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Development of a Highly Potent D_2/D_3 Agonist and a Partial Agonist from Structure−Activity Relationship Study of N^6-(2-(4-(1*H*-Indol-5-yl)piperazin-1-yl)ethyl)-N^6-propyl-4,5,6,7-tetrahydrobenzo[*d*]thiazole-2,6-diamine Analogues: Implication in the Treatment of Parkinson's Disease

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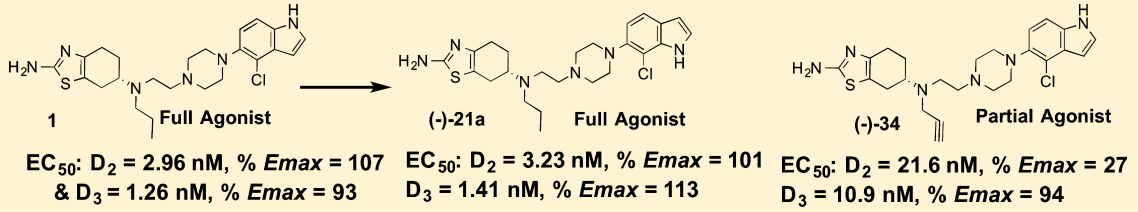
Supporting Information



ABSTRACT: Our structure−activity relationship studies with N^6-(2-(4-(1*H*-indol-5-yl)piperazin-1-yl)ethyl)-N^6-propyl-4,5,6,7-tetrahydrobenzo[*d*]thiazole-2,6-diamine derivatives led to development of a lead compound (−)-**21a** which exhibited very high affinity (K_i, D_2 = 16.4 nM, D_3 = 1.15 nM) and full agonist activity (EC_{50} (GTPγS); D_2 = 3.23 and D_3 = 1.41 nM) at both D_2 and D_3 receptors. A partial agonist molecule (−)-**34** (EC_{50} (GTPγS); D_2 = 21.6 (E_{max} = 27%) and D_3 = 10.9 nM) was also identified. In a Parkinson's disease (PD) animal model, (−)-**21a** was highly efficacious in reversing hypolocomotion in reserpinized rats with a long duration of action, indicating its potential as an anti-PD drug. Compound (−)-**34** was also able to elevate locomotor activity in the above PD animal model significantly, implying its potential application in PD therapy. Furthermore, (−)-**21a** was shown to be neuroprotective in protecting neuronal PC12 from toxicity of 6-OHDA. This report, therefore, underpins the notion that a multifunctional drug like (−)-**21a** might have the potential not only to ameliorate motor dysfunction in PD patients but also to modify disease progression by protecting DA neurons from progressive degeneration.

INTRODUCTION

Parkinson's disease (PD) is a multifaceted neurodegenerative disorder affecting 1.5% of the global population over 65 years of age and is characterized primarily by the deterioration of motor activities that are controlled by the nigrostriatal system. Among the clinical features of PD are motor impairments involving tremors at rest, rigidity, bradykinesia, and postural instability along with nonmotor symptoms such as autonomic, cognitive, and psychiatric problems.[1] The neuropathogenesis of PD is characterized by progressive, selective, and irreversible loss of dopaminergic neurons in the midbrain substantia nigra pars compacta (SNpc), which can eventually deplete the striatum of adequate levels of dopamine (DA), resulting in cardinal motor deficits.[2,3] Although extensive research on the basic and clinical aspects of PD has been done to elucidate the underlying molecular events leading to neuronal death, the exact etiology of the disease remains to be completely understood and thus PD remains a progressive and incurable condition.[4,5] There is a plethora of evidence to support the hypothesis that environmental factors converging on oxidative stress, mitochondrial dysfunction, nigral iron elevation, and aberrant protein aggregation account for most cases of PD.[5−7] The generally accepted hypothesis is that oxidative stress, via the generation of reactive oxygen species (ROS), is the major contributor in PD pathogenesis that harmfully affects proteins, lipids, and nucleic acids.[8] One of the major sources of ROS generation comes from inhibition of complex I of the mitochondrial electron transport chain, thereby generating superoxide and hydroxyl radical which cumulatively perturb cellular redox homeostasis and produce oxidative stress.[5] In addition, a common pathological hallmark of sporadic PD is the Lewy body, a rounded eosinophilic inclusion that is comprised mainly of a filamentous presynaptic protein, α-synuclein.[9,10] While α-synuclein is a natively unfolded protein, the monomeric form can misfold and aggregate into larger oligomeric and protofibrillar neurotoxic species.[11,12] α-synuclein

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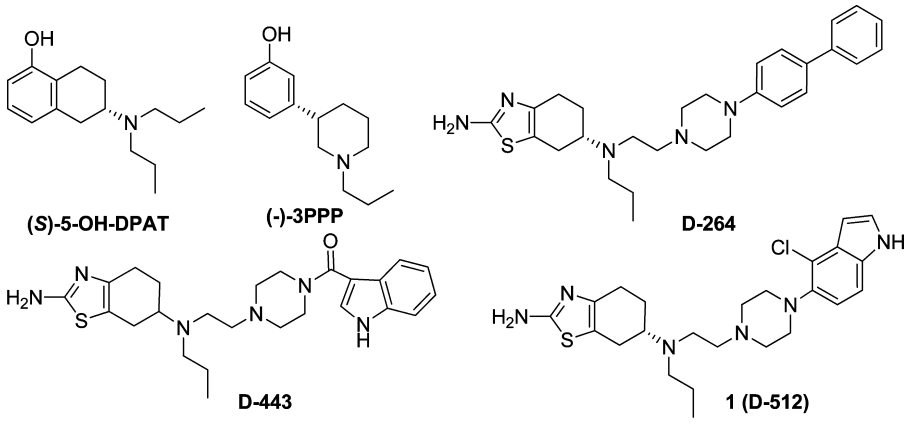
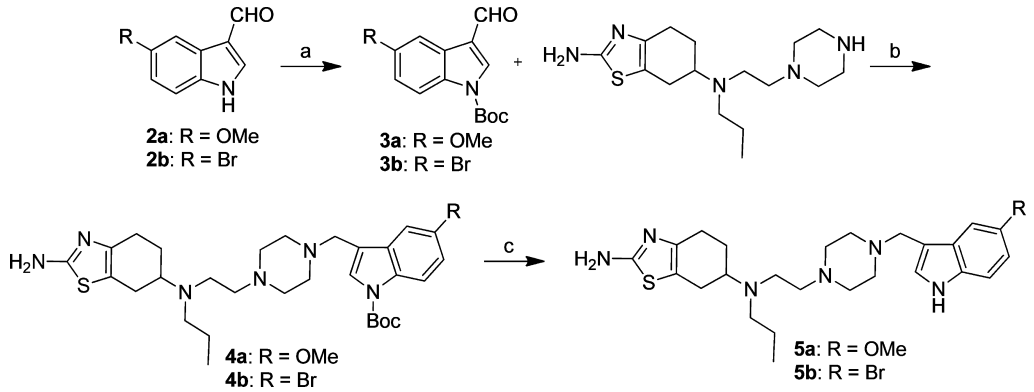


Figure 1. Molecular structures of D_2/D_3 agonists.

Scheme 1[a]



[a]Reagents and conditions: (a) $(Boc)_2O$, 4-DMAP, THF, rt, overnight; (b) $NaBH(OAc)_3$, CH_2Cl_2, rt, 48 h; (c) CF_3COOH, CH_2Cl_2, 0 °C to rt, 2 h.

protofibrils can cause vesicular dopamine leakage leading to increased oxidative stress, which might also promote protein misfolding and exacerbate α-synuclein aggregation in the cytoplasm.[13,14] Collectively, increased oxidative stress, elevated iron levels, compromised natural antioxidant defense, protein aggregation, and impaired mitochondrial functions are the mainstream predisposing factors implicated in the pathogenesis of PD.

DA exhibits a variety of pharmacological actions in the central nervous system (CNS) and also in the periphery. The DA receptor systems have been targeted for the development of pharmacotherapeutic agents for a number of CNS related disorders, including drug addiction, schizophrenia, depression, and PD. These effects are mediated by five DA receptors (D_1–D_5) grouped into D_1-like (D_1 and D_5) and D_2-like (D_2, D_3 and D_4) receptor subtypes which transduce signals via the effector molecule adenylate cyclase. Upon receptor activation, D_1-like receptors activate adenylate cyclase, whereas D_2-like receptors inhibit it.[15] The D_1 and D_5 receptors exhibit 80% homology in their transmembrane region. The sequence alignment between D_2-like receptors revealed overall 50% similarity between D_2 and D_3 receptors, and it increases up to 90% at the ligand binding site.[16,17]

Modulation of multiple targets along the same biological pathway can potentially lead to disease modification rather than just control of symptoms. For the successful treatment of multifactorial CNS diseases, like PD, a new paradigm has arisen to correct the underlying pathway, according to which multifunctional drugs having multiple pharmacological activities can be employed to address more than one pathological factor.[18] Currently, the mainstay treatment of PD focuses on the DA replacement strategies, which include levodopa (L-dopa, the precursor of DA) and DA receptor agonists as well as monoamine oxidase B (MAOB) inhibitors and catechol-*O*-methyltransferase inhibitors, thereby alleviating only the symptoms without affecting the course of the disease progression.[19] L-Dopa usage is unfortunately associated with the side effects including dyskinesia, and its long-term use can produce sudden "on–off" effects.[20] It has also been reported that the eventual oxidation of DA derived from L-dopa further augments oxidative stress leading to enhanced disease progression.[21,22] Therefore, beyond symptomatic relief, treatments that have neuroprotective or disease-modifying properties remain a critical unmet medical need. Unfortunately, no neuroprotective drugs have been identified or approved by the FDA so far for the treatment of PD,[23,24] thereby necessitating research for a breakthrough development.[25] Our approach in developing such agents involves alleviating symptoms of PD along with slowing or halting the neurodegeneration process. With this in mind, we initiated our drug discovery approach aimed at identifying novel multifunctional agents possessing D_2/D_3 agonist activity along with antioxidant, iron chelating, and neuroprotective properties. In this regard, we have recently reported the development of such multifunctional D_2/D_3 agonists derived from a novel molecular template which exhibited in vivo efficacy in PD animal models.[26−36] Some of our lead multifunctional D_2/D_3 agonist molecules, such as (*S*)-N^6-(2-(4-([1,1′-biphenyl]-4-yl)piperazin-1-yl)ethyl)-N^6-propyl-

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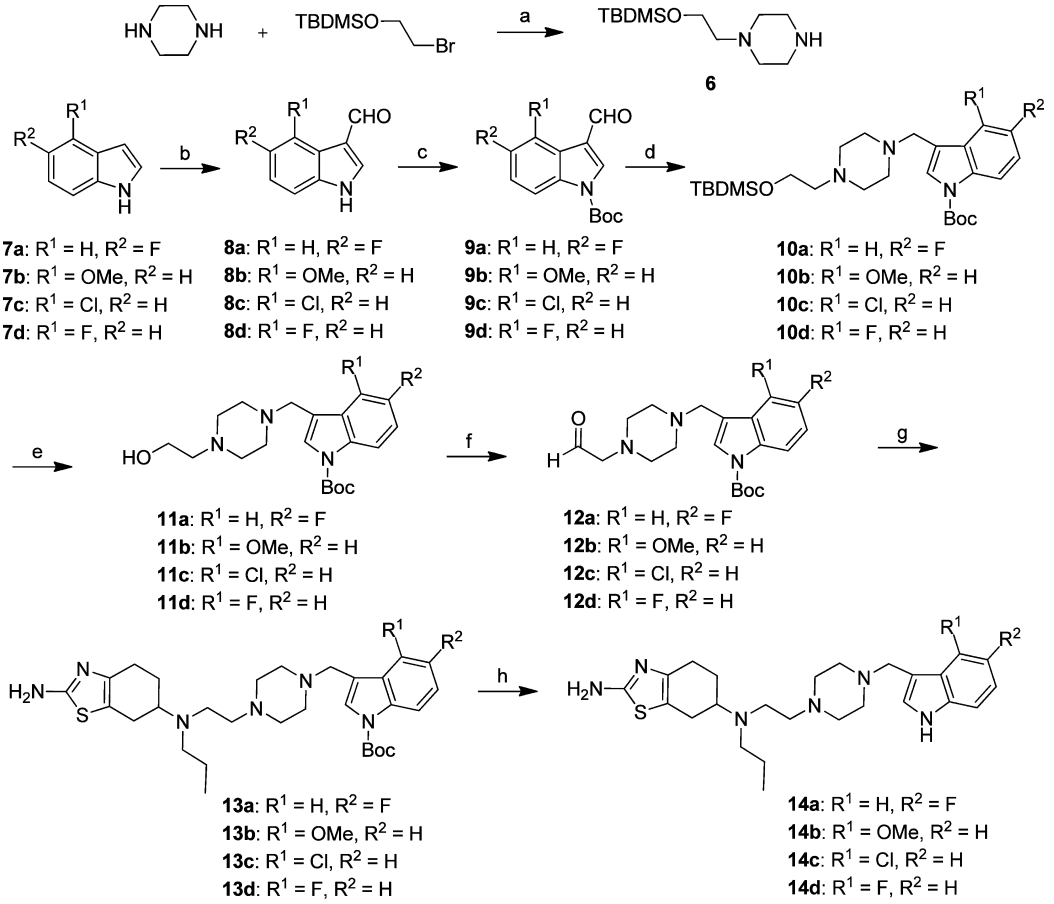
Scheme 2[a]



[a]Reagents and conditions: (a) K_2CO_3, CH_3CN, reflux, overnight; (b) (i) $POCl_3$, DMF, 0−40 °C, 1.5 h, (ii) ice, 2 M NaOH, (iii) reflux, 5 min; (c) $(Boc)_2O$, 4-DMAP, THF, rt, overnight; (d) **6**, $NaBH(OAc)_3$, CH_2Cl_2, rt, 48 h; (e) *n*-Bu_4NF, THF, 0 °C to rt, 2 h; (f) SO_3·py, CH_2Cl_2:DMSO (2:1), Et_3N, 0 °C to rt, 1.5 h; (g) (±)-pramipexole, $NaBH(OAc)_3$, CH_2Cl_2, rt, 48 h;(h) CF_3COOH, CH_2Cl_2, 0 °C to rt, 3 h.

4,5,6,7-tetrahydrobenzo[*d*]thiazole-2,6-diamine (D-264) and **1** (D-512) (Figure 1), exhibited neuroprotection in in vitro and in vivo experiments.[30,33,34] Additionally, we have reported development of multifunctional brain penetrant iron chelators with agonist activity at D_2/D_3 receptors.[29,31]

As part of our continued endeavor to design and develop D_2/D_3 receptor agonists based on our hybrid structure template,[37,38] which combines an agonist binding moiety (aminotetralin or bioisosteric equivalent) with arylpiperazine fragments via a linker of suitable length, we have recently reported a novel neuroprotective D_2/D_3 agonist, **1** (Figure 1), comprising an indole substituent that is expected to occupy the accessory binding site of the receptor.[32] As the compound turned out to be a promising candidate for PD through a number of experiments,[33,34] we further wanted to delve into a comprehensive structure−activity relationship (SAR) study and accordingly introduce, in the current work, several indole derivatives on the piperazine ring with varying chain length and position of attachment in the indole aromatic ring. We have also introduced a propargyl group in our core hybrid structure to shed light on its role in interacting with DA D_2/D_3 receptors. The lead molecule in this study not only exhibits potent in vivo activity in a PD animal model but also displays an array of interrelated activities pertinent to neuroprotection, such as potent antioxidant activity and inhibition of 6-hydroxydopamine (6-OHDA)-induced neuronal cell death.

CHEMISTRY

The first series of compounds discussed in the present work were prepared by combining the agonist headgroup with various halo- and methoxy-substituted indole derivatives in which the indole moieties were attached to the piperazine fragment via a methylene linker (Schemes 1 and 2). Scheme 1 describes the synthesis of final compounds (±)-**5a** and (±)-**5b**. *tert*-Butyl 3-formyl-5-methoxy-1*H*-indole-1-carboxylate (**3a**), prepared according to a procedure described previously,[39] was selectively condensed with N^6-(2-(piperazin-1-yl)ethyl)-N^6-propyl-4,5,6,7-tetrahydrobenzo[*d*]- thiazole-2,6-diamine[32] under reductive amination condition in the presence of sodium triacetoxyborohydride to give compound **4a**. Finally, the amine protecting *t*-Boc group was removed by using trifluoroacetic acid (TFA) to furnish the final compound (±)-**5a**. In a similar way, aldehyde **3b**[40] was reductively alkylated with the secondary amine to afford **4b**, which was then treated with TFA to give the final compound (±)-**5b**.

The syntheses of the target compounds (±)-**14a**, (±)-**14b**, (±)-**14c**, and (±)-**14d** are outlined in Scheme 2. The starting compound **6** was obtained by alkylating piperazine with (2-bromoethoxy)-*tert*-butyl-dimethylsilane in the presence of K_2CO_3 under reflux. Various commercially available indole derivatives were formylated in the presence of phosphorus oxychloride to give **8a**−**8d**, which were then *N*-protected using di-*tert*-butyl dicarbonate in the presence of 4-dimethylaminopyr-

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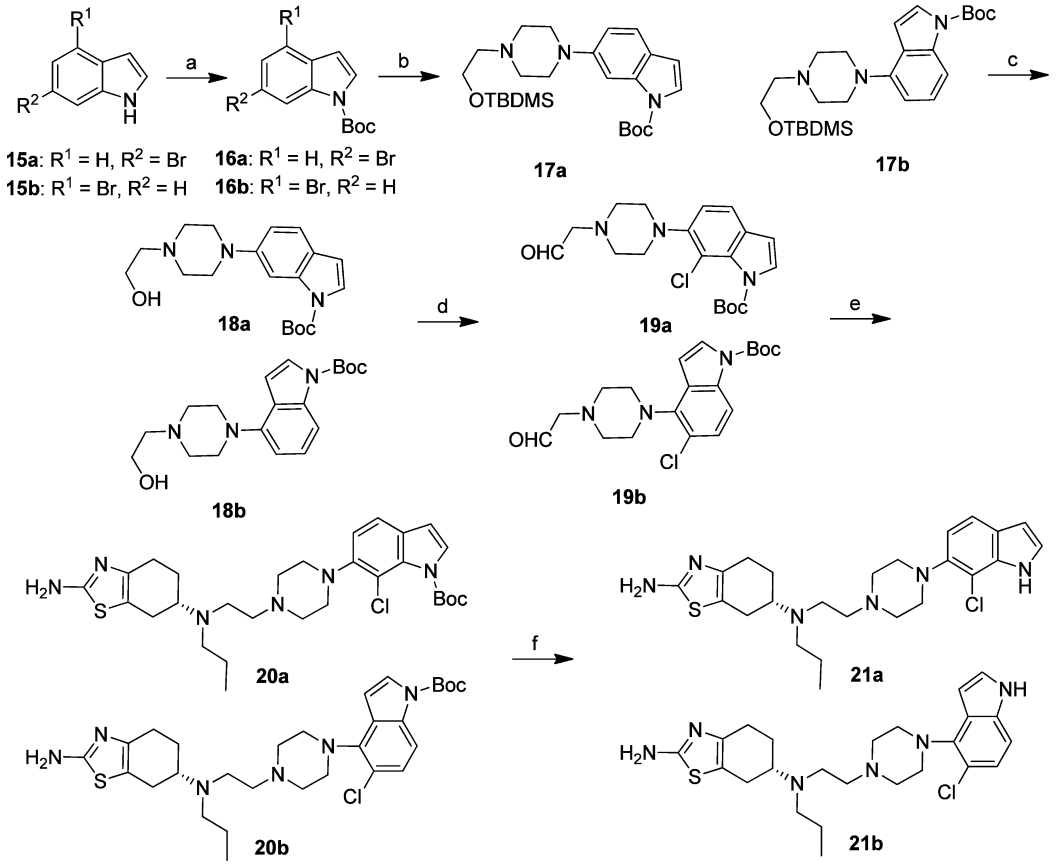
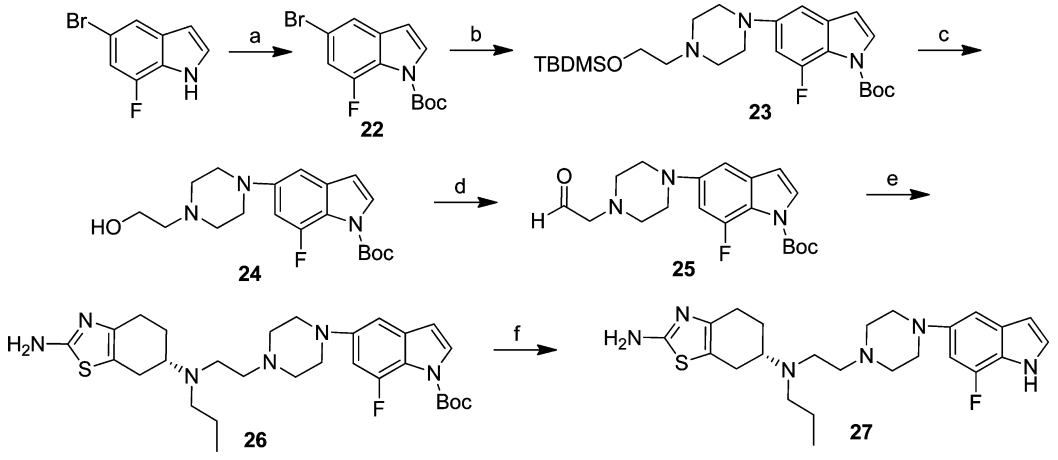
Scheme 3[a]



[a]Reagents and conditions: (a) $(Boc)_2O$, 4-DMAP, THF, rt, overnight; (b) **6**, $Pd(OAc)_2$, BINAP, Cs_2CO_3, toluene, reflux, 15 h; (c) *n*-Bu_4NF, THF, 0 °C to rt, 2 h; (d) oxalyl chloride, DMSO, Et_3N, CH_2Cl_2, −78 °C to rt, 2 h; (e) (−)-pramipexole, $NaBH(OAc)_3$, CH_2Cl_2, rt, 48 h; (f) CF_3COOH, CH_2Cl_2, 0 °C to rt, 3 h.

Scheme 4[a]



[a]Reagents and conditions: (a) $(Boc)_2O$, 4-DMAP, THF, rt, overnight; (b) **6**, $Pd(OAc)_2$, BINAP, Cs_2CO_3, toluene, reflux, 15 h; (c) *n*-Bu_4NF, THF, 0 °C to rt, 2 h; (d) SO_3·py, CH_2Cl_2:DMSO (2:1), Et_3N, 0 °C to rt, 1.5 h; (e) (−)-pramipexole, $NaBH(OAc)_3$, CH_2Cl_2, rt, 48 h; (f) CF_3COOH, CH_2Cl_2, 0 °C to rt, 3 h.

idine (4-DMAP) to afford **9a−9d**. Condensation of amine **6** with intermediates **9a−9d**, using $NaBH(OAc)_3$ as the reducing agent, yielded compounds **10a−10d**. The silyl groups of compounds **10a−10d** were removed by treatment with *n*-Bu_4NF (TBAF) in THF to afford compounds **11a−11d**. Subsequent oxidation of the hydroxyl groups using pyridine-sulfur trioxide furnished the corresponding aldehydes,[41] which were subsequently condensed with racemic pramipexole[42] under reductive amination conditions to give the penultimate compounds **13a−13d**. Finally, the amine protecting *t*-Boc groups were removed by treatment with TFA to furnish the final compounds (±)-**14a**, (±)-**14b**, (±)-**14c**, and (±)-**14d** as TFA salts.

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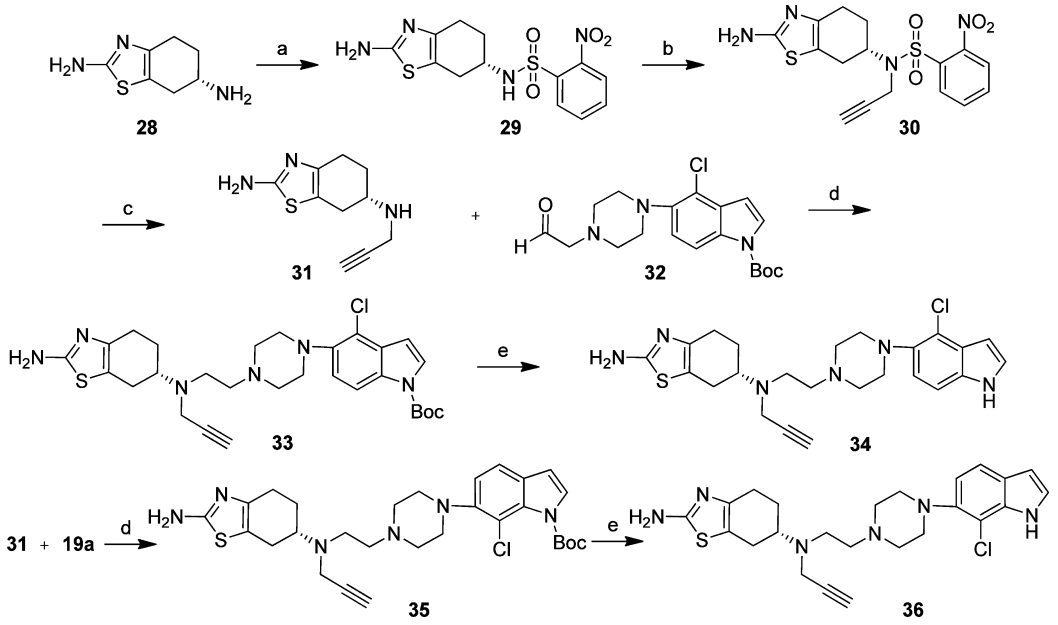
Scheme 5[a]



[a]Reagents and conditions: (a) 2-nitrobenzenesulfonyl chloride, Et_3N, THF, −10 °C to rt, 1.5 h; (b) propargyl bromide, K_2CO_3, CH_3CN, 50 °C, overnight; (c) thioglycolic acid, K_2CO_3, DMF, 0 °C to rt to 50 °C, 16 h; (d) $NaBH(OAc)_3$, CH_2Cl_2, rt, 40 h; (e) CF_3COOH, CH_2Cl_2, 0 °C to rt, 2 h.

Schemes 3 and 4 depict the syntheses of another series of compounds where the indole groups are directly attached to the piperazine moieties. As shown in Scheme 3, commercially available 6-bromoindole was *N*-protected with di-*tert*-butyl dicarbonate to give **16a**. Palladium-catalyzed cross coupling of **16a** with amine **6** in the presence of Cs_2CO_3 and BINAP in toluene under refluxing condition yielded intermediate **17a**, which on TBDMS deprotection afforded alcohol **18a** in excellent yield. Compound **18a** was then oxidized under Swern oxidation conditions[43] to aldehyde **19a**, which was subsequently condensed with *S*-(−)- pramipexole[27,42] under reductive amination conditions to give the compound (−)-**20a**. It is interesting to note that while carrying out the Swern oxidation reaction, a chlorine atom got inserted in the 7-position of indole aromatic ring of **19a**, a phenomenon observed previously by us and others.[44,45] The possible reaction mechanism might involve electrophilic chlorination of indole aromatic ring in the presence of excess dimethylchlorosulfonium chloride, the reactive intermediate formed during Swern oxidation.[46] Finally, deprotection of the indole moiety of (−)-**20a** under acidic condition afforded the target compound (−)-**21a** as TFA salt. Another final compound (−)-**21b** was also prepared in a similar fashion as described for (−)-**21a**, where 4-bromoindole was used as the starting material.

The synthesis of another target molecule (−)-**27** is shown in Scheme 4. The secondary amine of 5-bromo-7-fluoro-1*H*-indole was first protected with $(Boc)_2O$ to give **22**. The *N*-arylation was next performed by refluxing the mixture of **6**, **22**, Cs_2CO_3, BINAP, and $Pd(OAc)_2$ in toluene to give **23** in moderate yield. The silyl group of compound **23** was removed by treatment with TBAF in THF to afford alcohol **24**, which on subsequent oxidation in the presence of pyridine-sulfur trioxide yielded the corresponding aldehyde **25**. The aldehyde was then condensed with *S*-(−)- pramipexole[42] in the presence of $NaBH(OAc)_3$ to afford the compound (−)-**26**. Finally, deprotection of the indole amine of (−)-**26** was accomplished by treating with TFA to furnish the final compound (−)-**27**.

Scheme 5 shows the synthesis of the final series of compounds in which the *N*-propyl group of pramipexole is replaced with a propargyl moiety. The starting diamine **28**, prepared according to a procedure described previously,[47] was first treated with nitrobenzenesulfonyl chloride to selectively protect the aliphatic primary amine of **28** to afford **29**, which was next reacted with propargyl bromide in the presence of a base to give the intermediate **30**. Deprotection of the nitrobenzenesulfonyl group was achieved by treatment with thioglycolic acid to furnish optically active compound **31**. Secondary amine of **31** was next reductively alkylated with the aldehyde **32**[32] in the presence of $NaBH(OAc)_3$ to afford the compound (−)-**33**. Finally, removal of the Boc protecting group was accomplished under acidic condition to afford the target compound (−)-**34** as TFA salt. Another final compound (−)-**36** was also prepared in a similar fashion as described for (−)-**34**, where aldehyde **19a** was used as the partner for the reductive amination reaction with amine **31**.

RESULTS AND DISCUSSION

Affinity and Agonism at DA D_2 and D_3 Receptors. Our current study is aimed at investigating the molecular and chemical flexibility of the arylpiperazine fragment of our hybrid template as it relates to D_2/D_3 receptor binding and functional activity. The present series of compounds comprise various indole derivatives, as our previous studies have indicated that an indole substituent in the arylpiperazine region is well tolerated, producing molecules with high D_2/D_3 affinity and in number of instances a preference for D_3 receptor.[32,48] The structural modifications are mainly centered on the direct attachment of the piperazine nitrogen atom, distal to the agonist headgroup, to various positions of the indole ring. Other molecular alterations include substitution of the indole nucleus with halogen and methoxy group at different positions along with changes in linker

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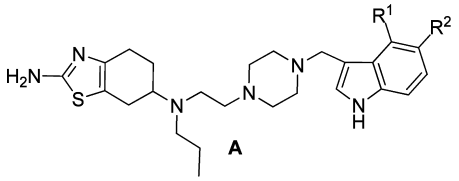
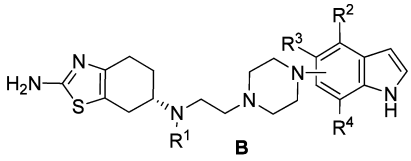
Table 1. Inhibition Constants Determined by Competition Experiments Assessing [^{3}H]Spiroperidol Binding to Cloned Rat D_{2L} and D_3 Receptors Expressed in HEK-293 Cells[a] (cLogP and tPSA Values Are Calculated Using ChemDraw)



5a: R^1 = H, R^2 = OMe
5b: R^1 = H, R^2 = Br
14a: R^1 = H, R^2 = F
14b: R^1 = OMe, R^2 = H
14c: R^1 = Cl, R^2 = H
14d: R^1 = F, R^2 = H



1: R^1 = propyl, R^2 = Cl, R^3 = H, R^4 = H
21a: R^1 = propyl, R^2 = H, R^3 = H, R^4 = Cl
21b: R^1 = propyl, R^2 = H, R^3 = Cl, R^4 = H
27: R^1 = propyl, R^2 = H, R^3 = H, R^4 = F
34: R^1 = propargyl, R^2 = Cl, R^3 = H, R^4 = H
36: R^1 = propargyl, R^2 = H, R^3 = H, R^4 = Cl

	K_i (nM)				
compd	D_{2L}, [^{3}H]spiroperidol	D_3, [^{3}H]spiroperidol	D_{2L}/D_3	cLogP	tPSA
dopamine[b]	990 ± 198	101 ± 14.9	9.8		
(−)-5-OH-DPAT	153 ± 32	2.07 ± 0.38	74		
D-443[c]	1503 ± 67	4.17 ± 0.30	360		
1[c]	39.0 ± 5.0	2.19 ± 0.39	18	5.1443	60.13
(±)-**5a**	1636 ± 219	15.9 ± 1.2	101	4.5221	69.36
(±)-**5b**	376 ± 38	12.9 ± 0.30	29	5.5321	60.13
(±)-**14a**	854 ± 51	12.1 ± 3.2	71	4.8121	60.13
(±)-**14b**	1883 ± 250	25.0 ± 2.4	75	4.5221	69.36
(±)-**14c**	1164 ± 214	13.8 ± 1.6	84	5.3821	60.13
(±)-**14d**	992 ± 82	8.87 ± 0.78	112	4.8121	60.13
(−)-**21a**	16.4 ± 2.8	1.15 ± 0.19	14	5.1443	60.13
(−)-**21b**	74.5 ± 6.7	0.87 ± 0.13	86	5.1443	60.13
(−)-**27**	156 ± 34	3.66 ± 0.66	43	4.5743	60.13
(−)-**34**	86.5 ± 15	14.4 ± 1.0	5.99	4.5063	60.13
(−)-**36**	172 ± 16	0.145 ± 0.035	1186	4.5063	60.13

[a]Results are the means ± SEM for 3−6 experiments each performed in triplicate. [b]Data obtained under almost identical assay conditions and taken from ref 49. [c]Data taken from ref 35.

length connecting the piperazine and indole moieties. We have carried out our binding studies with rat DA D_2 and D_3 (rD_2 and rD_3) receptors expressed in HEK-293 cells and functional characterization with human D_2 and D_3 receptors (hD_2 and hD_3) expressed in CHO cells. In our own findings, we did not see appreciable differences in the affinity of compounds for rat or human DA D_2/D_3 receptors, expressed in either HEK-293 or CHO cells (unpublished results and see also Zhen et al. 2015).[49] It has been shown that an overall high degree of homology exists between the two species; 95% for D_2 and greater than 78% for D_3, with a somewhat shorter third intracellular loop in the human version of the D_3 receptor.[50,51]

To evaluate receptor binding of the final compounds, a radioligand competition assay was conducted and the binding affinity profiles were compared with that of the reference agent (*S*)-5-OH-DPAT (Table 1). Binding potency was determined by inhibition of [^{3}H]spiroperidol binding to rat DA D_2 and D_3 receptors expressed in HEK-293 cells as described by us previously.[52,53] Table 1 summarizes the binding data for analogues that were synthesized. Compounds **5a−b** as well as **14a−d**, which incorporate the 2-aminothiazole headgroup and a methylene unit connecting piperazine ring to the 3 position of a variety of substituted indole ring, displayed high affinity for D_3 and low affinity for D_2 receptors. In this series of analogues, we introduced halogen or methoxy group either at the 4- or 5-position of the indole moiety to study the electronic effects of electron-withdrawing or donating groups on the indole nucleus as well as their possible effects on binding interactions in this region with D_2 and D_3 receptors. The 2-aminothiazole derived 5-methoxy indole derivative **5a** displayed high affinity and selectivity for the D_3 receptor (K_i, D_2 = 1636 nM, D_3 = 15.9 nM, D_2/D_3 = 101), while for the 5-bromo and 5-fluoro derivatives **5b** and **14a**, D_2 affinity improved and D_3 affinity remained the same (K_i, D_2 = 376 nM, D_3 = 12.9 nM, D_2/D_3 = 29 and D_2 = 854 nM, D_3 = 12.1 nM, D_2/D_3 = 71 for **5b** and **14a**, respectively). This data demonstrates the effect of halogen substitution at the 5-position of indole on imparting better interaction with D_2 receptor as opposed to 5-methoxy substituent in which, we speculate, the bulkier methoxy group or the unfavorable electronic effect might be producing some difficulty to interact with the D_2 receptor. Next, isomeric 4-substituted indole derivatives **14b−d** were synthesized. The 4-methoxy analogue **14b** exhibited low affinity for D_2 (K_i, D_2 = 1883 nM, D_3 = 25 nM, D_2/D_3 = 75) as observed with **5a**, while the 4-fluoro substituted compound **14d** (K_i, D_2 = 992 nM, D_3 = 8.87 nM, D_2/D_3 = 112) was similar in its binding profile for D_2/D_3 receptors as its isomeric counterpart **14a**. Compound **14c**, structurally different from **14d** only in having a chloride instead of a fluoride substituent, displayed **14d**-like affinities for D_2/D_3 receptors. Thus, affinity for the D_2 receptor varied among the isomeric compounds, with halo-substituted derivatives showing relatively higher affinity while maintaining similar D_3 selectivity.

Next, we investigated the effect of a methylene linker connecting the piparazine fragment, distal to the agonist headgroup, with the indole nucleus and compared it with a carbonyl linker containing compound (4-(2-((2-amino-4,5,6,7-

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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		
compd	[^{35}S]GTPγS EC_{50} (nM)	E_{max} (%)	[^{35}S]GTPγS EC_{50} (nM)	E_{max} (%)	D_2/D_3
dopamine (DA)	183 ± 14	100	5.34 ± 1.43	100	34.3
(−)-3PPP[b]	163 ± 6	19.8 ± 1.8	51.9 ± 3.6	52.0 ± 3.6	3.14
1[c]	2.96 ± 0.3	107 ± 3	1.26 ± 0.2	93.1 ± 4.4	2.35
(−)-**21a**	3.23 ± 0.78	101 ± 4	1.41 ± 0.08	113 ± 7	2.29
(−)-**21b**	32.7 ± 7.6	87.6 ± 4.1	3.93 ± 1.12	104 ± 9	8.32
(−)-**34**	21.6 ± 9.6	26.7 ± 6.4	10.9 ± 2.4	94 ± 3.5	1.98
(−)-**36**	39.4 ± 6.3	86.8 ± 3.8	0.84 ± 0.05	93.4 ± 3.6	46.9

[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. [b]Data from our previous study Zhen et al.[49] (see Supporting Information Tables S1a and S1b) [c]Data from our previous study Johnson et al.[32]

tetrahydrobenzo[*d*]-thiazol-6-yl)(propyl)amino)ethyl)-piperazin-1-yl)(1*H*-indol-3-yl)methanone (D-443) (Figure 1) we reported earlier. It was found that the introduction of a carbonyl group between piperazine and indole resulted in very high affinity and selectivity for D_3 receptor, and we hypothesized that an electronic effect of the carbonyl group might play an important role in deciphering the observed selectivity for the D_3 receptor.[32] In contrast, the present series of compounds **5a−b** and **14a−d** carrying a methylene rather than carbonyl linker also revealed low nanomolar and preferential affinity for the D_3 receptor comparable to that of the reference compound (K_i, D_2 = 1503 nM, D_3 = 4.17 nM, D_2/D_3 = 360; Table 1). This result indicated that introduction of an additional carbon, irrespective of any electronic effect, in the distal region of the hybrid structure might facilitate an enhanced access to the accessory binding sites in the D_3 receptor and thereby impart a significant effect in D_3 receptor affinity.

Our previous reports consistently demonstrated that in the 2-aminothiazole series of hybrid compounds, the (−)-isomeric version exhibited higher affinity for D_2/D_3 receptors compared to the (+)-isomer. Because the current SAR study is based on the promising results observed with **1** (Figure 1), we were interested in preparing only the (−)-isomeric version of a series of halogen-substituted indole analogues connected directly to the piperazine moiety. Among the molecules in this series, the 6-substituted indole derivative (−)-**21a** displayed the highest affinity for both D_2 and D_3 receptors along with low selectivity (K_i, D_2 = 16.4 nM, D_3 = 1.15 nM, D_2/D_3 = 14, Table 1) and the binding affinities were somewhat higher than the parent compound **1** (K_i, D_2 = 39 nM, D_3 = 2.19 nM, D_2/D_3 = 18). It is worth mentioning that the position of chlorine substitution imparted a trivial effect in DA receptor binding (7-chloro in (−)-**21a** vs 4-chloro in **1**). Thus, the direct attachment of the piperazine moiety at either 5- or 6-position of the indole nucleus did not result in a significant difference in terms of their affinity for D_2/D_3 receptors. In contrast, when the point of connection is at 4 position of indole as in the case of (−)-**21b**, the binding affinity for the D_2 receptor was reduced and that for the D_3 receptor was retained (K_i, D_2 = 74.5 nM, D_3 = 0.87 nM, D_2/D_3 = 86) compared to (−)-**21a**; however, the selectivity was increased by 6-fold (D_2/D_3, 86 vs 14 for (−)-**21b** vs (−)-**21a**). This suggests that the indole moiety alone does not give rise to selectivity for either receptor subtype but the position of connectivity to the piperazine fragment does. To further compare the effect of halogen substituent on the indole ring with the parent molecule, we next synthesized 5-substituted indole derivative (−)-**27** with a fluorine atom attached to the 7 position of the ring. As shown in Table 1, compound (−)-**27** displayed moderate affinity and selectivity for D_2/D_3 receptors (K_i, D_2 = 156 nM, D_3 = 3.66 nM, D_2/D_3 = 43) as opposed to the parent 4-chloro derivative **1**, which in comparison revealed low nanomolar affinity for both receptors along with 2.5-fold reduced selectivity. Taken altogether, chloro-derivatives have shown somewhat higher binding profiles than the corresponding fluoro-substituted compound.

To gain an insight into the effect of propargyl moiety on DA receptor binding, propargyl group was incorporated into 2-aminothiazole agonist headgroup to design compounds (−)-**34** and (−)-**36**. Interestingly, introduction of the propargyl group resulted in a diminished binding affinity for D_2/D_3 receptors (K_i, D_2 = 86.5 nM, D_3 = 14.4 nM for (−)-**34**) compared to the parent molecule **1** (Table 1), probably due to a less efficient interaction with the propyl binding cleft of the receptors.[54] Similarly, in comparing (−)-**36** with corresponding molecule (−)-**21a** also revealed lower binding affinity for the D_2 receptor; however, D_3 receptor affinity was increased suggesting a different role for the propyl binding cleft in D_2 and D_3 receptors.

On the basis of the binding results, functional activities of the optically active lead compounds for human DA D_2 and D_3 receptors expressed in CHO cells were measured by monitoring stimulation of [^{35}S]GTPγS binding in comparison to stimulation by the endogenous ligand DA. Comparison with the maximum stimulation (E_{max}), produced by the full agonist DA, indicates whether the compound is a full agonist, a partial agonist, or an antagonist. As shown in Table 2, (−)-**21a** demonstrated a very high potency (EC_{50} (GTPγS); D_2 = 3.23 and D_3 = 1.41 nM), correlating well with binding data, and full agonist activity at both D_2 and D_3 receptors (E_{max} > 100%). Compound (−)-**21b** was also found to be potent and efficacious in stimulating both receptors (EC_{50} (GTPγS); D_2 = 32.7 and D_3 = 3.93 nM; E_{max} = 88−104%). None of the compounds displayed appreciable selectivity for D_3 vs D_2, and their selectivity for D_3 receptor dropped considerably when compared to the binding data (Table 1). The propargyl compound (−)-**34** displayed high functional potency for both the receptors (EC_{50} (GTPγS); D_2 = 21.6 and D_3 = 10.9 nM) but was less potent compared to the corresponding **1**. Similar profile was also observed for (−)-**36** when compared with the corresponding (−)-**21a** (Table 2). Interestingly, (−)-**34** showed partial agonist activity at D_2 receptor (E_{max} = 27%) while exhibiting full agonism at D_3 receptor (E_{max} = 94%). Thus, full agonist **1** was transformed into a potent partial agonist (−)-**34** upon replacement of the propyl group by a propargyl group. On the other hand, compound (−)-**36** revealed full agonist activity at both D_2 and D_3 receptors (E_{max} ∼ 100%) and the selectivity for the D_3 receptor was also increased significantly (D_2/D_3 = 47) as

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expected from the binding data. This data indicates the effect of subtle structural variations resulting in alteration in receptor activation. In comparing results from functional GTPγS assays with [^{3}H]spiroperidol binding data, a number of factors need to be considered such as the sensitivity of agonist potency to receptor conformational state promoted by the binding buffer used[52] and the effect of receptor reserve in reducing a functional EC_{50} of a full agonist.[55] The D_2 and D_3 cell lines used in this work can show receptor reserve for full agonists such as dopamine; as alluded to in our recent report,[56] dopamine D_2 and D_3 receptor reserves in these cell lines are indicated by K_i/EC_{50} ratios for dopamine greater than unity (8.5 and 31.3, respectively). More work is needed to assess whether some of the full agonists identified in the present cell lines are partial agonists in brain tissue. It is well-known that in assays with cells heterologously expressing a receptor, partial agonism may not be observed as compared with in situ brain tissue preparations where receptor concentrations are generally lower in relation to available G proteins (for this phenomenon for dopamine receptors see Tadori et al. 2009).[55]

We have also calculated the cLogP and tPSA values for all the compounds (Table 1). The values are almost similar for all of the compounds. In general, the values indicate that these compounds should produce in vivo efficacy which we observed in case of (−)-**21a** and (−)-**34** (discussed later). Therefore, our current SAR results of a series of indole compounds indicate that the affinity and selectivity for the D_2/D_3 receptors are governed by the nature of linking moieties, molecular structure, as well as substitution groups in the indole nucleus. When piperazine and indole are connected via a methylene unit, as in the case of compounds **5a−b** and **14a−d**, the affinity and selectivity for D_3 receptor is appreciable. On the other hand, direct linking of piperazine and indole moieties results in compounds that have relatively increased D_2 receptor affinities. Moreover, halogen substitution on the indole ring somewhat increases D_2 affinity as compared with the corresponding methoxy substituent. We have also observed that incorporation of a propargyl group in the agonist part of structure resulted in the potent D_2 partial agonist compound, (−)-**34**.

Characterization of the Partial Agonist (−)-34 for its Antagonist Activity at D_2. Experiments were conducted to assess the potency of compound (−)-**34** in antagonizing the action of the full agonist dopamine at D_2. This was done in "shift" experiments yielding the antagonist K_e value based on the test compound's ability to reduce the apparent potency of dopamine in stimulating [^{35}S]GTPγS binding in CHO-D_2 cells.[57] As shown in Figure 2, 50 and 300 nM of compound (−)-**34** shifted the dopamine activation curve to the right as compared to the dopamine curve with vehicle (with corresponding EC_{50} values for DA of 423 ± 39 nM and 3.93 μM compared with 225 ± 45 nM, respectively, mean ± SEM), as did 200 nM and 1 μM of the well-known partial agonist (−)-3PPP (with corresponding EC_{50} values for DA of 376 ± 54 nM and 844 ± 63 nM compared with 225 ± 45 nM, respectively). The K_e values derived from these experiments are 29.0 nM for (−)-**34** and 372 nM for (−)-3PPP. These values are the average of the K_e values obtained at the two separate fixed concentrations of (−)-**34**, 50 and 300 nM: 37.2 ± 10.5 nM (n = 6) and 20.9 ± 2.8 nM (n = 5) (statistically not different, P = 0.203, Student's t test). At the two fixed concentrations of (−)-3PPP, 200 nM and 1 μM, the K_e values were 362 ± 89 nM (n = 7) and 383 ± 47 nM (n = 4) (statistically not different, P = 0.870, Student's t test). Antagonist activity of a partial agonist can be pharmacologically meaningful in attenuating the action of endogenous transmitter, in this case dopamine. While the partial agonist reduces the dopamine effect, it does not completely abolish it, but rather it has some effect itself in activating dopamine receptors, modulating dopamine transmission in a more subtle way than completely shutting it down. The antagonist K_e as computed in this work provides a measure of potency of the antagonist effect, whereas the E_{max} measured in the functional assay reflects the degree to which dopamine receptors can still be activated. The observed K_e values (average of 29.0 nM and 372 nM for (−)-**34** and (−)-3PPP, respectively) are somewhat greater than the EC_{50} values for partial agonism (21.6 and 163 nM,[49] Table 2). In comparing EC_{50} and K_e values, one should consider that, in principle, for a functional response by the full agonist dopamine not all receptors need to be occupied; in fact, in the CHO-D_2 cells used here, there is substantial receptor reserve for DA (see two paragraphs higher up).

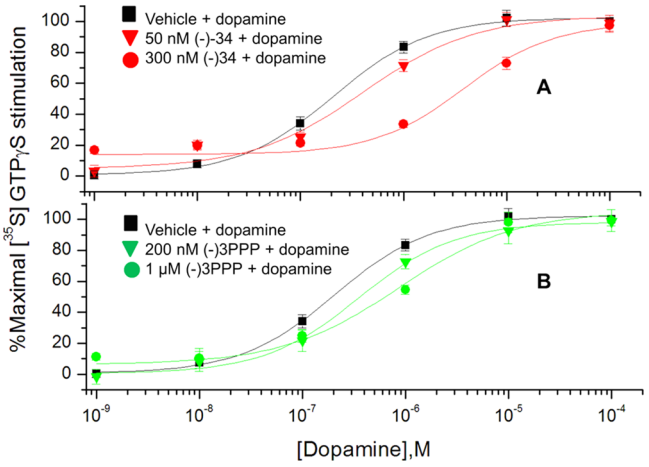

Figure 2. Rightward shift in dopamine curves for stimulation of [^{35}S]GTPγS binding in CHO-hD_2 cells by 50 nM and 300 nM (−)-**34** (A), or 200 nM and 1 μM (−)-3PPP (B). The indicated fixed concentrations of test compound were copresent with increasing concentrations of dopamine (colored curves). The two test compounds and vehicle with dopamine were tested in 4−7 independent experiments (carried out in triplicate); in order to show the shifts without clutter, the drugs are shown in separate panels (A,B) along with the same dopamine curve (averaged from all DA data obtained in two sets of experiments). The EC_{50} values calculated from the right-shifted curves were significantly larger than the EC_{50} of dopamine observed in the same set of experiments ($P < 0.05$, one-tailed Student's t test). For K_e values calculated from these shifts, see text. Data points are mean ± SEM.

Reversal of Reserpine-Induced Hypolocomotion in Rats by (−)-21a, (−)-34, (−)-Pramipexole, and Ropinirole. Reserpine induces depletion of catecholamine in nerve terminals, resulting in a cataleptic condition in rats, which is a well-established animal model for PD.[58,59] Significant inhibition of locomotion of rats was observed 18 h after the administration of reserpine (5 mg/kg, sc), which indicated the development of akinesia (Figure 3). Compound (−)-**21a** at the dose of 10 μMol/kg, ip, was not only highly efficacious in reversing akinesia in rats, compared to reserprine treatment alone, but also demonstrated significant enhancement of locomotion for the entire duration of the study of 6 h. Similar trend was also observed with the reference drug pramipexole; however, treatment with another reference drug ropinirole at the dose of 10 μMol/kg produced a quick onset of locomotor activation compared to control but with a much shorter duration of action compared with (−)-**21a**. The mechanism of the locomotor stimulation in this reserpine

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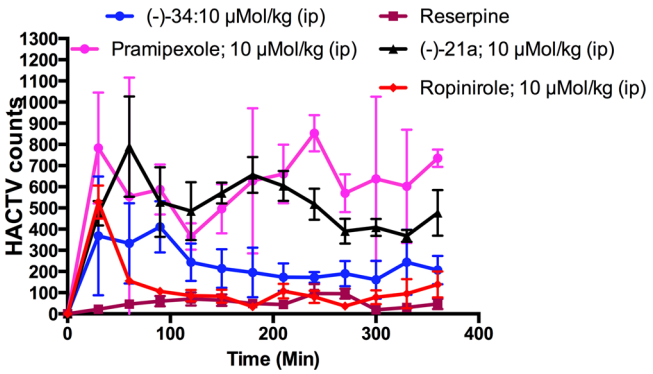

Figure 3. Effects of different drugs upon reserpine (5.0 mg/kg, sc, 18 h pretreatment) induced hypolocomotion in rats. Each point represents the mean ± SEM for three rats. Horizontal activity was measured as described in Experimental Section. Representation of horizontal locomotor activity is at discrete 30 min intervals after the administration of (−)-**21a** (10 μMol/kg), (−)-**34** (10 μMol/kg), (−)-Pramipexole (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 ($F(4, 60) = 31.62$ ($P < 0.0001$)): (****) $P < 0.001$ ((−)-**21a**), (**) $P < 0.05$ ((−)-**34**) or (****) $P < 0.001$ Pramipexole compared to reserpine control (Dunnett's multiple comparison test after one-way ANOVA).

model is likely to be mediated by postsynaptic D_2/D_3 receptor activation by (−)-**21a**. When the reserpinized rats were administered with partial agonist compound (−)-**34** at similar dosage, locomotor activity was enhanced compared to control but was less than that observed with (−)-**21a**. While being a partial agonist, significant activation of locomotion by (−)-**34** is impressive, indicating functional implication of this compound in an envirommment lacking little or no endogenous agonist. Such property will have a significant importance in PD therapy. Thus, the results suggest that these compounds behave like potent agonists, which cross the blood−brain barrier effectively and provide a sustained effect in the PD animal model.

Neuroprotection Against 6-OHDA-Induced Toxicity. To investigate the multifunctional property of the target molecules, we carried out in vitro antioxidant assay using 1,1-diphenyl-2-picrylhydrazyl (DPPH) (see the Supporting Information for details). As shown in Figure S1 in the Supporting Information, the target compound (−)-**21a** inhibited DPPH radical activity dose-dependently with an EC_{50} value of 21.72 μM. On the basis of the results of the in vivo activity as well as antioxidant assay, we next embarked on exploring the neuroprotective effect of (−)-**21a** in dopaminergic rat adrenal pheochromocytoma PC12 cells against 6-OHDA-induced cytotoxicity. 6-OHDA is a widely used toxin that mimics the generation of oxidative stress observed in PD and it induces neurotoxicity via its autooxidation and subsequent hydrogen peroxide generation.[60,61] Treatment of PC12 cells with 6-OHDA for 24 h resulted in a significant dose-dependent neurotoxicity and the cell viability was significantly decreased to ∼50% in cells exposed to 75 μM of 6-OHDA, and this concentration was used in subsequent in vitro experiments (Figure 4a). In contrast, cells treated with increasing concentrations of (−)-**21a** alone (0.01−30 μM) showed no cell loss at all compared to untreated controls and induced some cell proliferation instead at higher concentrations (Figure 4b), indicating the nontoxic profile of the compound at the doses tested. The potential neuroprotective effect of (−)-**21a** on 6-OHDA-induced toxicity was evaluated following pretreatment with the drug. Thus, when the cells were pretreated with (−)-**21a** for 24 h followed by exposure to 6-OHDA treatment for 24 h, the compound dose-dependently protected the cells from the neurotoxic insult and the greatest protective effect was obtained at concentration of 20 μM, which increased the cell survival by 30% compared to 6-OHDA (75 μM) treated alone (Figure 4c). These data strongly suggest the neuroprotective effect of (−)-**21a** on PC12 cell loss induced by 6-OHDA.

CONCLUSION

In binding and functional assays, the lead compound (−)-**21a** exhibited very high affinity and full agonist activity at both D_2 and D_3 receptors. It has also displayed efficient free radical quenching property, indicating its potent antioxidant activity. An interesting finding worth mentioning here that none of the methylene unit containing compounds showed any appreciable antioxidant property (Figures S1 and S2 in the Supporting Information). In a PD animal model, (−)-**21a** was highly efficacious in augmenting locomotor activity with a long duration of action, indicating its

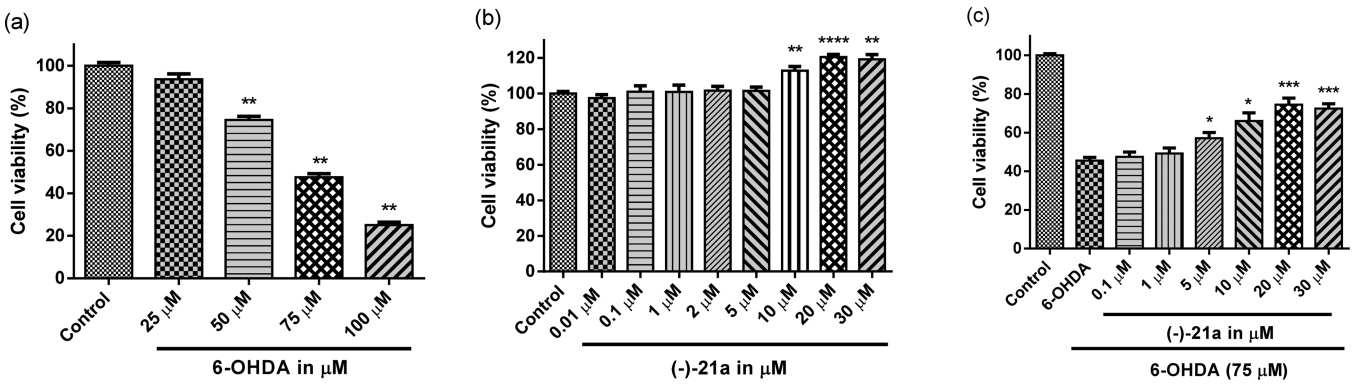

Figure 4. Dose-dependent effect on the viability of PC12 cells by the pretreatment of the cells with different concentrations of (−)-**21a** followed by treatment with 75 μM 6-OHDA. (a) PC12 cells were treated with different concentrations of 6-OHDA (25−100 μM). (b) Dose-dependent effect of (−)-**21a** on cell viability. (c) PC12 cells were pretreated with varying concentrations of (−)-**21a** for 24 h and then the media was replaced with fresh culture media followed by treatment with 75 μM 6-OHDA for another 24 h. The values shown are the mean ± SD of at least three independent experiments performed in four to six replicates. One way ANOVA analysis $F(4, 30) = 313.6$, $p < 0.0001$ for **4a**, $F(3, 24) = 18.23$, $p < 0.0001$ for **4b**, and $F(3, 18) = 118.7$, $p < 0.0001$ for **4c**. ANOVA was followed by Tukey's multiple comparison post hoc test (****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$ compared to control).

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potential as an anti-PD drug. The partial agonist compound (−)-**34** was also able to elevate locomotion significantly, implying its application in PD therapy. The data presented here also shows that (−)-**21a** is neuroprotective in an in vitro model of dopaminergic PC12 cells treated with the neurotoxin 6-OHDA. This report, therefore, underpins the notion that a multifunctional drug like (−)-**21a** has the potential not only to ameliorate motor dysfunction in PD patients but also to modify disease progression by protecting DA neurons from neurotoxic insults in addition to restoring their function. Further mechanistic studies to ascertain the disease-modifying effects of the compound are currently underway and will be published in due course.

EXPERIMENTAL SECTION

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

Procedure A: *tert*-Butyl 3-((4-(2-((2-Amino-4,5,6,7-tetrahydrobenzo[*d*]thiazol-6-yl)(propyl)amino)ethyl)-piperazin-1-yl)methyl)-5-methoxy-1*H*-indole-1-carboxylate (4a). Into a stirring solution of N^6-(2-(piperazin-1-yl)ethyl)-N^6-propyl-4,5,6,7-tetrahydrobenzo[*d*]thiazole-2,6-diamine (0.1 g, 0.30 mmol) in CH_2Cl_2 (5 mL) was added *tert*-butyl 3-formyl-5-methoxy-1*H*-indole-1-carboxylate (**3a**) (0.09 g, 0.32 mmol). After the mixture was stirred for 1.5 h, $NaBH(OAc)_3$ (0.13 g, 0.61 mmol) was added portionwise and the mixture was stirred for 48 h at room temperature. The reaction mixture was quenched with a saturated solution of $NaHCO_3$ at 0 °C and extracted with dicholoromethane (3 × 20 mL). The combined organic layer was dried over Na_2SO_4, and the solvent was removed under reduced pressure. Crude product was purified by column chromatography (EtOAc/MeOH, 20:1) to give compound **4a** (0.10 g, 60%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.99 (s, 1H), 7.45 (s, 1H), 7.16 (d, J = 2.4 Hz, 1H), 6.89 (dd, J_1 = 8.8 Hz, J_2 = 2.4 Hz, 1H), 5.21 (bs, −NH_2, 2H), 3.84 (s, 3H), 3.60 (s, 2H), 3.01−2.97 (m, 1H), 2.69−2.41 (m, 18H), 1.99−1.96 (m, 1H), 1.67−1.65 (m, 1H), 1.63 (s, 9H), 1.48−1.43 (m, 2H), 0.87 (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)methyl)-5-bromo-1H-indole-1-carboxylate (**4b**).* *tert*-Butyl 5-bromo-3-formyl-1*H*-indole-1-carboxylate (**3b**) (0.11 g, 0.31 mmol) was reacted with N^6-(2-(piperazin-1-yl)ethyl)-N^6-propyl-4,5,6,7-tetrahydrobenzo[*d*]thiazole-2,6-diamine (0.1 g, 0.3 mmol) and $NaBH(OAc)_3$ (0.13 g, 0.61 mmol) in CH_2Cl_2 (5 mL) according to procedure A. Crude product was purified by column chromatography (EtOAc/MeOH, 20:1) to give compound **4b** (0.097 g, 50%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.97 (s, 1H), 7.83 (d, J = 1.8 Hz, 1H), 7.45 (s, 1H), 7.37 (dd, J_1 = 8.4 Hz, J_2 = 1.8 Hz, 1H), 4.71 (bs, −NH_2, 2H), 3.58 (s, 2H), 3.0 (m, 1H), 2.71−2.39 (m, 17H), 1.96−1.94 (m, 1H), 1.74−1.65 (m, 1H), 1.63 (s, 9H), 0.87 (t, J = 7.2 Hz, 3H).

Procedure B: N^6-(2-(4-((5-Methoxy-1*H*-indol-3-yl)methyl)-piperazin-1-yl)ethyl)-N^6-propyl-4,5,6,7-tetrahydrobenzo[*d*]-thiazole-2,6-diamine (5a) (D-586). Into a stirred solution of **4a** (0.03 g, 0.05 mmol) in CH_2Cl_2 (5 mL) at 0 °C, trifluoroacetic acid (3 mL) was added slowly and the reaction mixture was stirred for another 2 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 **5a** (0.042 g, 90%). ^{1}H NMR (600 MHz, CD_3OD): δ 7.46 (s, 1H), 7.32 (d, J = 9 Hz, 1H), 7.20 (d, J = 2.4 Hz, 1H), 6.84 (dd, J_1 = 8.4 Hz, J_2 = 2.4 Hz, 1H), 4.52 (s, 2H), 3.83 (s, 3H), 3.51−3.49 (m, 3H), 3.41−3.31 (m, 2H), 3.26−3.15 (m, 8H), 3.04−3.01 (m, 1H), 2.88−2.65 (m, 7H), 2.49 (q, J = 12 Hz, 2H), 2.32−2.31 (m, 1H), 1.80−1.74 (m, 2H), 0.97 (t, J = 7.8 Hz, 3H). HRMS m/z $[M + H]^+$: calcd for $C_{26}H_{38}N_6OSH$, 483.2906; found, 483.2907; Anal. Calcd for $C_{26}H_{38}N_6OS{\cdot}3CF_3COOH{\cdot}CH_2Cl_2$: C, 43.57; H, 4.76; N, 9.24. Found: C, 44.39; H, 5.05; N, 8.83.

*N^6-(2-(4-((5-Bromo-1H-indol-3-yl)methyl)piperazin-1-yl)ethyl)-N^6-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine (**5b**) (D-589).* Compound **4b** (0.02 g, 0.03 mmol) was treated with trifluoroacetic acid (3 mL) in CH_2Cl_2 (5 mL) according to procedure B to furnish **5b** (0.027 g, 91%). ^{1}H NMR (600 MHz, CD_3OD): δ 7.91 (s, 1H), 7.55 (s, 1H), 7.38 (d, J = 8.4 Hz, 1H), 7.29 (dd, J_1 = 7.8 Hz, J_2 = 1.8 Hz, 1H), 4.52 (s, 2H), 3.88 (s, 1H), 3.49−3.23 (m, 11H), 3.04−3.02 (m, 1H), 2.88−2.31 (m, 8H), 1.80−1.76 (m, 2H), 0.99 (t, J = 7.2 Hz, 3H); Anal. Calcd for $C_{25}H_{35}BrN_6S{\cdot}3CF_3COOH{\cdot}CH_2Cl_2$: C, 40.10; H, 4.21; N, 8.77. Found: C, 40.79; H, 4.15; N, 8.65.

*1-(2-((tert-Butyldimethylsilyl)oxy)ethyl)piperazine (**6**).* A suspension of piperazine (10.0 g, 116.09 mmol), potassium carbonate (48.13 g, 348.27 mmol), and (2-bromoethoxy)(*tert*-butyl)dimethylsilane (11.1 g, 46.4 mmol) in acetonitrile (100 mL) was refluxed under N_2 for 15 h. The reaction mixture was filtered off, and the filtrate was evaporated under reduced pressure. The residue was then diluted with ether, washed with water, dried over sodium sulfate, filtered, and concentrated to afford the product **6** as a white colorless liquid (10.7 g, 95%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.73 (t, J = 6.0 Hz, 2H), 2.86 (t, J = 4.8 Hz, 4H), 2.50−2.46 (m, 6H), 0.86 (s, 9H), 0.03 (s, 6H).

Procedure C: 5-Fluoro-1*H*-indole-3-carbaldehyde (8a). Phosphorus oxychloride (0.25 mL, 2.66 mmol) was added dropwise to a stirred mixture of *N*,*N*-dimethylformamide (1.03 mL, 13.32 mmol) and indole **7a** (0.3 g, 2.2 mmol) at 0 °C. The resulting mixture was stirred at 0 °C for 0.5 h then at 40 °C for 1 h. Ice was added, followed by a solution of sodium hydroxide (2 M), and the mixture was heated under reflux for 5 min. On cooling, the solution was extracted with ethyl acetate (3 × 20 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, and concentrated. Column chromatography with hexane:ethyl acetate (1:1) gave a white solid (0.30 g, 85%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.86 (s, 1H), 7.86 (dd, J = 6.6, 2.4 Hz, 1H), 7.81 (s, 1H), 7.31 (q, J = 4.2 Hz, 1H), 6.97 (td, J = 9.0, 2.4 Hz, 1H).

*4-Methoxy-1H-indole-3-carbaldehyde (**8b**).* Indole **7b** (0.3 g, 2.04 mmol) was reacted with phosphorus oxychloride (0.23 mL, 2.45 mmol) in DMF (0.95 mL, 12.23 mmol) according to procedure C. The crude material was pure enough for use in the next step (0.27 g, 76%).

*4-Chloro-1H-indole-3-carbaldehyde (**8c**).* Indole **7c** (0.6 g, 3.96 mmol) was reacted with phosphorus oxychloride (0.44 mL, 4.75 mmol) in DMF (1.84 mL, 23.75 mmol) according to procedure C. The crude material was pure enough for use in the next step (0.61 g, 86%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 10.72 (s, 1H), 8.05 (s, 1H), 7.39 (d, J = 8.4 Hz, 1H), 7.28 (d, J = 8.4 Hz, 1H), 7.17 (t, J = 7.8 Hz, 1H).

Procedure D: *tert*-Butyl 5-Fluoro-3-formyl-1*H*-indole-1-carboxylazte (9a). Into a stirring solution of amine **8a** (0.3 g, 1.84 mmol) in THF (7 mL) were added $(Boc)_2O$ (0.44 g, 2.02 mmol) and DMAP (0.25 g, 2.02 mmol) 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 column chromatography over silica gel using hexane:ethyl acetate (5:1) as solvent to give compound **9a** (0.485 g, 100%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 10.05 (s, 1H), 8.24 (s, 1H), 8.09 (q, J = 4.2 Hz, 1H), 7.96 (dd, J = 6.0, 2.4 Hz, 1H), 7.13 (td, J = 9.0, 2.4 Hz, 1H), 1.69 (s, 9H).

*tert-Butyl 3-Formyl-4-methoxy-1H-indole-1-carboxylate (**9b**).* Amine **8b** (0.25 g, 1.43 mmol) was reacted with $(Boc)_2O$ (0.34 g,

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1.57 mmol) and DMAP (0.19 g, 1.57 mmol) in THF (5 mL) according to procedure D. The crude material was purified by silica gel column chromatography (hexane:EtOAc = 5:1) to yield compound **9b** (0.38 g, 95%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 10.54 (s, 1H), 8.22 (s, 1H), 7.83 (d, J = 8.4 Hz, 1H), 7.31 (t, J = 8.4 Hz, 1H), 6.80 (d, J = 8.4 Hz, 1H), 3.99 (s, 3H), 1.66 (s, 9H).

*tert-Butyl 4-Chloro-3-formyl-1H-indole-1-carboxylate (**9c**).* Amine **8c** (0.6 g, 3.34 mmol) was reacted with $(Boc)_2O$ (0.8 g, 3.68 mmol) and DMAP (0.45 g, 3.68 mmol) in THF (15 mL) according to procedure D. The crude material was purified by silica gel column chromatography (hexane:EtOAc = 5:1) to yield compound **9c** (0.86 g, 92%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 10.80 (s, 1H), 8.38 (s, 1H), 8.22 (d, J = 7.8 Hz, 1H), 7.38 (d, J = 7.8 Hz, 1H), 7.32 (t, J = 7.8 Hz, 1H), 1.69 (s, 9H).

*tert-Butyl 3-((4-(2-((tert-Butyldimethylsilyl)oxy)ethyl)piperazin-1-yl)methyl)-5-fluoro-1H-indole-1-carboxylate (**10a**).* Aldehyde **9a** (0.475 g, 1.8 mmol) was reacted with amine **6** (0.44 g, 1.8 mmol) and $NaBH(OAc)_3$ (0.69 g, 3.25 mmol) in CH_2Cl_2 (15 mL) according to procedure A. The crude product was purified by silica gel column chromatography (hexane:EtOAc = 1:1) to yield compound **10a** (0.83 g, 94%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.04 (bs, 1H), 7.50 (s, 1H), 7.39 (dd, J = 6.6, 2.4 Hz, 1H), 7.01 (td, J = 9.0, 2.4 Hz, 1H), 3.73 (t, J = 6.6 Hz, 2H), 3.57 (s, 2H), 2.52 (bt, J = 6.6 Hz, 10H), 1.64 (s, 9H), 0.87 (s, 9H), 0.03 (s, 6H).

*tert-Butyl 3-((4-(2-((tert-Butyldimethylsilyl)oxy)ethyl)piperazin-1-yl)methyl)-4-methoxy-1H-indole-1-carboxylate (**10b**).* Aldehyde **9b** (0.29 g, 1.05 mmol) was reacted with amine **6** (0.26 g, 1.05 mmol) and $NaBH(OAc)_3$ (0.4 g, 1.9 mmol) in CH_2Cl_2 (10 mL) according to procedure A. The crude material was purified by silica gel column chromatography (hexane:EtOAc = 2:3) to yield compound **10b** (0.435 g, 82%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.74 (bs, 1H), 7.38 (s, 1H), 7.18 (t, J = 8.4 Hz, 1H), 6.63 (d, J = 7.8 Hz, 1H), 3.88 (s, 3H), 3.85 (s, 2H), 3.74 (t, J = 6.6 Hz, 2H), 2.64−2.50 (m, 10H), 1.64 (s, 9H), 0.87 (s, 9H), 0.04 (s, 6H).

*tert-Butyl 3-((4-(2-((tert-Butyldimethylsilyl)oxy)ethyl)piperazin-1-yl)methyl)-4-chloro-1H-indole-1-carboxylate (**10c**).* Aldehyde **9c** (0.41 g, 1.47 mmol) was reacted with amine **6** (0.36 g, 1.47 mmol) and $NaBH(OAc)_3$ (0.63 g, 2.94 mmol) in CH_2Cl_2 (12 mL) according to procedure A. The crude material was purified by silica gel column chromatography (hexane:EtOAc = 3:2) to yield compound **10c** (0.54 g, 72%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.09 (bs, 1H), 7.51 (s, 1H), 7.18−7.17 (m, 2H), 3.85 (s, 2H), 3.76 (t, J = 6.6 Hz, 2H), 2.66−2.53 (m, 10H), 1.66 (s, 9H), 0.89 (s, 9H), 0.06 (s, 6H).

*tert-Butyl 3-((4-(2-((tert-Butyldimethylsilyl)oxy)ethyl)piperazin-1-yl)methyl)-4-fluoro-1H-indole-1-carboxylate (**10d**).* Aldehyde **9d**[62] (0.4 g, 1.52 mmol) was reacted with amine **6** (0.41 g, 1.67 mmol) and $NaBH(OAc)_3$ (0.64 g, 3.02 mmol) in CH_2Cl_2 (10 mL) according to procedure A. The crude material was purified by silica gel column chromatography (hexane:EtOAc = 20:1) to yield compound **10d** (0.55 g, 73%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.90 (s, 1H), 7.43 (s, 1H), 7.21−7.19 (m, 1H), 6.87 (dd, J_1 = 7.2 Hz, J_2 = 5.2 Hz, 1H), 3.74−3.72 (m, 4H), 2.57 (bs, 8H), 2.51 (t, J = 7.8 Hz, 2H), 1.69 (s, 9H), 0.86 (s, 9H), 0.03 (s, 6H).

Procedure E: *tert*-Butyl 5-Fluoro-3-((4-(2-hydroxyethyl)-piperazin-1-yl)methyl)-1*H*-indole-1-carboxylate (11a). Into a stirring solution of compound **10a** (0.69 g, 1.4 mmol) in THF (15 mL) was added *n*-tetrabutylammonium fluoride (2.2 mL, 2.1 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 (20 mL) and washed with a saturated solution of $NaHCO_3$. The water layer was extracted with CH_2Cl_2 (3 × 20 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 (CH_2Cl_2:MeOH = 9:1) to yield compound **11a** (0.495 g, 94%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.06 (bs, 1H), 7.52 (s, 1H), 7.39 (dd, J = 6.6, 2.4 Hz, 1H), 7.03 (td, J = 9.0, 2.4 Hz, 1H), 3.61 (t, J = 5.4 Hz, 2H), 3.60 (s, 2H), 2.56 (bt, J = 5.4 Hz, 10H), 1.66 (s, 9H).

*tert-Butyl 3-((4-(2-Hydroxyethyl)piperazin-1-yl)methyl)-4-methoxy-1H-indole-1-carboxylate (**11b**).* Compound **10b** (0.335 g, 0.67 mmol) was reacted with *n*-tetrabutylammonium fluoride (1.0 mL, 1.01 mmol, 1.0 M solution in THF) in THF (10 mL) according to procedure E. The crude product was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 9:1) to give compound **11b** (0.195 g, 75%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.76 (bs, 1H), 7.41 (s, 1H), 7.21 (t, J = 8.4 Hz, 1H), 6.66 (d, J = 7.8 Hz, 1H), 3.91 (s, 3H), 3.90 (s, 2H), 3.61 (t, J = 5.4 Hz, 2H), 2.70−2.56 (m, 10H), 1.66 (s, 9H).

*tert-Butyl 4-Chloro-3-((4-(2-hydroxyethyl)piperazin-1-yl)methyl)-1H-indole-1-carboxylate (**11c**).* Compound **10c** (0.52 g, 1.02 mmol) was reacted with *n*-tetrabutylammonium fluoride (2.1 mL, 2.05 mmol, 1.0 M solution in THF) in THF (8 mL) according to procedure E. The crude product was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 9:1) to give compound **11c** (0.38 g, 93%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.09 (bs, 1H), 7.52 (s, 1H), 7.20−7.18 (m, 2H), 3.86 (s, 2H), 3.63 (t, J = 5.4 Hz, 2H), 2.70−2.55 (m, 10H), 1.67 (s, 9H).

*tert-Butyl 4-Fluoro-3-((4-(2-hydroxyethyl)piperazin-1-yl)methyl)-1H-indole-1-carboxylate (**11d**).* Compound **10d** (0.72 g, 1.46 mmol) was treated with *n*-tetrabutylammonium fluoride (2.1 mL, 2.10 mmol, 1.0 M solution in THF) in THF (15 mL) according to procedure E. The crude product was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 19:1) to give compound **11d** (0.49 g, 89%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.92 (d, J = 8.0 Hz, 1H), 7.45 (s, 1H), 7.24−7.18 (m, 1H), 6.91 (dd, J_1 = 10.8 Hz, J_2 = 8 Hz, 1H), 3.76 (s, 2H), 3.59 (t, J = 4.8 Hz, 2H), 2.56−2.53 (m, 10H), 1.66 (s, 9H).

Procedure F. *tert*-Butyl 5-fluoro-3-((4-(2-oxoethyl)piperazin-1-yl)methyl)-1*H*-indole-1-carboxylate (12a). Into a stirring solution of compound **11a** (0.2 g, 0.53 mmol) in CH_2Cl_2 (4.5 mL) and DMSO (2.25 mL) was added Et_3N (0.52 mL, 3.71 mmol) at 0 °C. The reaction mixture was stirred for 5 min, followed by addition of SO_3·py complex (0.422 g, 2.65 mmol) at 0 °C. Ice bath was removed, and the reaction mixture was stirred at room temperature for 1.5 h. The reaction mixture was quenched by addition of water and extracted with CH_2Cl_2 (3 × 25 mL). The combined organic layer was dried using Na_2SO_4, and the solvent was removed under reduced pressure. Column chromatography with EtOAc:MeOH (19:1) afforded compound **12a** (0.17 g, 83%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.50 (s, 1H), 7.86 (bs, 1H), 7.33 (s, 1H), 7.21−7.19 (m, 1H), 6.84−6.81 (m, 1H), 4.46−4.42 (m, 2H), 3.47−3.41 (m, 2H), 3.22−3.16 (m, 2H), 3.00−2.99 (m, 1H), 2.55−2.31 (m, 5H), 1.48 (s, 9H).

*tert-Butyl 4-Methoxy-3-((4-(2-oxoethyl)piperazin-1-yl)methyl)-1H-indole-1-carboxylate (**12b**).* Alcohol **11b** (0.19 g, 0.49 mmol) was oxidized using SO_3·py complex (0.39 g, 2.45 mmol), DMSO (2 mL), and Et_3N (0.48 mL, 3.41 mmol) in CH_2Cl_2 (4 mL) according to procedure F. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 19:1) to give aldehyde **12b** (0.16 g, 82%). The purified aldehyde was used immediately for next step. ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.71 (s, 1H), 7.75 (bs, 1H), 7.38 (s, 1H), 7.20 (t, J = 8.4 Hz, 1H), 6.66 (d, J = 7.8 Hz, 1H), 3.90 (s, 3H), 3.87 (s, 2H), 3.38 (s, 2H), 2.62 (s, 8H), 1.66 (s, 9H).

*tert-Butyl 4-Chloro-3-((4-(2-oxoethyl)piperazin-1-yl)methyl)-1H-indole-1-carboxylate (**12c**).* Alcohol **11c** (0.35 g, 0.89 mmol) was oxidized using SO_3·py complex (0.707 g, 4.44 mmol), DMSO (3.5 mL), and Et_3N (0.87 mL, 6.22 mmol) in CH_2Cl_2 (7 mL) according to procedure F. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 19:1) to give aldehyde **12c** (0.29 g, 83%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.71 (s, 1H), 8.09 (bs, 1H), 7.51 (s, 1H), 7.19−7.17 (m, 2H), 4.66−4.61 (m, 2H), 3.86−3.84 (m, 2H), 3.43−3.38 (m, 2H), 3.19−3.18 (m, 1H), 2.63−2.61 (m, 5H), 1.67 (s, 9H).

*tert-Butyl 4-Fluoro-3-((4-(2-oxoethyl)piperazin-1-yl)methyl)-1H-indole-1-carboxylate (**12d**).* Alcohol **11d** (0.25 g, 0.66 mmol) was oxidized using SO_3·py complex (0.525 g, 3.30 mmol), DMSO (3 mL), and Et_3N (0.64 mL, 4.62 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 **12d** (0.18 g, 72%). The purified aldehyde was used immediately for next step. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.71 (s, 1H), 7.91 (d, J = 8.0 Hz, 1H), 7.42 (s, 1H), 7.22−7.17 (m, 1H), 6.93 (dd, J_1 = 10.8 Hz, J_2 = 8 Hz, 1H), 3.75 (s, 2H), 3.39 (s, 2H), 2.62−2.57 (m, 8H), 1.66 (s, 9H).

*tert-Butyl 3-((4-(2-((2-Amino-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)(propyl)amino)-ethyl)piperazin-1-yl)methyl)-5-fluoro-1H-indole-1-carboxylate (**13a**).* Compound **12a** (0.16 g, 0.43 mmol) was

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reacted with (±)-pramipexole (0.09 g, 0.43 mmol) and $NaBH(OAc)_3$ (0.16 g, 0.77 mmol) in CH_2Cl_2 (10 mL) according to procedure A. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 4:1) to afford compound **13a** (0.14 g, 56%). ^1H NMR (400 MHz, $CDCl_3$): δ 8.05 (bs, 1H), 7.51 (s, 1H), 7.39 (dd, J = 6.4, 2.4 Hz, 1H), 7.02 (td, J = 9.0, 2.4 Hz, 1H), 5.17 (bs, 2H), 3.59 (s, 2H), 3.04−2.98 (m, 1H), 2.72−2.41 (m, 18H), 1.97−1.94 (m, 1H), 1.71−1.61 (m, 10H), 1.49−1.40 (m, 2H), 0.87 (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)methyl)-4-methoxy-1H-indole-1-carboxylate (**13b**).* Compound **12b** (0.15 g, 0.39 mmol) was reacted with (±)-pramipexole (0.07 g, 0.35 mmol) and $NaBH(OAc)_3$ (0.16 g, 0.78 mmol) in CH_2Cl_2 (10 mL) according to procedure A. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 4:1) to afford compound **13b** (0.08 g, 39%). ^1H NMR (600 MHz, $CDCl_3$): δ 7.76 (bs, 1H), 7.42 (bs, 1H), 7.20 (t, J = 8.4 Hz, 1H), 6.65 (d, J = 7.8 Hz, 1H), 4.85 (bs, 2H), 3.90 (s, 3H), 3.89 (s, 2H), 3.03−2.98 (m, 1H), 2.75−2.42 (m, 18H), 1.97−1.96 (m, 1H), 1.70−1.62 (m, 10H), 1.48−1.42 (m, 2H), 0.87 (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)methyl)-4-chloro-1H-indole-1-carboxylate (**13c**).* Compound **12c** (0.2 g, 0.51 mmol) was reacted with (±)-pramipexole (0.1 g, 0.46 mmol) and $NaBH(OAc)_3$ (0.22 g, 1.02 mmol) in CH_2Cl_2 (10 mL) according to procedure A. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 5:1) to afford compound **13c** (0.12 g, 43%). ^1H NMR (600 MHz, $CDCl_3$): δ 8.09 (bs, 1H), 7.51 (s, 1H), 7.20−7.16 (m, 2H), 5.05 (bs, 2H), 3.86 (s, 2H), 3.03−2.98 (m, 1H), 2.71−2.42 (m, 18H), 1.98−1.96 (m, 1H), 1.71−1.66 (m, 10H), 1.48−1.42 (m, 2H), 0.87 (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)methyl)-4-fluoro-1H-indole-1-carboxylate (**13d**).* Compound **12d** (0.11 g, 0.26 mmol) was reacted with (±)-pramipexole (0.06 g, 0.26 mmol) and $NaBH(OAc)_3$ (0.13 g, 0.62 mmol) in CH_2Cl_2 (5 mL) according to procedure A. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 20:1) to afford compound **13d** (0.09 g, 60%). ^1H NMR (600 MHz, $CDCl_3$): δ 7.90 (s, 1H), 7.43 (s, 1H), 7.21−7.18 (m, 1H), 6.87 (dd, J_1 = 10.8 Hz, J_2 = 8.4 Hz, 1H), 4.67 (bs, $-NH_2$, 2H), 3.75 (s, 2H), 3.02 (bs, 2H), 2.65−2.45 (m, 18H), 1.96−1.94 (m, 1H), 1.65 (s, 9H), 1.43−1.41 (m, 2H), 0.85 (t, J = 7.2 Hz, 3H).

*N^6-(2-(4-((5-Fluoro-1H-indol-3-yl)methyl)piperazin-1-yl)ethyl)-N^6-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine (**14a**) (D-587).* Compound **13a** (0.12 g, 0.21 mmol) was treated with trifluoroacetic acid (2 mL) in CH_2Cl_2 (2 mL) according to procedure B to furnish the TFA salt of **14a** (0.18 g, 90%). ^1H NMR (400 MHz, CD_3OD): δ 7.59 (s, 1H), 7.43 (td, J = 8.8, 2.4 Hz, 2H), 6.98 (td, J = 8.8, 2.4 Hz, 1H), 4.53 (s, 2H), 3.90−3.88 (m, 1H), 3.51−3.34 (m, 4H), 3.28−3.03 (m, 7H), 2.92−2.72 (m, 5H), 2.52−2.51 (m, 2H), 2.36−2.33 (m, 1H), 2.11−2.03 (m, 1H), 1.83−1.77 (m, 2H), 1.00 (t, J = 7.2 Hz, 3H). HRMS m/z $[M + H]^+$: calcd for $C_{25}H_{35}FN_6SH$, 471.2706; found, 471.2722; Anal. Calcd for $C_{25}H_{35}FN_6S{\cdot}3CF_3COOH{\cdot}CH_2Cl_2$: C, 42.82; H, 4.49; N, 9.36. Found: C, 43.37; H, 4.74; N, 9.33.

*N^6-(2-(4-((4-Methoxy-1H-indol-3-yl)methyl)piperazin-1-yl)ethyl)-N^6-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine (**14b**) (D-623).* Compound **13b** (0.02 g, 0.034 mmol) was treated with trifluoroacetic acid (1.5 mL) in CH_2Cl_2 (1.5 mL) according to procedure B to furnish the TFA salt of **14b** (0.03 g, 91%). ^1H NMR (600 MHz, CD_3OD): δ 7.30 (d, J = 4.8 Hz, 1H), 7.05−7.02 (m, 1H), 6.98−6.96 (m, 1H), 6.55−6.53 (m, 1H), 4.59 (s, 2H), 3.89 (s, 3H), 3.88 (s, 2H), 3.45−3.26 (m, 5H), 3.18−2.94 (m, 6H), 2.82−2.61 (m, 6H), 2.49−2.45 (m, 2H), 2.26−2.25 (m, 1H), 2.01−1.95 (m, 1H), 1.74−1.66 (m, 2H), 0.92 (t, J = 7.2 Hz, 3H); Anal. Calcd for $C_{26}H_{38}N_6OS{\cdot}4CF_3COOH{\cdot}H_2O$: C, 42.68; H, 4.64; N, 8.78. Found: C, 42.69; H, 4.73; N, 8.45.

*N^6-(2-(4-((4-Chloro-1H-indol-3-yl)methyl)piperazin-1-yl)ethyl)-N^6-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine (**14c**) (D-624).* Compound **13c** (0.08 g, 0.13 mmol) was treated with trifluoroacetic acid (2 mL) in CH_2Cl_2 (2 mL) according to procedure B to furnish the TFA salt of **14c** (0.11 g, 91%). ^1H NMR (600 MHz, CD_3OD): δ 7.55 (bs, 1H), 7.34 (d, J = 7.8 Hz, 1H), 7.08−7.04 (m, 2H), 5.11 (bs, 2H), 4.75 (s, 2H), 3.84−3.81 (m, 1H), 3.49−3.28 (m, 6H), 3.19−2.96 (m, 6H), 2.82−2.60 (m, 6H), 2.29−2.27 (m, 1H), 2.02−1.95 (m, 1H), 1.76−1.70 (m, 2H), 0.93 (t, J = 7.2 Hz, 3H); Anal. Calcd for $C_{25}H_{35}ClN_6S{\cdot}3CF_3COOH{\cdot}H_2O$: C, 43.95; H, 4.76; N, 9.92. Found: C, 44.19; H, 4.75; N, 9.45.

*N^6-(2-(4-((4-Fluoro-1H-indol-3-yl)methyl)piperazin-1-yl)ethyl)-N^6-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine (**14d**) (D-590).* Compound **13d** (0.07 g, 0.12 mmol) was treated with trifluoroacetic acid (5 mL) in CH_2Cl_2 (5 mL) according to procedure B to furnish the TFA salt of **14d** (0.1 g, 90%). ^1H NMR (600 MHz, CD_3OD): δ 7.53 (s, 1H), 7.27 (d, J = 8.4 Hz, 1H), 7.14 (d, J = 4.8 Hz, 1H), 6.82 (dd, J_1 = 11.2 Hz, J_2 = 7.6 Hz, 1H), 4.60 (s, 2H), 3.51−3.01 (m, 10H), 3.01−2.52 (m, 11H), 2.31−1.77 (m, 4H), 0.98 (t, J = 6.4 Hz, 3H); Anal. Calcd for $C_{25}H_{35}FN_6S{\cdot}3CF_3COOH{\cdot}CH_2Cl_2$: C, 42.82; H, 4.49; N, 9.36. Found: C, 43.11; H, 4.74; N, 9.05.

*tert-Butyl 6-Bromo-1H-indole-1-carboxylate (**16a**).* 6-Bromo-1*H*-indole **15a** (2.0 g, 10.2 mmol) was reacted with $(Boc)_2O$ (2.45 g, 11.22 mmol) and DMAP (1.37 g, 11.22 mmol) in THF (20 mL) according to procedure D. The crude material was purified by silica gel column chromatography (hexane:EtOAc = 19:1) to afford compound **16a** (2.98 g, 99%). ^1H NMR (600 MHz, $CDCl_3$): δ 8.36 (bs, 1H), 7.55 (s, 1H), 7.40 (d, J = 8.4 Hz, 1H), 7.33 (dd, J = 7.2, 1.2 Hz, 1H), 6.52 (d, J = 4.2 Hz, 1H), 1.67 (s, 9H).

*tert-Butyl 4-Bromo-1H-indole-1-carboxylate (**16b**).* 4-Bromo-1*H*-indole **15b** (1.0 g, 5.1 mmol) was reacted with $(Boc)_2O$ (1.22 g, 5.61 mmol) and DMAP (0.69 g, 5.61 mmol) in THF (10 mL) according to procedure D. The crude material was used in the next step without any further purification (1.45 g, 96%).

Procedure G. *tert*-Butyl 6-(4-(2-((*tert*-Butyldimethylsilyl)oxy)-ethyl)piperazin-1-yl)-1*H*-indole-1-carboxylate (17a). To a mixture of compound **16a** (0.75 g, 2.53 mmol), **6** (1.24 g, 5.07 mmol), BINAP (0.16 g, 0.25 mmol), and Cs_2CO_3 (2.48 g, 7.60 mmol), toluene (12 mL) was added under N_2 atmosphere. The reaction mixture was degassed by bubbling N_2 for 5 min, and then $Pd(OAc)_2$ (0.043 g, 0.19 mmol) was added quickly. The system was degassed again and refluxed for 15 h under inert condition. The reaction mixture was cooled to room temperature, filtered through a pad of Celite, washed with dichloromethane, and concentrated in vacuo. The crude material was purified by silica gel column chromatography (hexane:EtOAc = 7:3) to give compound **17a** as light-brown oil (0.72 g, 62%). ^1H NMR (600 MHz, $CDCl_3$): δ 7.77 (bs, 1H), 7.44 (s, 1H), 7.40 (d, J = 8.4 Hz, 1H), 6.93 (dd, J = 6.6, 2.4 Hz, 1H), 6.45 (d, J = 3.6 Hz, 1H), 3.81 (t, J = 6.6 Hz, 2H), 3.25 (t, J = 4.8 Hz, 4H), 2.72 (t, J = 4.8 Hz, 4H), 2.60 (t, J = 6.0 Hz, 2H), 1.65 (s, 9H), 0.91 (s, 9H), 0.08 (s, 6H).

*tert-Butyl 4-(4-(2-((tert-Butyldimethylsilyl)oxy)ethyl)piperazin-1-yl)-1H-indole-1-carboxylate (**17b**).* A mixture of **16b** (1.0 g, 3.38 mmol), **6** (1.24 g, 5.08 mmol), $Pd(OAc)_2$ (0.03 g, 0.16 mmol), BINAP (0.16 g, 0.25 mmol), and Cs_2CO_3 (3.3 g, 10.16 mmol) in toluene (12 mL) was heated at 110 °C for 15 h according to procedure G. The crude residue was dissolved in ethyl acetate, washed with water, and evaporated to afford compound **17b**, which was purified by column chromatography (hexane:EtOAc = 5:1) (1.19 g, 76%). ^1H NMR (600 MHz, $CDCl_3$): δ 7.76 (d, J = 7.2 Hz, 1H), 7.43 (d, J = 3.0 Hz, 1H), 7.17 (t, J = 7.8 Hz, 1H), 6.70 (d, J = 7.8 Hz, 1H), 6.54 (d, J = 3.6 Hz, 1H), 3.77 (t, J = 6.0 Hz, 2H), 3.15 (t, J = 3.6 Hz, 4H), 2.72 (t, J = 3.6 Hz, 4H), 2.59 (t, J = 6.6 Hz, 1H), 1.62 (s, 9H), 0.86 (s, 9H), 0.04 (s, 6H).

*tert-Butyl 6-(4-(2-Hydroxyethyl)piperazin-1-yl)-1H-indole-1-carboxylate (**18a**).* Compound **17a** (0.7 g, 1.52 mmol) was reacted with *n*-tetrabutylammonium fluoride (3.0 mL, 3.05 mmol, 1.0 M solution in THF) in THF (10 mL) according to procedure E. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 5:1) to give compound **18a** (0.44 g, 84%). ^1H NMR (600 MHz, $CDCl_3$): δ 7.78 (bs, 1H), 7.45 (s, 1H), 7.41 (dd, J = 5.4, 3.0 Hz, 1H), 6.93−6.91 (m, 1H), 6.45 (d, J = 3.6 Hz, 1H), 3.68 (t, J = 6.0 Hz, 2H), 3.26−3.25 (m, 4H), 2.73−2.72 (m, 4H), 2.64−2.60 (m, 2H), 1.66 (s, 9H).

*tert-Butyl 4-(4-(2-Hydroxyethyl)piperazin-1-yl)-1H-indole-1-carboxylate (**18b**).* Compound **17b** (1.1 g, 2.39 mmol) was reacted with *n*-tetrabutylammonium fluoride (4.8 mL, 4.78 mmol, 1.0 M solution in THF) in THF (15 mL) according to procedure E. The crude product

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was purified by silica gel column chromatography (EtOAc:MeOH = 9:1) to yield compound **18b** (0.71 g, 86%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.83 (d, J = 7.2 Hz, 1H), 7.55 (d, J = 3.0 Hz, 1H), 7.23 (t, J = 7.8 Hz, 1H), 6.74 (d, J = 7.8 Hz, 1H), 6.58 (d, J = 3.6 Hz, 1H), 3.69 (t, J = 5.4 Hz, 2H), 3.21 (s, 4H), 2.77 (s, 4H), 2.66 (t, J = 5.4 Hz, 2H), 1.66 (s, 9H).

Procedure H. *tert*-Butyl 7-Chloro-6-(4-(2-oxoethyl)piperazin-1-yl)-1*H*-indole-1-carboxylate (19a). Into a stirring solution of oxalyl chloride (0.22 mL, 2.55 mmol) in CH_2Cl_2 (8 mL) at −78 °C was added DMSO (0.33 mL, 4.63 mmol). The reaction mixture was stirred for 0.5 h, followed by addition of compound **18a** (0.4 g, 1.16 mmol, in 5 mL of CH_2Cl_2). The reaction mixture was stirred at the same temperature for 0.5 h, followed by addition of Et_3N (0.97 mL, 6.95 mmol), and stirring was continued for another 1.5 h while allowing the reaction mixture to reach room temperature. The reaction mixture was quenched by addition of a saturated solution of $NaHCO_3$ at 0 °C and extracted with CH_2Cl_2 (3 × 25 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, 3:2) to afford compound **19a** (0.21 g, 48%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.76 (s, 1H), 7.44 (d, J = 3.6 Hz, 1H), 7.41 (d, J = 8.4 Hz, 1H), 7.02 (d, J = 8.4 Hz, 1H), 6.49 (d, J = 3.6 Hz, 1H), 3.29−3.24 (m, 4H), 3.20 (m, 4H), 2.76 (m, 2H), 1.64 (s, 9H).

*tert-Butyl 5-Chloro-4-(4-(2-oxoethyl)piperazin-1-yl)-1H-indole-1-carboxylate (**19b**).* Alcohol **18b** (0.2 g, 0.58 mmol) was oxidized with oxalyl chloride (0.11 mL, 1.27 mmol), DMSO (0.16 mL, 2.32 mmol), and Et_3N (0.48 mL, 3.47 mmol) in CH_2Cl_2 (7 mL) using procedure H. The crude residue was purified by column chromatography (hexane/EtOAc, 3:2) to afford compound **19b** (0.07 g, 32%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.78 (s, 1H), 7.86 (d, J = 7.8 Hz, 1H), 7.55 (d, J = 3.0 Hz, 1H), 7.27 (d, J = 9.0 Hz, 1H), 6.77 (d, J = 4.2 Hz, 1H), 3.41 (s, 4H), 3.28 (s, 2H), 2.73 (s, 4H), 1.66 (s, 9H).

*(S)-tert-Butyl 6-(4-(2-((2-Amino-4,5,6,7-tetrahydrobenzo[d]-thiazol-6-yl)(propyl)amino)ethyl)piperazin-1-yl)-7-chloro-1H-indole-1-carboxylate (**20a**).* Compound **19a** (0.18 g, 0.46 mmol) was reacted with (−)-pramipexole (0.09 g, 0.43 mmol) and $NaBH(OAc)_3$ (0.2 g, 0.93 mmol) in CH_2Cl_2 (10 mL) according to procedure A. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 5:1) to afford compound **20a** (0.14 g, 56%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.43 (d, J = 3.6 Hz, 1H), 7.39 (d, J = 8.4 Hz, 1H), 7.01 (d, J = 8.4 Hz, 1H), 6.48 (d, J = 3.6 Hz, 1H), 5.13 (bs, 2H), 3.24−3.15 (m, 4H), 3.07−3.03 (m, 1H), 2.77−2.66 (m, 8H), 2.57−2.46 (m, 6H), 2.01−1.99 (m, 1H), 1.75−1.71 (m, 1H), 1.64 (s, 9H), 1.51−1.45 (m, 2H), 0.90 (t, J = 7.2 Hz, 3H). $[\alpha]_D^{25}$ = −35.3 (c = 1.0 in CH_3OH).

*(S)-tert-Butyl 4-(4-(2-((2-Amino-4,5,6,7-tetrahydrobenzo[d]-thiazol-6-yl)(propyl)amino)ethyl)piperazin-1-yl)-5-chloro-1H-indole-1-carboxylate (**20b**).* Compound **19b** (0.07 g, 0.18 mmol) was reacted with (−)-pramipexole (0.04 g, 0.18 mmol) and $NaBH(OAc)_3$ (0.08 g, 0.37 mmol) in CH_2Cl_2 (5 mL) according to procedure A. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 9:1) to afford compound **20b** (0.07 g, 64%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.84 (d, J = 7.2 Hz, 1H), 7.53 (d, J = 3.0 Hz, 1H), 7.26 (d, J = 8.4 Hz, 1H), 6.77 (d, J = 3.0 Hz, 1H), 5.36 (bs, 2H), 3.37 (s, 4H), 3.09−3.05 (m, 1H), 2.80−2.67 (m, 8H), 2.61−2.47 (m, 6H), 2.02−2.00 (m, 1H), 1.76−1.69 (m, 1H), 1.65 (s, 9H), 1.52−1.46 (m, 2H), 0.90 (t, J = 7.2 Hz, 3H). $[\alpha]_D^{25}$ = −33.8 (c = 1.0 in CH_3OH).

*(S)-N^6-(2-(4-(7-Chloro-1H-indol-6-yl)piperazin-1-yl)ethyl)-N^6-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine (**21a**) (D-616).* Compound **20a** (0.07 g, 0.13 mmol) was treated with trifluoroacetic acid (2 mL) in CH_2Cl_2 (2 mL) according to procedure B to furnish the TFA salt of **21a** (0.11 g, 91%). ^{1}H NMR (600 MHz, CD_3OD): δ 7.44 (d, J = 8.4 Hz, 1H), 7.24 (d, J = 3.0 Hz, 1H), 6.91 (d, J = 8.4 Hz, 1H), 6.44 (d, J = 3.0 Hz, 1H), 3.60 (m, 1H), 3.51−3.42 (m, 8H), 3.29 (s, 4H), 2.97−2.89 (m, 3H), 2.78−2.66 (m, 3H), 2.24−2.22 (m, 1H), 1.97−1.95 (m, 1H), 1.74−1.67 (m, 2H), 0.99 (t, J = 7.2 Hz, 3H). $[\alpha]_D^{25}$ = −25.2 (c = 1.0 in CH_3OH). Anal. Calcd for $C_{24}H_{33}ClN_6S\cdot 4CF_3COOH$: C, 41.36; H, 4.01; N, 9.04. Found: C, 41.38; H, 4.34; N, 9.16.

*(S)-N^6-(2-(4-(5-Chloro-1H-indol-4-yl)piperazin-1-yl)ethyl)-N^6-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine (**21b**) (D-617).* Compound **20b** (0.07 g, 0.119 mmol) was treated with trifluoroacetic acid (2 mL) in CH_2Cl_2 (2 mL) according to procedure B to furnish the TFA salt of **21b** (0.1 g, 95%). ^{1}H NMR (600 MHz, CD_3OD): δ 7.23 (d, J = 3.6 Hz, 1H), 7.15 (d, J = 8.4 Hz, 1H), 7.05 (d, J = 7.8 Hz, 1H), 6.61 (d, J = 3.0 Hz, 1H), 3.56 (s, 4H), 3.45−3.41 (m, 7H), 3.29 (m, 3H), 2.92−2.80 (m, 3H), 2.74−2.70 (m, 2H), 2.21−2.20 (m, 1H), 1.92 (m, 1H), 1.68−1.62 (m, 2H), 0.97 (t, J = 7.2 Hz, 3H). $[\alpha]_D^{25}$ = −23.9 (c = 1.0 in CH_3OH). Anal. Calcd for $C_{24}H_{33}ClN_6S\cdot 3CF_3COOH\cdot H_2O$: C, 43.25; H, 4.60; N, 10.09. Found: C, 42.89; H, 4.69; N, 9.85.

*tert-Butyl 5-Bromo-7-fluoro-1H-indole-1-carboxylate (**22**).* 5-Bromo-7-fluoro-1*H*-indole (0.48 g, 2.22 mmol) was reacted with $(Boc)_2O$ (0.53 g, 2.44 mmol) and DMAP (0.3 g, 2.44 mmol) in THF (5 mL) according to procedure D. The crude material was purified by silica gel column chromatography (hexane:EtOAc = 19:1) to afford compound **22** in quantitative yield. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.64 (d, J = 3.6 Hz, 1H), 7.47 (d, J = 1.8 Hz, 1H), 7.17 (dd, J = 10.8, 1.2 Hz, 1H), 6.52 (q, J = 1.8 Hz, 1H), 1.65 (s, 9H).

*tert-Butyl 5-(4-(2-((tert-Butyldimethylsilyl)oxy)ethyl)piperazin-1-yl)-7-fluoro-1H-indole-1-carboxylate (**23**).* A mixture of **22** (0.69 g, 2.196 mmol), **6** (0.94 g, 3.84 mmol), $Pd(OAc)_2$ (0.04 g, 0.17 mmol), BINAP (0.14 g, 0.22 mmol), and Cs_2CO_3 (2.15 g, 6.59 mmol) in toluene (10 mL) was heated at 110 °C for 15 h according to procedure G. The crude residue was purified by column chromatography (hexane:EtOAc = 7:3) to afford compound **23** (0.61 g, 58%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.56 (d, J = 3.6 Hz, 1H), 6.79 (d, J = 1.8 Hz, 1H), 6.71 (dd, J = 12.6, 2.4 Hz, 1H), 6.46 (q, J = 1.8 Hz, 1H), 3.80 (t, J = 6.0 Hz, 2H), 3.19 (t, J = 4.8 Hz, 4H), 2.71 (t, J = 4.8 Hz, 4H), 2.60 (t, J = 6.0 Hz, 2H), 1.63 (s, 9H), 0.91 (s, 9H), 0.08 (s, 6H).

*tert-Butyl 7-Fluoro-5-(4-(2-hydroxyethyl)piperazin-1-yl)-1H-indole-1-carboxylate (**24**).* Compound **23** (0.35 g, 0.73 mmol) was reacted with *n*-tetrabutylammonium fluoride (1.45 mL, 1.46 mmol, 1.0 M solution in THF) in THF (7 mL) according to procedure E. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 5:1) to give compound **24** (0.23 g, 85%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.57 (d, J = 3.6 Hz, 1H), 6.79 (d, J = 1.8 Hz, 1H), 6.71 (dd, J = 12.6, 2.4 Hz, 1H), 6.47 (q, J = 1.8 Hz, 1H), 3.67 (t, J = 6.0 Hz, 2H), 3.20 (t, J = 4.8 Hz, 4H), 2.82 (bs, 1H), 2.69 (t, J = 4.8 Hz, 4H), 2.62 (t, J = 6.0 Hz, 2H), 1.63 (s, 9H).

*tert-Butyl 7-Fluoro-5-(4-(2-oxoethyl)piperazin-1-yl)-1H-indole-1-carboxylate (**25**).* Alcohol **24** (0.15 g, 0.41 mmol) was oxidized using SO_3·py complex (0.33 g, 2.06 mmol), DMSO (2 mL), and Et_3N (0.40 mL, 2.89 mmol) in CH_2Cl_2 (4 mL) according to procedure F. The crude product was purified by silica gel column chromatography using ethyl acetate as solvent to give aldehyde **25** (0.12 g, 84%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.75 (s, 1H), 7.57 (d, J = 3.6 Hz, 1H), 6.81−6.79 (m, 1H), 6.71 (dd, J = 13.2, 1.8 Hz, 1H), 6.47 (q, J = 1.8 Hz, 1H), 3.27−3.19 (m, 6H), 2.73−2.70 (m, 4H), 1.63 (s, 9H).

*(S)-tert-Butyl 5-(4-(2-((2-Amino-4,5,6,7-tetrahydrobenzo[d]-thiazol-6-yl)(propyl)amino)ethyl)piperazin-1-yl)-7-fluoro-1H-indole-1-carboxylate (**26**).* Aldehyde **25** (0.12 g, 0.33 mmol) was reacted with (−)-pramipexole (0.06 g, 0.30 mmol) and $NaBH(OAc)_3$ (0.14 g, 0.66 mmol) in CH_2Cl_2 (7 mL) according to procedure A. The crude product was purified by silica gel column chromatography (EtOAc:MeOH = 9:1) to afford compound **26** (0.09 g, 51%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.56 (d, J = 3.0 Hz, 1H), 6.79 (d, J = 2.4 Hz, 1H), 6.70 (dd, J = 13.8, 1.8 Hz, 1H), 6.46 (q, J = 1.8 Hz, 1H), 5.11 (bs, 2H), 3.19 (t, J = 4.8 Hz, 4H), 3.07−3.03 (m, 1H), 2.76−2.66 (m, 8H), 2.58−2.46 (m, 6H), 2.01−1.99 (m, 1H), 1.76−1.69 (m, 1H), 1.63 (s, 9H), 1.51−1.45 (m, 2H), 0.89 (t, J = 7.2 Hz, 3H). $[\alpha]_D^{25}$ = −36.4 (c = 1.0 in CH_3OH).

*(S)-N^6-(2-(4-(7-Fluoro-1H-indol-5-yl)piperazin-1-yl)ethyl)-N^6-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine (**27**) (D-615).* Compound **26** (0.08 g, 0.14 mmol) was treated with trifluoroacetic acid (2 mL) in CH_2Cl_2 (2 mL) according to procedure B to furnish the TFA salt of **27** (0.11 g, 85%). ^{1}H NMR (600 MHz, CD_3OD): δ 7.21 (d, J = 3.0 Hz, 1H), 6.94 (s, 1H), 6.69 (d, J = 13.2 Hz, 1H), 6.41 (t, J = 3.0 Hz, 1H), 3.57−3.53 (m, 1H), 3.30−3.16 (m, 7H), 3.09−2.85 (m, 9H), 2.76−2.69 (m, 2H), 2.16−2.15 (m, 1H), 1.96−1.89 (m, 1H), 1.71−1.65 (m, 2H), 0.98 (t, J = 7.2 Hz, 3H). $[\alpha]_D^{25}$ = −26.6 (c = 1.0 in CH_3OH). Anal. Calcd for $C_{24}H_{33}FN_6S\cdot 3CF_3COOH\cdot 2H_2O$: C, 43.17; H, 4.83; N, 10.07. Found: C, 42.95; H, 4.67; N, 9.62.

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*(S)-N-(2-Amino-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)-2-nitrobenzenesulfonamide (**29**).* 2-Nitrobenzenesulfonyl chloride (1.2 g, 5.41 mmol) was dissolved in 20 mL of THF, and the solution was cooled to approximately −10 °C. Et_3N (3.4 mL, 24.37 mmol) and (*S*)-4,5,6,7-tetrahydrobenzo[*d*]thiazole-2,6-diamine **28**[47] (1.0 g, 5.96 mmol) were added, and the resulting suspension was heated during mixing to approximately 25 °C and allowed to react for 1.5 h. Precipitated triethylammonium chloride was filtered off, and the filtrate was concentrated. Water was added and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layer was dried using Na_2SO_4, and the solvent was removed under reduced pressure to obtain sulfonamide **29** (1.8 g, 5.08 mmol) as a pale-yellow solid with a yield of 94%. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.05−8.02 (m, 1H), 7.93−7.91 (m, 1H), 7.84−7.81 (m, 2H), 6.63 (s, 2H), 3.53−3.48 (m, 1H), 2.60−2.56 (m, 1H), 2.45−2.41 (m, 2H), 2.37−2.32 (m, 1H), 1.76−1.64 (m, 2H).

*(S)-N-(2-Amino-4,5,6,7-tetrahydrobenzo[d]thiazol-6-yl)-2-nitro-N-(prop-2-yn-1-yl)benzene-sulfonamide (**30**).* Compound **29** (1.26 g, 3.56 mmol), potassium carbonate (3.44 g, 24.89 mmol) and propargyl bromide (0.54 mL, 7.11 mmol) were suspended in 20 mL of acetonitrile. The mixture was heated during stirring to about 50 °C and left to react overnight. The reaction mixture was cooled to room temperature; water was added and extracted with EtOAc (3 × 35 mL). The combined organic layer was dried using Na_2SO_4, and the solvent was removed under reduced pressure. Column chromatography with EtOAc:MeOH (19:1) afforded compound **30** (0.97 g, 69%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.17 (dd, J = 6.0, 1.2 Hz, 1H), 7.75−7.67 (m, 3H), 5.20 (bs, 2H), 4.29−4.24 (m, 1H), 4.20 (s, 2H), 3.01 (t, J = 12.6 Hz, 1H), 2.78 (dd, J = 10.8, 4.8 Hz, 1H), 2.72−2.63 (m, 2H), 2.23 (t, J = 2.4 Hz, 1H), 2.08−2.06 (m, 2H).

*(S)-N^6-(Prop-2-yn-1-yl)-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine (**31**).* Into a mixture of potassium carbonate (3.8 g, 27.52 mmol) in 15 mL of DMF, thioglycolic acid (1.09 mL, 15.29 mmol) was added slowly at 0 °C. The mixture was stirred at room temperature for approximately 1 h, followed by addition of compound **30** (1.2 g, 3.06 mmol, in 5 mL of DMF). The reaction mixture was heated during stirring to about 50 °C and allowed to react for an additional 15 h, after which it was quenched by addition of 1N NaOH and extracted with EtOAc (4 × 25 mL). The combined organic layer was dried using Na_2SO_4, and the solvent was concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (5−10% MeOH in EtOAc) to give compound **31** (0.43 g, 67%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.20 (bs, 2H), 3.55−3.49 (m, 2H), 3.28−3.22 (m, 1H), 2.86−2.83 (m, 1H), 2.67−2.57 (m, 2H), 2.43−2.39 (m, 1H), 2.24 (s, 1H), 2.02−1.96 (m, 1H), 1.75−1.69 (m, 1H). $[\alpha]_D^{25} = -89.4$ (c = 1.0 in CH_3OH).

*(S)-tert-Butyl 5-(4-(2-((2-Amino-4,5,6,7-tetrahydrobenzo[d]-thiazol-6-yl)(prop-2-yn-1-yl)amino)ethyl)piperazin-1-yl)-4-chloro-1H-indole-1-carboxylate (**33**).* *tert*-Butyl 4-chloro-5-(4-(2-oxoethyl)-piperazin-1-yl)-1*H*-indole-1-carboxylate **32**[32] (0.22 g, 0.58 mmol) was reacted with the compound **31** (0.11 g, 0.52 mmol) and $NaBH(OAc)_3$ (0.25 g, 1.16 mmol) in CH_2Cl_2 (12 mL) according to procedure A. The crude product was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 19:1) to afford compound **33** (0.08 g, 29%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.98 (bs, 1H), 7.59 (s, 1H), 7.09 (dd, J = 6.6, 2.4 Hz, 1H), 6.67 (d, J = 3.6 Hz, 1H), 5.07 (bs, 2H), 3.58 (d, J = 2.4 Hz, 2H), 3.15−3.11 (m, 5H), 2.89−2.82 (m, 3H), 2.74−2.68 (m, 4H), 2.65−2.56 (m, 4H), 2.51 (s, 1H), 2.24 (t, J = 2.4 Hz, 1H), 2.14−2.12 (m, 1H), 1.78−1.71 (m, 1H), 1.66 (s, 10H). $[\alpha]_D^{25} = -28.3$ (c = 1.0 in CH_2Cl_2).

*(S)-N^6-(2-(4-(4-Chloro-1H-indol-5-yl)piperazin-1-yl)ethyl)-N^6-(prop-2-yn-1-yl)-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine (**34**) (D-614).* Compound **33** (0.07 g, 0.12 mmol) was treated with trifluoroacetic acid (3 mL) in CH_2Cl_2 (3 mL) according to procedure B to furnish the TFA salt of **34** (0.1 g, 90%). ^{1}H NMR (600 MHz, CD_3OD): δ 7.29 (dd, J = 6.0, 3.0 Hz, 1H), 7.24 (dd, J = 6.6, 3.0 Hz, 1H), 7.00 (q, J = 8.4 Hz, 1H), 6.47 (d, J = 3.0 Hz, 1H), 3.59 (d, J = 2.4 Hz, 2H), 3.33 (s, 1H), 3.15−3.10 (m, 5H), 2.99−2.95 (m, 5H), 2.85−2.77 (m, 3H), 2.67−2.66 (m, 1H), 2.64−2.59 (m, 2H), 2.53−2.48 (m, 2H), 2.13−2.11 (m, 1H), 1.80−1.72 (m, 1H). $[\alpha]_D^{25} = -19.6$ (c = 1.0 in CH_3OH). LRMS m/z [M + H]$^+$: calcd for $C_{24}H_{29}ClN_6SH$, 469.19; found, 469.19. Anal. Calcd for $C_{24}H_{29}ClN_6S{\cdot}3CF_3COOH{\cdot}CH_2Cl_2$: C, 41.55; H, 3.82; N, 9.38. Found: C, 41.80; H, 4.21; N, 9.39.

*(S)-tert-Butyl 6-(4-(2-((2-Amino-4,5,6,7-tetrahydrobenzo[d]-thiazol-6-yl)(prop-2-yn-1-yl)amino)ethyl)piperazin-1-yl)-7-chloro-1H-indole-1-carboxylate (**35**).* Aldehyde **19a** (0.2 g, 0.53 mmol) was reacted with **31** (0.1 g, 0.48 mmol) and $NaBH(OAc)_3$ (0.22 g, 1.06 mmol) in CH_2Cl_2 (20 mL) according to procedure A. The crude product was purified by silica gel column chromatography (CH_2Cl_2:MeOH = 9:1) to afford compound **35** (0.06 g, 23%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.43 (d, J = 3.0 Hz, 1H), 7.39 (d, J = 7.8 Hz, 1H), 7.01 (d, J = 8.4 Hz, 1H), 6.48 (d, J = 3.6 Hz, 1H), 4.77 (bs, 2H), 3.58 (d, J = 2.4 Hz, 2H), 3.16−3.10 (m, 5H), 2.88−2.83 (m, 3H), 2.73−2.59 (m, 9H), 2.23 (t, J = 1.8 Hz, 1H), 2.15−2.13 (m, 1H), 1.79−1.73 (m, 1H), 1.64 (s, 9H). $[\alpha]_D^{25} = -28.0$ (c = 1.0 in CH_3OH).

*(S)-N^6-(2-(4-(7-Chloro-1H-indol-6-yl)piperazin-1-yl)ethyl)-N^6-(prop-2-yn-1-yl)-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine (**36**) (D-635).* Compound **35** (0.017 g, 0.03 mmol) was treated with trifluoroacetic acid (2 mL) in CH_2Cl_2 (2 mL) according to procedure B to furnish the TFA salt of **36** (0.017 g, 79%). ^{1}H NMR (600 MHz, CD_3OD): δ 7.48 (d, J = 7.8 Hz, 1H), 7.26 (d, J = 3.0 Hz, 1H), 6.95 (d, J = 8.4 Hz, 1H), 6.45 (d, J = 3.6 Hz, 1H), 3.63 (d, J = 2.4 Hz, 2H), 3.42 (t, J = 6.0 Hz, 2H), 3.34 (s, 3H), 3.30−3.29 (m, 5H), 3.24−3.18 (m, 3H), 2.80 (dd, J = 15.6, 4.8 Hz, 1H), 2.78 (t, J = 2.4 Hz, 1H), 2.71−2.67 (m, 2H), 2.62−2.58 (m, 1H), 2.19 (d, J = 10.8 Hz, 1H), 1.91−1.84 (m, 1H). $[\alpha]_D^{25} = -21.3$ (c = 1.0 in CH_3OH). HRMS m/z [M + H]$^+$: calcd for $C_{24}H_{29}ClN_6SH$, 469.1941; found, 469.1930. Anal. Calcd for $C_{24}H_{29}ClN_6S{\cdot}2CF_3COOH{\cdot}2H_2O$: C, 45.87; H, 4.81; N, 11.46. Found: C, 46.45; H, 4.49; N, 10.89.

Evaluation of Potency in Binding to and Activating Dopamine D_2 and D_3 Receptors. Binding potency was monitored by inhibition of [^{3}H]spiroperidol (15.3 Ci/mmol, PerkinElmer) 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, PerkinElmer) binding in comparison to stimulation by the full agonist DA as described by us previously.[52,53] Receptor expression was (pmol/mg of protein): HEK-D2, 2.41; HEK-D3, 1.08; CHO-D2, 0.64; CHO-D3, 6.76. Despite the order of magnitude difference in D_2 and D_3 expressionin CHO cells, the maximal stimulation of [^{35}S]GTPγS binding in absolute terms was similar for D_2 and D_3, likely due to the well- known low efficiency of D_3 receptor-G protein coupling.[49]

Evaluation of Antagonist Property of (−)-34. D_2 antagonist activity was assessed by testing a fixed concentration that by itself had little or no effect on baseline [^{35}S]GTPγS binding for its ability to shift the concentration curve of an agonist stimulating [^{35}S]GTPγS binding as described for opioid receptors by Sally and co-workers (2010).[57] The fixed concentration of compound (−)-**34** was 50 and 300 nM, and that of standard compound (−)-3PPP were studied for comparison, 200 nM and 1 μM. The agonist used was dopamine (0.001−100 μM). K_e is the functional K_i (equilibrium dissociation constant) of an antagonist and is calculated according to the equation: [test compound]/(EC_{50-2}/EC_{50-1} − 1), where EC_{50-2} is the EC_{50} value in the presence of the test compound and EC_{50-1} is the value in the absence of the test compound.

Animal Experiments: Drugs and Chemicals. The following commercially available drugs were used in the experiment: reserpine hydrochloride (Alfa Aesar) and ropinirole (Sigma-Aldrich). The TFA salt of (−)-**21a**, (−)-**34** and hydrochloride salt of (−)-pramipexole and ropinirole 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 intraperitoneal administration 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

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

Reversal of Reserpine-Induced Hypolocomotion in Rats. Administration of reserpine induces catalepsy in rodents primarily by blocking the vesicular monoamine transporter (VMAT) which helps in the internalization of monoamines into vesicles, resulting in metabolism of unprotected monoamines in the cytosol that ultimately causes depletion of monoamines in the synapse of the peripheral sympathetic nerve terminals.[58,63] The ability of compounds (−)-**21a** and (−)-**34** to reverse reserpine-induced hypolocomotion was investigated according to a reported procedure.[64] (−)-Pramipexle and ropinirole were used as standard reference compounds in this study. Reserpine (5.0 mg/kg, sc) was administered to anaesthetized rats 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 drug, standard drug, or vehicle. Immediately after administration of drug or vehicle, animals were individually placed in an 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 $p < 0.05$.

Cell Culture 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 serum, 5% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in 95% air/5% CO_2. Stock solutions of (−)-**21a** and 6-hydroxydopamine (6-OHDA) were prepared in dimethyl sulfoxide (DMSO), and aliquots were stored at −20 °C and −80 °C, respectively. For all experiments assessing neuroprotective effects of the test compound, PC12 cells were pretreated with indicated concentrations of (−)-**21a** for 24 h and then treated with 75 μM 6-OHDA only for another 24 h. The control cells were treated with above media containing 0.01% DMSO only.

Assessment of Cell Viability. To determine the neuroprotective effect of (−)-**21a** on 6-OHDA mediated cell death, 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 for 24 h. Cells were treated with varying concentrations of either 6-OHDA or the test compound to determine their direct effect on cell viability and to determine the optimum concentration of 6-OHDA in subsequent neuroprotection experiments. Neuroprotection experiments were conducted by treating cells for 24 h with varying concentrations of (−)-**21a**. 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. 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 °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 values were 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.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b01031.

Details of in vitro antioxidant assay procedures and results; physicochemical properties of the compounds (PDF)
Molecular formula strings (CSV)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

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; TFA, Trifluoroacetic acid; GTPγS, guanosine 5′-*O*-[γ-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; (−)-3PPP, (*S*)-(−)-3-(3-hydroxyphenyl)-*N*-propylpiperidine; ip, intraperitoneal; sc, subcutaneous; PD, Parkinson's disease; DA, dopamine

■ REFERENCES

(1) Gelb, D. J.; Oliver, E.; Gilman, S. Diagnostic criteria for Parkinson disease. *Arch. Neurol.* **1999**, *56*, 33−39.
(2) Schapira, A. H.; Olanow, C. W. Neuroprotection in Parkinson disease: mysteries, myths, and misconceptions. *JAMA* **2004**, *291*, 358−364.
(3) Olanow, C. W.; Stern, M. B.; Sethi, K. The scientific and clinical basis for the treatment of Parkinson disease. *Neurology* **2009**, *72*, S1−136.
(4) Siderowf, A.; Stern, M. Update on Parkinson disease. *Ann. Intern. Med.* **2003**, *138*, 651−658.
(5) Dawson, T. M.; Dawson, V. L. Molecular pathways of neurodegeneration in Parkinson's disease. *Science* **2003**, *302*, 819−822.
(6) Betarbet, R.; Sherer, T. B.; Di Monte, D. A.; Greenamyre, J. T. Mechanistic approaches to Parkinson's disease pathogenesis. *Brain Pathol.* **2002**, *12*, 499−510.
(7) Dunnett, S. B.; Bjorklund, A. Prospects for new restorative and neuroprotective treatments in Parkinson's disease. *Nature* **1999**, *399*, A32−39.
(8) Jenner, P. Oxidative stress in Parkinson's disease. *Ann. Neurol.* **2003**, *53* (Suppl 3), S26−S36 discussion S36−S38..
(9) Spillantini, M. G.; Schmidt, M. L.; Lee, V. M.; Trojanowski, J. Q.; Jakes, R.; Goedert, M. Alpha-synuclein in Lewy bodies. *Nature* **1997**, *388*, 839−840.
(10) Olanow, C. W.; Tatton, W. G. Etiology and pathogenesis of Parkinson's disease. *Annu. Rev. Neurosci.* **1999**, *22*, 123−144.

DOI: 10.1021/acs.jmedchem.5b01031
J. Med. Chem. XXXX, XXX, XXX−XXX

(11) El-Agnaf, O. M.; Salem, S. A.; Paleologou, K. E.; Curran, M. D.; Gibson, M. J.; Court, J. A.; Schlossmacher, M. G.; Allsop, D. Detection of oligomeric forms of alpha-synuclein protein in human plasma as a potential biomarker for Parkinson's disease. *FASEB J.* **2006**, *20*, 419−425.
(12) Lashuel, H. A.; Hartley, D.; Petre, B. M.; Walz, T.; Lansbury, P. T., Jr. Neurodegenerative disease: amyloid pores from pathogenic mutations. *Nature* **2002**, *418*, 291.
(13) Hashimoto, M.; Hsu, L. J.; Xia, Y.; Takeda, A.; Sisk, A.; Sundsmo, M.; Masliah, E. Oxidative stress induces amyloid-like aggregate formation of NACP/alpha-synuclein in vitro. *NeuroReport* **1999**, *10*, 717−721.
(14) Kim, K. S.; Choi, S. Y.; Kwon, H. Y.; Won, M. H.; Kang, T. C.; Kang, J. H. Aggregation of alpha-synuclein induced by the Cu,Zn-superoxide dismutase and hydrogen peroxide system. *Free Radical Biol. Med.* **2002**, *32*, 544−550.
(15) Beaulieu, J. M.; Gainetdinov, R. R. The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacol. Rev.* **2011**, *63*, 182−217.
(16) Gonnet, G. H.; Cohen, M. A.; Benner, S. A. Exhaustive matching of the entire protein sequence database. *Science* **1992**, *256*, 1443−1445.
(17) Carlsson, J.; Coleman, R. G.; Setola, V.; Irwin, J. J.; Fan, H.; Schlessinger, A.; Sali, A.; Roth, B. L.; Shoichet, B. K. Ligand discovery from a dopamine D3 receptor homology model and crystal structure. *Nat. Chem. Biol.* **2011**, *7*, 769−778.
(18) Cavalli, A.; Bolognesi, M. L.; Minarini, A.; Rosini, M.; Tumiatti, V.; Recanatini, M.; Melchiorre, C. Multi-target-directed ligands to combat neurodegenerative diseases. *J. Med. Chem.* **2008**, *51*, 347−372.
(19) Meissner, W. G.; Frasier, M.; Gasser, T.; Goetz, C. G.; Lozano, A.; Piccini, P.; Obeso, J. A.; Rascol, O.; Schapira, A.; Voon, V.; Weiner, D. M.; Tison, F.; Bezard, E. Priorities in Parkinson's disease research. *Nat. Rev. Drug Discovery* **2011**, *10*, 377−393.
(20) Marsden, C. D.; Parkes, J. D. "On-off" effects in patients with Parkinson's disease on chronic levodopa therapy. *Lancet* **1976**, *307*, 292−296.
(21) Basma, A. N.; Morris, E. J.; Nicklas, W. J.; Geller, H. M. L-dopa cytotoxicity to PC12 cells in culture is via its autoxidation. *J. Neurochem.* **1995**, *64*, 825−832.
(22) Fahn, S. Is levodopa toxic? *Neurology* **1996**, *47*, 184S−195S.
(23) Stocchi, F.; Olanow, C. W. Neuroprotection in Parkinson's disease: clinical trials. *Ann. Neurol.* **2003**, *53* (Suppl 3), S87−S97 discussion S97−S99..
(24) Mandel, S.; Grunblatt, E.; Riederer, P.; Gerlach, M.; Levites, Y.; Youdim, M. B. Neuroprotective strategies in Parkinson's disease: an update on progress. *CNS Drugs* **2003**, *17*, 729−762.
(25) Roth, B. L.; Sheffler, D. J.; Kroeze, W. K. Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia. *Nat. Rev. Drug Discovery* **2004**, *3*, 353−359.
(26) Biswas, S.; Zhang, S.; Fernandez, F.; Ghosh, B.; Zhen, J.; Kuzhikandathil, E.; Reith, M. E.; Dutta, A. K. Further structure-activity relationships study of hybrid 7-{[2-(4-phenylpiperazin-1-yl)ethyl]-propylamino}-5,6,7,8-tetrahydronaphthalen-2-o l analogues: identification of a high-affinity D3-preferring agonist with potent in vivo activity with long duration of action. *J. Med. Chem.* **2008**, *51*, 101−117.
(27) Biswas, S.; Hazeldine, S.; Ghosh, B.; Parrington, I.; Kuzhikandathil, E.; Reith, M. E.; Dutta, A. K. 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.* **2008**, *51*, 3005−3019.
(28) Ghosh, B.; Antonio, T.; Zhen, J.; Kharkar, P.; Reith, M. E.; Dutta, A. K. Development of (S)-N6-(2-(4-(isoquinolin-1-yl)piperazin-1-yl)ethyl)-N6-propyl-4,5,6,7-tetrahydro benzo[d]-thiazole-2,6-diamine and its analogue as a D3 receptor preferring agonist: potent in vivo activity in Parkinson's disease animal models. *J. Med. Chem.* **2010**, *53*, 1023−1037.
(29) Ghosh, B.; Antonio, T.; Reith, M. E.; Dutta, A. K. Discovery of 4-(4-(2-((5-Hydroxy-1,2,3,4-tetrahydronaphthalen-2-yl) (propyl)-amino)ethyl)piperaz in-1-yl)quinolin-8-ol and its analogues as highly potent dopamine D2/D3 agonists and as iron chelator: in vivo activity indicates potential application in symptomatic and neuroprotective therapy for Parkinson's disease. *J. Med. Chem.* **2010**, *53*, 2114−2125.
(30) Li, C.; Biswas, S.; Li, X.; Dutta, A. K.; Le, W. 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.* **2010**, *88*, 2513−2523.
(31) Gogoi, S.; Antonio, T.; Rajagopalan, S.; Reith, M.; Andersen, J.; Dutta, A. K. Dopamine D(2)/D(3) agonists with potent iron chelation, antioxidant and neuroprotective properties: potential implication in symptomatic and neuroprotective treatment of Parkinson's disease. *ChemMedChem* **2011**, *6*, 991−995.
(32) Johnson, M.; Antonio, T.; Reith, M. E.; Dutta, A. K. 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.* **2012**, *55*, 5826−5840.
(33) Santra, S.; Xu, L.; Shah, M.; Johnson, M.; Dutta, A. 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.* **2013**, *4*, 1382−1392.
(34) Shah, M.; Rajagopalan, S.; Xu, L.; Voshavar, C.; Shurubor, Y.; Beal, F.; Andersen, J. K.; Dutta, A. K. 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.* **2014**, *131*, 74−85.
(35) Modi, G.; Antonio, T.; Reith, M.; Dutta, A. 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.* **2014**, *57*, 1557−1572.
(36) Modi, G.; Voshavar, C.; Gogoi, S.; Shah, M.; Antonio, T.; Reith, M. E.; Dutta, A. K. Multifunctional D2/D3 agonist D-520 with high in vivo efficacy: modulator of toxicity of alpha-synuclein aggregates. *ACS Chem. Neurosci.* **2014**, *5*, 700−717.
(37) Dutta, A. K.; Fei, X. S.; Reith, M. E. A novel series of hybrid compounds derived by combining 2-aminotetralin and piperazine fragments: binding activity at D2 and D3 receptors. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 619−622.
(38) Dutta, A. K.; Venkataraman, S. K.; Fei, X. S.; Kolhatkar, R.; Zhang, S.; Reith, M. E. Synthesis and biological characterization of novel hybrid 7-[[2-(4-phenyl-piperazin-1-yl)-ethyl]-propyl-amino]-5,6,7,8-tetrahydro-naphthalen-2-ol and their heterocyclic bioisosteric analogues for dopamine D2 and D3 receptors. *Bioorg. Med. Chem.* **2004**, *12*, 4361−4373.
(39) Markey, S. J.; Lewis, W.; Moody, C. J. A new route to alpha-carbolines based on 6pi-electrocyclization of indole-3-alkenyl oximes. *Org. Lett.* **2013**, *15*, 6306−6308.
(40) Wang, W.; Xiong, C.; Yang, J.; Hruby, V. J. Practical, asymmetric synthesis of aromatic-substituted bulky and hydrophobic tryptophan derivatives. *Tetrahedron Lett.* **2001**, *42*, 7717−7719.
(41) Parikh, J. R.; Doering, W. v. E. Sulfur trioxide in the oxidation of alcohols by dimethyl sulfoxide. *J. Am. Chem. Soc.* **1967**, *89*, 5505−5507.
(42) Schneider, C. S.; Mierau, J. Dopamine autoreceptor agonists: resolution and pharmacological activity of 2,6-diaminotetrahydrobenzothiazole and an aminothiazole analogue of apomorphine. *J. Med. Chem.* **1987**, *30*, 494−498.
(43) Mancuso, A. J.; Huang, S.-L.; Swern, D. Oxidation of long-chain and related alcohols to carbonyls by dimethyl sulfoxide "activated" by oxalyl chloride. *J. Org. Chem.* **1978**, *43*, 2480−2482.
(44) Johnson, M.; Antonio, T.; Reith, M. E.; Dutta, A. K. Correction to 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

DOI: 10.1021/acs.jmedchem.5b01031
J. Med. Chem. XXXX, XXX, XXX−XXX

receptor agonists along with a highly potent D2/D3 agonist and their pharmacological characterization. *J. Med. Chem.* **2013**, *56*, 589−590.
(45) Langlois, Y.; Pouilhès, A.; Génin, D.; Andriamialisoa, R. Z.; Langlois, N. New approaches to the synthesis of eburnane alkaloids. *Tetrahedron* **1983**, *39*, 3755−3761.
(46) Yin, J.; Gallis, C. E.; Chisholm, J. D. Tandem oxidation/halogenation of aryl allylic alcohols under Moffatt−Swern conditions. *J. Org. Chem.* **2007**, *72*, 7054−7057.
(47) Živec, M.; Anžič, B.; Gobec, S. A novel scalable synthesis of pramipexole. *Org. Process Res. Dev.* **2010**, *14*, 1125−1129.
(48) Brown, D. A.; Mishra, M.; Zhang, S.; Biswas, S.; Parrington, I.; Antonio, T.; Reith, M. E.; Dutta, A. K. Investigation of various N-heterocyclic substituted piperazine versions of 5/7-{[2-(4-aryl-piperazin-1-yl)-ethyl]-propyl-amino}-5,6,7,8-tetrahydro-naphthale n-2-ol: effect on affinity and selectivity for dopamine D3 receptor. *Bioorg. Med. Chem.* **2009**, *17*, 3923−3933.
(49) Zhen, J.; Antonio, T.; Ali, S.; Neve, K. A.; Dutta, A. K.; Reith, M. E. Use of radiolabeled antagonist assays for assessing agonism at D2 and D3 dopamine receptors: comparison with functional GTPgammaS assays. *J. Neurosci. Methods* **2015**, *248*, 7−15.
(50) Giros, B.; Martres, M. P.; Sokoloff, P.; Schwartz, J. C. Gene cloning of human dopaminergic D3 receptor and identification of its chromosome. *C. R. Acad. Sci. III* **1990**, *311*, 501−508.
(51) Grandy, D. K.; Marchionni, M. A.; Makam, H.; Stofko, R. E.; Alfano, M.; Frothingham, L.; Fischer, J. B.; Burke-Howie, K. J.; Bunzow, J. R.; Server, A. C. Cloning of the cDNA and gene for a human D2 dopamine receptor. *Proc. Natl. Acad. Sci. U. S. A.* **1989**, *86*, 9762−9766.
(52) Zhen, J.; Antonio, T.; Dutta, A. K.; Reith, M. E. Concentration of receptor and ligand revisited in a modified receptor binding protocol for high-affinity radioligands: [3H]Spiperone binding to D2 and D3 dopamine receptors. *J. Neurosci. Methods* **2010**, *188*, 32−38.
(53) Ghosh, B.; Antonio, T.; Gopishetty, B.; Reith, M.; Dutta, A. 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.* **2010**, *18*, 5661−5674.
(54) Das, B.; Modi, G.; Dutta, A. Dopamine d3 agonists in the treatment of Parkinson's disease. *Curr. Top. Med. Chem.* **2015**, *15*, 908−926.
(55) Tadori, Y.; Forbes, R. A.; McQuade, R. D.; Kikuchi, T. Receptor reserve-dependent properties of antipsychotics at human dopamine D2 receptors. *Eur. J. Pharmacol.* **2009**, *607*, 35−40.
(56) Reith, M. E. A.; Zhen, J.; Antonio, T.; Jacob, J. C.; Grandy, D.; Dutta, A. K.; Selley, D. E. Influence of aromatic moieties linked to piperazine ring of tetrahydrobenzo[*d*]-thiazole based hybrid compounds impacts efficacy of dopamine D2/D3 agonists at D3 receptors. Society for Neuroscience Meeting, Chicago, October 17−21, 2015; in: abstract viewer and itinerary planner, Society for Neuroscience, online, no. 678.04.
(57) Sally, E. J.; Xu, H.; Dersch, C. M.; Hsin, L. W.; Chang, L. T.; Prisinzano, T. E.; Simpson, D. S.; Giuvelis, D.; Rice, K. C.; Jacobson, A. E.; Cheng, K.; Bilsky, E. J.; Rothman, R. B. Identification of a novel ″almost neutral″ micro-opioid receptor antagonist in CHO cells expressing the cloned human mu-opioid receptor. *Synapse* **2010**, *64*, 280−288.
(58) Carlsson, A.; Lindqvist, M.; Magnusson, T. 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. *Nature* **1957**, *180*, 1200.
(59) Skalisz, L. L.; Beijamini, V.; Joca, S. L.; Vital, M. A.; Da Cunha, C.; Andreatini, R. Evaluation of the face validity of reserpine administration as an animal model of depression–Parkinson's disease association. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **2002**, *26*, 879−883.
(60) Blum, D.; Torch, S.; Nissou, M. F.; Benabid, A. L.; Verna, J. M. Extracellular toxicity of 6-hydroxydopamine on PC12 cells. *Neurosci. Lett.* **2000**, *283*, 193−196.
(61) Soto-Otero, R.; Mendez-Alvarez, E.; Hermida-Ameijeiras, A.; Munoz-Patino, A. M.; Labandeira-Garcia, J. L. 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.* **2000**, *74*, 1605−1612.
(62) Bardiot, D.; Carlens, G.; Dallmeier, K.; Kaptein, S.; McNaughton, M.; Marchand, A.; Neyts, J.; Smets, W. 1,2-Amino-1-(indolyl)ethanone derivatives as viral replication inhibitors and their preparation of Flaviviridae infections. PCT Int. Appl. WO2013045516, 2013.
(63) Millan, M. J.; Di Cara, B.; Hill, M.; Jackson, M.; Joyce, J. N.; Brotchie, J.; McGuire, S.; Crossman, A.; Smith, L.; Jenner, P.; Gobert, A.; Peglion, J. L.; Brocco, M. S32504, a novel naphtoxazine agonist at dopamine D3/D2 receptors: II. Actions in rodent, primate, and cellular models of antiparkinsonian activity in comparison to ropinirole. *J. Pharmacol. Exp. Ther.* **2004**, *309*, 921−935.
(64) McCall, R. B.; Lookingland, K. J.; Bedard, P. J.; Huff, R. M. 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.* **2005**, *314*, 1248−1256.

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J. Med. Chem. XXXX, XXX, XXX−XXX