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Synthesis and pharmacological characterization of novel *trans*-cyclopropylmethyl-linked bivalent ligands that exhibit selectivity and allosteric pharmacology at the dopamine D₃ receptor (D₃R)

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Abstract

The development of bitopic ligands directed toward D₂-like receptors has proven to be of particular interest to improve the selectivity and/or affinity of these ligands and as an approach to modulate and bias their efficacies. The structural similarity between dopamine D₃ receptor (D₃R)-selective molecules that display bitopic or allosteric pharmacology and those that are simply competitive antagonists are subtle and intriguing. Herein we synthesized a series of molecules in which the primary and secondary pharmacophores were derived from the D₃R-selective antagonists SB269,652 (**1**) and SB277011A (**2**) whose structural similarity and pharmacological disparity provided the perfect templates for SAR investigation. Incorporating a *trans*-cyclopropylmethyl linker between pharmacophores and manipulating linker length resulted in the identification of two bivalent non-competitive D₃R-selective antagonists, **18a** and **25a**, which

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ASSOCIATED CONTENT

Supporting information. Elemental analysis results. This material is available free of charge via the Internet at <http://pubs.acs.org>.

further delineates SAR associated with allosterism at D₃R and provides leads toward novel drug development.

Keywords

dopamine D₃ receptor; dopamine D₂ receptor; antagonist; allosteric modulator; bitopic ligand; cyclopropyl; primary pharmacophore; secondary pharmacophore; synthon; orthosteric binding site; β -arrestin

INTRODUCTION

The neurotransmitter dopamine signals through a family of five G protein-coupled receptors (GPCRs), subdivided into dopamine D₁-like (D₁R and D₅R) and dopamine D₂-like (D₂R, D₃R and D₄R) families, based on sequence similarities and pharmacological actions.¹ Although D₂-like receptor antagonists have long been prescribed for the treatment of schizophrenia, lack of efficacy and potential extrapyramidal and/or metabolic syndrome-like side effects have precluded successful application to substance abuse disorders.^{2,3}

Since it was cloned and characterized, the D₃R has been a target of pharmacotherapeutic interest due to its relatively localized expression within mesolimbic neurocircuitry, including the nucleus accumbens, islands of Calleja and ventral striatum.^{4–7} As these brain regions are associated with rewarding and motivational characteristics of addictive drugs, the D₃R has been an appealing drug development target for substance abuse disorders.^{8–11}

Previous structure-activity relationships (SAR) developed for the D₃R have revealed 4-phenylpiperazine as a classical primary pharmacophore (PP), which binds to the orthosteric binding site (OBS) and is primarily responsible for receptor binding affinity and efficacy.^{12–15} A four-carbon linking chain with or without substituents (e.g. OH or F) or structural rigidity (e.g. *trans*-cyclohexyl or *trans*-olefin) can be attached to an extended aryl ring system, generally through an amide group, to create a secondary pharmacophore (SP) which, when suitably designed, results in D₃R selectivity.^{13,16–18}

Hybrid molecules that concomitantly engage both an orthosteric and an allosteric site on a receptor have been termed ‘bitopic’ or ‘dualsteric’ ligands. As such, bitopic ligands can be viewed as a specific class of bivalent ligands. The development of bitopic ligands has proven to be of particular interest as a means of improving the selectivity and/or affinity of ligands for GPCRs as well as an approach to modulate and bias their efficacy.^{19–23}

SB269,652 (**1**) is a potent and D₃R-preferential antagonist that has been shown to act at this receptor in a non-competitive or allosteric manner.^{15,24} Interestingly, this tetrahydroisoquinoline derivative is also an allosteric antagonist at the D₂R; a mode of action at this receptor has been proposed whereby **1** engages one protomer of a D₂R dimer in a bitopic manner to negatively modulate the binding of dopamine to the second protomer.^{24–27} Conversely, SB277011A (**2**) is a competitive D₃R-selective antagonist, first described in 2000 and extensively used as an *in vivo* tool to characterize the D₃R and its potential as a target for medication development.²⁸ The only structural differences between these two

compounds are the position of the CN group on the tetrahydroisoquinoline ring system (PP) and the replacement of the terminal 2-indole amide (SP) of **1** with a 4-quinoline amide on **2**. However, while both molecules display selectivity for the D₃R and display high structural similarity, they differ in their competitive versus allosteric pharmacology. Hence, it is of interest to explore the structural determinants of this divergent pharmacological profile.

Recently, another analog of **1**, compound **3** was described in which the indole moiety was replaced with a 7-azaindole.²⁶ This simple modification caused ~30-fold increase in binding affinity at the D₂R and it also displayed negative cooperativity, suggesting allosteric interactions with the D₂R. Another D₃R-selective partial agonist, BP1,4979 (**4**), has recently been evaluated for safety and efficacy in a clinical trial for smoking cessation and has structural similarities, but also differences from compounds **1** and **2**; notably a 3-CN-phenyl piperazine, instead of the CN-tetrahydroisoquinolines, and the lack of a terminal aryl amide.²⁹ In comparison, we reported PG622, (**5**, Fig. 1) as a moderately selective and high affinity D₃R weak partial agonist.³⁰ Its PP is the classic 2,3-diCl-phenylpiperazine. This compound is a structural analogue of the D₃R antagonist, PG01037 (**6**, Fig. 1), with the only difference being the *trans*-olefin in **6** was replaced with a *trans*-cyclopropylmethyl group. D₃R receptor binding affinities of **5** and **6** were virtually identical (~1 nM).³⁰ However, **6** had ~2-fold lower affinity at D₂R, resulting in a more D₃R-selective compound.

In the present study we deconstructed compounds **1**, **2** and **4** into “synthons” or functional fragments in order to: 1) investigate the binding affinities and functional activities of the PPs at both D₂R and D₃R; 2) test SP-derivatives of **1** for binding at both D₂R and D₃R receptors; 3) link these PPs and SPs with a conformationally-restricted *trans*-cyclopropylmethyl linking chain, as in compound **5**, and determine the binding and functional properties of the resulting compounds at both D₂R and D₃R and finally, 4) synthesize analogues with one additional methylene group in the linking chain in an attempt to improve D₃R selectivity, using either 2-indole or 7-azaindole as the SP. We hypothesized that the PPs would bind to both D₂R and D₃R and their affinities would improve with alkyl homologation. We expected the terminal aryl amide SPs to exhibit very low, if any, affinity for the D₂R or D₃R, but that linking these PPs and SPs with a *trans*-cyclopropylmethyl linking chain would result in compounds with high affinity and selectivity for D₃R. Moreover, we predicted that adding one additional methylene group to the linking chain would further decrease binding affinity at the D₂R resulting in a more D₃R-selective ligand. Finally, we wanted to determine if the structural rigidity conferred by the *trans*-cyclopropyl group on these novel ligands engenders allosteric interactions at D₃R, as has been reported for the *trans*-cyclohexyl linked parent compound **1** at the D₂R and D₃R.^{15,25–27}

CHEMISTRY

Scheme 1 describes the synthesis of both PP and SP synthons. To synthesize the PPs, 3-aminobenzonitrile (**7**) was cyclized to *N*-aryl piperazine (**8**)³¹ using bis(2-chloroethyl)amine hydrochloride in diglyme, as a solvent, at 150 °C.³² Piperazine derivative **8** and the 6- or 7-CN-substituted tetrahydroisoquinolines **9a**, **9b** were *N*-alkylated to the corresponding **10**, **11a** and **11b**, respectively, in the presence of K₂CO₃, under reflux conditions. To synthesize

the SP of **1**, indole-2-carboxylic acid (**12a**) was reacted with the corresponding amine, in the presence of CDI, as the coupling reagent, to afford the amides **13a**, **13b**,³³ **13c**³⁴ and **13d**.

Scheme 2 outlines the synthetic strategy used for the synthesis of the *trans*-methyl-aryl carboxamide derivatives **14a–f**. 2-(Aminomethyl)-*trans*-cyclopropylmethanol (**15**), was prepared according to a procedure described previously,^{30,35} and was coupled with **12a** or 4-quinoline carboxylic acid (**12b**) to afford the amides **16a** and **16b**, respectively, which were oxidized to the corresponding aldehydes **17a** and **17b**, using a Dess-Martin periodinane reagent in more than 80% yield.³⁶ Compounds **17a** and **17b** were reductively aminated using NaBH(OAc)₃ as the reducing agent, to give compounds **14a–f**.

The synthesis of target compounds **18a** and **18b** is outlined in Scheme 3. In these compounds, the *trans*-cyclohexyl linker of **1** and **3** was replaced with a *trans*-cyclopropylmethyl group, while keeping the same PP and SPs. In Scheme 3, ethyl 2-(hydroxymethyl)cyclopropane-1-carboxylate (**19**)³⁷ was prepared according to a procedure described previously and was converted to cyclopropylcarbinyl bromide (**20**)³⁸ using phosphorus tribromide under basic conditions.³⁹ Treatment of the bromide **20** with potassium cyanide in DMSO afforded the nitrile **21**,³⁸ which was reduced to the amine **22**, using LiAlH₄. Compound **22** was coupled to either the indole (**12a**) or 7-azaindole-2-carboxylic acid (**12c**) in the presence of the coupling reagent CDI to afford the alcohol amide intermediates, **23a** and **23b**, respectively. These intermediates were oxidized (**24a** and **24b**) and reductively aminated as described in Scheme 2 to afford the final compounds **18a** and **18b**.

In Scheme 4, two additional analogs of **1** were synthesized in which the *trans*-cyclopropyl ring of **18a** or **18b** was moved one methylene group closer to the indole or 7-azaindole ring. By adding a methylene group between the PP and the *trans*-cyclopropyl ring, the same linker length as in **18a** and **18b** was retained. To synthesize the target compound **25a**, tetrahydropyran (THP) protected ethyl-*trans*-cyclopropylmethanol **26** was prepared according to a procedure described previously⁴⁰ and was oxidized to the aldehyde **27**. Compound **27** was converted to intermediate **28** in the presence of *O*-benzylhydroxylamine HCl as a mixture of the *cis* and *trans* isomers of the resulting oxime (~1:1) in 59% yield.⁴¹ The benzyloxime **28** was reduced in the presence of LiAlH₄ to the amine **29** and coupled with **12a** to give the amide **30a**. The tetrahydropyranyl group was removed under acidic conditions to give the alcohol **31a**, which was oxidized to **32a**, and reductively aminated to give the target compound **25a**, as described in the previous scheme. The same procedure was used to synthesize the 7-azaindole derivative **25b** from **29** and **12c**, except that the THP group of **30b** was removed using pyridinium *p*-toluenesulfonate (PPTS) to provide **31b**.⁴²

PHARMACOLOGICAL RESULTS AND DISCUSSION

Radioligand binding assays

All ligands were evaluated in radioligand binding assays using HEK-293 cells stably transfected with human D₂RL and D₃R, as described previously.⁴³ The radioligand was the high-affinity D₂-like receptor-selective antagonist [³H]N-methylspiperone (NMS). K_i values are displayed in Table 1 for the synthons and Table 2 for the full-length ligands. In addition,

cLogP and polar surface area (PSA) values are provided in Table 2 as a relative measure of lipophilicity and predicted brain penetration, respectively.

On the basis of SAR and previous modeling studies, we first synthesized the PP of compounds **1**, **2** and **4**, the SP of **1** and additional alkylated derivatives. The simplest PPs, **8**, **9a** and **9b** (6/7-CN tetrahydroisoquinoline), were nominally D₃R-selective (D₂/D₃ 7.5–9.1) based on K_i values in the binding assays (Table 1). Importantly, as the *n*-butyl linking chain was introduced into these PPs, **10**, **11a**, and **11b**, binding affinities for both D₂R and D₃R increased (for D₃R, K_i range = 34–1090 nM; for D₂R, K_i range = 493–8300 nM). Consistent with previous SAR studies, this suggests that hydrophobic interactions between the linker and the receptor contribute to the progressive enhancement of affinity at both D₂R and D₃R. Although introduction of the *n*-butyl group enhanced affinity at both receptors, only a modest (5 to 14-fold) D₃R selectivity was observed. It is also noteworthy that these PPs have much lower affinities than the previously reported and privileged 2,3-dichlorophenylpiperazine PP, for which the N-*n*-butyl-substituted synthon had a K_i =1.9 nM.¹³ As shown previously with other SPs,^{13,15} **13a-d** showed no or very weak ability to displace [³H]N-methylspiperone (NMS) binding from either the D₂R or D₃R. Notably, however, in a separate study, at concentrations over 50 μ M, **13b** was able to partially inhibit (~20%) [³H]spiperone binding to the D₂R, a finding that might be consistent with an allosteric interaction.²⁷

As shown in Table 2, the linking chain between the PPs of compounds **1–4** and the SPs of **1** and **2** was replaced with the conformationally rigid *trans*-cyclopropylmethyl group of compound **5**. We had previously shown that the *trans*-olefin of **6** could be replaced with a *trans*-cyclopropyl group without diminishing binding affinity at D₃R.³⁰ We were interested to see if the *trans*-cyclohexyl linker in **1** and **2** could be replaced with the *trans*-cyclopropylmethyl group, which can often be bioisosteric,⁴⁴ and retain D₃R affinity and selectivity profiles of **1** when the PP and SP were identical. For comparison, we also synthesized compounds **14a** and **14b** to evaluate the SPs of compounds **1** and **2** with the 2,3-dichlorophenylpiperazine PP. We found that all of the full-length *trans*-cyclopropyl analogues preferred D₃R over D₂R and most showed high affinity (nM) D₃R binding (Table 2). Analogues **14a** (K_i = 0.45 nM) and **14b** (K_i = 1.51 nM) demonstrated the highest D₃R affinities among the **14**-series that have the same linker, consistent with the higher affinities for their PP compared to the others. Interestingly, compound **14d**, with the PP and SP of **2**, had the lowest D₃R affinity (K_i = 1 μ M) suggesting that the *trans*-cyclopropylmethyl linker is not as well tolerated as the parent compound's *trans*-cyclohexyl linker and that the binding mode of these tetrahydroisoquinolines differs from that of the 4-phenylpiperazines. In comparison to its regioisomer **14e**, in which the only structural difference is the position of the CN on the tetrahydroisoquinoline PP, D₃R binding affinity was improved by ~4-fold and a more dramatic improvement was seen when the 4-quinoline SP was replaced with the 2-indole in **14f** (K_i = 32 nM). Although D₂R affinity also increased by ~2-fold, D₃R selectivity was still higher with the indole SP. This SAR corresponds with our previously reported 4-phenylpiperazines, wherein the 2-indole analogues are typically more D₃R-selective than their quinoline derivatives.⁴⁵

Generally, the 6/7-CN-substituted tetrahydroisoquinolines **14d–f** and the CN-substituted-phenyl piperazine analogue **14c** showed lower affinities than the privileged 2,3-diCl-phenylpiperazines and this was particularly significant with the 4-quinoline analogues **14d** and **14e**. A similar pattern of lower affinity was previously observed at the D₂R for the tetrahydroisoquinoline containing compound **1** in comparison to analogues in which this moiety was swapped for 2,3-dichlorophenylpiperazine or 2-methoxyphenylpiperazine moieties.²⁶ In addition, the incorporation of these two privileged D₂R structures switched the pharmacological action from that of allosteric (compound **1**) to behavior that was best fit by a competitive model.²⁶ However, when an additional methylene group was added to the linking chain in **18a**, high affinity and selectivity for the D₃R receptor was regained with a $K_i = 0.89$ nM for D₃R and selectivity vs. D₂R of >200 (Table 2). The positional isomer, **25a**, was synthesized to further compare the effects on D₃R affinity and selectivity. Compared to **18a**, this analogue showed an 11-fold decrease in binding affinity for D₃R and a 2.6-fold decrease in D₂R affinity, resulting in lower D₃R selectivity (49-fold). When the indole ring of both **18a** and **25a** was replaced with 7-azaindole a decrease in the binding affinity was observed for D₃R with a simultaneous increase for D₂R, a change that caused both a significant increase in affinity and negative cooperativity with dopamine in a SAR study of compound **1** derivatives at the D₂R.²⁶

Of note, the cLogP value range of 3–5.3 and PSA range of 48–80 for all extended length compounds in Table 2 predict reasonable blood-brain barrier penetration, and thus support future in vivo investigation.

Functional profiling using a β -arrestin recruitment assay

Functional characterization of synthons—Functional profiling of the compounds at the D₂R and D₃R was achieved using a β -arrestin recruitment assay as previously employed.^{15,46–49} Each compound was tested dose-dependently in both “agonist mode”, which evaluated the compound’s ability to stimulate β -arrestin recruitment, and “antagonist mode” that evaluated the compound’s ability to inhibit β -arrestin recruitment stimulated by an EC₈₀ concentration of dopamine. The potency of dopamine at the D₂R and D₃R is different, therefore we used a derivation of the Gaddum/Schild model of competitive antagonism (equation 5 in Experimental Methods) to estimate a value of functional affinity (K_B) from these data that can be compared across the receptor subtypes to allow some insight into the functional selectivity of these compounds.

None of the compounds exhibited agonist activity up to 30 μ M (data not shown). The simplest PPs, **8**, **9a** and **9b** (6/7-CN tetrahydroisoquinoline), showed nonselective (in agreement with the aforementioned binding) functional antagonism at both D₂R and D₃R (Table 1). The functional affinity (in “antagonist mode”) of these compounds was low ($K_B > 2$ μ M) with **9a** having the lowest functional affinity of the series. The addition of the *n*-butyl linking chain in **10**, **11a**, and **11b** increased functional affinity (K_B) for both D₂R (75–1660 nM) and D₃R (32–1020 nM), with **10** displaying the highest potency. However, in agreement with the binding results, an *n*-butyl linking chain addition had minimal effects on D₃R vs. D₂R selectivity (Table 1).

We next examined the abilities of the SPs, **13a–d**, to influence dopamine receptor functions. Not surprisingly, given the above binding analysis, the compounds showed no functional inhibition of either the D₂R or D₃R at concentrations up to 100 μ M (Table 1). These findings are in agreement with both the currently presented binding results and previously published data for this SP wherein **13b** is reported as CS01-12.¹⁵ Notably, however, in a previous study, found that at concentrations over 50 μ M, **13b** acts as an allosteric antagonist of the D₂R with modest negative cooperativity with dopamine.²⁷ Together, these results suggest that the SPs have either no activity at, or very low affinity for, these dopamine receptors when not linked to a PP.

Functional characterization of extended compounds—Following characterization of the PP and SPs independently, we sought to examine, in functional assays, the extended length compounds containing both PP and SPs within the same chemical scaffold (Table 2). Compound **1** has previously been shown to act as a negative allosteric modulator at both the D₂R and D₃R.^{15,24,25} This allosteric action has been linked to a bitopic mode of interaction at the D₂R.²⁵ The above binding data show that the linkage of a SP to a PP enhances the affinity and selectivity of the extended-length compounds for the D₃R and this increase in affinity may be consistent with a bitopic mode of action.^{22,23} We therefore extended our pharmacological characterization to determine if these novel compounds might display allosteric pharmacology at the D₃R. We performed curve shift experiments in which the ability of increasing concentration of our novel compounds to modulate dopamine potency was measured. Increasing concentrations of **1** cause a limited rightward shift in D₃R dopamine dose-response curves (Fig. 2A). Analysis of these data with an allosteric ternary complex model (equation 3 in Experimental Methods) allowed estimations of affinity ($K_B = 19$ nM, $pK_B = 7.72 \pm 0.14$) and negative cooperativity with dopamine ($\alpha = 0.009$, $\text{Log}\alpha = -2.05 \pm 0.11$) that equates to a maximal 100-fold decrease in dopamine potency (Table 3). Equivalent curve shift experiments using cells expressing the D₂R revealed an allosteric mode of action for **1** but with both a lower affinity ($K_B = 2.5$ μ M, $pK_B = 5.60 \pm 0.10$) and weaker negative cooperativity with dopamine ($\alpha = 0.1$, $\text{Log}\alpha = -0.77 \pm 0.10$). Thus **1** displays 130-fold selectivity towards the D₃R (Table 2). In contrast, increasing concentrations of the D₂R/D₃R competitive antagonist sulpiride produces a limitless shift in the potency of dopamine for D₃R (Fig. 2B) and D₂R activation (not shown). These data could be fit to a Gaddum/Schild model of competitive antagonism with Schild slopes of unity (equation 5 in Experimental Methods) to derive affinities (K_B) of 40 nM ($pA_2 = 7.40 \pm 0.10$) and 3 nM ($pA_2 = 8.55 \pm 0.19$) at the D₃R and D₂R respectively. Interestingly, **2**, a structural congener of **1** (Fig. 1), exhibits the properties of a competitive antagonist in this curve-shift assay with 230-fold selectivity towards the D₃R (Fig. 2C, Table 2). These results suggest that subtle structural features in extended-length D₃R selective ligands can engender allosteric behavior.

We next tested all of the *trans*-cyclopropylmethyl-linked analogues to determine their mechanism of antagonism at the D₃R by performing curve-shift analyses (Figs. 3 and 4). These data were fit with a derivation of the operational model of allosterism (equation 3 in Experimental Methods) or a Gaddum/Schild model of competitive antagonism (equation 4

in Experimental Methods). For each compound, data were analyzed with both models and the best fit was determined by an F-test.

The **14**-series as described above contains both PP and SPs. As was shown in Fig. 2 for compounds **2** and sulpiride, increasing concentrations of compounds **14a**, **14b**, **14c**, **14d**, **14e**, and **14f** caused a limitless rightward displacement of dopamine concentration response curves with no effect on the $E_{\max}$. As such, these data, along with those for **2** and sulpiride, were best fit by a competitive Gaddum/Schild model to determine affinity (pA_2) and Schild slope (equation 5 in Experimental Methods, Table 2). Consistent with a competitive mode of interaction, the Schild slopes determined for all of the above compounds were not significantly different from unity. These data indicate that this novel series of compounds (**14a–f**) function as competitive antagonists at the D_3R . **14b** was found to display the highest functional affinity at both D_2R ($K_B = 17$ nM) and D_3R ($K_B = 51$ nM), while **14d** ($K_B = 1.1$ and 1.7 μ M for D_3R and D_2R , respectively) showed the lowest functional affinity of the **14**-series. These findings are consistent with the observed affinities in the binding studies. The regio-isomer of **14d**, compound **14e**, showed improved functional affinity for the D_2R ($K_B = 110$ nM) and D_3R ($K_B = 240$ nM) with no appreciable selectivity difference; although the binding analysis revealed a modest (6-fold) preference for D_3R . Similar results were obtained for compound **14f** suggesting that all of the **14**-series compounds were relatively nonselective for D_3R vs. D_2R .

As noted above, both **18a** and **25a** contain the same PP and SP, and same absolute cyclopropyl linker length, with the only difference being the length of the methylene chains on either side of the cyclopropyl group. Furthermore, whereas these compounds contain the same PP and SP as **1**, they contain a structurally rigid and shorter *trans*-cyclopropyl linker instead of the *trans*-cyclohexyl group. Hence, we reasoned that adding an extra methylene group to either side of the *trans*-cyclopropyl ring system, as compared to the **14**-series compounds, might improve D_3R selectivity and might also reveal allosterism as observed with compound **1**. Indeed, in contrast to the results observed with the compounds from the **14**-series, increasing concentrations of **18a** caused not only a decrease in dopamine potency but also a decrease in the $E_{\max}$ for dopamine suggesting non-surmountable or non-competitive antagonism (Fig. 4). Accordingly, these data were best fit by an allosteric operational model (equation 4 in Experimental Methods) to derive a value of affinity ($K_B = 4$ nM), negative cooperativity with dopamine affinity ($\alpha = 0.04$) and negative modulation of dopamine efficacy ($\beta = 0.005$, Table 3). Interestingly, compound **25a** behaved in a manner similar to **1** causing a limited rightward shift in the dopamine dose-response curve suggesting allosterism within its mechanism of antagonism (Fig. 4, Table 3). The more modest decrease in $E_{\max}$ of the dopamine concentration response curve produced by increasing concentrations of **25a** was reflected by a value of negative cooperativity with dopamine efficacy that was 6-fold smaller ($\beta = 0.03$) than that of **18a** whereas its negative cooperativity with dopamine affinity was similar ($\alpha = 0.03$, Fig. 4, Table 3). Thus, compounds **18a** and **25a** were the only *trans*-cyclopropyl-linked analogues found to antagonize the D_3R in an allosteric mechanism. It should be noted that the addition of a methylene group to the linking chain of **14f** to yield compound **18a**, caused a 67-fold increase in D_3R functional affinity yielding one of the most D_3R -selective compounds in this

series (Table 2). The positional isomer, **25a**, showed reduced D₂R and D₃R affinities, although D₃R-selectivity was retained (Table 2).

Finally, when the indole ring of both **18a** and **25a** was replaced with 7-azaindole (yielding **18b** and **25b** respectively), the potency of D₂R inhibition was increased (26-fold for both **18b** and **25b**) with either no effect (**18b**) or a decrease (**25b**) in D₃R potency resulting in a loss of D₃R selectivity (Table 2). In addition to a decrease in subtype selectivity, both **18b** and **25b** appeared to inhibit dopamine stimulation of the D₂R and D₃R in a manner similar to the competitive compounds (Fig. 4.)

CONCLUSION

In this study, we designed, synthesized and evaluated a series of novel compounds to extend the SAR for D₃R affinity and selectivity over D₂R. In addition, we investigated molecular determinants of competitive versus noncompetitive (or negative allosteric) functional antagonism at these receptor subtypes using the PPs and SPs of two D₃R-selective antagonists **1** and **2** that are structurally quite similar, but display significant differences in their pharmacological profiles. The synthesis and evaluation of their individual PPs and SPs provided insight into their roles in binding affinity for D₃R, however, none of these synthons demonstrated appreciable D₃R-selectivity over D₂R and the PPs were competitive antagonists in the β -arrestin recruitment assay. However, by linking these PPs and SPs via a *trans*-cyclopropyl-functionalized linker, D₃R selectivities emerged likely due to the bivalent nature of these compounds. Further, extending the linker length by an additional methylene group either before or after the *trans*-cyclopropylmethyl function engendered allostery within compounds **18a** and **25a** suggesting that they could be interacting with the receptor in a bitopic fashion. These allosteric effects appear to be enabled by a bivalent compound of sufficient length with the SP correctly oriented in a secondary binding pocket, which is dependent on the nature and conformation of the linker. Such a mode of action may be consistent with the bitopic mechanism proposed for the allosteric action of **1** at the D₂R. Although the PP clearly plays a role in D₃R (and D₂R) affinity, it is the combination of PP, SP and linker between them that determines functional activity. Taken together, the binding and functional data reported herein support the notion that the SPs and linkers within the extended-length compounds can modulate the selectivity of their antagonist activities at D₂R and D₃R. Furthermore, these data suggest that the specific orientation of the SP within the secondary binding pocket can confer non-competitive or allosteric modes of interaction, but that relatively subtle changes to structure and orientation of the SP can cause a switch between apparently allosteric and competitive antagonism.

EXPERIMENTAL METHODS

General

Reaction conditions and yields were not optimized. Anhydrous solvents were purchased from Aldrich and were used without further purification except for tetrahydrofuran, which was freshly distilled from sodium-benzophenone ketyl. All other chemicals and reagents were purchased from Sigma-Aldrich Co. LLC, Combi-Blocks, TCI America, Acros Organics, Maybridge, and Alfa Aesar. All amine final products were converted into the

oxalate salt. Spectroscopic data and yields refer to the free base form of compounds. Teledyne ISCO CombiFlash Rf or glass flash column chromatography was performed using silica gel (EMD Chemicals, Inc.; 230–400 mesh, 60 Å). ^1H and ^{13}C NMR spectra were acquired using a Varian Mercury Plus 400 spectrometer at 400 MHz and 100 MHz, respectively. Chemical shifts are reported in parts-per-million (ppm) and referenced according to deuterated solvent for ^1H spectra (CDCl_3 , 7.26, CD_3OD , 3.31 or $\text{DMSO}-d_6$, 2.50) and ^{13}C spectra (CDCl_3 , 77.2, CD_3OD , 49.0 or $\text{DMSO}-d_6$, 39.5). Gas chromatography-mass spectrometry (GC/MS) data were acquired (where obtainable) using an Agilent Technologies (Santa Clara, CA) 6890N GC equipped with an HP-5MS column (cross-linked 5% PH ME siloxane, 30 m $\times$ 0.25 mm i.d. $\times$ 0.25 μm film thickness) and a 5973 mass-selective ion detector in electron-impact mode. Ultrapure grade helium was used as the carrier gas at a flow rate of 1.2 mL/min. The injection port and transfer line temperatures were 250 and 280 $^\circ\text{C}$, respectively, and the oven temperature gradient used was as follows: the initial temperature (100 $^\circ\text{C}$) was held for 3 min and then increased to 295 $^\circ\text{C}$ at 15 $^\circ\text{C}/\text{min}$ over 13 min, and finally maintained at 295 $^\circ\text{C}$ for 10 min. Combustion analysis was performed by Atlantic Microlab, Inc. (Norcross, GA) and the results agree within $\pm 0.4\%$ of calculated values. cLogP and polar surface area (PSA) values were calculated using ChemDraw Professional Ultra 15.0. Melting point determination was conducted using a Thomas-Hoover melting point apparatus and are uncorrected. On the basis of NMR and combustion data, all final compounds are 95% pure.

3-(Piperazin-1-yl)benzonitrile (**8**)³¹

The reaction mixture of 3-aminobenzonitrile (**7**, 0.500 g, 4.23 mmol) and bis(2-chloroethyl)amine hydrochloride (0.831 g, 4.65 mmol) in diethylene glycol monomethyl ether (2.0 mL) was heated at 150 $^\circ\text{C}$ for 6 h. The reaction mixture was allowed to come to RT, MeOH (4 mL) was added, followed by dilution with ether (250 mL). The salt was filtered, suspended in CHCl_3 (20 mL) and neutralized with 2N NaOH to pH 8–9. The organic layer was collected, concentrated and purified by column chromatography using 3% MeOH/ CHCl_3 as eluent to provide 0.294 g (37%) of **8**.

3-(4-Butylpiperazin-1-yl)benzonitrile (**10**)

1-Bromobutane (0.263 g, 1.92 mmol) and K_2CO_3 (0.398 g, 2.88 mmol) were added to a solution of **8** (0.180 g, 0.96 mmol) in acetone (15 mL) and stirred at reflux overnight. The reaction mixture was filtered, concentrated and purified by flash chromatography using 4% acetone/ CHCl_3 as eluent to provide 0.150 g (65%) of **10** as an oil. ^1H NMR (400 MHz, CDCl_3) δ 7.32–7.28 (m, 1 H), 7.11–7.05 (m, 3H), 3.22 (t, $J = 5.2$ Hz, 4H), 2.59 (t, $J = 5.0$ Hz, 4H), 2.39 (t, $J = 7.6$ Hz, 2H), 1.55–1.47 (m, 2H), 1.33 (sextet, $J = 8.0$ Hz, 2H), 0.94 (t, $J = 7.2$ Hz, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 151.3, 129.8, 122.2, 119.7, 119.3, 118.2, 112.9, 58.3, 52.9, 48.2, 29.0, 20.7, 14.0. The oxalate salt was precipitated from acetone. Anal. ($\text{C}_{15}\text{H}_{21}\text{N}_3 \cdot \text{C}_2\text{H}_2\text{O}_4 \cdot 0.5\text{H}_2\text{O}$) C, H, N.

2-Butyl-1,2,3,4-tetrahydroisoquinoline-6-carbonitrile (**11a**)

The same procedure was used as described for compound **10** using **9a**. The crude product was purified using 15% EtOAc/hexanes as eluent to provide the product as an oil, in 71%

yield. ^1H NMR (400 MHz, CDCl_3) δ 7.38 (s, 1H), 7.37–7.36 (m, 1H), 7.11 (dd, J = 8.0, 0.8 Hz, 1H), 3.65 (s, 2H), 2.91 (t, J = 6.0 Hz, 2H), 2.73 (t, J = 6.0 Hz, 2H), 2.53–2.50 (m, 2H), 1.59–1.53 (m, 2H), 1.37 (sextet, J = 7.6 Hz, 2H), 0.94 (t, J = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 140.7, 136.0, 132.4, 129.1, 127.5, 119.1, 109.9, 58.1, 56.1, 50.3, 29.2, 28.9, 20.7, 14.1. GC-MS (EI) m/z 214.1 (M^+). The oxalate salt was precipitated from acetone. Anal. ($\text{C}_{14}\text{H}_{18}\text{N}_2 \cdot \text{C}_2\text{H}_2\text{O}_4 \cdot 0.5\text{H}_2\text{O}$) C, H, N. Mp 140–141 $^\circ\text{C}$.

2-Butyl-1,2,3,4-tetrahydroisoquinoline-7-carbonitrile (11b)

The same procedure was used as described for compound **10** using **9b**. The crude product was purified using 12% EtOAc/hexanes as eluent to provide the product as an oil, in 58% yield. ^1H NMR (400 MHz, CDCl_3) δ 7.37 (dd, J = 7.6, 1.6 Hz, 1H), 7.30 (s, 1H), 7.18 (d, J = 8.4 Hz, 1H), 3.60 (s, 2H), 2.94 (t, J = 5.6 Hz, 2H), 2.72 (t, J = 5.6 Hz, 2H), 2.53–2.49 (m, 2H), 1.60–1.53 (m, 2H), 1.37 (sextet, J = 7.2 Hz, 2H), 0.94 (t, J = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 140.4, 136.5, 130.3, 129.5, 129.4, 119.1, 109.3, 58.0, 55.6, 50.2, 29.4, 29.2. GC-MS (EI) m/z 214.2 (M^+). The oxalate salt was precipitated from acetone. Anal. ($\text{C}_{14}\text{H}_{18}\text{N}_2 \cdot \text{C}_2\text{H}_2\text{O}_4 \cdot 0.25\text{H}_2\text{O}$) C, H, N. Mp 167–168 $^\circ\text{C}$.

N-(Pentan-2-yl)-1*H*-indole-2-carboxamide (13a)

CDI (0.251 g, 1.55 mmol) was added to a solution of indole-2-carboxylic acid (**12a**, 0.250 g, 1.55 mmol) in THF (10 mL) under an Argon atmosphere. The reaction mixture was stirred for 4 h and cooled to 0–5 $^\circ\text{C}$. 2-Aminopentane (0.135 g, 1.55 mmol) was added drop wise in THF (10 mL). The reaction mixture was allowed to come to RT and then stirred overnight. The solvent was evaporated, the crude product was diluted with water (20 mL) and extracted with CHCl_3 (2x20 mL). The organic layer was dried, concentrated and purified by flash chromatography using 10% EtOAc/hexanes as eluent to provide 0.321 g (90%) of the desired product. ^1H NMR (400 MHz, CDCl_3) δ 9.75 (bs, 1H), 7.63 (dd, J = 8.0, 0.8 Hz, 1H), 7.45 (dd, J = 7.6, 0.8 Hz, 1H), 7.28 (dd, J = 7.2, 1.2 Hz, 1H), 7.15–7.11 (m, 1H), 6.82 (m, 1H), 5.96 (bs, 1H), 4.31–4.24 (m, 1H), 1.70–1.38 (m, 4H), 1.38–1.24 (m, 3H), 0.96 (t, J = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ 161.2, 136.5, 131.2, 127.8, 124.4, 121.9, 120.7, 112.2, 101.5, 45.6, 39.4, 21.3, 19.5, 14.1. Anal. ($\text{C}_{14}\text{H}_{18}\text{N}_2\text{O}$) C, H, N.

N-(Heptan-4-yl)-1*H*-indole-2-carboxamide (13d)

The same procedure was used as described for compound **13a** using *N*-heptan-4-ylamine. The crude product was purified using 15% EtOAc/hexanes as eluent to provide the product in 66% yield. ^1H NMR (400 MHz, CDCl_3) δ 9.75 (bs, 1H), 7.64 (dd, J = 8.0, 0.8 Hz, 1H), 7.45 (dd, J = 8.0, 0.8 Hz, 1H), 7.29–7.25 (m, 1H), 7.15–7.11 (m, 1H), 6.83 (m, 1H), 5.88 (d, J = 9.2 Hz, 1H), 4.24–4.21 (m, 1H), 1.64–1.39 (m, 8H), 0.95 (t, J = 7.2 Hz, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ 161.4, 136.4, 131.2, 127.8, 124.5, 121.9, 120.7, 112.1, 101.4, 49.4, 37.9, 19.4, 14.2. Anal. ($\text{C}_{16}\text{H}_{22}\text{N}_2\text{O}$) C, H, N.

N-(2-((4-(2,3-Dichlorophenyl)piperazin-1-yl)methyl)-*trans*-cyclopropylmethyl)-1*H*-indole-2-carboxamide (14a)

1-(2,3-Dichlorophenyl)piperazine (0.100 g, 0.43 mmol) and **17a** (0.105 g, 0.43 mmol) were mixed in 1,2-dichloroethane (10 mL) and treated with sodium triacetoxymethylborohydride (0.138

g, 0.64 mmol) and HOAc (0.2 mL) under an atmosphere of Argon. The reaction mixture was stirred at RT for 6 h. The reaction mixture was quenched with 2 N NaOH (10 mL) and extracted with EtOAc (3 x 20 mL). The organic layer was dried over Na₂SO₄, concentrated and purified by flash chromatography using 2% MeOH/CHCl₃ as eluent to provide 0.150 g (76%) of yellow solid product. Mp 215–216° C. ¹H NMR (400 MHz, CDCl₃) δ 11.53 (s, 1H), 8.57 (t, *J* = 6.0 Hz, 1H), 7.58 (d, *J* = 8.4 Hz, 1H), 7.40 (d, *J* = 8.4 Hz, 1H), 7.24–7.09 (m, 4H), 6.96 (dt, *J* = 7.2, 1.2 Hz, 1H), 6.76 (d, *J* = 6.8 Hz, 1H), 3.32–3.26 (m, 1H), 3.16–3.09 (m, 1H), 2.79 (bs, 4H), 2.53 (bs, 4H), 2.39 (dd, *J* = 12.4, 5.2 Hz, 1H), 2.07–2.02 (m, 1H), 0.91–0.83 (m, 2H), 0.49 (quintet, *J* = 4.8 Hz, 1H), 0.31 (quintet, *J* = 4.8 Hz, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 161.0, 151.1, 136.3, 132.5, 131.9, 128.3, 127.1, 125.8, 124.1, 123.1, 121.4, 119.6, 119.2, 112.2, 102.2, 61.6, 52.5, 50.8, 42.1, 18.2, 14.7, 9.3. Anal. (C₂₄H₂₆Cl₂N₄O•0.5H₂O) C, H, N.

***N*-(2-((4-(2,3-Dichlorophenyl)piperazin-1-yl)methyl)-*trans*-cyclopropylmethyl)quinoline-4-carboxamide (14b)**

The same procedure was used as described for compound **14a** from 2,3-dichloro, and **17b**. The crude product was purified by flash chromatography using 2% MeOH/CHCl₃ as eluent to provide the oily product in 96% yield. ¹H NMR (400 MHz, CDCl₃) δ 8.72 (d, *J* = 4.4 Hz, 1H), 8.14 (dd, *J* = 8.4, 0.8 Hz, 1H), 8.03 (d, *J* = 8.0 Hz, 1H), 7.69 (dt, *J* = 7.2, 1.6 Hz, 1H), 7.53 (dt, *J* = 7.2, 1.2 Hz, 1H), 7.30 (d, *J* = 7.2 Hz, 1H), 7.15–7.11 (m, 2H), 7.07 (bt, *J* = 5.2 Hz, 1H), 6.84 (dd, *J* = 7.6, 2.8 Hz, 1H), 3.46–3.41 (m, 1H), 3.35–3.30 (m, 1H), 2.98 (bs, 4H), 2.67 (bs, 4H), 2.42–2.37 (m, 1H), 2.32–2.27 (m, 1H), 0.97–0.92 (m, 2H), 0.58 (quintet, *J* = 5.2 Hz, 1H), 0.45 (quintet, *J* = 5.2 Hz, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 167.2, 151.1, 149.6, 148.4, 142.1, 133.9, 129.9, 129.6, 127.6, 127.5, 127.3, 125.2, 124.6, 124.4, 118.6, 118.4, 62.3, 53.2, 51.0, 43.9, 17.6, 15.2, 10.2. The oxalate salt was precipitated from MeOH/ether. Anal. (C₂₅H₂₆Cl₂N₄O•1.5 C₂H₂O₄•C₃H₆O•1.5H₂O) C, H, N. Mp 55–56 °C.

***N*-(2-((4-(3-Cyanophenyl)piperazin-1-yl)methyl)-*trans*-cyclopropylmethyl)quinoline-4-carboxamide (14c)**

The same procedure was used as described for compound **14a** from **8** and **17b**. The crude product was purified by flash chromatography using 50% acetone/ CHCl₃ as eluent. ¹H NMR (400 MHz, CDCl₃) δ 8.81 (d, *J* = 4.0 Hz, 1H), 8.20 (dd, *J* = 8.4, 1.0 Hz, 1H), 8.10 (d, *J* = 8.4 Hz, 1H), 7.76–7.72 (m, 1H), 7.60–7.56 (m, 1H), 7.36 (d, *J* = 4.4 Hz, 1H), 7.31–7.27 (m, 1H), 7.07–7.00 (m, 3H), 6.74 (bt, *J* = 5.6 Hz, 1H), 3.51 (quintet, *J* = 7.0 Hz, 1H), 3.98–3.33 (m, 1H), 3.14–3.11 (m, 4H), 2.63 (t, *J* = 5.0 Hz, 4H), 2.42–2.38 (m, 1H), 2.33–2.28 (m, 1H), 1.01–0.94 (m, 2H), 0.64–0.59 (m, 1H), 0.50–0.46 (m, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 167.3, 151.2, 149.8, 148.6, 142.1, 130.1, 130.0, 129.9, 127.7, 125.3, 124.4, 122.5, 119.8, 119.4, 118.4, 118.3, 112.9, 62.2, 52.8, 48.1, 44.0, 17.7, 15.3, 10.3. The oxalate salt was precipitated from acetone. Anal. (C₂₆H₂₇N₅O•C₂H₂O₄•2H₂O) C, H, N. Mp 125–126 °C.

***N*-(2-((6-Cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)methyl)-*trans*-cyclopropylmethyl)quinoline-4-carboxamide (14d)**

The same procedure was used as described for compound **14a** from **9a** and **17b**. The crude product was purified by flash chromatography using 40% acetone/ CHCl₃ as eluent to provide yellowish solid in 68% yield. ¹H NMR (400 MHz, CDCl₃) δ 8.86–8.85 (m, 1H), 8.23 (d, *J* = 8.4 Hz, 1H), 8.13 (d, *J* = 8.4 Hz, 1H), 7.77–7.31 (m, 1H), 7.59–7.55 (m, 1H), 7.40–7.31 (m, 3H), 7.01 (d, *J* = 8.0 Hz, 1H), 6.43 (d, *J* = 4.8 Hz, 1H), 3.76–3.67 (m, 2H), 3.59–3.52 (m, 1H), 3.45–3.39 (m, 1H), 2.88–2.76 (m, 4H), 2.57–2.53 (m, 1H), 2.46–2.41 (m, 1H), 1.09–1.03 (m, 2H), 0.69–0.65 (m, 1H), 0.58–0.53 (m, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 167.4, 149.9, 148.8, 142.1, 140.2, 135.7, 132.6, 130.1, 130.0, 129.2, 127.8, 127.5, 125.3, 124.5, 119.1, 118.5, 110.1, 62.0, 55.9, 50.4, 44.1, 28.8, 17.9, 15.7, 10.4. The oxalate salt was precipitated from EtOH/ ether. Anal. (C₂₅H₂₄N₄O•1.25C₂H₂O₄•2H₂O) C, H, N. Mp 65–66 °C.

***N*-(2-((7-Cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)methyl)-*trans*-cyclopropylmethyl)quinoline-4-carboxamide (14e)**

The same procedure was used as described for compound **14a** from **9b** and **17b**. The crude product was purified by flash chromatography using 40% acetone/ CHCl₃ as eluent. ¹H NMR (400 MHz, CDCl₃) δ 8.69 (d, *J* = 4.4 Hz, 1H), 8.15 (d, *J* = 8.0 Hz, 1H), 8.04 (d, *J* = 8.4 Hz, 1H), 7.69 (t, *J* = 8.0 Hz, 1H), 7.51 (t, *J* = 8.0 Hz, 1H), 7.34–7.11 (m, 4H), 7.04–7.00 (m, 1H), 3.65–3.59 (m, 2H), 3.51–3.32 (m, 2H), 2.86–2.75 (m, 4H), 2.50–2.39 (m, 2H), 1.0 (t, *J* = 6.0 Hz, 2H), 0.65–0.61 (m, 1H), 0.52–0.48 (m, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 167.3, 149.6, 148.4, 142.1, 140.1, 136.1, 130.3, 130.0, 129.9, 129.7, 129.5, 129.5, 127.5, 125.2, 124.3, 119.0, 118.4, 109.2, 61.8, 55.2, 50.1, 43.8, 29.3, 17.7, 15.5, 10.3. The oxalate salt was precipitated from acetone. Anal. (C₂₅H₂₄N₄O•1.25 C₂H₂O₄•2H₂O) C, H, N. Mp 82–83 °C.

***N*-(2-((7-Cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)methyl)-*trans*-cyclopropylmethyl)-1*H*-indole-2-carboxamide (14f)**

The same procedure was used as described for compound **14a** from **9b** and **17a**. The crude product was purified by flash chromatography using 25% acetone/ CHCl₃ as eluent to provide the oily product in 86% yield. ¹H NMR (400 MHz, CDCl₃) δ 10.15 (s, 1H), 7.59 (d, *J* = 8.4 Hz, 1H), 7.43 (dd, *J* = 9.6, 1.0 Hz, 1H), 7.33 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.28–7.17 (m, 2H), 7.14–7.09 (m, 2H), 6.85 (s, 1H), 6.73 (bt, *J* = 5.6 Hz, 1H), 3.67 (s, 2H), 3.52 (quintet, *J* = 6.0 Hz, 1H), 3.37 (quintet, *J* = 6.0 Hz, 1H), 2.91–2.75 (m, 4H), 2.62–2.56 (m, 1H), 2.36–2.31 (m, 1H), 1.05–0.99 (m, 2H), 0.64–0.60 (m, 1H), 0.52–0.50 (m, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 161.8, 140.1, 136.5, 136.0, 130.7, 130.3, 129.6, 129.5, 127.6, 124.4, 121.9, 120.6, 119.0, 112.1, 109.3, 102.2, 61.9, 55.3, 50.1, 43.5, 29.2, 18.2, 15.4, 9.9. The oxalate salt was precipitated from acetone. Anal. (C₂₄H₂₄N₄O• C₂H₂O₄) C, H, N. Mp 132–133 °C.

***N*-(2-Hydroxymethyl-*trans*-cyclopropylmethyl)-1*H*-indole-2-carboxamide (16a)**

CDI (0.184 g, 1.13 mmol) was added to a solution of **12a** (0.183 g, 1.13 mmol) in THF (10 mL) under an Argon atmosphere. The reaction mixture was stirred for 4 h and cooled to 0–5 °C. *trans*-(2-(Aminomethyl)cyclopropyl)methanol (**15**)^{30,35} (0.115 g, 1.13 mmol) was

added drop wise after dissolving in THF (10 mL). The reaction mixture was allowed to come to RT and then stirred overnight. The solvent was evaporated, the crude product was diluted with water (20 mL) and extracted with CHCl₃ (2x20 mL). The organic layer was dried, concentrated and purified by flash chromatography using 25% acetone/ CHCl₃ as eluent to provide 0.161 g (63%) of the desired product. ¹H NMR (400 MHz, CD₃OD) δ 8.46 (s, 1H), 7.61 (d, *J* = 8.0 Hz, 1H), 7.45 (d, *J* = 8.4 Hz, 1H), 7.22 (t, *J* = 8.0 Hz, 1H), 7.10–7.05 (m, 2H), 3.52–3.48 (m, 1H), 3.39–3.26 (m, 3H), 1.05 (m, 2H), 0.60–0.56 (m, 1H), 0.55–0.49 (m, 1H); ¹³C NMR (100 MHz, CD₃OD) δ 164.1, 138.3, 132.3, 129.0, 125.0, 122.7, 121.1, 113.0, 104.4, 66.4, 4.19, 20.8, 17.8, 9.1. GC-MS (EI) *m/z* 244.1 (M⁺).

***N*-(2-Hydroxymethyl-*trans*-cyclopropylmethyl)quinoline-4-carboxamide (16b)**

The same procedure was used as described for **16a** using 4-quinoline carboxylic acid (**12b**). The crude product was purified by flash chromatography using 30% acetone/ CHCl₃ as eluent to provide the desired product in 75% yield. ¹H NMR (400 MHz, (CDCl₃) δ 8.57 (bs, 1H), 7.95 (dd, *J* = 13.0, 8.4 Hz, 2H), 7.69 (t, *J* = 5.2 Hz, 1H), 7.64–7.60 (m, 1H), 7.46–7.42 (m, 1H), 7.11 (d, *J* = 4.4 Hz, 1H), 3.53–3.48 (m, 1H), 4.25 (bs, 1H), 3.93–3.33 (m, 1H), 2.98–2.90 (m, 2H), 0.86–0.77 (m, 2H), 0.38–0.30 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 167.4, 149.4, 148.0, 142.1, 130.0, 129.2, 127.5, 125.2, 124.2, 118.4, 65.5, 43.8, 20.1, 16.6, 8.4.

***N*-(2-Formyl-*trans*-cyclopropylmethyl)-1*H*-indole-2-carboxamide (17a)**

Dess-Martin periodinane (0.338 g, 0.79 mmol) was added in fractions to a solution of **16a** (0.130 g, 0.53 mmol) in THF (15 mL) and stirred for 30 min at –78° C, under Argon. The reaction mixture was allowed to come to RT and stirred for another 3h. The reaction mixture was concentrated and purified by flash chromatography using 10% acetone/ CHCl₃ as eluent to provide 0.110 g (86%) of the desired product. ¹H NMR (400 MHz, (CD₃)₂SO) δ 11.57 (s, 1H), 8.90 (d, *J* = 2.4 Hz, 1H), 8.63 (t, *J* = 6.0 Hz, 1H), 7.61 (dd, *J* = 8.0, 0.8 Hz, 1H), 7.42 (dd, *J* = 8.0, 0.8 Hz, 1H), 7.19–7.15 (m, 1H), 7.11–7.10 (m, 1H), 7.05–7.01 (m, 1H), 3.43–3.37 (m, 1H), 3.29–3.22 (m, 1H), 1.85–1.78 (m, 2H), 1.31–1.26 (m, 1H), 1.15–1.10 (m, 1H); ¹³C NMR (100 MHz, (CD₃)₂SO) δ 201.2, 161.2, 136.4, 131.5, 127.0, 123.3, 121.4, 119.7, 112.3, 102.5, 40.5, 28.6, 21.1, 12.1. GC-MS (EI) *m/z* 242.1 (M⁺).

***N*-(2-Formyl-*trans*-cyclopropylmethyl)quinoline-4-carboxamide (17b)**

The same procedure was used as described for **17a** from **16b**. The crude product was purified by flash chromatography using 25% acetone/ CHCl₃ as eluent to provide the desired product in 87% yield. ¹H NMR (400 MHz, (CD₃)₂SO) δ 8.96 (d, *J* = 4.4 Hz, 1H), 8.92 (d, *J* = 5.6 Hz, 1H), 8.06 (dd, *J* = 14, 8.0 Hz, 2H), 7.84–7.78 (m, 1H), 7.68–7.62 (m, 1H), 7.51 (d, *J* = 4.4 Hz, 1H), 3.50–3.44 (m, 1H), 3.30–3.25 (m, 2H), 1.88–1.80 (m, 2H), 1.33–1.29 (m, 1H), 1.17–1.12 (m, 1H); ¹³C NMR (100 MHz, (CD₃)₂SO) δ 201.1, 166.6, 150.2, 147.9, 142.0, 129.8, 129.4, 127.3, 125.3, 124.1, 119.0, 40.8, 28.5, 20.9, 12.2. GC-MS (EI) *m/z* 254.1 (M⁺).

***N*-(2-(7-Cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)methyl-*trans*-cyclopropylethyl)-1*H*-indole-2-carboxamide (18a)**

The same procedure was used as described for compound **14a** from **9b** and **24a**. The crude product was purified by flash chromatography using 25% acetone/ CHCl₃ as eluent to provide the oily product in 62% yield. ¹H NMR (400 MHz, CDCl₃) δ 9.88 (s, 1H), 7.52 (dd, *J* = 8.0, 0.8 Hz, 1H), 7.43 (dd, *J* = 8.4, 0.4 Hz, 1H), 7.33 (dd, *J* = 8.0, 2.0 Hz, 1H), 7.28–7.24 (m, 2H), 7.13–7.09 (m, 2H), 6.75 (dd, *J* = 2.4, 0.8 Hz, 1H), 6.71 (bt, *J* = 5.6 Hz, 1H), 3.74–3.61 (m, 3H), 3.58–3.50 (m, 1H), 2.93 (t, *J* = 6.0 Hz, 2H), 2.85–2.82 (m, 1H), 2.77–2.71 (m, 1H), 2.63–2.49 (m, 1H), 2.41–2.17 (m, 1H), 1.72–1.55 (m, 2H), 0.88–0.80 (m, 1H), 0.73–0.65 (m, 1H), 0.50–0.44 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 161.8, 140.1, 136.4, 136.0, 130.8, 130.3, 129.6, 129.5, 127.5, 124.3, 121.8, 120.6, 119.1, 112.0, 109.4, 101.9, 62.5, 55.6, 50.1, 39.6, 33.3, 29.2, 15.8, 15.7, 11.4. The oxalate salt was precipitated from acetone. Anal. (C₂₅H₂₆N₄O•1.5C₂H₂O₄) C, H, N. Mp 185–186 °C.

***N*-(2-(2-((7-Cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)methyl-*trans*-cyclopropyl)ethyl)-1*H*-pyrrolo[2,3-*b*]pyridine-2-carboxamide (18b)**

The same procedure was used as described for compound **14a** from **9b** and **24b**. The crude product was purified by flash chromatography using 50% acetone/ CHCl₃ as eluent to provide the product in 41% yield. ¹H NMR of oxalate salt (400 MHz, CD₃OD) δ 8.32 (dd, *J* = 4.4, 1.6 Hz, 1H), 8.07 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.63–7.56 (m, 3H), 7.44–7.39 (m, 2H), 7.16 (dd, *J* = 8.0, 4.8 Hz, 1H), 7.07 (s, 1H), 4.55–4.46 (m, 2H), 4.31 (s, 1H), 3.65–3.58 (m, 3H), 3.53–3.42 (m, 2H), 3.23–3.10 (m, 2H), 1.83–1.75 (m, 1H), 1.55–1.46 (m, 1H), 1.26–1.11 (m, 1H), 0.96 (m, 1H), 0.71–0.67 (m, 2H); ¹³C NMR of free base (100 MHz, CD₃OD) δ 161.5, 147.7, 145.2, 140.4, 139.9, 135.5, 131.7, 130.9, 120.4, 118.8, 116.7, 109.2, 102.2, 62.3, 55.1, 49.9, 39.6, 33.3, 28.6, 15.6, 15.5, 11.1. The oxalate salt was precipitated from acetone; mp 178–179 °C. Anal. (C₂₄H₂₅N₅O•2C₂H₂O₄) C, H, N.

Ethyl *trans*-2-(bromomethyl)cyclopropane-1-carboxylate (20)³⁸

A solution of PBr₃ (1.178 g, 4.35 mmol) in ether (20 mL) was added dropwise to a mixture of ethyl *trans*-2-(hydroxymethyl)cyclopropane-1-carboxylate (**19**)³⁷ (1.697 g, 11.77 mmol) and K₂CO₃ (3.248 g, 23.54 mmol) in ether (50 mL) at –78 °C under an atmosphere of Argon. The reaction mixture was allowed to come to RT, stirred overnight and filtered. The ether layer was washed with saturated NaHCO₃ solution, dried, concentrated and purified by flash chromatography using 1% EtOAc/ hexanes as eluent to provide 0.708 g (29%) of **20**.

***trans*-(2-(Aminoethyl)cyclopropyl)methanol (22)**

A suspension of LiAlH₄ (0.239 g, 6.31 mmol) in THF (30 mL) was stirred at RT for 30 min, cooled to 0 °C, and a solution of **21**³⁸ (0.483 g, 3.15 mmol) in THF (10 mL) was added slowly. The reaction mixture was brought to RT and then heated and stirred at reflux for 3 h. Upon cooling to 0 °C, the reaction mixture was carefully quenched by dropwise addition of water (1.0 mL). The reaction mixture was stirred further for 15 min, filtered and the residue was further washed with EtOAc (10 mL). The combined organic layer was concentrated, diluted with water and extracted in CHCl₃ to give the **22** as an oil, 0.363 g (89%), which was used without further purification in the next step.

***N*-(2-Hydroxymethyl-*trans*-cyclopropylethyl)-1*H*-indole-2-carboxamide (23a)**

The same procedure was used as described for **16a** from **12a** and **22**. The crude product was purified by prep. TLC using 60% EtOAc/ hexanes as eluent to provide the volatile product in 57%. ¹H NMR (400 MHz, CDCl₃) δ 9.58 (s, 1H), 7.61 (dd, *J* = 8.4, 0.8 Hz, 1H), 7.40 (dd, *J* = 8.4, 0.8 Hz, 1H), 7.27–7.23 (m, 1H), 7.12–7.08 (m, 1H), 7.06 (bt, *J* = 5.2 Hz, 1H), 6.97 (dd, *J* = 2.0, 0.8 Hz, 1H), 3.98–3.88 (m, 2H), 3.38–3.32 (m, 1H), 3.19 (bs, 1H), 1.99–1.93 (m, 2H), 1.12–1.03 (m, 1H), 0.97–0.90 (m, 1H), 0.71–0.65 (m, 1H), 0.43–0.34 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 162.1, 136.3, 130.9, 127.6, 124.4, 121.9, 120.5, 112.0, 102.5, 67.1, 39.8, 34.0, 20.9, 16.0, 9.0. GC-MS (EI) *m/z* 258.1 (*M*⁺).

***N*-(2-(2-(Hydroxymethyl-*trans*-cyclopropyl)ethyl)-1*H*-pyrrolo[2,3-*b*]pyridine-2-carboxamide (23b)**

The same procedure was used as described for the **16a** from **12c** and **22**. The crude product was used in the next step without further purification.

***N*-(2-Formyl-*trans*-cyclopropylethyl)-1*H*-indole-2-carboxamide (24a)**

The same procedure was used as described for the **17a** using **23a**. The crude product was purified by flash chromatography using 15% acetone/ CHCl₃ as eluent to provide the desired product in 54% yield. ¹H NMR (400 MHz, CDCl₃) δ 9.54 (s, 1H), 9.08 (d, *J* = 5.2 Hz, 1H), 7.64 (dd, *J* = 8.4, 0.8 Hz, 1H), 7.44 (dd, *J* = 8.4, 0.8 Hz, 1H), 7.29 (dd, *J* = 7.0, 0.8 Hz, 1H), 7.15–7.11 (m, 1H), 6.85 (m, 1H), 6.46 (bt, *J* = 5.2 Hz, 1H), 3.59 (dd, *J* = 12.8, 7.0 Hz, 2H), 1.76–1.68 (m, 3H), 1.58–1.53 (m, 1H), 1.37–1.32 (m, 1H), 1.02–0.98 (m, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 200.7, 161.8, 136.3, 130.5, 127.6, 124.6, 121.9, 120.7, 112.0, 102.0, 39.3, 32.8, 29.9, 20.2, 14.6.

***N*-(2-(2-Formyl-*trans*-cyclopropyl)ethyl)-1*H*-pyrrolo[2,3-*b*]pyridine-2-carboxamide (24b)**

The same procedure was used as described for the **17a** using **23b**. The crude product was purified by flash chromatography using 15% acetone/ CHCl₃ as eluent. ¹H NMR (400 MHz, CD₃OD + CDCl₃, 1:1) δ 8.93 (d, *J* = 5.2 Hz, 1H), 8.34 (d, *J* = 4.0 Hz, 1H), 7.90 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.07–6.97 (m, 3H), 6.93 (s, 1H), 3.47–3.43 (m, 2H), 1.66–1.55 (m, 3H), 1.50–1.46 (m, 1H), 1.26–1.22 (m, 1H), 0.94–0.89 (m, 1H); ¹³C NMR (100 MHz, CD₃OD + CDCl₃, 1:1) δ 201.4, 161.6, 147.8, 145.3, 131.5, 130.9, 120.3, 116.7, 102.1, 39.1, 32.4, 29.9, 20.4, 14.7.

***N*-((2-(2-(7-Cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)-*trans*-cyclopropyl)methyl)-1*H*-indole-2-carboxamide (25a)**

The same procedure was used as described for compound **14a** from **9b** and **32a**. The crude product was purified by flash chromatography using 22% acetone/ CHCl₃ as eluent to provide the desired product in 69% yield. ¹H NMR (400 MHz, CD₃OD + CDCl₃, 2:1) δ 7.88 (d, *J* = 8.4 Hz, 1H), 7.65 (d, *J* = 8.0 Hz, 1H), 7.61 (d, *J* = 8.0 Hz, 1H), 7.48–7.40 (m, 3H), 7.32 (t, *J* = 8.0 Hz, 1H), 7.26 (s, 1H), 3.77 (s, 2H), 3.68 (dd, *J* = 13.6, 6.4 Hz, 1H), 3.33 (dd, *J* = 13.6, 8.0 Hz, 1H), 3.15 (t, *J* = 6.0 Hz, 2H), 3.02–2.88 (m, 2H), 2.86–2.77 (m, 2H), 1.89 (sextet, *J* = 7.2 Hz, 1H), 1.61 (sextet, *J* = 7.2 Hz, 1H), 1.16–1.14 (m, 1H), 0.98–0.97 (m, 1H), 0.77–0.72 (m, 1H), 0.65–0.62 (m, 1H); ¹³C NMR (100 MHz, CD₃OD + CDCl₃, 2:1) δ

162.5, 140.0, 136.7, 135.5, 130.9, 130.3, 129.7, 129.5, 127.5, 124.0, 121.6, 120.1, 118.7, 111.9, 109.1, 103.4, 57.7, 55.2, 49.8, 43.4, 30.5, 28.5, 18.4, 15.6, 10.4. Anal. ($C_{25}H_{26}N_4O \cdot C_2H_2O_4 \cdot 0.25H_2O$) C, H, N. Mp 182–183°C.

***N*-((2-(2-(7-Cyano-3,4-dihydroisoquinolin-2(1*H*)-yl)ethyl)-*trans*-cyclopropyl)methyl)-1*H*-pyrrolo[2,3-*b*]pyridine-2-carboxamide (25b)**

The same procedure was used as described for compound **14a** from **9b** and **32b**. The crude product was purified by flash chromatography using 50% acetone/ $CHCl_3$ as eluent to provide the desired product in 44% yield. 1H NMR of oxalate salt (400 MHz, CD_3OD) δ 8.33 (d, J = 3.6 Hz, 1H), 8.08 (d, J = 7.2 Hz, 1H), 7.62 (m, 2H), 7.53 (m, 1H), 7.42 (d, J = 8.4 Hz, 1H), 7.34 (d, J = 8.4 Hz, 1H), 7.17 (dd, J = 8.0, 5.2 Hz, 1H), 7.06 (s, 1H), 4.44–4.40 (m, 4H), 3.57–3.45 (m, 5H), 3.41–3.16 (m, 1H), 1.90 (m, 1H), 1.70 (m, 1H), 1.01 (s, 1H), 0.84 (s, 1H), 0.62 (m, 1H), 0.52 (m, 1H); ^{13}C NMR of free base (100 MHz, $CDCl_3$) δ 161.4, 148.4, 145.7, 140.7, 140.3, 136.1, 131.9, 130.5, 129.4, 120.1, 119.1, 116.7, 109.2, 100.3, 57.9, 55.8, 49.7, 44.1, 30.9, 29.3, 18.2, 15.9, 10.7. The oxalate salt was precipitated from acetone; mp 189–190 °C. Anal. ($C_{24}H_{25}N_5O \cdot 2.5C_2H_2O_4 \cdot 1H_2O$) C, H, N.

2-(2-((Tetrahydro-2*H*-pyran-2-yl)oxy)ethyl)-*trans*-cyclopropane-1-carbaldehyde (27)

The same procedure was used as described for compound **17a** from **26**⁴⁰. The crude aldehyde was purified by flash chromatography using 15% EtOAc/hexanes as eluent to provide 0.159 g (80%) of the desired product. 1H NMR (400 MHz, $CDCl_3$) δ 8.93 (m, 1H), 4.50 (s, 1H), 3.75–3.70 (m, 2H), 3.42–3.36 (m, 2H), 1.74–1.64 (m, 1H), 1.62–1.43 (m, 9H), 1.25–1.22 (m, 1H), 0.92–0.89 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.8, 98.7, 66.4, 62.1, 32.7, 30.6, 30.0, 25.3, 19.8, 19.4, 14.3. GC-MS (EI) m/z 197.1 (M^+).

2-(2-((Tetrahydro-2*H*-pyran-2-yl)oxy)ethyl)-*trans*-cyclopropane-1-carbaldehyde *O*-benzyl oxime (28)

A mixture of **27** (1.210 g, 6.10 mmol), *O*-benzylhydroxylamine hydrochloride (1.948 g, 12.21 mmol), and molecular sieves 4A (1.000 g) in THF (20 mL) was stirred at RT overnight under Argon. The reaction mixture was filtered through a Florisil bed, and the filtrate was concentrated and purified by flash chromatography using 10% EtOAc/ hexanes as eluent to provide 1.09 g (59%) of an oily material - a mixture of *trans* and *cis* isomers (~1:1). 1H NMR (400 MHz, $CDCl_3$) δ 7.40–7.28 (m, 5H), 6.98 (d, J = 8.4 Hz, 0.5H) *trans*, 6.00 (dd, J = 8.8, 1.2 Hz, 0.5H) *cis*, 5.10 (s, 1H), 5.02 (s, 1H), 4.60–4.57 (m, 1H), 3.87–3.78 (m, 2H), 3.51–3.42 (m, 2H), 2.10–2.07 (m, 0.5H), 1.84–1.78 (m, 1H), 1.73–1.49 (m, 7H), 1.44–1.40 (m, 0.5H), 1.14–1.06 (m, 1H), 0.83–0.78 (m, 1.5H), 0.74–0.70 (m, 0.5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.6, 154.2, 138.2, 137.6, 128.4, 128.2, 127.9, 127.8, 127.7, 98.8, 98.7, 75.7, 75.6, 66.8, 62.3, 62.2, 33.4, 30.7, 25.5, 19.5, 18.1, 17.8, 17.3, 15.1, 15.0, 13.2, 13.1, 12.5, 12.4. ESI 304.1909 ($M+H$)⁺, 326.1725 ($M+Na$)⁺.

(2-(2-((Tetrahydro-2*H*-pyran-2-yl)oxy)ethyl)-*trans*-cyclopropyl)methanamine (29)

A suspension of $LiAlH_4$ (0.067 g, 1.78 mmol) in THF (10 mL) was stirred at RT for 30 min, cooled to 0 °C, and a solution of **28** (0.180 g, 0.59 mmol) in THF (10 mL) was added slowly. The reaction mixture was brought to RT and then heated and stirred at reflux for 3 h. It was

cooled to 0 °C and carefully quenched by dropwise addition of water (1.0 mL). The reaction mixture was stirred further for 15 min, filtered and the residue was further washed with EtOAc (10 mL). The combined organic layer was concentrated, diluted with water and extracted in CHCl₃ to give the title compound as an oil 0.093 g (79%), which was used without further purification in the next step. ¹H NMR (400 MHz, CDCl₃) δ 4.54–4.52 (m, 1H), 3.85–3.73 (m, 2H), 3.48–3.36 (m, 2H), 2–80–2.56 (bs, 2H), 2.46–2.34 (m, 1H), 1.81–1.72 (m, 1H), 1.69–1.62 (m, 2H), 1.55–1.46 (m, 6H), 0.61–0.52 (m, 2H), 0.34–0.24 (m, 2H); GC-MS (EI) m/z 198.2 (M⁺).

***N*-((2-(2-((Tetrahydro-2*H*-pyran-2-yl)oxy)ethyl)-*trans*-cyclopropyl)methyl)-1*H*-indole-2-carboxamide (30a)**

The same procedure was used as described for the **16a** from **12a** and **29**. The crude product was purified by flash chromatography using 20% EtOAc/ hexanes as eluent to provide 0.359 g (41%) of the product as an oil. ¹H NMR (400 MHz, CDCl₃) δ 10.73 (s, 1H), 7.56 (d, *J* = 8.4 Hz, 1H), 7.44 (d, *J* = 8.4 Hz, 1H), 7.20 (d, *J* = 7.6 Hz, 1H), 7.07–6.96 (m, 3H), 4.53 (d, *J* = 18.8 Hz, 1H), 3.86–3.74 (m, 2H), 3.53–3.43 (m, 3H), 3.23–3.18 (m, 1H), 1.74–1.32 (m, 8H), 0.88–0.87 (m, 1H), 0.75 (m, 1H), 0.46–0.44 (m, 1H), 0.35–0.33 (m, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 162.0, 136.8, 131.8, 127.6, 124.0, 121.7, 120.2, 112.3, 102.5, 99.2, 68.6, 62.6, 44.1, 33.6, 30.8, 27.7, 25.4, 22.0, 20.0, 19.8, 17.9, 15.9, 10.5. GC-MS (EI) m/z 342.2 (M⁺).

***N*-((2-(2-((Tetrahydro-2*H*-pyran-2-yl)oxy)ethyl)-*trans*-cyclopropyl)methyl)-1*H*-pyrrolo[2,3-*b*]pyridine-2-carboxamide (30b)**

The same procedure was used as described for the **16a** from **12c** and **29**. The crude product was purified by flash chromatography using 15% acetone/ CHCl₃ as eluent to provide 0.348 g (40%) of the product as oil. ¹H NMR (400 MHz, CDCl₃) δ 10.63 (bs, 1H), 8.51 (d, *J* = 3.2 Hz, 1H), 7.97 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.12 (dd, *J* = 8.4, 5.6 Hz, 1H), 6.94–6.88 (m, 1H), 6.60–6.57 (m, 1H), 4.62–4.56 (m, 1H), 3.95–3.75 (m, 2H), 3.68–3.58 (m, 2H), 3.53–3.50 (m, 1H), 3.16–3.06 (m, 1H), 1.90–1.24 (m, 8H), 0.90–0.83 (m, 1H), 0.80–0.74 (m, 1H), 0.51–0.49 (m, 1H), 0.46–0.40 (m, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 160.9, 148.1, 146.0, 130.3, 120.2, 116.9, 100.6, 99.5, 67.8, 63.1, 44.3, 33.5, 30.9, 25.3, 19.9, 17.7, 15.3, 10.4.

***N*-((2-(2-Hydroxyethyl)-*trans*-cyclopropyl)methyl)-1*H*-indole-2-carboxamide (31a)**

Aqueous HCl solution (10%, 6 mL) was added to a solution of **30a** in THF (4 mL) and stirred overnight. The crude product was extracted with EtOAc, concentrated and purified by flash chromatography using 40% EtOAc/hexanes as eluent to provide 0.178 g (67%) of a white solid product. ¹H NMR (400 MHz, CD₃OD + CDCl₃, 1:1) δ 10.41 (s, 1H), 7.99 (s, 1H), 7.59 (d, *J* = 7.6 Hz, 1H), 7.4 (d, *J* = 9.2 Hz, 1H), 7.20 (t, *J* = 7.6 Hz, 1H), 7.07–7.03 (m, 2H), 3.63 (t, *J* = 6.4 Hz, 2H), 3.51 (dd, *J* = 13.6, 6.0 Hz, 1H), 2.99 (dd, *J* = 14.0, 8.0 Hz, 1H), 1.67–1.59 (m, 1H), 1.29–1.21 (m, 1H), 0.86–0.79 (m, 1H), 0.75–0.67 (m, 1H), 0.47–0.43 (m, 1H), 0.37–0.32 (m, 1H); ¹³C NMR (100 MHz, CD₃OD + CDCl₃, 1:1) δ 162.6, 136.6, 130.8, 127.5, 124.1, 121.7, 120.1, 111.9, 103.6, 62.0, 43.9, 36.0, 17.6, 14.8, 9.8. GC-MS (EI) m/z 258.1 (M⁺).

***N*-((2-(2-Hydroxyethyl)-*trans*-cyclopropyl)methyl)-1*H*-pyrrolo[2,3-*b*]pyridine-2-carboxamide (31b)**

A solution of **30b** (0.268 g, 0.78 mmol) and pyridinium *p*-toluenesulfonate (PPTS) (0.019 g, 0.08 mmol) in EtOH (5 mL) was stirred at 55 °C overnight. The solvent was evaporated and the crude product was purified by column chromatography, using 45% acetone/ CHCl₃ as eluent to provide 0.142 g (70%) of white solid product. ¹H NMR (400 MHz, CD₃OD + CDCl₃, 1:1) δ 8.29 (d, *J* = 4.0 Hz, 1H), 8.13 (t, *J* = 5.2 Hz, 1H), 7.96 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.08–7.01 (m, 2H), 3.64 (t, *J* = 6.4 Hz, 2H), 3.56–3.50 (m, 1H), 3.01–2.95 (m, 1H), 1.67–1.59 (m, 1H), 1.28–1.19 (m, 1H), 0.85–0.80 (m, 1H), 0.73–0.68 (m, 1H), 0.47–0.43 (m, 1H), 0.37–0.32 (m, 1H); ¹³C NMR (100 MHz, CD₃OD + CDCl₃, 1:1) δ 161.6, 147.7, 145.1, 131.7, 131.0, 120.5, 116.6, 102.6, 62.1, 44.1, 35.9, 17.7, 14.8, 9.9.

***N*-((2-(2-Oxoethyl)-*trans*-cyclopropyl)methyl)-1*H*-indole-2-carboxamide (32a)**

The same procedure was used as described for **17a** from **31a**. The crude product was purified by flash chromatography using 5% acetone/ CHCl₃ as eluent to provide the desired product in 79% yield. ¹H NMR (400 MHz, acetone-*d*₆) δ 10.77 (bs, 1H), 9.79 (s, 1H), 7.96 (s, 1H), 7.63 (d, *J* = 8.0 Hz, 1H), 7.66 (d, *J* = 8.0 Hz, 1H), 7.24–7.16 (m, 2H), 7.07 (t, *J* = 7.6 Hz, 1H), 3.62–3.56 (m, 1H), 3.12–3.06 (m, 1H), 2.62 (dd, *J* = 18.0, 5.6 Hz, 1H), 2.30 (d, *J* = 18.0, 8.0 Hz, 1H), 0.99–0.93 (m, 2H), 0.64–0.60 (m, 1H), 0.49–0.44 (m, 1H); ¹³C NMR (100 MHz, acetone-*d*₆) δ 203.3, 162.0, 137.6, 132.9, 128.8, 124.4, 122.4, 120.8, 113.0, 102.6, 48.0, 44.1, 11.5, 10.5.

***N*-((2-(2-Oxoethyl)-*trans*-cyclopropyl)methyl)-1*H*-pyrrolo[2,3-*b*]pyridine-2-carboxamide (32b)**

The same procedure was used as described for **17a** from **31b**. The crude product was purified by flash chromatography using 30% acetone/ CHCl₃ as eluent to provide the desired product in 69% yield. ¹H NMR (400 MHz, (CD₃)₂SO) δ 12.01 (s, 1H), 8.48–8.46 (m, 1H), 8.28 (d, *J* = 4.4 Hz, 1H), 8.04–7.91 (m, 2H), 7.09–7.06 (m, 2H), 3.17–3.09 (m, 2H), 1.52–1.27 (m, 2H), 0.85–0.77 (m, 1H), 0.72–0.64 (m, 1H), 0.39–0.37 (m, 1H), 0.28–0.26 (m, 1H); ¹³C NMR (100 MHz, (CD₃)₂SO) δ 203.6, 168.1, 148.7, 145.6, 134.9, 126.8, 120.8, 116.8, 100.5, 47.1, 43.1, 18.3, 13.7, 10.4.

Radioligand binding assays

Binding at dopamine D₂-like receptors was determined using previously described methods.⁴³ Membranes were prepared from HEK293 cells expressing human D₂R, D₃R or D₄R, grown in a 50:50 mix of DMEM and Ham's F12 culture media, supplemented with 20 mM HEPES, 2 mM L-glutamine, 0.1 mM non-essential amino acids, 1X antibiotic/antimycotic, 10% heat-inactivated fetal bovine serum, and 200 µg/mL hygromycin (Life Technologies, Grand Island, NY) and kept in an incubator at 37°C and 5% CO₂. Upon reaching 80–90% confluence, cells were harvested using pre-mixed Earle's Balanced Salt Solution (EBSS) with 5 mM EDTA (Life Technologies) and centrifuged at 3000 rpm for 10 min at 21 °C. The supernatant was removed and the pellet was resuspended in 10 mL hypotonic lysis buffer (5 mM MgCl₂ · 6 H₂O, 5 mM Tris, pH 7.4 at 4 °C) and centrifuged at 20,000 rpm for 30 min at 4 °C. The pellet was then resuspended in fresh EBSS buffer made

from 8.7 g/L Earle's Balanced Salts without phenol red (US Biological, Salem, MA), 2.2 g/L sodium bicarbonate, pH to 7.4. A Bradford protein assay (Bio-Rad, Hercules, CA) was used to determine the protein concentration and membranes were diluted to 500 µg/mL and stored in a -80 °C freezer for later use.

Radioligand competition binding experiments were conducted using thawed membranes. Test compounds were freshly dissolved in 30% DMSO and 70% H₂O to a stock concentration of 100 µM. To assist the solubilization of free-base compounds, 10 µl of glacial acetic acid was added along with the DMSO. Each test compound was then diluted into 13 half-log serial dilutions using 30% DMSO vehicle; final test concentrations ranged from 10 µM to 10 pM. Previously frozen membranes were diluted in fresh EBSS to a 100 µg/mL (for hD₂R or hD₃R) or 200 µg/mL (hD₄R) stock for binding. Radioligand competition experiments were conducted in glass tubes containing 300 µl fresh EBSS buffer with 0.2 mM sodium metabisulfite, 50 µl of diluted test compound, 100 µl of membranes (10 µg total protein for hD₂R or hD₃R, 20 µg total protein for hD₄R), and 50 µl of [³H]N-methylspiperone (0.4 nM final concentration; Perkin Elmer). Nonspecific binding was determined using 10 µM (+)-butaclamol (Sigma-Aldrich, St. Louis, MO) and total binding was determined with 30% DMSO vehicle. All compound dilutions were tested in triplicate and the reaction incubated for one hour at room temperature. The reaction was terminated by filtration through Whatman GF/B filters, presoaked for one hour in 0.5% polyethylenimine, using a Brandel R48 filtering manifold (Brandel Instruments, Gaithersburg, MD). The filters were washed 3 times with 3 mL of ice cold EBSS buffer and transferred to scintillation vials. 3 mL CytoScint liquid scintillation cocktail (MP Biomedicals, Solon, OH) was added and vials were counted using a Perkin Elmer Tri-Carb 2910 TR liquid scintillation counter (Waltham, MA). IC₅₀ values for each compound were determined from dose-response curves and K_i values were calculated using the Cheng-Prusoff equation; these analyses were performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA).⁵⁰ Reported K_i values were determined from least three independent experiments.

β-Arrestin Recruitment Assays

Measurement of β-arrestin recruitment was conducted with minor modifications as previously published by our laboratory using the DiscoverX PathHunter technology (DiscoverX Inc., Fremont, CA).^{46,49,51,52} The amino-terminal fragment of β-galactosidase is fused to the DAR while the carboxyl-terminal fragment of the enzyme is fused to β-arrestin-2. Upon agonist activation, β-arrestin is recruited to the receptor resulting in the formation of an active β-galactosidase, which can be detected using a luminescent substrate. Briefly, CHO-K1 cells expressing either the D₂R long isoform or the D₃R were maintained in Ham's F12 supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 µg/ml streptomycin, 800 µg /ml G418, and 300 µg/ml hygromycin; at 37°C, 5% CO₂, and 90% humidity. Cells were seeded in Cell Plating Media 2 (DiscoverX) at a density of 2,625 cells/well in 384-well black, clear-bottom plates. Test compounds were diluted in PBS in the presence of 0.2 µM sodium metabisulfite for agonist mode assays and in an identical assay buffer with an EC₈₀ concentration of dopamine (1 µM for D₂R assays, and 30 nM for D₃R assays) for antagonist mode assays. For curve shift experiments, concentration response

curves of dopamine were generated with or without the indicated concentration of test compound. Cells were preincubated with the compounds at 37°C for 90 min followed by the addition of DiscoverX reagent followed by a 30 min incubation at room temperature. Luminescence was determined on a Hamamatsu FDSS μ Cell plate reader. Data were collected as RLUs and subsequently normalized to a percentage of the control luminescence seen with a maximum concentration of dopamine for agonist mode assays and as a percentage of the EC₈₀ dopamine concentration for antagonist mode assays.

Data analysis of functional experiments

Data analysis—GraphPad Prism 6.0b (San Diego, CA) was used for all statistical analysis, nonlinear regression, and simulations.

Radioligand binding data—Competition-binding curves between [³H]raclopride and **1** could be fit to the allosteric ternary complex model using the following equation:⁵³

$$Y = \frac{\frac{[A]}{K_A}}{\frac{[A]}{K_A} + \left(\frac{1 + \frac{[B]}{K_B}}{1 + \alpha \frac{[B]}{K_B}} \right)} \quad (1)$$

Where Y is percentage (vehicle control) binding; [A] and [B] are the concentrations of [³H]raclopride and **1**, respectively; K_A and K_B are the equilibrium dissociation constants of [³H]raclopride and **1**, respectively; α is the cooperativity between **1** and [³H]raclopride. Values of $\alpha > 1$ denote positive cooperativity; values < 1 (but > 0) denote negative cooperativity, and values = 1 denote neutral cooperativity.

Analysis of functional data—All concentration response (C/R) data were fitted to the following modified four-parameter Hill equation to derive potency estimates:⁵⁴

$$E = Basal + \frac{(E_{max} - Basal) \cdot [A]^{nH}}{[A]^{nH} + EC_{50}^{nH}} \quad (2)$$

Where E is the effect of the system, nH is the Hill slope, and EC₅₀ is the concentration of agonist [A] that gives the midpoint response between basal and maximal effect of dopamine or other agonists (E_{max}), which are the lower and upper asymptotes of the response, respectively.

Functional data describing the interaction between **1** and dopamine were globally analyzed according to the allosteric ternary complex model.

$$E = \frac{E_m \cdot [A]^{nH}}{[A]^{nH} + [EC_{50}]^{nH} \left(\frac{1 + \frac{[B]}{K_B}}{1 + \frac{\alpha\beta[B]}{K_B}} \right)} \quad (3)$$

Where E_m is the maximum possible cellular response, $[A]$ and $[B]$ are the concentrations of orthosteric and allosteric ligands, respectively, and K_B is the equilibrium dissociation constant of the allosteric ligand, $\alpha\beta$ is a composite cooperativity parameter between the orthosteric and allosteric ligand that includes effects upon orthosteric ligand affinity and efficacy and nH is the Hill slope of the orthosteric agonist concentration-response curve. Values of α and/or β greater than 1 denote allosteric potentiation, whereas values less than 1 (but greater than 0) denote allosteric inhibition.

Functional data describing the interaction between **25a & 18a** and dopamine at the D_3R were analyzed using a complete operational model of allosterism and agonism according to equation 3⁵⁵:

$$E = \frac{E_m (\tau_A [A] (K_B + \alpha\beta[B]) + \tau_B [B] K_A)^{nH}}{([A]K_B + K_A K_B + K_A [B] + \alpha[A][B])^{nH} + (\tau_A [A] (K_B + \alpha\beta[B]) + \tau_B [B] K_A)^{nH}} \quad (4)$$

Where E_m is the maximum possible cellular response, $[A]$ and $[B]$ are the concentrations of orthosteric and allosteric ligands, respectively, K_A and K_B are the equilibrium dissociation constant of the orthosteric and allosteric ligands, respectively, and τ_B (constrained to 0.001) are operational measures of orthosteric and allosteric ligand efficacy (which incorporate both signal efficiency and receptor density), respectively, α is the binding cooperativity parameter between the orthosteric and allosteric ligand, and β denotes the magnitude of the allosteric effect of the modulator on the efficacy of the orthosteric agonist. K_A was constrained to a value determined in a radioligand binding assay (0.3 μM).

A logistic equation of competitive agonist-antagonist interaction was globally fitted to data from functional experiments measuring the interaction between dopamine and test compounds which caused an unlimited rightward displacement of a dopamine dose-response curve and no decrease in E_{max} within the range of concentrations used (**2**, **14f**, **14a**, **14c**, **14e**, **14d**, **18b**, **25b**):⁵⁴

$$Response = Bottom + \frac{(E_{max} - Bottom)}{1 + \left(\frac{10^{-pEC_{50}} \left[1 + \left(\frac{[B]}{10^{-K_B}} \right)^s \right]^{nH}}{[A]} \right)} \quad (5)$$

Where s represents the Schild slope for the test compound and K_B is the equilibrium dissociation constant of the test compound, nH is the Hillslope, and EC_{50} is the

concentration of agonist [A] that gives the midpoint response between basal and maximal effect of dopamine or other agonists ($E_{\max}$), which are the lower and upper asymptotes of the response, respectively. For all cases where a competitive mode of interaction was preferred then the Schild slope did not differ significantly from unity as determined by an extra-sum-of-squares F-test, $P > 0.05$. For each of the novel compounds the two equations (models) were then compared for their fit using an extra-sum-of-squares F test, whereby the simpler model was selected unless the P value was less than 0.05.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS USED

DA	dopamine
SAR	structure activity relationship
TM	transmembrane
D₂R	D ₂ dopamine receptor
D₃R	D ₃ dopamine receptor
D₄R	D ₄ dopamine receptor
OBS	orthosteric binding site
PPTS	pyridinium <i>p</i> -toluenesulfonate
SBP	secondary binding pocket

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Figure 1.
Selective pharmacological tools for the dopamine D₃R.

Figure 2. Curve-shift assays contrasting the competitive vs. non-competitive behavior of known D3R antagonists

Dopamine-mediated β -arrestin recruitment assays were conducted by stimulating the receptor with a concentration response curve of dopamine at the indicated concentrations either with or without various concentrations of test compound. Data were measured as RLU and are expressed as a percentage of the maximum dopamine response seen in the absence of any test compound. Data points represent the mean $\pm$ SEM of three independent experiments performed in triplicate. Analysis of data fit is shown in Table 3.

Figure 3. Curve-shift assays indicate the 14-series compounds behave in a competitive manner at the D₃R

Dopamine-mediated β -arrestin recruitment assays were conducted by stimulating the receptor with a concentration response curve of dopamine at the indicated concentrations either with or without various concentrations of test compound. Data were measured as RLU and are expressed as a percentage of the maximum dopamine response seen in the absence of any test compound. Data points represent the mean $\pm$ SEM of two to four independent experiments performed in triplicate. Analysis of data fit is shown in Table 3.

Figure 4. Curve-shift assays indicate **18a** and **25a** behave in a non-competitive manner with dopamine at the D₃R

Dopamine-mediated β -arrestin recruitment assays were conducted by stimulating the receptor with a concentration response curve of dopamine at the indicated concentrations either with or without various concentrations of test compound. Data were measured as RLU and are expressed as a percentage of the maximum dopamine response seen in the absence of any test compound. **18a** and **25a** were best fit with a complete operational model of allosterism and agonism, indicating these compounds behave in a non-competitive manner at the D₃R (top row). The replacement of the indole ring in **18a** and **25a** with an azaindole ring as in **18b** and **25b** resulted in data that were best fit with a competitive Gaddum/Schild model (bottom row). Data points represent the mean $\pm$ SEM of three independent experiments performed in triplicate.

Scheme 1. Synthesis of synthons ^a

^aReagents and conditions: (a) bis(2-chloroethyl)amine.HCl, diethyleneglycol monoethylether, 150 °C, 6 h; (b) 1-bromobutane, K₂CO₃, acetone, reflux, overnight; (c) amine, CDI, THF, 0 °C to RT, overnight.

Scheme 2. Synthesis of analogues 14a–f ^a

^aReagents and conditions: (a) ArCOOH, CDI, THF, 0 °C to RT; overnight (b) Dess-Martin periodinane reagent, CH₂Cl₂, –78 °C to RT, 3 h ; (c) 2,3-dichlorophenyl piperazine or 3-cyanophenyl piperazine or 6-cyano-1,2,3,4-tetrahydroisoquinoline or 7-cyano-1,2,3,4-tetrahydroisoquinoline, NaBH(OAc)₃, AcOH, DCE, RT, overnight.

Scheme 3. Synthesis of analogues 18a and 18b ^a

^aReagents and conditions: (a) PBr_3 , K_2CO_3 , ether, -78°C to RT, overnight; (b) KCN , DMSO, 60°C , 3 h; (c) LAH, THF, reflux, 3 h; (d) **12a** or **12c**, CDI, DMF, 0°C to RT, overnight; (e) Dess-Martin periodinane reagent, CH_2Cl_2 , -78°C to RT, 3 h; (f) **9b**, $\text{NaBH}(\text{OAc})_3$, AcOH, DCE, RT, overnight.

Scheme 4. Synthesis of analogues 25a and 25b^a

^aReagents and conditions: (a) Dess-Martin periodinane reagent, CH₂Cl₂, -78 °C to RT, 3h; (b) BnONH₂.HCl, mol. sieves, THF, RT, overnight; (c) LAH, THF, reflux, 3 h; (d) **12a** or **12c**, CDI, THF, 0 °C to RT (e) 10% HCl, THF, RT, overnight or PPTS, THF, 55 °C, overnight; (f) Dess-Martin periodinane reagent, THF, -78 °C to RT, 3h; (g) **9b**, NaBH(OAc)₃, DCE, AcOH, RT, overnight.

Table 1*in vitro* pharmacological profile for synthons

Compd	Structure	D ₂ R	D ₃ R	D ₂ /D ₃ ^d	D ₂ R	D ₃ R
		Binding			β-Arrestin Recruitment	
		K _i ^a (nM) ± SEM			pK _B ^b ± SEM (K _B , nM)	
8		4890 ± 700	581 ± 70	8.4	5.68 ± 0.15 (2090)	5.42 ± 0.06 (3800)
9a		53800 ± 6800	5890 ± 1700	9.1	<5 (>10,000)	<5 (>10,000)
9b		11400 ± 1800	1520 ± 36	7.5	5.37 ± 0.10 (4270)	5.49 ± 0.22 (3240)
10		493 ± 28	34.7 ± 0.67	14	7.12 ± 0.13 (75)	7.50 ± 0.14 (32)
11a		8300 ± 1100	1090 ± 20	7.7	5.78 ± 0.13 (1660)	5.99 ± 0.09 (1020)

Compd	Structure	D ₂ R	D ₃ R	D ₂ /D ₃ ^d	D ₂ R	D ₃ R
		Binding			β-Arrestin Recruitment	
		K _i ^a (nM) ± SEM			pK _B ^b ± SEM (K _B , nM)	
11b		832 ± 190	149 ± 3.0	5.6	7.00 ± 0.19 (100)	6.70 ± 0.25 (200)
13a		>30 μM	>50 μM	0.42	NA ^c	NA
13b		>100 μM ± 0	>100 μM ± 0	0	NA	NA
13c		>100 μM ± 0	>100 μM ± 0	0	NA	NA
13d		13400 ± 1285	>100 μM ± 0	0	NA	NA

^a Estimate of the equilibrium dissociation constant $\pm$ standard error of the mean (SEM) determined by inhibition of [³H]N-methylspiperone binding in membranes harvested from HEK 293 cells stably expressing hD₂R or hD₃R as described in Experimental Methods.

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p Estimate of the negative logarithm of the equilibrium dissociation constant $\pm$ SEM determined in a β -arrestin assay using the DiscoverX PathHunter assay; by fitting IC₅₀ data to equation 5 as described in the Methods.

c NA indicates that “no activity” was observed at concentrations up to 100 μ M. Values represent the mean obtained from 3 individual experiments.

Table 2

in vitro pharmacological profile for extended length compounds

Compd	Structure	clogP	PSA	D ₂ R	D ₃ R	D ₂ /D ₃	D ₂ R	D ₃ R		
Radioligand Binding										
p <i>K</i> _B ± SEM (<i>K</i> _B , nM)										
R ₁	R ₂	m	n	<i>K</i> _i ^a (nM) ± SEM			β-Arrestin Recruitment			
1		4.78	68.16	485 ± 84	3.9 ± 1.6	124	5.60 ± 0.10 ^c (2510)	7.72 ± 0.14 ^c (19)		
2		4.82	68.49	5160±115	11.0±2.63	469	5.19 ± 0.19 ^b (6490)	7.55 ± 0.19 ^d (28)		
14a		1	1	5.29	47.61	8.41±0.52	0.45±0.06	19	6.70 ± 0.23 ^b (200)	7.13 ± 0.23 ^d (74)
14b		1	1	5.34	47.94	19.9±1.6	1.51±0.18	13	7.78 ± 0.26 ^b (17)	7.29 ± 0.12 ^d (51)
14c		1	1	3.75	71.73	2290±91	51.7±9.7	44	6.68 ± 0.16 ^b (209)	6.84 ± 0.10 ^d (144)

Compd	Structure	clogP	PSA	D ₂ R	D ₃ R	D ₂ /D ₃	Radioligand Binding		β-Arrestin Recruitment	
							K_i^H (nM) ± SEM		pK_B ± SEM (K_B , nM)	
14d		1	1	3.23	68.49	9500±3300	1040±290	9	5.76 ± 0.13 ^b (1740)	5.96 ± 0.19 ^d (1100)
14e		1	1	3.23	68.49	1440±270	256±35	6	6.96 ± 0.18 ^b (110)	6.62 ± 0.12 ^d (240)
14f		1	1	3.19	68.16	695 ±72	32.0±4.2	22	6.56 ± 0.19 ^b (275)	6.57 ± 0.10 ^d (269)
18a		1	2	3.72	68.16	182±3.20	0.890±0.02	204	6.88 ± 0.29 ^b (132)	8.36 ± 0.06 ^e (4)
18b		1	2	3.10	80.52	14.4±2.5	2.47±0.3	6	8.29 ± 0.13 ^b (5)	8.44 ± 0.11 ^d (4)
25a		2	1	3.72	68.16	476±19.4	9.81±1.6	49	5.76 ± 0.08 ^b (1740)	7.56 ± 0.23 ^e (28)

Compd	Structure	clogP	PSA	D ₂ R	D ₃ R	D ₂ /D ₃	D ₂ R	D ₃ R		
Radioligand Binding										
β-Arrestin Recruitment										
K_i^a (nM) ± SEM										
$pK_B \pm SEM$ (K_B , nM)										
25b		2	1	3.10	80.52	190±34	23.4±4.4	8	7.19 ± 0.11 ^b (65)	7.49 ± 0.11 ^d (32)

^a Estimate of the equilibrium dissociation constant ± standard error of the mean (SEM) determined by inhibition of [³H]N-methylspiperone binding in membranes harvested from HEK 293 cells stably expressing hD₂R or hD₃R as described in the Methods. Estimate of the equilibrium constant for **1** at D₂R and D₃R determined through application of equation 1.

^b Estimate of the negative logarithm of the equilibrium dissociation constant ± SEM determined in a β arrestin assay using the DiscoverX PathHunter assay, by; fitting IC₅₀ data to equation 5,

^c by fitting curve shift data to equation 3,

^d by fitting curve shift data to equation 5 as described in the methods, or

^e by fitting curve shift data to equation 4. Values represent the mean obtained from 2–3 individual experiments.

Table 3Affinity and cooperativity of allosteric compounds at D₃R.

Compd	$pK_B \pm SEM^a$ (K_B , nM)	$Log\alpha \pm SEM^b$ (α)	$Log\beta \pm SEM^c$ (β)
1	$7.72 \pm 0.14^*$ (19)	-2.05 ± 0.11 (0.009)	$= 0$ (1)
18a	$8.36 \pm 0.06^*$ (4)	-1.36 ± 0.08 (0.04)	-2.29 ± 0.10 (0.005)
25a	$7.56 \pm 0.23^*$ (28)	-1.54 ± 0.19 (0.03)	-1.57 ± 0.34 (0.03)

Estimate of the negative logarithm of the equilibrium dissociation constant^a, negative cooperativity with dopamine affinity^b and efficacy^c determined in a β -arrestin assay using the DiscoverX PathHunter assay by fitting IC₅₀ data to equation 3 (for **1**) or equation 4 (for **18a** and **25a**) as described in the Methods. Values represent the mean obtained from at least 3 individual experiments.

* Values also displayed in Table 2.