

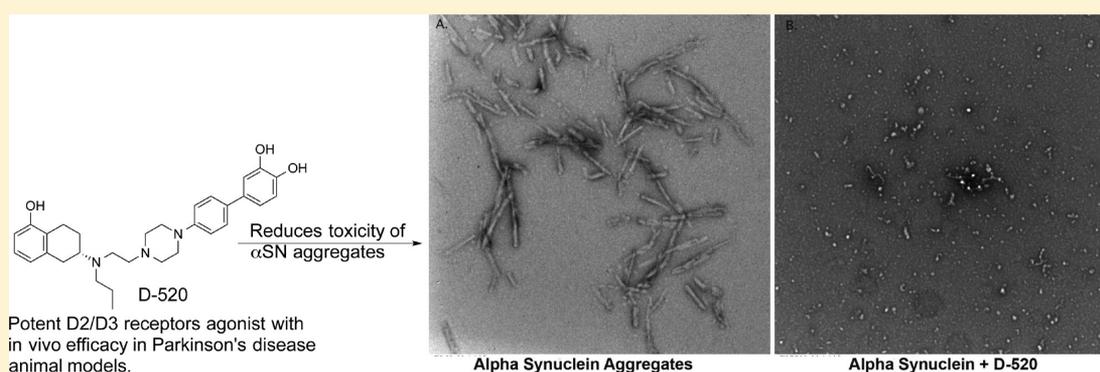
# Multifunctional D2/D3 Agonist D-520 with High in Vivo Efficacy: Modulator of Toxicity of Alpha-Synuclein Aggregates

Gyan Modi,<sup>†</sup> Chandrashekhar Voshavar,<sup>†</sup> Sanjib Gogoi,<sup>†</sup> Mrudang Shah,<sup>†</sup> Tamara Antonio,<sup>‡</sup> Maarten E. A. Reith,<sup>‡,§</sup> and Alope K. Dutta<sup>†,\*</sup>

<sup>†</sup>Department of Pharmaceutical Sciences, Wayne State University, Detroit, Michigan 48202, United States

<sup>‡</sup>Department of Psychiatry and <sup>§</sup>Department of Biochemistry and Molecular Pharmacology, New York University, New York, New York 10016, United States

## S Supporting Information



**ABSTRACT:** We have developed a series of dihydroxy compounds and related analogues based on our hybrid D2/D3 agonist molecular template to develop multifunctional drugs for symptomatic and neuroprotective treatment for Parkinson's disease (PD). The lead compound (–)-24b (D-520) exhibited high agonist potency at D2/D3 receptors and produced efficacious activity in the animal models for PD. The data from thioflavin T (ThT) assay and from transmission electron microscopy (TEM) analysis demonstrate that D-520 is able to modulate aggregation of alpha-synuclein ( $\alpha$ SN). Additionally, coinubation of D-520 with  $\alpha$ SN is able to reduce toxicity of preformed aggregates of  $\alpha$ SN compared to control  $\alpha$ SN alone. Finally, in a neuroprotection study with dopaminergic MN9D cells, D-520 clearly demonstrated the effect of neuroprotection from toxicity of 6-hydroxydopamine. Thus, compound D-520 possesses properties characteristic of multifunctionality conducive to symptomatic and neuroprotective treatment of PD.

**KEYWORDS:** Parkinson's disease, D2/D3 agonist, alpha-synuclein, neuroprotection, multifunctional drug

Parkinson's disease (PD) is a multifactorial progressive neurological disorder that results from the degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) region of the brain.<sup>1</sup> PD approximately affects 1–2% of people older than 65 years of age. Some of the common symptoms associated with PD include rigidity, bradykinesia, resting tremors, postural instability, and cognitive psychiatric complications.<sup>2,3</sup> The etiology of PD is not completely understood yet; however, recent research in the PD area has provided more insights into the basic pathogenetic factors of PD such as aggregation of alpha-synuclein ( $\alpha$ SN) protein, oxidative stress, and the presence of iron.<sup>4,5</sup> Pathological hallmarks of PD are characterized by the presence of  $\alpha$ SN aggregates called Lewy bodies (LBs) or Lewy neuritis (LN).<sup>6,7</sup>

The oxidative stress can potentially induce mitochondrial-derived reactive oxygen species, which in turn inhibit mitochondrial respiration and promote the aggregation of  $\alpha$ SN protein.<sup>4,8,9</sup> This also may lead to formation of LBs and LN.<sup>4</sup> Currently, it is not known how the aggregation of  $\alpha$ SN

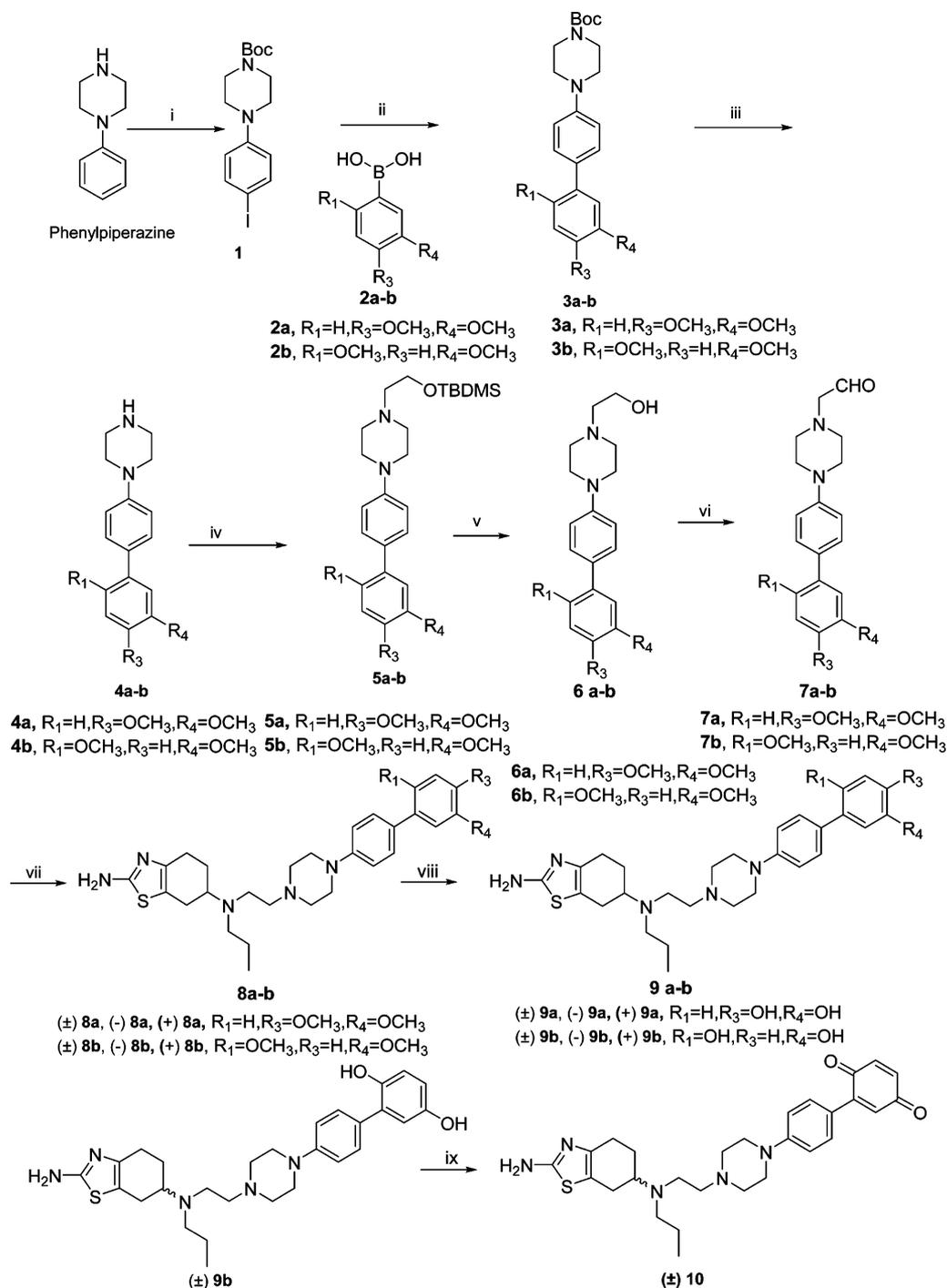
stimulates cell death. Elevated level of  $\alpha$ SN can also damage various cellular organelles.<sup>10</sup> Furthermore, increased human  $\alpha$ SN expression in transgenic flies (*Drosophila*) is associated with earlier onset of PD-like demise of dopaminergic neurons, including formation of inclusions bodies and locomotor dysfunction.<sup>11</sup>  $\alpha$ SN is expressed at a high level in the brain, but it is also found, without known functions, in erythrocytes and platelets.<sup>10</sup>  $\alpha$ SN is composed of 140 amino acids, and it can form an  $\alpha$ -helical structure upon lipid binding and thioflavin T (ThT) positive  $\beta$ -sheets upon mechanical shaking.<sup>10</sup> The middle part of  $\alpha$ SN, consisting of 61–95 amino acids, includes extremely hydrophobic residues and is known as NAC (non-A $\beta$  component of Alzheimer's disease amyloid), which is responsible for conversion of  $\alpha$ SN into  $\beta$ -sheets on prolonged periods of incubation.<sup>12</sup> Both wild type and mutants of  $\alpha$ SN

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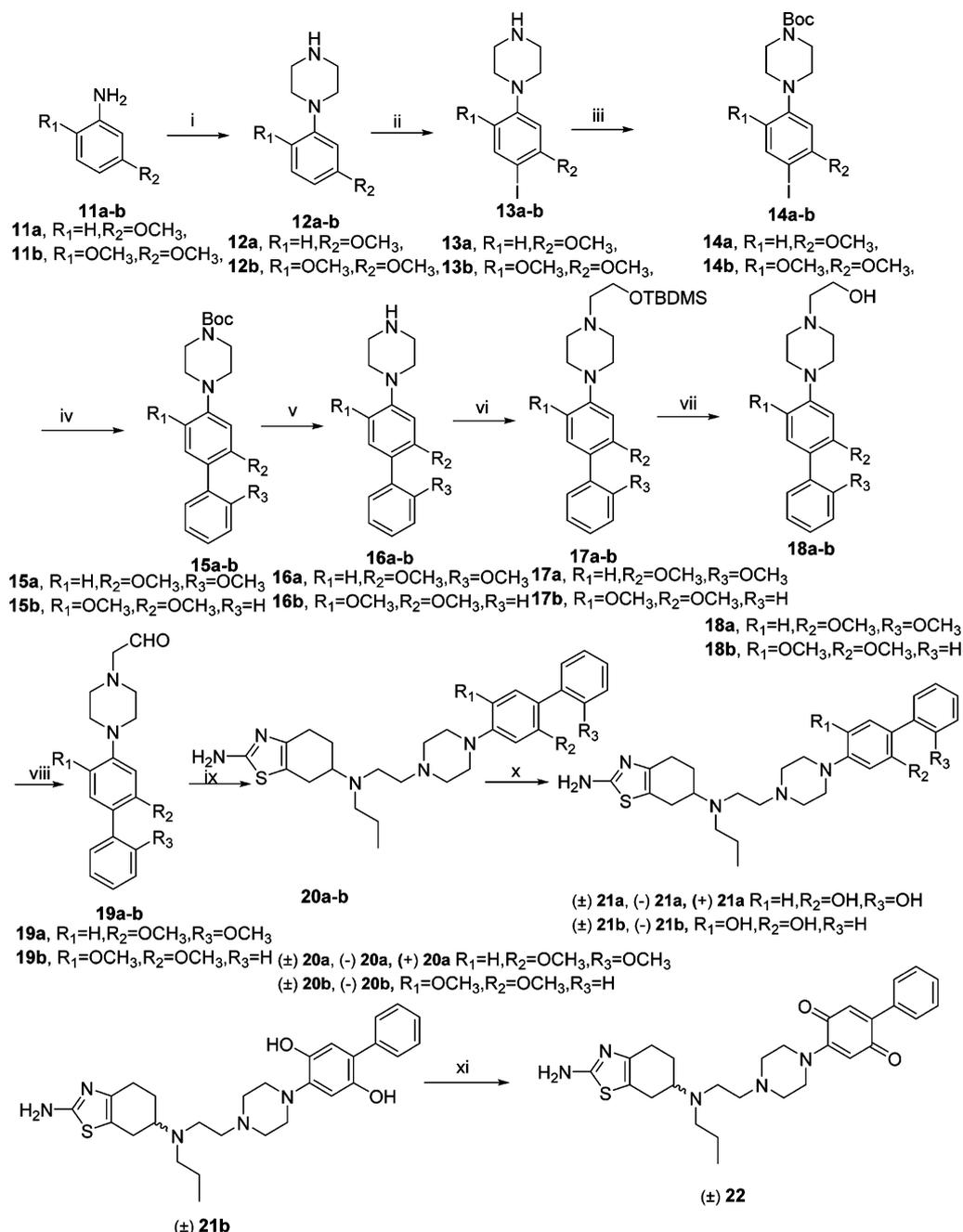
Scheme 1<sup>a</sup>

<sup>a</sup>Reagents and conditions: (i) (a) ICl, acetic acid, water, 55 °C, 1 h, 70–75%, (b) (Boc)<sub>2</sub>O, Et<sub>3</sub>N, dichloromethane, rt, 12 h, 80–85%; (ii) ArB(OH)<sub>2</sub>, Pd(PPh<sub>3</sub>)<sub>4</sub>, Na<sub>2</sub>CO<sub>3</sub>, dimethoxy ethane, ethanol, 95 °C, 2 h, 65–70%; (iii) TFA, dichloromethane, rt, 2 h, 90–95%; (iv) (2-bromoethoxy)(*tert*-butyl)dimethylsilane, K<sub>2</sub>CO<sub>3</sub>, acetonitrile, reflux, 14 h, 80–85%; (v) *n*-Bu<sub>4</sub>NF, THF, rt, 1.5 h, 90%; (vi) oxalyl chloride, DMSO, TEA, CH<sub>2</sub>Cl<sub>2</sub>, -78 °C, 2 h, 70–80%; (vii) (±)-pramipexole, (-)-pramipexole, (+)-pramipexole NaBH(OAc)<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, rt, 48 h, 60–70%; (viii) 48% aq HBr, reflux, 6 h, 60–70%; (ix) MnO<sub>2</sub>, dichloromethane, rt, 12 h, 50%.

(A53T and A30P) can lead to formation of aggregates in a concentration-dependent manner.<sup>4,13</sup> Dopamine (DA) and its metabolite DA-quinone (DAQ) inhibit fibril formation and accelerate the conversion of  $\alpha$ SN into oligomer form through interaction with lysine residues.<sup>14,15</sup> It has been shown in the literature that total nigral iron level is higher in PD brain compared to the normal brain with iron being the most

abundant metal of the body.<sup>16–19</sup> Metals, especially iron, have also been implicated in the formation of fibrilization of  $\alpha$ SN either via disruption of the interaction between the N- and C-termini regions of  $\alpha$ SN or by metal-catalyzed oxidation (MCO) of  $\alpha$ SN.<sup>20</sup>

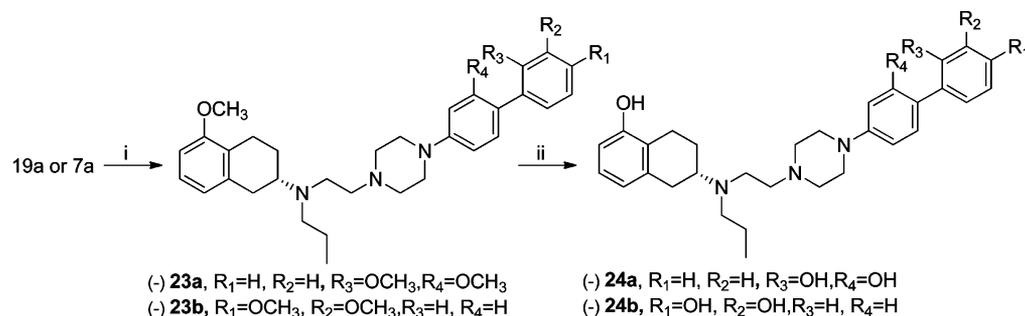
Current therapies of PD are limited to only symptomatic relief without addressing the underlying pathogenic factors of

Scheme 2<sup>a</sup>

<sup>a</sup>Reagents and conditions: (i) Bis(2-chloroethyl)amine, diethylene glycol monomethyl ether, 150 °C, 7 h, 65–70%; (ii) ICl, acetic acid, water, 55 °C, 1 h, 85–90%; (iii) (Boc)<sub>2</sub>O, Et<sub>3</sub>N, dichloromethane, rt, 12 h, 75–80%; (iv) phenyl boronic acid (for compound **15b**) and (2-methoxyphenyl)boronic acid (for compound **15a**); Pd(PPh<sub>3</sub>)<sub>4</sub>, Na<sub>2</sub>CO<sub>3</sub>, 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<sub>2</sub>CO<sub>3</sub>, CH<sub>3</sub>CN, reflux, 12 h, 80–85%; (vii) Bu<sub>4</sub>NE, THF, rt, 2 h, 70–75%; (viii) oxalyl chloride, DMSO, TEA, CH<sub>2</sub>Cl<sub>2</sub>, –78 °C, 2 h; 65–70%; (ix) (±)-pramipexole or (–)-pramipexole or (+)-pramipexole, NaBH(OAc)<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, rt, 48 h, 65–75%; (x) 48% aq HBr, reflux, 6 h, 70–75%; (xi) MnO<sub>2</sub>, dichloromethane, rt, 12 h, 60%.

the disease process like aggregation of  $\alpha$ SN, mitochondrial dysfunction, and oxidative stress. Consequently, the progression of the disease continues with the current therapies. It is now well recognized that, for complex neurodegenerative diseases such as PD and Alzheimers disease, molecules targeting more than one target site might prove to be more effective as disease modifying agents as opposed to single target based drugs.<sup>21,22</sup> Thus, it is hypothesized that multifunctional drugs having multiple pharmacological activities will be effective

as disease modifying agents in treatment of PD. Therefore, in our multifunctional drug development approach, we would like to combine symptomatic relief property in a drug along with other neuroprotective properties including antioxidant, iron chelating, and  $\alpha$ SN aggregation-modulating activities. With this in mind, we initiated our drug discovery approach aimed at identifying novel multifunctional agents possessing D2/D3 agonist activity along with antioxidant, iron chelating, and neuroprotective properties. In this regard, we have recently

Scheme 3<sup>a</sup>

<sup>a</sup>Reagents and conditions: (i) (S)-(5-methoxy-1,2,3,4-tetrahydronaphthalen-2-yl)-propyl-amine, NaBH(OAc)<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, rt, 48 h, 65–75%; (ii) 48% a. HBr, reflux, 6 h, 70–75%.

reported the development of such multifunctional D2/D3 agonists derived from a novel molecular template which exhibited in vivo efficacy in PD animal models.<sup>23–30</sup> Some of our multifunctional lead D2/D3 agonist molecules e.g. D-264 (Figure 1), exhibited neuroprotection in in vitro and in vivo experiments.<sup>23,24</sup> Additionally, we have reported development of multifunctional brain penetrant iron chelators with agonist activity at D2/D3 receptors.<sup>26</sup> One of our lead compounds in this series conferred in vivo neuroprotection in a mouse model of MPTP.<sup>26</sup>

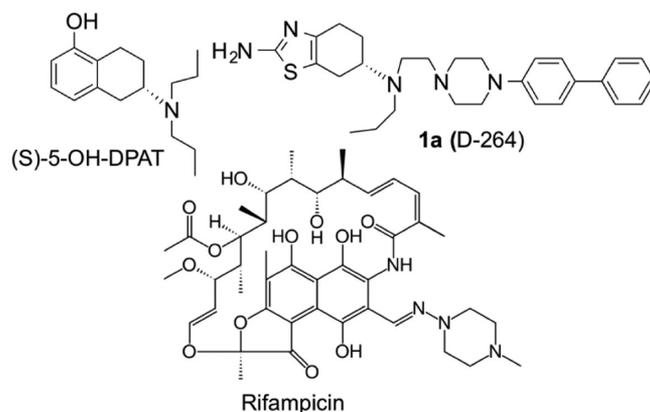
Given the importance of  $\alpha$ SN in the pathogenesis of PD, the modulation of its aggregation activity is emerging as an important therapeutic target to treat PD.<sup>3,7,9,10,31–37</sup> In this report, our major goal is to develop in vivo active multifunctional DA D2/D3 agonist molecules with an ability to modulate  $\alpha$ SN aggregation. Such molecules are hypothesized to address symptomatic effect along with production of neuroprotective effect to act as disease modifying agent. To achieve this, we have designed the incorporation of dihydroxyl groups with  $\alpha$ SN aggregation-modulating functionality at various positions on the accessory binding biphenyl ring of the hybrid molecule. Numerous studies have shown that compounds with dihydroxyl groups are able to effectively modulate  $\alpha$ SN aggregation.<sup>12,14,15,31,38–44</sup> Our recent structure–activity relationship (SAR) studies have demonstrated that monohydroxyl and -methoxy moieties are not only well tolerated but also produce highly efficacious in vivo active compounds. Thus, it was hypothesized that introduction of another hydroxyl group on the accessory binding site could produce compounds that not only retain high affinity for the D2/D3 receptor but also gain potent  $\alpha$ SN aggregation-modulating property. Based on this, various dihydroxy and quinone compounds were designed and synthesized. Selected lead compounds were tested in animal models of PD for their in vivo potency in reversing reserpine-induced hypolocomotion in rats and in producing rotational activity in 6-hydroxydopamine (6-OHDA) lesioned rats. We also assessed the ability of our in vivo active lead compound in a PD animal model to inhibit the aggregation of  $\alpha$ SN in a cell free preparation system and to determine the effect of our drug when cocubated with  $\alpha$ SN, in modulation of toxicity in a cellular system. Finally, the lead molecule was evaluated for its ability to provide neuroprotection against the toxicity induced by 6-OHDA in dopaminergic MN9D cells.

## CHEMISTRY

Scheme 1 describes the synthesis of final compounds ( $\pm$ )-**9a**, ( $\pm$ )-**9b**, and their enantiomers as well as compound ( $\pm$ )-**10**. To

synthesize intermediate **1**, iodination of phenyl piperazine was done using iodine monochloride followed by its reaction with Boc-anhydride. Boc protected intermediate, **1**, was then reacted with various commercially available substituted benzene boronic acids under Suzuki coupling reaction conditions.<sup>45,46</sup> The amine protecting Boc group was deprotected by using trifluoroacetic acid to yield the intermediates, **4a,b**, which were subjected to N-alkylation reaction with TBDMS protected bromoethanol to get intermediates (**5a,b**). The protective group, TBDMS, was removed by using tetrabutyl ammonium fluoride solution (TBAF) to get the alcohol intermediate (**6a,b**). These alcohol intermediates (**6a,b**) were oxidized under Swern oxidation conditions to get the arylpiperazine aldehydes (**7a,b**) which were further condensed with ( $\pm$ )-, S(-)-, or R(+)-pramipexole under reductive amination conditions to give ( $\pm$ )-**8a**, ( $\pm$ )-**8b** and their enantiomers. The demethylation of these intermediates by refluxing with freshly distilled aqueous hydrobromic acid (48%) yielded the final compounds **9a,b** and their enantiomers. One more final quinone compound ( $\pm$ )-**10** was generated by oxidation of ( $\pm$ )-**9b** in the presence of MnO<sub>2</sub>.

Scheme 2 shows the synthesis of final target compounds ( $\pm$ )-**21a**, ( $\pm$ )-**21b**, and their enantiomers as well as ( $\pm$ )-**22**.



**Figure 1.** Molecular structures of (S)-5-OH-DPAT, D-264, and rifampicin.

Various substituted methoxy anilines (**11a,b**) were subjected to cyclization by following the literature procedure to produce intermediate **12a,b**.<sup>47</sup> Further, iodination followed by Boc protection yielded Boc protected compound, **14a,b**. These amine protected compounds were exposed to Suzuki coupling reaction with commercially available benzene boronic acids, and subsequently, Boc group was removed by using TFA to yield

**16a,b.** The free amine intermediates, **16a,b**, were N-alkylated with (2-bromoethoxy)-*tert*-butyldimethylsilane to get compounds **17a,b**, which on TBDMS deprotection yielded alcohols **18a,b**. Compounds **18a,b** were converted into aldehyde derivatives **19a,b** under Swern oxidation conditions followed by condensation with ( $\pm$ )-, *S*-(-)-, or *R*-(+)-pramipexole under reductive amination conditions and subsequently treated with aqueous hydrobromic acid (48%) to yield the final compounds ( $\pm$ )-**21a**, ( $\pm$ )-**21b**, and respective enantiomers. One of the final compounds ( $\pm$ )-**21b** was oxidized in the presence of MnO<sub>2</sub> to yield final compound ( $\pm$ )-**22**.

In Scheme 3, we describe the synthesis of bioisosteric analogues of 2-aminothiazole moiety. The intermediates described in Schemes 1 and 2, the arylpiperazine aldehyde, **7a** and **19a**, were subjected under reductive amination conditions to react with (*S*)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine to get corresponding methoxy intermediates (-)-**23a** and (-)-**23b** and subsequently treated with aqueous hydrobromic acid (48%) to furnish the final compounds (-)-**24a** and (-)-**24b**.

## RESULTS AND DISCUSSION

In our approach to design neuroprotective multifunctional ligands which should not only act as agonist at dopamine D2/D3 receptors but also be able to chelate iron and modulate  $\alpha$ SN aggregation, we decided to introduce two hydroxyl groups in our hybrid template. Our SAR studies on this hybrid template have indicated that bulky aromatic substitutions located distally from the headgroup are well tolerated on the piperazine moiety. We have recently shown that monohydroxyl substitution on the biphenyl moiety not only was tolerated well but also produced highly efficacious compounds.<sup>48</sup> Our current design extends this finding by incorporating an additional hydroxyl group to enhance multifunctional property as described above. The final target compounds were tested in in vitro binding assays to determine their affinity toward DA D2 and D3 receptors (Table 1). In these assays, test compounds compete against the DA receptor antagonist [<sup>3</sup>H]spiperone for binding to hD2L and hD3 receptors expressed in HEK-293 cells. Binding and functional assays for dopamine receptors were carried out by Dr. Reith at New York University, School of Medicine.

The two compounds that were designed first, **9b** and **21b**, possess two hydroxyl groups in the para position to each other. Both compounds exhibited low nanomolar affinity for the D3 receptor (3.50 and 5.87 nM for **9b** and **21b**, respectively, Table 1). Compound **9b** was slightly more potent at D2 receptor compared to **21b** (1329 vs 2148 nM for **9b** and **21b**, respectively). These results indicated that two *p*-hydroxyl groups were well tolerated by the D3 receptor but weakly tolerated by D2. It is well established in the literature that conversion of dihydroxyl to quinone is critical for modulating  $\alpha$ SN protein.<sup>44</sup> Therefore, compounds **9b** and **21b** were converted into the corresponding quinone, which led to the generation of compounds **10** and **22**, respectively. Interestingly, there was no significant change in affinity of the **22** toward the D3 receptor (7.35 nM) compared to the dihydroxyl compound **21b** (5.87 nM) but affinity toward D2 receptor dropped 1.5-fold compared to **21b** (3210 and 2148 nM for **22** and **21b**, respectively). On the other hand, compound **10** showed lower affinity toward D3 receptor compared to the corresponding parent molecule **9b** (13.4 and 3.50 nM for **10** and **9b**, respectively) while the affinity to D2 receptor did not change significantly compared to **9b** (1719 and 1329 nM for **10** and

**Table 1. Inhibition Constants for Competition with [<sup>3</sup>H]Spiroperidol Binding to rD2L and rD3 Receptors Expressed in HEK-293 Cells<sup>a</sup>**

| compd                      | K <sub>i</sub> (nM), [ <sup>3</sup> H]spiperone |               | D2L/D3 |
|----------------------------|---|---------------|--------|
|                            | D2L   | D3            |        |
| (-)-5-OH-DPAT <sup>b</sup> | 58.8 ± 11.0                                     | 1.36 ± 0.28   | 43.2   |
| D-264 <sup>b</sup>         | 264 ± 40  | 0.92 ± 0.23   | 253    |
| <b>9a</b> (D-432)          | 320 ± 37  | 5.32 ± 1.17   | 60     |
| (-)- <b>9a</b> (D-519)     | 233 ± 29  | 1.42 ± 0.38   | 164    |
| (+)- <b>9a</b> (D-521)     | 2852 ± 458                                      | 18.3 ± 2.5    | 156    |
| <b>9b</b> (D-490)          | 1329 ± 182                                      | 3.50 ± 0.73   | 380    |
| (-)- <b>9b</b> (D-510)     | 762 ± 90  | 2.51 ± 0.50   | 303    |
| (+)- <b>9b</b> (D-511)     | 2542 ± 462                                      | 34.4 ± 6.0    | 74.0   |
| <b>10</b> (D-493)          | 1719 ± 33                                       | 13.4 ± 1.3    | 128    |
| <b>21a</b> (D-426)         | 4353 ± 802                                      | 5.23 ± 0.70   | 832    |
| (-)- <b>21a</b> (D-444)    | 2289 ± 522                                      | 1.68 ± 0.39   | 1362   |
| (+)- <b>21a</b> (D-496)    | 8316 ± 4710                                     | 92.2 ± 3.4    | 90     |
| <b>21b</b> (D-491)         | 2148 ± 349                                      | 5.87 ± 1.23   | 366    |
| (-)- <b>21b</b> (D-518)    | 654 ± 75  | 3.27 ± 0.51   | 200    |
| <b>22</b> (D-489)          | 3210 ± 572                                      | 7.35 ± 0.56   | 464    |
| (-)- <b>24a</b> (D-494)    | 185 ± 19  | 0.74 ± 0.14   | 250    |
| (-)- <b>24b</b> (D-520)    | 41.8 ± 11.2                                     | 0.350 ± 0.101 | 119    |

<sup>a</sup>Results are the mean ± SEM for three to six independent experiments, each performed in triplicate. <sup>b</sup>Data from ref 29.

**9b**, respectively). This could be due to complex interactions of the molecule in this region of D2 and D3 receptors but the major factor responsible for this change in affinity is not clear yet.

Next, we synthesized two enantiomerically pure forms of racemic **9b** to make compound (-)-**9b** (K<sub>i</sub>, D2 = 762 nM, D3 = 2.51 nM, D2/D3 = 303) and compound (+)-**9b** (K<sub>i</sub>, D2 = 2542 nM, D3 = 34.4 nM, D2/D3 = 74) to understand the underlying stereochemical features. In agreement with our earlier results, (-)-**9b** exhibited higher affinity at both D2 and D3 receptors compared to (+)-**9b**. Also compound (-)-**9b** showed higher affinity at both D2 and D3 receptors compared to the racemic **9b** (K<sub>i</sub>, D2 = 762, D3 = 2.51 nM vs K<sub>i</sub>, D2 = 1,329, D3 = 3.50 nM, respectively, for (-)-**9b** and ( $\pm$ )-**9b**, Table 1). Furthermore, we synthesized the enantiomerically pure (-)-isomer forms of racemic **21b**, that is, (-)-**21b** (K<sub>i</sub>, D2 = 654 nM, D3 = 3.27 nM, D2/D3 = 200). All compounds in this series showed nanomolar potency at D3 receptors in the competitive binding assay.

In our next design of compounds, we wanted to evaluate the positional effect of dihydroxyl group on binding affinity and selectivity. In this context, it has been shown in the literature that vicinal and *p*-dihydroxyl groups on an aromatic ring can provide better  $\alpha$ SN aggregation-modulating activity to flavonoids as well as iron chelating capability which is thought to bestow neuroprotective properties.<sup>14,38,40,49</sup> Therefore, we designed and synthesized ( $\pm$ )-**9a**, ( $\pm$ )-**21a**, and their enantiomers. Racemic compound ( $\pm$ )-**9a** with dihydroxyl group ortho to each other on the distal phenyl ring, attached to the piperazine ring, was found to exhibit higher affinity at D2/D3 receptors (K<sub>i</sub>, D2 = 320 nM; D3 = 5.32 nM) compared to ( $\pm$ )-**21a** (K<sub>i</sub>, D2 = 4353 nM, D3 = 5.23 nM). The enantiomer (-)-**9a** was more potent, exhibiting high affinity at both D2 and D3 receptors (K<sub>i</sub>, D2 = 233 nM, D3 = 1.42 nM). In comparison, (-)-**21a** with the dihydroxyl groups proximal to each other on the two distal phenyl rings proved to be most active and selective for D3 receptor (K<sub>i</sub>, D2 = 2289 nM, D3 =

Table 2. Stimulation of [<sup>35</sup>S]GTPγS Binding to hD2 and hD3 Receptors Expressed in CHO Cells<sup>a</sup>

| compd                  | CHO-D2   |                   | CHO-D3   |                   | D2/D3k |
|------------------------|--|-------------------|--|-------------------|--------|
|                        | EC <sub>50</sub> (nM) <sup>a</sup> [ <sup>35</sup> S]GTPγS | %E <sub>max</sub> | EC <sub>50</sub> (nM) <sup>a</sup> [ <sup>35</sup> S]GTPγS | %E <sub>max</sub> |        |
| dopamine               | 218 ± 12   | 100               | 10.6 ± 2.1   | 100               | 26.5   |
| (-)-D-264 <sup>b</sup> | 33.1 ± 6.6   | 104 ± 5           | 1.51 ± 0.02  | 90 ± 4.3          | 22.1   |
| (-)-D-510 ((-)-9b)     | 108 ± 20   | 96.0 ± 3.8        | 2.38 ± 0.22  | 97.2 ± 4.5        | 46.2   |
| (-)-D-519 ((-)-9a)     | 42.4 ± 13.6  | 98.2 ± 1.5        | 5.92 ± 0.74  | 82.4 ± 0.5        | 7.2    |
| (-)-D-520 ((-)-24b)    | 4.73 ± 0.44  | 80.9 ± 6.6        | 2.18 ± 0.30  | 58.3 ± 9.6        | 2.2    |
| (-)-D-444 ((-)-21a)    | 89.0 ± 14.8  | 83.3 ± 4.0        | 0.54 ± 0.15  | 100 ± 2           | 165    |

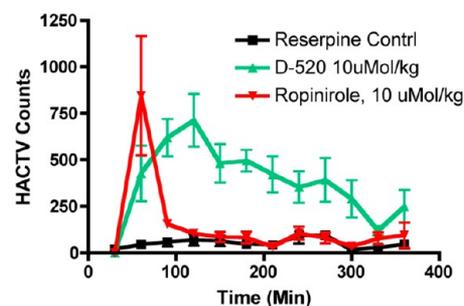
<sup>a</sup>EC<sub>50</sub> values (nM) are means ± SEM for three to six experiments each performed in triplicate. <sup>b</sup>Data from ref 29.

1.68 nM, D2/D3 = 1362). Compound (-)-21a was found to be one of the most selective ligands for D3 receptor known to date. In agreement with our previous finding, (+)-9a and (+)-21a showed moderate binding affinity at both D2 and D3 receptors compared to (-)-9a and (-)-21a (*K<sub>i</sub>*, D2 = 2852 nM, D3 = 18.3 nM and *K<sub>i</sub>*, D2 = 8316 nM, D3 = 92.2 nM for (+)-9a and (+)-21a, respectively).

The bioisosteric replacement of a thiazolidium moiety by aminotetraline was also performed. Specifically, the (-)-isomer of 5-hydroxy aminotetraline was synthesized, as our previous reports on 5-hydroxy compounds pointed to (-)-enantiomers exhibiting higher potency at both D2 and D3 receptors.<sup>30</sup> As expected from our earlier data, the aminotetraline analogues (-)-24a and (-)-24b were found to be more active at both D2 and D3 receptors compared to the thiazolidium derivatives (-)-9a and (-)-21a. However, (-)-24b exhibited higher affinity at D2 and D3 receptors compared to (-)-24a (*K<sub>i</sub>*, D2 = 185 nM, D3 = 0.74 nM and *K<sub>i</sub>*, D2 = 41.8, D3 = 0.35 nM for (-)-24a and (-)-24b (D-520), respectively).

Based on their high affinity and selectivity in the [<sup>3</sup>H]-spiroperidol binding analysis, compounds (-)-9a, (-)-9b, (-)-21a, and (-)-24b (D-520) were selected for functional assays to assess agonist activity at D2 and D3 receptors: Activation of [<sup>35</sup>S]GTPγS binding was compared with the full agonist DA (Table 2). The assays were carried out with hD2 and hD3 receptors expressed in CHO cells. In this study, (-)-9a as well as (-)-24b exhibited high potency for both D2 (EC<sub>50</sub> 42.4 nM and 4.73 nM for (-)-9a and (-)-24b, respectively) and D3 (EC<sub>50</sub> 5.92 and 2.18 nM for (-)-9a and (-)-24b, respectively) receptors, compared to the reference compound DA (EC<sub>50</sub> 218 nM for D2 and 10.6 nM for D3). Compounds (-)-9b and (-)-21a exhibited appreciable potency at D2 (EC<sub>50</sub> for D2, 108 and 89.0 nM, respectively) and high potency at D3 (EC<sub>50</sub> for D3 receptor, 2.38 and 0.54 nM respectively).

**Reversal of Reserpine-Induced Hypolocomotion in Rats.** Catalepsy in rats can be produced by reserpine-induced depletion of catecholamines in nerve terminals, which is a well established animal model for PD.<sup>50</sup> Significant reduction of locomotion of the rats was observed at 18 h after the administration of reserpine (5 mg/kg, s.c.) which indicated the development of akinesia in rats (Figure 2). Based on its high affinity and activity at D2 and D3 receptors, compound D-520 was selected for further biological evaluation in this assay and found to be highly efficacious in reversing locomotor activity of reserpinized rats. The locomotor activity following D-520 treatment remained elevated over that following vehicle treatment after 6 h (Figure 2). The reference drug ropinirole on the other hand exhibited a much shorter duration of action compared to D-520. In comparison, compound D-520 at the dose of 10 μmol/kg i.p. not only reversed reserpine induced



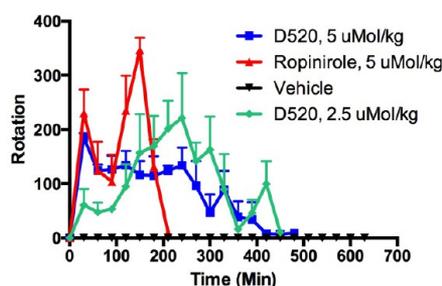
**Figure 2.** Effect of different drugs upon reserpine-induced (5.0 mg/kg, s.c.) hypolocomotion in rats. Data are means ± SEM, *n* = 3–4 rats per value. Horizontal activity was measured as described in Methods. The plots are the representation of horizontal locomotor activity at discrete 30 min intervals after the administration of D-520 (i.p.) and ropinirole (i.p.) at the dose of 10 μmol/kg compared to control reserpine treated rats in 18 h post reserpine treatment. One-way ANOVA analysis demonstrates significant effect among treatments  $F(2,35) = 10.06$  ( $P < 0.0001$ ). Dunnett's analysis following ANOVA showed that the effects of D-520 ( $P < 0.01$ ) and ropinirole ( $P < 0.01$ ) were significantly different compared to reserpine control.

hypokinesia, but also demonstrated significant enhancement of locomotion for the duration of study (Figure 2). The mechanism of the locomotor stimulation in reserpine model is likely mediated by postsynaptic D2/D3 receptor activation, suggesting that D-520 is a potent agonist that can cross the blood-brain barrier effectively.

#### In Vivo Pharmacology with 6-OHDA Lesioned Rats.

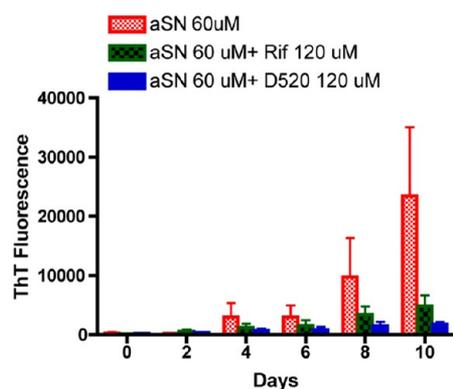
Based on the above locomotor activity results, compound D-520 was selected for in vivo evaluation in rats carrying a unilateral lesion in the medial forebrain bundle induced by application of the neurotoxin 6-OHDA. When these rats with supersensitized DA receptors are challenged with direct acting DA agonists, they show 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.<sup>51</sup> Compound D-520 was highly efficacious in producing large numbers of rotations at a dose of 2.5 μmol/kg (no. of rotations = 1180), and the activity lasted more than 5 h. At a higher dose of 5 μmol/kg, the rotational activity was initially higher compared to the lowest dose and the activity remained at high level after 5 h (Figure 3). The reference compound ropinirole at 5 μmol/kg produced fewer rotations, and its action lasted for a shorter time.

**Evaluation of Antifibrillar Activity of D-520 on αSN Aggregation.** αSN aggregates were generated with a goal to evaluate the effect of various extracellular αSN species on cell viability in PC12 cells. In this experiment, αSN aggregates were formed to yield β-sheet-positive fibrillar structures which were detected by thioflavin T (ThT). To achieve this, we incubated



**Figure 3.** Effect on turning behavior of two different doses of D-520 (i.p.), ropinirole, and vehicle in lesioned rats studied for maximum 12 h. Each point is the mean  $\pm$  SEM of three to four rats. The drugs were administered i.p. One-way ANOVA analysis demonstrates significant effect among treatments:  $F(3,62) = 16.79$  ( $P < 0.0001$ ). Dunnett's analysis showed that the effect of two doses of D-520, and ropinirole on rotations was significantly different compared to vehicle ( $P < 0.01$ ).

$\alpha$ SN (60  $\mu$ M) with mechanical agitation for 10 days and the time course of fibrilization process was monitored in the ThT assay. Next, we wanted to observe the effect of our lead compound with in vivo antiparkinsonian activity, D-520, and the reference drug rifampicin on aggregation of  $\alpha$ SN by monitoring the ThT activity. Figure 4 displays the activity of



**Figure 4.** Inhibition of  $\alpha$ SN fibrilization by D-520 and the reference drug rifampicin. The drugs were incubated with  $\alpha$ SN over a period of 10 days and the fibrilization was measured at different time intervals by ThT fluorescence assay. Data values shown are means  $\pm$  SEM of three independent experiments.

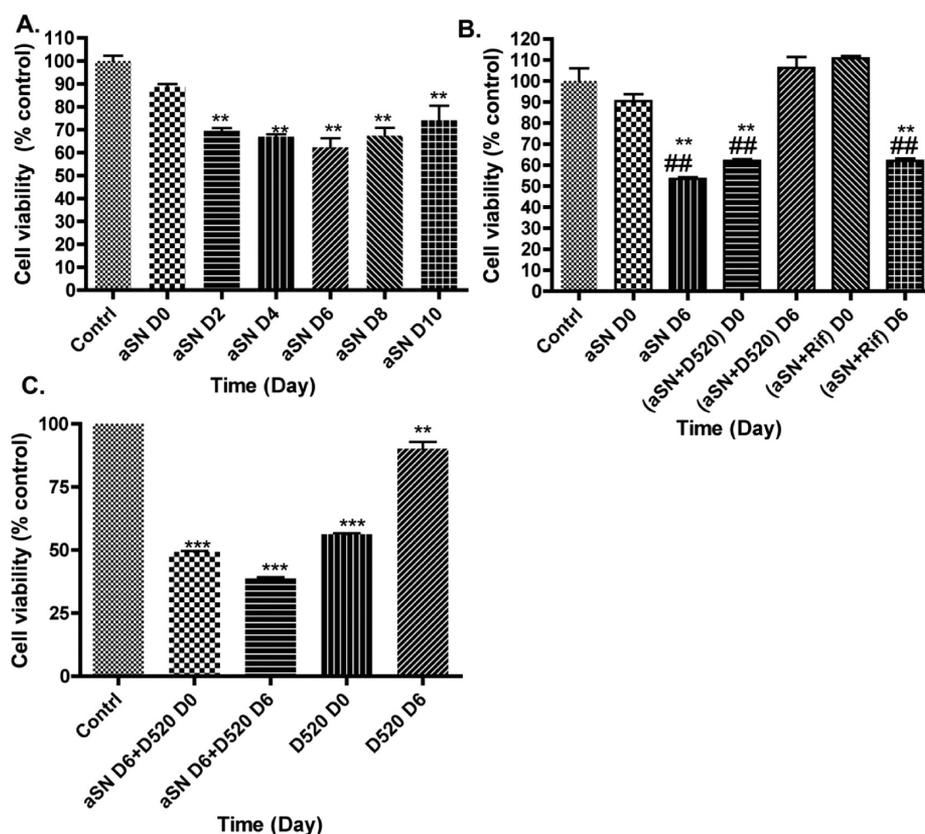
ThT when  $\alpha$ SN was shaken alone as well as in the presence of the drugs. An inhibition of ThT activity was observed in the presence of D-520 and rifampicin compared to  $\alpha$ SN alone, indicating alteration of the aggregation process in the presence of drugs.

In our next effort to evaluate toxicity of preformed aggregates of  $\alpha$ SN collected at various time points,  $\alpha$ SN (60  $\mu$ M) was incubated for 10 days by shaking followed by evaluation of toxicity of various time-dependent preformed aggregated species with PC12 cells (extracellular toxicity). The main objective of this experiment was to determine the time point which exhibits the highest cytotoxicity (ideally around  $\sim$ 50% cell death). Thus, PC12 cells were incubated with aliquots from various time-points at a final concentration of 10  $\mu$ M  $\alpha$ SN. It is evident from Figure 5A that  $\alpha$ SN (60  $\mu$ M) aggregates generated from incubation for 6 days are the most toxic to PC12 cells producing close to 40% cell death. We next assessed the ability of our lead compound D-520 and the reference drug (rifampicin) to alter cytotoxicity after coincubating  $\alpha$ SN (60

$\mu$ M) with these drugs (120  $\mu$ M) for 6 days followed by cell viability experiment with the PC12 cells. In order to maintain identical condition, cellular viability was determined by using samples from various experiments diluted to maintain a final concentration of 10  $\mu$ M of  $\alpha$ SN and 20  $\mu$ M of drugs. Treatment with preaggregated  $\alpha$ SN alone showed a reduction of cell viability by  $\sim$ 40% (Figure 5B). Interestingly, at a starting time point (day 0) of  $\alpha$ SN samples which were incubated with D-520, there was a significant reduction of cell viability. However, at a later time point (day 6), D-520 completely reversed the toxicity induced by  $\alpha$ SN alone at day 6. In comparison, the standard drug rifampicin was not able to confer significant protection from toxicity of  $\alpha$ SN at the day 6 time point (Figure 5B).

To obtain further insight into the role of D-520 in such protection (Figure 5B) against  $\alpha$ SN toxicity, we have carried out additional experiments. Thus, as shown in Figure 5C, addition of both fresh D-520 (day 0) and D-520 shaken for 6 days (day 6) to the prefabricated  $\alpha$ SN formed at day 6 significantly enhanced the toxicity compared to  $\alpha$ SN aggregates formed from incubation with D-520 at day 6 (Figure 5B vs C). These results clearly demonstrate lowering of toxicity of  $\alpha$ SN aggregates if incubated in the presence of D-520, indicating a possible covalent interaction between D-520 and  $\alpha$ SN. As reported previously, it is quite possible that the catechol moiety in D-520 upon incubation gets converted into a quinone moiety which in turn covalently labeled  $\alpha$ SN molecule via interaction with the basic lysine moieties in the  $\alpha$ SN molecules. Such a covalent interaction will result in formation of cross-linked aggregates with the drug which is evidenced in the transmission electron microscopy (TEM) picture (Figure 6D). Such involvement of the catechol moiety in alteration of the mode of  $\alpha$ SN aggregation has been reported earlier.<sup>14,15,39</sup> This might explain the reason for reduction of toxicity of aggregated  $\alpha$ SN when incubated with D-520. Furthermore, to evaluate the effect of D-520 (20  $\mu$ M) alone at day 0 (D0) and after shaking for 6 days at day 6 (D6), we carried out a cell viability experiment. As shown in Figure 5C, D-520 (20  $\mu$ M) at D0 produced significant cell toxicity. However, at day 6 (D6), toxicity of D-520 (20  $\mu$ M) was significantly reduced (Figure 5C). As described above, the addition of D-520 (D6) to preformed  $\alpha$ SN was not able to rescue the toxicity even though D-520 (D6) alone exhibited less toxicity. These results further strengthen the notion that D-520 is involved in a covalent association with  $\alpha$ SN when incubated together as addition of D-520 (D6) to preformed  $\alpha$ SN (D6) aggregates failed to rescue the toxicity of  $\alpha$ SN (D6) (Figure 5C).

The ThT and cytotoxicity results were further corroborated by TEM, which demonstrated the absence of significant amount of fibril in  $\alpha$ SN samples incubated in the presence of D-520 or rifampicin. The control samples ( $\alpha$ SN 60  $\mu$ M) at day 6 showed the presence of fibrillar structures tightly aligned to each other, which is complementary to data found in the ThT assay (Figure 6B). In contrast, D-520 showed distinct morphology of much smaller aggregates (Figure 6D). The  $\alpha$ SN incubated in the presence of rifampicin displayed amorphous and annular aggregates with few detectable fibril structures (Figure 6C). To provide a pictorial representation of our approach for development of multifunctional neuro-protective drug, We have included Figure 8 which depicts rationale for development dopamine D2/D3 agonist with potential activity to modulate  $\alpha$ SN aggregation to reduce toxicity.



**Figure 5.** Effect of treatment of time dependent prefabricated  $\alpha$ SN aggregates as well as  $\alpha$ SN aggregates formed in the presence of drugs on cell viability of PC12 cells. (A) PC12 cells were treated with  $10 \mu\text{M}$  prefabricated  $\alpha$ SN aggregates collected at different time points. (B) PC12 cells were treated with  $10 \mu\text{M}$  prefabricated  $\alpha$ SN aggregates formed in the presence of drugs collected at day 0 and day 6. Values shown are means  $\pm$  SEM of three independent experiments performed in four to six replicates. (C) PC12 cells were treated with  $10 \mu\text{M}$  prefabricated  $\alpha$ SN aggregates formed at day 6 and  $20 \mu\text{M}$  D-520 at day 0 as well as with  $20 \mu\text{M}$  D-520 after shaking for 6 days. One-way ANOVA analysis followed by Tukey's multiple comparison post hoc test was performed. (\*\* $p < 0.01$  and \*\*\* $p < 0.001$  compared to the control; ## $p < 0.01$  compared to the  $\alpha$ SN D0.)

**Neuroprotection against 6-OHDA.** 6-OHDA is known to cause cell death in a dose-dependent manner via production of reactive oxygen species. From our previous dose-dependent experiment of 6-OHDA, we chose  $75 \mu\text{M}$  6-OHDA which can induce 40–50% cell death for our study.<sup>23</sup> To examine whether D-520 can protect MN9D cells from the exposure to  $75 \mu\text{M}$  neurotoxin 6-OHDA, the cells were incubated with various concentrations of (30, 20, 10, 5, 1, 0.1, and  $0.01 \mu\text{M}$ ) D-520 for 1 h and then cotreated with  $75 \mu\text{M}$  6-OHDA for an additional 24 h. As shown in Figure 7, the data from the MTT assay clearly indicated that D-520 is able to protect the MN9D cells significantly from 6-OHDA toxicity in a dose-dependent manner. Significant protection by D-520 was conferred at concentrations of 5–30  $\mu\text{M}$ . The greatest protection was exhibited at concentrations of 5 and  $10 \mu\text{M}$  D-520, showing almost 25–30% protection from 6-OHDA toxicity.

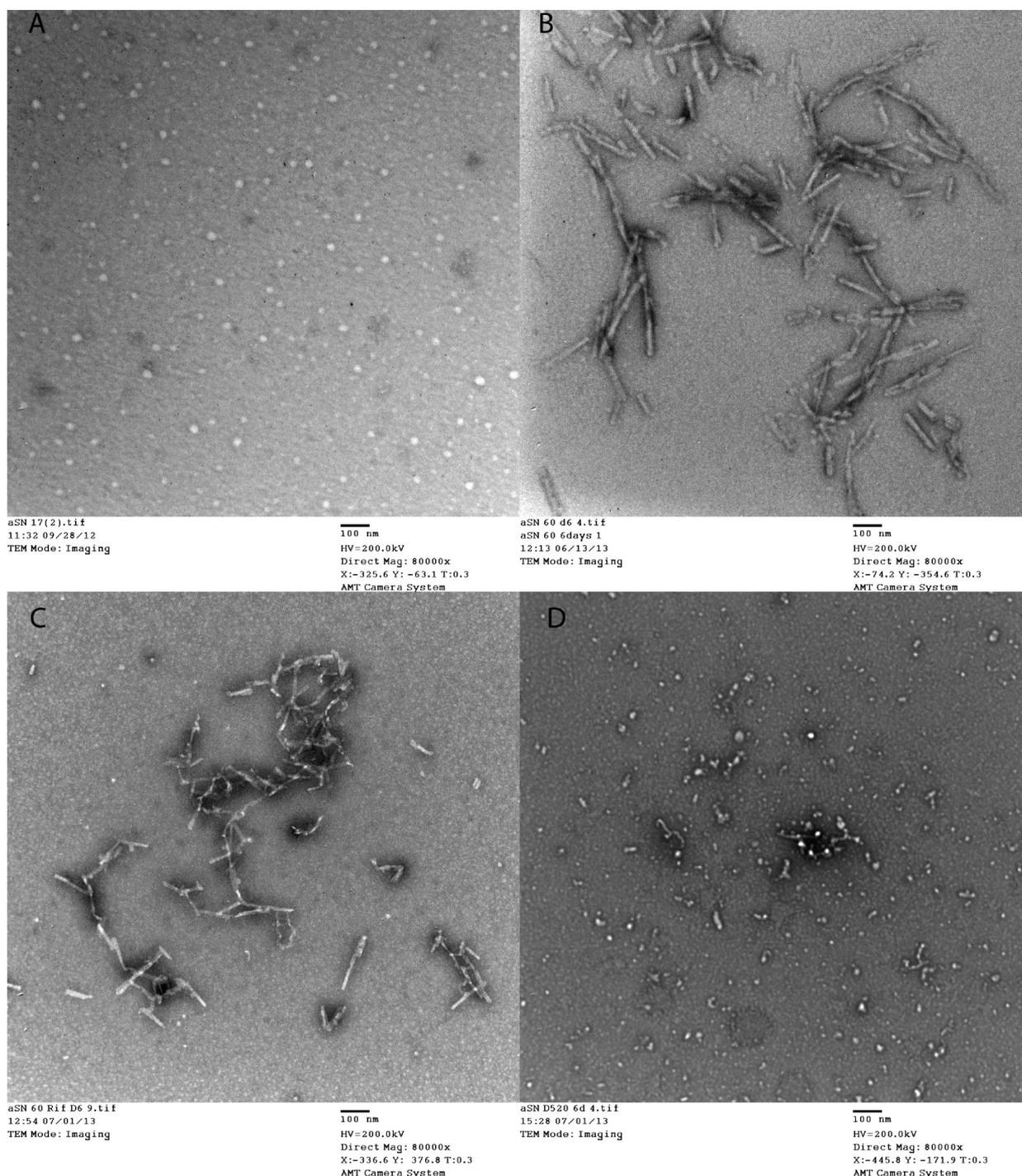
## CONCLUSION

Based on our hybrid template for DA D2/D3 agonists, we have developed a series of dihydroxy compounds and related analogues as a part of our approach to develop multifunctional drugs for PD. The dihydroxy groups were introduced distal to the agonist binding moiety and did not impact the agonist potency of the compounds toward D2/D3 receptor. The lead compound D-520 exhibited high agonist potency at D2/D3 receptors and produced efficacious activity in animal models of PD. To evaluate whether the lead compound can modulate

aggregation of  $\alpha$ SN and toxicity of preformed aggregates of  $\alpha$ SN, several assays were carried out with  $\alpha$ SN. The data from ThT activity and from TEM analysis demonstrate that D-520 is able to modulate aggregation of  $\alpha$ SN. Additionally, D-520 was able to reduce toxicity of preformed aggregates of  $\alpha$ SN compared to control  $\alpha$ SN alone. Finally, in a neuroprotection study with dopaminergic MN9D cells, D-520 clearly demonstrated an effect of neuroprotection from toxicity of 6-OHDA. Thus, compound D-520 possesses properties characteristic of multifunctionality conducive to symptomatic and neuroprotective treatment of PD.

## METHODS

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 ( $\text{N}_2$ ) 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.  $^1\text{H}$  NMR spectra were measured on a Varian 400 and 600 MHz FT NMR spectrometer using tetramethylsilane (TMS) as internal standard. The NMR solvent used was  $\text{CDCl}_3$  or  $\text{CD}_3\text{OD}$  as indicated. Optical rotations were recorded on PerkinElmer 241 polarimeter. Melting points were recorded using a MEL-TEMP II (Laboratory Devices Inc., Placerville, CA) capillary melting point apparatus and were uncorrected. Elemental analyses were performed by Atlantic Microlab, Inc.

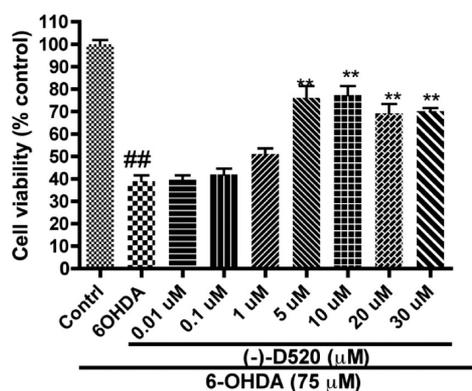


**Figure 6.** TEM analysis of  $\alpha$ SN. (A)  $\alpha$ SN at day 0, (B)  $60 \mu\text{M}$   $\alpha$ SN after 6 days, (C)  $60 \mu\text{M}$   $\alpha$ SN shaken in the presence of  $120 \mu\text{M}$  rifampicin after 6 days, and (D)  $60 \mu\text{M}$   $\alpha$ SN shaken in the presence of  $120 \mu\text{M}$  D-520 after 6 days. Scale bar = 100 nm.

**Procedure A. *tert*-Butyl 4-(4-iodophenyl)piperazine-1-carboxylate (1).** To a stirring solution of 1-phenylpiperazine (10.9 g, 67.0 mmol) in acetic acid/water (3:1, 21 mL), a suspension of iodine monochloride (12.0 g, 74.0 mmol) in acetic acid/water (3:1, 21 mL) was added at  $55^\circ\text{C}$ . The reaction was stirred at  $55^\circ\text{C}$  for 1 h and then at room temperature for 1 h. The solution was poured into 200 mL of crushed ice, and the pH was adjusted to 13 with 4 N NaOH. The product was then extracted with dichloromethane ( $3 \times 100$  mL). The combined organic layer was dried over  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated in vacuo to provide the free amine of compound **1** as a pale yellow solid (13.16 g, 74%) which was converted to Boc derivative without further purification. To a stirring solution of this amine (13.0

g, 45.12 mmol) in dichloromethane (40 mL),  $(\text{Boc})_2\text{O}$  (10.49 g, 48.04 mmol) and  $\text{Et}_3\text{N}$  (16.25 mL, 120.1 mmol) were added at room temperature. The reaction mixture was stirred at the same temperature for 12 h and was extracted with  $\text{CH}_2\text{Cl}_2$  ( $3 \times 100$  mL), washed with water, dried over  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated in vacuo. The crude material was purified by column chromatography over silica gel (methanol/EtOAc, 0.5:9.5) to give compound **1** (16.82 g, 96%).  $^1\text{H}$  ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  1.48 (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 B. *tert*-Butyl 4-(3',4'-dimethoxy-[1,1'-biphenyl]-4-yl)piperazine-1-carboxylate (3a).** A suspension of (3,4-dimethoxyphenyl)boronic acid, **2a** (2.34 g, 12.88 mmol), iodo



**Figure 7.** Dose dependent effect of combination of pretreatment followed by cotreatment of D-520 with 75  $\mu\text{M}$  6-OHDA on cell viability of MN9D cells from toxicity of 75  $\mu\text{M}$  6-OHDA. MN9D cells were pretreated with different doses of D-520 for 1 h followed by cotreatment with 75  $\mu\text{M}$  6-OHDA for 24 h. The values shown are mean  $\pm$  SEM of three independent experiments performed in four to six replicates. One-way ANOVA analysis followed by Tukey's multiple comparison post hoc test was performed. (\*\* $p < 0.001$  compared to the 6-OHDA control. ## $p < 0.001$  compared to the control.)

compound **1** (5.0 g, 12.88 mmol),  $\text{Na}_2\text{CO}_3$  (2.73 g, 25.76 mmol, 2 M solution in water), and  $\text{Pd}(\text{PPh}_3)_4$  (731 mg, 0.63 mmol) in dimethoxy ethane/ethanol (20 mL/19 mL) was refluxed for 1 h. The solvents were removed in vacuo, and the crude product was purified by flash chromatography using solvent system ethyl acetate/hexane (1:4) to yield compound **3a** (3.43 g, 67%).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  1.49 (s, 9H), 3.17 (t,  $J = 4.8$  Hz, 4H), 3.60 (t,  $J = 4.8$  Hz, 4H), 3.91 (s, 3H), 3.94 (s, 3H), 6.88–7.14 (m, 5H), 7.48 (d,  $J = 8.8$  Hz, 2H).

**tert-Butyl 4-(2',5'-dimethoxybiphenyl-4-yl)piperazine-1-carboxylate (3b).** Commercially available (2,5-dimethoxyphenyl)-boronic acid, **2b** (4.68 g, 25.70 mmol), was reacted with iodo compound **1** (9.97 g, 25.70 mmol),  $\text{Na}_2\text{CO}_3$  (5.44 g, 51.40 mmol, 2 M solution in water) and  $\text{Pd}(\text{PPh}_3)_4$  (1.47 g, 1.28 mmol) in dimethoxy ethane/ethanol (40 mL/40 mL) by following procedure B to yield compound **3b** (6.64 g, 65%).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  1.49 (s, 9H), 3.18 (bs, 4H), 3.58 (t,  $J = 4.4$  Hz, 4H), 3.74 (s, 3H), 3.84 (s, 3H), 6.83 (dd,  $J = 2.8$  Hz, 9.2 Hz, 1H), 6.88–6.90 (m, 2H), 6.96 (d,  $J = 8.4$  Hz, 2H), 7.47 (d,  $J = 8.0$  Hz, 2H).

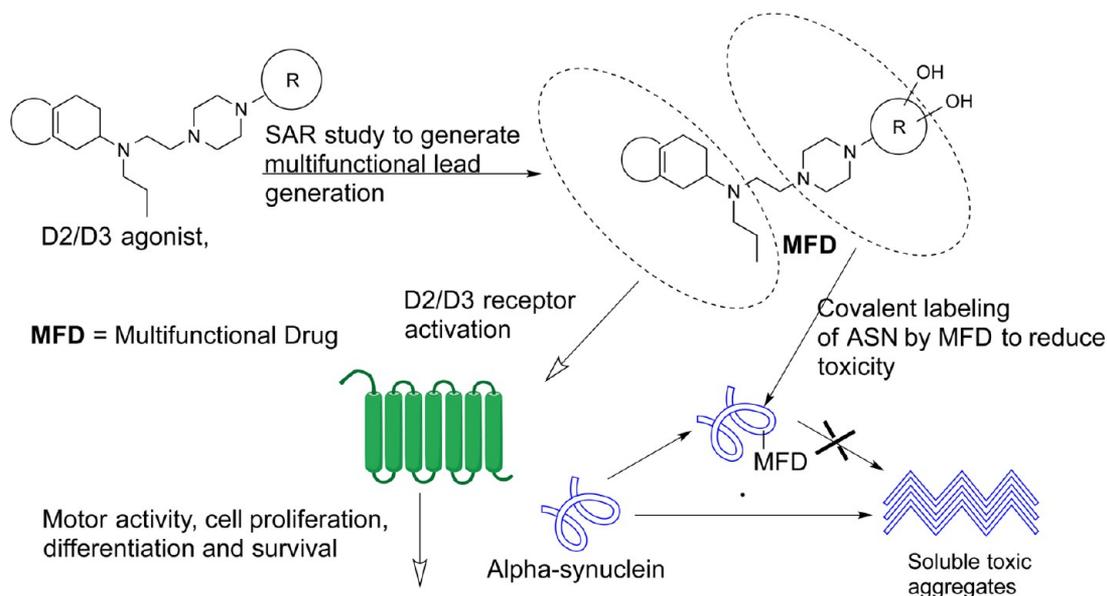
**Procedure C. 1-(3',4'-Dimethoxy-[1,1'-biphenyl]-4-yl)-piperazine (4a).** To a stirring solution of compound **3a** (3.2 g, 8.03 mmol) in  $\text{CHCl}_3$  (20 mL), TFA (20 mL) was added slowly at room temperature and the reaction mixture was stirred for 4 h. Unreacted TFA and the solvent  $\text{CHCl}_3$  were removed in vacuo, and the salt formed was washed with diethyl ether. Saturated solution of sodium bicarbonate was added to the salt, and the free base was extracted with dichloromethane ( $50 \times 3$  mL). The combined organic layer was dried over  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated in vacuo to provide the compound **4a** (2.37 g, 99%).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  1.70 (bs, 1H, NH), 3.06 (t,  $J = 4.4$  Hz, 4H), 3.19 (t,  $J = 4.4$  Hz, 4H), 3.92 (s, 3H), 3.94 (s, 3H), 6.92 (d,  $J = 7.2$  Hz, 1H), 6.99 (d,  $J = 7.2$  Hz, 2H), 7.04–7.14 (m, 2H), 7.48 (d,  $J = 7.2$  Hz, 2H).

**1-(2',5'-Dimethoxybiphenyl-4-yl)piperazine (4b).** Compound **3b** (5.4 g, 14.65 mmol) was reacted with TFA (30 mL) in  $\text{CHCl}_3$  (40 mL) using the procedure C to give compound **4b** (3.41 g, 87%).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz): 3.20 (bs, 8H), 3.75 (s, 3H), 3.85 (s, 3H), 6.62 (s, 1H), 6.85 (s, 1H), 7.31 (t,  $J = 7.2$  Hz, 1H), 7.40 (t,  $J = 7.2$  Hz, 2H), 7.52 (d,  $J = 8.0$  Hz, 2H).

**Procedure D. 1-(2-((tert-Butyldimethylsilyloxy)ethyl)-4-(3',4'-dimethoxy-[1,1'-biphenyl]-4-yl)piperazine (5a).** A mixture of compound **4a** (2.37 g, 7.95 mmol), (2-bromo-ethyl)-*tert*-butyldimethylsilane (2.28 g, 9.56 mmol), and  $\text{K}_2\text{CO}_3$  (3.17 g, 22.9 mmol) in  $\text{CH}_3\text{CN}$  (30 mL) was refluxed for 14 h. Acetonitrile was evaporated in vacuo, and the crude material was purified by silica gel column chromatography (hexane/EtOAc, 3:2) to give compound **5a** (3.41 g, 94%).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  0.09 (s, 6H), 0.92 (s, 9H), 2.64 (t,  $J = 6.4$  Hz, 2H), 2.75 (t,  $J = 4.8$  Hz, 4H), 3.27 (t,  $J = 4.8$  Hz, 4H), 3.83 (t,  $J = 6.4$  Hz, 2H), 3.92 (s, 3H), 3.95 (s, 3H), 6.93 (d,  $J = 8$  Hz, 1H), 6.99 (d,  $J = 8.8$  Hz, 2H), 7.04–7.14 (m, 2H), 7.47 (d,  $J = 8.4$  Hz, 2H).

**1-(2-((tert-Butyldimethylsilyloxy)ethyl)-4-(2',5'-dimethoxybiphenyl-4-yl)piperazine (5b).** Compound **4b** (3.0 g, 10.06 mmol) was reacted with (2-bromo-ethyl)-*tert*-butyldimethylsilane (2.88 g, 12.07 mmol) and  $\text{K}_2\text{CO}_3$  (4.17 g, 30.18 mmol) in  $\text{CH}_3\text{CN}$  (60 mL) by following procedure D to afford compound **5b** (3.90 g, 85%).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  0.06 (s, 6H), 0.87 (s, 9H), 2.61 (t,  $J = 6.8$  Hz, 2H), 2.74 (bs, 4H), 3.16 (bs, 4H), 3.74 (s, 3H), 3.80 (t,  $J = 6.4$  Hz, 2H), 3.85 (s, 3H), 6.83 (dd,  $J = 2.4$  Hz, 8.0 Hz, 1H), 6.98 (d,  $J = 8.0$  Hz, 2H), 7.09 (t,  $J = 2.4$  Hz, 1H), 7.14 (d,  $J = 7.2$  Hz, 1H), 7.50 (d,  $J = 8.0$  Hz, 2H).

**Procedure E. 2-(4-(3',4'-Dimethoxy-[1,1'-biphenyl]-4-yl)-piperazin-1-yl)ethanol (6a).** Into a stirring solution of compound **5a** (3.4 g, 7.46 mmol) in anhydrous THF (30 mL), *n*-



**Figure 8.** Schematic description of development of a multifunctional neuroprotective drug.

tetrabutylammonium fluoride (1.95 g, 7.46 mmol, 1.0 M solution in THF) was added at 0 °C. The reaction mixture was then stirred at room temperature for 1 h. THF was evaporated in vacuo, and the residue was diluted with CH<sub>2</sub>Cl<sub>2</sub> (50 mL) and washed with water. The water layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 75 mL). The combined organic layer was washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and evaporated in vacuo. The crude product was purified by silica gel column chromatography (EtOAc/MeOH, 9:1) to yield compound **6a** (2.30 g, 90%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 2.63 (t, *J* = 5.2 Hz, 2H), 2.70 (t, *J* = 4.0 Hz, 4H), 3.25 (t, *J* = 4.0 Hz, 4H), 3.68 (t, *J* = 5.2 Hz, 2H), 3.91 (s, 3H), 3.94 (s, 3H), 6.92 (d, *J* = 8.4 Hz, 1H), 6.98 (d, *J* = 8.4 Hz, 2H), 7.02–7.16 (m, 2H), 7.47 (d, *J* = 8.4 Hz, 2H).

**2-(4-(2',5'-Dimethoxybiphenyl-4-yl)piperazin-1-yl)ethanol (6b).** Compound **5b** (3.8 g, 8.93 mmol) was reacted with *n*-tetrabutylammonium fluoride (2.33 g, 8.93 mmol, 1.0 M solution in THF) in THF (80 mL) by following the procedure E to yield compound **6b** (2.27 g, 80%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 2.62 (t, *J* = 5.0 Hz, 2H), 2.69 (t, *J* = 4.4 Hz, 4H), 3.26 (t, *J* = 4.8 Hz, 4H), 3.67 (t, *J* = 5.0 Hz, 2H), 3.74 (s, 3H), 3.84 (s, 3H), 6.80 (dd, *J* = 9.2 Hz, 3.6 Hz, 1H), 6.89–6.95 (m, 2H), 6.96 (d, *J* = 8.8 Hz, 2H), 7.46 (d, *J* = 8.4 Hz, 2H).

**Procedure F. 2-(4-(3',4'-Dimethoxy-[1,1'-biphenyl]-4-yl)piperazin-1-yl)acetaldehyde (7a).** Into a stirred solution of oxalyl chloride (0.25 mL, 2.92 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (40 mL) at –78 °C, dimethyl sulfoxide (DMSO; 0.42 mL, 5.84 mmol) was added. The reaction mixture was stirred for 10 min followed by addition of compound **6a** (500 mg, 1.46 mmol, solution in 5 mL of CH<sub>2</sub>Cl<sub>2</sub>). The reaction mixture was stirred at the same temperature for 12 min. Then Et<sub>3</sub>N (1.2 mL, 8.77 mmol) was added next and stirring was continued for another 1 h and 20 min while allowing the reaction mixture temperature to reach room temperature. The reaction mixture was quenched by addition of water and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 25 mL). The combined organic layer was washed with water and brine and finally purified by silica gel column chromatography (EtOAc/MeOH, 9.5:0.5) to yield compound **7a** (402 mg, 81%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 2.40–2.80 (m, 4H), 2.82–3.50 (m, 6H), 3.92 (s, 3H), 3.95 (s, 3H), 6.93 (d, *J* = 8 Hz, 1H), 6.98 (d, *J* = 7.6 Hz, 2H), 7.03–7.16 (m, 2H), 7.48 (d, *J* = 6.8 Hz, 2H).

**2-(4-(2',5'-Dimethoxybiphenyl-4-yl)piperazin-1-yl)acetaldehyde (7b).** Compound **6b** (1.2 g, 3.50 mmol) was reacted with oxalyl chloride (0.60 mL, 7.0 mmol), DMSO (1.0 mL, 14.0 mmol), and Et<sub>3</sub>N (2.91 mL, 21.0 mmol) in dichloromethane (50 mL) by following procedure F to yield compound **7b** (850 mg, 72%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz): δ 2.69–2.71 (m, 4H), 3.24 (s, 2H), 3.29–3.30 (m, 4H), 3.74 (s, 3H), 3.79 (s, 3H), 6.78–6.80 (dd, *J* = 8.7 Hz, 3.3 Hz, 1H), 6.87–6.89 (dd, *J* = 7.8 Hz, 4.2 Hz, 2H), 6.95–6.97 (d, *J* = 9.0 Hz, 2H), 7.45–7.47 (d, *J* = 9.0 Hz, 2H), 9.75 (s, 1H).

**Procedure G. N<sup>6</sup>-(2-(4-(3',4'-Dimethoxy-[1,1'-biphenyl]-4-yl)piperazin-1-yl)ethyl)-N<sup>6</sup>-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine ((±)-8a).** Into a stirring solution of compound **7a** (402 mg, 1.18 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL), (±)-pramipexole (275 mg, 1.30 mmol) was added at room temperature. The reaction mixture was stirred for 1 h, and then NaBH(OAc)<sub>3</sub> (451 mg, 2.13 mmol) was added into the solution. After stirring for 48 h, saturated solution of NaHCO<sub>3</sub> was added into the reaction mixture and the compound was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 50 mL). The combined organic layer was washed with water and brine and finally purified by silica gel column chromatography (EtOAc/MeOH, 9:1) to yield compound (±)-**8a** (443 mg, 70%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 0.89 (t, *J* = 7.6 Hz, 3H), 1.38–1.60 (m, 2H), 1.62–1.82 (m, 1H), 1.86–2.10 (m, 2H), 2.35–2.84 (m, 13 H), 2.94–3.62 (m, 5H), 3.90 (s, 3H), 3.93 (s, 3H), 5.1 (bs, 2H), 6.91 (d, *J* = 8 Hz, 1H), 6.90 (d, *J* = 8.4 Hz, 2H), 7.04–7.14 (m, 2H), 7.46 (d, *J* = 8.8 Hz, 2H).

**(S)-N<sup>6</sup>-(2-(4-(3',4'-Dimethoxybiphenyl-4-yl)piperazin-1-yl)ethyl)-N<sup>6</sup>-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine ((–)-8a).** Compound **7a** (200 mg, 0.587 mmol) was reacted with (–)-pramipexole (124 mg, 0.587 mmol) and NaBH(OAc)<sub>3</sub> (225 mg, 1.06 mmol) in dichloromethane (30 mL) using procedure G to yield compound (–)-**8a** (218 mg, 70%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz): δ 0.86 (t, *J* = 7.2 Hz, 3H); 1.45–1.49 (m, 2H); 1.70–1.73 (m,

1H); 1.98–2.02 (m, 2H), 2.48–2.57 (m, 6 H), 2.66–2.72 (m, 7 H), 3.05 (s, 1H), 3.22–3.24 (m, 4H), 3.89 (s, 3H), 3.93 (s, 3H), 4.81 (bs, 2H), 6.91 (d, *J* = 8.4 Hz, 1H); 6.96 (d, *J* = 8.4 Hz, 2H), 7.04–7.08 (m, 2H), 7.46 (d, *J* = 8.4 Hz, 2H).

**(R)-N<sup>6</sup>-(2-(4-(3',4'-Dimethoxybiphenyl-4-yl)piperazin-1-yl)ethyl)-N<sup>6</sup>-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine ((+)-8a).** Compound **7a** (100 mg, 0.293 mmol) was reacted with (+)-pramipexole (51 mg, 0.293 mmol) and NaBH(OAc)<sub>3</sub> (112 mg, 0.53 mmol) in dichloromethane (20 mL) using procedure G to yield compound (+)-**8a** (100 mg, 64%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz): δ 0.87 (t, *J* = 7.2 Hz, 3H), 1.43–1.49 (m, 2H), 1.66–1.74 (m, 1H), 1.97–2.01 (m, 2H), 2.46–2.55 (m, 6 H), 2.66–2.73 (m, 7 H), 3.03 (s, 1H), 3.22–3.26 (m, 4H), 3.89 (s, 3H), 3.93 (s, 3H), 4.8 (bs, 2H), 6.89–6.90 (m, 1H), 6.96 (d, *J* = 7.8 Hz, 2H), 7.04–7.08 (m, 2H), 7.45 (d, *J* = 7.8 Hz, 2H).

**N<sup>6</sup>-(2-(4-(2',5'-Dimethoxybiphenyl-4-yl)piperazin-1-yl)ethyl)-N<sup>6</sup>-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine ((±)-8b).** Compound **7b** (200 mg, 0.585 mmol) was reacted with (±)-pramipexole (123.62 mg, 0.585 mmol) and NaBH(OAc)<sub>3</sub> (223 mg, 1.05 mmol) in dichloromethane (10 mL) by following procedure G to yield compound (±)-**8b** (205 mg, 65%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 0.92 (t, *J* = 7.6 Hz, 3H), 1.52–1.61 (m, 2H), 1.73–1.77 (m, 1H), 2.03 (d, *J* = 4.0 Hz, 2H), 2.41–2.89 (m, 13 H), 3.00–3.17 (m, 5H), 3.73 (s, 3H), 3.84 (s, 3H), 4.76 (bs, 2H), 6.79 (dd, *J* = 8.8 Hz, 3.2 Hz, 1H), 6.87–6.95 (m, 2H), 6.94 (d, *J* = 8.8 Hz, 2H), 7.44 (d, *J* = 8.8 Hz, 2H).

**(S)-N<sup>6</sup>-(2-(4-(2',5'-Dimethoxybiphenyl-4-yl)piperazin-1-yl)ethyl)-N<sup>6</sup>-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine ((–)-8b).** Compound **7b** (435 mg, 1.27 mmol) was reacted with (–)-pramipexole (268 mg, 1.27 mmol) and NaBH(OAc)<sub>3</sub> (485 mg, 2.28 mmol) in dichloromethane (50 mL) by following procedure G to yield compound (–)-**8b** (424 mg, 60%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 0.92 (t, *J* = 7.6 Hz, 3H), 1.52–1.61 (m, 2H), 1.73–1.77 (m, 1H), 2.03 (d, *J* = 4.0 Hz, 2H), 2.41–2.89 (m, 13 H), 3.00–3.17 (m, 5H), 3.73 (s, 3H), 3.78 (s, 3H), 5.11 (bs, 2H), 6.79 (dd, *J* = 8.8 Hz, 3.2 Hz, 1H), 6.87–6.95 (m, 2H), 6.94 (d, *J* = 8.8 Hz, 2H), 7.44 (d, *J* = 8.8 Hz, 2H).

**(R)-N<sup>6</sup>-(2-(4-(2',5'-Dimethoxy-[1,1'-biphenyl]-4-yl)piperazin-1-yl)ethyl)-N<sup>6</sup>-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine ((+)-8b).** Compound **7b** (200 mg, 0.585 mmol) was reacted with (±)-pramipexole (123.62 mg, 0.585 mmol) and NaBH(OAc)<sub>3</sub> (223 mg, 1.05 mmol) in dichloromethane (10 mL) by following procedure G to yield compound (+)-**8b** (203 mg, 65%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz): δ 0.89 (t, *J* = 7.2 Hz, 3H), 1.46–1.52 (m, 2H), 1.71–1.78 (m, 1H), 2.01 (bs, 2H), 2.49–2.57 (m, 6 H), 2.68–2.75 (m, 7 H), 3.01–3.21 (m, 5H), 3.74 (s, 3H), 3.84 (s, 3H), 4.76 (bs, 2H), 6.79 (dd, *J* = 8.8 Hz, 3.2 Hz, 1H), 6.87–6.95 (m, 2H), 6.94 (d, *J* = 8.8 Hz, 2H), 7.44 (d, *J* = 8.8 Hz, 2H).

**Procedure H. 4'-(4-(2-((2-Amino-4,5,6,7-tetrahydro-benzothiazol-6-yl)propyl-amino)ethyl)piperazin-1-yl)-biphenyl-3,4-diol ((±)-9a).** A mixture of compound **8a** (200 mg, 0.37 mmol) and 48% aqueous HBr (10 mL) was refluxed for 6 h. The reaction mixture was then evaporated to dryness in vacuo. The crude mixture was then washed with diethyl ether and finally recrystallized from ethanol to afford compound (±)-**9a** as a blue solid (145 mg, 65%). mp 209–214 °C. <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz): δ 1.07 (t, *J* = 7.2 Hz, 3H), 1.88–2.06 (m, 2H), 2.12–2.30 (m, 1H), 2.55 (d, *J* = 10 Hz, 1H), 2.74–2.90 (m, 2H), 2.95–3.46 (m, 4H), 3.52–4.20 (m, 13H), 6.82 (d, *J* = 8.0 Hz, 1H), 6.93 (dd, *J* = 8.4 Hz, 1.6 Hz, 1H), 7.03 (d, *J* = 1.6 Hz, 1H), 7.27 (d, *J* = 8.4 Hz, 2H), 7.54 (d, *J* = 8.8 Hz, 2H). mp 209–214 °C. Anal. (C<sub>28</sub>H<sub>37</sub>N<sub>5</sub>O<sub>2</sub>S·5.0HBr·2.0H<sub>2</sub>O): C, H, N.

**(S)-4'-(4-(2-((2-Amino-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)(propyl)amino)ethyl)piperazin-1-yl)biphenyl-3,4-diol ((–)-9a).** Compound (–)-**8a** (200 mg, 0.37 mmol) and 48% aqueous HBr (10 mL) were refluxed for 12 h by following procedure H to afford compound (–)-**9a** as a blue solid (219 mg, 65%), which was recrystallized from ether and ethanol mixture. <sup>1</sup>H NMR (CD<sub>3</sub>OD, 600 MHz): δ 1.06 (t, *J* = 7.8 Hz, 3H), 1.93–1.97 (m, 2H), 2.16–2.26 (m, 1H), 2.51–2.55 (m, 1H), 2.76–2.90 (m, 2H), 3.02–3.10 (m, 1H), 3.22–3.47 (m, 5H), 3.57–3.86 (m, 11H), 4.08 (bs, 1H), 6.80 (d, *J* = 7.8 Hz, 1H), 6.90 (dd, *J* = 9.0 Hz, 1.8 Hz, 1H), 7.0 (d, *J* = 2.4 Hz, 1H),

7.20 (bs, 2H), 7.51 (d,  $J = 8.4$  Hz, 2H).  $[\alpha]_D^{25} = -18.56$  ( $c = 0.6$ , CH<sub>3</sub>OH). Hydrobromide salt, mp 260–265 °C. Anal. (C<sub>28</sub>H<sub>37</sub>N<sub>5</sub>O<sub>2</sub>S·5.0HBr·1.0C<sub>2</sub>H<sub>5</sub>OC<sub>2</sub>H<sub>5</sub>): C, H, N. MS(ES<sup>+</sup>):  $m/z$  calculated for C<sub>28</sub>H<sub>37</sub>N<sub>5</sub>O<sub>2</sub>S [M + H<sup>+</sup>]: calculated, 507.27; found, 508.6.

**(R)-4'-(4-(2-((2-Amino-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)(propyl)amino)ethyl)piperazin-1-yl)biphenyl-3,4-diol ((+)-9a).** Compound (+)-8a (100 mg, 0.19 mmol) and 48% aqueous HBr (10 mL) were refluxed for 12 h by following procedure H to afford compound (+)-9a as a blue solid (119 mg, 70%, recrystallized from ethanol). <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz):  $\delta$  1.08 (t,  $J = 7.2$  Hz, 3H), 1.88–2.06 (m, 2H), 2.12–2.30 (m, 1H), 2.51–2.55 (m, 1H), 2.74–2.90 (m, 2H), 2.95–3.46 (m, 4H), 3.52–4.20 (m, 13H), 6.78–6.83 (m, 1H), 6.93 (dd,  $J = 8.4$  Hz, 1.6 Hz, 1H), 7.08–7.10 (m, 1H), 7.23 (d,  $J = 8.0$  Hz, 2H), 7.46–7.54 (m, 2H). C, H, N.  $[\alpha]_D^{25} = +20.69$  ( $c = 0.6$ , CH<sub>3</sub>OH). Hydrobromide salt, mp 257–262 °C. Anal. (C<sub>28</sub>H<sub>37</sub>N<sub>5</sub>O<sub>2</sub>S·5.0HBr·1.0C<sub>2</sub>H<sub>5</sub>OC<sub>2</sub>H<sub>5</sub>): C, H, N.

**4'-(4-(2-((2-Amino-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)(propyl)amino)ethyl)piperazin-1-yl)biphenyl-2,5-diol ((±)-9b).** Compound (±)-8b (200 mg, 0.37 mmol) and 48% aqueous HBr (10 mL) were refluxed for 12 h by following procedure H to afford compound (±)-9b as a gray solid (243 mg, 71%, recrystallized from ethanol). <sup>1</sup>H NMR of HBr salt (CD<sub>3</sub>OD, 600 MHz):  $\delta$  1.07 (t,  $J = 7.6$  Hz, 3H), 1.92–1.96 (m, 2H), 2.18–2.21 (m, 1H), 2.51–2.53 (bs, 1H), 2.82 (bs, 2H), 3.01–3.24 (m, 4H), 3.54–4.08 (m, 13H), 6.58 (dd,  $J = 8.4$  Hz, 2.4 Hz, 1H), 6.68–6.80 (m, 2H), 7.10–7.28 (m, 2H), 7.48–7.63 (m, 2H). Hydrobromide salt, mp 265–270 °C. Anal. (C<sub>28</sub>H<sub>37</sub>N<sub>5</sub>O<sub>2</sub>S·5.0HBr·1.0C<sub>2</sub>H<sub>5</sub>OC<sub>2</sub>H<sub>5</sub>): C, H, N.

**(S)-4'-(4-(2-((2-Amino-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)(propyl)amino)ethyl)piperazin-1-yl)biphenyl-2,5-diol ((-)-9b).** Compound (-)-8b (400 mg, 0.75 mmol) and 48% aqueous HBr (25 mL) were refluxed for 12 h by following procedure H to afford compound (-)-9b as a blue solid (439 mg, 65%, recrystallized from ethanol). <sup>1</sup>H NMR of HBr salt (CD<sub>3</sub>OD, 400 MHz):  $\delta$  1.07 (t,  $J = 7.6$  Hz, 3H), 1.91–1.97 (m, 2H), 2.12–2.30 (m, 1H), 2.50 (d,  $J = 8.0$  Hz, 1H), 2.81 (m, 2H), 3.0–3.12 (m, 4H), 3.54–4.08 (m, 13H), 6.58 (dd,  $J = 8.8$  Hz, 3.2 Hz, 1H), 6.68–6.72 (m, 2H), 7.20–7.28 (m, 2H), 7.56 (d,  $J = 8.0$  Hz, 2H).  $[\alpha]_D^{25} = -18.0$  ( $c = 0.1$ , CH<sub>3</sub>OH). Hydrobromide salt, mp 265–270 °C. Anal. (C<sub>28</sub>H<sub>37</sub>N<sub>5</sub>O<sub>2</sub>S·5.0HBr·1.3H<sub>2</sub>O): C, H, N.

**(R)-4'-(4-(2-((2-Amino-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)(propyl)amino)ethyl)piperazin-1-yl)-[1,1'-biphenyl]-2,5-diol ((+)-9b).** Compound (+)-8d (150 mg, 0.280 mmol) and 48% aqueous HBr (5 mL) were refluxed for 12 h by following procedure H to afford compound (+)-9d as a blue solid (180 mg, 70%, recrystallized from ethanol). <sup>1</sup>H NMR of HBr salt (CD<sub>3</sub>OD, 400 MHz):  $\delta$  1.08 (t,  $J = 7.6$  Hz, 3H), 1.91–2.01 (m, 2H), 2.16–2.27 (m, 1H), 2.54 (d,  $J = 10.0$  Hz, 1H), 2.81 (m, 2H), 2.98–3.11 (m, 2H), 3.23–3.28 (m, 2H), 3.36–4.10 (m, 13H), 6.58–6.63 (m, 1H), 6.69–6.73 (m, 2H), 7.23–7.25 (m, 2H), 7.57 (d,  $J = 8.0$  Hz, 2H).  $[\alpha]_D^{25} = +15.0$  ( $c = 0.1$ , CH<sub>3</sub>OH). Hydrobromide salt, mp 260–265 °C. Anal. (C<sub>28</sub>H<sub>37</sub>N<sub>5</sub>O<sub>2</sub>S·5.0HBr·1.0C<sub>2</sub>H<sub>5</sub>OC<sub>2</sub>H<sub>5</sub>): C, H, N.

**Procedure I. 2-(4-(4-(2-((2-Amino-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)(propyl)amino)ethyl)piperazin-1-yl)phenyl)cyclohexa-2,5-diene-1,4-dione ((±)-10).** Into the solution of free base of compound (±)-9b (200 mg, 0.396 mmol) in dichloromethane (10 mL) was added MnO<sub>2</sub> (140 mg, 1.58 mmol), and the mixture stirred for 1 h at room temperature. The reaction mixture was filtered through Celite. The crude compound thus obtained was further purified through silica gel column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 9:2) to afford compound (±)-10 as an oil (129 mg, 65%). NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  0.88 (t,  $J = 7.2$  Hz, 3H), 1.47–1.53 (m, 2H), 1.71–1.75 (m, 1H), 2.01 (d,  $J = 10.0$  Hz, 1H), 2.54–2.73 (m, 14H), 3.10–3.30 (m, 5H), 4.85 (bs, 2H), 6.77–6.81 (m, 3H), 6.90 (d,  $J = 9.2$  Hz, 2H), 7.46 (d,  $J = 8.8$  Hz, 2H). The product was converted into corresponding hydrochloride salt, mp 265–270 °C. Anal. (C<sub>28</sub>H<sub>35</sub>N<sub>5</sub>O<sub>2</sub>S·5.0HCl·1.0 C<sub>2</sub>H<sub>5</sub>O C<sub>2</sub>H<sub>5</sub>·3.0H<sub>2</sub>O): C, H, N.

**Procedure J. 1-(3-Methoxy-phenyl)-piperazine (12a).** A stirring solution of 3-methoxy-phenylamine, 11a (15.0 g, 121.95 mmol), and bis(2-chloroethyl)amine was heated at 150 °C in diethylene glycol monomethyl ether (10 mL) for 6 h. After cooling to room temperature, the mixture was dissolved in MeOH (4 mL)

followed by addition of Et<sub>2</sub>O (150 mL). The precipitate was filtered off and washed with Et<sub>2</sub>O to provide HCl salt. The HCl salt was further converted to free amine by treatment with Na<sub>2</sub>CO<sub>3</sub> solution and extracted with EtOAc (2 × 100 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuo to provide the pure free amine product 12a (17.8 g, 76%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  3.07 (t,  $J = 4.2$  Hz, 4H), 3.19 (t,  $J = 4.2$  Hz, 4H), 3.79 (s, 3H), 6.40 (dd,  $J = 8.4$  Hz, 1.6 Hz, 1H), 6.45 (d,  $J = 8.4$  Hz, 1.6 Hz, 1H), 6.51 (dd,  $J = 8.4$  Hz, 1.6 Hz, 1H), 7.15 (t,  $J = 8.4$  Hz, 1H).

**1-(2,5-Dimethoxyphenyl)piperazine (12b).** Commercially available 2,5-dimethoxyaniline 11c (10.0 g, 65.32 mmol) and bis(2-chloroethyl)amine (11.65 g, 65.32 mmol) were heated at 150 °C in diethylene glycol monomethyl ether (100 mL) by following procedure J to yield compound 12a (9.28 g, 65% yield). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  3.07 (d,  $J = 5.2$  Hz, 8H), 3.75 (s, 3H), 3.81 (s, 3H), 6.46–6.54 (m, 2H), 6.76 (d,  $J = 8.4$  Hz, 1H).

**1-(4-Iodo-3-methoxy-phenyl)-piperazine (13a).** Amine 12a (10.0 g, 52.0 mmol) was reacted with ICl (8.45 g, 52.0 mmol) in acetic acid/water (3:1, 21 mL) by following procedure A. The crude product 13a (14.74 g, 89%) thus obtained was converted into its Boc derivative without further purification.

**1-(4-Iodo-2,5-dimethoxyphenyl)piperazine (13b).** Amine 12b (8.0 g, 36.0 mmol) was reacted with ICl (5.84 g, 36.0 mmol) in acetic acid/water (3:1, 100 mL) by following procedure A. The crude product 13b (9.98 g, 80%) thus obtained was converted into its Boc derivative without further purification.

**Procedure K. 4-(4-Iodo-3-methoxy-phenyl)-piperazine-1-carboxylic Acid tert-Butyl Ester (14a).** Into a stirring solution of amine 13a (14.0 g, 44.03 mmol) in dichloromethane (40 mL), (Boc)<sub>2</sub>O (9.62 g, 44.03 mmol) and Et<sub>3</sub>N (17.61 mL, 132.09 mmol) were added at room temperature. The reaction mixture was stirred at the same temperature for 12 h and was extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 100 mL), washed with water, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The crude material was purified by column chromatography over silica gel (methanol/EtOAc, 0.5:9.5) to give compound 14a (16.74 g, 91%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  1.49 (s, 9H), 3.14 (t,  $J = 4.8$  Hz, 4H), 3.58 (t,  $J = 5.2$  Hz, 4H), 3.86 (s, 3H), 6.32 (dd,  $J = 8.4$  Hz, 2.4 Hz, 1H), 6.42 (d,  $J = 2.4$  Hz, 1H), 7.58 (d,  $J = 8.8$  Hz, 1H).

**tert-Butyl 4-(4-iodo-2,5-dimethoxyphenyl)piperazine-1-carboxylate (14b).** Amine, 13b (9.0 g, 25.85 mmol) dissolved in dichloromethane (100 mL) along with (Boc)<sub>2</sub>O (5.64 g, 25.85 mmol) and Et<sub>3</sub>N (10.75 mL, 77.57 mmol) were reacted by following procedure K to give compound 14b (11.0 g, 95%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  1.49 (s, 9H), 2.98 (bs, 4H), 3.61 (t,  $J = 4.0$ , 4H), 3.81 (s, 3H), 3.83 (s, 3H), 6.52 (s, 1H), 7.01 (s, 1H).

**4-(2,2'-Dimethoxy-biphenyl-4-yl)-piperazine-1-carboxylic Acid tert-Butyl Ester (15a).** A stirring solution of iodo compound 14a (10.0 g, 23.92 mmol), 2-methoxybenzene boronic acid (3.68 g, 23.92 mmol), Pd(PPh<sub>3</sub>)<sub>4</sub> (789 mg, 0.68 mmol), and Na<sub>2</sub>CO<sub>3</sub> (5.0 g, 47.17 mmol) in a mixture of solvent dimethoxy ethane and ethanol (40 mL: 40 mL) were heated at 95 °C for 2 h as described in procedure B to afford compound 15a (7.05 g, 74%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  1.49 (s, 9H), 3.19 (t,  $J = 4.8$  Hz, 4H), 3.60 (t,  $J = 4.8$  Hz, 4H), 3.76 (s, 3H), 3.77 (s, 3H), 6.52–6.64 (m, 2H), 6.96 (d,  $J = 8.4$  Hz, 2.4 Hz, 1H), 6.99 (d,  $J = 7.2$  Hz, 1H), 7.15 (d,  $J = 9.2$  Hz, 1H), 7.20–7.36 (m, 2H).

**tert-Butyl 4-(2,5-dimethoxybiphenyl-4-yl)piperazine-1-carboxylate (15b).** Commercially available benzene boronic acid (2.72 g, 22.31 mmol) was reacted with iodo compound 14b (10.0 g, 22.31 mmol), Pd(PPh<sub>3</sub>)<sub>4</sub> (1.28 g, 1.11 mmol) and Na<sub>2</sub>CO<sub>3</sub> (4.72 g, 44.62 mmol) in a mixture of solvent dimethoxy ethane and ethanol (40 mL/40 mL) by following procedure B to afford compound 15b (6.16 g, 70%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  1.49 (s, 9H), 3.07 (t,  $J = 4.4$  Hz, 4H), 3.62 (t,  $J = 5.2$  Hz, 4H), 3.75 (s, 3H), 3.87 (s, 3H), 6.60 (s, 1H), 6.85 (s, 1H), 7.23–7.31 (m, 1H), 7.39 (t,  $J = 8.0$ , 2H), 7.53 (d,  $J = 7.2$ , 2H).

**1-(2,2'-Dimethoxy-biphenyl-4-yl)piperazine (16a).** Compound 15a (3.5 g, 8.79 mmol) was reacted with TFA (20 mL) in CHCl<sub>3</sub> (20 mL) by following procedure C to give compound 16a (2.54 g, 97%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  1.98 (bs, 1H), 3.06 (t,

$J = 4.8$  Hz, 4H), 3.21 (t,  $J = 4.4$  Hz, 4H), 3.76 (s, 3H), 3.77 (s, 3H), 6.52–6.64 (m, 2H), 6.96 (d,  $J = 8.0$  Hz, 2.4 Hz, 1H), 6.99 (d,  $J = 7.2$  Hz, 1H), 7.14 (d,  $J = 8.8$  Hz, 1H), 7.20–7.34 (m, 2H).

**1-(2,5-Dimethoxy-[1,1'-biphenyl]-4-yl)piperazine (16b).** Compound **15b** (6.0 g, 15.06 mmol) was reacted with TFA (20 mL) in  $\text{CHCl}_3$  (20 mL) by following procedure C to give compound **16b** (3.6 g, 80%).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  3.20 (bs, 8H), 3.75 (s, 3H), 3.85 (s, 3H), 6.62 (s, 1H), 6.85 (s, 1H), 7.30 (t,  $J = 7.2$  Hz, 1H), 7.40 (t,  $J = 8.4$  Hz, 2H), 7.52 (t,  $J = 8.0$  Hz, 2H).

**1-[2-(tert-Butyl-dimethyl-silanyloxy)-ethyl]-4-(2,2'-dimethoxy-biphenyl-4-yl)piperazine (17a).** Compound **16a** (2.37 g, 7.95 mmol) was reacted with (2-bromo-ethyl)-tert-butyl dimethylsilane (2.28 g, 9.56 mmol) and  $\text{K}_2\text{CO}_3$  (3.17 g, 22.9 mmol) in  $\text{CH}_3\text{CN}$  (30 mL) by following procedure D. The crude residue was purified by column chromatography (ethyl acetate/hexane, 2:3) to afford compound **17a** (3.45 g, 95%).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  0.09 (s, 6H), 0.92 (s, 9H), 2.61 (t,  $J = 6.4$  Hz, 2H), 2.71 (t,  $J = 4.8$  Hz, 4H), 3.26 (t,  $J = 4.8$  Hz, 2H), 3.77 (s, 3H), 3.78 (s, 3H), 3.82 (t,  $J = 6.4$  Hz, 2H), 6.53–6.61 (m, 2H), 6.93–7.02 (m, 2H), 7.14 (d,  $J = 8.8$  Hz, 1H), 7.22–7.33 (m, 2H).

**1-(2-(tert-Butyldimethylsilyloxy)ethyl)-4-(2,5-dimethoxybiphenyl-4-yl)piperazine (17b).** Compound **16b** (3.0 g, 10.06 mmol) was reacted with (2-bromo-ethyl)-tert-butyl dimethylsilane (2.88 g, 12.07 mmol) and  $\text{K}_2\text{CO}_3$  (3.20 g, 30.18 mmol) in  $\text{CH}_3\text{CN}$  (50 mL) by following procedure D. The crude residue was purified by column chromatography (ethyl acetate/hexane, 2:3) to afford compound **17b** (3.90 g, 82%).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  0.09 (s, 6H), 0.92 (s, 9H), 2.61 (t,  $J = 6.8$  Hz, 2H), 2.75 (bs, 4H), 3.16 (bs, 4H), 3.74 (s, 3H), 3.78 (t,  $J = 6.8$  Hz, 2H), 3.84 (s, 3H), 6.34 (s, 1H), 6.84 (s, 1H), 7.25–7.31 (m, 1H), 7.39 (t,  $J = 7.6$  Hz, 2H), 7.53 (d,  $J = 6.8$  Hz, 2H).

**2-[4-(2,2'-Dimethoxy-biphenyl-4-yl)-piperazin-1-yl]-ethanol (18a).** Compound **17a** (3.4 g, 7.46 mmol) was reacted with *n*-tetrabutylammonium fluoride (1.95 g, 7.46 mmol, 1.0 M solution in THF) in THF (30 mL) by following procedure E. The crude product was purified by silica gel column chromatography (EtOAc/MeOH, 9:1) to yield compound **18a** (2.38 g, 94%).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  2.64 (t,  $J = 5.2$  Hz, 2H), 2.70 (t,  $J = 5.2$  Hz, 4H), 2.81 (bs, 1H), 3.28 (t,  $J = 4.8$  Hz, 4H), 3.69 (q,  $J = 4.8$  Hz, 2H), 3.77 (s, 3H), 3.78 (s, 3H), 6.53–6.62 (m, 2H), 6.97 (d,  $J = 8.0$  Hz, 1H), 7.00 (dd,  $J = 7.6$  Hz, 1.2 Hz, 1H), 7.15 (d,  $J = 8.8$  Hz, 1H), 7.22–7.27 (m, 1H), 7.30 (dt,  $J = 8.0$  Hz, 1.6 Hz, 1H).

**2-[4-(2,5-Dimethoxybiphenyl-4-yl)piperazin-1-yl]-ethanol (18b).** Compound **17b** (3.8 g, 8.32 mmol) was reacted with *n*-tetrabutylammonium fluoride (2.17 g, 8.32 mmol, 1.0 M solution in THF) in THF (30 mL) by following procedure E. The crude product was purified by silica gel column chromatography (EtOAc/MeOH, 9:1) to yield compound **18b** (2.21 g, 86%).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  2.62 (t,  $J = 5.6$  Hz, 2H), 2.73 (bs, 5H), 3.16 (bs, 4H), 3.65 (t,  $J = 5.6$  Hz, 2H), 3.74 (s, 3H), 3.84 (s, 3H), 6.62 (s, 1H), 6.83 (s, 1H), 7.28–7.30 (m, 1H), 7.38 (t,  $J = 8.0$  Hz, 2H), 7.51 (d,  $J = 6.4$  Hz, 2H).

**[4-(2,2'-Dimethoxy-biphenyl-4-yl)-piperazin-1-yl]-acetaldehyde (19a).** Compound **18a** (600 mg, 1.75 mmol) was reacted with oxalyl chloride (0.30 mL, 3.50 mmol), DMSO (0.50 mL, 6.95 mmol), and  $\text{Et}_3\text{N}$  (1.44 mL, 10.52 mmol) in dichloromethane (30 mL) by following procedure F. The crude product was purified by silica gel column chromatography (EtOAc/MeOH, 9.5:0.5) to yield compound **19a** (420 mg, 85%).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  2.60–2.80 (m, 4H), 2.92–3.24 (m, 6H), 3.75 (s, 3H), 3.78 (s, 3H), 6.66 (d,  $J = 7.6$  Hz, 1H), 6.96 (dd,  $J = 8.0$  Hz, 1H), 7.00 (dd,  $J = 7.6$  Hz, 0.8 Hz, 1H), 7.20 (dt,  $J = 5.6$  Hz, 3.6 Hz, 1H), 7.23 (s, 1H), 7.32 (t,  $J = 7.6$  Hz, 1H), 7.40–7.26 (m, 1H), 9.76 (s, 1H).

**2-[4-(2,5-Dimethoxybiphenyl-4-yl)piperazin-1-yl]-acetaldehyde (19b).** Compound **18b** (1.2 g, 3.50 mmol) was reacted with oxalyl chloride (0.60 mL, 7.01 mmol), DMSO (1.0 mL, 14.0 mmol), and  $\text{Et}_3\text{N}$  (2.91 mL, 21.0 mmol) in dichloromethane (30 mL) by following procedure F. The crude product was purified by silica gel column chromatography (EtOAc/MeOH, 9.5:0.5) to yield compound **19b** (834 mg, 70%).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  2.76–2.83 (m, 4H), 3.22 (m, 4H), 3.75 (s, 2H), 3.85 (s, 3H), 6.64 (s, 1H), 6.85 (s,

1H), 7.27–7.32 (t,  $J = 7.6$  Hz, 1H), 7.37–7.41 (t,  $J = 8.0$  Hz, 2H), 7.52–7.54 (d,  $J = 8.0$  Hz, 2H), 9.76 (s, 1H).

**$N^6$ -[2-[4-(2,2'-Dimethoxy-biphenyl-4-yl)-piperazin-1-yl]-ethyl]- $N^6$ -propyl-4,5,6,7-tetrahydro-benzothiazole-2,6-diamine (( $\pm$ )-20a).** Compound **19a** (200 mg, 0.58 mmol) was reacted with ( $\pm$ )-pramipexole (138 mg, 0.65 mmol) and  $\text{NaBH}(\text{OAc})_3$  (226 mg, 1.06 mmol) in dichloromethane (10 mL) by following procedure G. The crude product was purified by silica gel column chromatography (EtOAc/MeOH, 9:1) to yield compound **20a** (222 mg, 70%).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  0.90 (t,  $J = 7.2$  Hz, 3H), 1.40–1.59 (m, 2H), 1.62–1.85 (m, 1H), 1.92–2.14 (m, 1H), 2.36–3.30 (m, 19H), 3.75 (s, 3H), 3.78 (s, 3H), 5.09 (s, 2H), 6.67 (d,  $J = 8$  Hz, 1H), 6.96 (d,  $J = 8.0$  Hz, 0.8 Hz, 1H), 7.00 (d,  $J = 7.6$  Hz, 3.6 Hz, 1H), 7.16–7.44 (m, 4H).

**(S)- $N^6$ -[2-[4-(2,2'-Dimethoxy-biphenyl-4-yl)-piperazin-1-yl]-ethyl]- $N^6$ -propyl-4,5,6,7-tetrahydro-benzothiazole-2,6-diamine ((-)-20a).** Compound **19a** (200 mg, 0.58 mmol) was reacted with (-)-pramipexole (138 mg, 0.65 mmol) and  $\text{NaBH}(\text{OAc})_3$  (226 mg, 1.06 mmol) in dichloromethane (10 mL) by following procedure G. The crude product was purified by silica gel column chromatography (EtOAc/MeOH, 9:1) to yield compound (-)-**20a** (235 mg, 74%).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  0.90 (t,  $J = 7.2$  Hz, 3H), 1.40–1.59 (m, 2H), 1.64–1.87 (m, 1H), 1.90–2.15 (m, 1H), 2.36–3.30 (m, 19H), 3.75 (s, 3H), 3.78 (s, 3H), 5.09 (s, 2H), 6.67 (d,  $J = 8$  Hz, 1H), 6.96 (d,  $J = 8.0$  Hz, 0.8 Hz, 1H), 7.00 (d,  $J = 7.6$  Hz, 3.6 Hz, 1H), 7.17–7.47 (m, 4H).

**(R)- $N^6$ -[2-[4-(2,2'-Dimethoxy-biphenyl-4-yl)-piperazin-1-yl]-ethyl]- $N^6$ -propyl-4,5,6,7-tetrahydro-benzothiazole-2,6-diamine ((+)-20a).** Compound **19a** (200 mg, 0.58 mmol) was reacted with (+)-pramipexole (138 mg, 0.65 mmol) and  $\text{NaBH}(\text{OAc})_3$  (226 mg, 1.06 mmol) in dichloromethane (10 mL) by following procedure G. The crude product was purified by silica gel column chromatography (EtOAc/MeOH, 9:1) to yield compound (+)-**20a** (238 mg, 75%).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  0.92 (t,  $J = 7.2$  Hz, 3H), 1.41–1.59 (m, 2H), 1.64–1.87 (m, 1H), 1.90–2.15 (m, 1H), 2.36–3.30 (m, 19H), 3.75 (s, 3H), 3.78 (s, 3H), 5.09 (s, 2H), 6.67 (d,  $J = 8$  Hz, 1H), 6.96 (d,  $J = 8.0$  Hz, 0.8 Hz, 1H), 7.01 (d,  $J = 7.6$  Hz, 3.6 Hz, 1H), 7.17–7.47 (m, 4H).

**$N^6$ -(2-[4-(2,5-Dimethoxybiphenyl-4-yl)piperazin-1-yl]ethyl)- $N^6$ -propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine (( $\pm$ )-20b).** Compound **19b** (300 mg, 0.88 mmol) was reacted with ( $\pm$ )-pramipexole (185 mg, 0.88 mmol) and  $\text{NaBH}(\text{OAc})_3$  (334 mg, 1.58 mmol) in dichloromethane (10 mL) by following procedure G. The crude product was purified by silica gel column chromatography (EtOAc/MeOH, 9:1) to yield compound ( $\pm$ )-**20b** (456 mg, 65%).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  0.89 (t,  $J = 7.2$  Hz, 3H), 1.45–1.48 (m, 2H), 1.69–1.74 (m, 1H), 1.97–2.02 (m, 1H), 2.41–2.81 (m, 14H), 3.12–3.22 (m, 5H), 3.72 (s, 3H), 3.85 (s, 3H), 5.22 (bs, 2H), 6.62 (s, 1H), 6.83 (s, 1H), 7.26 (t,  $J = 6.8$  Hz, 1H), 7.37 (t,  $J = 7.6$  Hz, 2H), 7.51 (d,  $J = 7.2$  Hz, 2H).

**(S)- $N^6$ -(2-[4-(2,5-Dimethoxybiphenyl-4-yl)piperazin-1-yl]-ethyl)- $N^6$ -propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine ((-)-20b).** Compound **19b** (500 mg, 1.46 mmol) was reacted with (-)-pramipexole (310 mg, 1.46 mmol) and  $\text{NaBH}(\text{OAc})_3$  (556 mg, 2.62 mmol) in dichloromethane (10 mL) by following procedure G. The crude product was purified by silica gel column chromatography (EtOAc/MeOH, 9:1) to yield compound (-)-**20b** (511 mg, 65%).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 600 MHz):  $\delta$  0.88 (t,  $J = 7.2$  Hz, 3H), 1.49–1.51 (m, 2H), 1.70–1.76 (m, 1H), 1.97–2.02 (m, 1H), 2.53–2.57 (m, 6H), 2.67–2.76 (m, 8H), 3.08–3.26 (m, 5H), 3.73 (s, 3H), 3.78 (s, 3H), 5.22 (bs, 2H), 6.77 (dd,  $J = 3.0$  Hz, 9.0 Hz, 1H), 6.86–6.88 (m, 2H), 6.94 (d,  $J = 8.4$  Hz, 2H), 7.43–7.45 (m, 2H).

**4-[4-(2-[(2-Amino-4,5,6,7-tetrahydro-benzothiazol-6-yl)-propyl-aminol]-ethyl)-piperazin-1-yl]-biphenyl-2,2'-diol (( $\pm$ )-21a).** Compound ( $\pm$ )-**20a** (200 mg, 0.37 mmol) and 48% aqueous HBr (10 mL) were refluxed for 6 h by following procedure H to afford compound ( $\pm$ )-**21a** as a yellow solid (151 mg, 71%, recrystallized from ethanol).  $^1\text{H NMR}$  ( $\text{CD}_3\text{OD}$ , 400 MHz):  $\delta$  1.07 (t,  $J = 7.2$  Hz, 3H), 1.82–2.06 (m, 2H), 2.10–2.30 (m, 1H), 2.54 (d,  $J = 10.8$  Hz, 1H), 2.71–2.84 (m, 2H), 2.86–3.16 (m, 1H), 3.17–4.20 (m, 16 H), 6.82 (d,  $J = 8.4$  Hz, 1H), 6.88–6.96 (m, 2H), 7.12–7.32 (m,

4H). mp 248–252 °C. Anal. (C<sub>28</sub>H<sub>37</sub>N<sub>5</sub>O<sub>2</sub>S·5.0 HBr·0.6H<sub>2</sub>O): C, H, N.

**(S)-4-(4-{2-[(2-Amino-4,5,6,7-tetrahydro-benzothiazol-6-yl)-propyl-amino]-ethyl}-piperazin-1-yl)-biphenyl-2,2'-diol ((-)-21a).** Compound (-)-20a (200 mg, 0.37 mmol) and 48% aqueous HBr (10 mL) were refluxed for 6 h by following procedure H to afford compound (-)-21a as a yellow solid (155 mg, 73%, recrystallized from ethanol). <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz): δ 1.08 (t, J = 7.2 Hz, 3H), 1.83–2.07 (m, 2H), 2.10–2.30 (m, 1H), 2.55 (d, J = 10.8 Hz, 1H), 2.71–2.84 (m, 2H), 2.86–3.16 (m, 1H), 3.18–4.20 (m, 16 H), 6.82 (d, J = 8.4 Hz, 1H), 6.88–6.96 (m, 2H), 7.12–7.32 (m, 4H). mp 205–210 °C. [α]<sub>D</sub> = -18.0 (c = 0.1, CH<sub>3</sub>OH). Anal. (C<sub>28</sub>H<sub>37</sub>N<sub>5</sub>O<sub>2</sub>S·5.0HBr·1.2H<sub>2</sub>O): C, H, N.

**(R)-4-(4-{2-[(2-Amino-4,5,6,7-tetrahydro-benzothiazol-6-yl)-propyl-amino]-ethyl}-piperazin-1-yl)-biphenyl-2,2'-diol ((+)-21a).** Compound (+)-20a (200 mg, 0.37 mmol) and 48% aqueous HBr (10 mL) were refluxed for 6 h by following procedure H to afford compound (+)-21a (155 mg, 73%, recrystallized from ethanol). <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz): δ 1.09 (t, J = 7.2 Hz, 3H), 1.85–2.07 (m, 2H), 2.10–2.30 (m, 1H), 2.53 (d, J = 10.8 Hz, 1H), 2.71–2.84 (m, 2H), 2.86–3.16 (m, 1H), 3.18–4.20 (m, 16 H), 6.82 (d, J = 8.4 Hz, 1H), 6.88–6.96 (m, 2H), 7.12–7.32 (m, 4H). mp 205–210 °C. [α]<sub>D</sub> = +16.3 (c = 0.5, CH<sub>3</sub>OH). Anal. (C<sub>28</sub>H<sub>37</sub>N<sub>5</sub>O<sub>2</sub>S·5.0HBr·1.0H<sub>2</sub>O): C, H, N.

**4-(4-{2-[(2-Amino-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)-(propylamino)ethyl] piperazin-1-yl}-[1,1'-biphenyl]-2,5-diol ((±)-21b).** A mixture of compound (±)-20b (300 mg, 0.560 mmol) and 48% aqueous HBr (10 mL) was refluxed for 12 h followed by procedure H to afford compound (±)-21b as a light brown solid (380 mg, 75%). <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz): δ 1.07 (t, J = 7.2 Hz, 3H), 1.90–1.99 (m, 2H), 2.16–2.23 (m, 1H), 2.48–2.56 (m, 1H), 2.81 (bs, 2H), 2.98–3.25 (m, 4H), 3.54–4.08 (m, 13H), 6.89–7.01 (m, 2H), 7.17–7.56 (m, 5H). Hydrobromide salt, mp 265–270 °C. Anal. (C<sub>28</sub>H<sub>37</sub>N<sub>5</sub>O<sub>2</sub>S·5.0HBr·0.4C<sub>2</sub>H<sub>5</sub>OC<sub>2</sub>H<sub>5</sub>): C, H, N.

**(S)-4-(4-{2-[(2-Amino-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)-(propylamino)ethyl] piperazin-1-yl}-[1,1'-biphenyl]-2,5-diol ((-)-21b).** A mixture of compound (-)-20b (60 mg, 0.746 mmol) and 48% aqueous HBr (10 mL) was refluxed for 12 h followed by procedure H to afford compound (-)-21b as a blue solid (73 mg, 70%). <sup>1</sup>H NMR (CD<sub>3</sub>OD, 400 MHz): δ 1.07 (t, J = 7.2 Hz, 3H), 1.90–1.99 (m, 2H), 2.16–2.23 (m, 1H), 2.48–2.56 (m, 1H), 2.81 (bs, 2H), 2.98–3.25 (m, 4H), 3.54–4.08 (m, 13H), 6.89–6.90 (m, 2H), 7.17–7.55 (m, 5H). [α]<sub>D</sub> = -22.18 (c = 0.55, CH<sub>3</sub>OH). Hydrobromide salt, mp 248–253 °C. Anal. (C<sub>28</sub>H<sub>37</sub>N<sub>5</sub>O<sub>2</sub>S·5.0HBr·1.2H<sub>2</sub>O): C, H, N.

**2-(4-{2-[(2-Amino-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)-(propylamino)ethyl]piperazin-1-yl}-5-phenylcyclohexa-2,5-diene-1,4-dione ((±)-22).** Into the solution of free base of compound (±)-21b (100 mg, 0.20 mmol) in dichloromethane (10 mL) was added MnO<sub>2</sub> (140 mg, 0.8 mmol), and the mixture was stirred for 1 h at room temperature by following procedure I to yield compound (±)-22 (59 mg, 60%) as an oil. NMR (CDCl<sub>3</sub>, 400 MHz): δ 0.89 (t, J = 6.4 Hz, 3H), 1.52–2.03 (m, 4H), 2.54–2.73 (m, 15H), 3.47 (bs, 4H), 4.85 (bs, 2H), 5.84 (s, 1H), 6.64 (s, 1H), 7.30–7.46 (m, 5H). The product was converted into corresponding hydrochloride salt, mp 235–240 °C. Anal. (C<sub>28</sub>H<sub>35</sub>N<sub>5</sub>O<sub>2</sub>S·5.0HCl·2.0C<sub>2</sub>H<sub>5</sub>OC<sub>2</sub>H<sub>5</sub>·1.0H<sub>2</sub>O): C, H, N.

**(S)-N-(2-(4-(2,2'-Dimethoxy-[1,1'-biphenyl]-4-yl)piperazin-1-yl)ethyl)-5-methoxy-N-propyl-1,2,3,4-tetrahydronaphthalen-2-amine ((-)-23a).** Compound 19a (70 mg, 0.21 mmol) was reacted with (S)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (50 mg, 0.23 mmol) and NaBH(OAc)<sub>3</sub> (79 mg, 0.37 mmol) in dichloromethane (10 mL) by following procedure G. The crude product was purified by silica gel column chromatography (EtOAc/hexane, 3:2) to yield compound (-)-23a (67 mg, 75%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 0.92 (t, J = 7.2 Hz, 3H), 1.40–1.68 (m, 3H), 2.01–2.18 (m, 1H), 2.36–3.28 (m, 19H), 3.79 (s, 3H), 3.81 (s, 3H), 3.82 (s, 3H), 6.68 (t, J = 9.2 Hz, 1H), 6.73 (d, J = 8 Hz, 1H), 6.90–7.04 (m, 2H), 7.10 (t, J = 8 Hz, 1H), 7.16–7.29 (m, 1H), 7.30–7.43 (m, 4H).

**(S)-N-(2-(4-(3',4'-Dimethoxybiphenyl-4-yl)piperazin-1-yl)ethyl)-5-methoxy-N-propyl-1,2,3,4-tetrahydronaphthalen-2-amine ((-)-23b).** Compound 7b (200 mg, 0.59 mmol) was reacted with (S)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (130 mg, 0.59 mmol) and NaBH(OAc)<sub>3</sub> (225 mg, 1.06 mmol) in dichloromethane (50 mL) by following procedure G. The crude product was purified by silica gel column chromatography (EtOAc/hexane, 4:1) to yield compound (-)-23b (205 mg, 65%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 0.92 (t, J = 7.2 Hz, 3H), 1.40–1.68 (m, 3H), 2.01–2.18 (m, 1H), 2.36–3.28 (m, 19H), 3.81 (s, 3H), 3.91 (s, 3H), 3.93 (s, 3H), 6.65 (d, J = 8.4 Hz, 1H), 6.70 (d, J = 7.2 Hz, 1H), 6.92 (d, J = 8.0 Hz, 1H), 6.98 (d, J = 8.0 Hz, 2H), 7.06–7.11 (m, 3H), 7.46 (d, J = 8.8 Hz, 2H).

**(S)-4-(4-{2-[(5-Hydroxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amino]-ethyl}-piperazin-1-yl)-biphenyl-2,2'-diol ((-)-24a).** Compound (-)-23a (80 mg, 0.15 mmol) dissolved in 48% aqueous HBr (10 mL) was refluxed for 6 h by following procedure H to afford compound (-)-24a as a yellow solid (62 mg, 73%, recrystallized from ethanol). <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD): δ 1.08 (t, J = 7.8 Hz, 3H), 1.80–2.04 (m, 3H), 2.36–2.48 (m, 1H), 2.60–2.84 (m, 1H), 3.00–4.02 (m, 18 H), 6.62 (d, J = 7.6 Hz, 1H), 6.68 (d, J = 7.6 Hz, 1H), 6.80–7.04 (m, 3H), 7.05–7.4 (m, 5H). [α]<sub>D</sub> = -23.2 (c = 1, CH<sub>3</sub>OH). mp 175–180 °C. Anal. (C<sub>31</sub>H<sub>39</sub>N<sub>3</sub>O<sub>3</sub>S·3.0HBr·1.5CH<sub>2</sub>Cl<sub>2</sub>): C, H, N.

**(S)-4'-(4-(2-[(5-Hydroxy-1,2,3,4-tetrahydronaphthalen-2-yl)-(propylamino)ethyl]piperazin-1-yl)biphenyl-3,4-diol ((-)-24b) (D-520).** Compound (-)-23b (100 mg, 0.183 mmol) and 48% aqueous HBr (5 mL) were refluxed for 12 h by following procedure H to afford compound (-)-24b as a blue solid (95 mg, 70%, recrystallized from ether and ethanol mixture). <sup>1</sup>H NMR of HBr salt (400 MHz, CD<sub>3</sub>OD): δ 1.08 (t, J = 7.8 Hz, 3H), 1.80–2.04 (m, 3H), 2.41–2.51 (m, 1H), 2.60–2.80 (m, 1H), 3.09–3.97 (m, 18 H), 6.62 (d, J = 8.4 Hz, 1H), 6.68 (d, J = 8.0 Hz, 1H), 6.80 (d, J = 8.0 Hz, 1H), 6.90–7.01 (m, 3H), 7.18 (d, J = 8.0 Hz, 2H), 7.51 (d, J = 8.8 Hz, 2H). [α]<sub>D</sub> = -20.5 (c = 1.0, CH<sub>3</sub>OH). Hydrobromide salt, mp 250–255 °C. Anal. (C<sub>31</sub>H<sub>39</sub>N<sub>3</sub>O<sub>3</sub>S·3.0HBr·0.5H<sub>2</sub>O): C, H, N.

**DA D2 and D3 Receptor Assays.** Binding potency was monitored by inhibition of [<sup>3</sup>H]spiroperidol (16.2 Ci/mmol, PerkinElmer) binding to DA rD2 and rD3 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.<sup>28,52</sup> Observed IC<sub>50</sub> values were converted to inhibition constants (K<sub>i</sub>) by using the Cheng–Prusoff equation.<sup>27</sup> Functional activity of test compounds in activating DA hD2 and hD3 receptors expressed in CHO cells was measured by stimulation of [<sup>35</sup>S]GTPγS (1,250 Ci/mmol, PerkinElmer) binding in comparison to stimulation by the full agonist DA as described by us previously.<sup>28</sup>

**Animal Experiments. Drugs and Chemicals.** The following commercially available drugs were used in the experiment: reserpine hydrochloride (Alfa Aesar) and ropinirole (Sigma-Aldrich). The hydrobromide salt of (-)-24b was dissolved in 1–5% β-hydroxypropyl cyclodextrin solution, and ropinirole was 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 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 D-520 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, s.c.) or saline (s.c.) was administered 18 h before the injection of drug or vehicle (i.p.). 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 an Opto-Varimex-4 Auto tracker animal activity monitor chamber (44.5 cm 44.5 cm; Columbus Instruments, 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  $\pm$  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 postlesion, 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. The test drugs were dissolved in saline. The drugs D-520 and ropinirole were administered i.p. The rotations were measured over 6 h. For control, vehicle was administered alone. Rotations were measured in the Rotomax rotometry system (AccuScan Instruments, Inc., Columbus, OH) equipped with a Rotomax analyzer, high resolution sensor, and animal chambers with harnesses. Data were analyzed with the 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 using the GraphPad (version 4, San Diego, CA) program. All drugs produced contralateral rotations in all lesioned rats, which lasted over 3–7 h.

**Cell Culture Experiments and  $\alpha$ SN Assays.**  $\alpha$ SN was purchased from rpeptide (Bogart, GA). Rifampicin, sodium thiosulfate, silver nitrate, sodium hydroxide, formalin (36.5–38% formaldehyde in water), and thioflavin-T were purchased from Sigma-Aldrich (St. Louis, MO). Sodium carbonate, methanol, glacial acetic acid, sodium phosphate, ethanol, and sodium chloride were purchased from Fisher Scientific (Fair Lawn, NJ). PC12 Adh (ATCC CRL1721.1) cells, a rat adrenal pheochromocytoma cell line, were purchased from ATCC. RPMI 1640, heat-inactivated horse serum, fetal bovine serum, penicillin-streptomycin, and trypsin were purchased from GIBCO (Grand Island, NY). PC12 cells were cultured in T-75 flasks (Sarstedt Inc., Newtown, NC) and maintained in RPMI 1640 medium supplemented with 10% heat-inactivated horse serum, 5% fetal bovine serum, penicillin (100 units/mL), and streptomycin (100ug/mL) at 37 °C in 5% CO<sub>2</sub> atmosphere. 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 flasks (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% FetalClone III serum, penicillin (50 units/ml), and streptomycin (50  $\mu$ g/mL) at 37 °C under 5% CO<sub>2</sub> atmosphere. Stock solution of D-520

was 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 75  $\mu$ M 6-OHDA (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.

**Confirmation of  $\beta$ -Sheet Positive Protein Structure by Thioflavin-T Assay.** A volume of 42  $\mu$ L of 500  $\mu$ M (1.1 mg of ThT in 7 mL of phosphate-buffered saline (PBS)) ThT solution was mixed with 479  $\mu$ L of PBS to obtain 40  $\mu$ M ThT solution. Then 10  $\mu$ L of protein (60  $\mu$ M) from each time point was mixed with 10  $\mu$ L of 40  $\mu$ M ThT into a black 384-well plate (solid bottom, Corning), and fluorescence was measured using the Synergy Hybrid H1 fluorescence microplate reader (BioTek) at 440 nm excitation and 485 nm emission wavelength with autosensitivity mode. Control wells received 10  $\mu$ L of PBS and 10  $\mu$ L of 40  $\mu$ M ThT.

**Transmission Electron Microscopy.** A 4  $\mu$ L aliquot (60  $\mu$ M) was removed from  $\alpha$ SN aggregation incubation and absorbed onto a Formvar-coated, carbon-stabilized copper grid (400 mesh) for 4 min. The grid was then rinsed briefly with distilled water twice, negatively stained with 2% aqueous uranyl acetate, air-dried, and examined with a JEOL (JEM 2010) transmission electron microscope at an accelerating voltage of 200 kV and 80 000 magnification.

**Generation of  $\alpha$ SN Aggregates to Assess Extracellular Toxicity in Cell Culture Models.** All samples were prepared in 1 $\times$  PBS. Shaking experiments were conducted on a Thermomix R shaker (Eppendorf, Hamburg, Germany) at 1400 rpm and 37 °C. The amount of 1 mg of  $\alpha$ SN was dissolved in 576.3  $\mu$ L of 1 $\times$  PBS to yield 120  $\mu$ M stock solution which was filtered through a 0.2  $\mu$ m filter to remove any preformed aggregates. Then 250  $\mu$ L  $\alpha$ SN (120  $\mu$ M) was mixed with 250  $\mu$ L of 1 $\times$  PBS to yield 60  $\mu$ M  $\alpha$ SN. A 70  $\mu$ L aliquot was taken from the mixture in the beginning of the experiment (day 0), and then the solution was shaken for 10 days. Aliquots of 70  $\mu$ L were collected each time at days 2, 4, 6, 8, and 10. Aliquots were used to perform cytotoxicity and thioflavin-T assays (10  $\mu$ L).

**Evaluation of Cytotoxicity of Extracellular  $\alpha$ SN Aggregates (preformed) in Cell Culture System.** Aliquots obtained from experiments mentioned above were used to evaluate the effect of (preformed) various species generated from  $\alpha$ SN aggregation experiments on PC12 cell viability (extracellular toxicity). PC12 cells were seeded at 17 000 cells/well density in 100  $\mu$ L of medium in a 96-well plate. Cells were allowed to adhere to the plate for 24 h. Aliquots of 40  $\mu$ L (60  $\mu$ M  $\alpha$ SN) from various time points were diluted with 200  $\mu$ L of PC12 cell medium to make the final concentration of  $\alpha$ SN 10  $\mu$ M for cell culture experiments. Media was removed, and the adhered PC12 cells were treated with 55  $\mu$ L of  $\alpha$ SN (10  $\mu$ M) containing medium. Control cells were treated with appropriately diluted PC12 medium. Treatment with extracellular  $\alpha$ SN was conducted for 24 h. After incubation, 6  $\mu$ L of 5 mg/mL MTT was added to the cells and the plate was incubated for another 3 h at 37 °C under 5% CO<sub>2</sub> 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  $\mu$ L 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 and 690 nm using an Epoch microplate reader (BioTek, Winooski, VT). 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, CA).

**Assessment of D-520 and Rifampicin to Alter Cytotoxicity Induced by Extracellular  $\alpha$ SN.**  $\alpha$ SN alone (60  $\mu$ M) was able to induce around  $\sim$ 40% cell death after shaking for 6 days. Therefore, we assessed the ability of our lead compound D-520 and a reference drug (rifampicin) to alter cytotoxicity induced by  $\alpha$ SN (60  $\mu$ M) after shaking together for 6 days. All solutions were prepared in 1 $\times$  PBS. Shaking experiments were conducted on a Thermomix R shaker (Eppendorf, Hamburg, Germany) at 1400 rpm and 37 °C. Then 120  $\mu$ M  $\alpha$ SN was prepared by dissolving 1 mg of  $\alpha$ SN in 576.3  $\mu$ L of 1 $\times$  PBS following filtration through 0.2  $\mu$ m filter to remove any

preformed aggregates. The solution was next diluted to 60  $\mu\text{M}$ . Aliquots for  $\alpha\text{SN}$  alone from days 0 and 6 were collected and frozen at  $-20\text{ }^\circ\text{C}$  until further use. From the day 0 aliquot, 40  $\mu\text{L}$  was used for cell viability assay, whereas from the day 6 aliquot 40  $\mu\text{L}$  was used for cell viability assay, and the remaining volume was used for TEM.

Similarly, 70  $\mu\text{L}$  of  $\alpha\text{SN}$  (120  $\mu\text{M}$ ) was mixed with 70  $\mu\text{L}$  of rifampicin (240  $\mu\text{M}$ ) or 70  $\mu\text{L}$  of D-520 (240  $\mu\text{M}$ ) to yield 60  $\mu\text{M}$   $\alpha\text{SN}$  and 120  $\mu\text{M}$  rifampicin or D-520, and each solution mixture was incubated with shaking for 6 days. A 50  $\mu\text{L}$  aliquot was taken from the mixture at time 0 (day 0), and the remaining mixture was shaken for 6 days. After 6 days, the mixture was frozen at  $-20\text{ }^\circ\text{C}$  until further use. From the day 0 aliquot, 40  $\mu\text{L}$  was used for cell viability assay, whereas from the day 6 aliquot 40  $\mu\text{L}$  was used for cell viability assay, and the remaining volume was used for TEM. The aliquots were diluted with PC12 medium to get the final concentration of  $\alpha\text{SN}$  to 10  $\mu\text{M}$  in PC12 medium. MTT assay was carried out as described above to evaluate the effect of compounds on cytotoxicity of extracellular  $\alpha\text{SN}$  aggregates (preformed) in the cell culture system.

**Assessment of Toxicity of Addition of Fresh (D0) and 6 Day Shaken (D6) D-520 to Preformed  $\alpha\text{SN}$  Aggregates (D6) in Cell Culture System.** We wanted to evaluate whether D-520 imparts protection against cytotoxic effects of  $\alpha\text{SN}$  by interfering with its aggregation process during shaking or interacting with the preformed aggregates by some other mechanism in the cell culture system. Therefore, we have cogenerated 6 days shaken preformed  $\alpha\text{SN}$  aggregates with fresh D-520 (day 0) and D-520 shaken alone for 6 days. All solutions were prepared in 1 $\times$  PBS. Shaking experiments were conducted on a Thermomix R shaker (Eppendorf, Hamburg, Germany) at 1400 rpm and 37  $^\circ\text{C}$ .  $\alpha\text{SN}$  of 120  $\mu\text{M}$  was prepared by dissolving 1 mg of  $\alpha\text{SN}$  in 576.3  $\mu\text{L}$  of 1 $\times$  PBS following filtration through a 0.2  $\mu\text{m}$  filter to remove any preformed aggregates. The solution was next diluted to 60  $\mu\text{M}$ . Similarly, 120  $\mu\text{M}$  D-520 was prepared in 1 $\times$  PBS. Solutions of 60  $\mu\text{M}$   $\alpha\text{SN}$  and 120  $\mu\text{M}$  D-520 were shaken separately for 6 days. Collected aliquots were frozen at  $-20\text{ }^\circ\text{C}$  until further use. On the day of treatment, 120  $\mu\text{M}$  D-520 was prepared in 1 $\times$  PBS and used as fresh solution (day 0). For cell viability assay, 40  $\mu\text{L}$  of  $\alpha\text{SN}$  (60  $\mu\text{M}$ ) from the 6 day shaken aliquot was used to cotreat with either 120  $\mu\text{M}$  freshly prepared D-520 (day 0) or the aliquot from 6 day shaking. Alternately, PC12 cells were also treated with D-520 alone (both fresh and 6 day shaken). The aliquots were diluted with PC12 medium to get the final concentration of  $\alpha\text{SN}$  to 10  $\mu\text{M}$  and D-520 to 20  $\mu\text{M}$ . MTT assay was carried out as described above to compare the protective effect of D-520 with fresh and 6 day shaken solutions against cytotoxicity of preformed  $\alpha\text{SN}$  aggregates in PC12 cells.

**Assessment of Cell Viability.** The quantitative and colorimetric MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) tetrazolium salt assay was used to assess cell viability. MN9D cells were seeded into poly-L-lysine coated 96-well plates at  $1 \times 10^4$  cells/well in 100  $\mu\text{L}$  of medium. After the plate was equilibrated for 40 h, old medium was taken out from each well and 160  $\mu\text{L}$  of fresh medium (containing 0.01% DMSO) was added to control wells and wells which were to be treated with 6-OHDA. A solution of 160  $\mu\text{L}$  of D-520 in the above medium without DMSO in 30, 20, 10, 5, 1, 0.1, 0.01  $\mu\text{M}$  was added to wells which would be cotreated with 6-OHDA. The plate was incubated for 1 h at 37  $^\circ\text{C}$  under 5%  $\text{CO}_2$  atmosphere. At the end of incubation, required amount of 6-OHDA was added to each well (except the control wells) to maintain a final concentration of 75  $\mu\text{M}$ . The plate was then incubated for 24 h at 37  $^\circ\text{C}$  under 5%  $\text{CO}_2$  atmosphere. Next, 20  $\mu\text{L}$  of MTT stock solution (prepared in Dulbecco's PBS) 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  $^\circ\text{C}$  under 5%  $\text{CO}_2$  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  $\mu\text{L}$  of a 1:1 mixture of DMSO/methanol 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). 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, CA).

## ■ ASSOCIATED CONTENT

### 📄 Supporting Information

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

## ■ AUTHOR INFORMATION

### Corresponding Author

\*Mailing 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](mailto:adutta@wayne.edu).

### Author Contributions

A.K.D. was involved with all aspects of the studies including design of compounds and biological (in vitro and in vivo) experiments reported in the manuscript. G.M. and S.G. were involved with synthesis of compounds. C.V. and G.M. were involved with in vitro cell culture and in vivo biological studies. C.V., M.S. and G.M. were involved with in vitro alpha synuclein protein studies. M.E.R. and T.A. were involved with screening of compounds for dopamine D2 and D3 receptors.

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### Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS

GTP $\gamma$ S, guanosine 5'-[ $\gamma$ -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; PD, Parkinson's disease; DA, dopamine; s.c., subcutaneous;  $\alpha\text{SN}$ , alpha-synuclein

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