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8,9-Dihydroxy-1,2,3,11b-tetrahydrochromeno[4,3,2,-*de*]isoquinoline (dinoxyline), a high affinity and potent agonist at all dopamine receptor isoforms

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Abstract—The synthesis and preliminary pharmacological evaluation of 8,9-dihydroxy-1,2,3,11b-tetrahydrochromeno[4,3,2,-*de*]-isoquinoline (**5,** now named dinoxyline) is described. This molecule was designed as a potential bioisostere that would conserve the essential elements of our β-phenyldopamine D_1 pharmacophore (i.e., position and orientation of the nitrogen, hydroxyls, and phenyl rings). Previously, we have rigidified these elements using alkyl bridges, as exemplified in the dopamine D_1 full agonist molecules dihydrexidine (**1**) and dinapsoline (**2**). This approach has been modified and we now show that it is possible to tether these elements using an ether linkage. Preliminary pharmacology has revealed that **5** is a potent full D_1 agonist ($K_{0.5} < 10$ nM; $EC_{50} = 30$ nM), but also has high affinity for brain D_2-like and cloned D_2 and D_3 receptors. Interestingly, whereas **1** and **2** and their analogues have only moderate affinity for the human D_4 receptor, **5** also has high affinity for this isoform. Moreover, although *N*-alkylation of **1** and **2** increases D_2 affinity, the *N*-allyl (**15**) and *N*-*n*-propyl (**17**) derivatives of **5** had decreased D_2 affinity. Therefore, **5** may be engaging different amino acid residues than do **1** and **2** when they bind to the D_2 receptor. This is the first example of a ligand with high affinity at all dopamine receptors, yet with functional characteristics similar to dopamine. These rigid ligands also will be useful tools to determine specific residues of the receptor transmembrane domains that are critical for agonist ligand selectivity for the D_4 receptor.

1. Introduction

The diverse physiological actions of dopamine are mediated by at least five distinct G-protein coupled receptors, the molecular biology of which has been reviewed by numerous authors and in numerous books.[1–5] The D_1-like receptor subtypes (D_1 or D_{1A} and D_5 or D_{1B}) come from intron-less genes (albeit D_5 pseudogenes exist), and the receptors typically couple to the G proteins G_S and G_{olf} and activate adenylate cyclase. The D_2-like subfamily comes from three genes (D_2, D_3, and D_4) that are prototypic of G protein-coupled receptors that inhibit adenylate cyclase and activate K^+ channels. The D_2 and D_3 receptors vary in certain tissues and species as a result of alternative splicing, and there are interesting polymorphisms of the human D_4 receptor gene that have attracted great interest.[6,7]

Our major goal has been the development of isoform-selective agonists, with a primary focus on drugs for the D_1 and D_5 receptors, and a secondary one on D_3 and D_4 selective agonists. We have been interested in drugs that have particular characteristics that allow them to be used for the structural elucidation of specific dopamine receptor isoforms, and also as neuropharmacological research tools. Thus, dihydrexidine (**1**) and other D_1 agonists have been valuable research tools in studying the roles of D_1 receptors, both in vivo[8,9] and in vitro.[10,11] These same drugs have clear and novel uses in several neurological and psychiatric disorders.

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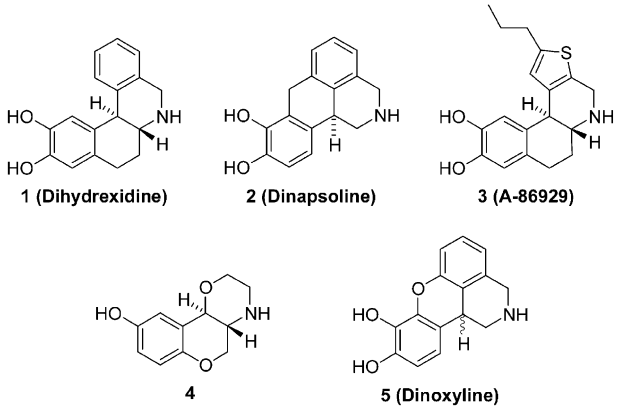
For example, Parkinson's disease (PD), a dopamine degenerative disorder,[12] still has 'dopamine replacement' as the most widely used and accepted treatment.[13] While the field has accepted for decades a crucial role for D_2-like receptors in the therapy of PD, recently our group and others have shown the critical role of D_1 receptor activation in the therapy of Parkinson's disease.[14–16] Thus, dihydrexidine (**1**), the first full D_1 agonist, was used to provide the first demonstration of the profound antiparkinsonian effects of full D_1 agonists in primates resistant to L-DOPA or D_2 agonists.[14] Confirmatory results emerged from studies of several drugs,[17,18] including some developed by Abbott Laboratories.[19] Despite their efficacy, each of these drugs was eliminated as a clinical agent by bioavailability, tolerance, or seizure issues. Clearly, a full D_1 agonist without these side effects is likely to have great utility, a view supported by numerous recent studies that have elucidated new aspects of basal ganglia circuitry and the role of D_1-like receptors.[20,21]

In addition, D_1 and D_5 receptors play a crucial role in the dopaminergic modulation of cognition and motor behavior.[22,23] For example, Goldman-Rakic and colleagues carried out elegant studies that have localized the various message and proteins of the dopamine receptors in various lamina of the primate prefrontal cortex, and have demonstrated how working memory performance is influenced by activation of D_1-like dopamine receptors in the prefrontal cortex.[24–27] Similar studies with D_1 agonists in a number of tests of cognitive function have determined their potential utility.[27,28] Indeed, a recent NIMH Consensus Symposium overwhelmingly voted drugs with D_1 agonist properties as the most promising future therapy for the most difficult-to-treat aspects of schizophrenia (see www.matrics.ucla.edu). There have also been extensive studies on the importance of D_1-like receptors for normal hippocampal function.[29,30] D_1/D_5 receptor agonists can affect long-term potentiation (LTP) by inducing a protein synthesis-dependent late potentiation in the CA1 region of the hippocampus,[31] and can increase the magnitude of LTP in a synapse-specific and cAMP dependent manner.[32] Such data, coupled with other related studies,[33–36] suggest that dopamine D_1/D_5 receptor agonists (selective or non-selective) might have therapeutic utility.[37]

As this brief review indicates, the essential role of D_1-like receptors in critical CNS processes makes it imperative to develop drugs to study these functions, also with an aim of improved clinical treatments. The current model that led to the synthesis of the selective full D_1 agonists dihydrexidine (**1**)[38] and dinapsoline (**2**),[39] has been referred to as the *trans*-β-phenyldopamine D_1 agonist pharmacophore. The essential elements of this pharmacophore include dopamine in the *trans*-β-rotameric conformation with a β-accessory ring (typically an aromatic ring).[40] By contrast, apomorphine, an older structurally rigid dopamine agonist, incorporates dopamine in a *trans*-α-rotameric conformation and is a partial agonist at the D_1 receptor.[41]

Other accessory rings such as thiophene have been substituted for the phenyl group of dihydrexidine, and **3** (A-86929) was shown to be a potent and selective D_1 ligand.[42] The geometry of the pharmacophore is defined by a catechol ring that is close to planarity with the accessory ring and a nitrogen atom that is 7 Å away from the *meta*-hydroxyl. Both **1** and **2** are rigid compounds that have a limited range of low energy conformations and meet the requirements of the proposed pharmacophore.[43,44] Conformational restriction of dopaminergic pharmacophores also has been accomplished using isochromans, and compound **4** is a potent and selective D_3 agonist.[45]

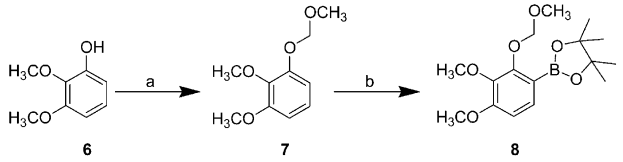
The conformational restriction of dinapsoline (**2**) was accomplished using a methylene linkage, which in fact is somewhat difficult to construct. Using a bioisosteric approach, it was hypothesized that an ether linkage could be substituted for this methylene tether, anticipating that it might be easier to introduce the oxygen than the methylene in **2**, and that **5** (dinoxyline; DNX) might possess useful and interesting dopaminergic properties. This hypothesis was tested and the results are presented here.



2. Chemistry and pharmacology

The synthesis (Scheme 2) of **5** (dinoxyline) was based on an alternative formal synthesis of dinapsoline (**2**) described by Qandil et al..[46] The biaryl bond of **10** was constructed using the Miyaura–Suzuki[47,48] cross-coupling reaction and the ether linkage of **5** was generated by phenol displacement of the isoquinoline nitro group. The major modification from the Qandil synthesis involved cyclization while the isoquinoline nucleus remained unreduced and therefore did not require the previously-needed protection and deprotection sequence.

The coupling partners **8** (Scheme 1) and **9** for the Suzuki reaction were readily synthesized and required minimal purification. Nitration of 4-bromoisoquinoline using potassium nitrate and sulfuric acid, conditions described previously by Osborn et al.[49] for nitration of 5-bromoisoquinoline, gave 4-bromo-5-nitroisoquinoline (**9**) in excellent yield. The borolane derivative **8** was obtained in two steps: protection of 2,3-dimethoxyphenol (**6**) as the



Scheme 1. (a) (i) NaH; (ii) $ClCH_2OCH_3$; (b) (i) *n*-BuLi; (ii) 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane.

methoxymethyl derivative **7**,[50] followed by lithiation[51] and reaction with 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (Scheme 1).

After obtaining the Suzuki coupling partners, the synthesis of dinoxyline (**5**) was completed as shown in Scheme 2. Suzuki coupling between **8** and **9** using conditions described by Zoltewicz et al.[48] gave **10** in high yield. After isolation of **10** by column chromatography, the methoxymethyl protecting group was hydrolyzed with *p*-toluenesulfonic acid.[52] The resulting nitrophenol **11** was isolated and treated with potassium carbonate in DMF at 80 °C to afford the tetracyclic chromenoisoquinoline **12**.[53] Reduction of the isoquinoline ring to tetrahydroisoquinoline **13** was accomplished by catalytic hydrogenation over platinum oxide in acetic acid containing HCl.[54] Boron tribromide was then used to cleave the methoxy groups to obtain **5**.

The *N*-allyl **15** and *N*-propyl **17** derivatives of **5** were prepared from **13** as shown in Scheme 3.[55] The tertiary amine **14,** was obtained by stirring **13** with potassium carbonate and allyl bromide in acetone. Demethylation of **14** with boron tribromide gave **15**. Catalytic hydrogenation of **14** was followed by treatment with boron tribromide to obtain **17**.

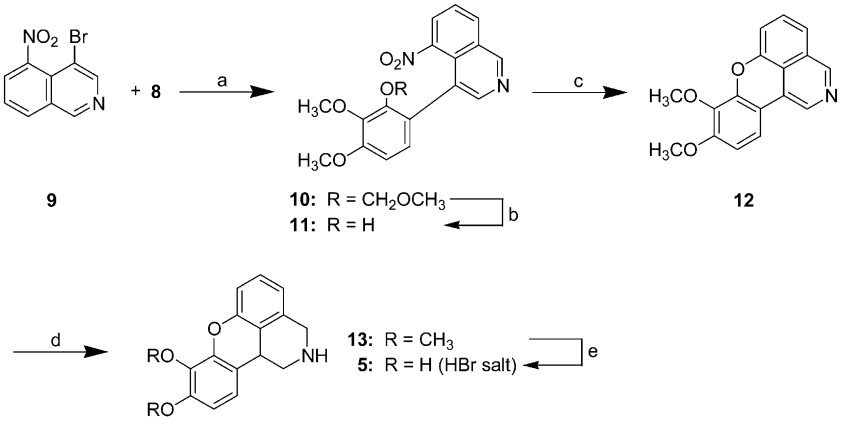
3. Pharmacology

In order to assess the receptor binding characteristics, we first evaluated **5** and its *N*-alkyl analogues at rat striatal D_1 and D_2 receptors. For comparison, we also tested our first two full D_1 agonist compounds, dihydrexidine (DHX, **1**) and dinapsoline (DNS, **2**). To generate a more comprehensive pharmacological profile, we then evaluated the affinity of **5** and its analogues at the cloned dopamine receptors. Again, **1** and **2** were included as reference compounds.

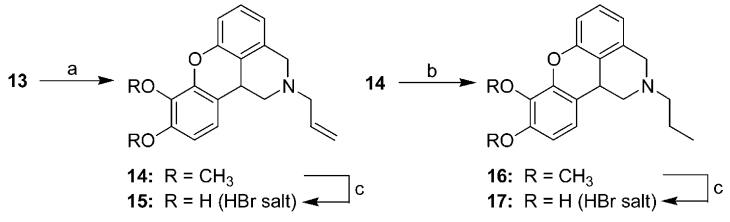
Finally, the functional profile of **5** and the two *N*-alkylated analogues was evaluated by assessing their ability to modulate adenylate cyclase production using a cAMP accumulation assay in rat striatal membranes and in the cloned dopamine receptors. Activity at the D_3 receptor was not evaluated because it is poorly coupled in this assay system, and available assay systems (e.g., mitogenesis or MAP-K) are very indirect. The endogenous neurotransmitter dopamine was used to define full agonist activity, and **1** and **2** were again included for comparison.

4. Results and discussion

The receptor binding characteristics of the new compounds at rat striatal D_1 and D_2 receptors are presented in Table 1 and Figures 1 and 2. At striatal D_1-like receptors, the parent compound **5** had high affinity ($K_{0.5}$ = 8.3 nM), similar to that observed with **1** and **2**



Scheme 2. (a) $Pd(PPh_3)_4$, KOH, $Bu_4N^+Cl^-$, H_2O, DME, reflux; (b) $TsOH{\cdot}H_2O$, MeOH; (c) K_2CO_3, DMF, 80 °C; (d) PtO_2, AcOH, HCl, H_2; (e) BBr_3, CH_2Cl_2, −78 °C.



Scheme 3. (a) allyl bromide, acetone, K_2CO_3; (b) H_2, Pd-C, EtOH; (c) BBr_3, CH_2Cl_2.

Table 1. Binding affinities of 5 and analogues for striatal dopamine receptors[a]

Test Ligand	D_1-like $K_{0.5}$ (nM)	D_2-like $K_{0.5}$ (nM)
Chlorpromazine	—	0.92±0.12[b]
SCH23390	0.52[c]	—
(±)-**1** (DHX)	4.6±0.3[b]	43±3[b]
(±)-**2** (DNS)	5.9±0.7[b]	31±4[b]
(±)-**5** (DNX)	8.3±6.4	6.2±1.8
(±)-**15** (*N*-allyl-DNX)	260±42	54±12
(±)-**17** (*N*-pr-DNX)	250±78	17±1

[a] The Hill slope n_H for the antagonists was near unity, whereas those for other compounds ranged from ~0.9 for **15** and **17**, to 0.65 for **1**, **2**, and **5**. Data represent the mean and standard error from a minimum of three independent assays.
[b] From Ghosh et al.[39]
[c] From Knoerzer et al.[56]

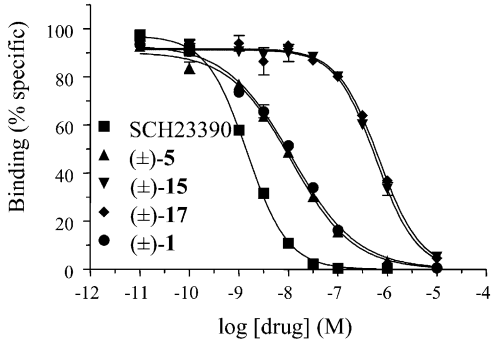


Figure 1. Representative competition binding curves for **5** (DNX) and its *N*-alkyl analogues at D_1-like receptors in rat striatal membranes. The high-affinity, full agonist **1** (DHX) and the prototype antagonist SCH23390 are included for comparison.

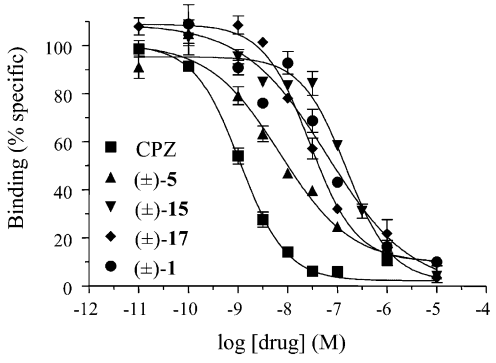


Figure 2. Representative competition binding curves for **5** (DNX) and its *N*-alkyl analogues at D_2-like receptors in rat striatal membranes. The high-affinity, full agonist **1** (DHX) and the prototype antagonist chlorpromazine are included for comparison.

($K_{0.5}$ = 4.6 nM and 5.9 nM, respectively). The affinity of **5** at striatal D_2-like receptors was, however, surprisingly high ($K_{0.5}$ = 6.2 nM) and comparable to its D_1-like affinity. By contrast, both **1** and **2** had lower affinity ($K_{0.5}$ = 43 nM and 31 nM, respectively) at D_2-like receptors compared to D_1-like receptors. Surprisingly, despite the fact that **1**, **2**, and **5** all embody the same pharmacophore (at least as defined for the D_1 receptor), the location and nature of the tethering moieties clearly introduce subtle binding interactions within the different dopamine receptors that affect isoform selectivity and potency. These findings reinforce our previous conclusions that substitutions on the pendant phenyl ring of either **1** or **2** can target the substituted molecule to different receptor isoforms.

Previous studies with **1** had demonstrated that *N*-alkyl substituents change the D_1/D_2 affinity profile such that the resulting compounds have selectivity for various D_2-, rather than D_1-like receptors.[56,57] We synthesized the *N*-allyl and *N*-*n*-propyl analogues of **5** and tested their affinities at striatal D_1 and D_2 receptors (Table 1 and Fig. 1). As was observed with **1**, either an *N*-allyl or *N*-*n*-propyl group on **5** reversed the D_1/D_2 affinity profile, making these compounds 5- to 15-fold selective for D_2 receptors, respectively. At D_1-like receptors, addition of either *N*-alkyl substituent caused a 40-fold decrease in affinity ($K_{0.5}$ = 260 nM and 250 nM for **15** and **17**, respectively), indicative of low steric tolerance in this region of the D_1-like receptors. At D_2-like receptors, addition of either N-alkyl substituent caused less than a 9-fold decrease in affinity ($K_{0.5}$ = 54 nM and 17 nM for **15** and **17**, respectively). Overall, the reversal in D_1/D_2 selectivity observed with the *N*-alkyl substituents can be attributed to the substantial decrease in D_1 binding compared to the small decrease in D_2 binding. Although in the current work we did not resolve racemic **5** into its enantiomers, the D_1 full agonist pharmacophore we have described has correctly predicted the absolute configuration of all D_1 full agonists (e.g., **1**, **2**, and **3**).[44] In the case of **5**, this configuration would be 12a*S*, homochiral with the active enantiomer shown for **2**. In addition, we have previously shown for **1** and **2** that the same enantiomer possesses both the D_1 and D_2-like activity, and it would be extremely surprising if this were not also the case for **5** and its analogues.

At the cloned dopamine receptors (Table 2) **5** had high affinity at all of the dopamine receptor isoforms ($K_{0.5}$ ranging from 1.0 nM to 3.9 nM) with the exception of D_{2L} ($K_{0.5}$ = 86 nM). Compared to **1**, the binding affinity of **5** was similar at D_1 and D_5 receptors but was approximately 15-fold higher at both D_3 and D_4 receptors. It is particularly interesting that the introduction of the oxygen tether into **5** leads to increased D_4 affinity and potency relative to **1** and **2**.[55,56] Similar to what was observed in striatal homogenate, *N*-alkyl substituents had dramatic effects on affinity, particularly for the D_1-like family of receptors. Consistent with proposed low steric tolerance at D_1-like receptors,[40,44] *N*-alkyl substituents dramatically decreased affinity at D_1 and D_5 receptors. The most significant decrease was observed at the D_5 receptor ($K_{0.5}$ = 1,500 nM and 570 nM for **15** and **17**, respectively). At the D_1 receptor, affinity was decreased approximately 30- to 50-fold ($K_{0.5}$ = 110 nM and 190 nM for **15** and **17**, respectively). At the D_2-like receptors, the *N*-alkyl substituents had very little effect on affinity compared to **5**. The *N*-allyl (**15**) substituent decreased affinity less than 10-fold ($K_{0.5}$ = 290 nM, 9.0 nM, and 6.3 nM for D_{2L}, D_3, and D_4, respectively) and the *N*-*n*-propyl (**15**) substituent had essentially no effect ($K_{0.5}$ = 98 nM, 0.93 nM, and 2.6 nM for D_{2L}, D_3, and D_4, respectively).

Table 2. Binding affinities of 5 and analogues for cloned dopamine receptors[a]

Test Ligand	$K_{0.5}$ (nM)±SEM				
	D_1 (C-6)	D_{2L} (C-6)	D_3 (C-6)	D_4 (CHO)	D_5 (HEK)
Chlorpromazine	—	0.74±0.09[b]	0.86±0.03[b]	20±2	—
SCH23390	0.57±0.05[c]	—	—	—	1.2±0.3
(±)-**1** (DHX)	2.2±0.4[c]	180±21[b]	15±3[b]	14±2	14±3
(±)-**2** (DNS)	5.5±3[c]	140±21[d]	10±1	60±18	10±7
(±)-**5** (DNX)	3.9±0.6	86±19	1.0±0.2	1.1±0.3	3.8±0.5
(±)-**15** (*N*-allyl-DNX)	110±2	290±40	9.0±1.7	6.3±0.9	1500±550
(±)-**17** (*N*-pr-DNX)	190±6	98±29	0.93±0.10	2.6±0.6	570±66

[a] The Hill slope n_H for the antagonists was near unity, whereas those for other compounds ranged from 0.8–1 for **15** and **17**, to 0.65–0.8 for **1**, **2** and **5**. Data represent the mean and standard error from a minimum of three independent assays.
[b] From Watts et al.[10]
[c] From Lewis et al.[59]
[d] From Ghosh et al.[39]

Table 3. Potency of 5 and analogues at dopamine receptors[a]

Test Ligand	EC_{50} (nM)±SEM				
	D_1-like (striatum)	D_1 (C6)	D_{2L} (C6)	D_4 (CHO)	D_5 (HEK)
Dopamine	5000[b]	530±260[c]	20±3.2[d]	1800±680	110±18
(±)-**1** (DHX)	70[b]	34±22[c]	110±21[d]	1400±480	12±4
(±)-**2** (DNS)	30[e]	44±11[c]	550±120[d]	N.R.	9.7±2.2
(±)-**5** (DNX)	87±14	8.6±3.2	26±11	52±16	12±6
(±)-**15** (*N*-allyl-DNX)	N.R.	610±200	>10 μM	170±120	N.R.
(±)-**17** (*N*-pr-DNX)	N.R.	760±220	99±9	25±17	2600±970

[a] Data represent the mean and standard error from a minimum of three independent assays. N.R. = No Response.
[b] From Brewster et al.[38]
[c] From Lewis et al.[59]
[d] From Watts et al.[11]
[e] From Ghosh et al.[39]

In the functional assays used to measure effects on cAMP, **5** had high potency (EC_{50} = 8.6 nM to 52 nM) and intrinsic activity equal to dopamine at all of the cloned dopamine receptors tested (Table 3 and Fig. 3). It was slightly less potent in striatal D_1 receptor preparations (EC_{50} = 87 nM). Whereas **5** was a potent full agonist at all of the dopamine receptors, **1** and **2** displayed slightly decreased potency at D_{2L} (EC_{50} = 110 nM and 550 nM for **1** and **2**, respectively) and D_4 receptors (EC_{50} ca. 1,400 nM for **1** and no activity detected for **2**).

Addition of *N*-alkyl substituents to **5** produced dramatic effects on functional effects in terms of adenylate cyclase activity. Both the *N*-allyl (**15**) and *N*-*n*-propyl (**17**) substituents lost all agonist activity at striatal D_1-like receptors. At cloned D_1 receptors, the *N*-allyl (**15**) and *N*-*n*-propyl (**17**) substituents significantly decreased potency (EC_{50} = 610 nM and 760 nM, respectively) compared to the parent compound **5** (EC_{50} = 8.6 nM). At D_{2L} receptors, the *N*-allyl substituent (**15**) markedly attenuated agonist activity (EC_{50} > 10 μM), whereas the *N*-*n*-propyl derivative (**17**) had only slightly decreased potency (EC_{50} = 99 nM) compared to **5** (EC_{50} = 26 nM) at this receptor. At D_4 receptors, the *N*-allyl (**15**) substituent slightly decreased potency (EC_{50} = 170 nM) whereas the *N*-propyl (**17**) substituent, by contrast, slightly increased potency (**17** EC_{50} = 25 nM) compared to the parent compound (**5** EC_{50} = 52 nM). At D_5 receptors, both *N*-alkyl substituents dramatically decreased potency (EC_{50} = 2600 nM for **17** and no response for **15**) compared to **5** (EC_{50} = 12 nM). In terms of efficacy, **15** was a weak partial agonist at all the dopamine receptors tested except for D_4, where it had significant efficacy. In contrast, **17** retains near full agonist activity at all cloned receptors except the D_5 (Fig. 3). Taken together, this preliminary structure–activity information about **5** and its *N*-allyl and *N*-n-propyl substituted analogues should prove useful in the design

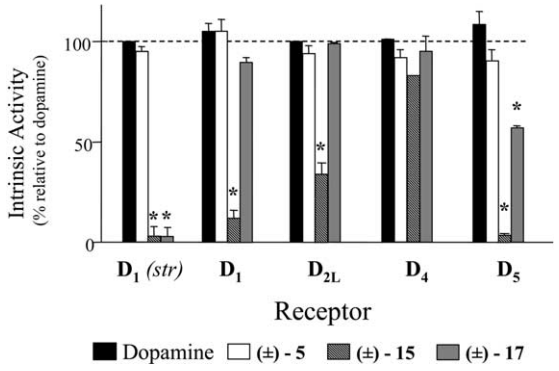


Figure 3. Maximal stimulation or inhibition of cAMP accumulation produced by **5** (DNX) and its *N*-alkyl analogues at D_1-like and D_2-like receptors. Compounds were evaluated in rat striatal tissue (D_1-like) or in cells expressing the cloned receptors (monkey D_1 stably expressed in C-6 glioma, rat D_{2L} stably expressed in C-6 glioma, human D_4 stably expressed in CHO, or human D_5 transiently expressed in HEK 293). Emax values were determined from full dose-response curves. Data represent mean±SEM from at least two experiments. * = significantly different from effects of dopamine ($P < 0.05$).

of new dopamine agonists by guiding our ability to target specific receptor isoforms.

5. Conclusions

The current work demonstrates yet another approach to tethering the β-phenyldopamine pharmacophore to afford potent and useful new dopamine receptor agonists. Although our initial hypothesis was that **5** would possess dopamine D_1/D_5 selectivity, surprisingly, we obtained a potent agonist with activity at *all* of the dopamine receptor isoforms. The subtle differences in isoform selectivity and potency for substituted derivatives of **1**, **2**, and **5** should be extremely useful information in conjunction with docking studies of these ligands within homology models of the receptors, to identify key residues that are differentially engaged in the different receptors by these structurally similar rigid analogues. Compound **5** and its analogues also may represent the first drugs that can be considered true high affinity dopamine replacements, and as such may have interesting biological properties.

6. Experimental

6.1. Chemistry

6.1.1. General procedures. All reagents were commercially available and were used without further purification, unless otherwise indicated. Dry THF and diethyl ether were obtained by distillation from benzophenone-sodium under nitrogen immediately before use. Column chromatography was carried out using silica gel 60 (230-400 mesh). J.T. Baker flexible thin layer chromatography sheets (silica gel IB2-F) were used to monitor reactions. Melting points were determined using a Thomas-Hoover apparatus and are uncorrected. ^{1}H NMR spectra were recorded using a 300 MHz Bruker ARX-300 NMR spectrometer. Chemical shifts are reported in δ values ppm relative to an internal reference (0.03%, v/v) of tetramethylsilane (TMS) in $CDCl_3$, except where noted. Chemical ionization mass spectra (CIMS) using isobutane as a carrier gas were obtained with a Finnigan 4000 spectrometer. Elemental analyses were performed by the Purdue University Microanalysis Laboratory. All the reactions were carried out under an inert atmosphere of argon.

6.1.2. 1,2-Dimethoxy-3-(methoxymethoxy)benzene (7). A stirring suspension of sodium hydride was prepared by adding 1000 mL of dry THF to 7.06 g (0.18 mol) of sodium hydride (60% dispersion in mineral oil) under an argon atmosphere at 0 °C. To the suspension, neat 2,3-dimethoxyphenol (23.64 g, 0.153 mol) was added with a syringe. The resulting mixture was allowed to warm to room temperature and stir for 2 h. The black solution was cooled to 0 °C and 13.2 mL (14 g, 0.173 mol) of chloromethyl methyl ether was slowly added by syringe. The solution was allowed to reach room temperature and stirred for an additional 8 h. The yellow mixture was concentrated to an oil that was dissolved in 1000 mL of diethyl ether. The resulting solution was washed with water (500 mL), 2N NaOH (3×400 mL), dried ($MgSO_4$), filtered, and concentrated under vacuum. After Kugelrohr distillation (90–100 °C, 0.3 atm), 24.6 g (84%) of a clear oil was obtained: ^{1}H NMR: (300 MHz, $CDCl_3$): δ 6.97 (t, 1H, J = 8.7 Hz); 6.79 (dd, 1H, J = 7.2, 1.8 Hz); 6.62 (dd, 1H, J = 6.9, 1.2 Hz); 5.21 (s, 2H); 3.87 (s, 3H); 3.85 (s, 3H); 3.51 (s, 3H). CIMS m/z: 199 ($M+H^+$, 50%); 167 ($M+H^+$-CH_3OH, 100%). Anal. cald ($C_{10}H_{14}O_4$): C, H, N.

6.1.3. 2-(3,4-Dimethoxy-2-methoxymethoxyphenyl)-4,4,5,5-tetramethyl[1,3,2]dioxaborolane (8). The MOM-protected phenol **7** (10 g, 0.0505 mol) was dissolved in 1000 mL of dry diethyl ether and cooled to −78 °C. A solution of *n*-butyl lithium in hexane (22.2 mL of 2.5 M, 0.0555 mol) was then added by syringe. The cooling bath was removed and the solution was allowed to warm to room temperature. While stirring the solution at room temperature for 2 h, a yellow precipitate formed. The mixture was cooled to −78 °C, and 2-isopropoxy-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (15 mL, 0.080 mol) was added by syringe. The cooling bath was removed after 2 h. Stirring was continued for four hours at room temperature. The mixture was then poured into 300 mL of water and extracted with diethyl ether (3×300 mL), dried (Na_2SO_4), and concentrated to a yellow oil (12.37g, 76%) that was used without further purification; ^{1}H NMR: (300 MHz, $CDCl_3$): δ 7.46 (d, 1H, J = 8.4 Hz); 6.69 (d, 1H, J = 8.4 Hz); 5.15 (s, 2H); 3.87 (s, 3H); 3.83 (s, 3H); 1.327 (s, 12H).

6.1.4. 4-Bromo-5-nitroisoquinoline (9). Potassium nitrate (5.34 g, 0.052 mol) was added to 20 mL of concentrated sulfuric acid and slowly dissolved by careful heating. The resulting solution was added dropwise to a solution of 4-bromoisoquinoline (10 g, 0.048 mol) dissolved in 40 mL of concentrated sulfuric acid at 0 °C. After removal of the cooling bath, the solution was stirred for 1 h at room temperature. The reaction mixture was then poured onto crushed ice (400 g) and made basic with ammonium hydroxide. The resulting yellow precipitate was collected by filtration and the filtrate was extracted with diethyl ether (3×500 mL), dried (Na_2SO_4), and concentrated to give a yellow solid that was combined with the initial precipitate. Recrystallization from methanol gave 12.1 g (89%) of slightly yellow crystals: mp 172–174 C; ^{1}H NMR: (300 MHz, $CDCl_3$): δ 9.27 (s, 1H); 8.87 (s, 1H); 8.21 (dd, 1H, J = 6.6, 1.2 Hz); 7.96 (dd, 1H, J = 6.6, 1.2 Hz); 7.73 (t, 1H, J = 7.5 Hz). CIMS m/z: 253 ($M+H^+$, 100%); 255 ($M+H^++2$, 100%). Anal. ($C_9H_5BrN_2O_2$) C, H, N.

6.1.5. 4-(3,4-Dimethoxy-2-methoxymethoxyphenyl)-5-nitroisoquinoline (10). Pinacol boronate ester **8** (5.56 g, 0.0172 mol), isoquinoline **9** (3.36 g, 0.0143 mol), and 1.0 g (6 mol%) of $Pd(PPh_3)_4$ were suspended in 100 mL of dimethoxyethane (DME). Potassium hydroxide (3.6 g, 0.064 mol), and 0.46 g (10 mol%) of tetrabutylammonium bromide were dissolved in 14.5 mL of water and were added to the DME mixture. The resulting suspension was degassed for 30 min with argon and then heated at reflux for 4 h. The resulting black solution was allowed to cool to room temperature, poured into 500 mL of water, extracted with diethyl ether

(3×500 mL), dried (Na_2SO_4), and concentrated. The product was then purified by column chromatography (silica gel, 50% ethyl acetate:hexane) giving 5.29 g of yellow crystals (80%); mp 138–140 °C; ^{1}H NMR: (300 MHz, $CDCl_3$): δ 9.33 (s, 1H); 8.61 (s, 1H); 8.24 (dd, 1H, J = 7.2, 0.9 Hz); 8.0 (dd, 1H, J = 6.3, 1.2 Hz); 7.67 (t, 1H, J = 7.8 Hz); 7.03 (d, 1H, J = 9.6 Hz); 6.81 (d, 1H, J = 8.1 Hz); 4.86 (d, 1H, J = 6 Hz); 4.70 (d, 1H, J = 5.4 Hz); 3.92 (s, 3H); 3.89 (s, 3H); 2.613 (s, 3H). CIMS m/z: 371 ($M+H^+$, 100%). Anal. calcd ($C_{19}H_{18}N_2O_6$) C, H, N.

6.1.6. 2,3-Dimethoxy-6-(5-nitroisoquinolin-4-yl)phenol (11). After dissolving isoquinoline **10** (5.285 g, 0.014 mol) in 200 mL of methanol by gentle heating, *p*-toluenesulfonic acid monohydrate (8.15 g, 0.043 mol) was added in several portions. Stirring was continued for four h at room temperature. After completion of the reaction (monitored by TLC), the solution was made basic by adding saturated sodium bicarbonate. The product was then extracted with dichloromethane (3×250 mL), dried (Na_2SO_4), and concentrated. The resulting yellow solid (4.65 g, 98%) was used directly in the next reaction. An analytical sample was recrystallized from methanol: mp 170–174 °C; ^{1}H NMR: (300 MHz, $CDCl_3$): δ 9.33 (s, 1H); 8.62 (s, 1H); 8.24 (dd, 1H, J = 7.2, 0.9 Hz); 7.99 (dd, 1H, J = 6.3, 1.2 Hz); 7.67 (t, 1H, J = 7.8 Hz); 6.96 (d, 1H, J = 8.7 Hz); 6.59 (d, 1H, J = 8.7 Hz); 5.88 (bs, 1H); 3.94 (s, 3H); 3.92 (s, 3H). CIMS m/z: 327 ($M+H^+$, 100%). Anal. calcd ($C_{17}H_{14}N_2O_5$) C, H, N.

6.1.7. 8,9-Dimethoxychromeno[4,3,2-*de*]isoquinoline (12). Phenol **11** (4.65 g, 0.014 mol) was dissolved in 100 mL of dry DMF. The solution was degassed with argon for thirty min. Potassium carbonate (5.80 g, 0.042 mol) was added to the yellow solution in one portion. After heating the mixture at 80 °C for 1 h, the mixture had turned brown and no more starting material remained. After cooling to room temperature, 200 mL of water was added. The aqueous layer was extracted with dichloromethane (3×500 mL), which was washed with water (3×500 mL), dried (Na_2SO_4), and concentrated. A white powder (3.65 g 92%) was obtained that was used in the next reaction without further purification. An analytical sample was recrystallized from ethyl acetate:hexane: mp 195–196 °C; ^{1}H NMR: (300 MHz, $CDCl_3$): δ 9.02 (s, 1H); 8.82 (s, 1H); 7.87 (d, 1H, J = 8.7 Hz); 7.62 (m, 3H); 7.32 (dd, 1H, J = 6.0, 1.5 Hz); δ 6.95 (d, J = 9.6 Hz); 3.88 (s, 3H); δ 3.82 (s, 3H). CIMS m/z: 280 ($M+H^+$, 100%). Anal. calcd($C_{17}H_{13}NO_3$) C, H, N. HRCIMS m/z: Calc'd: 280.0974; Found: 280.0975.

6.1.8. 8,9-Dimethoxy-1,2,3,11b-tetrahydrochromeno[4,3,2-*de*]isoquinoline (13). Platinum (IV) oxide (200 mg) was added to a solution containing 50 mL of acetic acid and isoquinoline **12** (1.0 g, 3.5 mmol). After adding 2.8 mL of concentrated HCl, the mixture was shaken for 24 h under 60 psi H_2 in a Parr hydrogenator. When the reaction was complete the green solution was filtered through Celite to remove the catalyst, and most of the acetic acid was removed by rotary evaporation. The remaining acid was neutralized using a saturated sodium bicarbonate solution, and the product was extracted into diethyl ether (3×250 mL), dried (Na_2SO_4), and concentrated. The resulting oil (0.997 g, 99%) was used without further purification: ^{1}H NMR: (300 MHz, $CDCl_3$): δ 7.10 (t, 1H, J = 7.5 Hz); δ 7.00 (d, 1H, J = 8.4 Hz); δ 6.78 (m, 2H); δ 6.60 (d, 1H, J = 9 Hz); δ 4.10 (s, 2H); δ 3.84 (m, 8H); δ 2.93 (t, 1H, J = 12.9 Hz).

6.1.9. 8,9-Dihydroxy-1,2,3,11b-tetrahydrochromeno[4,3,2-*de*]isoquinoline hydrobromide (5). Crude **13** (0.834 g, 3.0 mmol) was dissolved in 50 mL of anhydrous dichloromethane. The solution was cooled to −78 °C and 15.0 mL of a boron tribromide solution (1.0 M in dichloromethane) was slowly added. The solution was stirred overnight, while the reaction slowly warmed to room temperature. Then, after cooling the solution to −78 °C, 50 mL of methanol was slowly added to quench the reaction. The solution was then concentrated to dryness. Methanol (50 mL) was added and the solution was concentrated. This process was repeated three times. The resulting brown solid was treated with activated charcoal, filtered through Celite, and recrystallized from ethanol as yellow crystals (0.72 g, 72%): mp 298–302 °C dec; ^{1}H NMR: (300 MHz, D_2O): δ 7.12 (t, 1H, J = 9 Hz); 6.88 (d, 1H, J = 7.5 Hz); 6.83 (d, 1H, J = 7.5 Hz); 6.46 (d, 1H, J = 6 Hz); 6.40 (d, 1H, J = 6 Hz); 4.20 (q, 2H, J = 15 Hz); 4.07 (m, 1H); 3.93 (m, 1H); 2.91 (t, 1H, J = 12 Hz). Anal. calcd ($C_{15}H_{14}BrNO_3 \cdot H_2O$) C, H, N.

6.1.10. *N*-Allyl-8,9-dimethoxy-1,2,3,11b-tetrahydrochromeno[4,3,2-*de*]isoquinoline (14). The tetrahydroisoquinoline **13** (1.273 g, 4.5 mmol) was dissolved in 150 mL of acetone. Potassium carbonate (0.613 g, 4.5 mmol) and 0.4 mL (4.6 mmol) of allyl bromide were added. The reaction was stirred at room temperature for 4 h. The mixture was then filtered and the filtered solids washed several times with ether. The filtrate was concentrated and purified by flash chromatography (silica gel, 50% ethyl acetate:hexane) to afford 1.033 g (71%) of a yellow oil that was used without further purification: ^{1}H NMR: (300 MHz, $CDCl_3$): δ 7.15 (t, 1H, J = 9 Hz); 7.04 (d, 1H, J = 9 Hz); 6.83 (m, 2H); 6.65 (d, 1H, J = 6 Hz); 5.98 (m, 1H); 5.27 (m, 2H); 4.10 (m, 3H); 3.95 (s, 3H); 3.86 (s, 3H); 3.46 (d, 1H, J = 15 Hz); 3.30 (d, 2H, J = 6 Hz); 2.56 (t, 1H, J = 12 Hz).

6.1.11. *N*-Allyl-8,9-dihydroxy-1,2,3,11b-tetrahydrochromeno[4,3,2-*de*]isoquinoline (15). The *N*-Allylamine **14** (0.625 g, 1.93 mmol) was dissolved in 50 mL of dichloromethane. The solution was cooled to −78 °C and 10.0 mL of a boron tribromide solution (1.0 M in dichloromethane) was slowly added. The solution was stirred overnight, while the reaction slowly warmed to room temperature. After cooling the solution to −78 °C, 50 mL of methanol was slowly added to quench the reaction. The reaction was then concentrated to dryness. Methanol (50 mL) was added and the solution was concentrated. This process was repeated three times. Recrystallization of the brown solid from ethanol gave 0.68 g (61%) of a white solid: mp 251–253 °C dec; ^{1}H NMR: (300 MHz, D_2O): δ 10.55 (s, 1H); 10.16 (s, 1H); 8.61 (t, 1H, J = 9 Hz); 8.42 (d, 1H, J = 9 Hz); 8.31 (d, 1H, J = 9 Hz); 7.87 (d, 1H, J = 9 Hz); 7.82 (d, 1H, J = 9

Hz); 7.36 (q, 1H, $J=9$ Hz); 6.89 (m, 2H); 6.85 (d, 1H, $J=15$ Hz); 5.58 (m, 3H); 5.28 (m, 2H); 3.76 (d, 1H, $J=3$ Hz). Anal. calcd($C_{18}H_{18}BrNO_3$): C, H, N. HRCIMS *m*/*z*: Calc'd: 295.1208; Found: 295.1214.

6.1.12. *N*-*n*-Propyl-8,9-dimethoxy-1,2,3,11b-tetrahydrochromeno[4,3,2-*de*]isoquinoline (16). The *N*-Allyl amine **14** (1.033 g, 3.2 mmol) was dissolved in 50 mL of ethanol. Palladium (10% dry weight; 0.103 g) was then added. The mixture was shaken for 3 h under 60 psi H_2 in a Parr hydrogenator. After TLC showed the absence of starting material, the mixture was filtered through Celite and concentrated to give 0.95 g (91%) of an oil that was used without further purification: ^{1}H NMR: (300 MHz, $CDCl_3$): δ 7.15 (t, 1H, $J=7.2$ Hz); 7.04 (d, 1H, $J=8.1$ Hz); 6.84 (t, 2H, $J=7.5$ Hz); 6.65 (d, 1H, $J=8.4$ Hz); 4.07 (m, 2H); 3.95 (s, 3H); 3.86 (s, 3H); 3.71 (q, 1H, $J=5.1$ Hz); 3.42 (d, 2H, $J=15.6$ Hz); 2.62 (m, 2H); 2.471 (t, $J=10.5$ Hz); 1.69 (h, 2H, $J=7.2$ Hz); 0.98 (t, 3H, $J=7.5$ Hz). CIMS *m*/*z*: 326 ($M+H^+$, 100%).

6.1.13. *N*-*n*-Propyl-8,9-dihydroxy-1,2,3,11b-tetrahydrochromeno[4,3,2-*de*]isoquinoline (17). The *N*-propyl amine **16** (0.90 g, 2.8 mmol) was dissolved in 200 mL of dichloromethane and cooled to −78 °C. In a separate 250 mL round bottom flask, 125 mL of dry dichloromethane was cooled to −78 °C, and 1.4 mL (14.8 mmol) of BBr_3 was added by syringe. The BBr_3 solution was then transferred using a cannula to the flask containing the solution of **16**. The reaction was stirred overnight, while slowly warming to room temperature. After cooling the solution to −78 °C, 50 mL of methanol was slowly added to quench the reaction. The reaction was then concentrated to dryness. Methanol (50 mL) was added and the solution was concentrated. This process was repeated three times. The resulting tan solid was suspended in hot isopropyl alcohol. Slowly cooling to room temperature resulted in a fine yellow precipitate. The solid was collected by filtration (0.66 g, 63%): mp 259–264 °C dec; ^{1}H NMR: (300 MHz, D_2O): δ 7.16 (t, 1H, $J=9$ Hz); 6.97 (d, 1H, $J=12$ Hz); 6.83 (d, 1H, $J=9$ Hz); 6.55 (d, 1H, $J=9$ Hz); 6.46 (d, 1H, $J=9$ Hz); 4.45 (d, 1H, $J=15$ Hz); 4.10 (m, 3H); 3.17 (q, 2H, $J=6$ Hz); 3.04 (t, 1H, $J=9$ Hz); 1.73 (q, 2H, $J=9$ Hz); 0.90 (t, 3H, $J=6$ Hz). Anal. calcd($C_{18}H_{20}BrNO_3$) C, H, N.

6.2. Pharmacology methods

6.2.1. Materials. [^{3}H]-SCH23390 (specific activity ≈70 Ci/mmol) was synthesized according to the method of Wyrick et al.[58] [^{3}H]-Spiperone (specific activity = 65-140 Ci/mmol) was purchased from Amersham (Piscataway, NJ). Dihydrexidine (**1**) and dinapsoline (**2**) were synthesized according to published methods.[39,56] Chlorpromazine HCl was a gift from SmithKline Beecham and other ligands were purchased from Research Biochemicals, Inc. (Natick, MA). Antibodies were purchased from Advanced Magnetics (Cambridge MA). All reagents and supplies for cell culture were obtained from known commercial sources.

6.2.2. Receptor binding and functional assays. Novel compounds were evaluated for binding affinity and functional activity at D_1-like and D_2-like receptors, as described previously,[10,59,60] utilizing both rat striatum and cell lines expressing the various receptor subtypes. D_1 and D_5 binding affinities were evaluated in the same manner, essentially as described in Watts et al.[10] for rat striatum and Lewis et al.[59] for cell line assays. D_2-like receptor binding and functional assays were performed essentially as described previously.[60]

For radioligand binding studies at dopamine receptors in membranes prepared from rat striatum, receptors were labeled with 0.3 nM [^{3}H]-SCH23390 (for D_1-like) or 0.07 nM [^{3}H]-spiperone (for D_2-like) with 100 nM ketanserin to mask 5-HT_2 receptors. Total binding was defined in the absence of test ligand and nonspecific binding was defined by the addition of 1 μM SCH23390 (for D_1-like) or 1 μM chlorpromazine (for D_2-like). Test ligands were incubated with membranes (150–200 μg protein/assay tube) for 15 min prior to harvesting by vacuum filtration.

Radioligand binding studies were also carried out in membranes prepared from cell lines expressing the cloned receptors (monkey D_1, rat D_{2L}, or rat D_3 stably expressed in C-6 glioma; human D_4 stably expressed in CHO; human D_5 transiently transfected into HEK 293 cells using Lipofectamine, following the manufacturer's recommended guidelines). Receptors were labeled with either 0.3 or 1.0 nM [^{3}H]-SCH23390 (for D_1 and D_5, respectively) or 0.07 nM [^{3}H]-spiperone (for D_2-like). Test ligands were incubated with membranes (10-40 μg protein) for 15 min prior to harvesting by vacuum filtration.

Functional potency was measured as follows. D_1-like functional assays were done as described in Watts et al.[10] using broken cell membranes from both rat striatum and cell lines, followed by RIA to determine cAMP levels. For D_1-like receptors, adenylate cyclase activity was determined in membranes prepared from frozen rat striatal tissue. For cloned receptors expressed in cells, adenylate cyclase activity was measured in whole cell preparations (for D_{2L} and D_4) or membranes (for D_{1A} and D_5). Tissue samples (20 μg protein for striatal tissue or 10–20 μg protein/well for cell membranes) were incubated with 100 mM HEPES (pH 7.4), 100 mM NaCl, 4 mM $MgCl_2$, 2 mM EDTA, 500 μM isobutyl methylxanthine, 0.01% ascorbic acid, 10 μM pargyline, 2 mM ATP, 5 μM GTP, 20 mM phosphocreatine, 5 units of creatine phosphokinase, and test ligand. After incubating for 15 min at 30 °C, the reaction was terminated by addition of 0.1 N HCl. The concentrations of accumulated cAMP were assessed by radioimmunoassay.

6.2.3. Data and statistical analyses. Receptor binding data were analyzed using algorithms in Prism 3.03 (GraphPad, Inc, San Diego, CA). The data were fit to a sigmoidal curve with variable slope to provide a slope coefficient. Final affinity data are expressed as $K_{0.5}$ by correcting the experimentally-derived IC_{50} values for radioligand concentration using the one-site Cheng–Prusoff equation.[61]

For functional assays, data were expressed as fmol/mg protein. Dose–response curves were plotted as a percentage of the maximal dopamine response versus log concentration of test ligand. Data were analyzed as a sigmoidal curve with variable slope (GraphPad Prism) to determine EC_{50} and E_{max}. Competition curves were analyzed by nonlinear regression to determine estimates for $K_{0.5}$ and Hill slope (n_H). For each receptor, efficacy data (i.e., E_{max}) for **5** and its analogues were compared to the full agonist dopamine using an unpaired Student's *t*-test to determine statistical significance. A $p < 0.01$ was considered significant (as denoted by * in Fig. 3).

Elemental analysis data

Compd	%C		%H		%N	
	Calcd	Found	Calcd	Found	Calcd	Found
5	50.87	51.18	4.55	4.31	3.82	3.95
7	60.59	60.93	7.12	7.16	NA	NA
9	42.72	42.59	1.99	1.76	11.07	10.87
10	61.62	61.66	4.90	4.81	7.56	7.49
11	62.57	62.18	4.32	4.38	8.58	8.35
12	73.11	72.85	4.69	4.40	5.02	4.86
15	57.46	56.82	4.82	4.80	3.72	3.61
17	57.16	56.78	5.33	5.26	3.70	3.65

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