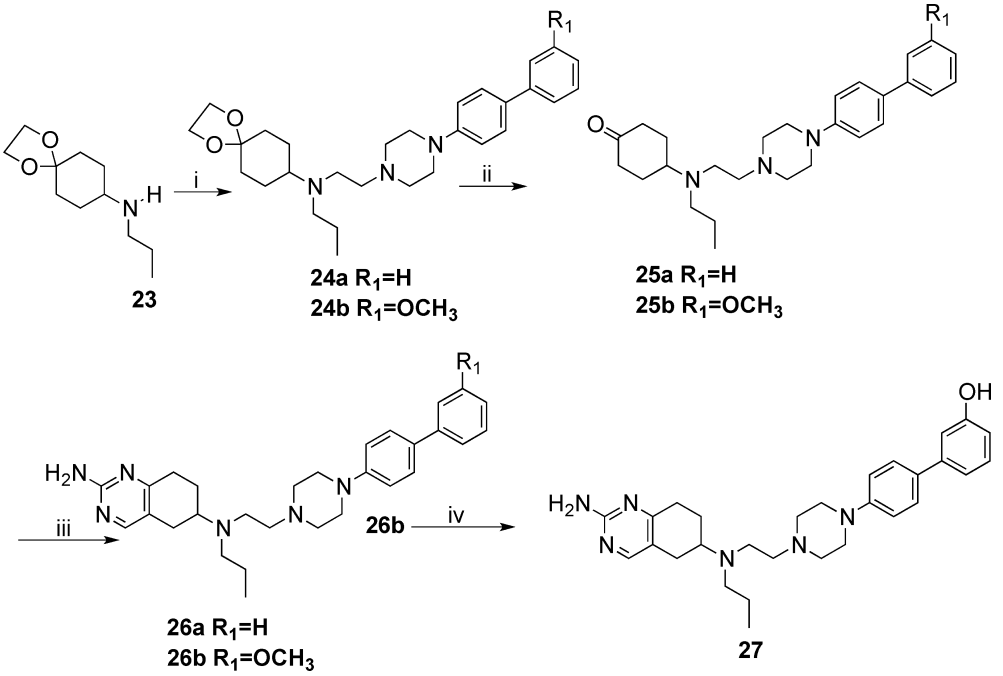
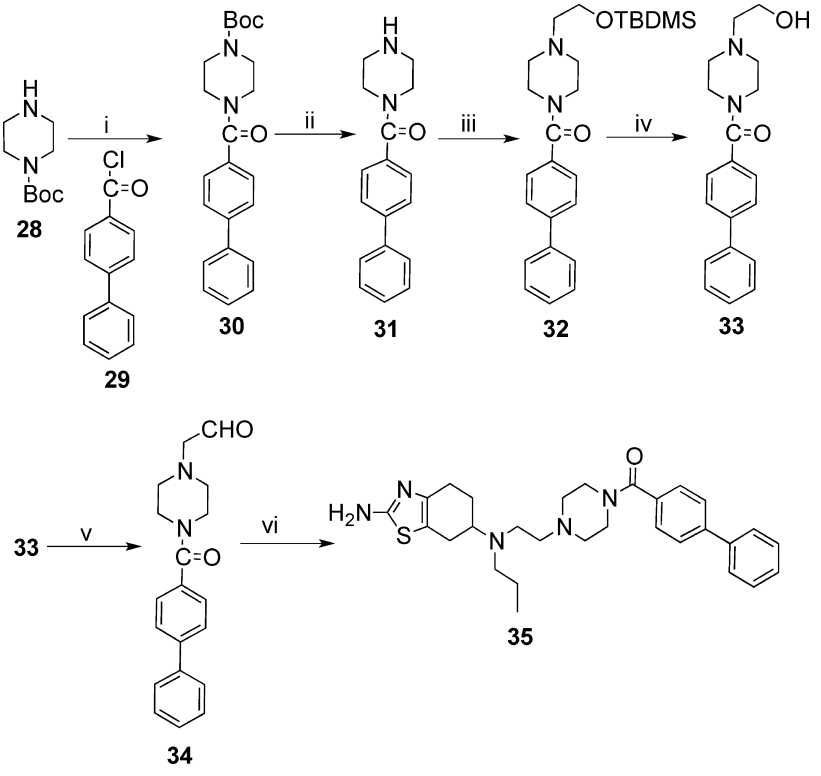
Scheme 3[a]



[a]Reagents and conditions: (i) **7d**, **7b**, $NaBH(OAc)_3$, HOAC, $ClCH_2CH_2Cl$, rt, 48 h, 60−70%; (ii) 2 N HCl, THF, reflux, 2 h, 85−90%; (iii) Tris(dimethylamino)methane, toluene, reflux, guanidine carbonate/EtOH, reflux, 7 h,70−75% ; (iv) 48% aq. HBr, reflux, 8 h, 65%.

Scheme 4[a]



[a]Reagents and conditions: (i) Diisopropylethylamine, THF, rt, overnight, 80%; (ii) TFA, dichloromethane, rt, 4 h, 80%; (iii) (2-bromo-ethyl)-*tert*-butyldimethylsilane, K_2CO_3, acetonitrile, reflux, 14 h, 70%; (iv) *n*-Bu_4NF, THF, rt, 1.5 h, 90%; (v) oxalyl chloride, DMSO, TEA, CH_2Cl_2, −78 °C, 2 h, 85%; (vi) (±)-pramipexole, $NaBH(OAc)_3$, CH_2Cl_2, rt, 48 h,70%.

point (K_i, D_2 = 347, D_3 = 1.20 nM, D_2/D_3 = 289). On the other hand, **22** with the hydroxyl group at the ortho position of the phenyl ring proximal to the piperazine ring proved to be the most potent of the compounds tested at this point for D_2 (K_i, D_2 = 70.6, D_3 = 2.35 nM, D_2/D_3 = 30). Compound **8b**, a methoxy analogue of **9b**, exhibited a somewhat lower binding affinity at D_2

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Table 1. Inhibition of [^{3}H]Spiroperidol Binding to rD_{2L} and rD_3 Receptors Expressed in HEK-293 Cells[a]

compound	K_i (nM), rD_{2L} [^{3}H]spiroperidol	K_i (nM), rD_3 [^{3}H]spiroperidol	D_{2L}/D_3
(−)-5-OH-DPAT[b]	58.8 ± 11.0	1.36 ± 0.28	43.2
1a[b]	186 ± 34	2.10 ± 0.34	86
1b[b]	1,073 ± 92	1.84 ± 0.51	583
8a	213 ± 12	1.41 ± 0.12	151
8b	464 ± 93	2.11 ± 0.34	220
(−)-**8b**	343 ± 65	2.33 ± 0.26	147
8c	274 ± 45	3.57 ± 0.44	78
9a	230 ± 50	1.17 ± 0.37	196
9b	347 ± 54	1.20 ± 0.14	289
(−)-**9b**	369 ± 39	1.73 ± 0.14	213
(+)-**9b**	1507 ± 312	19.7 ± 2.1	76
9c	208 ± 15	1.80 ± 0.38	115
9d	567 ± 83	9.43 ± 1.14	60
(−)-**11**	27.8 ± 1.8	0.77 ± 0.030	36
22	70.6 ± 10.2	2.35 ± 0.13	30
26a	735 ± 198	3.65 ± 0.64	201
27	13,121 ± 4539	67 ± 7.8	196
35	1666 ± 282	9.58 ± 1.18	174

[a]Results are the means ± SEM for 3−6 experiments each performed in triplicate. [b]From previous ref 44.

receptor compared to **9b**, while D_3 affinity decreased approximately 2-fold in comparison to **9b** (K_i, D_2 = 464, D_3 = 2.11 nM, D_2/D_3 = 220 for **8b**). These results indicated that introduction of monomethoxy and monohydroxyl groups is well tolerated on the distal phenyl ring of **1a** and actually increases selectivity for the D_3 receptor.

Among the racemic compounds with D_3 affinity in the nanomolar range, **9b** exhibited the highest selectivity for D_3; we therefore chose this racemic compound for synthesizing both the (−)- and (+)-enantiomer to evaluate the differential potency and selectivity of the enantiomers at DA receptors. In agreement with our earlier results on stereoselectivity in this type of compounds, (−)-**9b** (K_i, D_2 = 369 nM, D_3 = 1.73 nM, D_2/D_3 = 213) exhibited higher potency at both D_2 and D_3 receptors compared to (+)-**9b** (K_i, D_2 = 1507 nM, K_i D_3 = 19.7 nM, D_2/D_3 = 76). In compound (−)-**9b**, an additional hydroxyl functionality is present compared with the parent compound **1a**, resulting in retention of high binding affinity at D_3 with slightly reduced affinity for D_2, that is, overall higher selectivity of (−)-**9b** for D_3 compared to **1a** (K_i; D_2/D_3 = 213 vs D_2/D_3 = 86 for (−)-**9b** and **1a**, respectively). The (−)-isomer of **8b** was made to evaluate whether the free hydroxyl group in (−)-**9b** is critical for activity (Table 1). Compound (−)-**8b**, which is a methoxy analogue maintained D_2 receptor affinity similar to (−)-**9b** (K_i; D_2 = 343 nM vs D_2 = 369 nM for (−)-**8b** and (−) **9b**, respectively), while the binding affinity toward D_3 dropped slightly (K_i; D_3 = 2.33 nM vs D_3 = 1.73 nM for (−)-**8b** and (−)-**9b**, respectively); this resulted in a somewhat lower selectivity for D_3 over D_2 receptors (D_2/D_3 = 147 vs D_2/D_3 = 213 for (−)-**8b** and (−)-**9b**, respectively). All compounds in this series showed nanomolar binding potency for the D_3 receptor.

In our next series of compounds, aminotetraline and amino pyrimidine moieties were incorporated as bioisosteric replacement of the thiazolidium moiety of pramipexole in **9b** or **1a**, resulting in (−)-**11**, **26a**, and **27**.[50] It was hypothesized that in both cases H-bonding interaction of the parent amino group with serine-192 at the DA receptor should be maintained.[51] Specifically, the (−) isomer of 5-hydroxy aminotetraline was synthesized, as we have shown in our previous reports that the (−)-enantiomer exhibits the highest affinity compared to the (+)-isomer for both D_2 and D_3 receptors. As expected, the 5-hydroxy aminotetraline analogue (−)-**11** exhibited higher affinity, compared to (−)-**9b**, for both D_2 and D_3 receptors with overall less selectivity for D_3 receptor (K_i, D_2 = 27.8, D_3 = 0.77 nM, D_2/D_3 = 36). In our previous report, the phenolic moiety of 5-hydroxy aminotetraline was replaced by an amino pyrimidine moiety, which is a known bioisostere of a phenolic group. Here we wanted to explore this further with linearly fused biphenyl rings at the other side of the molecule. Incorporation of amino pyrimidine to this moiety in compound **26a** resulted in reduced potency for both D_2/D_3 receptors (K_i, D_2 = 735 nM, D_3 3.65 nM) with decreased in selectivity (D_2/D_3 = 201) compared to **9b**. Next, in compound **27** we introduced a hydroxyl group on the biphenyl ring of **26a** (targeting the accessory binding domain of the receptor). Compound **27**, which is a bioisosteric analogue compound **9b**, exhibited significantly decreased binding affinity, compared to **9b**, at both D_2 and D_3 receptors (K_i, D_2 = 13121 nM, D_3 = 67 nM, vs D_2 = 235 nM, D_3 0.70 nM for **27** vs **9b**, respectively). This suggests the combination of either 2-aminothiazole or hydroxytetralin, and a linearly fused biphenyl moiety gives rise to D_2 and D_3 potency and D_2/D_3 selectivity. Next, we increased the length of the two-carbon linker in **9b** to four carbons in compound **9d**. In agreement with our previous results with a four-methylene linker at this position, compound **9d** (K_i, D_2 = 567 nM, D_3 = 9.43 nM) displayed lower potency at both D_2 and D_3 receptors compared to **9b**.[38] The reason behind characterizing bio-isosteric ((−)-**11**, **26a**, and **27**) and higher chain length (**9d**) compound is to expand the SAR study to better understand molecular interaction of our hybrid molecules with D_2/D_3 receptors.

Finally, in an earlier publication, we reported compound (*S*)-(4-{2-[(2-amino-4,5,6,7-tetrahydrobenzothiazol-6-yl)-propylamino]ethyl}piperazin-1-yl)-(1H-indol-2-yl)methanone (**D-440**) as one of the most potent and selective agonists for D_3 receptor known to date, and this compound contains a carbonyl bond between the piperazine nitrogen atom and 5-position of indole, distal to the agonist headgroup.[44] So, to probe the impact of introduction of a carbonyl group on D_3 receptor selectivity in our first generation hybrid compound **1a**, we incorporated a carbonyl bond between the piperazine nitrogen and the accessory binding biphenyl ring of **1a**. This modification generated compound **35**, Scheme 4, which exhibited lower binding affinity for D_2/D_3

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Table 2. Stimulation of [^{35}S]GTPγS Binding to hD_2 and hD_3 Receptors Expressed in CHO Cells

	CHO−D_2		CHO−D_3		
compd	EC_{50} (nM)[a] [^{35}S]GTPγS	$\%E_{max}$	EC_{50} (nM)[a] [^{35}S]GTPγS	$\%E_{max}$	D_2/D_3
dopamine	218 ± 12	100	10.6 ± 2.1	100	26.5
1a[b]	33.1 ± 6.6	104 ± 5	1.51 ± 0.02	90 ± 4.3	22.1
(−)-**8b**	36.8 ± 7.2	105 ± 6	3.42 ± 1.01	67.3 ± 5.6	10.8
(−)-**9b**	15.9 ± 1.8	116 ± 10	0.10 ± 0.02	95.8 ± 3.7	159

[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 (D_2) or 100 uM (D_3) of the full agonist DA ($\%E_{max}$). Results are the means ± SEM for 3−6 experiments each performed in triplicate. [b]From previous ref 43.

receptors (K_i, D_2 = 1666 nM, D_3 = 9.58 nM), and its selectivity was increased (D_2/D_3 = 174) compared to parent compound **1a**. Thus, introduction of a carbonyl group between the piperazine nitrogen and the biphenyl ring in this series of compounds impacted D_3 affinity and selectivity in opposite fashion.

On the basis of the binding results, selected compounds (−)-**8b** and (−)-**9b** were subjected to the GTPγS binding functional assay for D_2 and D_3 receptors and compared with endogenous ligand DA and the parent compound **1a**. The functional assay measures quantitatively the ability of the compound to stimulate the receptor as an agonist. Comparison with the maximum stimulation (E_{max}), produced by the full agonist DA, indicates whether the compound is a full agonist, a partial agonist, or an antagonist. The assays were carried out with cloned human D_2 and D_3 receptors expressed in CHO cells. Compound (−)-**9b** displayed higher functional potency for D_2/D_3 and selectivity for D_3 receptor in comparison to **1a** and dopamine (Table 2). (−)-**9b** displayed a 15-fold increase in D_3 functional potency in comparison to **1a** (EC_{50} = 15.9 nM vs 33.1 nM for D_2 and 0.1 nM vs 1.51 nM for D_3, for (−)-**9b** vs **1a**, respectively) and a 7-fold increase in functional selectivity (D_2/D_3 = 159 vs 22.1 for (−)-9b vs **1a**). Compounds (−)-**9b** and **1a** exhibited full agonist activity at D_2 and D_3 receptors, while their selectivity for D_3 receptor dropped significantly when compared to the binding data (Table 1). On the other hand, compound (−)-**8b** turned out to be functionally 2-fold less potent at D_3 receptor (EC_{50} = 3.42 nM) in comparison to **1a** and was a partial agonist at D_3 but full agonist at D_2 receptor. The functional potency of compound (−)-**8b** for D_2 was comparable to **1a** (EC_{50} = 36.8 nM vs 33.1 nM for (−)-**8b** vs **1a**, respectively).

Evaluation of Free Radical Scavenging Activity. Scavenging of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical by **1a**, (−)-**9b**, (−)-**8b**, (−)-**11**, ropinirole, and ascorbic acid was monitored (Figure 2). As shown in Figure 2, all compounds inhibited DPPH radical activity dose dependently. Overall, all of the compounds exhibited similar antioxidant efficacy as ascorbic acid except ropinirole which exhibited poor activity in this assay. Interestingly, (−)-**11** exhibited less antioxidant activity in this assay than (−)-**9b**, indicating a more efficacious antioxidant activity of the thiazolidum moiety compared to aminotetraline.

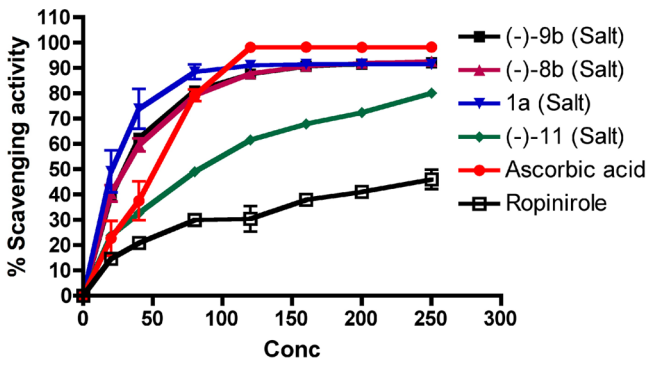

Figure 2. DPPH radical scavenging activity by **1a**, (−)-**9b**, (−)-**8b**, (−)-**11**, ropinirole, and ascorbic acid.

Reversal of Reserpine-Induced Hypolocomotion in Rats by 1a, (−)-8b, (−)-9b, and Ropinirole. Reserpine induces depletion of catecholamine in nerve terminals, resulting in a cataleptic condition in rats, which is a well established animal model for PD.[52,53] Significant inhibition of locomotion of rats was observed 18 h after the administration of reserpine (5 mg/kg, s.c.) which indicated the development of akinesia. Compounds (−)-**8b** and (−)-**9b** at a dose of 5 μmol/kg, i.p., in DI water, were highly efficacious in reversing akinesia (Figure 3), while **1a** at the same dose (5 μmol/kg, i.p., in DI water) failed to produce any significant effect in reversing akinesia in reserpine-treated rats. However, **1a** was more effective when it was dissolved in 10% beta-hydroxy cyclodextrin solution (Figure 3). The reference drug Ropinirole exhibited much shorter duration of action compared to the test compounds (Figure 3). The locomotor activity of (−)-**8b** at the end of 6h remained high compared to (−)-**9b**. It is evident from the result that compounds (−)-**8b** and (−)-**9b** were more efficacious in producing reversal of akinesia than **1a** ((−)-**8b** > (−)-**9b** > **1a**). Thus, the results indicate that compounds (−)-**8b** and (−)-**9b** exhibited higher in vivo efficacy

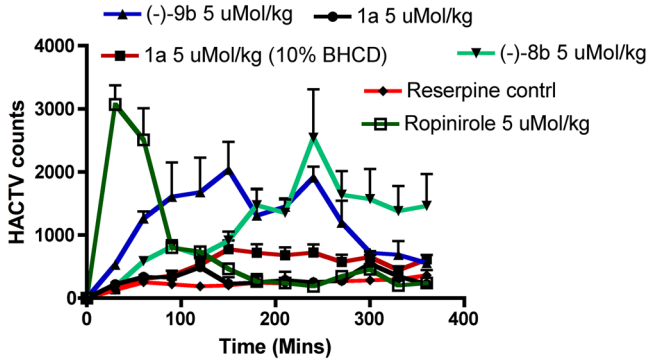

Figure 3. Effect of different drugs upon reserpine (5.0 mg/kg, s.c.)-induced hypolocomotion in rats. Data are means ± SEM, n = 4 per value. Horizontal activity was measured as described under materials and methods. The plots are the representation of horizontal locomotor activity at discrete 30-min intervals after the administration of (−)-**9b** (i.p.), (−)-**8b** (i.p.), ropinirole (s.c) and **1a** (i.p.) at the dose of 5 μmol/kg compared to control reserpine treated rats in 18 h post reserpine treatment. One way ANOVA analysis demonstrates significant effect among treatments F (5,95) = 14.16 (P < 0.0001). Dunnett's analysis following ANOVA showed that the effects of (−)**9b** (P < 0.01), (−)-**8b** (P < 0.01), and ropinirole (P < 0.01) were significantly different compared to reserpine control.

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which might be due to efficient crossing of the blood brain barrier. Compound **1a** was able to produce in vivo activity only if dissolved in 10% beta-hydroxy propyl cyclodextrin solution, indicating limitations in brain uptake for this compound when administered by itself. Interestingly, all three compounds displayed a long duration of action (Figure 3).

In Vivo Pharmacology in 6-OHDA Lesioned Rats. On the basis of the above locomotor data, compounds (−)-**8b** and (−)-**9b** as well as the reference Ropinirole were selected for in vivo evaluation in rats carrying an unilateral lesion in the medial forebrain bundle; the lesion was induced by application of the neurotoxin 6-hydroxydopamine (6-OHDA), resulting in the production of supersensitized DA receptors on the lesioned side. Such rats, when challenged with direct acting DA agonists, respond with 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 activity.[54] Both compounds (−)-**8b** and (−)-**9b** produced potent rotational activity in a dose-dependent manner when administered intraperitoneally (i.p). At a 10 μmol/kg dose, both (−)-**8b** (6.56 mg/kg) and (−)-**9b** (9.28 mg/kg) produced potent rotation that lasted for more than 10 h (Figure 4). Compound

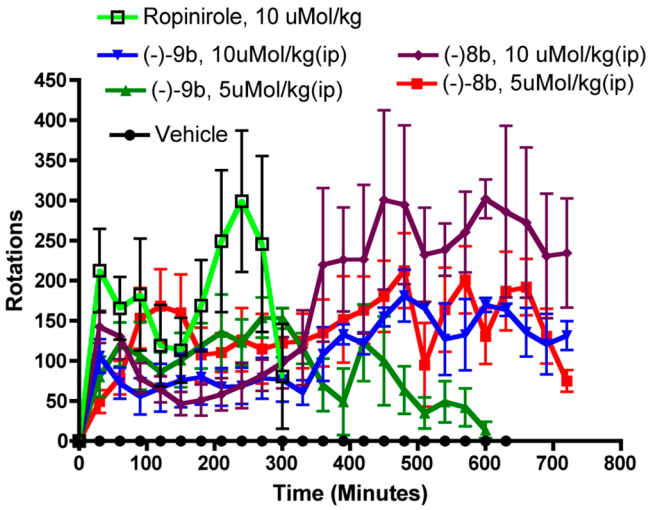

Figure 4. Effect on turning behavior of two different doses of (−)-**9b** (i.p.), (−)-**8b** (i.p.), ropinirole and vehicle in lesioned rats studied for maximum 12 h. Each point is the mean ± SEM of 3−4 rats. The drugs were administered i.p. One way ANOVA analysis demonstrates significant effect among treatments: F (5, 95) = 29.70 (P < 0.0001). Dunnett's analysis showed that the effect of (−)-**9b**, (−)-**8b** and ropinirole on rotations at two doses was significantly different compared to vehicle (P < 0.01).

(−)-**8b** was more efficacious in producing rotation compared to (−)-**9b** (total of 5866 vs 2653 rotations for (−)-**8b** vs (−)-**9b**, respectively). Peak effect of both compounds was reached at 7.5 h. This is an indication of long duration of action of both compounds in producing contralateral rotation. When tested at a lower doses (5 μmol/kg), both compounds, (−)-**8b** (3.28 mg/kg) and (−)-**9b** (4.64 mg/kg), produced a lower total number of rotations (3333 and 1839 for (−)-**8b** and (−)-**9b**, respectively) than at the 10 μmol/kg dose. The rotation in this case lasted for more than 7 h (Figure 4). Interestingly, both compounds produced initial increase of rotational activity followed by a brief decrease of activity before exhibiting a steady increase of rotational activity. At present, the reason for such biphasic activity is unknown. At both tested doses (−)-**8b** was more efficacious than (−)-**9b** in the rotation test, just as in the locomotor activity study with reserpinized rats (Figure 3). The reference drug Ropinirole at a higher dose (10 μmol/kg) exhibited much shorter duration of action. As we have reported in the Supporting Information section, pretreatment studies with the potent DA receptor antagonist haloperidol demonstrated block of the production of rotation by our hybrid D_2/D_3 agonist, indicating site-specific interaction at the target D_2/D_3 receptor sites.

Neuroprotection against MPP+ Toxicity. The dose-dependent effect of treatment of **1a** and (−)-**9b** in reversing the toxicity of MPP+ to dopaminergic MN9D cells is demonstrated in Figure 5. From our previous dose−effect experiment with MPP+, we chose 100 μM of MPP+ which can induce 50−60% cell death, for our study.[55] To test whether **1a** and (−)-**9b** can protect dopaminergic MN9D cells from MPP+ induced toxicity, the cells were pretreated with various concentrations of (20, 10, 5, 1, 0.1, 0.01, and 0.001 μM) of either **1a** or (−)-**9b** for 1 h and then cotreated with 100 μM MPP+ for an additional 24 h. The data from the MTT assay indicated that both **1a** and (−)-**9b** are able to protect the MN9D cells in a dose-dependent manner. For **1a**, significant protection from toxicity of MPP+ was conferred by 1, 5, 10, and 20 μM doses, and this result correlates well with in vivo neuroprotection result that we published earlier.[45] For (−)-**9b**, significant neuroprotection was conferred at 5 and 10 μM doses. It seems **1a** is relatively more potent and efficacious than (−)-**9b** in this neuroprotection assay. Interestingly, (−)-**8b** did not show any neuroprotection when the assay was carried out under identical condition (see Supporting Information).

CONCLUSION

In this paper, we describe an SAR study based on our earlier lead molecule **1a**, with some highly potent agonist molecules for D_2 and D_3 receptors with enhanced blood brain barrier crossing ability compared to the parent molecule **1a**. SAR results have demonstrated that hydroxyl derivatives of **1a** have higher affinity for the D_3 receptor. In both binding and functional assays, compound (−)-**9b** exhibited the highest selectivity for D_3 over D_2 receptors. Lead molecules also exhibited potent free radical quenching property, indicating their antioxidant property. Furthermore, lead molecules were tested in two PD animal models and compared with parent molecule **1a**. Compounds (−)-**9b** and (−)-**8b** exhibited significant, long-lasting reversal of hypolocomotion in reserpinized rats; on the other hand, **1a** was efficacious in this model only if dissolved in 10% BHCD solution. Similarly, in 6-OHDA animal model studies, compounds (−)-**8b** and (−)-**9b** produced extensive rotational activity with long duration of action. In vitro neuroprotection experiments with dopaminergic MN9D cells treated with **1a** and (−)-**9b** indicated protection from toxicity of MPP+.

EXPERIMENTAL SECTION

Reagents and solvents were purchased from commercial suppliers and used as received unless otherwise indicated. Dry solvent was obtained according to the standard procedure. All reactions were performed under inert atmosphere (N_2) unless otherwise noted. Analytical silica gel 60 F254-coated TLC plates were obtained from EMD Chemicals, Inc. and were visualized with UV light or by treatment with phosphomolybdic acid (PMA), Dragendorff's reagent, or ninhydrin. Flash column chromatographic purifications were performed using Whatman Purasil 60A silica gel 230−400 mesh. The proton nuclear magnetic resonance (^{1}H NMR) spectra were measured on a Varian 400 MHz FT NMR spectrometer using tetramethylsilane (TMS) as an internal standard. The NMR solvent used was $CDCl_3$ or CD_3OD as indicated. Optical rotations were recorded on Perkin-Elmer 241 polarimeter. Melting points were recorded using MEL-TEMP II

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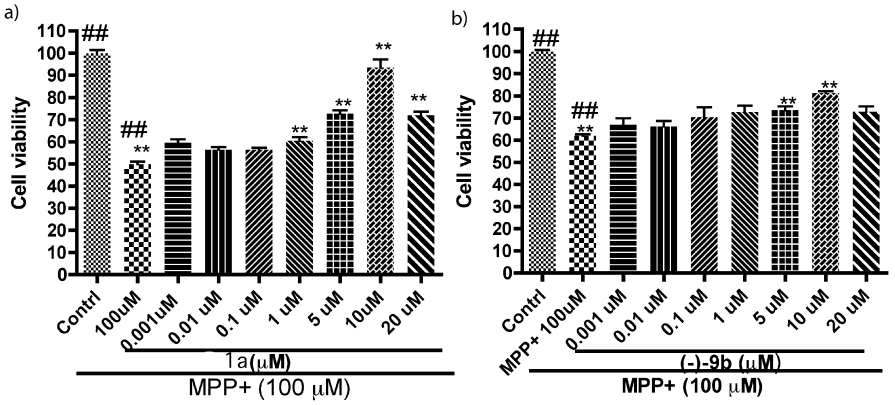

Figure 5. Dose-dependent effect of combination of pretreatment followed by cotreatment of **1a** and (−)-**9b** with 100 μM MPP+ on cell viability of MN9D cells from toxicity of 100 μM MPP+. (A, B) MN9D cells were pretreated with different doses of **1a** and (−)-**9b** for 1 h followed by cotreatment with 100 μM MPP+ for 24 h. The values shown are means ± SDs of three independent experiments performed in 4−6 replicates. One-way ANOVA analysis followed by Tukey's multiple comparison post hoc test were performed (**p < 0.01 compared to the MPP+ group. ##p < 0.001 compared to the control group).

(Laboratory Devices Inc., USA) capillary melting point apparatus and were uncorrected. Elemental analyses were performed by Atlantic Microlab, Inc.

***t*-Butyl 4-(4-Iodophenyl)piperazine-1-carboxylate (1).** Into a stirring solution of 1-phenylpiperazine (21.8 g, 134.0 mmol) in acetic acid/water (3:1, 42 mL), a suspension of iodine monochloride (24.0 g, 148.0 mmol) in acetic acid/water (3:1, 42 mL) was added at 55 °C. The reaction was stirred at 55 °C for 1 h and then at room temperature for another 1 h. The solution was poured into 400 mL of crushed ice, and the pH was adjusted to 13 with 4 N NaOH. The product was then extracted with dichloromethane (3 × 100 mL). The combined organic layer was dried over Na_2SO_4, filtered, and evaporated in vacuo to provide the free amine of compound **1** as a pale yellow solid (28.69 g, 74%) which was converted to *t*-Boc derivative without further purification.

Into a stirring solution of this amine (28.0 g, 97.17 mmol) in dichloromethane (80 mL), $(Boc)_2O$ (25.44 g, 116.60 mmol) and Et_3N (35.26 mL, 252.64 mmol) were added at room temperature. The reaction mixture was stirred at the same temperature for 12 h and was extracted with CH_2Cl_2 (3 × 100 mL), washed with water, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude material was purified by column chromatography over silica gel (hexane/EtOAc, 9.0:1.0) to give compound **1** (34.70 g, 92%). ^{1}H ($CDCl_3$, 400 MHz): δ1.48 (s, 9 H), 3.10 (t, J = 4.8 Hz, 4H), 3.56 (t, J = 4.8 Hz, 4H), 6.68 (d, J = 8.8 Hz, 2H), 7.53 (d, J = 9.2 Hz, 2H).

Procedure A. *t*-Butyl 4-(4′-methoxybiphenyl-4-yl)piperazine-1-carboxylate (3a). A suspension of (4-methoxyphenyl)boronic acid **2a** (2.34 g, 15.49 mmol), iodo compound **1** (6.01 g, 15.49 mmol), Na_2CO_3 (3.28 g, 30.98 mmol, 2 M solution in water), and $Pd(PPh_3)_4$ (875 mg, 0.75 mmol) in dimethoxy ethane/ethanol (1:1) was refluxed for 1 h. The solvents were removed in vacuo, and the crude product was purified by flash chromatography using the solvent system hexane/ethyl acetate (4.0:1.0) to yield compound **3a** (3.82 g, 67%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.49 (s, 9H), 3.17 (t, J = 4.8 Hz, 4H), 3.61 (t, J = 4.8 Hz, 4H), 3.85 (s, 3H), 6.96 (d, J = 8.8 Hz, 2H), 6.98 (d, J = 8.8 Hz, 2H), 7.48 (d, J = 8.8 Hz, 2H), 7.49 (d, J = 8.8 Hz, 2H).

***t*-Butyl 4-(3′-Methoxybiphenyl-4-yl)piperazine-1-carboxylate (3b).** Commercially available (3-methoxyphenyl)boronic acid, **2b** (4.60 g, 30.44 mmol) was reacted with iodo compound **1** (11.81 g, 30.44 mmol), Na_2CO_3 (6.45 g, 60.88 mmol, 2 M solution in water), and $Pd(PPh_3)_4$ (1.16 g, 1.01 mmol) in dimethoxy ethane/ethanol (46 mL:46 mL) as followed in procedure **A** to yield compound **3b** (6.95 g, 62% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.49 (s, 9H), 3.18 (t, J = 4.0 Hz, 4H), 3.59 (t, J = 4.4 Hz, 4H), 3.85 (s, 3H), 6.82 (dd, J = 8.0 Hz, 1.6 Hz, 1H), 6.99 (d, J = 8.0 Hz, 2H), 7.09 (bs, 1H), 7.15 (d, J = 7.2 Hz, 1H), 7.32 (t, J = 7.8 Hz, 1H), 7.52 (d, J = 8.0 Hz, 2H).

***t*-Butyl-4-(2′-methoxybiphenyl-4-yl)piperazine-1-carboxylate (3c).** Commercially available (2-methoxyphenyl)boronic acid, **2c** (2.10 g, 13.90 mmol) was reacted with iodo compound **1** (5.4 g, 13.90 mmol), Na_2CO_3 (2.94 g, 27.80 mmol, 2 M solution in water), and $Pd(PPh_3)_4$ (560 mg, 0.484 mmol) in dimethoxy ethane/ethanol (20 mL/20 mL) by following procedure **A** to yield compound **3c** (3.58 g, 70% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.50 (s, 9H), 3.19 (t, J = 4.8 Hz, 4H), 3.60 (t, J = 4.8 Hz, 4H), 3.81 (s, 3H), 6.96−7.04 (m, 4H), 7.25−7.32 (m, 2H), 7.48 (d, J = 9.2 Hz, 2H).

***t*-Butyl 4-([1,1′-Biphenyl]-4-yl)piperazine-1-carboxylate (3d).** Commercially available benzeneboronic acid, **2d** (2.5 g, 20.48 mmol), was reacted with iodo compound **1** (7.95 g, 20.48 mmol), Na_2CO_3 (4.34 g, 40.96 mmol, 2 M solution in water), and $Pd(PPh_3)_4$ (1.18 g, 1.02 mmol) in dimethoxy ethane/ethanol (25 mL/25 mL) by following procedure **A** to yield compound **3d** (1.74 g, 80%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.49 (s, 9H), 3.07 (bs, 4H), 3.61 (t, J = 4.8 Hz, 4H), 6.96 (d, J = 8.0 Hz, 2H), 7.23 (d, J = 7.2 Hz, 1H), 7.41 (t, J = 8.0 Hz, 2H), 7.49 (d, J = 8.0 Hz, 2H), 7.52 (d, J = 7.2 Hz, 2H).

Procedure B. 1-(4′-Methoxy-biphenyl-4-yl)piperazine (4a). Into a stirring solution of compound **3a** (3.4 g, 9.23 mmol) in CH_2Cl_2 (30 mL), TFA (20 mL) was added slowly at room temperature, and the reaction mixture was stirred for 4 h. Unreacted TFA and solvent CH_2Cl_2 were removed in vacuo, and the salt formed was washed with diethyl ether. Saturated solution of sodium bicarbonate was added to the salt, and it was extracted with dichloromethane (50 × 3 mL). The combined organic layer was dried over Na_2SO_4, filtered, and evaporated in vacuo to provide the compound **4a** (2.22 g, 90%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.63 (bs, 1H); 3.06 (t, J = 4.4 Hz, 4H); 3.19 (t, J = 4.6 Hz, 4H), 3.84 (s, 3H); 6.95 (d, J = 8.4 Hz, 2H), 6.98(d, J = 8.8 Hz, 2H), 7.47 (d, J = 8.0 Hz, 2H), 7.49 (d, J = 8.0 Hz, 2H).

1-(3′-Methoxy-biphenyl-4-yl)piperazine (4b). Compound **3b** (4.6 g, 12.5 mmol) was reacted with TFA (30 mL) in CH_2Cl_2 (20 mL) by following procedure **B** to give compound **4b** (3.34 g, 99%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.07 (t, J = 4.8 Hz, 4H), 3.21 (t, J = 7.2 Hz, 4H), 3.85 (s, 3H), 6.83 (dd, J = 2.4 Hz, 8.0 Hz 1H), 6.98 (d, J = 8.8 Hz, 2H), 7.09 (t, J = 2.8 Hz, 1H), 7.15 (d, J = 7.2 Hz, 1H), 7.32 (t, J = 8.2 Hz, 1H), 7.52 (d, J = 8.8 Hz, 2H).

1-(2′-Methoxy-biphenyl-4-yl)piperazine (4c). Compound **3c** (3.4 g, 9.23 mmol) was reacted with TFA (15 mL) in CH_2Cl_2 (20 mL) by following procedure **B** to give compound **4c** (2.47 g, 99%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.08 (bs, 4H); 3.23 (bs, 4H), 3.81 (s, 3H); 6.93−7.06 (m, 4H), 7.25−7.35 (m, 2H), 7.40−7.49 (m, 2H).

1-(1,1′-Biphenyl]-4-yl)piperazine (4d). Compound **3d** (1.7 g, 5.02 mmol) was reacted with TFA (10 mL) in CH_2Cl_2 (20 mL) by following procedure **B** to give compound **4d** (2.18 g, 90%). ^{1}H NMR ($CDCl_3$, 400 MHz): 3.18 (t, J = 6.8 Hz, 4H), 3.28 (t, J = 4.8 Hz, 4H), 6.98 (d, J = 8.0 Hz, 2H), 7.27 (d, J = 6.4 Hz, 1H), 7.41 (t, J = 8.0 Hz, 2H), 7.52 (d, J = 8.0 Hz, 2H), 7.58 (d, J = 7.2 Hz, 2H).

Procedure C. 1-(2-(*t*-Butyldimethylsilyloxy)ethyl)-4-(4′-methoxybiphenyl-4-yl)piperazine (5a). A mixture of compound **4a** (1.5 g, 5.59 mmol), (2-bromo-ethyl)-*tert*-butyldimethylsilane (1.57 g, 6.56 mmol), and K_2CO_3 (2.27 g, 16.44 mmol) in CH_3CN (30 mL) was refluxed for 14 h. Acetonitrile was evaporated under vacuo and the crude material was purified by silica gel column chromatography

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(hexane/EtOAc, 1:4) to give compound **5a** (1.90 g, 80%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.09 (s, 6H), 0.92 (s, 9H), 2.61 (t, J = 6.0 Hz, 2H), 2.72 (t, J = 5.0 Hz, 4H), 3.24 (t, J = 4.80 Hz, 4H), 3.80 (t, J = 6.4 Hz, 2H), 3.83 (s, 3H), 6.95 (d, J = 9.2 Hz, 2H), 6.98(d, J = 8.8 Hz, 2H), 7.46 (d, J = 8.8 Hz, 2H), 7.48 (d, J = 8.4 Hz, 2H).

1-(2-(*t*-Butyldimethylsilyloxy)ethyl)-4-(3′-methoxybiphenyl-4-yl)piperazine (5b). Compound **4b** (3.20 g, 11.94 mmol), was reacted with (2-bromo-ethyl)-*tert*-butyldimethylsilane (3.42 g, 14.32 mmol), and K_2CO_3 (4.94 g, 35.74 mmol) in CH_3CN (80 mL) by following the procedure **C** to furnish **5b** (4.06 g, 80%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.06 (s, 6H), 0.90 (s, 9H), 2.49 (t, J = 7.2 Hz, 2H), 2.63 (bs, 4H), 3.26 (t, J = 4.8 Hz, 4H), 3.68 (t, J = 4.0 Hz, 2H), 3.83 (s, 3H), 6.84 (dd, J = 8.0 Hz, 2.4 Hz, 1H), 6.99 (d, J = 8.8 Hz, 2H), 7.09 (t, J = 2.4 Hz, 1H), 7.15 (d, J = 8.0 Hz, 1H), 7.32 (t, J = 8.0 Hz, 1H), 7.51 (d, J = 8.0 Hz, 2H).

1-(2-(*t*-Butyldimethylsilyloxy)ethyl)-4-(2′-methoxybiphenyl-4-yl)piperazine (5c). Compound **4c** (2.20 g, 8.20 mmol) was reacted with (2-bromo-ethyl)-*tert*-butyldimethylsilane (2.34 g, 9.84 mmol), and K_2CO_3 (3.39 g, 24.60 mmol) in CH_3CN (30 mL) by following procedure **C** to afford compound **5c** (2.70 g, 80%). ^{1}H NMR ($CDCl_3$, 400 MHz): 0.06 (s, 6H), 0.90 (s, 9H), 2.58 (t, J = 5.6 Hz, 2H), 2.69 (bs, 4H), 3.24 (bs, 4H), 3.78 (t, J = 4.8 Hz, 2H), 3.83 (s, 3H), 6.95−7.06 (m, 4H), 7.24−7.27 (m, 2H), 7.44−7.46 (m, 2H).

1-([1,1′-Biphenyl]-4-yl)-4-(2-((*tert*-butyldimethylsilyl)oxy)-ethyl)piperazine(5d). Compound **4d** (2.1 g, 8.81 mmol) was reacted with (2-bromo-ethyl)-*tert*-butyldimethylsilane (2.52 g, 10.58 mmol), and K_2CO_3 (3.65 g, 26.43 mmol) in CH_3CN (30 mL) by following procedure **C** to afford compound **5d** (2.79 g, 80%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.09 (s, 6H), 0.92 (s, 9H), 2.69 (t, J = 5.6 Hz, 2H), 3.18 (t, J = 6.8 Hz, 4H), 3.30 (t, J = 4.8 Hz, 2H), 3.72 (t, J = 5.6 Hz, 4H), 6.98 (d, J = 8.0 Hz, 2H), 7.25−7.30 (m, 1H), 7.40 (t, J = 8.0 Hz, 2H), 7.51−7.56 (m, 4H).

1-(4-((*t*-Butyldimethylsilyl)oxy)butyl)-4-(3′-methoxy-[1,1′-biphenyl]-4-yl)piperazine (5e). Compound **4b** (2.0 g, 7.45 mmol) was reacted with (4-bromobutoxy)(*tert*-butyl)dimethylsilane (2.38 g, 8.94 mmol), and K_2CO_3 (3.08 g, 22.35 mmol) in CH_3CN (40 mL) by following procedure **C** to afford compound **5e** (2.80 g, 85%).^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.09 (s, 6H), 0.92 (s, 9H), 1.62 (t, J = 8.0 Hz, 4H), 2.41 (t, J = 7.2 Hz, 2H), 2.62 (t, J = 7.6 Hz, 4H), 3.27 (t, J = 6.8 Hz, 4H), 3.64 (t, J = 7.2 Hz, 2H), 3.85 (s, 3H), 6.85 (dd, J = 1.6 Hz, 8.0 Hz, 1H), 6.99 (d, J = 8.8 Hz, 2H), 7.09 (bs, 1H), 7.15 (d, J = 8.0 Hz, 1H), 7.32 (t, J = 8.0 Hz, 1H), 7.51 (d, J = 8.0 Hz, 2H).

Procedure D. 2-(4-(4′-Methoxybiphenyl-4-yl)piperazin-1-yl)-ethanol (6a). Into a stirring solution of compound **5a** (1.5 g, 3.52 mmol) in anhydrous THF (30 mL), *n*-tetrabutylammonium fluoride (0.92 g, 3.52 mmol, 1.0 M solution in THF) was added at 0 °C. The reaction mixture was then stirred at room temperature for 1.5 h. THF was evaporated in vacuo, and the residue was diluted with CH_2Cl_2 (50 mL) and washed with water. The water layer was extracted with CH_2Cl_2 (3 × 75 mL). The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated in vacuo. The crude product was purified by silica gel column chromatography (EtOAc) to yield compound **6a** (1.04 g, 95%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.62 (t, J = 5.2 Hz, 2H), 2.70 (t, J = 4.8 Hz, 4H), 3.25 (t, J = 4.8 Hz, 4H), 3.67 (t, J = 5.4 Hz, 2H), 3.83 (s, 3H), 6.95 (d, J = 9.2 Hz, 2H), 6.98 (d, J = 8.0 Hz, 2H), 7.46 (d, J = 8.0 Hz, 2H), 7.48 (d, J = 9.2 Hz, 2H).

2-(4-(3′-Methoxybiphenyl-4-yl)piperazin-1-yl)ethanol (6b). Compound **5b** (4.0 g, 9.39 mmol) was reacted with *n*-tetrabutylammonium fluoride (2.44 g, 9.39 mmol, 1.0 M solution in THF) in anhydrous THF (100 mL) by following procedure **D** to yield compound **6b** (2.62 g, 90%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.61 (t, J = 5.2 Hz, 2H), 2.69 (t, J = 4.0 Hz, 4H), 3.25 (t, J = 4.8 Hz, 4H), 3.67 (t, J = 6.0 Hz, 2H), 3.83 (s, 3H), 6.83 (dd, J = 8.0 Hz, 2.4 Hz, 1H), 6.99 (d, J = 8.0 Hz, 2H), 7.09 (bs, 1H), 7.15 (d, J = 7.2 Hz, 1H), 7.31 (t, J = 8.2 Hz, 1H), 7.51 (d, J = 8.0 Hz, 2H).

2-(4-(2′-Methoxybiphenyl-4-yl)piperazin-1-yl)ethanol (6c). Compound **5c** (2.5 g, 5.87 mmol) was reacted with *n*-tetrabutylammonium fluoride (1.53 g, 5.87 mmol, 1.0 M solution in THF) in anhydrous THF (50 mL) by following procedure **D** to yield compound **6c** (1.57 g, 86%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.66 (t, J = 5.4 Hz, 2H), 2.74 (t, J = 4.0 Hz, 4H), 3.29 (t, J = 5.0 Hz, 4H), 3.70 (t, J = 5.6 Hz, 2H), 3.83 (s, 3H), 6.95−7.02 (m, 4H), 7.27−7.31 (m, 2H), 7.46 (d, J = 8.8 Hz, 2H).

2-(4-([1,1′-Biphenyl]-4-yl)piperazin-1-yl)ethanol (6d). Compound **5d** (2.5 g, 6.30 mmol) was reacted with *n*-tetrabutylammonium fluoride (1.65 g, 6.30 mmol, 1.0 M solution in THF) in THF (50 mL) by following procedure **D** to yield compound **6d** (1.50 g, 80%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.69 (t, J = 8.0 Hz, 4H), 2.78 (t, J = 4.0 Hz, 2H), 3.30 (t, J = 4.8 Hz, 4H), 3.72 (t, J = 5.6 Hz, 2H), 6.97−7.02 (m, 2H), 7.25−7.30 (m, 1H), 7.38−7.42 (m, 2H), 7.51−7.56 (m, 4H).

4-(4-(3′-Methoxy-[1,1′-biphenyl]-4-yl)piperazin-1-yl)butan-1-ol (6e). Compound **5e** (2.6 g, 5.72 mmol) was reacted with *n*-tetrabutylammonium fluoride (1.50 g, 5.72 mmol, 1.0 M solution in THF) in THF (40 mL) by following procedure **D** to yield compound **6e** (1.40 g, 72%). ^{1}H NMR ($CDCl_3$, 400 MHz): 1.62 (t, J = 8.0 Hz, 4H), 2.41 (t, J = 7.2 Hz, 2H), 2.62 (t, J = 7.6 Hz, 4H), 3.27 (t, J = 6.8 Hz, 4H), 3.64 (t, J = 7.2 Hz, 2H), 3.85 (s, 3H), 6.85 (dd, J = 1.6 Hz, 8.0 Hz, 1H), 6.99 (d, J = 8.8 Hz, 2H), 7.09 (bs, 1H), 7.15 (d, J = 8.0 Hz, 1H), 7.32 (t, J = 8.0 Hz, 1H), 7.51 (d, J = 8.0 Hz, 2H).

Procedure E. 2-(4-(4′-Methoxybiphenyl-4-yl)piperazin-1-yl)-acetaldehyde (7a). Into a stirred solution of oxalyl chloride (0.324 mL, 2.56 mmol) in CH_2Cl_2 (40 mL) at −78 °C, DMSO (0.40 mL, 5.12 mmol) was added. The reaction mixture was stirred for 10 min followed by addition of compound **6a** (400 mg, 1.28 mmol, dissolved in 5 mL of CH_2Cl_2). The reaction mixture was stirred at the same temperature for 15 min. Then Et_3N (0.78 mL, 7.68 mmol) was added next, and stirring was continued for another 1 h and 20 min while allowing the reaction mixture to reach at room temperature. The reaction mixture was quenched by addition of water and extracted with CH_2Cl_2 (3 × 25 mL). The combined organic layer was washed with brine and concentrated to yield the compound **7a** (321 mg, 81%), which was used without purification in the next step.

2-(4-(3′-Methoxybiphenyl-4-yl)piperazin-1-yl)acetaldehyde (7b). Compound **6b** (2.6 g, 8.32 mmol) was reacted with oxalyl chloride (1.43 mL, 16.65 mmol), DMSO (2.36 mL, 33.28 mmol), and Et_3N (6.91 mL, 49.92 mmol) in dichloromethane (50 mL) by following procedure **E** to yield compound **7b** (2.18 g, 85%).

2-(4-(2′-Methoxybiphenyl-4-yl)piperazin-1-yl)acetaldehyde (7c). Compound **6c** (500 mg, 1.60 mmol) was reacted with oxalyl chloride (0.41 mL, 3.20 mmol), DMSO (0.50 mL, 6.40 mmol), and Et_3N (0.97 mL, 9.60 mmol) in dichloromethane (40 mL) by following procedure **E** to yield compound **7c** (372 mg, 75%).

2-(4-([1,1′-Biphenyl]-4-yl)piperazin-1-yl)acetaldehyde (7d). Compound **6d** (1.3 g, 4.60 mmol) was reacted with oxalyl chloride (0.79 mL, 9.21 mmol), DMSO (1.30 mL, 18.4 mmol), and Et_3N (3.82 mL, 27.60 mmol) in dichloromethane (40 mL) by following procedure **E** to yield compound **7d** (1.0 g, 78%).

4-(4-(3′-Methoxy-[1,1′-biphenyl]-4-yl)piperazin-1-yl)butanal (7e). Compound **6e** (1.2 g, 3.52 mmol) was reacted with oxalyl chloride (0.60 mL, 7.05 mmol), DMSO (1.00 mL, 14.08 mmol), and Et_3N (2.92 mL, 21.12 mmol) in dichloromethane (30 mL) by following procedure **E** to yield compound **7e** (0.89 g, 75%).

Procedure F. *N*6-(2-(4-(4′-Methoxybiphenyl-4-yl)piperazin-1-yl)ethyl)-*N*6-propyl-4,5,6,7-tetrahydrobenzo[*d*]thiazole-2,6-diamine (±)-(8a). Into a stirring solution of compound **7a** (321 mg, 1.03 mmol) in CH_2Cl_2 (10 mL), (±)-pramipexole (219 mg, 1.03 mmol) was added at room temperature. The reaction mixture was stirred for 1 h, and then $NaBH(OAc)_3$ (393 mg, 1.85 mmol) was added into the reaction mixture. After the reaction was stirred for 48 h, a saturated solution of $NaHCO_3$ was added into the reaction mixture, and it was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layer was washed with brine and finally purified by silica gel column chromatography (EtOAc/MeOH, 9:1) to yield compound (±)-**8a** (313 mg, 60%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.91 (t, J = 7.2 Hz, 3H), 1.52−1.56 (m, 2H), 1.76−1.79 (m, 1H), 2.06 (d, J = 8.8 Hz, 1H), 2.59−2.80 (m, 13H), 3.17−3.26 (m, 6H), 3.84 (s, 3H), 6.91−7.01(m, 4H), 7.42−7.49 (m, 4H). The product was converted into corresponding hydrochloride salt, m.p. 268 °C. Anal. ($C_{29}H_{39}N_5OS$·4.0HCl·2.0H_2O): C, H, N.

***N*6-(2-(4-(3′-Methoxybiphenyl-4-yl)piperazin-1-yl)ethyl)-*N*6-propyl-4,5,6,7-tetrahydrobenzo[*d*]thiazole-2,6-diamine ((±)-8b).** Compound **7b** (250 mg, 0.80 mmol) was reacted with (±)-pramipexole

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(169 mg, 0.80 mmol) and $NaBH(OAc)_3$ (305.19 mg, 1.44 mmol) in dichloromethane (30 mL) by following procedure **F** to yield compound (±)-**8b** (263 mg, 65%).^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.89 (t, J = 7.2 Hz, 3H), 1.46−1.51 (m, 2H), 1.63−1.77 (m, 1H), 1.90 (d, J = 11.6 Hz, 1H), 2.42−2.73 (m, 13H), 3.06−3.26 (m, 6H), 3.84 (s, 3H), 5.05 (bs, 2H), 6.82 (d, J = 8.0 Hz, 1H), 6.96 (d, J = 8.4 Hz, 2H), 7.08 (bs, 1H), 7.14 (d, J = 7.6 Hz, 1H), 7.31 (t, J = 8.0 Hz, 1H), 7.50 (d, J = 8.4 Hz, 2H). The product was converted into corresponding hydrochloride salt, m.p. 255 °C. Anal. ($C_{29}H_{39}N_5OS$·4.0 HCl·1.0 H_2O): C, H, N.

(*S*)-*N*6-(2-(4-(3′-Methoxybiphenyl-4-yl)piperazin-1-yl)ethyl)-*N*6-propyl-4,5,6,7-tetrahydrobenzo[*d*]thiazole-2,6-diamine ((−)-8b). Compound **7b** (500 mg, 1.61 mmol) was reacted with (−)pramipexole (340.24 mg, 1.61 mmol) and $NaBH(OAc)_3$ (612.50 mg, 2.89 mmol) in dichloromethane (100 mL) by following procedure **F** to yield compound (−)-**8b** (526 mg, 65%). $[\alpha]^{25}_D$ = −34.6 (c = 1, CH_3OH). Spectral data matching with compound (±)-**8b**. The product was converted into corresponding hydrochloride salt, m.p. 245 °C. Anal. ($C_{29}H_{39}N_5OS$·4.0 HCl·1.0H_2O): C, H, N.

(*R*)-*N*6-(2-(4-(3′-Methoxybiphenyl-4-yl)piperazin-1-yl)ethyl)-*N*6-propyl-4,5,6,7-tetrahydrobenzo[*d*]thiazole-2,6-diamine ((+)-8b). Compound **7b** (100 mg, 0.322 mmol) was reacted with (+)-pramipexole (68.04 mg, 0.322 mmol) and $NaBH(OAc)_3$ (122.84 mg, 0.579 mmol) in dichloromethane (20 mL) by following procedure **F** to yield compound (+)-**8b** (105 mg, 65%). Spectral data matching with compound (±)-**8b**.

***N*6-(2-(4-(2′-Methoxybiphenyl-4-yl)piperazin-1-yl)ethyl)-*N*6-propyl-4,5,6,7-tetrahydrobenzo[*d*]thiazole-2,6-diamine ((±)-8c).** Compound **7c** (372 mg, 1.19 mmol) was reacted with (±)-pramipexole (251.48 mg, 1.19 mmol) and $NaBH(OAc)_3$ (453.60 mg, 2.14 mmol) in dichloromethane (40 mL) by following procedure **F** to yield compound (±)-**8c** (391 mg, 65%).^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.89 (t, J = 7.2 Hz, 3H), 1.42−1.50 (m, 2H), 1.66−1.77 (m, 1H), 1.99 (d, J = 10.8 Hz, 1H), 2.41−2.76 (m, 13H), 3.02−3.27 (m, 6H), 3.79 (s, 3H), 4.83 (bs, 2H), 6.94−6.97 (m, 2H), 6.99 (dd, J = 1.2 Hz, J = 7.6 Hz, 2H), 7.24−7.31 (m, 2H), 7.43−7.46 (m, 2H). The product was converted into corresponding hydrochloride salt, m.p. 255 °C. Anal. ($C_{29}H_{39}N_5OS$·4.0 HCl·1.0$CH_3COOCH_2CH_3$): C, H, N. MS (ES+): m/z calculated for $C_{29}H_{39}N_5OS$ [M + H^+]: calculated 505.29; found 506.56.

***N*6-(4-(4-(3′-Methoxy-[1,1′-biphenyl]-4-yl)piperazin-1-yl)-butyl)-*N*6-propyl-4,5,6,7-tetrahydrobenzo[*d*]thiazole-2,6-diamine ((±)-8d).** Compound **7e** (600 mg, 1.77 mmol) was reacted with (±)-pramipexole (375 mg, 1.77 mmol) and $NaBH(OAc)_3$ (675 mg, 3.18 mmol) in dichloromethane (25 mL) by following procedure **F** to yield compound (±)-**8d** (586 mg, 62%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.89 (t, J = 7.2 Hz, 3H), 1.39−1.74 (m, 6H), 2.01−2.21 (m, 1H), 2.44−2.68 (m, 13H), 3.11−3.26 (m, 6H), 3.46 (s, 1H), 3.84 (s, 3H), 4.91 (bs, 2H), 6.82 (dd, J = 2.4 Hz, 8.0 Hz, 1H), 6.97 (d, J = 8.8 Hz, 2H), 7.08 (bs, 1H), 7.14 (d, J = 8.0 Hz, 1H), 7.31 (t, J = 7.6 Hz, 1H), 7.50 (d, J = 8.8 Hz, 2H).

Procedure G. 4′-(4-(2-((2-Amino-4,5,6,7-tetrahydrobenzo[*d*]-thiazol-6-yl)(propyl)amino)ethyl)piperazin-1-yl)-[1,1′-biphenyl]-4-ol ((±)-9a). Into a stirring solution of compound (±)-**8a** (60 mg, 0.11 mmol) in anhydrous CH_2Cl_2 (10 mL) at −78 °C, boron tribromide (1.1 mL, 1.1 mmol, 1 M solution in CH_2Cl_2) was added. The reaction mixture was allowed to come to room temperature and was stirred for 48 h. The reaction was quenched by addition of saturated $NaHCO_3$ solution, and the mixture was extracted with CH_2Cl_2. The combined organic layer was dried over Na_2SO_4 and evaporated under a vacuum, and the crude product was purified by flash chromatography (CH_2Cl_2/MeOH = 9:1) to afford compound (±)-**9a** (0.029 g, 50%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.96 (t, J = 7.2 Hz, 3H), 1.60−1.64 (m, 2H), 1.81−1.85 (m, 1H), 2.08 (d, J = 7.2 Hz, 1H), 2.52−3.04 (m, 13H), 3.11−3.24 (m, 6H), 6.81(d, J = 8.4 Hz, 2H), 7.00 (d, J = 8.8 Hz, 2H), 7.38 (d, J = 8.4 Hz, 2H), 7.43 (d, J = 8.8 Hz, 2H). The product was converted into corresponding hydrochloride salt, m.p. 272 °C. Anal. ($C_{28}H_{37}N_5OS$·4.0 HCl·1.0H_2O): C, H, N.

4′-(4-(2-((2-Amino-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)-(propyl)amino)-ethyl)piperazin-1-yl)-[1,1′-biphenyl]-3-ol ((±)-9b). A mixture of compound (±)-**8b** (70 mg, 0.13 mmol) in anhydrous CH_2Cl_2 (10 mL) at −78 °C, boron tribromide (1.04 mL, 1.04 mmol, 1 M solution in CH_2Cl_2) was added as followed by procedure **G** to yield (±)-**9b** (40 mg, 60%). The product was converted into corresponding hydrochloride salt, m.p. 265 °C. ^{1}H NMR of HCl salt ($CDCl_3$, 400 MHz): δ 1.07 (t, J = 7.2 Hz, 3H), 1.89−1.95 (m, 2H), 2.13−2.21 (m, 1H), 2.49 (d, J = 12.0 Hz, 1H) 2.80 (m, 2H), 2.96−3.32 (m, 4H), 3.38−3.99 (m, 13H), 6.71 (dd, J = 8.0 Hz, 1.6 Hz, 1H), 6.98 (bs, 1H), 7.03 (d, J = 7.2 Hz, 1H), 7.13−7.24 (m, 3H), 7.55 (d, J = 8.8 Hz, 2H). Anal. ($C_{28}H_{37}N_5OS$·4.0 HCl·1.0 H_2O): C, H, N. MS (ES+): m/z calculated for $C_{28}H_{37}N_5OS$ [M + H^+]: calculated 491.27; found 492.52.

Procedure H. (*S*)-4′-(4-(2-((2-Amino-4,5,6,7-tetrahydrobenzo-[*d*]thiazol-6-yl)(propyl)amino)ethyl)piperazin-1-yl)biphenyl-3-ol ((−)-9b). Compound (−)-**8b** (200 mg, 0.98 mmol) and 48% aqueous HBr (15 mL) was refluxed for 12 h. The reaction mixture was then evaporated to dryness in vacuo. The crude mixture was then washed with diethylether and finally recrystallized from ethanol to afford compound (−)-**9b** (246 mg, 70%, recrystallized from ethanol). ^{1}H NMR of HBr salt (CD_3OD, 400 MHz): δ 1.08 (t, J = 7.2 Hz, 3H), 1.94−2.06 (m, 2H), 2.22−2.27 (m, 1H), 2.55 (d, J = 10.4 Hz, 1H) 2.83 (m, 2H), 2.99−3.39 (m, 4H), 3.52−4.20 (m, 13H), 6.73 (dd, J = 8.0 Hz, 1.6 Hz, 1H), 7.00 (bs, 1H), 7.04 (d, J = 7.6 Hz, 1H), 7.20−7.24 (m, 3H), 7.57 (d, J = 8.8 Hz, 2H). $[\alpha]^{25}_D$ = −21.0 (c = 0.5, CH_3OH). Hydrobromide salt, m.p. 270 °C. Anal. ($C_{28}H_{37}N_5OS$·5.0 HBr): C, H, N.

(*R*)-4′-(4-(2-((2-Amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)ethyl)piperazin-1-yl)biphenyl-3-ol ((+)-9b). Compound (+)-**8b** (100 mg, 0.20 mmol) and 48% aqueous HBr (10 mL) was refluxed for 12 h by following procedure **H** to afford compound (+)-**9b** (105 mg, 60%, recrystallized from ethanol). Spectral data matching with compound (−)-**9b**. $[\alpha]^{25}_D$ = +16.0 (c = 0.5, CH_3OH). Hydrobromide salt, m.p. 270 °C. Anal. ($C_{28}H_{37}N_5OS$·5.0 HBr·1.0H_2O): C, H, N. m/z calculated for $C_{28}H_{37}N_5OS$ [M + H^+]: calculated 491.27; found 492.27.

4′-(4-(2-((2-Amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)-(propyl)amino)ethyl)-piperazin-1-yl)biphenyl-2-ol ((±)-9c). Into the mixture of compound (±)-**8c** (100 mg, 0.197 mmol) in anhydrous CH_2Cl_2 (10 mL) at −78 °C, boron tribromide (1.38 mL, 1.38 mmol, 1 M solution in CH_2Cl_2) was added as followed by procedure **G** to yield (±)-**9c** (53 mg, 55%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.90 (t, J = 7.2 Hz, 3H), 1.45−1.51 (m, 2H), 1.66−1.77 (m, 1H), 1.93−2.02 (m, 1H), 2.62−3.12 (m, 13H), 3.16−3.66 (m, 6H), 6.84−6.89 (m, 2H), 7.01 (d, J = 8.8 Hz, 2H), 7.07−7.11 (m, 1H), 7.20 (dd, J = 1.2 Hz, J = 7.6 Hz, 1H),), 7.47(d, J = 8.4 Hz, 2H). The product was neutralized and converted into corresponding hydrochloride salt, m.p. 270 °C. Anal. ($C_{28}H_{37}N_5OS$·4.0 HCl·2.0 H_2O): C, H, N.

4′-(4-(4-((2-Amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)-(propyl)amino)-butyl)piperazin-1-yl)-[1,1′-biphenyl]-3-ol ((±)-9d). Compound (±)-**8d** (200 mg, 0.38 mmol) and 48% aqueous HBr (5 mL) was refluxed for 12 h using procedure **H** to afford compound (±)-**9d** (248 mg, 72%, recrystallized from ethanol). ^{1}H NMR of HBr salt (CD_3OD, 400 MHz): δ 1.05 (t, J = 7.2 Hz, 3H), 1.86−2.16 (m, 6H), 2.45 (bs, 1H), 2.79 (bs, 2H), 2.96−3.94 (m, 18H), 6.73 (dd, J = 2.4 Hz, 8.0 Hz, 1H), 7.00 (bs, 1H), 7.04 (d, J = 8.0 Hz, 1H), 7.20−7.24 (m, 3H), 7.57 (t, J = 7.2 Hz, 2H). m.p.245 °C. Anal. ($C_{30}H_{41}N_5OS$·5.0HBr·2.0H_2O·1.0 $C_2H_5OC_2H_5$): C, H, N. MS (ES+): m/z calculated for $C_{30}H_{41}N_5OS$ [M + H^+]: calculated 519.30; found 520.59.

(*S*)-5-Methoxy-*N*-(2-(4-(3′-methoxybiphenyl-4-yl)piperazin-1-yl)ethyl)-*N*-propyl-1,2,3,4-tetrahydronaphthalen-2-amine (−)(10). Compound **7b** (350 mg, 1.13 mmol) was reacted with (*S*)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (247.83 mg, 1.13 mmol) and $NaBH(OAc)_3$ (430.23 mg, 2.03 mmol) in dichloromethane (60 mL) by following procedure **F**. The crude product was purified by silica gel column chromatography (EtOAc/hexane, 3:2) to yield compound (−)-**10** (347 mg, 60%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.90 (t, J = 7.2 Hz, 3H), 1.46−1.64 (m, 3H), 2.04−2.25 (m, 1H); 2.51−3.28 (m, 19H), 3.74 (s, 3H); 3.81 (s, 3H), 6.65 (d, J = 8.0 Hz, 1H); 6.71 (d, J = 7.6 Hz, 1H); 6.82−6.86 (m, 1H), 7.0 (d, J = 8.4 Hz, 2H); 7.07−7.15 (m, 3H), 7.31 (t, J = 8.0 Hz, 1H); 7.50 (d, J = 8.4 Hz, 2H).

(S)-6-((2-(4-(3′-Hydroxybiphenyl-4-yl)piperazin-1-yl)ethyl)-(propyl)amino)-5,6,7,8-tetrahydronaphthalen-1-ol (−)(11). Compound (−)-**10** (300 mg, 0.58 mmol) and 48% aqueous HBr (15 mL) was refluxed for 10 h by following procedure **H** to afford compound (−)-**11** (296 mg, 70%, recrystallized from ethanol). ^{1}H NMR of HBr salt (400 MHz, CD_3OD): δ 1.04 (t, J = 7.2 Hz, 3H), 1.73−1.93 (m, 3H),

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2.30−2.69 (m, 2H), 3.07−3.83 (m, 18 H), 6.60 (d, J = 5.2 Hz, 1H), 6.69 (d, J = 6.4 Hz, 1H), 6.73 (d, J = 7.6 Hz, 1H), 6.96−7.04 (m, 3H), 7.19−7.23 (m, 3H), 7.56 (d, J = 7.2 Hz, 2H). $[\alpha]^{25}_{D}$ = −41.0 (c = 1.0, CH_3OH). Hydrobromide salt, m.p. 290 °C. Anal. ($C_{31}H_{39}N_3O_2S$·3.0 HBr·2.0 H_2O): C, H, N.

Procedure I. 1-(2-Methoxyphenyl)piperazine (13). A stirring solution of 2-methoxyaniline **12** (31.60 g, 256.91 mmol) and bis-(2-chloroethyl)amine (45.85 g, 256.91 mmol) was heated at 150 °C in diethylene glycol monomethyl ether (100 mL) for 6 h. After being cooled to room temperature, the mixture was dissolved in MeOH (4 mL) followed by addition of Et_2O (300 mL). The precipitate was filtered off and washed with Et_2O to provide HCl salt. The HCl salt was further converted to free amine by treatment with Na_2CO_3 solution and extracted with EtOAc (2 × 100 mL). The combined organic layers were dried over Na_2SO_4, and concentrated in vacuo to provide the pure free amine product **13** (34.34 g, 70%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 3.12 (t, J = 7.6 Hz, 4H), 3.37 (t, J = 6.4 Hz, 4H), 3.79 (s, 3H), 6.86 (t, J = 7.6 Hz, 1.6 Hz, 1H), 6.93 (t, J = 4.8 Hz, 2H), 6.94−7.07 (m, 1H).

Procedure J. 1-(4-Bromo-2-methoxyphenyl)piperazine (14). Amine **13** (15.0 g, 78.07 mmol) was dissolved in CH_2Cl_2 (200 mL) and cooled to 0 °C. Bromine (4.02 mL, 78.07 mmol) was added dropwise into the above solution. After 2 h, reaction mixture was washed with 1 N sodium hydroxide, and the organic layer was separated, dried over Na_2SO_4, and concentrated in vacuo to yield **14** (16.86 g, 80% yield). The crude product **14** thus obtained was converted into its *t*-Boc derivative without further purification.

Procedure K. *t*-Butyl 4-(4-bromo-2-methoxyphenyl)-piperazine-1-carboxylate (15). Into a stirring solution of amine **14** (14.0 g, 51.84 mmol) in dichloromethane (40 mL), $(Boc)_2O$ (11.31 g, 51.84 mmol) and Et_3N (21.55 mL, 155.52 mmol) were added at room temperature. The reaction mixture was stirred at the same temperature for 12 h and was extracted with CH_2Cl_2 (3 × 100 mL), washed with water, dried over Na_2SO_4, filtered, and concentrated. The crude material was purified by column chromatography over silica gel (hexane/EtOAc, 8:2) to give compound (**15**) (16.30 g, 85%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.49 (s, 9H), 2.95 (t, J = 4.4 Hz, 4H), 3.58 (t, J = 5.2 Hz, 4H), 3.86 (s, 3H), 6.75 (d, J = 8.4 Hz, 1H), 6.97 (d, J = 2.0 Hz, 1H), 7.58 (dd, J = 8.4 Hz, 2.0 Hz, 1H).

***t*-Butyl 4-(3-Methoxybiphenyl-4-yl)piperazine-1-carboxylate (16).** Commercially available, phenylboronic acid (2.63 g, 21.61 mmol) was reacted with bromo compound **15** (8.0 g, 21.61 mmol), Na_2CO_3 (4.58 g, 43.22 mmol, 2 M solution in water), and $Pd(PPh_3)_4$ (1.24 g, 0.75 mmol) in dimethoxy ethane/ethanol (30 mL:30 mL) by follwing procedure **A** to yield compound **16** (5.17 g, 65% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.49 (s, 9H), 3.04 (t, J = 4.4 Hz, 4H), 3.63 (t, J = 4.8 Hz, 4H), 3.92 (s, 3H), 6.96 (d, J = 8.0 Hz, 1H), 7.08 (d, J = 2.0.0 Hz, 1H), 7.15 (dd, J = 8.4 Hz, 2.0 Hz, 1H), 7.29−7.32 (m, 1H), 7.41 (t, J = 7.6 Hz, 2H), 7.56 (d, J = 7.20 Hz, 2H).

1-(3-Methoxy-[1,1′-biphenyl]-4-yl)piperazine (17). Compound **16** (6.2 g, 16.83 mmol) was reacted with TFA (20 mL) in CH_2Cl_2 (20 mL) by following procedure **B** to give compound **17** (4.0 g, 90%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.77 (bs, 1H), 3.08 (t, J = 4.8 Hz, 4H), 3.18 (t, J = 5.6 Hz, 4H), 3.92 (s, 3H), 6.84 (dd, J = 2.4 Hz, 8.0 Hz, 1H), 6.94 (d, J = 8.0 Hz, 1H), 6.98 (t, J = 4.8 Hz, 1H), 7.29−7.34 (m, 1H), 7.44 (t, J = 7.2 Hz, 2H), 7.56 (d, J = 7.6 Hz, 2H).

1-(2-((*t*-Butyldimethylsilyl)oxy)ethyl)-4-(3-methoxy-[1,1′-biphenyl]-4-yl)piperazine (18). Compound **17** (3.5 g, 13.05 mmol) was reacted with (2-bromo-ethyl)-*tert*-butyldimethylsilane (3.74 g, 15.66 mmol), and K_2CO_3 (4.14 g, 39.15 mmol) in CH_3CN (50 mL) by following procedure **C**. The crude residue was purified by column chromatography (ethylacetate/hexane, 2:3) to afford compound **18** (5.0 g, 90%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.08 (s, 6H), 0.90 (s, 9H), 2.53 (t, J = 6.4 Hz, 2H), 3.08 (t, J = 4.8 Hz, 4H), 3.18 (t, J = 5.6 Hz, 4H), 3.26 (t, J = 4.8 Hz, 2H), 3.92 (s, 3H), 6.84 (dd, J = 8.0 Hz, 2.4 Hz, 1H), 6.94 (d, J = 8.0 Hz, 1H), 6.98 (t, J = 4.8 Hz, 1H), 7.29−7.34 (m, 1H), 7.44 (t, J = 7.2 Hz, 2H), 7.56 (d, J = 7.6 Hz, 2H).

2-(4-(3-Methoxybiphenyl-4-yl)piperazin-1-yl)ethanol (19). Compound **18** (4.5 g, 11.72 mmol) was reacted with *n*-tetrabutylammonium fluoride (3.06 g, 11.72 mmol, 1.0 M solution in THF) in THF (30 mL) by following procedure **D**. The crude product was purified by silica gel column chromatography (EtOAc/MeOH, 9:1) to yield compound **19** (2.25 g, 76%). ^{1}H NMR ($CDCl_3$, 400 MHz): ^{1}H NMR ($CDCl_3$, 400 MHz): 2.65 (t, J = 5.6 Hz, 2H), 2.76 (bs, 4H), 3.15 (bs, 4H), 3.67(t, J = 5.6 Hz, 2H), 3.93 (s, 3H), 6.84 (d, J = 2.6 Hz, 1H), 7.08 (d, J = 2.4 Hz, 1H), 7.14 (dd, J = 8 Hz, 2.4 Hz, 1H), 7.31 (t, 7.2 Hz, 1H), 7.42 (t, J = 7.2 Hz, 2H), 7.56 (d, J = 7.6 Hz, 2H).

2-(4-(3-Methoxybiphenyl-4-yl)piperazin-1-yl)acetaldehyde (20). Compound **19** (500 mg, 1.60 mmol) was reacted with oxalyl chloride (0.28 mL, 3.20 mmol), DMSO (0.45 mL, 6.40 mmol) and Et_3N (1.33 mL, 9.6 mmol) in dry dichloromethane (30 mL) by following procedure **E**. The crude product was purified by silica gel column chromatography (EtOAc/MeOH, 9.5:0.5) to yield compound **20** (397 mg, 80%).

***N*6-(2-(4-(3-Methoxybiphenyl-4-yl)piperazin-1-yl)ethyl)-*N*6-propyl-4,5,6,7-tetrahydrobenzo[*d*]thiazole-2,6-diamine (±)(21).** Compound **20** (350 mg, 1.12 mmol) reacted with (±)-pramipexole (238 mg, 1.12 mmol) and $NaBH(OAc)_3$ (427 mg, 2.01 mmol) in dichloromethane (10 mL) by following procedure **F**. The crude product was purified by silica gel column chromatography (EtOAc/MeOH, 9:1) to yield compound (±)-**21** (370 mg, 65%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.89 (t, J = 7.2 Hz, 3H), 1.45−1.59 (m, 2H), 1.70−1.75 (m, 2H), 1.98−2.04 (m, 1H), 2.49−3.15 (m, 18H), 3.93 (s, 3H), 4.70 (s, 2H), 6.9 (d, J = 8.4 Hz, 1H), 7.07 (bs, 1H), 7.13−7.17 (m, 1H), 7.28−7.33 (m, 1H), 7.42 (t, J = 7.2 Hz, 2H), 7.56 (d, J = 8.0 Hz, 2H).

4-(4-(2-((2-Amino-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)-(propyl)amino)ethyl)-piperazin-1-yl)-[1,1′-biphenyl]-3-ol (±)-(22). A mixture of compound (±)-**21** (200 mg, 0.395 mmol) and 48% aqueous HBr (10 mL) was refluxed for 12 h by following procedure **H** to afford compound (±)-**22** (281 mg, 80%). ^{1}H NMR of free base ($CDCl_3$, 400 MHz): δ 0.90 (t, J = 7.2 Hz, 3H), 1.45−1.59 (m, 2H), 1.68−1.75 (m, 2H), 2.0 (d, J = 10.0 Hz, 1H), 2.48−3.05 (m, 18H), 4.75 (bs, 2H), 7.10 (d, J = 8.0 Hz, 1H), 7.16−7.23 (m, 2H), 7.31−7.34 (m, 1H), 7.42 (t, J = 8.0 Hz, 2H), 7.55 (d, J = 8.0 Hz, 2H).The product was converted into corresponding hydrochloride salt, m.p. 270 °C. Anal. ($C_{28}H_{37}N_5OS$·4.0 HCl·2.0 H_2O): C, H, N.

Procedure L. *N*-(2-(4-([1,1′-Biphenyl]-4-yl)piperazin-1-yl)-ethyl)-*N*-propyl-1,4-dioxaspiro[4.5]decan-8-amine (24a). Into a stirring solution of compound **7d** (750 mg, 2.55 mmol) in $ClCH_2CH_2Cl$ (40 mL), amine **23** (510 mg, 2.55 mmol), $NaBH(OAc)_3$ (973 mg, 4.59 mmol), and HOAc (153 mg, 2.55 mmol) were added at room temperature. After the reaction was stirred for 48 h, a saturated solution of $NaHCO_3$ was added into the reaction mixture, and it was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layer was washed with brine and finally purified by silica gel column chromatography (EtOAc/MeOH, 9:1) to yield (670 mg, 60%) of compound **24a**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.90 (t, J = 6.8 Hz, 3H), 1.46−1.50 (m, 2H), 1.69−1.79 (m, 2H), 2.04−2.07 (m, 2H), 2.38−2.55 (m, 8H), 2.65−2.70 (m, 6H), 3.01 (t, J = 7.2 Hz, 1H), 3.26 (t, J = 4.4 Hz, 4H), 3.92 (s, 4H), 6.98 (d, J = 8.4 Hz, 2H), 7.27 (d, J = 7.6 Hz, 1H), 7.41 (t, J = 8.0 Hz, 2H), 7.51 (d, J = 8.8 Hz, 2H), 7.55 (d, J = 8.0 Hz, 2H).

***N*-(2-(4-(3′-Methoxy-[1,1′-biphenyl]-4-yl)piperazin-1-yl)-ethyl)-*N*-propyl-1,4-dioxaspiro[4.50]decan-8-amine (24b).** Into a stirring solution of compound **7b** (700 mg, 2.25 mmol) in $ClCH_2CH_2Cl$ (40 mL), amine **23** (450 mg, 2.25 mmol), $NaBH(OAc)_3$ (858 mg, 4.05 mmol), and HOAc (135 mg, 2.25 mmol) were added at room temperature using procedure **L** to yield (710 mg, 70%) of compound **24b**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.89 (t, J = 7.2 Hz, 3H), 1.52−1.62 (m, 2H), 1.79−1.82 (m, 4H), 2.59−2.82 (m, 15H), 3.24 (t, J = 4.8 Hz, 4H), 3.88 (s, 3H), 3.93 (s, 4H), 6.82 (dd, J = 1.2 Hz, 8.4 Hz, 1H), 6.97 (d, J = 8.8 Hz, 2H), 7.08 (t, J = 2.4 Hz, 1H), 7.13−7.15 (m, 1H), 7.30 (t, J = 8.0 Hz, 1H), 7.50 (d, J = 7.2 Hz, 2H).

Procedure M. 4-((2-(4-([1,1′-Biphenyl]-4-yl)piperazin-1-yl)-ethyl)(propyl)amino)-cyclohexanone (25a). A solution of ketal **24a** (600 mg, 1.29 mmol) in THF (50 mL) and 1 N HCl (10 mL) was stirred at 80 °C under N_2 for 2 h. THF was removed under vacuo, and saturated $NaHCO_3$ solution was added slowly. The mixture was extracted with CH_2Cl_2 (4 × 100 mL), and the combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated to give the crude product, which was purified by silica gel column chromatography (EtOAc/MeOH, 9:2) to yield (490 mg, 90%) of compound **25a**. ^{1}H NMR

($CDCl_3$, 400 MHz): δ 0.89 (t, J = 7.6 Hz, 3H), 1.46−1.60 (m, 2H), 1.80−1.83 (m, 2H), 2.04−2.37 (m, 2H), 2.39−2.55 (m, 8H), 2.65−2.70 (m, 6H), 3.15 (t, J = 7.2 Hz, 1H), 3.25 (t, J = 4.4 Hz, 4H), 6.98 (d, J = 8.4 Hz, 2H), 7.28 (d, J = 7.2 Hz, 1H), 7.40 (t, J = 8.0 Hz, 2H), 7.51 (d, J = 7.2 Hz, 2H), 7.55 (d, J = 8.8 Hz, 2H).

4-((2-(4-(3′-Methoxy-[1,1′-biphenyl]-4-yl)piperazin-1-yl)-ethyl)(propyl)amino)-cyclohexanone (25b). A solution of ketal **24b** (700 mg, 1.41 mmol) in THF (50 mL) and 1 N HCl (10 mL) was stirred at 80 °C under N_2 for 2 h followed by procedure **M** to yield (540 mg, 85%) of compound **25b**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.89 (t, J = 7.2 Hz, 3H), 1.45−1.52 (m, 2H), 1.82−1.91 (m, 2H), 2.04−2.12 (m, 2H), 2.30−2.52 (m, 8H), 2.66−2.70 (m, 6H), 3.15 (t, J = 7.2 Hz, 1H), 3.67 (t, J = 6.0 Hz, 4H), 3.85 (s, 3H), 6.83 (m, 1H), 6.98 (d, J = 8.8 Hz, 2H), 7.14 (bs, 1H), 7.13−7.15 (m, 1H), 7.31 (t, J = 8.0 Hz, 1H), 7.50 (d, J = 8.4 Hz, 2H).

Procedure N. *N*6-(2-(4-([1,1′-Biphenyl]-4-yl)piperazin-1-yl)-ethyl)-*N*6-propyl-5,6,7,8-tetrahydroquinazoline-2,6-diamine (26a). Into a solution of ketone **25a** (450 mg, 1.07 mmol) in dry toluene (20 mL), tris(dimethylamino)methane (780 mg, 5.36 mmol) was added, and the mixture was stirred under nitrogen at 90 °C for 4 h. The solvent was removed under vacuo, and the residue was dissolved in EtOH (50 mL). Guandine carbonate (460 mg, 2.55 mmol) was added next. The mixture was then refluxed for 17 h. The solvent was evaporated in vacuo, and the residue was diluted with CH_2Cl_2 and washed with brine. The organic layer was dried over Na_2SO_4 and evaporated to yield crude product, which was purified by purified by silica gel column chromatography (EtOAc/MeOH, 7:3) to yield (378 mg, 75%) of compound **26a**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.87 (t, J = 6.4 Hz, 3H), 1.57−1.76 (m, 3H), 2.05−2.13 (m, 1H), 2.64−2.98 (m, 15H), 3.27 (t, J = 4.4 Hz, 4H), 4.93 (s, 2H), 6.97 (d, J = 8.8 Hz, 2H), 7.28 (d, J = 7.6 Hz, 1H), 7.41 (t, J = 7.6 Hz, 2H), 7.50 (d, J = 8.4 Hz, 2H), 7.54 (d, J = 7.2 Hz, 2H), 8.07 (s, 1H). The product was converted into corresponding hydrochloride salt, m.p. 232 °C. Anal. ($C_{29}H_{38}N_6$·4.0HCl·1.0$CH_3COOCH_2CH_3$): C, H, N. MS (ES+): m/z calculated for $C_{29}H_{38}N_6$ [M + H^+]: calculated 470.32; found 471.52.

***N*6-(2-(4-(3′-Methoxy-[1,1′-biphenyl]-4-yl)piperazin-1-yl)-ethyl)-*N*6-propyl-5,6,7,8-tetrahydroquinazoline-2,6-diamine (26b).** Into a solution of ketone **25b** (500 mg, 1.11 mmol) in dry toluene (30 mL), tris(dimethylamino)methane (807 mg, 5.56 mmol) was added, and the mixture was stirred under nitrogen at 90 °C for 4 h. The solvent was removed under vacuo, and the residue was dissolved in EtOH (50 mL). Guandine carbonate (500 mg, 2.77 mmol) was added next followed by procedure **N** to yield (390 mg, 70%) of compound **26b**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.90 (t, J = 7.2 Hz, 3H), 1.45−1.51 (m, 2H), 1.67−1.75 (m, 2H), 2.04−2.12 (m, 1H), 2.50−2.93 (m, 14H), 3.24 (t, J = 4.8 Hz, 4H), 3.85 (s, 3H), 6.83 (dd, J = 2.4 Hz, 7.6 Hz, 1H), 6.97 (d, J = 8.8 Hz, 2H), 7.09 (bs, 1H), 7.14 (d, J = 8.0 Hz, 2H), 7.31 (t, J = 7.6 Hz, 1H), 7.50 (d, J = 8.8 Hz, 2H), 8.01 (bs, 2H).

4′-(4-(2-((2-Amino-5,6,7,8-tetrahydroquinazolin-6-yl)-(propyl)amino)ethyl)piperazin-1-yl)-[1,1′-biphenyl]-3-ol (27). Compound **26b** (100 mg, 0.59 mmol) and 48% aqueous HBr (10 mL) was refluxed for 8 h using procedure **H** to afford compound **27** (118 mg, 65%, recrystallized from ether). ^{1}H NMR of HBr salt (CD_3OD, 400 MHz): δ 1.08 (t, J = 7.2 Hz, 3H), 1.95−2.01 (m, 2H), 2.27−2.29 (m, 1H), 2.58−2.64 (m, 1H), 3.01−3.39 (m, 6 H), 3.48 (bs, 8H), 3.91−4.01 (m, 4H), 4.14 (bs, 1H), 6.71 (d, J = 8.0 Hz, 1H), 6.99 (bs, 1H), 7.03 (d, J = 7.6 Hz, 2H), 7.19−7.26 (m, 3H), 7.56 (d, J = 8.4 Hz, 2H), 8.67 (bs, 1H). Hydrobromide salt, m.p. 255 °C. Anal. ($C_{29}H_{38}N_6O$·6.0 HBr·3.0H_2O): C, H, N.

Procedure O. *t*-Butyl 4-(biphenylcarbonyl)piperazine-1-carboxylate (30). To a stirring solution of *t*-Boc-piperazine, **28** (1.5 g, 8.05 mmol) in THF (25 mL), 4-biphenyl carbonyl chloride, **29** (1.6 g, 7.24 mmol) and diisopropylethylamine (2.53 mL, 14.49 mmol) were added at room temperature. The reaction mixture was stirred at the same temperature for overnight and partitioned between brine and ethyacetate. The organic layer was separated and washed with brine, dried over Na_2SO_4, and concentrated. The crude material was purified by column chromatography over silica gel (hexane/EtOAc, 8.0:2.0) to give compound **30** (2.16 g, 80%). ^{1}H ($CDCl_3$, 400 MHz): δ1.47 (s, 9 H), 3.47 (bs, 4H), 3.74 (bs, 4H), 7.37 (t, J = 7.2 Hz, 1H), 7.43−7.49 (m, 4H), 7.59 (d, J = 7.2 Hz, 2H), 7.59 (d, J = 7.2 Hz, 2H).

Biphenyl-4-yl(piperazin-1-yl)methanone (31). Compound **30** (2.1 g, 5.73 mmol) was reacted with TFA (20 mL) in CH_2Cl_2 (30 mL) by following procedure **B** to give compound **31** (1.44 g, 95%). ^{1}H ($CDCl_3$, 400 MHz): 3.47 (bs, 4H), 3.74 (bs, 4H), 7.38 (t, J = 7.2 Hz, 1H), 7.44−7.49 (m, 4H), 7.59 (d, J = 7.2 Hz, 2H), 7.63 (d, J = 8.4 Hz, 2H).

Biphenyl-4-yl(4-(2-(*tert*-butyldimethylsilyloxy)ethyl)-piperazin-1-yl)methanone (32). A mixture of compound **31** (1.2 g, 4.50 mmol), was reacted with (2-bromo-ethyl)-*tert*-butyldimethylsilane (1.30 g, 5.41 mmol), and K_2CO_3 (1.86 g, 13.50 mmol) in CH_3CN (50 mL) by following the procedure **C** to furnish **32** (1.30 g, 70%).^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.05 (s, 6H), 0.88 (s, 9H), 2.51−2.62 (m, 6H), 3.47−3.54 (m, 2H), 3.76 (t, J = 5.6 Hz, 4H), 7.37 (t, J = 7.2 Hz, 1H), 7.43−7.49 (m, 4H), 7.59 (d, J = 7.2 Hz, 2H), 7.62 (d, J = 8.0 Hz, 2H).

Biphenyl-4-yl(4-(2-hydroxyethyl)piperazin-1-yl)methanone (33). Compound **32** (1.2 g, 2.82 mmol) was reacted with *n*-tetrabutylammonium fluoride (0.8 g, 2.82 mmol, 1.0 M solution in THF) in anhydrous THF (20 mL) by following procedure **D** to yield compound **33** (790 mg, 90%). ^{1}H NMR ($CDCl_3$, 400 MHz): 2.50−2.61 (m, 6H), 3.46−3.54 (m, 2H), 3.65 (t, J = 4.8 Hz, 4H), 7.37 (t, J = 8.8 Hz, 1H), 7.43−7.49 (m, 4H), 7.58 (d, J = 7.2 Hz, 2H), 7.62 (d, J = 8.0 Hz, 2H).

2-(4-(Biphenylcarbonyl)piperazin-1-yl)acetaldehyde (34). Compound **33** (500 mg, 1.61 mmol) was reacted with oxalyl chloride (0.29 mL, 3.22 mmol), DMSO (0.46 mL, 6.44 mmol), and Et_3N (1.33 mL, 9.66 mmol) in dichloromethane (20 mL) by following procedure **E** to yield compound **34** (420 mg, 85%).

(4-(2-((2-Amino-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)-(propyl)amino)ethyl)-piperazin-1-yl)(biphenyl-4-yl)-methanone(±)(35). Compound **34** (400 mg, 1.29 mmol) was reacted with (±)-pramipexole (275 mg, 1.29 mmol) and $NaBH(OAc)_3$ (492.12 mg, 2.32 mmol) in dichloromethane (20 mL) using procedure **F** to yield compound (±)-**35** (420 mg, 70%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.86 (t, J = 7.2 Hz, 3H), 1.41−1.46 (m, 2H), 1.57−1.70 (m, 1H), 1.95 (d, J = 11.6 Hz, 1H), 2.40−2.70 (m, 13H), 3.01−3.06 (m, 1H), 3.48 (bs, 2H), 3.78 (bs, 2H), 4.02−4.12 (m, 1H), 4.96 (bs, 2H), 7.35 (t, J = 7.2 Hz, 1H), 7.41−7.47 (m, 4H), 7.56−7.61 (m, 4H). The product was converted into corresponding hydrochloride salt, m.p. 255 °C. Anal. ($C_{29}H_{37}N_5OS$·5.0 HCl·1.0 $C_2H_5OC_2H_5$): C, H, N.

Evaluation of Antioxidant Activity. DPPH Radical Scavenging Assay. To a 96-well plate, an amount of 100 μL of drug solutions (dissolved in methanol) ranging from 20 to 250 μM was added. Next 100 μL of 200 μM methanolic solution of 1,1-DPPH was added, and the plate was shaken vigorously at 30 °C for 25 min. Control wells received 100 μL of methanol and 100 μL of 200 μM methanolic DPPH solution. Wells containing only 200 μL of methanol served as a background correction. The change in absorbance of all samples and standard (ascorbic acid) was measured at 517 nm. Radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula: % scavenging activity = (absorbance of control − absorbance of sample)/ (absorbance of control)] × 100.

Animal Experiments. Drugs and Chemicals. The following commercially available drugs were used in the experiment: reserpine hydrochloride (Alfa Aesar), Ropinirole (Sigma Aldrich). The hydrochloride salts of (−)-**8b** and hydrobromide salt of (−)-**9b** were dissolved in water for both locomotor and 6-OH-DA rotational experiments. Reserpine was dissolved in 10−25 μL of glacial acetic acid and further diluted with 5.5% glucose solution. All compounds for this study were administered in a volume of 0.1−0.2 mL for subcutaneous administration and 0.5−0.7 mL for intraperitoneal administration into each rat.

Animals. In rodent studies, animals were male Sprague−Dawley rats from Harlan (Indianapolis, IN) weighing 220−225 g unless otherwise specified. The lesioned rats (290−320 g) were purchased from Charles River (Rensselaer, NY), and their unilateral lesion was checked twice by apomorphine challenge following the surgery. Animals were maintained in sawdust-lined cages in a temperature and humidity controlled environment at 22 ± 1 °C and 60 ± 5%, respectively, with a 12 h light/dark cycle, with lights on from 6:00 a.m. to 6:00 p.m. They were group-housed with unrestricted access to food and water. All experiments were

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performed during the light component. All animal use procedures were in compliance with the Wayne State University Animal Investigation Committee consistent with AALAC guidelines.

Reversal of Reserpine-Induced Hypolocomotion in Rats. Administration of reserpine induces catalepsy in rodents primarily by blocking the vesicular monoamine transporter (VMAT) which helps in the internalization of monoamines into vesicles, resulting in metabolism of unprotected monoamines in the cytosol that ultimately causes depletion of monoamines in the synapse of the peripheral sympathetic nerve terminals. The ability of the compound (−)-**8b**, (−)-**9b**, **1a** and ropinirole to reverse the reserpine induced hypolocomotion was investigated. Prior to administration of reserpine animals were anaesthetized using isoflurane. Reserpine (5.0 mg/kg, sc) or saline (sc) was administered 18 h before the injection of drug or vehicle (ip). The rats were placed individually in chambers for 1 h for acclimatization purposes before the administration of the test drug, standard drug, or vehicle. Immediately after administration of drug or vehicle, animals were individually placed in VersaMax animal activity monitor chamber (45 cm 30 cm 20 cm) (AccuScan Instruments, Inc. Columbus, OH) to start measuring locomotor activity. Locomotion was monitored for 6 h. Consecutive interruption of two infrared beams situated 24 cm apart and 4 cm above the cage floor in the monitor chamber recorded movement. The data were presented as horizontal counts (HACTV). The effect of the individual doses of drugs on locomotor activity was compared with respect to saline treated controls (mean ± SEM). The data were analyzed by one way analysis of variance (ANOVA) followed by Dunnett's post hoc test. The effect was considered significant if the difference from control group was observed at $p < 0.05$.

In Vivo Rotational Experiment with 6-OH-DA Lesioned Rats. In the first 14 days post lesion challenge with apomorphine was done with lesioned animals to observe a complete rotation session post administration. 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 of the medial forebrain bundle in the brain, and the coordinates used from Bregma are the following: AP, −4.3; ML, p1.2; DV, −8.3. The rotations produced upon agonist challenge were clockwise. In this study, apomorphine was also used as a reference compound. The test drugs were dissolved in saline. The drugs (−)-**8b**, **1a**, and (−)-**9b** were administered i.p. The rotations were measured over 7−12 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 Windows software program. 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 GraphPad (version 4, San Diego, CA) program. All drugs produced contralateral rotations in all lesioned rats, which lasted over 3−10 h.

Cell Culture and Treatments. The hybridoma dopaminergic MN9D cells are derived from the somatic infusion of rostral mesencephalic neurons from embryonic C57BL/6J (E14) mice with N18TG2 mouse cells. They were cultured in T-75 flask (Greiner Bio One, Frickenhausen, Germany) coated with 1 mg/mL poly-L-lysine and maintained in DMEM (high glucose with phenol red) supplemented with 10% Fecal Clone III serum, penicillin (50 units/mL), and streptomycin (50 μg/mL) at 37 °C under 5% CO_2 atmosphere. Stock solution of **1a** and (−)-**9b** were prepared in DMSO and stored at −20 °C for the period of experiments. MN9D cells were pretreated with various concentrations of drugs for 1 h and then cotreated with 100 μM MPP+ (prepared freshly before addition from a stock solution in DMSO stored at −20 °C) for 24 h. The control cells were treated with the above medium having 0.01% DMSO only.

Assessment of Cell Viability. To evaluate the neuroprotection ability of the test compounds in the presence of the neurotoxins MPP+, the quantitative and colorimetric MTT (3−4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazoliumbromide) tetrazolium salt assay was used to assess cell viability. MN9D cells were seeded into poly-L-lysine coated 96-well plates at 1×10^4 cells/well in 100 μL medium. After the plate was equilibrated for 40 h, old medium was taken out from each well, and 160 μL of fresh medium (containing 0.01% DMSO) was added to control wells and wells which were to be treated with MPP+. A solution of 160 μL of **1a** or (−)-**9b** in the above medium without DMSO in 20, 10, 5, 1, 0.1, 0.01, 0.001 μM were added to wells which would be cotreated with MPP+. The plate was incubated for 1 h at 37 °C under 5% CO_2 atmosphere. At the end of incubation, required amount of MPP+ was added to each well (except the control wells) to maintain a final concentration of 100 μM. The plate was then incubated for 24 h at 37 °C under 5% CO_2 atmosphere. Next, 20 μL of MTT stock solution (prepared in Dulbecco's phosphate-buffered saline) was added to each well to maintain a final concentration of 0.5 mg/mL, and the plate was incubated for another 3 h at 37 °C under 5% CO_2 atmosphere. Next, the plate was centrifuged at 1500 rpm for 10 min, and the supernatants were removed carefully. The formazan crystals were dissolved in 100 μL of a 1:1 mixture of DMSO/methanol solution by shaking gently at 400 rpm for 30 min at room temperature on a Thermomix R shaker (Eppendorf, Hamburg, Germany). Then, the absorbance was measured at 570 nM and 690 nM using an Epoch microplate reader (BioTek, Winooski, VT, USA). Background corrected values (570−690 nM) were used to plot the graph. Data from at least three experiments were analyzed using GraphPad software (Version 4, San Diego, USA).

DA D_2 AND D_3 RECEPTOR ASSAYS

Binding potency was monitored by inhibition of [^{3}H]spiroperidol (16.2 Ci/mmol, Perkin-Elmer) binding to dopamine rD_2 and rD_3 receptors expressed in HEK-293 cells, in a buffer containing 0.9% NaCl under conditions corresponding to our "high [radioligand] protocol" as described by us previously.[40,56] Observed IC_{50} values were converted to inhibition constants (K_i) by the Cheng−Prusoff equation (see ref 39).[39] Functional activity of test compounds in activating dopamine hD_2 and hD_3 receptors expressed in CHO cells was measured by stimulation of [^{35}S]GTPγS (1250 Ci/mmol, Perkin-Elmer) binding in comparison to stimulation by the full agonist dopamine as described by us previously.[40]

ASSOCIATED CONTENT

Supporting Information

Additional in vitro and in vivo data for the lead molecules and the elemental analysis report for all final targets are included. This material is available free of charge via the Internet at http://pubs.acs.org

AUTHOR INFORMATION

Corresponding Author

*Address: Department of Pharmaceutical Sciences Eugene Applebaum College of Pharmacy & Health Sciences, Rm# 3128 Detroit, MI 48202. Tel: 1-313-577-1064. Fax: 1-313-577-2033. E-mail: adutta@wayne.edu.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

GTPγS, guanosine 5′-[g-thio]triphosphate; 5-OH-DPAT, 5-hydroxy-2-(dipropylamino)tetralin; CHO, chinese hamster ovary; HEK, human embryonic kidney; L-DOPA, (*S*)-(3,4-

dihydroxyphenyl) alanine; DPPH, 1,1-diphenyl-2-picryl-hydrazyl; i.p., intraperitoneal; PD, Parkinson's disease; DA, dopamine; s.c., subcutaneous

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