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Design, Synthesis and Pharmacological Characterization of Carbazole Based Dopamine Agonists as Potential Symptomatic and Neuroprotective Therapeutic Agents for Parkinson's Disease

Asma Elmabruk,[1$] Banibrata Das,[1$] Deepthi Yedlapudi,[1] Liping Xu,[1] Tamara Antonio,[2] Maarten E.A. Reith,[2] Aloke K. Dutta[1*]

1. Department of Pharmaceutical Sciences, Wayne State University, Detroit, MI 48202
2. Department of Psychiatry, New York University, New York, NY 10016

[§]Both authors contributed equally to this work

***Corresponding Author:**
Aloke K. Dutta, Ph.D.
Department of Pharmaceutical Sciences
Eugene Applebaum College of Pharmacy & Health Sciences
Wayne State University
Detroit, MI 48202
Tel: 1-313-577-1064, Fax: 1-313-577-2033, e-mail: adutta@wayne.edu

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Abstract

We have developed a series of carbazole-derived compounds based on our hybrid D_2/D_3 agonist template to design multifunctional compounds for the symptomatic and disease-modifying treatment of Parkinson's disease (PD). The lead molecules (-)-**11b** (**D-636**), (-)-**15a** (**D-653**) and (-)-**15c** (**D-656**) exhibited high affinity for both D_2 and D_3 receptors and in GTPγS functional assay, the compounds showed potent agonist activity at both D_2 and D_3 receptors (EC_{50} (GTPγS); D_2 = 48.7 nM, D_3 = 0.96 nM for **11b**, D_2 = 0.87 nM, D_3 = 0.23 nM for **15a** and D_2 = 2.29 nM, D_3 = 0.22 nM for **15c**). In an animal model of PD, the test compounds exhibited potent *in vivo* activity in reversing hypolocomotion in reserpinized rats with a long duration of action compared to the reference drug ropinirole. In a cellular antioxidant assay, compounds (-)-**11b**, (-)-**15a** and (-)-**15c** exhibited potent activity in reducing oxidative stress induced by neurotoxin 6-hydroxydopamine (6-OHDA). Also, in a cell-based PD neuroprotection model, these lead compounds significantly increased cell survival from toxicity of 6-OHDA, thereby, producing a neuroprotective effect. Additionally, compounds (-)-**11b** and (-)-**15a** inhibited aggregation and reduced toxicity of recombinant alpha synuclein protein in a cell based *in vitro* assay. These observations suggest that the lead carbazole-based dopamine agonists may be promising multifunctional molecules for a viable symptomatic and disease modifying therapy of PD and should be further investigated.

Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disease that develops from gradual depletion of dopamine (DA) in the basal ganglia with extensive loss of

dopaminergic neurons in the substantia nigra pars compacta (SNc) of the midbrain accompanied by accumulation of presynaptic protein α-synuclein (α-syn) known as Lewy bodies.[1-3] PD has been identified as the second most common neurodegenerative disorder after Alzheimer's disease and it affects 2-3% of the population ≥65 years of age. The four cardinal symptoms associated with PD are tremor, rigidity, bradykinesia, and postural instability.[4] Although progression and development of PD increase with age, the exact pathogenesis and etiology remain unclear. Monogenic mutations account for less than 10% of PD cases.[5] On the other hand environmental factors and genetic susceptibility are thought to contribute to development of sporadic PD cases.[4] Several factors have been implicated in the pathogenesis of PD including protein aggregation, oxidative stress, mitochondrial dysfunction, metal toxicity, and exposure to pesticides.[4] Compromised antioxidant defense mechanism could lead to cell death because increased oxidative stress leads to misfolding of proteins such as α-syn and parkin in the substantia nigra.[4,6]

The current treatment of Parkinson's disease can be classified into four categories: levodopa, DA agonists, monoamine oxidase inhibitors (MAO-Is), and catechol-o-methyl transferase inhibitors (COMT-Is).[7,8] DA agonists activate mostly the postsynaptic DA receptors. These receptors are divided into D_1-like (D_1 and D_5) which activate the adenylate cyclase and D_2-like (D_2, D_3, and D_4) which inhibit the adenylate cyclase.[8,9] COMT-Is are also used in combination with levodopa to treat the wearing off symptoms of PD, which occur after prolonged therapy with levodopa. Even though several medications are available on the market to treat PD, there is still no cure of the disease.[4,10] Moreover, the current Parkinsonism treatments act by reducing the symptoms only but

they do not slow or stop the progression of the disease process, and they also produce severe side effects such as dyskinesia and motor fluctuation with long-term therapy.[6,11] New strategies have been adopted to overcome the current concerns with PD therapy by developing multifunctional agents to target multiple aspects of the pathogenesis of the disease. The rationale behind this strategy was built upon the fact that PD has been identified as multifactorial in nature, thereby, development of a multifunctional agent could be beneficial to treat patients with PD; however, no such agents are available in the clinic.[12,13]

Our efforts to design such multifunctional compounds to address multiple pathogenic factors of PD as well as to address the symptomatic aspect led us to embark upon a drug discovery approach focused on development of novel multifunctional dopamine D2/D3 agonist molecules. Specifically, as reported by us earlier, our hybrid structure strategy which combines D_2/D_3 agonist head groups to other moieties via piperazine linker, produced potent agonists for D2/D3 receptors.[14,15] It seems the piperazine moiety not only assists in producing D2/D3 potency but also provides suitable pharmacokinetic properties. Thus, such hybrid molecules containing suitable moieties to modulate the pathogenic pathway of PD, led to development of molecules to validate our proof of concept.[16-21] We have shown in our recent studies that lead molecule like D-512 and D-607 not only have the potential to provide symptomatic effects but also produced potent neuroprotective effects in various *in vitro* and *in vivo* experiments.[16,21-23] In addition, we have demonstrated that the compound D-512 (Figure 1) exhibits superior antiparkinsonian effects *in vivo* over a clinically approved drug, ropinirole.[24] In our current study, we carried out structure activity relationship (SAR) studies on carbazole-based

molecules that have previously been identified to have neuroprotective properties.[25-28] Previous studies have shown that carbazole-containing compounds exert neuroprotective properties by enhancing the formation of neurons in the subgranular zone of the dental gyrus.[29,30] Moreover, it has been reported that carbazole analogues exhibit the ability to regenerate neurons in the substantia nigra in neurodegenerative disease.[27,30,31] Thus, aminopropyl carbazole P7C3 and its analogue P7C3A20 (Figure 1) have shown proneurogenic and neuroprotective properties in aged rats, stabilized mitochondrial membrane potential and inhibited newborn hippocampal neuron apoptosis.[29] In addition, tricyclic neuroleptic phenothiazines and tricyclic antidepressants like nortriptyline have shown properties to interact with α-syn.[32-34] Carbazole moiety bearing a close resemblance to such tricyclic structures may also produce an interaction with α-syn protein. Based on these findings, we designed and developed a number of multifunctional molecules by covalently attaching D_2/D_3 agonist head groups such as pramipexole and 5-OH-MPAT to various carbazole moieties through a piperazine linker (Schemes 1-3).

In our current work, a series of compounds were synthesized, and the selected compounds were characterized by both *in vitro* and *in vivo* assays. The lead molecules (-)-**11b**, (-)-**15a** and (-)-**15c** exhibited high affinity and functional potency at both D_2 and D_3 receptors. The target compounds also exhibited potent *in vivo* activity in reserpinized rats and demonstrated significant dose-dependent neuroprotection effect in 6-hydroxydopamine treated cells. This study will, therefore, shed additional light on the importance of a carbazole moiety [25] as a potential molecular component in hybrid drug design for developing disease-modifying therapeutics for PD.

Results and Discussion

In this study, a series of compounds were synthesized by incorporating the aminotetralin or bioisosteric equivalent agonist head group with carbazole functionality via ethylpiperazine linker (scheme 1, 2 and 3). Scheme 1 describes the syntheses of final compounds (±)-**10a**, (±)-**10b**, (±)-**10c** and (-)-**11a**, (-)-**11b**, (-)-**11c**. A palladium catalyzed coupling of (4-bromophenyl)boronic acid and 1-bromo-2-nitrobenzene afforded 4'-bromo-2-nitro-1,1'-biphenyl (**2a**). This compound was then cyclized in the presence of PPh_3 to produce 2-bromo-9*H*-carbazole (**3a**)[35] which then underwent *N*-protection using di-*tert*-butyl dicarbonate in the presence of 4-dimethylaminopyridine (4-DMAP) to yield **4a**. Palladium-catalyzed mediated cross coupling of **4a** with 1-(2-((*tert*-butyldimethylsilyl)oxy)-ethyl)piperazine[18] under refluxing conditions in the presence of Cs_2CO_3 and BINAP in toluene produced intermediate **5a**. Treatment with *n*-Bu_4NF (TBAF) in THF removed the silyl protecting group of compound **5a** to produce the alcohol **6a**, which in the presence of pyridine-sulfur trioxide was oxidized to yield the corresponding aldehyde **7a**. Reductive amination of the aldehyde with either (±) or (-)-pramipexole in the presence of $NaBH(OAc)_3$ afforded compounds **8a** and **9a**, respectively. In the final step, the amine protecting *t*-Boc groups were removed by treatment with trifluoroacetic acid to produce the final compounds (±)-**10a** and (-)-**11a** as TFA salts. The other final compounds (±)-**10b**, (±)-**10c**, (-)-**11b** and (-)-**11c** were also synthesized in the similar fashion as described above, where 3-Bromo-9*H*-carbazole and 4-Bromo-9*H*-carbazole were used as the starting materials, respectively.

The syntheses of the final compounds (±)-**14a**, (±)-**14b**, (±)-**14c** and (-)-**15a**, (-)-**15b**, (-)-**15c** is shown in Scheme 2. To prepare these compounds, we employed (±) and (-)-(5-

methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine and reductively alkylated with intermediate aldehydes **7a-7c** in the presence of $NaBH(OAc)_3$ to produce compounds (±)-**12a**, (±)-**12b**, (±)-**12c,** (-)-**13a**, (-)-**13b**, and (-)-**13c**. Finally, demethylation and removal of the protecting *t*-Boc groups were carried out in one step by refluxing with aq. HBr to give the final compounds (±)-**14a**, (±)-**14b**, (±)-**14c,** (-)-**15a**, (-)-**15b**, and (-)-**15c** as HBr salts.

The syntheses of two more target compounds (±)-**20** and (-)-**21** are illustrated in scheme 3. *N*-alkylation was first performed by refluxing carbazole with dibromoethane in presence of a mixture of K_2CO_3, KOH and TBAB to afford 9-(2-bromoethyl)-9*H*-carbazole **16**. Base-catalyzed condensation of 1-(2-((*tert*-butyldimethylsilyl)-oxy)ethyl)piperazine with intermediate **16** yielded compound **17**, which on TBDMS deprotection in the presence of TBAF in THF afforded alcohol **18** in excellent yield. Pyridine-sulfur trioxide was used to oxidize alcohol **18** next to yield the corresponding aldehyde **19**, which was then underwent reductive amination with either (±) or (-)- pramipexole in the presence of $NaBH(OAc)_3$ to produce the final compounds (±)-**20** and (-)-**21**. These two molecules were converted to their corresponding HCl salts by treatment with ethereal HCl. All the final compounds were characterized by ^1H and ^{13}C NMR as well as elemental analysis.

A well-established radioligand competition assay was carried out to evaluate binding affinity of the test compounds and were compared with that of the reference agent (*S*)-5-OH-DPAT (Table 1). Binding affinity to rat DA D_2 and D_3 receptors expressed in HEK-293 cells was determined as described by us previously.[17] Table 1 lists the binding data of new compounds. Compounds (±)-**10a-c**, which incorporate racemic 2-aminothiazole head group and a piperazine ring connected to the different positions of the carbazole ring, exhibited high affinity for D_3 and low to moderate affinity for D_2 receptors. When the

positions of attachment are at carbon 2 and 3 of the carbazole moiety for compounds **10a** and **10b,** respectively, both the compounds displayed low affinity for D_2 and high affinity for the D_3 receptors with high selectivity (K_i, D_2 = 902 nM, D_3 = 6.18 nM, D_2/D_3 = 146 and D_2 = 612 nM, D_3 = 3.12 nM, D_2/D_3 = 196 for **10a** and **10b,** respectively). Interestingly, covalent attachment at position 4 of the carbazole ring dramatically improved the affinity for D_2 while that for D_3 receptor remained the same (K_i, D_2 = 76.9 nM, D_3 = 7.8 nM, D_2/D_3 = 9.86 for **10c**). This indicated highest tolerance of the 4-substituted carbazole derivative for interaction with the D2 and D3 receptors. As expected, we observed a 2-4-fold improvement in binding affinity when enantiomerically pure aminothiazole moiety was attached to the carbazole as in (-)-**11a** and (-)-**11b** compared to their racemic counterparts (K_i, D_2 = 504 nM, D_3 = 3.94 nM, D_2/D_3 = 128 and D_2 = 135 nM, D_3 = 3.80 nM, D_2/D_3 = 35 for (-)-**11a** and (-)-**11b** respectively). However, for (-)-**11c** we did not observe much difference from its racemic version.

Next, the effect of bioisosteric replacement of the aminothiazole moiety with aminotetraline functionality on the receptor binding of target compounds was evaluated. In corroboration with our previous results,[16,38] aminotetraline substituted compounds (±)-**14a-c** and (-)-**15a-c** exhibited high affinity at both D_2 and D_3 receptors. For instance, the aminotetraline analogue **(-)-15a** has been found to have very high affinity for D_2 while displaying subnanomolar affinity for D_3 receptor compared to the corresponding thiazolidium counterpart (-)-**11a** (K_i, D_2 = 71.2 nM, D_3 = 0.40 nM, D_2/D_3 = 177 for (-)-**15a** vs D_2 = 504 nM, D_3 = 3.94 nM, D_2/D_3 = 128 for (-)-**11a**). Among the three enantiomerically pure isomers (-)-**15a-c**, which differ only in the substitution positions at the carbazole moiety, positions 2, 3 and 4, showed variable binding affinity at both D_2 and D_3 receptors

(K_i, D_2 = 71.2 nM, D_3 = 0.40 nM for (-)-**15a** (; D_2 = 61.6 nM, D_3 = 1.94 nM for (-)-**15b** and D_2 = 16.9 nM, D_3 = 0.36 nM for (-)-**15c**). As discussed before, substitution at the 4-position of the carbazole aromatic ring resulted in compounds **10c**, (-)-**11c**, **14c** and (-)-**15c**) with better D_2/D_3 binding affinities in comparison to other isomeric analogues with compound (-)-**15c** exhibiting the highest affinity among all the molecules, underscoring the importance of positional attachment to the carbazole ring. Finally, the binding affinities were evaluated for another series of compounds in which the piperazine ring of the agonist fragment was appended directly to the carbazole nitrogen atom through a methylene linker. As shown in Table 1, enantiomeric compound (-)-**21** displayed relatively higher binding affinity at D_2 and comparable affinity at D_3 receptor with moderate selectivity compared to the racemic compound (±)-**20** (K_i, D_2 = 435 nM, D_3 = 6.60 nM, D_2/D_3 = 65.9 and D_2 = 82.6 nM, D_3 = 7.18 nM, D_2/D_3 = 12 for **20** and (-)-**21,** respectively). This structural modification suggests no significant differences in DA receptor interaction between compounds where the carbazole moiety is attached either at the 2/3 positions of the aromatic ring or through the nitrogen atom; however, a prominent difference exists for compounds where the carbazole nitrogen is sterically free to probably participate in additional receptor interaction (e.g. (-)-**15c** vs (-)-**15b** and (-)-**21**).

Based on binding data, functional activities of the selected optically active lead compounds for human DA D_2 and D_3 receptors expressed in CHO cells were measured by using [^{35}S]GTPγS binding assay.[17] Agonist or partial agonist activity was judged by comparing with the maximum stimulation (E_{max}), produced by the full agonist DA. As shown in Table 2, aminothiazole containing compounds (-)-**11b** and (-)-**11c** demonstrated moderate potency at both D_2 and D_3 receptors (EC_{50} (GTPγS); D_2 = 48.7, D_3 = 0.96 nM

and D_2 = 22.2, D_3 = 1.67 nM, respectively), correlating well with binding data. While (-)-**11b** showed full agonist activity at both D_2 and D_3 receptors (E_{max} = 87-93%), compound (-)-**11c** revealed partial agonist activity at D_2 and full agonism at D_3 receptor (E_{max} = 57% vs 82%, respectively for D_2 and D_3). On the other hand, aminotetraline compound (-)-**15a** displayed very high functional potency (EC_{50} (GTPγS); D_2 = 0.87 and D_3 = 0.23 nM) and full agonism (E_{max} = 85-92%) at both the receptors. Compound (-)-**15c** was also found to be highly potent and efficacious in stimulating both receptors (EC_{50} (GTPγS); D_2 = 2.29 and D_3 = 0.22 nM; E_{max} = 74-88%). Neither compounds exhibited appreciable selectivity for D_3 over D_2 (Table 2) and their selectivity for D_3 receptor was much less when compared to the binding data (Table 1). The values for the ClogP and tPSA for all the compounds were calculated as shown in Table 1. In general, these values predict that these compounds should cross the blood brain barrier to produce *in vivo* CNS efficacy which we observed in case of (-)-**11b**, (-)-**15a** and (-)-**15c** (see below). Therefore, our SAR results of a series of carbazole compounds show that the affinity and selectivity for the D_2/D_3 receptors are governed by the nature of covalent attachment to the carbazole moiety and the structure of agonist binding head group in the hybrid molecule.

In vivo evaluation of the compounds (-)-**11b**, (-)-**15a** and (-)-**15c** in a reserpine-based PD animal model was performed next. In this well-established animal model for PD, depletion of catecholamines by reserpine results in catalepsy in rats.[39,40] Reserpine produced a significant inhibition of locomotion in rats 18 h after the administration of the drug (5 mg/kg, sc), which indicated the development of akinesia (Figure 2). Compounds (-)-**11b**, (-)-**15a** and (-)-**15c** at the dose of 10 μMol/kg, ip, significantly reversed akinesia in rats compared to reserpine treatment alone. The compounds also demonstrated

significant enhancement of locomotion for the duration of the study of 6 h. The total average number of horizontal locomotor counts generated by (-)-15a, (-)-15c, (-)-11b and Ropinirole were 3718, 2680, 2408 and 1528, respectively. Thus, among the molecules tested, (-)-**15a** was found to have the highest *in vivo* activity and this finding correlates with *in vitro* functional assay where the compound exhibited subnanomolar potency for stimulation of D_2/D_3 receptors along with full agonist property. On the other hand, the reference drug ropinirole at 10 μMol/kg, ip, produced a fast onset of activation of locomotion compared to the control but had a shorter duration of action compared to the test compounds. It is also important to note that pharmacokinetic properties of these compounds should also play an important role in efficacy. It has been shown that the locomotor stimulation in this reserpine model is mediated by postsynaptic D_2/D_3 receptor activation by the compounds.

Antioxidant Activity

Experimental detection of reactive oxygen species (ROS) produced by 6-OHDA was carried out by DCFDA assay. 6-OHDA, a well known neurotoxin, produces oxidative stress similar to what is observed in PD and the mechanism of neurotoxicity of 6-OHDA takes place via its auto-oxidation and subsequent generation of hydrogen peroxide.[41,42] DCFDA is a non-fluorogenic dye but in the presence of ROS, it is oxidized to produce DCF which produces florescence. We have previously shown that 6-OHDA causes cell death in a dose-dependent manner.[43] We chose 75 μM 6-OHDA as we have shown that this dose induces 40-50% cell death. To examine whether our compounds (-)-**11b**, (-)-**15a** and (-)-**15c** can protect PC12 cells from the ROS produced by the exposure to 75 μM 6-OHDA, the PC12 cells were treated with 6-OHDA after pretreatment with various

concentrations of drugs (5, 10, 20 μM) for 24 h, and compared with 6-OHDA alone treated cells. As shown in Figure 3, a well over two-fold increase in ROS was observed in cells treated with 6-OHDA (75 μM) alone compared to the control untreated cells. However, the test compounds could dose dependently decrease, albeit without any significant differences, the production of ROS induced by 6-OHDA (75 μM) in PC12 cells. In this regard, highest dose of all the three compounds was the most efficacious in producing significant antioxidant effect. Thus at 20 μM, a reduction in 76%, 93% and 36% ROS were induced by (-)-**11b**, (-)-**15a** and (-)-**15c**, respectively (Figure 3A-C).

Cellular Neuroprotection Activity

To investigate the neuroprotection property of the selected target molecules (-)-**11b**, (-)-**15a** and (-)-**15c** against 6-OHDA-induced cytotoxicity, we used dopaminergic rat adrenal Pheochromocytoma PC12 cells. As reported above, treatment of PC12 cells with 6-OHDA at 75 μM for 24 h produced ~ 50% loss in cells viability and this concentration was used in subsequent experiments.[22] In contrast, it was observed that the cells treated with increasing concentrations of either (-)-**11b** or (-)-**15c** alone (0.01–30 μM) showed no cells loss compared to untreated controls (Figure 4A and 4C, respectively), indicating no toxicity of the compounds at the doses tested. However, (-)-**15a** produced some incremental toxicity starting at 20 μM dose (Figure 4B). The potential neuroprotective effects of (-)-**11b**, (-)-**15a** and (-)-**15c** against 6-OHDA-induced toxicity were evaluated next. Thus, pre-treatment of the cells with the test compounds for 24 h followed by exposure to 6-OHDA 75 μM for another 24 h produced dose-dependent protection of the cells from the neurotoxic insult and the greatest protective effect was observed at 5 μM for (-)-**11b** and 20 μM for (-)-**15c.** At those concentration both drugs increased cell survival

by ~20% compared to 6-OHDA (75 μM) treated alone (Figure 4D and 4F), indicating neuroprotective effect of these drugs. Compound (-)-**15a** also revealed dose-dependent neuroprotection and the highest effect was observed at the dose of 10 μM (15%) but the effect was not observed at the higher doses (Figure 4E). This could be due to the fact that the compound by itself produced toxicity to the cells at doses ≥20 μM and the presence of 6-OHDA enhanced the toxicity (Figure 4B).

Effect of (-)-11b and (-)-15a on the aggregation of α-syn with 0.5% seeding

The α-syn assay was carried out by following the procedures developed by us earlier.[37] The compounds (-)-**11b** and (-)-**15a** were incubated with α-syn seeded with 0.5% PFFs for a period of 30D at 37 °C without agitation. Seeding of monomeric α-syn with 0.5% PFFs leads to a significant increase in aggregation characterized by a 15-fold increase in ThT fluorescence at 30D. Both (-)-**11b** and (-)-**15a** lead to a significant decrease in the ThT fluorescence ($p \leq 0.0001$) when compared to α-syn seeded with 0.5% PFFs alone at 30D (Fig. 5A). (-)-**11b** and (-)-**15a** showed a 16-fold and 3-fold decrease in ThT fluorescence respectively, when compared to seeded α-syn at 30D.

When PC12 cells were treated with the aggregates formed by 0.5% PFFs seeding at 30D, it was observed that the viability went down to 37% when compared to control. However, the seeded samples formed in the presence of (-)-**11b** and (-)-**15a** showed significant less toxicity when compared to seeded α-syn without compounds. The viability values of PC12 cells when treated with α-syn seeded samples in the presence of (-)-**11b** and (-)-**15a** were 71% and 72% at 30D when compared to control (Figure 5B). We have previously shown that the parent compounds to our hybrid molecules, Pramipexole and

5-OHDPAT were far less effective in decreasing ThT fluorescence and reducing toxicities when compared to our multifunctional hybrid agonist.[37]

In conclusion, the present study represents the development of a novel series of carbazole-based multifunctional DA D_2/D_3 receptor agonists. Compounds (-)-**11b**, (-)-**15a** and (-)-**15c** exhibited high binding affinity and full agonist activity at the both D_2 and D_3 receptors. The lead molecules were highly efficacious in a PD animal model by reversing hypo-locomotion activity with a long duration of action, indicating their potential in relieving motor dysfunction in PD. In a cellular model, the lead compounds exhibited potent anti-oxidant activity produced by neurotoxin 6-OHDA. To gain further insight into their possible multifunctional property, the data presented here also demonstrates that all three compounds (-)-**11b, (-)-15a** and (-)-**15c** exhibited neuroprotective property in an *in vitro* cellular model of PD by protecting neuronal PC12 cells from neurotoxin 6-OHDA. Furthermore, in vitro assays with recombinant α-syn protein demonstrated potent modulatory effect of **(-)-11b** and **(-)-15a** on aggregation and toxicity of α-syn protein. In summary, we are able to demonstrate that multifunctional drugs like (-)-**11b**, (-)-**15a** and (-)-**15c** have the potential not only to alleviate motor dysfunction in PD patients, but also to modify disease progression. More in depth future studies to establish the disease-modifying effects of the compounds will shed more light into their potential therapeutic application.

Experimental Section

Reagents and solvents were obtained from commercial suppliers and used as received unless otherwise indicated. Dry solvent was obtained according to the standard procedure. All reactions were performed under N_2 atmosphere unless otherwise

indicated. Analytical silica gel 60 F254-coated TLC plates were purchased from EMD Chemicals, Inc. and were visualized with UV light or by treatment with phosphomolybdic acid (PMA), or ninhydrin. Flash column chromatographic purification was done using Whatman Purasil 60A silica gel 230−400 mesh. Proton nuclear magnetic resonance (^{1}H NMR) spectra and carbon nuclear magnetic resonance (^{13}C NMR) spectra were measured on Varian 400 and 600 MHz NMR spectrometer with tetramethylsilane (TMS) as an internal standard. The NMR solvent used was either $CDCl_3$ or CD_3OD unless otherwise indicated. Autopol III automatic polarimeter (Rudolph Research Analytical) was used to record the optical rotations. MEL-TEMP II (Laboratory Devices Inc., U.S.) capillary melting point apparatus was used to record the melting points. Purity of the compounds was determined by elemental analysis and was within ±0.4% of the theoretical value (≥95% purity). Elemental analyses were performed by Atlantic Microlab, Inc., GA, USA.

Procedure A. 4'-Bromo-2-nitro-1,1'-biphenyl (2a). To a stirring solution of 1-bromo-2-nitrobenzene (2.0 g, 9.9 mmol) and (4-bromophenyl)boronic acid (2.19 g, 10.89 mmol) in THF (25 mL) were added $Pd(PPh_3)_4$ (0.572 g, 0.50 mmol) followed by 2M K_2CO_3 (5.53 g in 20 mL water) at room temperature. The reaction mixture was stirred at 90 °C for 12 h after which it was cooled and extracted with CH_2Cl_2. The combined organic layer was dried over Na_2SO4, filtered, and concentrated in vacuo. The crude material was purified by column chromatography over silica gel using hexane:ethyl acetate (9:1) as solvent to give compound **2a** (2.7 g, 98%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 7.88 (d, J = 8.4 Hz, 1H), 7.64-7.61 (m, 1H), 7.59-7.55 (m, 2H), 7.53-7.50 (m, 1H), 7.42-7.40 (m, 1H), 7.20-7.18 (m, 2H).

2-Bromo-2'-nitro-1, 1'-biphenyl (2c). To a stirring solution of 1-bromo-2-nitrobenzene (2.50 g, 12.37 mmol) and (2-bromophenyl)boronic acid (2.73 g, 13.61 mmol) in THF (30 mL) were added $Pd(PPh_3)_4$ (0.715 g, 0.61 mmol) followed by 2M K_2CO_3 (5.53 g in 20 mL water) according to procedure A to give compound **2c** (2.18 g, 94%). **^{1}H NMR** (600 MHz, $CDCl_3$): *δ* 7.87 (d, *J* = 8.4 Hz, 1H), 7.44 (t, *J* = 7.2 Hz, 1H), 7.40 (d, *J* = 9 Hz, 1H), 7.30 (d, *J* = 7.8 Hz, 1H), 7.14 (t, *J* = 7.2 Hz, 1H), 7.10 (d, *J* = 7.2 Hz, 1H), 7.03 (d, *J* = 7.2 Hz, 2H).

Procedure B. 2-Bromo-9*H*-carbazole (3a). Compound **2a** and PPh_3 were dissolved in 1,2-dichlorobenzene and the resulting solution was stirred at 170 °C for 12 h after which it was cooled and extracted with CH_2Cl_2/H_2O. The combined organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude material was purified by column chromatography over silica gel using hexane:ethyl acetate (9:1) to yield compound **3a** (1.57 g, 89%). **^{1}H NMR** (600 MHz, $CDCl_3$): *δ* 8.04 (d, *J* = 8.4 Hz, 1H), 7.92 (d, *J* = 8.4 Hz, 1H), 7.62-7.58 (m, 2H), 7.52-7.47 (m, 2H), 7.39 (d, *J* = 8.4 Hz, 1H).

4-Bromo-9*H*-carbazole (3c). Compound **2c** and PPh_3 were reacted in 1,2-dichlorobenzene according to procedure B to yield compound **3c** (1.25 g, 87%). **^{1}H NMR** (600 MHz, $CDCl_3$): *δ* 8.81 (d, *J* = 7.8 Hz, 1H), 7.97 (s, 1H), 7.50 (t, *J* = 7.2 Hz, 1H), 7.44 (d, *J* = 7.2 Hz, 1H), 7.35 (t, *J* = 7.8 Hz, 1H), 7.30 (d, *J* = 7.8 Hz, 1H), 7.25-7.20 (m, 1H).

Procedure C. *tert*-Butyl 2-bromo-9*H*-carbazole-9-carboxylate (4a). To a stirring solution of compound **3a** (1.5 g, 6.09 mmol) in THF (20 mL) were added $(Boc)_2O$ (1.46 g, 6.7 mmol) and DMAP (0.819 g, 6.7 mmol) in THF (20 mL) at room temperature. The reaction mixture was stirred at the same temperature for 12 h. The crude mixture was evaporated under reduced pressure, followed by extraction with EtOAc (3 × 20 mL) in

water. The combined organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude material was purified by silica gel column chromatography (hexane:EtOAc = 19:1) to yield compound **4a** (1.78 g, 84%). **^{1}H NMR** (600 MHz, $CDCl_3$): *δ* 8.48 (d, *J* = 8.4 Hz, 1H), 8.07 (d, *J* = 8.4 Hz, 1H), 7.24 (t, *J* = 7.8 Hz, 1H), 7.18 (d, *J* = 7.8 Hz, 1H), 7.11 (t, *J* = 7.2 Hz, 1H), 6.97 (t, *J* = 8.10 Hz, 1H), 1.53 (s, 9H).

***tert*-Butyl 3-bromo-9*H*-carbazole-9-carboxylate (4b).** Commercially available 3-Bromo-9*H*-carbazole (**3b**) (2.0 g, 8.13 mmol) was reacted with $(Boc)_2O$ (1.95 g, 8.94 mmol) and DMAP (1.09 g, 8.94 mmol) in THF (20 mL) according to procedure C. The crude material was purified by column chromatography over silica gel using hexane:ethyl acetate (19:1) as solvent to give compound **4b** (2.8 g, ~100%). **^{1}H NMR** (600 MHz, $CDCl_3$): *δ* 8.27 (d, *J* = 8.4 Hz, 1H), 8.19 (d, *J* = 8.4 Hz, 1H), 8.08 (d, *J* = 2.4 Hz, 1H), 7.92 (qd, *J* = 7.8, 0.6 Hz, 1H), 7.55 (dd, *J* = 6.6, 2.4 Hz, 1H), 7.49 (td, *J* = 8.4, 1.2 Hz, 1H), 7.36 (td, *J* = 8.4, 1.2 Hz, 1H), 1.76 (s, 9H).

***tert*-Butyl 4-bromo-9*H*-carbazole-9-carboxylate (4c).** Compound **3c** (1.5 g, 6.09 mmol) was reacted with $(Boc)_2O$ (1.46 g, 6.7 mmol) and DMAP (0.819 g, 6.7 mmol) in THF (20 mL) according to procedure C. The crude material was purified by silica gel column chromatography (hexane:EtOAc = 19:1) to yield compound **4c** (1.78 g, 84%). **^{1}H NMR** (600 MHz, $CDCl_3$): *δ* 8.53 (s, 1H), 8.26 (d, *J* = 8.4 Hz, 1H), 7.94 (d, *J* = 7.8 Hz, 1H), 7.82 (d, *J* = 8.4 Hz, 1H), 7.49-7.46 (m, 2H), 7.36 (t, *J* = 7.2 Hz, 1H), 1.77 (s, 9H).

Procedure D. *tert*-Butyl 2-(4-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (5a). To a mixture of compounds **4a** (0.8 g, 2.31 mmol), 1-(2-((tert-butyldimethylsilyl)oxy)-ethyl)piperazine[18] (1.13 g, 4.62 mmol), BINAP (0.144 g, 0.23 mmol) and Cs_2CO_3 (2.26 g, 6.93 mmol), toluene (15 mL) was added under N_2

atmosphere. The reaction mixture was degassed by bubbling N_2 for 5 min and then $Pd(OAc)_2$ (0.039 g, 0.17 mmol) was added quickly. The system was degassed again and refluxed for 24 h under inert condition. The reaction mixture was cooled to room temperature, filtered through a pad of celite, washed with dichloromethane and concentrated in vacuum. The crude residue was purified by column chromatography (hexane:EtOAc = 4:1) to afford compound **5a** (0.97 g, 82%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.23 (d, J = 7.8 Hz, 1H), 7.92 (s, 1H), 7.84 (d, J = 7.2 Hz, 1H), 7.81 (d, J = 9.0 Hz, 1H), 7.35 (t, J = 7.2 Hz, 1H), 7.29 (t, J = 7.2 Hz, 1H), 7.00 (dd, J = 9.0, 1.8 Hz, 1H), 3.82 (t, J = 6.6 Hz, 2H), 3.32 (t, J = 4.8 Hz, 4H), 2.74 (t, J = 4.8 Hz, 4H), 2.62 (t, J = 6.6 Hz, 2H), 1.75 (s, 9H), 0.91 (s, 9H), 0.09 (s, 6H).

***tert*-Butyl 3-(4-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (5b).** A mixture of compound **4b** (1.2 g, 3.47 mmol), 1-(2-((tert-butyldimethylsilyl)oxy)ethyl)piperazine (1.27 g, 5.20 mmol), $Pd(OAc)_2$ (0.058 g, 0.26 mmol), BINAP (0.216 g, 0.35 mmol) and Cs_2CO_3 (3.39 g, 10.4 mmol) in toluene (25 mL) was heated at 110 ^{o}C for 24 h according to procedure D. The crude material was purified by silica gel column chromatography (hexane:EtOAc = 4:1) to give compound **5b** (1.4 g, 79%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.27 (d, J = 8.4 Hz, 1H), 8.16 (d, J = 8.4 Hz, 1H), 7.93 (d, J = 7.8 Hz, 1H), 7.48 (d, J = 2.4 Hz, 1H), 7.44 (t, J = 7.8 Hz, 1H), 7.32 (t, J = 7.8 Hz, 1H), 7.13 (dd, J = 6.6, 2.4 Hz, 1H), 3.83 (t, J = 6.6 Hz, 2H), 3.28 (t, J = 4.8 Hz, 4H), 2.77 (t, J = 4.8 Hz, 4H), 2.63 (t, J = 6.6 Hz, 2H), 1.75 (s, 9H), 0.92 (s, 9H), 0.09 (s, 6H).

***tert*-Butyl 4-(4-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (5c).** A mixture of **4c** (2.70 g, 7.80 mmol), 1-(2-((tert-butyldimethylsilyl)oxy)-ethyl)piperazine (3.24 g, 13.25 mmol), $Pd(OAc)_2$ (0.0.13 g, 0.59 mmol), BINAP (0.49 g,

0.78 mmol) and Cs_2CO_3 (7.62 g, 23.4 mmol) in toluene (30 mL) was heated at 110°C for 24 h according to procedure D. The crude residue was purified by column chromatography (hexane:EtOAc = 4:1) to afford compound **5c** (3.24 g, 82%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.34 (dd, J = 8.1,4.8 Hz, 2H), 8.08 (d, J = 8.4 Hz, 1H), 7.45 (t, J = 7.2 Hz, 1H), 7.41-7.36 (m, 2H), 7.04 (d, J = 8.4 Hz, 1H), 3.85 (t, J = 6.6 Hz, 2H), 3.33 (d, J = 5.4 Hz, 2H), 2.99 (t, J = 5.4 Hz, 2H), 2.71 (t, J = 6.6 Hz, 4H), 1.75 (s, 9H), 0.91 (s, 9H), 0.10 (s, 6H).

Procedure E. *tert*-Butyl 2-(4-(2-hydroxyethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (6a). Into a stirring solution of compound **5a** (0.95 g, 1.86 mmol) in THF (10 mL) was added *n*-tetrabutylammonium fluoride (2.8 mL, 2.8 mmol, 1.0 M solution in THF) at 0 °C. The reaction mixture was then stirred at room temperature for 2 h. THF was evaporated in vacuo, and the residue was diluted with CH_2Cl_2 (25 mL) and washed with a saturated solution of $NaHCO_3$. The water layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 9:1) to give compound **6a** (0.595 g, 81%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.23 (d, J = 7.8 Hz, 1H), 7.94 (s, 1H), 7.85 (d, J = 7.8 Hz, 1H), 7.82 (d, J = 9.0 Hz, 1H), 7.36 (t, J = 7.2 Hz, 1H), 7.30 (t, J = 7.2 Hz, 1H), 7.00 (dd, J = 6.6, 1.8 Hz, 1H), 3.68 (t, J = 5.4 Hz, 2H), 3.34 (t, J = 4.8 Hz, 4H), 2.73 (t, J = 4.8 Hz, 4H), 2.64 (t, J = 5.4 Hz, 2H), 1.76 (s, 9H).

***tert*-Butyl 3-(4-(2-hydroxyethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (6b).** Compound **5b** (1.2 g, 2.35 mmol) in THF (15 mL) was reacted with *n*-tetrabutylammonium fluoride (4.71 mL, 4.71 mmol, 1.0 M solution in THF) according to procedure E. The crude

product was purified by silica gel column chromatography (EtOAc:MeOH = 9:1) to yield compound **6b** (0.78 g, 84%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.28 (d, J = 8.4 Hz, 1H), 8.18 (d, J = 8.4 Hz, 1H), 7.93 (d, J = 7.8 Hz, 1H), 7.49 (d, J = 2.4 Hz, 1H), 7.44 (t, J = 7.8 Hz, 1H), 7.33 (t, J = 7.8 Hz, 1H), 7.13 (dd, J = 6.6, 2.4 Hz, 1H), 3.69 (t, J = 5.4 Hz, 2H), 3.29 (t, J = 4.8 Hz, 4H), 2.76 (t, J = 4.8 Hz, 4H), 2.66 (t, J = 5.4 Hz, 2H), 1.75 (s, 9H).

***tert*-Butyl 4-(4-(2-hydroxyethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (6c).** Compound **5c** (3.30 g, 6.47 mmol) was reacted with *n*-tetrabutylammonium fluoride (9.70 mL, 9.70 mmol, 1.0 M solution in THF) in THF (30 mL) according to procedure E. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 9:1) to give compound **6c** (2.01 g, 80%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.27 (d, J = 6.6 Hz, 1H), 8.17 (d, J = 7.8 Hz, 1H), 7.92 (d, J = 7.8 Hz, 1H), 7.47 (s, 1H), 7.43 (t, J = 7.2 Hz, 1H), 7.31 (t, J = 7.2 Hz, 1H), 7.11 (d, J = 9.0 Hz, 1H), 3.69 (t, J = 5.4 Hz, 2H), 3.26 (s, 4H), 2.76 (s, 4H), 2.62 (t, J = 5.4 Hz, 2H), 1.73 (s, 9H).

Procedure F. *tert*-Butyl 2-(4-(2-oxoethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (7a). Into a stirring solution of compound **6a** (0.30 g, 0.76 mmol) in CH_2Cl_2 (6 mL) and DMSO (3 mL), was added Et_3N (0.74 mL, 5.31 mmol) at 0 °C. The reaction mixture was stirred for 5 min followed by addition of SO_3.py complex (0.604 g, 3.79 mmol) at 0 °C. Ice bath was removed and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was quenched by addition of water and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layer was dried using Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (hexane:EtOAc = 3:7) to give aldehyde **7a** (0.25 g, 84%). The purified aldehyde was used immediately for next step. **^{1}H NMR** (600 MHz, $CDCl_3$): δ 9.74 (s, 1H),

8.23 (d, *J* = 8.4 Hz, 1H), 7.94 (s, 1H), 7.84 (d, *J* = 7.8 Hz, 1H), 7.80 (d, *J* = 8.4 Hz, 1H), 7.36 (td, *J* = 7.2, 1.2 Hz, 1H), 7.29 (td, *J* = 7.2, 1.2 Hz, 1H), 6.97 (dd, *J* = 6.6, 1.8 Hz, 1H), 3.36 (t, *J* = 4.8 Hz, 4H), 3.24 (t, *J* = 1.2 Hz, 2H), 2.73 (t, *J* = 4.8 Hz, 4H), 1.75 (s, 9H).

***tert*-Butyl 3-(4-(2-oxoethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (7b).** Compound **6b** (0.45 g, 1.14 mmol) in CH_2Cl_2 (10 mL) and DMSO (5 mL), was oxidized using SO_3.py complex (0.905 g, 5.69 mmol) and Et_3N (1.11 mL, 7.96 mmol) according to procedure F. The crude product was purified by silica gel column chromatography (EtOAc) to yield compound **7b** (0.35 g, 78%). **^{1}H NMR** (600 MHz, $CDCl_3$): *δ* 9.76 (s, 1H), 8.28 (d, *J* = 8.4 Hz, 1H), 8.18 (d, *J* = 8.4 Hz, 1H), 7.93 (d, *J* = 7.8 Hz, 1H), 7.48 (d, *J* = 2.4 Hz, 1H), 7.44 (t, *J* = 7.2 Hz, 1H), 7.32 (t, *J* = 7.2 Hz, 1H), 7.12 (dd, *J* = 6.6, 2.4 Hz, 1H), 3.31 (t, *J* = 4.2 Hz, 4H), 3.25 (t, *J* = 1.2 Hz, 2H), 2.74 (t, *J* = 4.2 Hz, 4H), 1.74 (s, 9H).

***tert*-Butyl 4-(4-(2-oxoethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (7c).** Alcohol **6c** (0.35 g, 0.88 mmol) was oxidized using SO_3.py complex (0.704 g, 4.425 mmol), DMSO (9 mL) and Et_3N (0.86 mL, 6.19 mmol) in CH_2Cl_2 (6 mL) according to procedure F. The crude product was purified by silica gel column chromatography (hexane:EtOAc = 3:7) to give aldehyde **7c** (0.31 g, 89%). The purified aldehyde was used immediately for next step. **^{1}H NMR** (600 MHz, $CDCl_3$): *δ* 9.78 (s, 1H), 8.33 (d, *J* = 7.8 Hz, 1H), 8.27 (d, *J* = 7.8 Hz, 1H), 7.84 (d, *J* = 7.8 Hz, 1H), 8.09 (d, *J* = 8.4 Hz, 1H), 7.44-7.40 (m, 2H), 7.35 (t, *J* = 7.2 Hz, 1H), 7.06 (dd, *J* = 7.8, 1.8 Hz, 1H), 3.36-3.34 (m, 4H), 3.06 (t, *J* = 11.1 Hz, 2H), 2.73 (d, *J* = 11.4 Hz, 2H), 2.71 (d, *J* = 10.2 Hz, 2H), 1.75 (s, 9H).

Procedure G. *tert*-Butyl 2-(4-(2-((2-amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)-ethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (8a). Into a stirring solution of racemic N^6-propyl-4,5,6,7-tetrahydrobenzo[*d*]thiazole-2,6-diamine

(0.058 g, 0.27 mmol) in CH_2Cl_2 (7 mL) was added aldehyde **7a** (0.12 g, 0.31 mmol). After the mixture was stirred for 1.5 h, $NaBH(OAc)_3$ (0.13 g, 0.61 mmol) was added and the mixture was stirred for another 46 h at room temperature. The reaction mixture was quenched with a saturated solution of $NaHCO_3$ at 0 °C and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layer was dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 19:1) to afford compound **8a** (0.06 g, 38%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.23 (d, J = 8.4 Hz, 1H), 7.92 (s, 1H), 7.85 (d, J = 7.8 Hz, 1H), 7.81 (d, J = 8.4 Hz, 1H), 7.35 (td, J = 7.2, 1.2 Hz, 1H), 7.29 (td, J = 7.2, 1.2 Hz, 1H), 7.00 (dd, J = 6.0, 2.4 Hz, 1H), 4.77 (bs, 2H), 3.33 (t, J = 4.8 Hz, 4H), 3.07 (m, 1H), 2.78–2.68 (m, 8H), 2.60–2.47 (m, 6H), 2.02–2.00 (m, 1H), 1.75 (s, 10H), 1.51–1.47 (m, 2H), 0.90 (t, J = 7.2 Hz, 3H).

***tert*-Butyl 3-(4-(2-((2-amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino) ethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (8b).** Aldehyde **7b** (0.15 g, 0.38 mmol) in CH_2Cl_2 (10 mL) was reacted with racemic N^6-propyl-4,5,6,7-tetrahydrobenzo[*d*]thiazole-2,6-diamine (0.073 g, 0.34 mmol) and $NaBH(OAc)_3$ (0.162 g, 0.76 mmol) according to procedure G. Crude product was purified by column chromatography (EtOAc:MeOH = 9:1) to afford compound **8b** (0.065 g, 32%). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.27 (d, J = 8.0 Hz, 1H), 8.16 (d, J = 9.2 Hz, 1H), 7.93 (d, J = 7.6 Hz, 1H), 7.48 (d, J = 2.4 Hz, 1H), 7.44 (t, J = 7.2 Hz, 1H), 7.32 (t, J = 7.2 Hz, 1H), 7.12 (dd, J = 7.2, 2.4 Hz, 1H), 5.12 (bs, 2H), 3.28 (t, J = 4.8 Hz, 4H), 3.07–3.02 (m, 1H), 2.78–2.67 (m, 8H), 2.58–2.45 (m, 6H), 2.00–1.98 (m, 1H), 1.74 (s, 10H), 1.52–1.46 (m, 2H), 0.90 (t, J = 7.2 Hz, 3H).

***tert*-Butyl 4-(4-(2-((2-amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino) ethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (8c).** Aldehyde **7c** (0.15 g, 0.38 mmol) was reacted with racemic N^6-propyl-4,5,6,7-tetrahydrobenzo[*d*]thiazole-2,6-diamine (0.073 g, 0.34 mmol) and $NaBH(OAc)_3$ (0.162 g, 0.76 mmol) in CH_2Cl_2 (10 mL) according to procedure G. The crude product was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 19:1) to afford compound **8c** (0.91 g, 41%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.23 (d, *J* = 6.6 Hz, 1H), 8.28 (d, *J* = 6.6 Hz, 1H), 8.06 (d, *J* = 6.6 Hz, 1H), 7.43 (d, *J* = 5.4 Hz, 1H), 7.37 (d, *J* = 5.4 Hz, 2H), 7.02 (d, *J* = 6.0 Hz, 1H), 5.03 (s, 2H), 3.10-3.05 (m, 3H), 3.07-2.98 (m, 2H), 2.76–2.69 (m, 4H), 2.62–2.60 (m, 8H), 2.01–2.00 (m, 1H), 1.73 (s, 10H), 1.50–1.46 (m, 2H), 0.90 (t, *J* = 7.2 Hz, 3H).

(*S*)-*tert*-Butyl 2-(4-(2-((2-amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl) amino)ethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (9a). Aldehyde **7a** (0.125 g, 0.32 mmol) was reacted with (-)-pramipexole (0.06 g, 0.29 mmol) and $NaBH(OAc)_3$ (0.135 g, 0.64 mmol) in CH_2Cl_2 (8 mL) according to procedure G. The crude product was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 19:1) to afford compound **9a** (0.067 g, 40%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.23 (d, *J* = 8.4 Hz, 1H), 7.92 (s, 1H), 7.85 (d, *J* = 7.2 Hz, 1H), 7.81 (d, *J* = 8.4 Hz, 1H), 7.35 (td, *J* = 7.2, 1.2 Hz, 1H), 7.29 (td, *J* = 7.2, 1.2 Hz, 1H), 7.00 (dd, *J* = 6.6, 2.4 Hz, 1H), 4.76 (bs, 2H), 3.33 (t, *J* = 4.8 Hz, 4H), 3.07 (m, 1H), 2.79–2.68 (m, 8H), 2.61–2.48 (m, 6H), 2.02–2.00 (m, 1H), 1.75 (s, 10H), 1.51–1.48 (m, 2H), 0.90 (t, *J* = 7.2 Hz, 3H); $[\alpha]_D^{25}$= -44.0 (*c*=1.0 in CH_2Cl_2).

(*S*)-*tert*-Butyl 3-(4-(2-((2-amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl) amino)ethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (9b). Aldehyde **7b** (0.30 g, 0.76 mmol) was reacted with (-)-pramipexole (0.145 g, 0.69 mmol) and $NaBH(OAc)_3$

(0.323 g, 1.52 mmol) in CH_2Cl_2 (15 mL) according to procedure G. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 9:1) to afford compound **9b** (0.158 g, 39%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.27 (d, *J* = 7.2 Hz, 1H), 8.16 (d, *J* = 8.4 Hz, 1H), 7.93 (d, *J* = 7.8 Hz, 1H), 7.48 (d, *J* = 2.4 Hz, 1H), 7.43 (t, *J* = 7.2 Hz, 1H), 7.32 (t, *J* = 7.2 Hz, 1H), 7.12 (dd, *J* = 6.6, 2.4 Hz, 1H), 5.21 (bs, 2H), 3.28 (t, *J* = 4.8 Hz, 4H), 3.07–3.02 (m, 1H), 2.78–2.66 (m, 8H), 2.59–2.46 (m, 6H), 2.01–1.99 (m, 1H), 1.74 (s, 10H), 1.52–1.46 (m, 2H), 0.90 (t, *J* = 7.2 Hz, 3H).

(*S*)-*tert*-Butyl 4-(4-(2-((2-amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl) amino)ethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (9c). Aldehyde **7c** (0.150 g, 0.38 mmol) was reacted with (-)-pramipexole (0.73 g, 0.54 mmol) and $NaBH(OAc)_3$ (0.162 g, 0.76 mmol) in CH_2Cl_2 (10 mL) according to procedure G. The crude product was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 19:1) to afford compound **9c** (0.097 g, 43%). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.32 (d, *J* = 7.2 Hz, 1H), 8.28 (d, *J* = 7.8 Hz, 1H), 8.06 (d, *J* = 7.8 Hz, 1H), 7.43 (d, *J* = 7.2 Hz, 1H), 7.40-7.35 (m, 2H), 7.30 (d, *J* = 7.2 Hz, 1H), 4.83 (bs, 2H), 3.33 (d, *J* = 8.4 Hz, 2H), 3.06 (m, 3H), 2.99 (m, 2H), 2.76–2.70 (m, 4H), 2.60 (m, 7H), 1.74 (s, 10H), 1.51–1.48 (m, 2H), 0.90 (t, *J* = 6.6 Hz, 3H); $[\alpha]_D^{25}$= -27.20 (*c*=1.0 in CH_2Cl_2).

Procedure H. N^6-(2-(4-(9*H*-carbazol-2-yl)piperazin-1-yl)ethyl)-N^6-propyl-4,5,6,7-tetrahydrobenzo-[*d*]thiazole-2,6-diamine (10a) (D-652). To a stirred solution of **8a** (0.055 g, 0.09 mmol) in CH_2Cl_2 (3 mL) at 0 ^{o}C, trifluoroacetic acid (3 mL) was added slowly and the reaction mixture was stirred for 3 h at room temperature. Unreacted TFA and solvent were removed under reduced pressure and the obtained TFA salt was washed with ether for several times followed by drying to yield **10a** (0.079 g, 90%). **^{1}H**

NMR (600 MHz, CD_3OD): δ 7.99 (d, J = 8.4 Hz, 1H), 7.96 (d, J = 7.8 Hz, 1H), 7.42 (d, J = 7.8 Hz, 1H), 7.32 (t, J = 7.2 Hz, 1H), 7.25 (s, 1H), 7.13 (t, J = 7.2 Hz, 1H), 7.03 (d, J = 8.4 Hz, 1H), 3.84 (m, 1H), 3.64–3.55 (m, 5H), 3.48-3.42 (m, 3H), 3.36 (s, 4H), 3.23-3.14 (m, 2H), 3.01-2.99 (m, 1H), 2.84-2.79 (m, 1H), 2.71–2.65 (m, 2H), 2.32-2.31 (m, 1H), 2.02-1.95 (m, 1H), 1.83–1.79 (m, 2H), 1.03 (t, J = 7.2 Hz, 3H); **^{13}C NMR** (150 MHz, CD_3OD): δ 170.28, 160.68, 145.32, 140.57, 132.69, 125.56, 124.53, 122.50, 121.20, 120.14, 119.84, 119.33, 118.79, 118.29, 117.08, 115.15, 111.37, 110.77, 109.73, 59.75, 52.49, 51.56, 50.08, 22.29, 21.88, 21.39, 18.01, 10.19; Anal. Calcd for $C_{28}H_{36}N_6S.4CF_3COOH$: C, 45.77; H, 4.27; N, 8.90. Found: C, 45.71; H, 4.64; N, 8.87.

***N*^{6}-(2-(4-(9*H*-carbazol-3-yl)piperazin-1-yl)ethyl)-*N*^{6}-propyl-4,5,6,7-tetrahydrobenzo [*d*]thiazole-2,6-diamine (10b) (D-627).** Compound **8b** (0.05 g, 0.09 mmol) in CH_2Cl_2 (2 mL) at 0 ^{o}C, was treated with trifluoroacetic acid (2 mL) according to procedure H to obtain the TFA salt of compound **10b** (0.052 g, 84%). **^{1}H NMR** (400 MHz, CD_3OD): δ 8.01 (d, J = 8.0 Hz, 1H), 7.66 (d, J = 1.6 Hz, 1H), 7.41-7.36 (m, 2H), 7.32 (t, J = 7.2 Hz, 1H), 7.16-7.14 (m, 1H), 7.11 (t, J = 7.2 Hz, 1H), 3.34 (s, 1H), 3.19 (s, 4H), 2.98–2.91 (m, 2H), 2.80-2.51 (m, 12H), 2.07–2.04 (m, 1H), 1.81-1.77 (m, 1H), 1.63–1.53 (m, 2H), 0.95 (t, J = 7.2 Hz, 3H); **^{13}C NMR** (100 MHz, CD_3OD): δ 168.63, 144.25, 143.69, 143.37, 140.69, 135.84, 125.07, 123.21, 122.92, 119.50, 118.21, 118.00, 113.45, 110.87, 110.41, 107.90, 58.85, 55.41, 53.24, 53.12, 51.07, 25.37, 24.70, 23.79, 20.35, 10.43; Anal. Calcd for $C_{28}H_{36}N_6S.2CF_3COOH$: C, 53.62; H, 5.34; N, 11.73. Found: C, 53.64; H, 5.83; N, 11.34.

***N*^{6}-(2-(4-(*9H*-carbazol-4-yl)piperazin-1-yl)ethyl)-*N*^{6}-propyl-4,5,6,7-tetrahydrobenzo [*d*]thiazole-2,6-diamine (10c) (D-658).** Compound **8c** (0.082 g, 0.14 mmol) was treated with trifluoroacetic acid (3 mL) in CH_2Cl_2 (3 mL) according to procedure H to furnish the

TFA salt of **10c** (0.091g, 90%). **^{1}H NMR** (600 MHz, CD_3OD): δ 8.08 (d, J = 7.8 Hz, 1H), 7.45 (d, J = 7.8 Hz, 1H), 7.36 (t, J = 7.8 Hz, 1H), 7.31 (t, J = 7.8 Hz, 1H), 7.22 (d, J = 7.8 Hz, 1H), 7.19 (t, J = 7.2 Hz, 1H), 6.82 (d, J = 7.8 Hz, 1H), 3.94–3.89 (m, 1H), 3.83 (d, J = 7.2 Hz, 2H), 3.77-3.59 (m, 8H), 3.29 (d, J = 1.2 Hz, 1H), 3.24-3.20 (m, 3H), 3.06-3.04 (m, 1H), 2.92-2.88 (m, 1H), 2.78–2.71 (m, 2H), 2.37-2.36 (m, 1H), 2.11-2.04 (m, 1H), 1.86–1.82 (m, 2H), 1.04 (t, J = 7.2 Hz, 3H); **^{13}C NMR** (150 MHz, CD_3OD): δ 170.32, 161.19, 146.42, 141.50, 139.78, 132.81, 125.91, 124.74, 121.78, 121.34, 118.62, 115.64, 111.66, 110.23, 107.39, 59.17, 53.36, 53.25, 52.80, 50.81, 48.60, 48.01, 44.95, 22.51, 22.03, 21.49, 18.34, 9.79; Anal. Calcd for $C_{28}H_{36}N_6S$, $3CF_3COOH$, CH_3OH, H_2O : C, 47.73; H, 5.15; N, 9.54. Found: C, 47.63; H, 4.67; N, 9.23.

(*S*)-*N*^{6}-(2-(4-(9*H*-carbazol-2-yl)piperazin-1-yl)ethyl)-*N*^{6}-propyl-4,5,6,7-tetrahydro-benzo[*d*]thiazole-2,6-diamine (11a) (D-651). Compound **9a** (0.065 g, 0.11 mmol) was treated with trifluoroacetic acid (3 mL) in CH_2Cl_2 (3 mL) according to procedure H to furnish the TFA salt of **11a** (0.085 g, 93%). **^{1}H NMR** (600 MHz, CD_3OD): δ 7.99 (d, J = 8.4 Hz, 1H), 7.96 (d, J = 7.8 Hz, 1H), 7.42 (d, J = 7.8 Hz, 1H), 7.33 (t, J = 7.2 Hz, 1H), 7.26 (s, 1H), 7.13 (t, J = 7.2 Hz, 1H), 7.04 (d, J = 8.4 Hz, 1H), 3.86 (m, 1H), 3.65–3.56 (m, 5H), 3.49-3.42 (m, 3H), 3.37 (s, 4H), 3.25-3.16 (m, 2H), 3.02-3.00 (m, 1H), 2.85-2.81 (m, 1H), 2.73–2.66 (m, 2H), 2.34-2.32 (m, 1H), 2.04-1.97 (m, 1H), 1.83–1.80 (m, 2H), 1.03 (t, J = 7.2 Hz, 3H); **^{13}C NMR** (150 MHz, CD_3OD): δ 170.29, 160.41, 145.25, 140.58, 132.67, 125.55, 124.55, 122.49, 121.21, 120.15, 119.82, 119.32, 118.82, 118.24, 116.97, 115.06, 111.39, 110.76, 109.70, 59.75, 52.50, 51.55, 50.17, 22.31, 21.89, 21.40, 17.98, 10.18; $[\alpha]_D^{25}$= -24.8 (c=1.0 in CH_3OH); Anal. Calcd for $C_{28}H_{36}N_6S.3CF_3COOH.2H_2O$: C, 47.11; H, 5.00; N, 9.70. Found: C, 47.63; H, 4.67; N, 9.23.

(*S*)-*N*^{6}-(2-(4-(9*H*-carbazol-3-yl)piperazin-1-yl)ethyl)-*N*^{6}-propyl-4,5,6,7-tetrahydro-benzo[*d*]thiazole-2,6-diamine (11b) (D-636). Compound **9b** (0.15 g, 0.25 mmol) was treated with trifluoroacetic acid (8 mL) in CH_2Cl_2 (8 mL) according to procedure H to furnish the TFA salt of **11b** (0.20 g, 96%). **^{1}H NMR** (600 MHz, CD_3OD): δ 8.27 (s, 1H), 8.11 (d, *J* = 7.2 Hz, 1H), 7.59-7.54 (m, 2H), 7.49 (d, *J* = 8.4 Hz, 1H), 7.44 (t, *J* = 7.2 Hz, 1H), 7.21 (t, *J* = 7.2 Hz, 1H), 3.96 (m, 1H), 3.80 (s, 4H), 3.57–3.45 (m, 2H), 3.34-3.27 (m, 2H), 3.20-3.10 (m, 7H), 2.97-2.92 (m, 1H), 2.83-2.75 (m, 2H), 2.44–2.42 (m, 1H), 2.17-2.10 (m, 1H), 1.90–1.84 (m, 2H), 1.07 (t, *J* = 7.2 Hz, 3H); **^{13}C NMR** (150 MHz, CD_3OD): δ 170.35, 160.54, 141.04, 139.17, 135.08, 132.80, 126.39, 123.44, 122.29, 119.95, 119.05, 117.60, 111.67, 111.55, 111.45, 110.90, 59.31, 54.18, 53.61, 51.21, 50.61, 22.42, 22.09, 21.50, 17.98, 9.82; $[\alpha]_D^{25}$= -17.4 (*c*=1.0 in CH_3OH); Anal. Calcd for $C_{28}H_{36}N_6S.3CF_3COOH.H_2O$: C, 48.11; H, 4.87; N, 9.90. Found: C, 48.25; H, 4.99; N, 9.58.

***(S)*-*N*^{6}-(2-(4-(9H-carbazol-4-yl)piperazin-1-yl)ethyl)-*N*^{6}-propyl-4,5,6,7-tetrahydro-benzo[*d*]thiazole-2,6-diamine (11c) (D-657).** Compound **9c** (0.83 g, 0.14 mmol) was treated with trifluoroacetic acid (7 mL) in CH_2Cl_2 (7 mL) according to procedure H to furnish the TFA salt of **11c** (0.085 g, 93%). **^{1}H NMR** (600 MHz, CD_3OD): δ 8.07 (d, *J* = 7.2 Hz, 1H), 7.44 (d, *J* = 7.8 Hz, 1H), 7.36 (d, *J* = 7.8 Hz, 1H), 7.31 (t, *J* = 7.8 Hz, 1H), 7.22 (d, *J* = 7.8 Hz,, 1H), 7.19 (t, *J* = 7.2 Hz, 1H), 6.81 (d, *J* = 8.4 Hz, 1H), 3.90 (s, 1H) 3.84 (d, *J* = 6.6 Hz, 2H), 3.78–3.71 (m, 4H), 3.56-3.43 (m, 4H), 3.29 (s, 2H), 3.26-3.21 (m, 3H), 3.06-3.04 (m, 1H), 2.92-2.88 (m, 1H), 2.78–2.71 (m, 2H), 2.36 (bs, 1H), 2.09-2.04 (m, 1H), 1.86–1.82 (m, 2H), 1.04 (t, *J* = 7.2 Hz, 3H); **^{13}C NMR** (150 MHz, CD_3OD): δ 170.32, 161.06, 146.42, 139.77, 132.80, 126.38, 124.29, 122.26, 121.30, 119.075, 118.18, 115.62, 111.61, 110.74, 109.72, 59.72, 58.72, 53.27, 52.76, 50.70, 44.91, 22.99,

22.34, 21.44, 18.30, 10.07, 9.46; $[\alpha]_D^{25}$= -25.0 (*c*=1.0 in CH_3OH); Anal. Calcd for $C_{28}H_{36}N_6S.3CF_3COOH$: C, 49.16; H, 4.73; N, 10.12. Found: C, 48.93; H, 4.84; N, 9.77.

***tert*-Butyl 2-(4-(2-((5-methoxy-1,2,3,4-tetrahydronaphthalen-2-yl)(propyl)amino) ethyl)-piperazin-1-yl)-9*H*-carbazole-9-carboxylate (12a).** Compound **7a** (0.10 g, 0.25 mmol) was reacted with (±)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (0.056 g, 0.25 mmol) and $NaBH(OAc)_3$ (0.108 g, 0.51 mmol) in CH_2Cl_2 (7 mL) according to procedure G. The crude product was purified by silica gel column chromatography (hexane:EtOAc = 1:3) to afford compound **12a** (0.115 g, 77%). **^{1}H NMR** (600 MHz, $CDCl_3$): *δ* 8.23 (d, *J* = 7.8 Hz, 1H), 7.91 (s,1H), 7.83 (d, *J* = 7.8 Hz, 1H), 7.79 (d, *J* = 8.4 Hz, 1H), 7.34 (t, *J* = 7.2, Hz, 1H), 7.28 (t, *J* = 7.2 Hz, 1H), 7.08 (t, *J* = 7.8 Hz, 1H), 6.98 (dd, *J* = 6.6, 1.8 Hz, 1H), 6.72 (d, *J* = 7.2, Hz, 1H), 6.64 (d, *J* = 7.8, Hz, 1H), 3.79 (s, 3H), 3.32 (t, *J* = 4.8 Hz, 4H), 3.02–2.93 (m, 2H), 2.88-2.85 (m, 1H), 2.79–2.75 (m, 3H), 2.70 (t, *J* = 4.8 Hz, 4H), 2.56–2.50 (m, 5H), 2.09–2.06 (m, 1H), 1.74 (s, 9H), 1.62–1.55 (m, 1H), 1.53-1.47 (m, 2H), 0.91 (t, *J* = 7.2 Hz, 3H).

***tert*-Butyl 3-(4-(2-((5-methoxy-1,2,3,4-tetrahydronaphthalen-2-yl)(propyl)amino) ethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (12b).** Compound **7b** (0.170 g, 0.43 mmol) was reacted with (±)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (0.095 g, 0.43 mmol) and $NaBH(OAc)_3$ (0.183 g, 0.86 mmol) in CH_2Cl_2 (10 mL) according to procedure G. The crude product was purified by silica gel column chromatography with EtOAc to afford compound **12b** (0.11 g, 43%). **^{1}H NMR** (600 MHz, $CDCl_3$): *δ* 8.27 (d, *J* = 7.8 Hz, 1H), 8.16 (d, *J* = 8.4 Hz, 1H), 7.92 (d, *J* = 7.8 Hz, 1H), 7.47 (d, *J* = 2.4 Hz, 1H), 7.43 (t, *J* = 7.2, Hz, 1H), 7.31 (t, *J* = 7.2 Hz, 1H), 7.12 (dd, *J* = 6.6, 2.4 Hz, 1H), 7.08 (t, *J* = 8.4 Hz, 1H), 6.72 (d, *J* = 7.2, Hz, 1H), 6.64 (d, *J* = 8.4 Hz, 1H), 3.79 (s, 3H), 3.27 (t, *J* =

4.8 Hz, 4H), 3.03–2.98 (m, 2H), 2.90-2.87 (m, 1H), 2.81–2.77 (m, 3H), 2.73 (t, *J* = 4.8 Hz, 4H), 2.59–2.51 (m, 5H), 2.11–2.08 (m, 1H), 1.74 (s, 9H), 1.63–1.57 (m, 1H), 1.55-1.49 (m, 2H), 0.91 (t, *J* = 7.2 Hz, 3H).

***tert*-Butyl 4-(4-(2-((5-methoxy-1,2,3,4-tetrahydronaphthalen-2-yl)(propyl)amino) ethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (12c).** Compound **7c** (0.10 g, 0.25 mmol) was reacted with (±)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (0.055 g, 0.25 mmol) and $NaBH(OAc)_3$ (0.108 g, 0.50 mmol) in CH_2Cl_2 (8 mL) according to procedure G. The crude product was purified by silica gel column chromatography (hexane:EtOAc = 1:3) to afford compound **12c** (0.112 g, 74%). **^{1}H NMR** (600 MHz, $CDCl_3$): *δ* 8.23 (d, *J* = 8.4 Hz, 1H), 8.16 (d, *J* = 8.4, Hz, 1H), 8.09 (d, *J* = 7.2 Hz, 1H), 7.47 (d, *J* = 7.8 Hz, 1H), 7.39 (m, 2H), 7.13 (t, *J* = 7.8 Hz, 1H), 7.00 (d, *J* = 7.8 Hz, 1H), 6.70 (q, *J* = 7.8 Hz, 2H), 3.92 (bs, 2H), 3.79 (s, 3H), 3.58–3.48 (m, 4H), 2.29 (m, 2H), 3.06-3.04 (m, 5H), 2.62, (m, 1H), 2.37 (bs, 1H), 2.09–2.06 (m, 1H), 1.74 (s, 9H), 1.62–1.55 (m, 1H), 1.53-1.47 (m, 2H), 0.92 (t, *J* = 7.2 Hz, 3H).

(*S*)-*tert*-Butyl 2-(4-(2-((5-methoxy-1,2,3,4-tetrahydronaphthalen-2-yl)(propyl) amino)ethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (13a). Compound **7a** (0.10 g, 0.25 mmol) was reacted with (-)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (0.056 g, 0.25 mmol) and $NaBH(OAc)_3$ (0.108 g, 0.51 mmol) in CH_2Cl_2 (7 mL) according to procedure G. The crude product was purified by silica gel column chromatography (hexane:EtOAc = 1:3) to afford compound **13a** (0.11 g, 74%). **^{1}H NMR** (600 MHz, $CDCl_3$): *δ* 8.23 (d, *J* = 8.4 Hz, 1H), 7.91 (s,1H), 7.82 (d, *J* = 7.8 Hz, 1H), 7.78 (d, *J* = 8.4 Hz, 1H), 7.34 (t, *J* = 7.2, Hz, 1H), 7.28 (t, *J* = 7.2 Hz, 1H), 7.08 (t, *J* = 7.8 Hz, 1H), 6.97 (dd, *J* = 6.6, 1.8 Hz, 1H), 6.71 (d, *J* = 7.2, Hz, 1H), 6.63 (d, *J* = 7.8, Hz, 1H),

3.78 (s, 3H), 3.32 (t, *J* = 4.8 Hz, 4H), 3.02–2.94 (m, 2H), 2.88-2.85 (m, 1H), 2.79–2.74 (m, 3H), 2.70 (t, *J* = 4.8 Hz, 4H), 2.56–2.50 (m, 5H), 2.08–2.06 (m, 1H), 1.74 (s, 9H), 1.61–1.54 (m, 1H), 1.52-1.47 (m, 2H), 0.91 (t, *J* = 7.2 Hz, 3H); $[\alpha]_D^{25}$= -28.6 (*c*=1.0 in CH_2Cl_2).

(*S*)- *tert*-Butyl 3-(4-(2-((5-methoxy-1,2,3,4-tetrahydronaphthalen-2-yl)(propyl)amino) ethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (13b). Compound **7b** (0.140 g, 0.456 mmol) was reacted with (-)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (0.070 g, 0.32 mmol) and $NaBH(OAc)_3$ (0.151 g, 0.71 mmol) in CH_2Cl_2 (10 mL) according to procedure G. The crude product was purified by silica gel column chromatography with EtOAc to afford compound 13b (0.07 g, 33%). **^{1}H NMR** (600 MHz, $CDCl_3$): *δ* 8.18 (d, *J* = 6 Hz, 1H), 8.09 (m, 1H), 7.83 (d, *J* = 7.2 Hz, 1H), 7.38-7.32 (m, 2H), 7.23 (q, *J* = 9.6, 7.5 Hz, 1H), 7.03 (d, *J* = 9 Hz, 1H), 6.99 (t, *J* = 7.8 Hz, 1H), 6.63 (d, *J* = 6.6, Hz, 1H), 6.55 (d, *J* = 7.8, Hz, 1H), 3.70 (s, 3H), 3.18 (t, *J* = 4.2 Hz, 4H), 3.27–3.26 (m, 1H), 3.12-3.10 (m, 2H), 2.93–2.90 (m, 2H), 2.81–2.78 (m, 1H), 2.69-2.64 (m, 5H), 2.48–2.43 (m, 4H), 2.03–2.00 (m, 1H), 1.65 (s, 9H), 1.54–1.47 (m, 1H), 1.45-1.37 (m, 2H), 0.82 (t, *J* = 7.2 Hz, 3H).

(*S*)-*tert*-Butyl 4-(4-(2-((5-methoxy-1,2,3,4-tetrahydronaphthalen-2-yl)(propyl) amino)ethyl)piperazin-1-yl)-9*H*-carbazole-9-carboxylate (13c). Compound **7c** (0.10 g, 0.25 mmol) was reacted with (-)-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propyl-amine (0.055 g, 0.25 mmol) and $NaBH(OAc)_3$ (0.108 g, 0.50 mmol) in CH_2Cl_2 (8 mL) according to procedure G. The crude product was purified by silica gel column chromatography (hexane:EtOAc = 1:3) to afford compound **13c** (0.124 g, 82%). **^{1}H NMR** (600 MHz, $CDCl_3$): *δ* 8.35-8.30 (m, 2H), 7.07 (d, *J* = 7.8 Hz, 1H), 7.44-7.41 (m, 1H), 7.41-

7.39 (m, 2H), 7.08 (d, *J* = 7.8 Hz, 1H), 7.04 (d, *J* = 7.8 Hz, 1H), 6.73 (d, *J* = 7.2, Hz, 1H), 6.64 (d, *J* = 8.4 Hz, 1H), 3.79 (s, 3H), 3.53-3.48 (m, 4H), 3.29 (m, 2H), 3.16-3.04 (m, 5H), 2.67-2.60 (m, 1H), 2.37 (bs, 1H), 2.03–2.01 (m, 2H), 1.85–1.84 (m, 3H), 1.75 (s, 9H), 1.26-1.24 (m, 3H), 0.92 (t, *J* = 7.2 Hz, 3H); $[\alpha]_D^{25}$= -20.3 (*c*=1.0 in CH_2Cl_2).

Procedure I. 6-((2-(4-(9*H*-carbazol-2-yl)piperazin-1-yl)ethyl)(propyl)amino)-5,6,7,8-tetrahydronaphthalen-1-ol (14a) (D-654). A mixture of compound **12a** (0.10 g, 0.17 mmol) and 48% aqueous HBr (8 mL) was refluxed at 130 °C for 4 h. The reaction mixture was evaporated to dryness, washed with ether followed by vacuo drying to yield HBr salt of **14a** (0.095 g, 78%). **^{1}H NMR** (600 MHz, CD_3OD): *δ* 8.11 (d, *J* = 8.4 Hz, 1H), 8.03 (d, *J* = 7.8 Hz, 1H), 7.67-7.62 (m, 1H), 7.46 (d, *J* = 7.8 Hz, 1H), 7.38 (t, *J* = 7.2 Hz, 1H), 7.34-7.29 (m, 1H), 7.17 (t, *J* = 7.2 Hz, 1H), 6.96 (t, *J* = 7.8 Hz, 1H), 6.67 (d, *J* = 7.8, Hz, 1H), 6.62 (d, *J* = 7.8, Hz, 1H), 3.99-3.86 (m, 5H), 3.84–3.72 (m, 5H), 3.68-3.63 (m, 2H), 3.39 (m, 1H), 3.33-3.29 (m, 2H), 3.16-3.08 (m, 2H), 2.71-2.66 (m, 1H), 2.63 (m, 1H), 2.49-2.47 (m, 1H), 2.00–1.93 (m, 3H), 1.07 (t, *J* = 7.2 Hz, 3H); **^{13}C NMR** (150 MHz, CD_3OD): *δ* 154.74, 140.88, 140.12, 133.27, 129.28, 126.63, 125.85, 122.06, 121.59, 121.12, 120.99, 119.93, 119.78, 119.11, 117.61, 112.08, 110.61, 110.26, 61.26, 52.93, 51.47, 50.73, 50.47, 50.32, 45.16, 29.29, 23.55, 22.35, 18.26, 9.92, 8.31; Anal. Calcd for $C_{31}H_{38}N_4O.3HBr$: C, 51.33; H, 5.70; N, 7.72. Found: C, 51.50; H, 5.92; N, 7.46.

6-((2-(4-(9*H*-carbazol-3-yl)piperazin-1-yl)ethyl)(propyl)amino)-5,6,7,8-tetrahydro-naphthalen-1-ol (14b) (D-650). A mixture of compound **12b** (0.08 g, 0.13 mmol) and 48% aqueous HBr (7 mL) was reflexed according to procedure I to yield HBr salt of **14b** (0.095 g, 98%). **^{1}H NMR** (600 MHz, CD_3OD): *δ* 8.58 (s, 1H), 8.14 (d, *J* = 7.8 Hz, 1H), 7.76 (d, *J* = 8.4 Hz, 1H), 7.61 (d, *J* = 9.0 Hz, 1H), 7.50 (d, *J* = 7.8 Hz, 1H), 7.44 (t, *J* = 7.2 Hz, 1H),

7.21 (t, *J* = 7.2 Hz, 1H), 6.94 (t, *J* = 7.8 Hz, 1H), 6.66 (d, *J* = 7.8 Hz, 1H), 6.61 (d, *J* = 7.8, Hz, 1H), 4.30 (s, 4H), 4.11-4.04 (m, 6H), 3.93–3.89 (m, 2H), 3.78 (s, 1H), 3.46-3.39 (m, 1H), 3.33-3.29 (m, 2H), 3.22-3.16 (m, 1H), 3.09-3.05 (m, 1H), 2.72-2.69 (m, 1H), 2.52-2.47 (m, 1H), 2.02–1.94 (m, 3H), 1.07 (t, *J* = 7.2 Hz, 3H); **^{13}C NMR** (150 MHz, CD_3OD): *δ* 154.67, 141.05, 139.88, 133.32, 132.51, 127.22, 126.31, 123.45, 122.11, 121.60, 120.77, 120.15, 119.76, 117.91, 116.92, 112.40, 112.24, 111.96, 111.54, 111.40, 110.53, 60.95, 53.09, 50.47, 49.91, 29.34, 22.81, 22.37, 18.30, 10.26, 9.73; Anal. Calcd for $C_{31}H_{38}N_4O.3HBr.2H_2O$: C, 48.90; H, 5.96; N, 7.36. Found: C, 49.30; H, 5.86; N, 7.24.

6-((2-(4-(9*H*-carbazol-4-yl)piperazin-1-yl)ethyl)(propyl)amino)-5,6,7,8-tetrahydro-naphthalen-1-ol (14c) (D-655). Compound **12c** (0.11 g, 0.18 mmol) was refluxed with 48% aqueous HBr (8 mL) according to procedure I to yield HBr salt of **14c** (0.097 g, 78%). **^{1}H NMR** (600 MHz, CD_3OD): *δ* 8.11 (d, *J* = 7.8 Hz, 1H), 8.07 (d, *J* = 7.8 Hz, 1H), 7.46-7.43 (m, 1H), 7.37-7.32 (m, 1H), 7.31-7.27 (m, 1H), 7.25-7.19 (m, 1H), 6.95 (t, *J* = 7.8 Hz, 1H), 6.87 (d, *J* = 7.8 Hz, 1H), 6.79 (d, *J* = 6.6, Hz, 1H), 6.61 (d, *J* = 7.8, Hz, 1H), 4.02 (s, 3H), 3.90–3.85 (m, 5H), 3.58-3.57 (m, 3H), 3.39 (m, 2H), 3.33-3.29 (m, 2H), 3.29-3.17 (m, 2H), 3.11-3.08 (m, 1H), 2.72-2.70 (m, 1H), 2.48 (m, 1H), 1.99–1.94 (m, 3H), 1.07 (t, *J* = 7.2 Hz, 3H); **^{13}C NMR** (150 MHz, CD_3OD): *δ* 154.71, 141.47, 139.70, 133.20, 126.61, 125.85, 124.74, 121.97, 121.81, 121.58, 121.25, 119.96, 118.76, 118.66, 115.57, 112.07, 110.24, 110.21,107.23 61.40, 52.89, 50.25, 48.57, 47.09, 45.57, 43.81, 29.23, 23.57, 22.30, 18.45, 9.91; Anal. Calcd for $C_{31}H_{38}N_4O.3HBr$: C, 51.33; H, 5.70; N, 7.72. Found: C, 51.07; H, 5.91; N, 8.17.

(*S*)-6-((2-(4-(9*H*-carbazol-2-yl)piperazin-1-yl)ethyl)(propyl)amino)-5,6,7,8-tetrahydronaphthalen-1-ol (15a) (D-653). Compound **13a** (0.10 g, 0.17 mmol) was

refluxed with 48% aqueous HBr (8 mL) according to procedure I to yield HBr salt of **15a** (0.10 g, 82%). **^{1}H NMR** (600 MHz, CD_3OD): *δ* 8.11 (d, *J* = 8.4 Hz, 1H), 8.03 (d, *J* = 7.8 Hz, 1H), 7.62 (m, 1H), 7.46 (d, *J* = 7.8 Hz, 1H), 7.38 (t, *J* = 7.2 Hz, 1H), 7.34-7.28 (m, 1H), 7.17 (t, *J* = 7.2 Hz, 1H), 6.96 (t, *J* = 7.8 Hz, 1H), 6.68 (d, *J* = 7.8, Hz, 1H), 6.62 (d, *J* = 7.8, Hz, 1H), 3.99 (s, 3H), 3.93-3.85 (m, 2H), 3.82–3.63 (m, 7H), 3.39 (m, 1H), 3.33-3.29 (m, 2H), 3.17-3.08 (m, 2H), 2.70 (m, 1H), 2.63-2.62 (m, 1H), 2.49-2.47 (m, 1H), 2.01–1.94 (m, 3H), 1.07 (t, *J* = 7.2 Hz, 3H); **^{13}C NMR** (150 MHz, CD_3OD): *δ* 154.73, 140.86, 140.14, 133.28, 129.27, 126.62, 125.82, 122.08, 121.60, 121.09, 120.97, 119.93, 119.76, 119.10, 117.58, 112.08, 110.60, 110.26, 72.29, 61.27, 52.94, 51.83, 51.45, 50.78, 50.49, 41.61, 29.31, 23.48, 22.35, 18.26, 9.92, 8.31; $[\alpha]_D^{25}$= -21.5 (*c*=1.0 in CH_3OH); Anal. Calcd for $C_{31}H_{38}N_4O.3HBr$: C, 51.33; H, 5.70; N, 7.72. Found: C, 51.44; H, 5.93; N, 7.47.

(*S*)-6-((2-(4-(9*H*-carbazol-3-yl)piperazin-1-yl)ethyl)(propyl)amino)-5,6,7,8-tetra-hydronaphthalen-1-ol (15b) (D-659). Compound **13b** (0.07 g, 0.12 mmol) was refluxed with 48% aqueous HBr (7 mL) according to procedure I to yield HBr salt of **15b** (0.085 g, 97%). **^{1}H NMR** (600 MHz, CD_3OD): *δ* 8.58 (s, 1H), 8.15 (q, *J* = 7.8 Hz, 2H), 7.76 (d, *J* = 6.6 Hz, 1H), 7.61 (d, *J* = 8.4 Hz, 1H), 7.57 (d, *J* = 7.8 Hz, 1H), 7.43 (q, *J* = 7.8 Hz, 1H), 7.21 (d, *J* = 7.8 Hz, 1H), 6.94 (t, *J* = 7.2 Hz, 1H), 6.66 (d, *J* = 7.2 Hz, 1H), 6.61 (d, *J* = 7.8, Hz, 1H), 4.17 (s, 4H), 3.91-3.85 (m, 6H), 3.87–3.85 (m, 2H), 3.57 (s, 1H), 3.50-3.49 (m, 1H), 3.32-3.28 (m, 2H), 3.17-3.14 (m, 1H), 3.08-3.05 (m, 1H), 2.69-2.67 (m, 1H), 2.51-2.46 (m, 1H), 1.96–1.93 (m, 3H), 1.48 (t, *J* = 6.0 Hz, 3H); **^{13}C NMR** (150 MHz, CD_3OD): *δ* 157.19, 151.08, 147.936, 147.513.32, 138.93, 137.85, 133.55, 132.78, 129.08,127.16, 126.92, 122.87, 122.74, 121.64, 119.43, 118.42, 117.40, 107.78, 106.90, 106.54, 57.16, 55.21, 51.85, 50.41, 48.10, 40.131, 29.71, 25.60, 23.87, 22.08, 11.92; $[\alpha]_D^{25}$= -22.2 (*c*=1.0

in CH_3OH); Anal. Calcd for $C_{31}H_{38}N_4O.4HBr$: C, 46.18; H, 5.25; N, 6.95. Found: C, 46.13; H, 5.66; N, 7.59.

(*S*)-6-((2-(4-(9*H*-carbazol-4-yl)piperazin-1-yl)ethyl)(propyl)amino)-5,6,7,8-tetra-hydronaphthalen-1-ol (15c) (D-656). Compound **13c** (0.112 g, 0.18 mmol) was refluxed with 48% aqueous HBr (9 mL) according to procedure I to yield HBr salt of **15c** (0.10 g, 82%). **^{1}H NMR** (600 MHz, CD_3OD): δ 8.09 (d, J = 7.2 Hz, 1H), 7.34 (d, J = 8.4 Hz, 1H), 7.33 (t, J = 7.2 Hz, 1H), 7.26 (dd, J = 4.8, 2.4 Hz, 1H), 7.20 (d, J = 8.4 Hz, 2H), 6.92 (t, J = 7.8 Hz, 1H), 6.72 (d, J = 7.2 Hz, 1H), 6.65 (d, J = 7.2 Hz, 1H), 6.62 (d, J = 7.8, Hz, 1H), 4.02 (s, 3H), 3.90-3.84 (m, 6H), 3.59–3.56 (m, 2H), 3.40 (m, 2H), 3.30-3.28 (m, 2H), 3.19-3.14 (m, 1H), 3.06-3.05 (m, 1H), 2.71-2.68 (m, 1H), 2.47 (bs, 1H), 1.96–1.92 (m, 3H), 1.05 (t, J = 7.2 Hz, 3H); **^{13}C NMR** (150 MHz, CD_3OD): δ 154.69, 141.41, 139.73, 133.24, 127.24, 125.30, 124.28, 122.56, 121.57, 121.21,120.28, 119.63, 119.28, 118.33, 115.52, 112.43, 111.75, 110.77, 109.71, 107.68, 61.27, 52.94, 51.83, 60.95, 52.82, 50.31, 44.47, 43.68, 22.53, 18.97, 9.92, ; $[\alpha]_D^{25}$= -17.8 (c=1.0 in CH_3OH); Anal. Calcd for) $C_{31}H_{38}N_4O.2HBr.\ CH_2Cl_2$: C, 52.69; H, 5.80; N, 7.68. Found: C, 52.23; H, 6.18; N, 7.96.

9-(2-bromoethyl)-9*H*-carbazole (16). A suspension of carbazole (1.0 g, 5.98 mmol), K_2CO_3 (1.82 g, 13.16 mmol), tetrabutylammonium bromide (0.039 g, 0.12 mmol) and KOH (2.25 g, 40.07 mmol) in dibromoethane (10 mL, 119.61 mmol) was stirred at 50 °C under N_2 overnight. The reaction mixture was filtered off and diluted with CH_2Cl_2. The organic layer was washed with water, dried over sodium sulfate, filtered and concentrated. The crude product was purified by silica gel column chromatography with petroleum ether to afford compound **16** (0.52 g, 32%). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.10 (d, J = 7.6 Hz, 2H), 7.51-7.42 (m, 4H), 7.29-7.25 (m, 2H), 4.71 (t, J = 7.2 Hz, 2H), 3.68 (t, J = 7.2 Hz, 2H).

9-(2-(4-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)piperazin-1-yl)ethyl)-9*H*-carbazole (17). A mixture of compound **16** (0.7 g, 2.55 mmol), 1-(2-((tert-butyldimethylsilyl)-oxy)ethyl)piperazine (0.75 g, 3.06 mmol), and K_2CO_3 (1.06 g, 7.66 mmol) in acetonitrile (20 mL) was refluxed for 24 h under inert condition. The reaction mixture was cooled to room temperature, filtered, washed with EtOAc and concentrated in vacuo. The crude material was purified by silica gel column chromatography (hexane:EtOAc = 3:2) to give compound **17** (0.81 g, 73%). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.09 (d, J = 7.2 Hz, 2H), 7.49-7.41 (m, 4H), 7.25-7.21 (m, 2H), 4.45 (t, J = 7.2 Hz, 2H), 3.76 (t, J = 6.8 Hz, 2H), 2.76 (t, J = 7.2 Hz, 2H), 2.68-2.57 (m, 6H), 2.54 (t, J = 6.4 Hz, 4H), 0.90 (s, 9H), 0.06 (s, 6H).

2-(4-(2-(9*H*-carbazol-9-yl)ethyl)piperazin-1-yl)ethanol (**18**). Compound **17** (0.875 g, 2.0 mmol) was treated with *n*-tetrabutylammonium fluoride (4.0 mL, 4.0 mmol, 1.0 M solution in THF) in THF (12 mL) according to procedure E. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 5:1) to give compound **18** (0.525 g, 81%). **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.09 (d, J = 7.2 Hz, 2H), 7.48-7.41 (m, 4H), 7.25-7.21 (m, 2H), 4.44 (t, J = 7.2 Hz, 2H), 3.62 (t, J = 5.4 Hz, 2H), 2.82-2.76 (m, 4H), 2.61-2.55 (m, 8H).

2-(4-(2-(9*H*-carbazol-9-yl)ethyl)piperazin-1-yl)acetaldehyde (19). Alcohol **18** (0.30 g, 0.93 mmol) was oxidized using SO_3.py complex (0.74 g, 4.64 mmol), DMSO (3 mL) and Et_3N (0.90 mL, 6.49 mmol) in CH_2Cl_2 (6 mL) according to procedure F. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 9:1) to give aldehyde **19** (0.23 g, 77%). The purified aldehyde was used immediately for next step. **^{1}H NMR** (600 MHz, $CDCl_3$): δ 9.67 (s, 1H), 8.07 (d, J = 7.8 Hz, 2H), 7.47-7.41 (m, 4H), 7.23-7.21 (m, 2H), 4.44-4.42 (m, 2H), 3.38-3.36 (m, 2H), 2.78-2.74 (m, 4H), 2.60-2.56 (m, 6H).

N^6-(2-(4-(2-(9*H*-carbazol-9-yl)ethyl)piperazin-1-yl)ethyl)-N^6-propyl-4,5,6,7-tetrahydrobenzo[*d*]thiazole-2,6-diamine (20) (D-626). Compound **19** (0.14 g, 0.44 mmol) was reacted with (±)-pramipexole (0.083 g, 0.39 mmol) and $NaBH(OAc)_3$ (0.185 g, 0.87 mmol) in CH_2Cl_2 (10 mL) according to procedure G. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 4:1) to afford compound **20** (0.115 g, 57%). The compound was converted to HCl salt. **^{1}H NMR** (400 MHz, $CDCl_3$): δ 8.07 (d, J = 8.4 Hz, 2H), 7.47-7.39 (m, 4H), 7.23-7.20 (m, 2H), 5.20 (bs, 2H), 4.41 (t, J = 7.2 Hz, 2H), 3.04–2.97 (m, 1H), 2.75 (t, J = 7.2 Hz, 2H), 2.69–2.48 (m, 14H), 2.45 (t, J = 7.2 Hz, 4H), 1.97–1.94 (m, 1H), 1.73-1.63 (m, 1H), 1.49–1.40 (m, 2H), 0.87 (t, J = 7.2 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 166.01, 144.77, 140.29, 125.70, 122.90, 120.39, 118.96, 116.88, 108.54, 58.52, 58.08, 56.12, 53.59, 53.48, 48.28, 40.94, 26.46, 25.82, 25.09, 22.33, 11.85; Anal. Calcd for $C_{30}H_{40}N_6S.5HCl.CH_3OH$: C, 50.25; H, 6.61; N, 11.72. Found: C, 50.81; H, 7.00; N, 11.33.

(*S*)-N^6-(2-(4-(2-(9*H*-carbazol-9-yl)ethyl)piperazin-1-yl)ethyl)-N^6-propyl-4,5,6,7-tetrahydrobenzo[*d*]thiazole-2,6-diamine (21) (D-637). Compound **19** (0.19 g, 0.59 mmol) was reacted with (-)-pramipexole (0.112 g, 0.53 mmol) and $NaBH(OAc)_3$ (0.25 g, 1.18 mmol) in CH_2Cl_2 (12 mL) according to procedure G. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 4:1) to afford compound **21** (0.13 g, 47%). The compound was converted to HCl salt. **^{1}H NMR** (600 MHz, $CDCl_3$): δ 8.07 (d, J = 7.8 Hz, 2H), 7.45 (t, J = 7.8 Hz, 2H), 7.40 (d, J = 7.8 Hz, 2H), 7.22 (t, J = 7.8 Hz, 2H), 5.11 (bs, 2H), 4.41 (t, J = 7.2 Hz, 2H), 3.03–2.98 (m, 1H), 2.74 (t, J = 7.2 Hz, 2H), 2.69–2.48 (m, 14H), 2.45 (t, J = 7.2 Hz, 4H), 1.96–1.94 (m, 1H), 1.71-1.64 (m, 1H), 1.48–1.41 (m, 2H), 0.87 (t, J = 7.2 Hz, 3H); **^{13}C NMR** (150 MHz, $CDCl_3$): δ 165.85, 144.91,

140.28, 125.68, 122.89, 120.39, 118.95, 117.00, 108.53, 58.58, 58.08, 56.13, 53.63, 53.52, 48.33, 40.95, 26.52, 25.84, 25.10, 22.35, 11.85; $[\alpha]_D^{25}$= -19.8 (*c*=1.0 in CH_2Cl_2); Anal. Calcd for $C_{30}H_{40}N_6S.4HCl.2CH_3OH$: C, 52.89; H, 7.21; N, 11.57. Found: C, 53.16; H, 7.14; N, 11.68.

Evaluation of binding affinity and functional potencies at dopamine D_2 and D_3 receptors. Binding affinity was evaluated by inhibition of [^{3}H]spiroperidol (15.0 Ci/mmol, Perkin-Elmer) binding to DA rD_2 and rD_3 receptors expressed in HEK-293 cells in a buffer containing 0.9% NaCl. Functional activity of test compounds in activating dopamine hD_2 and hD_3 receptors expressed in CHO cells was measured by stimulation of [^{35}S]GTPγS (1250 Ci/mmol, Perkin-Elmer) binding in comparison to stimulation by the full agonist DA. All these procedures were described by us previously.[17]

***In vivo* study of (-)-11b, (-)-15a and (-)-15c in parkinsonian rats**

Drugs and chemicals. The following commercially available drug was used in the experiment: reserpine hydrochloride (Alfa Aesar). The TFA salt of (-)-**11b** (**D-636)** and HBr salts of (-)-**15a** (**D-653**) and (-)-**15c** (**D-656**) were dissolved in water. Reserpine was dissolved in 20 µL of glacial acetic acid and further diluted with 5.5% glucose solution. The 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 interaperitoneal adminstration into each rat.

Animals. In rodent studies, animals were male and female Sprague-Dawley rats from Harlan (Indianapolis, IN) weighing 220-225 g unless otherwise specified. Animals were maintained in sawdust-lined cages in a temperature and humidity controlled environment

at 22 ± 1 °C and 60 ± 5% humidity, respectively. A 12 h light/dark cycle was maintained, 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. The ability of compounds (-)-**11b**, (-)-**15a** and (-)-**15c** to reverse reserpine-induced hypolocomotion was investigated according to a reported procedure.[44] Reserpine (5.0 mg/kg, sc) was administered 18 h before the injection of drug or vehicle. The rats were placed individually in the chambers for 1 h for acclimatization before administration of the test drugs or vehicle. Immediately after administration of drug or vehicle, animals were individually placed in Opto-Varimex 4 animal activity monitor chamber (Columbus Instruments, Ohio, USA) to start measuring locomotor activity. Locomotion was monitored for 6 h. Consecutive interruption of two infrared beams, situated 50 cm apart and 4 cm above the cage floor, in the monitor chamber recorded movement. The data were presented as horizontal activity (HACTV). The effect of individual doses of drugs on locomotor activity was compared with respect to saline treated controls (mean ± SEM). The data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. The effect was considered significant if the difference from control group was observed at $p < 0.05$.

Assessment of anti-oxidant activity:

Cell Cultures and Treatments. PC12 cells (ATCC CRL1721.1, Manassas, VA, USA), a rat adrenal pheochromocytoma cell line, were cultured in T-75 flasks (Greiner Bio One, Frickenhausen, Germany) and maintained in RPMI 1640 medium supplemented with

10% heat inactivated horse sérum, 5% fetal bovine sérum, 100 U/ ml penicillin, and 100 μg/mL streptomycin at 37 ^{o}C in 95% air/5% CO_2. Stock solutions of (-)-**11b**, (-)-**15a** and (-)-**15c** were prepared in dimethylsulfoxide (DMSO) and stored at -20 ^{o}C, a stock solution of 6-Hydroxydopamine (6-OHDA) was stored at -80 ^{o}C, a solution of 6-carboxy-2',7'-dichlorodihydroflourescence diacetate (carboxy-DCFDA) was prepared fresh in DMSO before addition. All stock solution were stored for the period of the experiments.

Measurement of antioxidant activity: To determine the effects of (-)-**11b**, (-)-**15a** and (-)-**15c** in decreasing reactive oxygen species (ROS) in PC12 cells produced by the neurotoxin 6-OHDA, a quantitative fluorometric ROS assay was performed. PC12 cells were plated at 30,000 cells/well density in 100 μL media in 96-well black plates and incubated at 37 ^{o}C under 5% CO_2 atmosphere for 24 h. The cells were treated for 24 h with various concentrations of compounds (-)-**11b**, (-)-**15a** and (-)-**15c**. Then the drugs containing media were removed and replaced with DCFDA 20 μm for 30 min in an incubator (37 ^{o}C, 5% CO_2). The DCFDA containing media was then removed and the cells were washed with PBS buffer to remove the traces of the dye. Fresh culture media was added followed by treatment with 75 μM of 6-OHDA alone for an additional 1 h under the same conditions. After incubation for 1 h, the fluorescence was measured using spectrophotometer fluorescence generated microplate reader (Biotek Epoch, Winooski, VT, USA) at excitation 497 nm and emission at 527nm. Data from at least three experiments were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test using GraphPad software (version 6, San Diego, CA, USA). The specific fluorescence emission was calculated after subtraction of the DCFDA untreated control cells from the DCFDA treated cells for both the background

and 1h treatment conditions. This was followed by the division of the 1h treatment data by the background activity which was determined from vials before treatment with 6-OHDA, to derive the final data point.

Assessment of Cell Viability:

Cell Cultures and Treatments. PC12 cells (ATCC CRL1721.1, Manassas, VA, USA), a rat adrenal pheochromocytoma cell line, were cultured in T-75 flasks (Greiner Bio One, Frickenhausen, Germany) and maintained in RPMI 1640 medium supplemented with 10% heat inactivated horse sérum, 5% fetal bovine sérum, 100 U/ml penicillin, and 100 μg/mL streptomycin at 37 ^{o}C in 95% air/5% CO_2. Stock solutions of (-)-**11b**, (-)-**15a**, (-)-**15c**, and 6-hydroxydopamine (6-OHDA) were prepared in dimethylsulfoxide (DMSO) and aliquots were stored at -20 ^{o}C and -80 ^{o}C, respectively. For all experiments assessing neuroprotective effects of the test compounds, PC12 cells were pretreated with indicated concentrations of (-)-**11b**, (-)-**15a** and (-)-**15c** for 24 h and then treated with 75 μM 6-OHDA for another 24 h. The control cells were treated with above media containing 0.01% DMSO only.

To determine the neuroprotective effects of (-)-**11b**, (-)-**15a** and (-)-**15c** in the presence of neurotoxin 6-OHDA, a quantitative colorimetric MTT assay was performed. PC12 cells were plated at 17000 cells/well density in 100 μL of media in 96-well plates and incubated at 37 ^{o}C under 5% CO_2 atmosphere for 24 h. Cells were treated with varying concentrations of the test compounds to determine their direct effect on cell viability. Neuroprotection experiments were conducted by treating cells for 24 h with varying concentrations of (-)-**11b**, (-)-**15a** and (-)-**15c**. Then the drug containing media was replaced with fresh culture media followed by treatment with 75 μM of 6-OHDA alone for

an additional 24 h under the same condition. After incubation for 24 h, 5 mg/mL MTT solution (prepared in Dulbecco's phosphate-buffered saline) was added to the cells (to a final concentration of 0.5 mg/mL) and the plates were further incubated at 37 ^{o}C in 95% air/5% CO_2 atmosphere for 3-4 h to produce dark-blue formazan crystals. Afterward, the plates were centrifuged at 450 *g* for 10 min and the supernatants were carefully removed. Formazan crystals were dissolved by adding 100 μL of methanol:DMSO (1:1) mixture to each well and shaking gently at 400 rpm for 30 min at room temperature on a Thermomix R shaker (Eppendorf, Hamburg, Germany). The absorbance was measured on a microplate reader (Biotek Epoch, Winooski, VT, USA) at 570 nm with background correction performed at 690 nm. Data from at least three experiments were analyzed using GraphPad software (version 6, San Diego, CA, USA). Cell viability was defined as percentage reduction in absorbance compared to untreated controls. The data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test.

Assessment of α- synuclein aggregation inhibition activity:

The experimental conditions were followed as described in our earlier publication.[37] Briefly, α-syn fibrils were formed by incubating purified α-syn at a concentration of 5 mg/mL at 37 °C under constant agitation at 1000 rpm in a Thermomix R shaker (Eppendorf, Hamburg, Germany) for a period of 5 days. These fibrils at a concentration of 0.5% were used to seed the aggregation of α-syn at 1.25 mg/ml (86.45 μM) with or without compounds **D-636** or **D-653** at a concentration of 172.9 μM without agitation at 37°C for a period of 30 days. Samples were collected at 0D and 30D. Thioflavin T (ThT)

assay was performed using these samples as described previously to evaluate the extent of aggregation.

Further, the cytotoxicity of the aggregates formed above was evaluated in PC12 cells. Briefly, PC12 cells were treated with α-syn aggregates such that its concentration is 10 μM and the concentration of drug is 20 μM. These solutions were prepared after appropriate dilution of the above drug and α-syn solution collected at different time points. 24h following treatments, viability was measured by MTT assay as described previously.

Acknowledgements:

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Abbreviations:

Cs_2CO_3, cesium carbonate; BINAP, 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl; $Pd(OAc)_2$, palladium(II) acetate; TBAF, tetrabutylammonium fluoride; SO_3.py, sulfur trioxide pyridine; $NaBH(OAc)_3$, sodium triacetoxyborohydride; GTPγS: guanosine 5'-O-[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; i.p.: intraperitoneal; s.c.: subcutaneous; PD: Parkinson's disease; DA: dopamine.

Corresponding Author

*E-mail: adutta@wayne.edu. Phone: (+1) 313-577-1064. Fax: (+1) 313-577-2033.

References

(1) Surmeier, D. J.; Obeso, J. A.; Halliday, G. M. *(***2017)** Selective neuronal vulnerability in Parkinson disease.Nat Rev Neurosci, *18*, 101-113.

(2) Schapira, A. H.; Olanow, C. W. *(***2004)** Neuroprotection in Parkinson disease: mysteries, myths, and misconceptions.Jama, *291*, 358-364.

(3) Alexander, G. E. *(***2004)** Biology of Parkinson's disease: pathogenesis and pathophysiology of a multisystem neurodegenerative disorder.Dialogues Clin Neurosci, *6*, 259-280.

(4) Poewe, W.; Seppi, K.; Tanner, C. M.; Halliday, G. M.; Brundin, P.; Volkmann, J.; Schrag, A. E.; Lang, A. E. *(***2017)** Parkinson disease.Nat Rev Dis Primers, *3*, 17013.

(5) Klein, C.; Westenberger, A. *(***2012)** Genetics of Parkinson's disease.Cold Spring Harb Perspect Med, *2*, a008888.

(6) Przedborski, S. *(***2017)** The two-century journey of Parkinson disease research.Nat Rev Neurosci, *18*, 251-259.

(7) Das, B.; Modi, G.; Dutta, A. *(***2015)** Dopamine D3 agonists in the treatment of Parkinson's disease.Curr Top Med Chem, *15*, 908-926.

(8) Salat, D.; Tolosa, E. *(***2013)** Levodopa in the treatment of Parkinson's disease: current status and new developments.J Parkinsons Dis, *3*, 255-269.

(9) Hearn, M. G.; Ren, Y.; McBride, E. W.; Reveillaud, I.; Beinborn, M.; Kopin, A. S. *(***2002)** A Drosophila dopamine 2-like receptor: Molecular characterization and identification of multiple alternatively spliced variants.Proc Natl Acad Sci U S A, *99*, 14554-14559.

(10) Mandel, S.; Grunblatt, E.; Riederer, P.; Gerlach, M.; Levites, Y.; Youdim, M. B. *(***2003)** Neuroprotective strategies in Parkinson's disease : an update on progress.CNS Drugs, *17*, 729-762.

(11) Cenci, M. A.; Ohlin, K. E.; Odin, P. *(***2011)** Current options and future possibilities for the treatment of dyskinesia and motor fluctuations in Parkinson's disease.CNS Neurol Disord Drug Targets, *10*, 670-684.

(12) Youdim, M. B.; Oh, Y. J. *(***2013)** Promise of neurorestoration and mitochondrial biogenesis in Parkinson's disease with multi target drugs: an alternative to stem cell therapy.Exp Neurobiol, *22*, 167-172.

(13) Cavalli, A.; Bolognesi, M. L.; Minarini, A.; Rosini, M.; Tumiatti, V.; Recanatini, M.; Melchiorre, C. *(***2008)** Multi-target-directed ligands to combat neurodegenerative diseases.J Med Chem, *51*, 347-372.

(14) Dutta, A. K.; Fei, X. S.; Reith, M. E. *(***2002)** A novel series of hybrid compounds derived by combining 2-aminotetralin and piperazine fragments: binding activity at D2 and D3 receptors.Bioorg Med Chem Lett, *12*, 619-622.

(15) Dutta, A. K.; Venkataraman, S. K.; Fei, X. S.; Kolhatkar, R.; Zhang, S.; Reith, M. E. *(***2004)** Synthesis and biological characterization of novel hybrid 7-[[2-(4-phenyl-piperazin-1-yl)-ethyl]-propyl-amino]-5,6,7,8-tetrahydro-na phthalen-2-ol and their

heterocyclic bioisosteric analogues for dopamine D2 and D3 receptors.Bioorg Med Chem, *12*, 4361-4373.

(16) Das, B.; Kandegedara, A.; Xu, L.; Antonio, T.; Stemmler, T.; Reith, M. E. A.; Dutta, A. K. *(***2017)** A Novel Iron(II) Preferring Dopamine Agonist Chelator as Potential Symptomatic and Neuroprotective Therapeutic Agent for Parkinson's Disease.ACS Chem Neurosci, *8*, 723-730.

(17) Biswas, S.; Hazeldine, S.; Ghosh, B.; Parrington, I.; Kuzhikandathil, E.; Reith, M. E.; Dutta, A. K. *(***2008)** Bioisosteric heterocyclic versions of 7-{[2-(4-phenyl-piperazin-1-yl)ethyl]propylamino}-5,6,7,8-tetrahydronaphthalen-2- ol: identification of highly potent and selective agonists for dopamine D3 receptor with potent in vivo activity.J Med Chem, *51*, 3005-3019.

(18) Das, B.; Vedachalam, S.; Luo, D.; Antonio, T.; Reith, M. E.; Dutta, A. K. *(***2015)** Development of a Highly Potent D2/D3 Agonist and a Partial Agonist from Structure-Activity Relationship Study of N(6)-(2-(4-(1H-Indol-5-yl)piperazin-1-yl)ethyl)-N(6)-propyl-4,5,6,7-tetrahydroben zo[d]thiazole-2,6-diamine Analogues: Implication in the Treatment of Parkinson's Disease.J Med Chem, *58*, 9179-9195.

(19) Modi, G.; Antonio, T.; Reith, M.; Dutta, A. *(***2014)** Structural modifications of neuroprotective anti-Parkinsonian (-)-N6-(2-(4-(biphenyl-4-yl)piperazin-1-yl)-ethyl)-N6-propyl-4,5,6,7-tetrahydrobe nzo[d]thiazole-2,6-diamine (D-264): an effort toward the improvement of in vivo efficacy of the parent molecule.J Med Chem, *57*, 1557-1572.

(20) Luo, D.; Sharma, H.; Yedlapudi, D.; Antonio, T.; Reith, M. E.; Dutta, A. K. *(***2016)** Novel multifunctional dopamine D2/D3 receptors agonists with potential neuroprotection and anti-alpha synuclein protein aggregation properties.Bioorg Med Chem, *24*, 5088-5102.

(21) Johnson, M.; Antonio, T.; Reith, M. E.; Dutta, A. K. *(***2012)** Structure-activity relationship study of N(6)-(2-(4-(1H-Indol-5-yl)piperazin-1-yl)ethyl)-N(6)-propyl-4,5,6,7-tetrahydroben zo[d]thiazole-2,6-diamine analogues: development of highly selective D3 dopamine receptor agonists along with a highly potent D2/D3 agonist and their pharmacological characterization.J Med Chem, *55*, 5826-5840.

(22) Shah, M.; Rajagopalan, S.; Xu, L.; Voshavar, C.; Shurubor, Y.; Beal, F.; Andersen, J. K.; Dutta, A. K. *(***2014)** The high-affinity D2/D3 agonist D512 protects PC12 cells from 6-OHDA-induced apoptotic cell death and rescues dopaminergic neurons in the MPTP mouse model of Parkinson's disease.J Neurochem, *131*, 74-85.

(23) Das, B.; Rajagopalan, S.; Joshi, G. S.; Xu, L.; Luo, D.; Andersen, J. K.; Todi, S. V.; Dutta, A. K. *(***2017)** A novel iron (II) preferring dopamine agonist chelator D-607 significantly suppresses alpha-syn- and MPTP-induced toxicities in vivo.Neuropharmacology, *123*, 88-99.

(24) Lindenbach, D.; Das, B.; Conti, M. M.; Meadows, S. M.; Dutta, A. K.; Bishop, C. *(***2017)** D-512, a novel dopamine D2/3 receptor agonist, demonstrates greater anti-Parkinsonian efficacy than ropinirole in Parkinsonian rats.Br J Pharmacol, *174*, 3058-3071.

(25) Gluszynska, A. *(***2015)** Biological potential of carbazole derivatives.Eur J Med Chem, *94*, 405-426.

(26) Wu, X.; Kosaraju, J.; Zhou, W.; Tam, K. Y. *(***2017)** Neuroprotective Effect of SLM, a Novel Carbazole-Based Fluorophore, on SH-SY5Y Cell Model and 3xTg-AD Mouse Model of Alzheimer's Disease.ACS Chem Neurosci, *8*, 676-685.

(27) Wang, S. N.; Xu, T. Y.; Wang, X.; Guan, Y. F.; Zhang, S. L.; Wang, P.; Miao, C. Y. *(***2016)** Neuroprotective Efficacy of an Aminopropyl Carbazole Derivative P7C3-A20 in Ischemic Stroke.CNS Neurosci Ther, *22*, 782-788.

(28) MacMillan, K. S.; Naidoo, J.; Liang, J.; Melito, L.; Williams, N. S.; Morlock, L.; Huntington, P. J.; Estill, S. J.; Longgood, J.; Becker, G. L.; McKnight, S. L.; Pieper, A. A.; De Brabander, J. K.; Ready, J. M. *(***2011)** Development of proneurogenic, neuroprotective small molecules.J Am Chem Soc, *133*, 1428-1437.

(29) Pieper, A. A.; Xie, S.; Capota, E.; Estill, S. J.; Zhong, J.; Long, J. M.; Becker, G. L.; Huntington, P.; Goldman, S. E.; Shen, C. H.; Capota, M.; Britt, J. K.; Kotti, T.; Ure, K.; Brat, D. J.; Williams, N. S.; MacMillan, K. S.; Naidoo, J.; Melito, L.; Hsieh, J.; De Brabander, J.; Ready, J. M.; McKnight, S. L. *(***2010)** Discovery of a proneurogenic, neuroprotective chemical.Cell, *142*, 39-51.

(30) Loris, Z. B.; Pieper, A. A.; Dietrich, W. D. *(***2017)** The neuroprotective compound P7C3-A20 promotes neurogenesis and improves cognitive function after ischemic stroke.Exp Neurol, *290*, 63-73.

(31) Yoon, H. J.; Kong, S. Y.; Park, M. H.; Cho, Y.; Kim, S. E.; Shin, J. Y.; Jung, S.; Lee, J.; Farhanullah; Kim, H. J.; Lee, J. *(***2013)** Aminopropyl carbazole analogues as potent enhancers of neurogenesis.Bioorg Med Chem, *21*, 7165-7174.

(32) Collier, T. J.; Srivastava, K. R.; Justman, C.; Grammatopoulous, T.; Hutter-Paier, B.; Prokesch, M.; Havas, D.; Rochet, J. C.; Liu, F.; Jock, K.; de Oliveira, P.; Stirtz, G. L.; Dettmer, U.; Sortwell, C. E.; Feany, M. B.; Lansbury, P.; Lapidus, L.; Paumier, K. L. *(***2017)** Nortriptyline inhibits aggregation and neurotoxicity of alpha-synuclein by enhancing reconfiguration of the monomeric form.Neurobiol Dis, *106*, 191-204.

(33) Jeannotte, A. M.; McCarthy, J. G.; Redei, E. E.; Sidhu, A. *(***2009)** Desipramine modulation of alpha-, gamma-synuclein, and the norepinephrine transporter in an animal model of depression.Neuropsychopharmacology, *34*, 987-998.

(34) Yu, L.; Cui, J.; Padakanti, P. K.; Engel, L.; Bagchi, D. P.; Kotzbauer, P. T.; Tu, Z. *(***2012)** Synthesis and in vitro evaluation of alpha-synuclein ligands.Bioorg Med Chem, *20*, 4625-4634.

(35) Kim, M.; Lee, J. Y. *(***2013)** Synthesis of 2- and 4-substituted carbazole derivatives and correlation of substitution position with photophysical properties and device performances of host materials.Organic Electronics, *14*, 67-73.

(36) Li, C.; Biswas, S.; Li, X.; Dutta, A. K.; Le, W. *(***2010)** Novel D3 dopamine receptor-preferring agonist D-264: Evidence of neuroprotective property in Parkinson's disease animal models induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and lactacystin.J Neurosci Res, *88*, 2513-2523.

(37) Yedlapudi, D.; Joshi, G. S.; Luo, D.; Todi, S. V.; Dutta, A. K. *(***2016)** Inhibition of alpha-synuclein aggregation by multifunctional dopamine agonists assessed by a novel in vitro assay and an in vivo Drosophila synucleinopathy model.Scientific reports, *6*, 38510.

(38) Ghosh, B.; Antonio, T.; Gopishetty, B.; Reith, M.; Dutta, A. *(***2010)** Further delineation of hydrophobic binding sites in dopamine D(2)/D(3) receptors for N-4 substituents on the piperazine ring of the hybrid template 5/7-{[2-(4-aryl-piperazin-1-yl)-ethyl]-propyl-amino}-5,6,7,8-tetrahydro-naphthale n-2-ol.Bioorg Med Chem, *18*, 5661-5674.

(39) Carlsson, A.; Lindqvist, M.; Magnusson, T. *(***1957)** 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists.Nature, *180*, 1200.

(40) Skalisz, L. L.; Beijamini, V.; Joca, S. L.; Vital, M. A.; Da Cunha, C.; Andreatini, R. *(***2002)** Evaluation of the face validity of reserpine administration as an animal model of depression--Parkinson's disease association.Prog Neuropsychopharmacol Biol Psychiatry, *26*, 879-883.

(41) Blum, D.; Torch, S.; Nissou, M. F.; Benabid, A. L.; Verna, J. M. *(***2000)** Extracellular toxicity of 6-hydroxydopamine on PC12 cells.Neurosci Lett, *283*, 193-196.

(42) Soto-Otero, R.; Mendez-Alvarez, E.; Hermida-Ameijeiras, A.; Munoz-Patino, A. M.; Labandeira-Garcia, J. L. *(***2000)** Autoxidation and neurotoxicity of 6-hydroxydopamine in the presence of some antioxidants: potential implication in relation to the pathogenesis of Parkinson's disease.J Neurochem, *74*, 1605-1612.

(43) Santra, S.; Xu, L.; Shah, M.; Johnson, M.; Dutta, A. *(***2013)** D-512 and D-440 as novel multifunctional dopamine agonists: characterization of neuroprotection properties and evaluation of in vivo efficacy in a Parkinson's disease animal model.ACS Chem Neurosci, *4*, 1382-1392.

(44) McCall, R. B.; Lookingland, K. J.; Bedard, P. J.; Huff, R. M. *(***2005)** Sumanirole, a highly dopamine D2-selective receptor agonist: in vitro and in vivo pharmacological characterization and efficacy in animal models of Parkinson's disease.J Pharmacol Exp Ther, *314*, 1248-1256.

Figure Legends

Figure 1. Structures of carbazole compounds and D_2/D_3 receptor multifunctional agonists.

Figure 2. Effects of different drugs upon reserpine (5.0 mg/kg, sc, 18 h pretreatment) induced hypolocomotion in rats. Each point represents the mean ± SEM for three rats. Horizontal activity was measured as described in Experimental Section. Representation of horizontal locomotor activity is at discrete 30 min intervals after the administration of (-)-**11b (D-636)** (10 μMol/kg), (-)-**15a (D-653)** (10 μMol/kg), (-)-**15c (D-656)** (10 μMol/kg), and ropinirole (10 μMol/kg) compared to control rats, 18 h after reserpine treatment. Differences among treatments were significant by one-way ANOVA analysis ($F(4, 60) = 8.861$; ($P < 0.0001$)). Dunnett's analysis following ANOVA revealed that the effect of the

compounds (-)-**11b**, (-)-**15a** and (-)-**15c** ($p < 0.0001$) was significantly different when compared to reserpine control.

Figure 3: Detection of intracellular ROS using DCFDA-based fluorescence assay: PC12 cells were pretreated with different doses of (-)-**11b** (**D-636)** (A), (-)-**15a (D-653)** (B), and (-)-**15c (D-656)** (C) for 24 h followed by treatment with DCFDA (20 μM, 2% serum) for 30 min. The DCFDA containing media was removed, and replaced with fresh media, followed by treatment with 75 μM 6-OHDA, and incubated for 1 h. Data represents mean ± SDs of three independent experiments in four to six replicates. One-Way ANOVA analysis followed by Tukey's multiple comparison post hoc test was performed. (*P < 0.05, **P < 0. 01, ***P < 0. 001, and ****P < 0.0001 compared to the 6-OHDA, ####P < 0.0001 compared to the control).

Figure 4. Dose-dependent effect on the viability of PC12 cells by 24 h pretreatment of compounds (-)-**11b (D-636)**, (-)-**15a (D-653)**, and (-)-**15c (D-656)** at various concentrations followed by single treatment of 75 μM 6-OHDA. (a, c and e) Dose-dependent effect of (-)-**11b**, (-)-**15a**, and (-)-**15c** on cell viability, respectively. (b, d and f) PC12 cells were pretreated with varying concentrations of (-)-**11b**, (-)-**15a**, and (-)-**15c**, respectively for 24 h followed by treatment with 75 μM 6-OHDA for another 24 h. The results shown are mean ± SEM of three independent experiments performed in seven to eight replicates. One way ANOVA analysis $F(7, 54) = 21.64$, $p < 0.0001$ for (-)-**11b**; $F(7, 56) = 167.5$, $p < 0.0001$ for (-)-**15a**; and $F(7, 56) = 39.78$, $p < 0.0001$ for (-)-**15c**. ANOVA

was followed by Tukey's multiple comparison post hoc test (****p < 0.0001, ***p < 0.001, **p<0.01 compared to control).

Figure 5: Effect of (-)-**11b (D-636)** and (-)-**15a (D-653)** on the aggregation of α-syn induced by seeding with 0.5% PFFs: 1.25 mg/mL α-syn was incubated with 0.5% PFFs for a period of 30D without shaking in the presence of (-)-**11b** or (-)-**15a** at a concentration of 172.9 μM. Fibrillation was measured by ThT assay at 30D (A). Values are represented in terms of % 0D Synuclein. Viability of PC12 cells was measured by MTT assay after 24 h treatment with α-syn seeded samples collected at 30D (B). Values are represented in terms of % control. Data values shown are means ±SD of three independent experiments. One-way ANOVA analysis followed by Tukey's multiple comparison post hoc test was performed, **p≤0.01, ****p≤0.0001compared to Syn +0.5%-0D; ####p≤0.0001 compared to Syn +0.5%-30D.

Figure 1

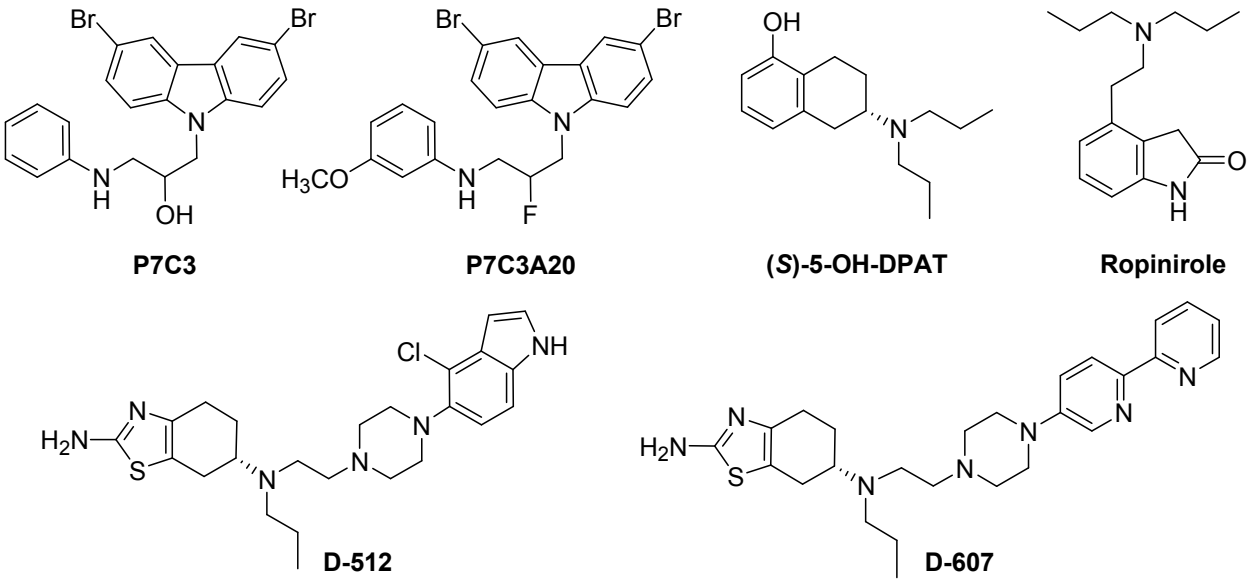

P7C3
P7C3A20
(S)-5-OH-DPAT
Ropinirole
D-512
D-607

Figure 2

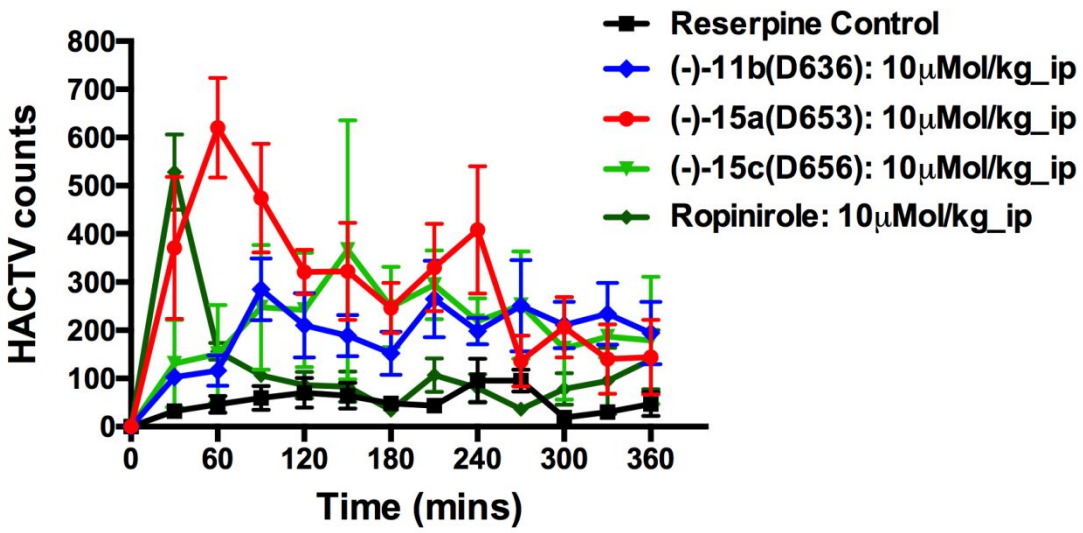

Reserpine Control
(-)-11b(D636): 10μMol/kg_ip
(-)-15a(D653): 10μMol/kg_ip
(-)-15c(D656): 10μMol/kg_ip
Ropinirole: 10μMol/kg_ip
HACTV counts
Time (mins)

Figure 3

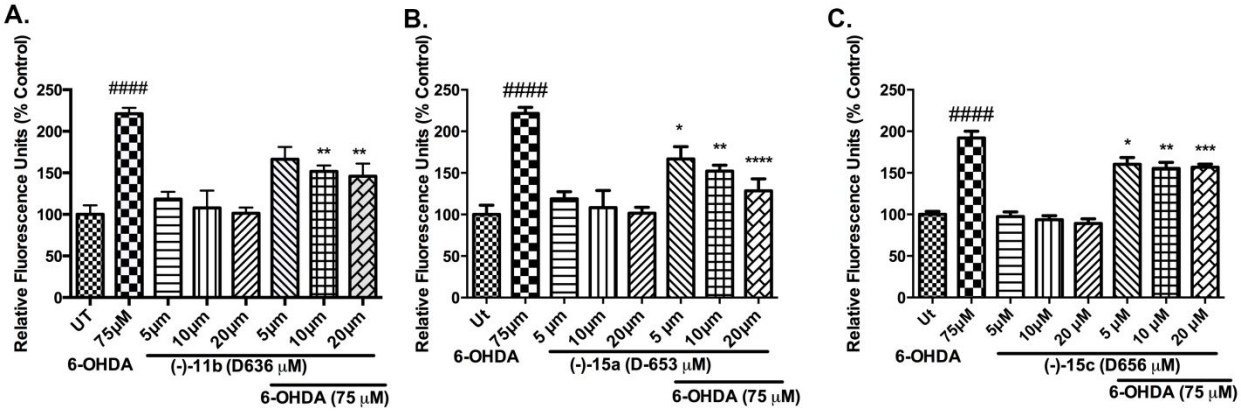


Figure 4

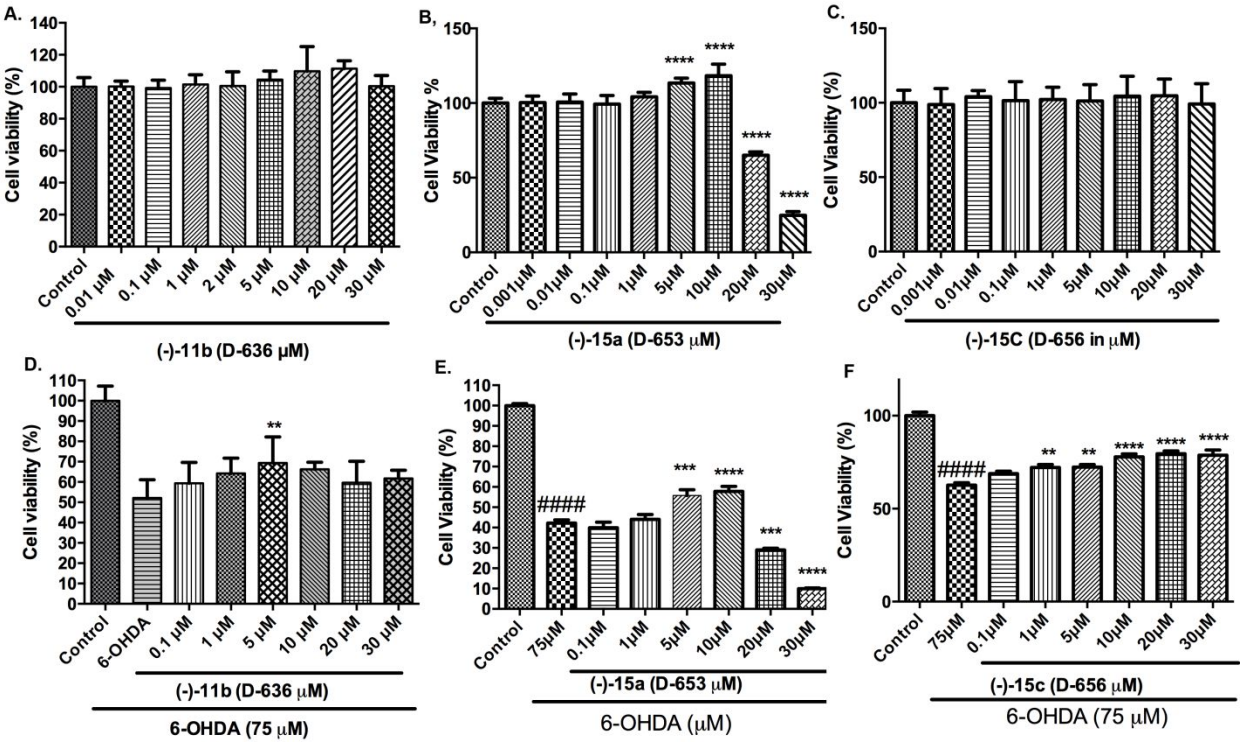

A.
Cell viability (%)
Control
0.01 µM
0.1 µM
1 µM
2 µM
5 µM
10 µM
20 µM
30 µM
(-)-11b (D-636 µM)
B,
Cell Viability %
Control
0.001µM
0.01µM
0.1µM
1µM
5µM
10µM
20µM
30µM
(-)-15a (D-653 µM)
C.
Cell Viability (%)
Control
0.001µM
0.01µM
0.1µM
1µM
5µM
10µM
20µM
30µM
(-)-15C (D-656 in µM)
D.
Cell viability (%)
Control
6-OHDA
0.1 µM
1 µM
5 µM
10 µM
20 µM
30 µM
(-)-11b (D-636 µM)
6-OHDA (75 µM)
E.
Cell Viability (%)
####
Control
75µM
0.1µM
1µM
5µM
10µM
20µM
30µM
(-)-15a (D-653 µM)
6-OHDA (µM)
F
Cell Viability (%)
####
Control
75µM
0.1µM
1µM
5µM
10µM
20µM
30µM
(-)-15c (D-656 µM)
6-OHDA (75 µM)

Figure 5

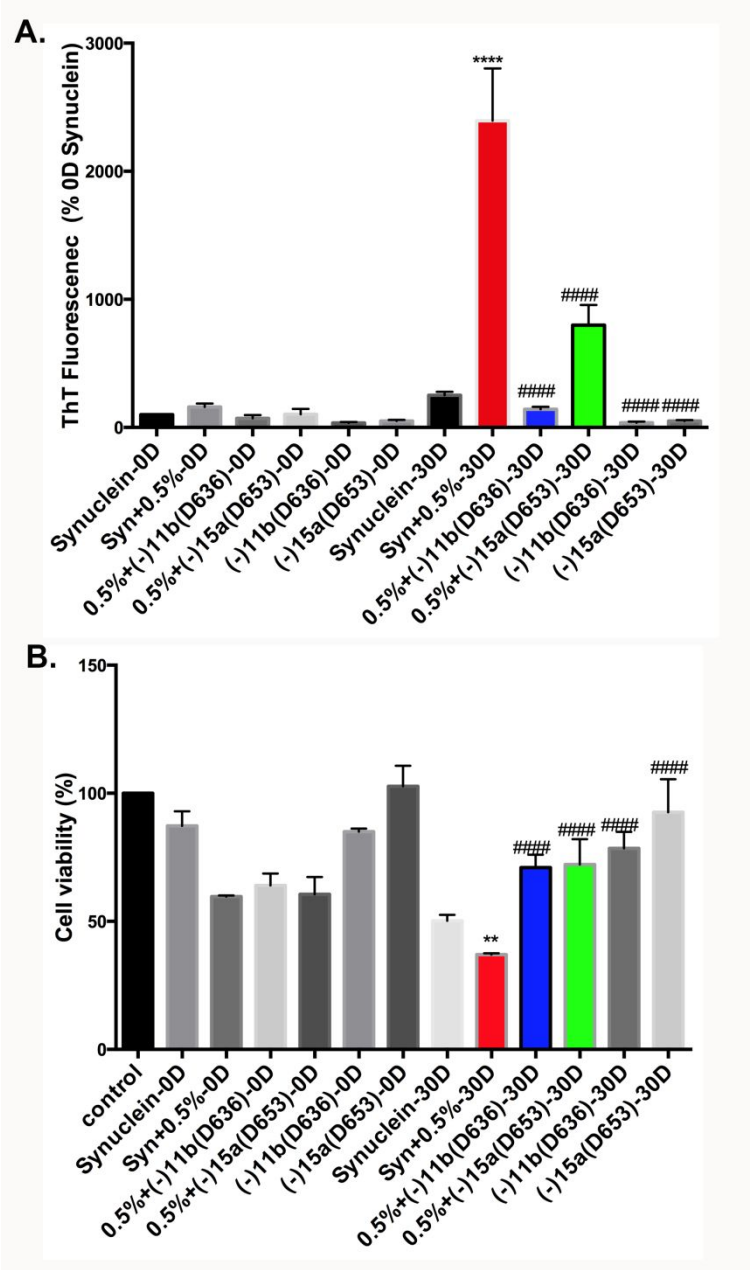
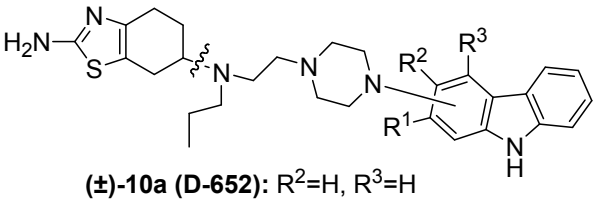
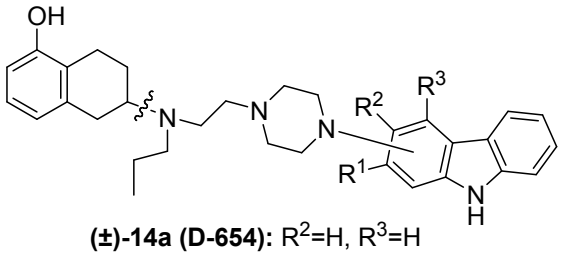

A.
ThT Fluorescenec (% 0D Synuclein)
3000
2000
1000
0
****
####
####
####
Synuclein-0D
Syn+0.5%-0D
0.5%+(-)11b(D636)-0D
0.5%+(-)15a(D653)-0D
(-)11b(D636)-0D
(-)15a(D653)-0D
Synuclein-30D
Syn+0.5%-30D
0.5%+(-)11b(D636)-30D
0.5%+(-)15a(D653)-30D
(-)11b(D636)-30D
(-)15a(D653)-30D
B.
Cell viability (%)
150
100
50
0
**
####
####
####
####
control
Synuclein-0D
Syn+0.5%-0D
0.5%+(-)11b(D636)-0D
0.5%+(-)15a(D653)-0D
(-)11b(D636)-0D
(-)15a(D653)-0D
Synuclein-30D
Syn+0.5%-30D
0.5%+(-)11b(D636)-30D
0.5%+(-)15a(D653)-30D
(-)11b(D636)-30D
(-)15a(D653)-30D

Table 1. Inhibition constants determined by competition experiments assessing [^{3}H]spiroperidol binding to cloned rat D_{2L} and D_3 receptors expressed in HEK-293 cells[a]. cLogP values are calculated by using SwissADME data base, tPSA values and MW are calculated by using ChemDraw.



(±)-10a (D-652): R^2=H, R^3=H
(±)-10b (D-627): R^1=H, R^3=H
(±)-10c (D-658): R^1=H, R^2=H
(-)-11a (D-651): R^2=H, R^3=H
(-)-11b (D-636): R^1=H, R^3=H
(-)-11c (D-657): R^1=H, R^2=H



(±)-14a (D-654): R^2=H, R^3=H
(±)-14b (D-650): R^1=H, R^3=H
(±)-14c (D-655): R^1=H, R^2=H
(-)-15a (D-653): R^2=H, R^3=H
(-)-15b (D-659): R^1=H, R^3=H
(-)-15c (D-656): R^1=H, R^2=H

Compound	K_i (nM)		D_{2L}/D_3	cLogP	tPSA	MW
	D_{2L}, [^{3}H]spiroperidol	D_3, [^{3}H]spiroperidol				
(-)-**5-OH-DPAT**	153 ± 32	2.07 ± 0.38	74	4.01	23.47	247.38
(-)-**Pramipexole**	6740 ± 510	11.7 ± 2.5	576	1.17	50.94	211.33
(±)-**10a**	902 ± 132	6.18 ± 0.91	146	4.30	60.13	488.69
(±)-**10b**	612 ± 92	3.12 ± 0.62	196	4.28	60.13	488.69
(±)-**10c**	76.9 ± 5.2	7.8 ± 1.17	9.86	4.31	60.13	488.69
(-)-**11a**	504 ± 50	3.94 ± 0.62	128	4.30	60.13	488.69
(-)-**11b**	135 ± 12	3.8 ± 0.38	35.4	4.28	60.13	488.69
(-)-**11c**	92.4 ± 8.5	4.18 ± 0.47	22.2	4.31	60.13	488.69
(±)-**14a**	62.1 ± 7.3	2.85 ± 0.62	21.8	4.89	41.98	482.66

(±)-**14b**	37.8 ± 4.7	1.87 ± 0.41	20.2	4.89	41.98	482.66
(±)-**14c**	29.4 ± 1.3	3.61± 0.28	8.13	4.83	41.98	482.66
(-)-**15a**	71.2 ± 9.6	0.400 ± 0.038	177	4.89	41.98	482.66
(-)-**15b**	61.6 ± 3.8	1.94 ± 0.18	31.8	4.89	41.98	482.66
(-)-**15c**	16.9 ± 1.9	0.362 ± 0.032	46.9	4.83	41.98	482.66
(±)-**20**	435 ± 90	6.60 ± 1.13	65.9	4.41	53.56	516.74
(-)-**21**	82.6 ± 13.8	7.18 ± 0.86	11.5	5.11	53.56	516.74

[a]Results are expressed as means ± SEM for 3-6 experiments each performed in triplicate.

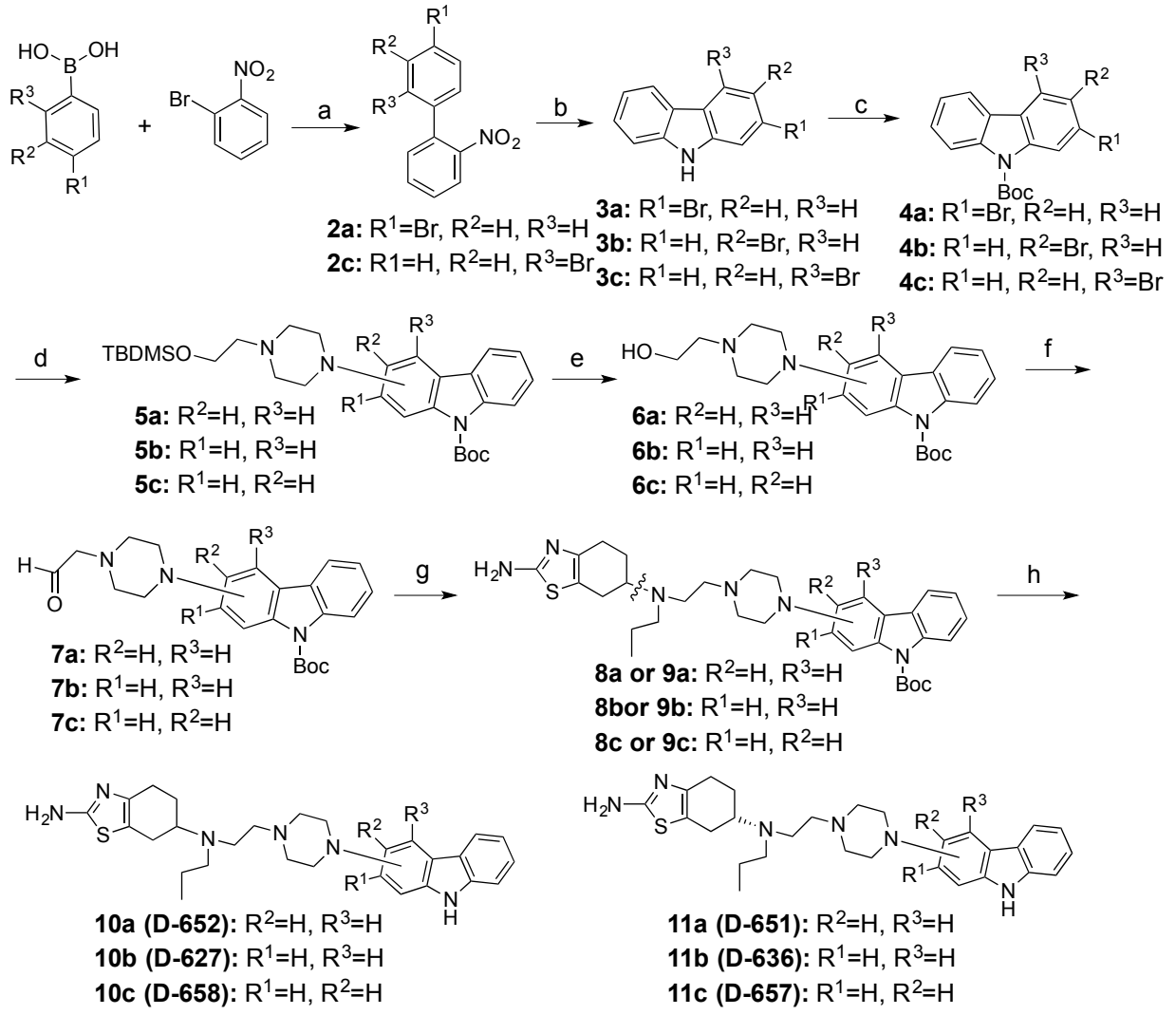
Table 2. Stimulation of [^{35}S]GTPγS binding to cloned human D_2 and D_3 receptors expressed in CHO cells[a]

	hCHO-D_2		hCHO-D_3		
	[^{35}S]GTPγS		[^{35}S]GTPγS EC_{50}		
Compound	EC_{50} (nM)	E_{max} (%)	(nM)	E_{max} (%)	D_2/D_3
Dopamine (DA)	146 ± 24	100	1.95 ± 0.62	100	75.0
(-)-**5-OH-DPAT**	41 ± 6	80 ± 4	0.63 ± 0.08	75 ± 4	65
(-)-**Pramipexole**	251 ± 16	96.6 ± 4.9	4.08 ± 1.00	96.9 ± 0.8	61.5
(-)-11b	48.7 ± 6.3	87.3 ± 2.1	0.96 ± 0.25	93.4 ± 4.4	50.7
(-)-11c	22.2 ± 6.9	56.7 ± 5.1	1.67 ± 0.30	82.0 ± 7.0	13.3
(-)-15a	0.87 ± 0.098	85.2 ± 4.7	0.23 ± 0.02	92.2 ± 3.3	3.79
(-)-15c	2.29 ± 0.70	73.6 ± 10.1	0.22 ± 0.06	87.9 ± 3.8	10.3

[a]EC_{50} is the concentration producing half maximal stimulation. For each compound, maximal stimulation (E_{max}) is expressed as a percent of the E_{max} observed with 1 mM (D_2) or 100 μM (D_3) of the full agonist DA (E_{max}, %). Results are the mean ± SEM for 3–6

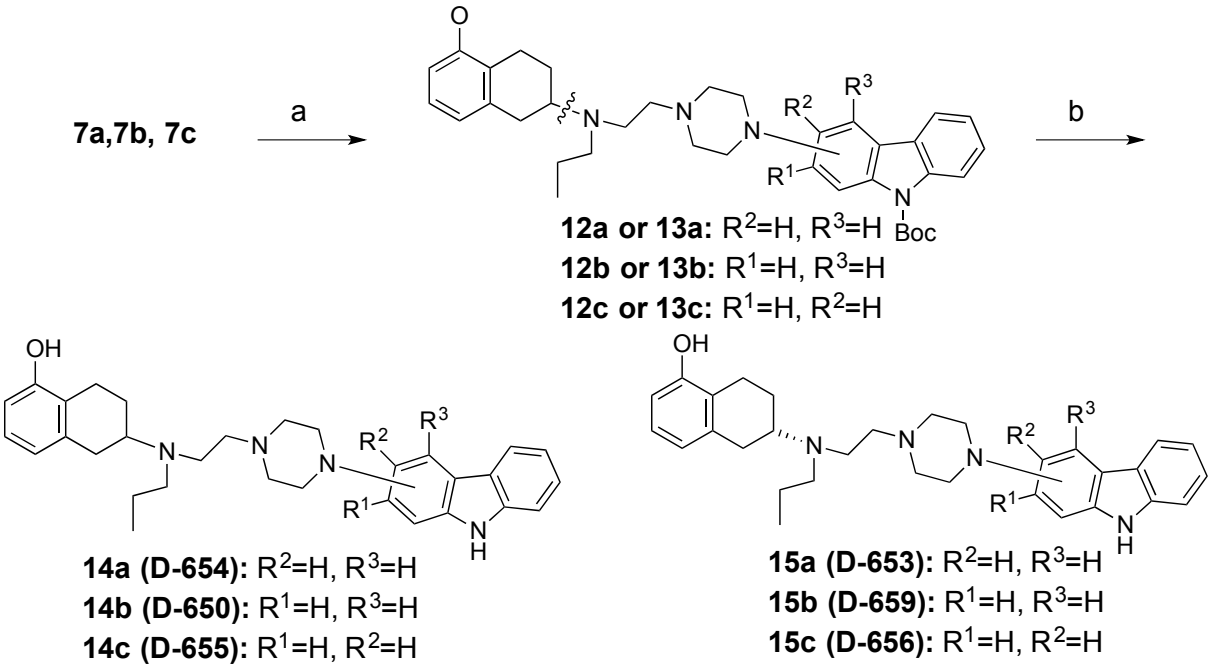
experiments, each performed in triplicate.

Scheme 1



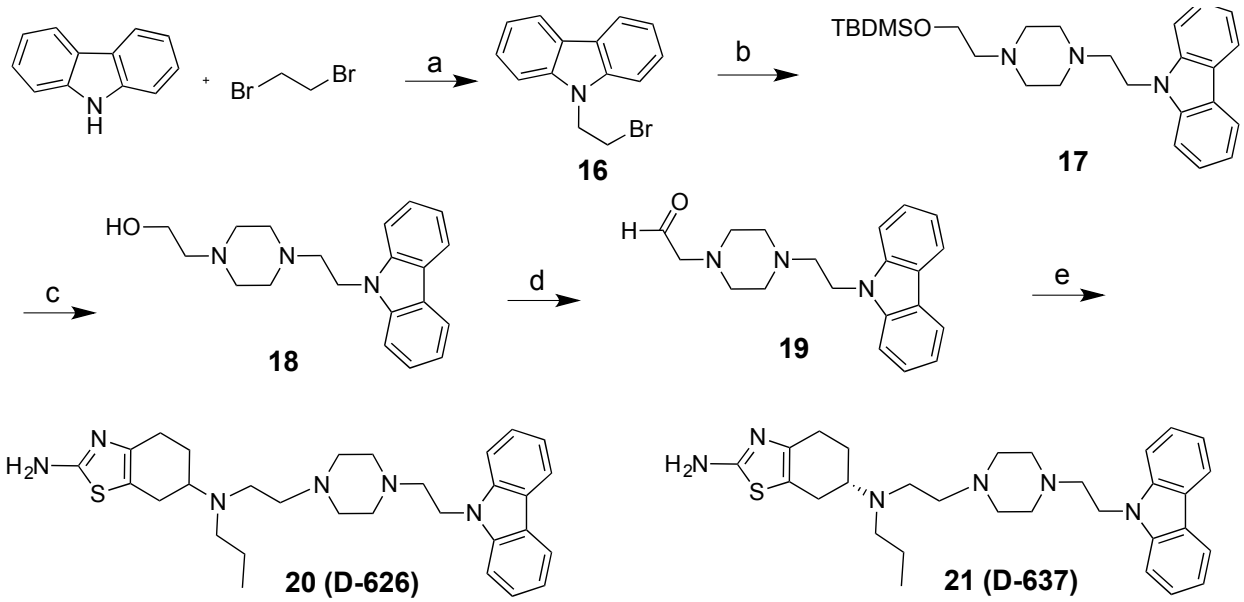
Reagents and conditions: a) $Pd(PPh_3)_4$, 2M K_2CO_3, THF, 90 °C, 12 h, (98% for 2a, 94% for 2c); b) PPh_3, 1,2-dichlorobenzene, 170 °C, 12 h, (89% for 3a, 87% for 3c); c) $(Boc)_2O$, 4-DMAP, THF, rt, overnight, (84% for 4a, 100% for 4b, 84% for 4c); d) 1-[2-(*tert*-butyl-dimethyl-silanyloxy)-ethyl]-piperazine, $Pd(OAc)_2$, BINAP, Cs_2CO_3, toluene, reflux, 24 h, (82% for 5a, 79% for 5b, 82% for 5c); e) *n*-Bu_4NF, THF, 0 °C to rt, 2 h, (81% for 6a, 84% for 6b, 80% for 6c); f) SO_3.py, CH_2Cl_2 :DMSO (2:1), Et_3N, 0 °C to rt, 2 h, (84% for 7a, 78% for 7b, 89% for 7c); g) (±) or (-)-pramipexole, $NaBH(OAc)_3$, CH_2Cl_2, rt, 48 h, (38% for 8a, 32% for 8b, 41% for 8c and 40% for 9a, 39% for 9b, 43% for 9c); h) CF_3COOH, CH_2Cl_2, 0 °C to rt, 3 h, (90% for 10a, 84% for 10b, 90% for 10c and 93% for 11a, 96% for 11b, 93% for 11c).

Scheme 2

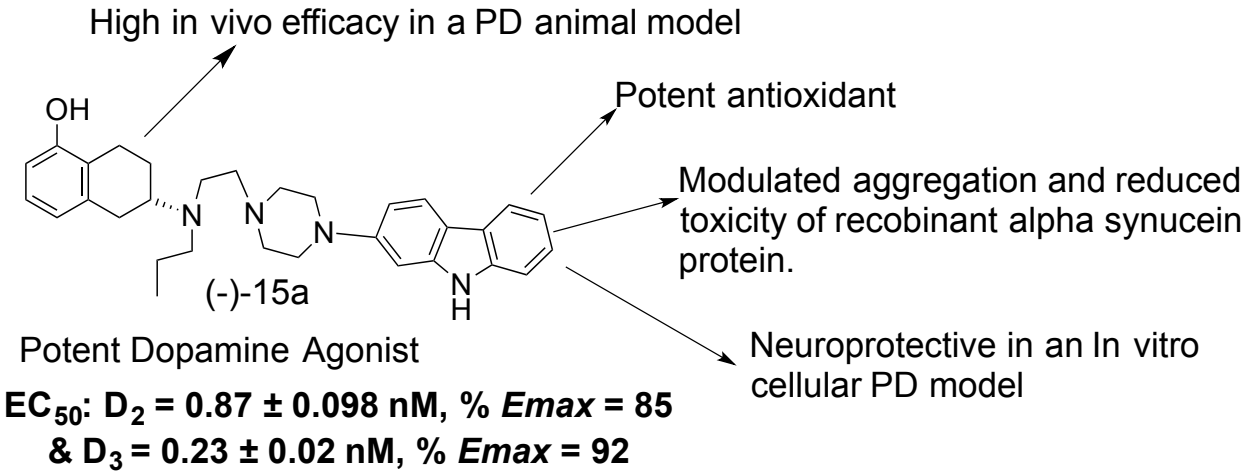


Reagents and conditions: a) (±) or (-)-MPAT, $NaBH(OAc)_3$, CH_2Cl_2, rt, 48 h, (77% for 12a, 43% for 12b, 74% for 12c and 74% for 13a, 33% for 13b, 82% for 13c); b) 48% aq. HBr, reflux, 5 h, (78% for 14a, 98% for 14b, 78% for 14c and 82% for 15a, 97% for 15b, 82% for 15c).

Scheme 3



Reagents and conditions: a) K_2CO_3, KOH, TBAB, DBE, 50 °C, overnight, 32%; b) 1-[2-(*tert*-butyl-dimethyl-silanyloxy)-ethyl]-piperazine, K_2CO3, CH_3CN, reflux, 24 h, 73%; c) *n*-Bu_4NF, THF, 0 °C to rt, 3 h, 81%; d) SO_3.py, CH_2Cl_2:DMSO (2:1), Et_3N, 0 °C to rt, 2 h, 77%; e) (±) or (-)-pramipexole, $NaBH(OAc)_3$, CH_2Cl_2, rt, 48 h, 57% for 20 and 47% for 21, respectively.


High in vivo efficacy in a PD animal model
OH
N
N
N
N
H
(-)-15a
Potent antioxidant
Modulated aggregation and reduced toxicity of recobinant alpha synucein protein.
Neuroprotective in an In vitro cellular PD model
Potent Dopamine Agonist
EC50: D2 = 0.87 ± 0.098 nM, % Emax = 85
& D3 = 0.23 ± 0.02 nM, % Emax = 92