

Novel Bivalent Ligands for D2/D3 Dopamine Receptors: Significant Cooperative Gain in D2 Affinity and Potency

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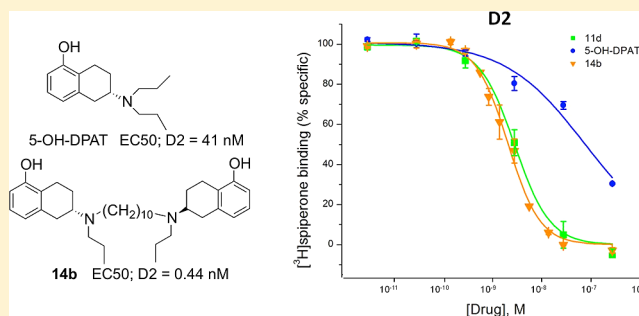
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Supporting Information

ABSTRACT: This report describes development of a series of novel bivalent molecules with a pharmacophore derived from the D2/D3 agonist 5-OH-DPAT. The spacer length in the bivalent compounds had a pronounced influence on affinity for D2 receptors. A 23-fold increase of D2 affinity was observed at a spacer length of 9 or 10 (compounds **11d** and **14b**) as compared to monovalent 5-OH-DPAT (K_i ; 2.5 and 2.0 vs 59 nM for **11d** and **14b** vs 5-OH-DPAT, respectively). The functional potency of **11d** and **14b** indicated a 24- and 94-fold increase in potency at the D2 receptor as compared to 5-OH-DPAT (EC_{50} ; 1.7 and 0.44 vs 41 nM for **11d** and **14b** vs 5-OH-DPAT, respectively). These are the most potent bivalent agonists for the D2 receptor known to date. This synergism is consonant with cooperative interaction at the two orthosteric binding sites in the homodimeric receptor.

KEYWORDS: bivalent ligands, D2/D3 dopamine receptors, cooperative gain, affinity, potency, synergism



GPCRs make up one of the largest and diverse family of mammalian proteins. Consequently, GPCRs are targeted most frequently in therapeutics development for numerous diseases.^{1,2} The prototypical GPCR structure consists of seven trans membrane domains as the central core, connected by extracellular and intracellular loops. The traditional concept of participation of a monomeric receptor in the signal transduction process has been increasingly challenged by evidence for the existence of homo- and heterodimeric receptors.³ A number of GPCRs have been shown to form dimers, which include opioid receptors,⁴ adrenergic receptors,⁵ and muscarinic receptors.⁶ Dopamine receptors are also G protein-coupled and act via modulation of adenylylase cyclase activity. Both dopamine D2 and D3 receptors can exist as homodimers in cell lines expressing these receptors.^{7,8} Furthermore, the dopamine D2 receptor has been shown to form a dimer in human and rat brain tissues.⁹ Recently, dopamine D1 and D2 receptors also have been shown to form heterodimers.¹⁰

Bivalent ligands for GPCRs have been reported going back more than a decade.^{4,11,12} Bivalent ligands typically consist of two pharmacophoric moieties connected by a spacer with an appropriate length, which then allows the two "heads" to interact either with two orthosteric binding sites of receptors on adjacent protomers of a dimer or with another relevant, allosteric site at the same receptor. Bivalents are classified as either homo- or heterobivalent ligands depending on the nature of pharmacophores.¹³ Such simultaneous cooperative inter-

action results in increased affinity theoretically equivalent to the product of the binding contribution of both putative sites.^{14,15} This can also give rise to higher functional potency, greater selectivity, and better therapeutic agents.¹⁶ Bivalent ligands with varying linker lengths for dopamine receptors and other GPCR have been reported.^{17–21}

In our quest to probe dimeric forms of either D2 or D3 receptors in cells expressing these cloned receptors, we have designed a prototypical structure of bivalent molecules by linking the well-known dopamine D2/D3 agonist molecule 5-hydroxy-2-(dipropylamino)tetralin (5-OH-DPAT) via a methylene spacer of varying length (Figure 1). Site-directed mutagenesis has demonstrated that the amino tetralin moiety interacts with an agonist binding domain involving TM-3 and TM-5 for activation of either D2 or D3 receptors.^{22,23} In our own study, we have demonstrated the critical importance of basic nitrogen and hydroxyl functionalities in TM3 and TM5 for binding activity of 5-OH-DPAT.²⁴ Suitable potent bivalent agonists or antagonists for dopamine receptors might find potential therapeutic use for treatment of neuropsychiatric and neurodegenerative diseases like Parkinson's disease.

Scheme 1 describes synthesis of target compound **5**. Optically active (–)-5-methoxy aminotetralin²⁵ was acylated

Received: July 23, 2012

Accepted: October 26, 2012

Published: October 26, 2012

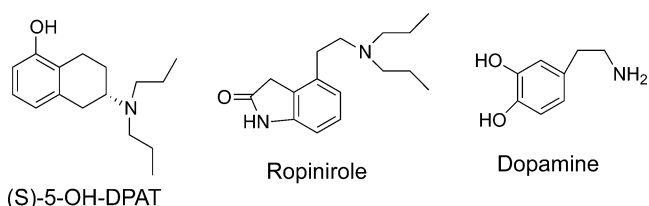


Figure 1. Molecular structures of known dopamine D2 and D3 agonists.

with chloroacetyl chloride under basic conditions to produce intermediate **2**. Further N-alkylation of **1** with intermediate **2** in presence of a base provided **3**, which upon reduction with lithium aluminum hydride (LAH) produced **4**. Finally, demethylation in presence of boron tribromide at $-40\text{ }^{\circ}\text{C}$ produced the final bivalent target compound **5**.

Similarly, Scheme 2 describes synthesis of compounds **11a–d**. Compound **1** was treated with either appropriate acid chloride or acid to yield intermediates **7a–d**. Hydrolysis of ester by lithium hydroxide produced acid intermediate **8a–d**. Acid coupling with amine **1** under standard 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide-hydrochloride (EDCI) coupling conditions in the presence of hydroxybenzotriazole (HOBt) produced amide intermediate **9a–d**. Reduction of amide followed by demethylation yielded the final compounds **11a–d**.

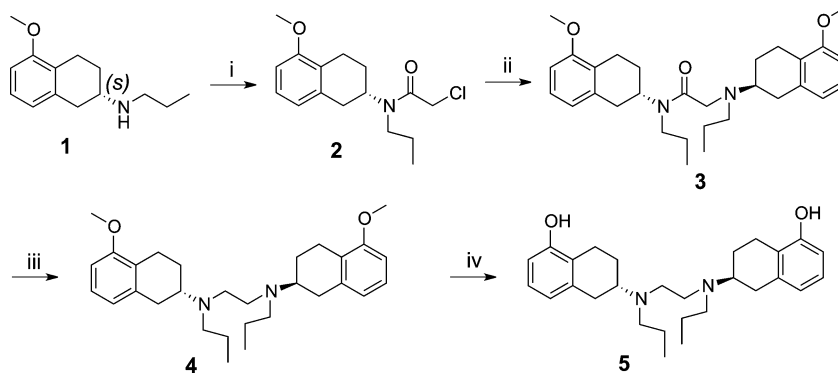
Scheme 3 describes synthesis of compounds **14a–d**. Two molecules of amine **1** were coupled with the appropriate diacid to produce intermediate diamides **12a–d**, which on reduction with LAH produced amines **13a–d**. The final targets **14a–d** were synthesized after demethylation with boron tribromide. Scheme 4 describes the synthesis of compounds **16** and **19**. In both cases, straightforward synthesis approaches were adopted.

In our design of this series of bivalent ligands, we incorporated methylene units to build the spacer separating the two pharmacophoric head groups, with a spacer length varying from 2 to 14 methylene units (Table 1). We included the agonist 5-OH-DPAT as the parent compound for comparison purposes. The four-linker **11a** exhibited similar affinity at the D2 receptor as 5-OH-DPAT (K_i ; 70 vs 59 nM for **11a** vs 5-OH-DPAT, respectively) (Table 1). Moving from a 4-linker to a 5-linker did not affect the affinity of **11b** significantly as compared to **11a**. Interestingly, switching from the 5-linker (**11b**) to the 6-linker (**11c**) induced a significant, 12-fold

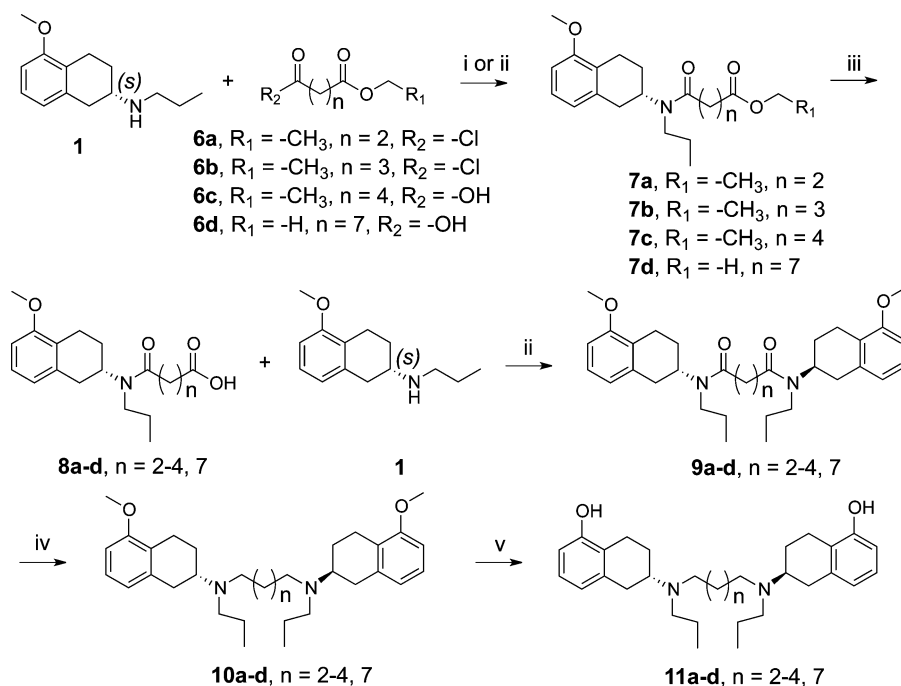
increase, consonant with a cooperative interaction resulting in synergistic enhancement of affinity at D2. The synthesis of 7-, 9- and 10-linkers produced **14a**, **11d**, and **14b**, which exhibited further improvement in affinity, with 9- and 10-linkers producing the highest affinity for D2 receptors. A further increase of the spacer length to 12 methylene units (**14c**) decreased the affinity to a level that was comparable to compound **5** with a linker length of 2. Upon further increase of the spacer length to 14 (**14d**), the affinity dropped to that of 5-OH-DPAT (see also Figure 2). At the D3 receptor, less dramatic changes occurred upon varying the linker length, and the 4-linker compound **11a** started the bivalent compounds of this spacer length at an affinity lower (higher K_i) than the parent compound 5-OHDPAT (Table 1 and Figure 2). Increasing the length from four linkers up gradually (but modestly) increased D3 affinity, with the 6-linker compound displaying the first significant affinity enhancement, just as observed for D2 affinity. The highest D3 affinity was seen with a linker length of nine methylene units [**11d**, K_i (D3); 0.91 nM], whereas further elongation (**14b**, **14c**, and **14d**) resulted in a lowering of affinity. In comparison, in the case of the D2 receptor, **11d** along with **14b** with a 10-linker spacer, produced the highest affinity [K_i (D2); 2.0–2.5 nM; statistically the two compounds were indistinguishable at D2]. Compounds **11d** and **14b** were selected for further functional analysis (see below).

The gain in affinity for **11d** and **14b** at D2 (and to a lesser extent at D3) is consonant with a cooperative effect of simultaneous interaction of the two heads with two distal binding sites. As pointed out by Kuhhorn et al.,¹⁷ binding of bivalent ligands to two sites will induce cooperativity with binding of the first head facilitating binding of the second head due to being placed in the vicinity of the second binding site. This binding cooperativity can be reflected in increased Hill numbers (as compared with unity for binding of monovalent neutral antagonists). Thus, neutral antagonist binding to D2 receptors displays Hill numbers up to 2.0 in the case of the bivalent compounds in the study of Kuhhorn et al.¹⁷ However, McRobb et al.¹⁹ report no increases in Hill numbers over unity for clozapine propylamine bivalent antagonists at D2 receptors, indicating that this is a ligand-dependent phenomenon. Full monovalent agonists, recognizing high-affinity D2 states, generally display Hill numbers below unity (0.5–0.7).¹⁷ Our compounds are full agonists (Table 2) and display increases in

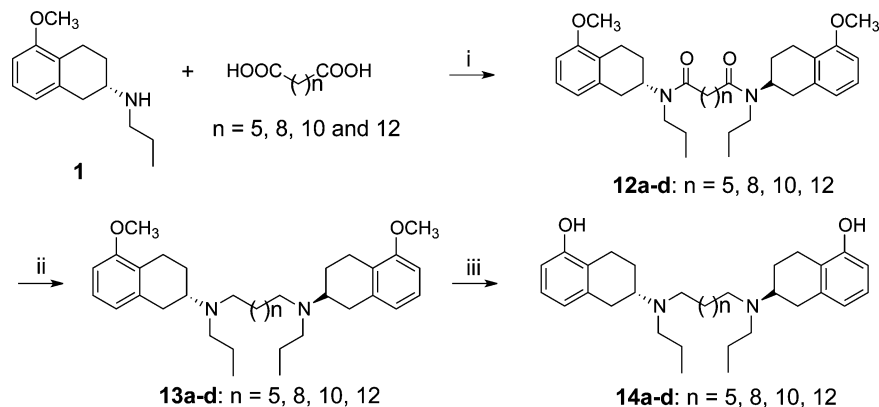
Scheme 1^a



^aReagents and conditions: (i) Chloroacetyl chloride, Et₃N, CH₂Cl₂, 0 °C to RT, 2 h. (ii) (S)-5-Methoxy-1,2,3,4-tetrahydro-naphthalen-2-yl)-propylamine (**1**), K₂CO₃, acetonitrile, reflux, 3 h. (iii) LiAlH₄, THF, 80 °C, 3 h. (iv) BBr₃, CH₂Cl₂, $-40\text{ }^{\circ}\text{C}$ to RT, 12 h.

Scheme 2^a

^aReagents and conditions: (i) Et_3N , CH_2Cl_2 , 0°C to RT, 3 h. (ii) EDCl , HOBT , Et_3N , CH_2Cl_2 , RT, 12 h. (iii) LiOH , $\text{MeOH}:\text{H}_2\text{O}$ (3:2), RT, 12 h. (iv) LiAlH_4 , THF , 80°C , 3 h. (v) BBr_3 , CH_2Cl_2 , -40°C to RT, 12 h.

Scheme 3^a

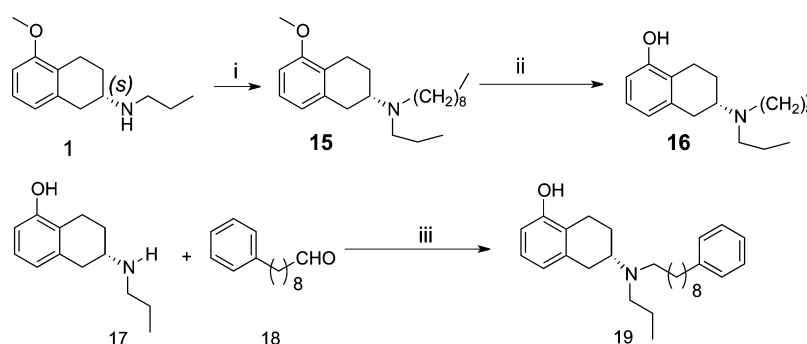
^aReagents and conditions: (i) For compound **12a**: (1,5)-heptanedioic acid, $\text{EDCl}\cdot\text{HCl}$, HOBT , Et_3N , CH_2Cl_2 , rt, 24 h; for compound **12b**: (1,8)-decanedioic acid, $\text{EDCl}\cdot\text{HCl}$, HOBT , Et_3N , CH_2Cl_2 , rt, 24 h; for compound **12c**: (1,10)-dodecanedioic acid, $\text{EDCl}\cdot\text{HCl}$, HOBT , Et_3N , CH_2Cl_2 , rt, 24 h; for compound **12d**: (1,12)-tetradecanedioic acid, $\text{EDCl}\cdot\text{HCl}$, HOBT , Et_3N , CH_2Cl_2 , rt, 24 h. (ii) LiAlH_4 , THF , reflux, 3 h. (iii) BBr_3 , CH_2Cl_2 , -78°C to rt, 12 h.

Hill number as a function of bivalent binding to D2 (Table 1). Thus, **11a**, the 4-linker compound, has a Hill less than unity as does 5-OH-DPAT, and with increasing linker length, the Hill numbers go up in the 1.3–1.8 range. The two bivalent compounds with the highest D2 potency, **11d** and **14b**, had Hill numbers of 1.3 and 1.4, with clearly steeper binding inhibition curves as compared with 5-OH-DPAT with an average Hill of 0.8. Therefore, our Hill numbers strongly indicate cooperative binding of our lead bivalent compounds.

To assess any contribution of a nine methylene spacer, by itself, to affinity for D2/D3 receptors, monovalent **16** with a nine methylene spacer length was synthesized and monitored for D2/D3 receptor binding. It is apparent that **16** exhibited similar affinity for D2 as 5-OH-DPAT (K_i ; 59 vs 68.8 nM for 5-

OH-DPAT vs **16**, respectively) and exhibited lower affinity for D3 as compared to 5-OH-DPAT. In our effort to further evaluate any contribution of hydrophobic or related effect in production of high affinity in **11d** and **14b**, nine-linker **19** with a terminal phenyl group was designed and synthesized. The binding data indicate much weaker activity of **19** at D2 ($K_i = 141$) and D3 receptors (23 nM). This clearly indicates that the methylene spacer units did not contribute toward the enhanced affinity of **11d** and **14b**. It furthermore indicates that enhanced interaction of lead bivalent compounds does not result from enhanced hydrophobic or π -stacking interaction.

Following binding analysis, selected lead **11d** and **14b** were subjected to the $[35\text{S}]\text{GTP}\gamma\text{S}$ (guanosine 5'-[γ -thio]-triphosphate) functional assay for D2 and D3 receptors and

Scheme 4^a

^aReagents and conditions: (i) Nonanal, NaCNBH₃, acetic acid, 1,2-dichloroethane, 12 h, rt. (ii) 48% aqueous HBr, reflux, 6 h. (iii) NaCNBH₃, CH₃OH, room temperature, overnight.

Table 1. Inhibition Constants for Competing for [³H]Spiperone Binding to Cloned rD_{2L} and rD₃ Receptors Expressed in HEK Cells^a

D-compound	K _i (nM)			D2/ D3
	HEK-rD2	Hill slopes HEK-rD2	HEK-rD3	
parent				
5-OH-DPAT	59 ± 11	0.83 ± 0.07 ^b	1.4 ± 0.3	43
bivalent (spacer length)				
5 (2)	16 ± 2	1.1 ± 0.1	1.1 ± 0.3	14
11a (4)	70 ± 22	0.56 ± 0.04	4.0 ± 1.0	17
11b (5)	75 ± 19	0.72 ± 0.06	3.6 ± 0.6	21
11c (6)	5.9 ± 0.2	1.1 ± 0.01	2.2 ± 0.4	2.7
14a (7)	3.9 ± 0.7	1.5 ± 0.2	1.1 ± 0.4	3.6
11d (9)	2.5 ± 0.5	1.3 ± 0.1	0.9 ± 0.3	2.7
14b (10)	2.0 ± 0.4	1.4 ± 0.1	1.8 ± 0.6	1.1
14c (12)	11 ± 2	1.8 ± 0.2	10 ± 3	1.0
14d (14)	55 ± 11	1.4 ± 0.2	31 ± 5	1.7
monovalent				
16	69 (4) ± 9		7.3 ± 1.1	9.4
19	140 ± 30		23 ± 4	6.1

^aResults are means ± SEMs for 3–7 experiments each performed in triplicate. ^bAverage of conglomerate of 27 separate experiments (including data sets collected previously under the same conditions).

compared with the full agonists dopamine, ropinirole, and 5-OH-DPAT (Table 2). The assays were carried out with the cloned human D2 and D3 receptors expressed in Chinese hamster ovary (CHO) cells. Dopamine and ropinirole, as observed by previously, were more potent in activating D3 than D2. The results also indicated that **11d** and **14b** potently stimulate both D2 and D3 receptors. In this regard, **11d** and **14b** exhibited more than 24- and 94-fold potency as compared to its monovalent counterpart 5-OH-DPAT in activating the D2 receptor (EC₅₀ values of 1.7 and 0.44 nM, respectively, vs 41 nM for 5-OH-DPAT, Table 2 and Figure 2), gains similar to or exceeding those observed for binding affinity. The 94-fold increase for **14b** indicates remarkable synergistic enhancement of potency for this compound for the D2 receptor. The linker length of our lead bivalent compounds is ~13.5 Å (between the two pharmacophoric N-atoms) or ~22.6 Å (between the two pharmacophoric –OH groups), which is different from the bivalent compounds reported by others. In this regard, there are a number of different linker lengths for bivalent interactions that have been reported in the literature. The antagonist/

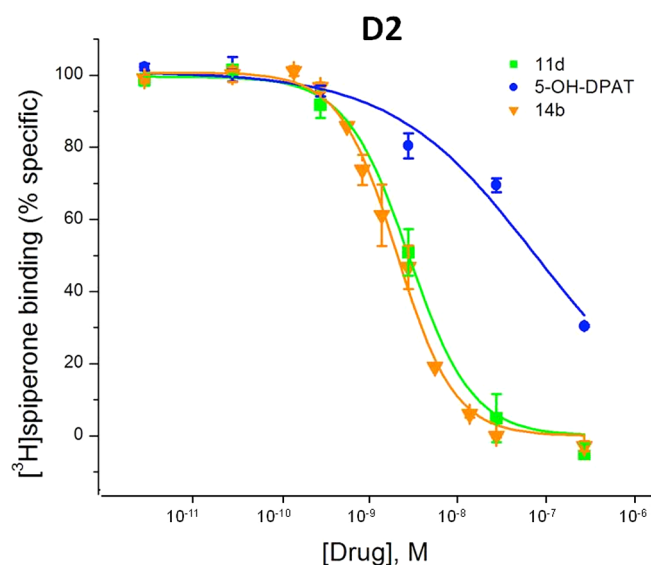


Figure 2. Interaction of **11d**, **14b**, and 5-OH-DPAT with rD2 receptors expressed in HEK-293 cells. Isotherms were obtained in competition experiments with [³H]spiperone as described in the experimental section (see the Supporting Information). The Hill slopes for the curve **11d** (n_H = 1.31) and **14b** (n_H = 1.43) were higher than monovalent 5-OH-DPAT (n_H = 0.83), indicating a possible cooperative binding interaction with the D2 receptor dimer. Each point is the average ± SEM (vertical bar) of 4–6 independent experiments assayed in triplicate. For K_i values for each curve, see Table 1. The averages shown for 5-OH-DPAT are from a subset of six experiments. The x-axis concentrations are expressed as K_i taking into account the conversion from IC₅₀ as indicated in the methods (see the Supporting Information).

antagonist bivalent ligands reported by Gmeiner et al. for interaction at the D2 receptor were based on aryl piperazine and aminoindane moieties.^{17,18} In a recent report by McRobb et al. on a bivalent antagonist, clozapine-like compound at the D2 receptor, a different, shorter linker length was reported.¹⁹ In addition, for agonist interaction at other GPCRs, a shorter linker length was also reported for optimal interaction.²¹ At this point, it is not known what dominant interaction forces might play a role in initiating the binding interaction of these varying bivalent ligands with the D2 homodimer.

In the case of the D3 receptor, there was not the same gain for bivalent interaction as observed for D2 (Table 2). The present bivalent ligands have higher receptor affinities for D3 receptor to begin with (as compared with their corresponding

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

compd	hCHO-D2		hCHO-D3		D2/D3
	EC ₅₀ (nM) ^b [³⁵ S]GTPγS	% E _{max}	EC ₅₀ (nM) ^b [³⁵ S]GTPγS	% E _{max}	
dopamine	240 ± 40	100	5.8 ± 1.3	100	42
ropinirole ^b	300 ± 10	74 ± 0.9	10 ± 2	67 ± 8	30
5-OH-DPAT	41 ± 6	80 ± 4	0.63 ± 0.08	75 ± 4	65
11d	1.7 ± 0.1	100 ± 3	0.53 ± 0.04	92 ± 1	3.2
14b	0.44 ± 0.09	97 ± 4	0.94 ± 0.28	74 ± 5	0.46

^aEC₅₀ values (nM) are means ± SEMs for 3–6 experiments each performed in triplicate. ^bData from ref 27.

D2 affinities and potencies, Tables 1 and 2); therefore, it may be more difficult to show additional gains in potency through bivalent interactions. D3 receptors also couple to G protein less effectively than D2 receptors,²⁶ and we speculate that this underlies the generally higher Hill numbers observed for D3 binding (data not shown).

In summary, we have designed a novel series of bivalent ligands for interaction with dopamine D2 and D3 receptors. The results demonstrate an effect of chain length on affinity and potency of our bivalent molecules for their interaction with dopamine receptors, primarily of the D2 subtype (see Figure 2). At the critical chain length of six methylene groups in **11c**, a significant increase in affinity for D2 receptors took place as compared to spacer length of five methylene groups in **11b**. As mentioned earlier, such a 12-fold increase in affinity might indicate initiation of synergistic cooperative binding. Further optimization led to bivalent **11d** and **14b** with enhanced affinity at both D2 and D3 receptors. Such interaction was further substantiated by results on the functional activity of **11d** and **14b**, which indicated a significant 26–94-fold enhancement of D2 potency as compared to monovalent 5-OH-DPAT. These bivalent compounds, **14b** in particular, are the most potent bivalent D2 agonists known to date. The significant enhancement in D2 potency is in agreement with interaction at the two orthosteric binding sites at two different D2 protomers in the homodimeric receptor.

■ ASSOCIATED CONTENT

Supporting Information

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

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Funding

This work is supported by the National Institute of Neurological Disorders and Stroke/National Institute of Health (NS047198, A.K.D.).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to Dr. K. Neve, Oregon Health and Science University (Portland, OR), for D2L and D3 expressing HEK cells. We are also grateful to Dr. J. Shine, Garvan Institute for Medical Research (Sydney, Australia), for D2L expressing CHO cells.

■ ABBREVIATIONS

GTPγS, guanosine 5'-[γ-thio]triphosphate; 5-OH-DPAT, 5-hydroxy-2-(dipropylamino)tetralin; CHO, Chinese hamster ovary; HEK, human embryonic kidney

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