Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

Structure−Functional Selectivity Relationship Studies of β-Arrestin-Biased Dopamine D_2 Receptor Agonists

Xin Chen,[†,⊥] Maria F. Sassano,[‡,⊥] Lianyou Zheng,[†,§] Vincent Setola,[‡] Meng Chen,[∥] Xu Bai,[§] Stephen V. Frye,[†] William C. Wetsel,[∥] Bryan L. Roth,*[,‡] and Jian Jin*[,†]

[†]Center for Integrative Chemical Biology and Drug Discovery, Division of Chemical Biology and Medicinal Chemistry, UNC Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, United States

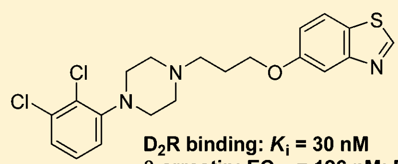
[‡]Department of Pharmacology and National Institute of Mental Health Psychoactive Drug Screening Program, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, United States

[§]Center for Combinatorial Chemistry and Drug Discovery, Jilin University, Changchun, Jilin 130012, China

[∥]Departments of Psychiatry and Behavioral Sciences, Cell Biology, and Neurobiology, Duke University Medical Center, Durham, North Carolina 27710, United States

S *Supporting Information*

ABSTRACT: Functionally selective G protein-coupled receptor (GPCR) ligands, which differentially modulate canonical and noncanonical signaling, are extremely useful for elucidating key signal transduction pathways essential for both the therapeutic actions and side effects of drugs. However, few such ligands have been created, and very little purposeful attention has been devoted to studying what we term: "structure−functional selectivity relationships" (SFSR). We recently disclosed the first β-arrestin-biased dopamine D_2 receptor (D_2R) agonists UNC9975 (**44**) and UNC9994 (**36**), which have robust in vivo antipsychotic drug-like activities. Here we report the first comprehensive SFSR studies focused on exploring four regions of the aripiprazole scaffold, which resulted in the discovery of these β-arrestin-biased D_2R agonists. These studies provide a successful proof-of-concept for how functionally selective ligands can be discovered.



D_2R binding: K_i = 30 nM
β-arrestin: EC_{50} = 126 nM; E_{max} = 88%
c-AMP: inactive

INTRODUCTION

Classical notions of receptor pharmacology imply that when a ligand interacts with a receptor, only a unitary outcome is possible. Accordingly, a full or partial agonist can activate only a single signal transduction pathway while an antagonist can only block the actions of an agonist. The key theoretical construct underlying this model was the concept of intrinsic efficacy.[1] According to this conceptualization, a full agonist has maximum intrinsic efficacy and maximally stimulates ***all*** cellular responses induced by ligand binding. A partial agonist possesses a lower degree of intrinsic efficacy and partially activates all cellular responses induced by an agonist. Antagonists, according to this schema, are neutral entities which possess no intrinsic activity but are able to block the receptor and preclude activation by full or partial agonists.[1] An extension of this model has been that a single G protein-coupled receptor (GPCR) interacts with a single G protein subtype and that full and partial agonists activate only a single signal transduction pathway.

For many decades, however, it has been clear that these simplistic notions of GPCR function cannot account for the myriad of actions induced by agonist and antagonist binding. This was first convincingly demonstrated for the serotonin and dopamine families of receptors. For example, it has been demonstrated that: (1) agonists differentially activate distinct signal transduction pathways,[2−9] (2) antagonists can possess negative intrinsic activity (e.g., function as inverse agonists) or be silent and thereby block the actions of an inverse agonist (e.g., neutral antagonists),[10−14] and (3) antagonists can also induce receptor down regulation[15] and receptor internalization in vitro[16] and in vivo,[17,18] properties typically associated with agonists.

Functional selectivity[19,20] refers to the process by which GPCR ligands differentially modulate canonical pathways involving heterotrimeric large G proteins and noncanonical G protein-independent pathways involving other signaling proteins including β-arrestins.[6,9,21−24] GPCR ligands with distinct functional selectivity patterns will be extremely useful tools for elucidating the key signaling pathways essential for both the therapeutic actions and the side effects of GPCR targeted drugs.[20] Understanding which signaling pathways contribute to antipsychotic efficacy and side effects, for instance, should enable the design of better antipsychotic drug candidates and may, ultimately, lead to safer and more effective therapies for patients. Despite the importance of functionally selective ligands, only a limited number have been reported.[6,19,20,24−27] In addition, to our knowledge, scant attention has been devoted to studying structure−functional selectivity relationships (SFSR). We recently reported the first β-arrestin-biased dopamine D_2 receptor (D_2R) agonists

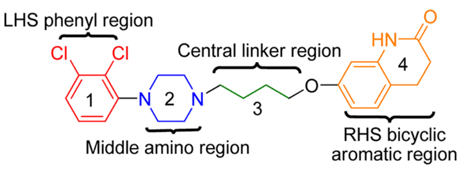
Received: May 1, 2012
Published: July 30, 2012

   dx.doi.org/10.1021/jm300603y | *J. Med. Chem.* 2012, 55, 7141−7153

UNC9975 (**44**) and UNC9994 (**36**), which are simultaneously inactive toward G_i-regulated cyclic adenosine monophosphate (cAMP) production and partial agonists for D_2R/β-arrestin-2 interactions.[28] These β-arrestin-biased D_2R agonists displayed robust antipsychotic drug-like activities in wild-type mice, and the antipsychotic drug-like activities were significantly attenuated or completely abolished in β-arrestin-2 knockout mice, suggesting that β-arrestin recruitment and signaling can be a significant contributor to antipsychotic efficacy.[28] Here we report our SFSR studies that resulted in the discovery of these β-arrestin-biased D_2R agonists. We describe the design, synthesis, and in vitro and in vivo pharmacological evaluation of novel compounds that explore four regions of the scaffold represented by aripiprazole (**1**), an FDA-approved atypical antipsychotic drug.[24,29,30] These first comprehensive SFSR studies provide a successful proof-of-concept for how functionally selective ligands of GPCRs can be discovered.

■ RESULTS AND DISCUSSION

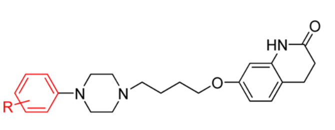
We selected compound **1** as the starting point for the following reasons: (1) **1** is an FDA-approved drug with excellent pharmacokinetic (PK) properties including high oral bioavailability and central nervous system (CNS) penetration,[28,31] PK properties likely to be retained by its close analogues,[28] (2) although some structure−activity relationships (SAR) have been reported,[32−34] the SFSR of the scaffold represented by compound **1** have not been studied, and (3) this core template is amenable to rapid multidimensional chemical modifications and optimization, which is ideal for exploring SFSR. To determine whether modifying various structural motifs of compound **1** could result in biased compounds that favor either cAMP or β-arrestin signaling, we intensively investigated the following four regions of compound **1**: (1) the left-hand side (LHS) phenyl ring with various mono- or disubstitution, (2) the middle cyclic amino moiety, (3) the central linker, and (4) the right-hand side (RHS) bicyclic aromatic moiety (Figure 1).



1

Figure 1. Four regions of compound **1** investigated for SFSR.

SFSR of the LHS Phenyl Moiety. To determine the effects of substituents in the LHS phenyl ring on D_2R functional selectivity, we designed the compounds outlined in Figure 2. Because Oshiro and co-workers reported that 2-substituents at the LHS phenyl were preferred for D_2R and enhanced in vivo activity,[33] we explored a number of electron donating or withdrawing groups at the 2-position (compounds **2**−**10**). In addition, 3- and 4-substitution and 2,3-disubsitution were also investigated (compounds **1**, **11**−**13**).



R = 2,3-(Cl)$_2$	**1**		
R = 2-OCH$_3$	**2**	R = 2-CN	**8**
R = 2-OEt	**3**	R = 2-CH$_3$	**9**
R = 2-OiPr	**4**	R = 2-CF$_3$	**10**
R = 2-H	**5**	R = 3-OEt	**11**
R = 2-F	**6**	R = 4-OEt	**12**
R = 2-Cl	**7**	R = 2,3-(CH$_3$)$_2$	**13**

Figure 2. Compounds designed to explore the LHS phenyl moiety.

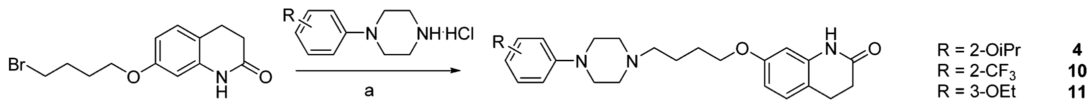
A representative synthesis of these compounds is shown in Scheme 1. Compounds **4**, **10**, and **11** were produced via nucleophilic displacement of commercially available 7-(4-bromobutoxy)-3,4-dihydroquinolin-2(1*H*)-one with the corresponding 2-subsituted phenylpiperazines in moderate to good yields. The 2-isopropoxy-,[35] 2-trifluoromethyl-,[36] and 3-ethoxyphenyl-[37] piperazine intermediates were prepared according to literature procedures. Synthesis of compounds **1**−**3**, **5**−**9**, **12**, and **13** were described previously.[33,34]

The synthesized compounds were evaluated in: (1) D_2R radioligand binding assay to assess their binding affinity to the receptor, (2) D_2R-mediated cAMP accumulation assay, which measures inhibition of isoproterenol-stimulated cAMP production via the G_i-coupled signaling pathway, and (3) D_2R-mediated β-arrestin-2 recruitment Tango assay to determine their potency and efficacy in activating β-arrestin translocation. Quinpirole, a full agonist of D_2R,[38] was used as the positive control in both cAMP accumulation and β-arrestin-2 recruitment Tango assays. Details of these binding and functional assays were described in our recent paper.[28]

Similar to compound **1**, which potently activated both D_2R-mediated G_i-regulated cAMP accumulation and β-arrestin-2 recruitment, compounds with or without a 2-subsituent (compounds **2**−**10**) had high D_2R binding affinities (K_i < 10 nM) and high potencies for activating both G_i signaling and β-arrestin-2 recruitment (Table 1). These compounds did not display apparent bias for activating either G_i signaling or β-arrestin-2 recruitment, suggesting that neither the electronic nature (electron donating or withdrawing, compounds **2**, **5**−**10**) nor the steric bulkiness (compounds **2**−**4**) of the 2-substituent significantly modulates functional selectivity profiles. Interestingly, compounds **5** and **6** activated both G_i and β-arrestin signaling with very high potencies (EC_{50} < 1 nM) and efficacies (E_{max} similar to the positive control quinpirole). On the other hand, compound **2** was a potent, low efficacy partial agonist (E_{max} = ∼40%) at both signaling pathways. In addition, compounds **3**, **4**, and **10** were about 10-fold more potent at activating β-arrestin than G_i signaling. Effects of the substitution patterns were also evaluated. Moving the ethoxy group from the *ortho*-position (compound **3**) to the *meta*- (compound **11**) or *para*-position (compound **12**) resulted in significant decreases in binding affinities and agonist potencies. However, these modifications did not lead to significant changes in functional selectivity patterns. Additionally, compound **13**, which possesses a 2,3-dimethyl phenyl group, displayed similar efficacies (e.g., E_{max} values) for activating both G_i and β-arrestin pathways (similar to compound **1**), although compound **13** was significantly more potent at activating β-arrestin than G_i signaling. Overall, the electronic nature and steric bulkiness of substituents on the LHS phenyl ring and various patterns of substitution did not significantly affect patterns of D_2R functional selectivity for the tested compounds.

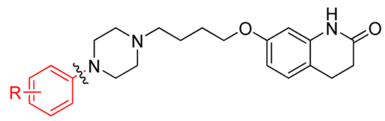
SFSR of the Middle Amino Moiety. We next investigated the middle amino moiety of compound **1**. Compounds **18** and **19** (Scheme 2) were designed and synthesized to modulate the basicity of the inner nitrogen and the ring size of the cyclic amino motif, respectively. 1,4-Addition of the in situ generated Grignard reagent to activated pyridinium species gave the intermediate **14** in 76% yield, which was then converted to the desired 4-aryl piperidine **16** via hydrogenation and subsequent deprotection in good overall yield. The nucleophilic displacement of the commercially available 7-(4-bromobutoxy)-3,4-

Scheme 1. Synthesis of Compounds with Various Substituents on the LHS Phenyl Ring[a]



[a]Reagents and conditions: (a) NaI/K_2CO_3, CH_3CN, reflux, 6 h, 40−70%.

Table 1. SFSR of the LHS Phenyl Moiety[a]



			β-arrestin		cAMP	
Cmpd	R	D_2R K_i (nM)	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)
1	2,3-$(Cl)_2$	3.9	4.0	62	1.0	51
2	2-OCH_3	0.3	0.6	46	2.5	40
3	2-OEt	2.8	0.8	66	7.9	46
4	2-OiPr	3.1	2.0	63	25	43
5	2-H	4.3	0.4	87	0.8	90
6	2-F	5.5	0.8	89	0.3	93
7	2-Cl	3.7	2.0	82	7.9	68
8	2-CN	2.9	0.8	77	5.0	78
9	2-CH_3	5.9	3.2	81	16	56
10	2-CF_3	4.2	2.5	79	25	77
11	3-OEt	21	10	40	158	76
12	4-OEt	53	50	82	251	69
13	2,3-$(CH_3)_2$	8.1	5.0	74	200	65

[a]K_i, EC_{50}, and E_{max} values are the average of at least two duplicate experiments with standard deviations (SD) values that are 3-fold less than the average.

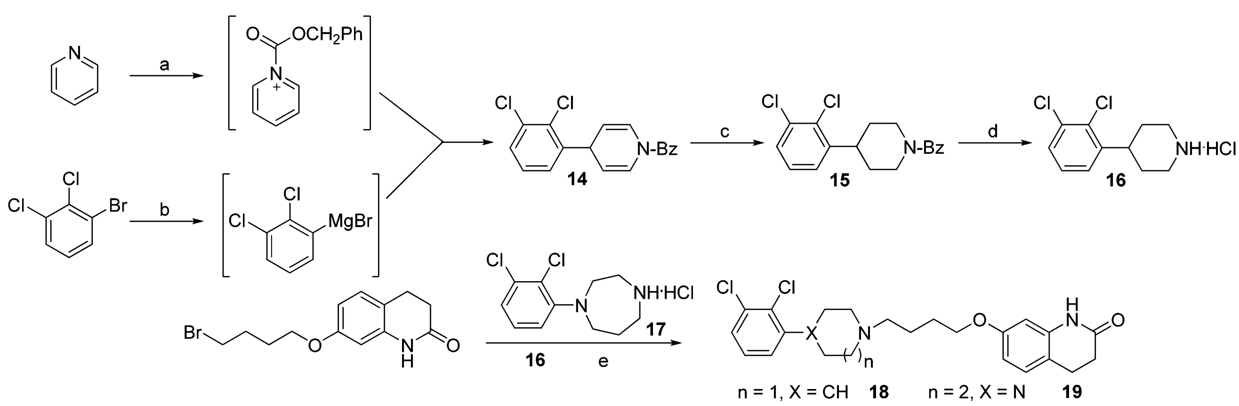
dihydroquinolin-2(1*H*)-one with the intermediate **16** afforded the targeted compound **18** in good yield. Synthesis of the intermediate **17** and the targeted compound **19** was reported in our previous paper.[28]

These compounds were evaluated in the D_2R radioligand binding, cAMP accumulation, and β-arrestin-2 recruitment assays to assess their binding affinities and patterns of D_2R functional selectivity. Replacing the piperazine group (compound **1**) with the more basic piperidine group (compound **18**) did not result in significant changes in the efficacy of either G_i or β-arrestin signaling although compound **18** was significantly more potent at activating β-arrestin than G_i signaling (Table 2). On the other hand, replacing the piperazine group (compound **1**) with the homopiperazine group (compound **19**), which leads to substantial conformation changes, resulted in significant bias for β-arrestin over G_i signaling. Compound **19** was a potent partial agonist at activating β-arrestin-2 recruitment (EC_{50} = 2.0 nM, E_{max} = 41%) and was simultaneously inactive at G_i signaling. The effects of the homopiperazine moiety on biasing for β-arrestin signaling were also observed with other analogues (e.g., compounds **44** and **45** in Table 5). Both compounds **18** and **19** retained high binding affinity to D_2R.

SFSR of the Central Linker. To examine effects of the central linker on patterns of D_2R functional selectivity, we designed the compounds outlined in Scheme 3, which contain either shorter or conformationally constrained linkers. Compounds **22−24** were synthesized by a two-step sequence similar to that previously developed for compound **21**.[39] Thus, the commercially available 7-hydroxy-3,4-dihydroquinolin-2(1*H*)-one was refluxed with various dibromides or dichlorides to give the bromo or chloro intermediates, which were then reacted with commercially available 1-(2,3-dichlorophenyl)piperazine hydrochloride (**20**) to afford the target compounds **22−24**. Synthesis of compounds **25** and **26** started from the piperazine **20** reacting with 1-bromo-3-(bromomethyl)benzene and 1-bromo-4-(bromomethyl)benzene to give the intermediates **27** and **28**, which were then treated with 7-hydroxy-3,4-dihydroquinolin-2(1*H*)-one to afford the target compounds **25** and **26**, respectively.

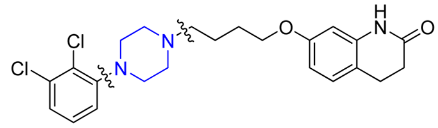
Results of these compounds in D_2R binding, cAMP accumulation, and β-arrestin-2 recruitment assays are summarized in Table 3. Compound **21**, which contains a shorter linker (3-carbon versus 4-carbon in compound **1**), had slightly higher efficacy at activating β-arrestin-2 recruitment than G_i signaling although compound **21** had a lower binding affinity and agonist

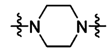
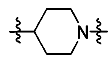
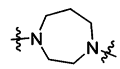
Scheme 2. Synthesis of Compounds for Exploring the Middle Amino Moiety[a]



[a]Reagents and conditions: (a) $ClCOOCH_2Ph$, CuI, THF, −10 °C; (b) *i*-PrMgCl, THF, −20 °C, 76%; (c) H_2, $RhCl(PPh_3)_3$, toluene, 70 °C, 4 d, 95%; (d) 6 N HCl, reflux, 93%; (e) NaI/K_2CO_3, CH_3CN, reflux, 6 h, 60% for **18**, 62% for **19**.

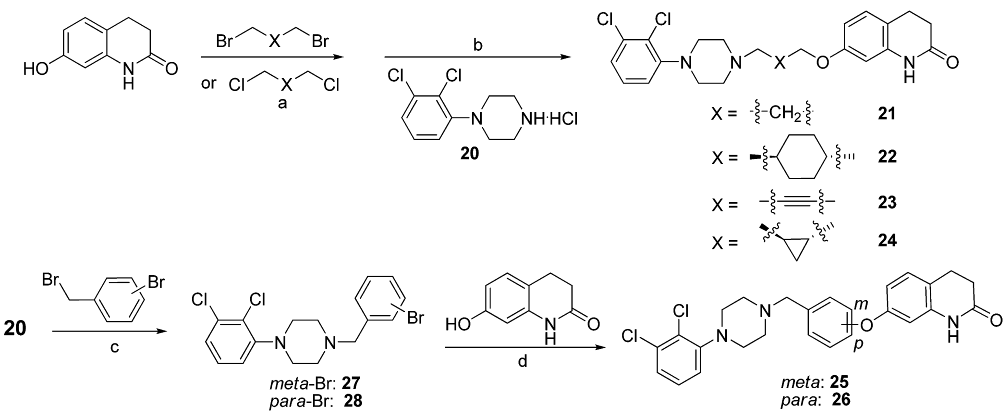
Table 2. SFSR of the Middle Amino Moiety[a]



Cmpd	Middle Amino Moiety	D_2R K_i (nM)	β-arrestin		cAMP	
			EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)
1		3.9	4.0	62	1.0	51
18		11	2.0	70	363	80
19		3.6	2.0	41	N/A	< 20

[a] K_i, EC_{50}, and E_{max} values are the average of at least two duplicate experiments with standard deviations (SD) values that are 3-fold less than the average.

Scheme 3. Synthesis of Compounds for Exploring the Central Linker[a]



[a] Reagents and conditions: (a) K_2CO_3, EtOH, reflux, overnight, 35–80%; (b) NaI/K_2CO_3, CH_3CN, reflux, 6 h, 40–70%; (c) Et_3N, CH_3CN, reflux, 4 h, 70–75%; (d) CuCl, Cs_2CO_3, TMHD, NMP, 120 °C, 7.5 h, 40–50%.

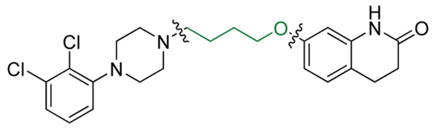
potency than compound **1**. Interestingly, the cyclohexyl group containing compound **22**, the acetylene containing compound **23**, the *meta*-benzyl group containing compound **25**, and the *para*-benzyl group containing compound **26** were all biased ligands for *β*-arrestin: partial agonists at *β*-arrestin-2 recruitment and simultaneously inactive at G_i signaling. However, all four compounds suffered from significant lower binding affinities and agonist potencies. On the other hand, the cyclopropyl group containing compound **24** did not display significant bias for activating *β*-arrestin-2 recruitment over G_i signaling. Taken together, these results suggest that the central linker plays an important role in modulating functional selectivity and potency of these compounds. This finding is consistent with previously reported results: the linker flexibility significantly affects ligand binding affinities and intrinsic efficacies.[32−34,40−42]

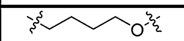
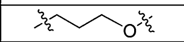
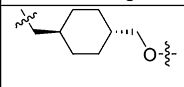
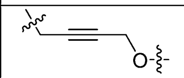
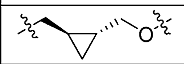
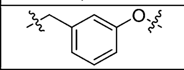
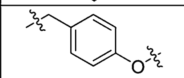
SFSR of the RHS Bicyclic Aromatic Moiety. We next explored the RHS bicyclic aromatic moiety of compound **1**. We selected six different bicyclic aromatic groups (outlined in Scheme 4) to evaluate their effects on patterns of D_2R functional selectivity. The previously described two-step nucleophilic displacement sequence for synthesizing compounds **29** and **30**[28] was applied to the preparation of compounds **31**–**34** (Scheme 4). The corresponding commercially available phenols were reacted with butyl-1,4-dibromide to give the bromo intermediates, which were then reacted with the piperazine **20** to yield the target compounds **31**–**34**.

Results of these compounds in D_2R binding, cAMP accumulation, and *β*-arrestin-2 recruitment assays are summarized in Table 4. In contrast to compound **1**, compound **29**, which contains a quinoline-2-one moiety, exhibited a higher efficacy for activating D_2R mediated *β*-arrestin translocation than D_2R-mediated G_i activity. Similar effects were observed with compound **30**, which contains a dihydro-1,8-naphthyridin-2-one moiety. Importantly, replacing the dihydroquinolin-2-one (**1**) with the dihydroisoquinolinone (compound **31**), indazole (compound **32**), benzimidazolone (compound **33**), or benzothiazole (compound **34**) resulted in apparent bias for D_2R-mediated *β*-arrestin over G_i signaling. These four compounds were partial agonists at D_2R-mediated *β*-arrestin-2 recruitment, with moderate to high potencies and simultaneously inactive at D_2R-mediated G_i signaling. In general, the RHS bicyclic aromatic moiety plays a critical role in modulating functional selectivity of these analogues. All six investigated RHS aryl groups exhibited some degree of bias for *β*-arrestin over G_i signaling.

Synthesis and in Vitro Pharmacological Evaluation of Combination Compounds. After exploring the above four regions of compound **1** and studying their SFSR, we next designed and synthesized a number of combination compounds

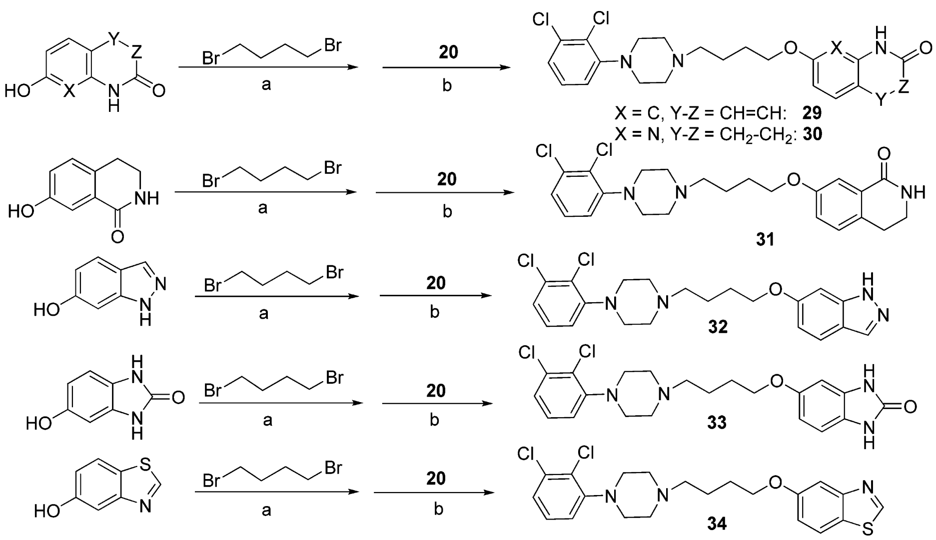
Table 3. SFSR of the Central Linker[a]



Cmpd	Middle Linker	D_2R K_i (nM)	β-arrestin		cAMP	
			EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)
1		3.9	4.0	62	1.0	51
21		21	79	82	251	58
22		145	1,580	45	N/A	< 20
23		1,004	199	45	N/A	< 20
24		235	1,260	30	N/A	< 20
25		113	316	57	N/A	< 20
26		108	501	48	N/A	< 20

[a] K_i, EC_{50}, and E_{max} values are the average of at least two duplicate experiments with standard deviations (SD) values that are 3-fold less than the average.

Scheme 4. Synthesis of Compounds with Various RHS Bicyclic Aromatic Groups[a]



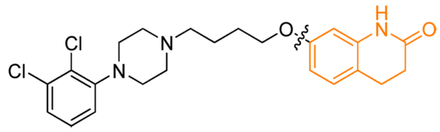
[a] Reagents and conditions: (a) K_2CO_3, EtOH, reflux, 6 h, 30−80%; (b) NaI/K_2CO_3, CH_3CN, reflux, 6 h, 40−70%.

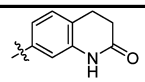
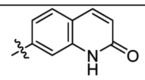
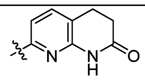
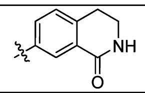
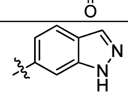
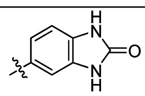
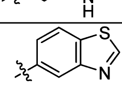
(outlined in Scheme 5), which combined the structural motifs that contribute to the bias for β-arrestin recruitment into single molecules. We selected all three middle amino groups, 3- and 4-carbon linkers, and all six RHS aromatic groups for this study. The synthetic routes for these combination compounds were shown in Scheme 5. These compounds (**35**−**45**) were prepared following the same synthetic approach developed for compounds **29**−**34** using the corresponding amino groups, central linkers, and RHS aromatic groups.

We next evaluated compounds **35**−**45** in the D_2R radioligand binding, cAMP accumulation, and β-arrestin-2 recruitment assays (results are summarized in Table 5). As expected, these combination compounds were all significantly biased for D_2R-mediated β-arrestin over G_i signaling. With the exception of compound **40**, all compounds were agonists at D_2R mediated β-arrestin-2 recruitment with moderate to high potencies and simultaneously inactive at D_2R-mediated G_i signaling. Most notably, compounds **36** and **37** were the most biased for β-arrestin, with E_{max} values similar to the positive control quinpirole. Notably, compounds **44** and **45** had extremely high potencies (EC_{50} < 5 nM) but relatively low efficacies (E_{max} = 40−50%) at D_2R-mediated β-arrestin translocation. These unique patterns of D_2R-mediated functional selectivity profiles will be extremely useful for elucidating which signaling pathways contribute to antipsychotic efficacies and side effects.

From these SFSR studies, we observed the following general trends: (1) the electronic nature (e.g., electron donating or

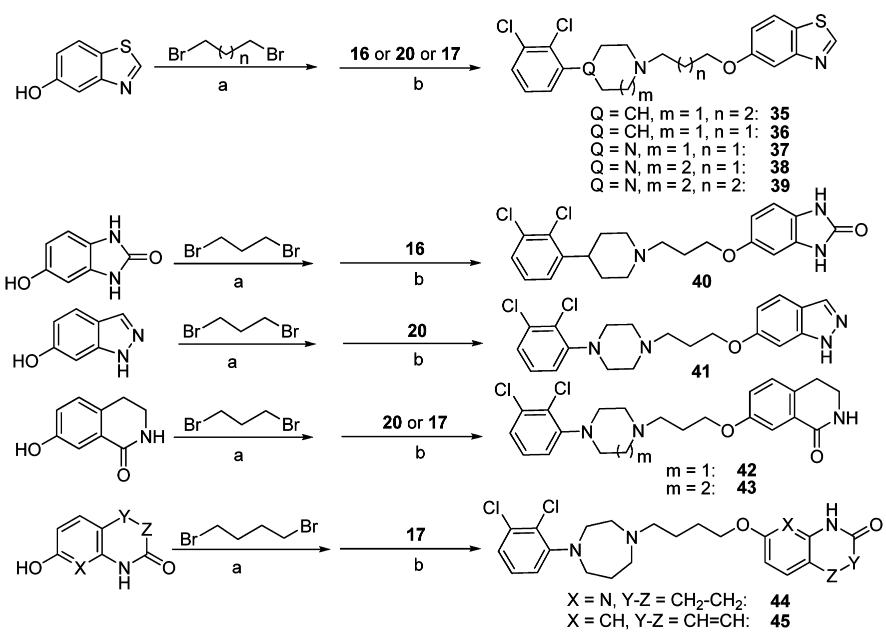
Table 4. SFSR of the RHS Bicyclic Aromatic Moiety[a]



Cmpd	RHS Aryl Group	D_2R K_i (nM)	β-arrestin		cAMP	
			EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)
1		3.9	4.0	62	1.0	51
29		7.3	6.3	79	158	29
30		3.4	3.2	73	N/A	41
31		18	4.0	46	N/A	< 20
32		66	100	61	N/A	< 20
33		15	16	65	N/A	< 20
34		17	63	63	N/A	< 20

[a] K_i, EC_{50}, and E_{max} values are the average of at least two duplicate experiments with standard deviations (SD) values that are 3-fold less than the average.

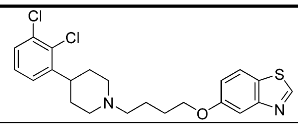
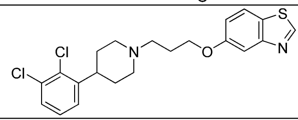
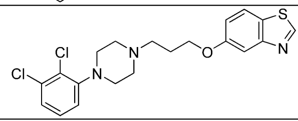
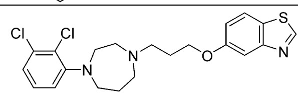
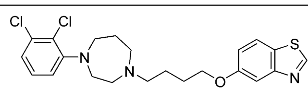
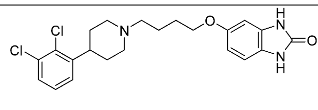
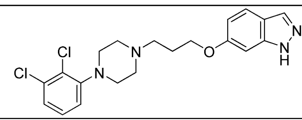
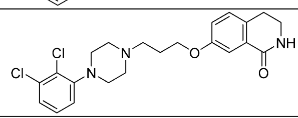
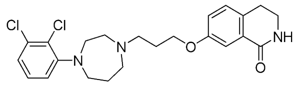
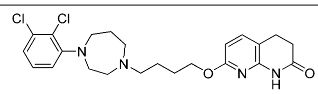
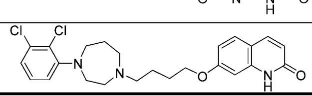
Scheme 5. Synthesis of Combination Compounds[a]



[a] Reagents and conditions: (a) K_2CO_3, EtOH, reflux, 6 h, 30−80%; (b) NaI/K_2CO_3, CH_3CN, reflux, 6 h, 40−70%.

withdrawing), steric bulkiness, and substitution pattern of substituents on the LHS phenyl ring did not, apparently, have significant effects on patterns of D_2R functional selectivity, (2) the homopiperazine group as a middle amino moiety reduced efficacy for activating both β-arrestin and G_i pathways, (3) several conformationally constrained central linkers could lead to significant bias for D_2R-mediated β-arrestin-2 over G_i activities. However, these central linkers resulted in significant losses of potency, and (4) a number of RHS aromatic groups such as benzothiazole, dihydroisoquinolinone, indazole, and

Table 5. SFSR of Combination Compounds[a]

Cmpd	Structure	D_2R K_i (nM)	β-arrestin		cAMP	
			EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)
35		42	79	78	N/A	< 20
36		75	50	97	N/A	< 20
37		30	126	88	N/A	< 20
38		20	63	71	N/A	< 20
39		18	25	36	N/A	< 20
40		11	20	80	N/A	32
41		104	200	78	N/A	< 20
42		18	20	84	N/A	< 20
43		5.7	6.3	41	N/A	< 20
44		1.2	1.6	47	N/A	< 20
45		3.4	2.5	49	N/A	< 20

[a] K_i, EC_{50}, and E_{max} values are the average of at least two duplicate experiments with standard deviations (SD) values that are 3-fold less than the average.

benzimidazolone led to significant bias for D_2R-mediated *β*-arrestin-2 over G_i activities. Importantly, we observed that subtle structural changes could result in substantial changes in functional selectivity.

We subsequently confirmed that compounds **19**, **36**, and **44** were *β*-arrestin-biased agonists in several orthologous *β*-arrestin-2 translocation and signaling assays.[28]

In addition, we determined selectivity of compounds **19**, **35**, **36**, and **44** against a number of dopamine and serotonin receptors. Compound **19** displayed high affinities to D_2 and D_3 receptors and low affinities to D_1, D_4, and D_5 receptors, while compound **35** displayed high affinities to D_2, D_3, and D_4 receptors and low affinities to D_1 and D_5 receptors (Supporting Information Table S1). At serotonin receptors, although compounds **19** and **35** displayed moderate to high binding affinities (K_i's: 0.6−138 nM) for 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, and 5-HT_{1A} (Supporting Information Table S1), compound **19** was significantly less potent in functional assays (Ca^{2+} mobilization fluorometric imaging plate reader (FLIPR) or cAMP biosensor).[28] Similarly, compounds **36** and **44** displayed much lower functional potencies compared to their binding affinities to 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, and 5-HT_{1A} receptors.[28] Selectivity of compounds **36** and **44** against dopamine D_1−D_5 receptors was reported previously.[28]

In Vivo Behavioral Studies in Mice. We previously reported that compounds **36** and **44** displayed robust antipsychotic drug-like activities and did not induce catalepsy in wild-type mice.[28] In *β*-arrestin-2 knockout mice, however, the antipsychotic drug-like activities of these compounds were significantly attenuated or completely abolished.[28] To extend and confirm these findings, we evaluated the effect of two additional *β*-arrestin-biased D_2R agonists (compound **19** and **35**) on phencyclidine (PCP)-induced hyperlocomotion[43] in *β*-arrestin-2 knockout mice and the wild-type littermate controls.[44] Compound **19** (2 mg/kg, intraperitoneal (ip) administration) markedly inhibited PCP-induced hyperloco-

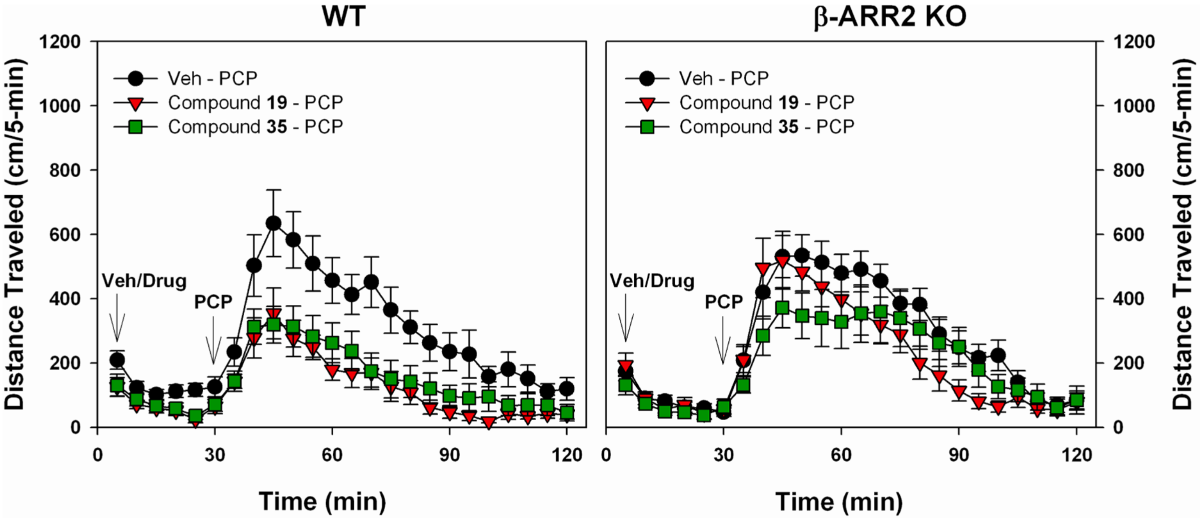


Figure 3. Compounds **19** and **35** exhibit potent antipsychotic-like activities in mouse hyperlocomotion studies, which are completely abolished or significantly attenuated in *β*-arrestin-2 knockout mice. Locomotor activities shown as 5 min binned intervals for wild-type (WT) or *β*-arrestin-2 knockout (*β*-ARR2 KO) littermate mice given vehicle or 2.0 mg/kg **19** or **35** (ip), followed 30 min later with 6 mg/kg phencyclidine (PCP, ip). n = 8−13 WT and *β*-ARR2 KO pairs/group.

motion in wild-type mice. Importantly, this significant antipsychotic-like activity of compound **19** was completely abolished in *β*-arrestin-2 knockout mice (Figure 3). Similarly, compound **35** (2 mg/kg, ip) significantly reduced PCP-induced hyperlocomotion in wild-type mice and this antipsychotic-like activity was significantly attenuated in *β*-arrestin-2 knockout mice (Figure 3). Taken together, these results suggest that *β*-arrestin recruitment and signaling can be a significant contributor to antipsychotic efficacy.

■ CONCLUSION

In summary, we designed and synthesized a series of novel compounds for exploring four regions of the scaffold represented by compound **1**. Comprehensive evaluation of these compounds in D_2R radioligand binding, cAMP accumulation, and *β*-arrestin-2 recruitment assays revealed a number of important SFSR findings. Combining the best structural motifs identified from these studies into single molecules resulted in the discovery of extremely *β*-arrestin-biased D_2R agonists **35**−**37** and high potency and low efficacy *β*-arrestin-biased D_2R agonists **19** and **44**. Findings from our in vivo studies of these *β*-arrestin-biased D_2R agonists in wild-type and *β*-arrestin-2 knockout mice suggest that *β*-arrestin recruitment and signaling can be a significant contributor to antipsychotic efficacy. Our combined medicinal chemistry and pharmacological profiling approach provides the biomedical community a successful proof-of-concept for how functionally selective ligands can be discovered.

■ EXPERIMENTAL SECTION

Chemistry General Procedures. Unless stated to the contrary, where applicable, the following conditions apply: all commercial grade reagents were used without further purification. MeCN and CH_2Cl_2 were distilled from CaH_2 under a N_2 atmosphere before use, THF was distilled from Na/benzophenone under N_2. All other dry solvents were of anhydrous quality purchased from Sigma-Aldrich. Brine (NaCl), $NaHCO_3$, and NH_4Cl refer to saturated aqueous (satd aq) solutions. Column chromatography was performed on silica gel G (200−300 mesh) with reagent grade solvents. Melting points were uncorrected. NMR spectra were recorded on a Varian spectrometer (400 or 300 MHz for ^1H NMR and 100 MHz or 75 MHz for ^{13}C NMR, respectively) at ambient temperature. All ^1H and ^{13}C chemical shifts are reported in ppm (δ) relative to $CDCl_3$ (7.26 and 77.16, respectively) or CD_3OD (3.30 and 49.00, respectively).[45] HPLC data for all compounds were acquired using an Agilent 6110 series system with a UV detector set to 220 nm. Samples were injected (<10 μL) onto an Agilent Eclipse Plus 4.6 mm × 50 mm, 1.8 μm, C18 column at room temperature (rt) at a flow rate of 1.0 mL/min. A linear gradient from 10% to 100% (v/v) B over 5.0 min followed by 2.0 min at 100% B with a mobile phase of (A) H_2O + 0.1% acetic acid and (B) MeOH + 0.1% acetic acid was used. Mass spectra (MS) data were acquired in positive ion mode using an Agilent 6110 series single quadrupole mass spectrometer with an electrospray ionization (ESI) source. High-resolution (positive ion) mass spectrum (HRMS) for compound **35** was acquired using a Shimadzu LCMS-IT-Tof time-of-flight mass spectrometer. HRMS (positive ion) for compounds **37**, **38**, **41**, and **42** were recorded on Agilent 6210 ESI-LCT-TOF mass spectrometer with dual source for reference and sample. HPLC was used to establish the purity of targeted compounds. All compounds that were evaluated in biological assays had >95% purity using the HPLC methods described above.

Compounds **1**−**3**, **5**−**9**, **12**, and **13** were synthesized as previously reported.[31,33]

7-(4-(4-(2-Isopropoxyphenyl)piperazin-1-yl)butoxy)-3,4-dihydroquinolin-2(1*H*)-one (4). A mixture of intermediate 7-(4-bromobutoxy)-3,4-dihydroquinolin-2(1*H*)-one (119 mg, 0.4 mmol) and NaI (119.6 mg, 0.8 mmol) in CH_3CN was heated to reflux for 30 min and then cooled to rt. Compound 1-(2-isopropoxyphenyl)-piperazine (97.0 mg, 0.44 mmol) and anhydrous K_2CO_3 (121.6 mg, 0.88 mmol) were added to the mixture. The resulting mixture was heated to reflux and stirred for 6 h. Precipitated crystals were filtered off, and the filtrate was evaporated under reduced pressure. The residue was extracted with EtOAc. The combined EtOAc layers were washed with brine, dried over anhydrous Na_2SO_4, concentrated in vacuo, and purified by flash chromatography on silica gel column (elution with DCM/MeOH = 30:1) to give compound **4** as light-yellow solid (75.3 mg, 43%). ^1H NMR (400 MHz, $CDCl_3$): δ 8.59 (br s, 1H), 8.24 (d, J = 8.0 Hz, 1H), 7.41 (app t, J = 7.6 Hz, 1H), 7.06 (d, J = 8.4 Hz, 1H), 7.02−6.96 [m, 2H, containing a d at δ 7.00 (J = 8.4 Hz)], 6.49 (s, 1H), 6.46 (d, J = 8.4 Hz, 1H), 4.99−4.86 (m, 2H), 4.80 (sept, J = 6.0 Hz, 1H), 4.63−4.49 (m, 2H), 4.01−3.92 (m, 2H), 3.76−3.61 (m, 4H), 3.31−3.20 (m, 2H), 2.85 (app t, J = 7.4 Hz, 2H), 2.56 (app t, J = 7.4 Hz, 2H), 2.20−2.08 (m, 2H), 1.91−1.81 (m, 2H), 1.56 (d, J = 6.0 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.7, 158.2, 151.1, 138.5, 132.3, 128.8, 128.2, 124.7, 121.4, 116.2, 114.7, 108.9, 102.6, 72.7, 67.2, 57.3, 49.8, 48.0, 31.2, 26.4, 24.7, 21.9, 20.9. HPLC 99%, RT 4.39 min. MS (ESI) m/z 438.3 $[M + H]^+$.

7-(4-(4-(2-(Trifluoromethyl)phenyl)piperazin-1-yl)butoxy)-3,4-dihydroquinolin-2(1*H*)-one (10). Compound **10** (188 mg) was prepared as white solid by the same procedure as preparing **4**, yield 62%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.17 (br s, 1H), 7.62 (d, J = 8.0 Hz, 1H), 7.52 (app t, J = 7.8 Hz, 1H), 7.40 (d, J = 8.0 Hz, 1H), 7.23 (app t, J = 7.6 Hz, 1H), 7.04 (d, J = 8.4 Hz, 1H), 6.52 (dd, J = 2.4, 8.4 Hz, 1H), 6.34 (d, J = 2.4 Hz, 1H), 4.01−3.92 (m, 2H), 3.18−2.93 (m, 4H), 2.92−2.44 [m, 10H, containing an app t at δ 2.89 (J = 7.6 Hz) and an app t at δ 2.61 (J = 7.6 Hz)], 1.93−1.71 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.8, 158.7, 138.3, 133.0, 129.3, 128.8, 127.5 (q, J_{CF} = 29.0 Hz), 127.3 (q, J_{CF} = 5.4 Hz), 125.2, 124.3, 124.2 (q, J_{CF} = 274.4 Hz), 115.9, 108.8, 102.3, 67.9, 58.2, 53.5, 53.0, 31.2, 27.3, 24.8, 23.1. HPLC 99%, RT 4.36 min. MS (ESI) m/z 448.3 $[M + H]^+$.

7-(4-(4-(3-Ethoxyphenyl)piperazin-1-yl)butoxy)-3,4-dihydroquinolin-2(1*H*)-one (11). Compound **11** (48 mg) was prepared as white solid by the same procedure as preparing compound **4**, yield 57%. ^{1}H NMR (400 MHz, CDCl3): δ 7.76 (s, 1H), 7.15 (t, J = 8.1 Hz, 1H), 7.04 (d, J = 8.1 Hz, 1H), 6.56−6.49 (m, 2H), 6.47 (s, 1H), 6.40 (d, J = 8.1 Hz, 1H), 6.29 (s, 1H), 4.06−3.91 (m, 4H), 3.25−3.14 (m, 4H), 2.90 (t, J = 7.4 Hz, 2H), 2.67−2.56 (m, 6H), 2.46 (t, J = 7.2 Hz, 2H), 1.88−1.77 (m, 2H), 1.76−1.66 (m, 2H), 1.40 (t, J = 6.7 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 171.6, 160.1, 158.8, 152.8, 138.2, 129.9, 128.9, 115.9, 108.9, 108.8, 105.1, 103.2, 102.3, 68.1, 63.5, 58.4, 53.4, 49.19, 31.3, 27.4, 24.8, 23.6, 15.1. HPLC 99%, RT 4.26 min. MS (ESI) m/z 424.3 $[M + H]^+$.

(4-(2,3-Dichlorophenyl)pyridin-1(4*H*)-yl)(phenyl)methanone (14). To a −30 °C solution of 1-bromo-2,3-dichlorobenzene (10 g, 44 mmol) in THF (120 mL) was added *i*-PrMgCl (2.0 M in THF, 35 mL, 70 mmol) at a rate such that the temperature <−20 °C. Meanwhile, to a −10 °C solution of CuI (420 mg, 2.2 mmol) in THF (120 mL) was added pyridine (7.1 mL, 88 mmol) and then benzyl carbonochloridate (9.7 mL, 68 mmol) such that the temperature <0 °C. To this heterogeneous mixture was added the initially formed Grignard at a rate such that the temperature <0 °C. The resulting solution was stirred at 0 °C for 30 min and then allowed to warm to rt. The reaction was then quenched with 10% aq NH_4Cl. EtOAc was added, and the blue aqueous layer was removed. The organic layer was washed with 10% aq NH_4Cl, 1 N HCl, and a 20% aq NaCl solution. The organic layer was then concentrated, and the residue was dissolved and crystallized from MeOH. The slurry was filtered and the filtercake washed with MeOH to give benzyl 4-(2,3-dichlorophenyl)pyridine-1(4*H*)-carboxylate (**14**) (12 g, 76%) as an off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.42−7.21 (m, 8H), 7.04 (d, J = 9.2 Hz, 1H), 6.92 (d, J = 6.4 Hz, 1H), 5.25 (s, 2H), 5.02 (d, J = 6.4 Hz, 1H), 4.93 (d, J = 9.2 Hz, 1H), 4.73−4.71 (m, 1H). HPLC: 99%, RT 4.069 min. MS (ESI) m/z 360.0 $[M + H]^+$.

(4-(2,3-Dichlorophenyl)piperidin-1-yl)(phenyl)methanone (15). To a solution of intermediate **15** (7.0 g, 19.5 mmol) in toluene (150 mL) was added $RhCl(PPh_3)_3$ (2.1 g, 2.0 mmol) as a slurry in toluene (50 mL). The reaction was subjected to an atmosphere of H_2 at 40 psi and heated to 70 °C. After 6 h, the reaction was filtered through silica gel and washed with 1:9 EtOAc/toluene. The filtrate was dissolved in toluene, concentrated in vacuo, and purified by flash chromatography on silica gel column (elution with PE/EtOAc = 50:1) to give benzyl 4-(2,3-dichlorophenyl)piperidine-1-yl)(phenyl)-methanone (**15**) (6.8 g, 96%) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.41−7.28 (m, 5H), 7.26−7.25 (m, 1H), 7.18 (t, J = 7.8 Hz, 1H), 7.13−7.10 (m, 1H), 5.16 (s, 2H), 4.35 (d, J = 12.0 Hz, 2H), 3.24−3.19 (m, 1H), 2.93 (t, J = 12.6 Hz, 2H), 1.86 (d, J = 13.0 Hz, 2H), 1.59 (t, J = 10.5 Hz, 2H). HPLC: 99%, RT 3.881 min. MS (ESI) m/z 364.0 $[M + H]^+$.

4-(2,3-Dichlorophenyl)piperidine Hydrochloride (16). To 6N HCl (30 mL) was added a solution of compound **15** (5.2 g, 14 mmol) in THF (10 mL). The mixture was heated to reflux for 3 h and then concentrated in vacuo. The residue was washed with Et_2O to give 4-(2,3-dichlorophenyl)piperidine hydrochloride (**16**) (3.5 g, 92%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.48−7.42 (m, 1H), 7.36−7.31 (m, 2H), 3.51−3.41 (m, 3H), 3.33−3.15 (m, 3H), 2.11−2.07 (m, 2H), 1.99−1.85 (m, 2H). HPLC: 99%, RT 2.090 min. MS (ESI) m/z 230.0 $[M + H]^+$.

7-(4-(4-(2,3-Dichlorophenyl)piperidin-1-yl)butoxy)-3,4-dihydroquinolin-2(1H)-one (18). Compound **18** (106.8 mg) was prepared as white solid by the same procedure as preparing compound **4** from intermediate 7-(4-bromobutoxy)-3,4-dihydroquinolin-2(1*H*)-one (119 mg, 0.4 mmol) and compound **16** (117.3 mg, 0.44 mmol), yield 60%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.85 (s, 1H), 7.33 (d, J = 8.1 Hz, 1H), 7.23−7.17 (m, 2H), 7.05 (d, J = 8.1 Hz, 1H), 6.54 (dd, J = 2.1 Hz, 8.1 Hz, 1H), 6.31 (d, J = 2.1 Hz, 1H), 3.96 (t, J = 6.0 Hz, 2H), 3.15−3.05 (m, 3H), 2.90 (t, J = 7.3 Hz, 2H), 2.62 (t, J = 7.3 Hz, 2H), 2.50 (t, J = 7.2 Hz, 2H), 2.30−2.10 (m, 2H), 1.91−1.72 (m, 8H). HPLC: 99%, RT 2.504 min. MS (ESI) m/z 447.2 $[M + H]^+$.

Intermediate **17** and compounds **19** and **21** were prepared according to previous procedures.[27,37,39]

***trans*-7-((4-((4-(2,3-Dichlorophenyl)piperazin-1-yl)methyl)-cyclohexyl)methoxy)-3,4-dihydroquinolin-2(1*H*)-one (22).** A mixture of 7-hydroxy-3,4-dihydroquinolin-2(1*H*)-one (105 mg, 0.65 mmol), 1,4-bis(bromomethyl)cyclohexane (523 mg, 1.9 mmol), and anhydrous K_2CO_3 (89 mg, 0.65 mmol) was dissolved in EtOH, and the solution was heated to reflux for 6 h. The solution was diluted with water and extracted with EtOAc. The combined organic layers were washed with satd aq $NaHCO_3$, brine, dried over anhydrous Na_2SO_4, concentrated in vacuo, and purified by flash chromatography on silica gel column to give 7-((4-(bromomethyl)cyclohexyl)methoxy)-3,4-dihydroquinolin-2(1*H*)-one (70 mg, 30%, 0.2 mmol) as a white solid, which was redissolved in CH_3CN. To this mixture was added NaI (60 mg, 0.4 mmol), and the reaction mixture was heated to reflux for 30 min and then cooled to rt. The commercial available compound **20** (81 mg, 0.3 mmol) and anhydrous K_2CO_3 (110 mg, 0.8 mmol) were added to the mixture. The resulting mixture was heated to reflux and stirred for 6 h. Precipitated crystals were filtered off, and the filtrate was evaporated under reduced pressure. The residue was extracted with EtOAc. The combined EtOAc layers was washed with brine, dried over anhydrous Na_2SO_4, concentrated in vacuo, and purified by flash chromatography on silica gel column (elution with DCM/MeOH = 50:1) to give *trans*-7-((4-((4-(2,3-dichlorophenyl)piperazin-1-yl)-methyl)cyclohexyl)methoxy)-3,4-dihydroquinolin-2(1*H*)-one (**22**) as a white solid (63 mg, yield 63%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.06 (s, 1H), 7.15−7.13 (m, 2H), 7.04 (d, J = 8.4 Hz, 1H), 6.97−6.94 (m, 1H), 6.52 (dd, J = 2.4 Hz, 8.4 Hz, 1H), 6.32 (d, J = 2.1 Hz, 1H), 3.74−3.71 (m, 2H), 3.07−3.05 (m, 4H), 2.92−2.87 (m, 2H), 2.64−2.59 (m, 6H), 2.24 (d, J = 6.9 Hz, 2H), 1.83 (d, J = 6.9 Hz, 2H), 1.53−1.69 (m, 4H), 1.10−0.94 (m, 4H). HPLC: 99%, RT 2.652 min. MS (ESI) m/z 502.2 $[M + H]^+$.

7-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)but-2-ynyloxy)-3,4-dihydroquinolin-2(1*H*)-one (23). Compound **23** (280 mg) was prepared as yellow solid by the same procedure as preparing **22**, yield 79%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62 (s, 1H), 7.20−7.11 (m, 2H), 7.07 (d, J = 8.3 Hz, 1H), 6.96 (dd, J = 7.1, 2.5 Hz, 1H), 6.62 (dd, J = 8.3, 2.5 Hz, 1H), 6.39 (d, J = 2.4 Hz, 1H), 4.72 (t, J = 1.7 Hz, 2H), 3.41 (t, J = 1.7 Hz, 2H), 3.08 (bs, 4H), 2.93−2.85 (m, 2H), 2.78−2.68 (m, 2H), 2.63−2.57 (m, 2H). HPLC: 99%, RT 2.403 min. MS (ESI) m/z 444.1 $[M + H]^+$.

***trans*-7-((2-((4-(2,3-Dichlorophenyl)piperazin-1-yl)methyl)-cyclopropyl)methoxy)-3,4-dihydroquinolin-2(1*H*)-one (24).** Compound **24** (82 mg) was prepared as white solid by the same procedure as prepaing **22**, yield 48%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (s, 1H), 7.19−7.09 (m, 2H), 7.03 (d, J = 8.2 Hz, 1H), 6.98−6.91 (m, 1H), 6.57 (d, J = 8.4 Hz, 1H), 6.35 (s, 1H), 3.89 (s, 2H), 3.04 (s, 4H), 2.88 (t, J = 7.3 Hz, 2H), 2.80−2.56 (m, 6H), 2.46 (s, 2H), 0.65 (s, 2H), 0.48 (s, 2H). ^{13}C NMR (101 MHz, CDCl3): δ 171.6, 159.2, 151.5, 138.1, 134.2, 128.7, 127.6, 127.6, 124.6, 118.7, 115.8, 109.1, 102.5, 72.3, 62.8, 53.7, 51.4, 31.3, 24.8, 18.0, 9.5, 2.1. HPLC: 99%, RT 2.541 min. MS (ESI) m/z 460.1 $[M + H]^+$.

1-(3-Bromobenzyl)-4-(2,3-dichlorophenyl)piperazine (27). A mixture of 1-(2,3-dichlorophenyl)piperazine hydrochloride (**20**) (294 mg, 1.1 mmol), 1-bromo-3-(bromomethyl)benzene (250 mg, 1 mmol), and anhydrous triethylamine (253 mg, 2.5 mmol) was dissolved in CH_3CN, and the solution was heated to reflux for 4 h.

dx.doi.org/10.1021/jm300603y | J. Med. Chem. 2012, 55, 7141−7153

The solution was diluted with water and extracted with EtOAc. The combined organic layers were washed with sd aq $NaHCO_3$, brine, dried over anhydrous Na_2SO_4, concentrated in vacuo, and purified by flash chromatography on silica gel column (elution with PE/EtOAc = 8:1) to give 1-(3-bromobenzyl)-4-(2,3-dichlorophenyl)piperazine (**27**) (220 mg, 74%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.54 (s, 1H), 7.40 (d, *J* = 7.8 Hz, 1H), 7.28 (d, *J* = 7.8 Hz, 1H), 7.21 (d, *J* = 7.8 Hz, 1H), 7.17–7.10 (m, 2H), 6.97–6.94 (m, 1H), 3.55 (s, 2H), 3.07 (br, 4H), 2.64 (br, 4H). HPLC: 99%, RT 2.542 min. MS (ESI) *m/z* 399.1 [M + H]$^+$.

7-(3-((4-(2,3-Dichlorophenyl)piperazin-1-yl)methyl)-phenoxy)-3,4-dihydroquinolin-2(1*H*)-one (25). To a solution of 7-hydroxy-3,4-dihydroquinolin-2(1*H*)-one (196 mg, 1.2 mmol) in NMP was added Cs_2CO_3 (391 mg, 1.2 mmol). The slurry was degassed by evacuating and filling the reaction flask with N_2 three times. Compound **27** (240 mg, 0.6 mmol) and TMHD (11 mg, 0.06 mmol) were added, followed by the addition of CuCl (60 mg, 0.6 mmol). The reaction mixture was degassed by evacuating and filling the reaction flask with N_2 three times and then warmed to 120 °C under N_2 for 7.5 h. The reaction mixture was cooled to rt and diluted with Et_2O. The slurry was filtered, and the filtercake was washed with Et_2O. Combined filtrates were washed with 2 N HCl, 0.6 N HCl, 2 M NaOH, and 10% aq NaCl. The resulting organic layer was dried, concentrated, and purified by flash chromatography on a silica gel column (elution with PE/EtOAc = 1:1) to give 7-(3-((4-(2,3-dichlorophenyl)piperazin-1-yl)methyl)phenoxy)-3,4-dihydro quinolin-2(1*H*)-one (Compound **25**) (off-white solid, 110 mg, 38%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.62 (s, 1H), 7.31 (d, *J* = 7.2 Hz, 2H), 7.15–7.05 (m, 4H), 6.96–6.89 (m, 2H), 6.62 (dd, *J* = 2.1 Hz, 8.1 Hz, 1H), 6.41 (d, *J* = 2.1 Hz, 1H), 3.57 (s, 2H), 3.06 (m, 4H), 2.94 (t, *J* = 7.5 Hz, 2H), 2.66–2.61 (m, 5H). HPLC: 99%, RT 2.637 min. MS (ESI) *m/z* 482.1 [M + H]$^+$. Mp: 182–183 °C.

1-(4-Bromobenzyl)-4-(2,3-dichlorophenyl)piperazine (28). Compound **28** (220 mg) was prepared as white solid from **20** (294 mg, 1.1 mmol), 1-bromo-4-(bromomethyl) benzene (250 mg, 1.0 mmol), and anhydrous triethylamine (253 mg, 2.5 mmol) by the same procedure as preparing **27**, yield 74%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.46 (d, *J* = 8.1 Hz, 2H), 7.27–7.23 (m, 2H), 7.15–7.13 (m, 2H), 6.96–6.93 (m, 2H), 3.54 (s, 2H), 3.06 (br, 4H), 2.63 (m, 4H). HPLC: 99%, RT 2.541 min. MS (ESI) *m/z* 398.9 [M + H]$^+$.

7-(4-((4-(2,3-Dichlorophenyl)piperazin-1-yl)methyl)-phenoxy)-3,4-dihydroquinolin-2(1*H*)-one (26). Compound **26** (120 mg) was prepared as off-white solid by the same procedure as preparing **25**, yield 35%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.67 (s, 1H), 7.32 (d, *J* = 8.7 Hz, 2H), 7.16–7.09 (m, 3H), 6.98–6.94 (m, 3H), 6.64–6.61 (m, 1H), 6.41 (d, *J* = 3.0 Hz, 1H), 3.56 (s, 2H), 3.07 (m, 4H), 2.94 (t, *J* = 6.6 Hz, 2H), 2.66–2.61 (m, 6H). HPLC: 99%, RT 2.626 min. MS (ESI) *m/z* 482.1 [M + H]$^+$. Melting point: 185–186 °C.

Compounds **29** and **30** were synthesized as previously described.[27,32]

7-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)butoxy)-3,4-dihydroisoquinolin-1(2*H*)-one (31). Compound **31** (215 mg) was prepared as white solid by the same procedure as preparing **22**, yield 65%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.58 (d, *J* = 2.7 Hz, 1H), 7.14–7.11 (m, 3H), 7.01–6.94 (m, 2H), 6.18 (brs, 1H), 4.05 (t, *J* = 6.3 Hz, 2H), 3.56–3.51 (m, 2H), 3.07 (m, 4H), 2.90 (t, *J* = 8.1 Hz, 2H), 2.66 (m, 4H), 2.49–2.46 (m, 2H), 1.86–1.68 (m, 4H). HPLC: 99%, RT 2.374 min. MS (ESI) *m/z* 448.3 [M + H]$^+$.

6-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)butoxy)-1*H*-indazole (32). Compound **32** (off-white solid, 35 mg) was prepared by the same procedure as preparing **22**, yield 45%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.97 (s, 1H), 7.61 (d, *J* = 8.7 Hz 1H), 7.17–7.15 (m, 2H), 6.96 (dd, *J* = 3.0 Hz, 6.6 Hz, 1H), 6.86–6.81 (m, 2H), 4.05 (t, *J* = 5.4 Hz, 2H), 3.16 (s, 4H), 2.79 (s, 4H), 2.64–2.60 (m, 2H), 1.89–1.83 (m, 4H). HPLC: 99%, RT 2.491 min. MS (ESI) *m/z* 419.2 [M + H]$^+$. Melting point: 101–103 °C.

5-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)butoxy)-1*H*-benzo[*d*]imidazol-2(3*H*)-one (33). Compound **33** (54 mg) was prepared as white solid by the same procedure as preparing **22**, yield 42%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.53 (s, 1H), 9.24 (s, 1H), 7.17–7.13 (m, 2H), 6.97–6.91 (m, 2H), 6.66–6.60 (m, 2H), 3.95 (t, *J* = 5.7 Hz, 2H), 3.20–3.01 (m, 4H), 2.80–2.61 (m, 4H), 2.52 (t, *J* = 7.5 Hz, 2H), 1.82–1.73 (m, 4H). HPLC: 99%, RT 2.325 min. MS (ESI) *m/z* 435.1 [M + H]$^+$.

6-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)butoxy)benzo[*d*]-thiazole (34). Compound **34** (light-yellow soild, 98 mg) was prepared by the same procedure as preparing **22**, yield 60%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.97 (s, 1H), 7.79 (d, *J* = 8.7 Hz, 1H), 7.61 (s, 1H), 7.16–7.08 (m, 3H), 6.97–6.94 (m, 1H), 4.11 (t, *J* = 5.7 Hz, 2H), 3.09 (br, 4H), 2.68 (brs, 4H), 2.55–2.50 (m, 2H), 1.92–1.76 (m, 4H). HPLC: 99%, RT 2.651 min. MS (ESI) *m/z* 436.3 [M + H]$^+$. Melting point: 93–94.5 °C.

5-(4-(4-(2,3-Dichlorophenyl)piperidin-1-yl)butoxy)benzo[*d*]-thiazole (35). Compound **35** (144 mg) was prepared as light-yellow solid by the same procedure as preparing **22**, yield 66%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.97 (s, 1H), 7.79 (d, *J* = 8.8 Hz, 1H), 7.61 (d, *J* = 2.4 Hz, 1H), 7.31 (dd, *J* = 7.6, 1.8 Hz, 1H), 7.23–7.12 (m, 2H), 7.09 (dd, *J* = 8.8, 2.5 Hz, 1H), 4.10 (t, *J* = 6.3 Hz, 2H), 3.16–3.01 (m, 3H), 2.55–2.45 (m, 2H), 2.14 (t, *J* = 10.9 Hz, 2H), 1.96–1.68 (m, 8H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 158.6, 155.0, 154.8, 133.2, 131.9, 128.2, 127.5, 125.6, 125.5, 122.1, 116.6, 106.6, 68.3, 58.7, 54.4, 39.9, 32.1, 27.4, 23.7. HPLC: 99%, RT 2.689 min. MS (ESI) *m/z* 435.3 [M + H]$^+$. HRMS *m/z* [M + H]$^+$ calcd for $C_{22}H_{25}Cl_2N_2OS$ 435.1065, found 435.1039. Melting point:79–81 °C.

Synthesis of compound **36** was described previously.[27]

5-(3-(4-(2,3-Dichlorophenyl)piperazin-1-yl)propoxy)benzo-[*d*]thiazole (37). Compound **37** (light-yellow solid, 209 mg) was prepared by the same procedure as preparing **22**, yield 62%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.97 (s, 1H), 7.80 (d, *J* = 8.8 Hz, 1H), 7.62 (d, *J* = 2.4 Hz, 1H), 7.15 (dd, *J* = 7.0, 4.7 Hz, 2H), 7.10 (dd, *J* = 8.8, 2.5 Hz, 1H), 6.97 (dd, *J* = 6.6, 2.8 Hz, 1H), 4.15 (t, *J* = 6.3 Hz, 2H), 3.11 (bs, 4H), 2.81–2.61 (m, 6H), 2.15–2.05 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 158.5, 155.1, 154.8, 151.3, 134.2, 127.7, 127.6, 125.7, 124.8, 122.2, 118.8, 116.5, 106.7, 66.7, 55.3, 53.4, 51.3, 26.7. HPLC: 99%, RT 2.607 min. MS (ESI) *m/z* 422.0 [M + H]$^+$. HRMS *m/z* [M + H]$^+$ calcd for $C_{20}H_{22}Cl_2N_3OS$ 422.0861, found 422.0885. Melting point: 127–129 °C.

5-(3-(4-(2,3-Dichlorophenyl)-1,4-diazepan-1-yl)propoxy)-benzo[*d*]thiazole (38). Compound **38** (light-brown solid, 98 mg) was prepared by the same procedure as preparing **22**, yield 60%. ^{1}H NMR (400 MHz, CD_3OD): δ 9.91 (s, 1H), 8.10 (t, *J* = 8.7 Hz, 1H), 7.65 (s, 1H), 7.34 (t, *J* = 7.7 Hz, 1H), 7.29–7.15 (m, 3H), 4.31 (t, *J* = 5.2 Hz, 2H), 3.87–3.71 (m, 2H), 3.67–3.43 (m, 6H), 3.42–3.29 (m, 2H), 2.48–2.24 (m, 4H). ^{13}C NMR (101 MHz, CD_3OD): δ 162.1, 160.9, 152.9, 134.9, 129.1, 128.6, 126.4, 125.8, 125.2, 122.1, 119.5, 119.2, 103.6, 67.2, 56.5, 56.3, 54.6, 53.4, 50.9, 25.8, 25.5. HPLC: 99%, RT 2.601 min. MS (ESI) *m/z* 436.3 [M + H]$^+$. HRMS *m/z* [M + H]$^+$ calcd for $C_{21}H_{24}Cl_2N_3OS$ 436.1017, found 436.1038. mp: 93–94 °C.

5-(4-(4-(2,3-Dichlorophenyl)-1,4-diazepan-1-yl)butoxy)-benzo[*d*]thiazole (39). Compound **39** (light-brown solid, 98 mg) was prepared by the same procedure as preparing **22**, yield 60%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.97 (s, 1H), 7.80 (d, *J* = 8.7 Hz, 1H), 7.60 (d, *J* = 3.0 Hz, 1H), 7.11–7.07 (m, 3H), 7.01–6.98 (m, 1H), 4.10 (t, *J* = 5.7 Hz, 2H), 3.76–3.68 (m, 2H), 3.33–3.27 (m, 4H), 2.96–2.95 (m, 4H), 2.73–2.71 (m, 2H), 2.06–2.04 (m, 2H), 1.92–1.80 (m, 2H). HPLC: 99%, RT 2.702 min. MS (ESI) *m/z* 450.1 [M + H]$^+$. Melting point: 82–84 °C.

5-(4-(4-(2,3-Dichlorophenyl)piperidin-1-yl)butoxy)-1*H*-benzo[*d*]imidazol-2(3*H*)-one (40). Compound **40** (61 mg) was prepared as off-white solid by the same procedure as preparing **22**, yield 40%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.59 (brs, 1H), 9.29 (brs, 1H), 7.33–7.30 (m, 2H), 7.20–7.13 (m, 1H), 6.92 (d, *J* = 8.7 Hz, 1H), 6.65–6.59 (m, 2H), 3.94–3.91 (m, 2H), 3.15–3.04 (m, 3H), 2.51–2.49 (m, 2H), 2.15(t, *J* = 11.1 Hz, 2H), 1.90–1.74 (m, 8H). HPLC: 99%, RT 2.332 min. MS (ESI) *m/z* 434.0 [M + H]$^+$. Melting point: 180–182 °C.

6-(3-(4-(2,3-Dichlorophenyl)piperazin-1-yl)propoxy)-1*H*-indazole (41). Compound **41** (90 mg) was prepared by the same procedure as preparing **22**, yield 71%. ^{1}H NMR (400 MHz, $CDCl_3$): δ

10.38 (bs, 1H), 7.97 (s, 1H), 7.60 (d, J = 8.7 Hz, 1H), 7.20−7.08 (m, 2H), 6.95 (dd, J = 7.1, 2.5 Hz, 1H), 6.90−6.78 (m, 2H), 4.08 (t, J = 6.3 Hz, 2H), 3.09 (bs, 4H), 2.77−2.61 (m, 6H), 2.12−2.00 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 159.2, 151.3, 141.5, 135.0, 134.2, 127.6, 127.5, 124.7, 121.7, 118.7, 118.1, 113.5, 91.7, 66.6, 55.3, 53.5, 51.4, 26.8. HPLC: 99%, RT 2.462 min. MS (ESI) m/z 405.2 $[M + H]^+$. HRMS m/z $[M + H]^+$ calcd for $C_{22}H_{26}Cl_2N_3OS$ 405.1249, found 405.1273. Melting point: 159−161 °C.

7-(3-(4-(2,3-Dichlorophenyl)piperazin-1-yl)propoxy)-3,4-dihydroisoquinolin-1(2*H*)-one (42). Compound **42** (88 mg) was prepared as a white solid by the same procedure as preparing **22**, yield 52%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60 (d, J = 2.7 Hz, 1H), 7.18−7.09 (m, 3H), 7.01 (dd, J = 8.3, 2.8 Hz, 1H), 6.96 (dd, J = 6.2, 3.4 Hz, 1H), 6.15 (bs, 1H), 4.10 (t, J = 6.4 Hz, 2H), 3.54 (td, J = 6.6, 2.8 Hz, 2H), 3.08 (bs, 4H), 2.93 (t, J = 6.6 Hz, 2H), 2.73−2.57 (m, 6H), 2.06−1.97 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 166.4, 158.3, 151.5, 134.1, 131.1, 129.9, 128.5, 127.7, 127.6, 124.7, 120.4, 118.8, 112.0, 66.7, 55.2, 53.5, 51.4, 40.7, 27.7, 26.9. HPLC: 99%, RT 2.612 min. MS (ESI) m/z 434.1 $[M + H]^+$. HRMS m/z $[M + H]^+$ calcd for $C_{22}H_{26}Cl_2N_3O_2$ 434.1402, found 434.1425. Melting point: 162−164 °C.

7-(3-(4-(2,3-Dichlorophenyl)-1,4-diazepan-1-yl)propoxy)-3,4-dihydroisoquinolin-1(2*H*)-one (43). Compound **43** (125 mg) was prepared as a white solid by the same procedure as preparing **22**, yield 71%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.60 (d, J = 2.1 Hz, 1H), 7.14−7.08 (m, 3H), 7.02−7.00 (m, 2H), 6.02 (br., 1H), 4.10 (t, J = 6.3 Hz, 2H), 3.56−3.53 (m, 2H), 3.31−3.27 (m, 4H), 2.96−2.88 (m, 6H), 2.77−2.72 (m, 2H), 2.01(m, 4H). HPLC: 99%, RT 2.578 min. MS (ESI) m/z 448.1 $[M + H]^+$. Melting point: 116−117 °C.

Synthesis of compound **44** was described previously.[27]

7-(4-(4-(2,3-Dichlorophenyl)-1,4-diazepan-1-yl)butoxy)-quinolin-2(1*H*)-one (45). Compound **45** (50 mg) was prepared as a white solid by the same procedure as preparing **22**, yield 46%. ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.54 (br, 1H), 7.71 (d, J = 6.9 Hz, 1H) 7.44 (d, J = 8.1 Hz, 1H), 7.09−7.07 (m, 2H), 7.01−6.97 (m, 1H), 6.82−6.78 (m, 2H), 6.53 (d, J = 9.3 Hz, 1H), 4.10 (t, J = 6.6 Hz, 2H), 3.30 (d, J = 5.1 Hz, 4H), 2.87−2.81 (m, 4H), 2.62 (t, J = 7.5 Hz, 2H), 2.01−1.99 (m, 2H), 1.92−1.82 (m, 2H), 1.76−1.68 (m, 2H). HPLC: 99%, RT 2.461 min. MS(ESI) m/z 460.2 $[M + H]^+$. mp: 55−57 °C.

Experimental Procedures for in Vitro Biochemical Assays. *General Procedures.* Experimental procedures for the radioligand binding assays for the GPCRs listed in Supporting Information Table S1 (including D_1, D_3, D_4, D_5, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, and 5-HT_{1A}) are available online through the Psychoactive Drug Screening Program (PDSP) Web site: http://pdsp.med.unc.edu/. The PDSP Assay Protocol book is freely available at http://pdsp.med.unc.edu/UNC−CH%20Protocol%20Book.pdf. The dopamine D_2 radioligand binding, cAMP biosensor, and β-arrestin recruitment Tango assays are detailed below.

CHO−D_2 Membrane Preparation and Radioligand Binding Assay. CHO−D_2 membrane preparation. Cells stably expressing human D_{2L} receptors (CHO−D_{2L}) were plated in 15 cm dishes (in DMEM containing 10% FBS) and grown to 90% confluence. Then cells were washed with PBS, pH 7.4, and harvested by scraping into PBS, pH 7.4. Harvested cells were centrifuged at 1000*g* for 10 min and then hypotonically lysed by resuspension into ice-cold 50 mM Hepes, 1% BSA, pH 7.4. Membranes were isolated by centrifugation at 21000*g* for 20 min. The supernatant was removed, and the membrane pellets were stored at −80 °C until used for radioligand binding assays.

Radioligand Binding Assay. Membranes prepared as above were resuspended to 1 μg protein/μL (measured by Bradford assay using BSA as standard), and 50 μL was added to each well of a polypropylene 96-well plate containing (per well) 50 μL of buffer (20 mM Hepes, 10 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, 100 mM *N*-methyl-D-gluconate, pH 7.4), 50 μL of 1.5 nM [^{3}H]*N*-methylspiperone (final concentration 0.3 nM), and reference or D_2 test ligands at various concentrations ranging from 50 pM to 50 μM (final concentrations ranging from 10 pM to 10 μM, triplicate determinations for each concentration of D_2 test ligand). After a 1.5 h incubation in the dark at room temperature, the reactions were harvested onto 0.3% PEI-soaked Filtermax GF/A filters (Wallac) and washed three times with ice-cold 50 mM Tris, pH 7.4, using a Perkin-Elmer Filtermate 96-well harvester. The filters were subsequently dried and placed on a hot plate (100 °C), and Melitilex-A (Wallac) scintillant was applied. The filters were then removed from the hot plate and allowed to cool. The filters were counted on a Wallac TriLux microbeta counter (3 min/well). Residual [^{3}H]*N*-methylspiperone binding to filtered membranes was plotted as a function of log [reference] or log [D_2 test ligand], and the data were regressed using the one-site competition model built into Prism 4.0 (GraphPad software).

D_2-Mediated cAMP Assay. HEK293T cells coexpressing the cAMP biosensor GloSensor-22F (Promega) and hD_{2L} receptors were seeded (20000 cells/20 μL/well) into white, clear-bottom, tissue culture plates in HBSS, 20 mM Hepes, pH 7.4. After 30 min of recovery, cells were treated with 10 μL of 3× test or reference drug prepared in HBSS, 20 mM Hepes, pH 7.4. After 30 min, cells were treated with 10 μL of 1200 nM (4×) isoproterenol in 8% (4×) GloSensor reagent. Luminescence per well per second was read on a Wallac TriLux microbeta plate counter. Data were normalized to the isoproterenol response (100%) and the maximal quinpirole-induced inhibition thereof (0%) and regressed using the sigmoidal dose−response function built into GraphPad Prism 4.0. Notably, HEK293T cells expressing the GloSensor-22F alone (no hD_2) were assayed in parallel and displayed no inhibition of isoproterenol-stimulated cAMP, either by quinpirole or by the test compounds, suggesting that the effect observed in hD_{2L}-expressing cells was due to compound acting via the recombinant receptor.

D_2 β-Arrestin Recruitment Tango Assay. Recruitment of β-arrestin to agonist-stimulated D_{2L} receptors was performed using a previously described "Tango"-type assay.[46] Briefly, HTLA cells stably expressing β-arrestin-TEV protease and a tetracycline transactivator-driven luciferase were plated in 15 cm dishes in DMEM containing 10% FBS and transfected (via calcium phosphate) with 16 μg of a D_2V_2-TCS-tTA construct.[46] The next day, cells were plated in white, clear-bottom, 384-well plates (Greiner; 15000 cells/well, 50 μL/well) in DMEM containing 1% dialyzed FBS. The following day, the cells were challenged with 10 μL/well of reference agonist (6 μM) or D_2 test ligand (6 μM) prepared in HBSS, 20 mM Hepes, pH 7.4, and 6% DMSO (final ligand concentrations are 1 μM, final DMSO concentration is 1%). After 18 h, the medium was removed and replaced with 1× BriteGlo reagent (Promega), and luminescence per well was read using a TriLux plate reader (1 s/well). Data were normalized to vehicle (0%) and quinpirole (100%) controls and regressed using the sigmoidal dose−response function built into GraphPad Prism 4.0.0

Experimental Procedures for in Vivo Studies in Mice. *General Procedures.* All experiments were approved by the Institutional Animal Care and Use Committees at the University of North Carolina, Chapel Hill, and Duke University. Wild-type and β-arrestin-2 knockout mice were housed under standard conditions −14 h light/dark cycle (lights on 0600 h) with food and water provided ad libitum. Adult, age-matched male and female wild-type and β-arrestin-2 knockout drug-naive mice were used for all behavioral testing.

*Locomotor Activity of Compounds **19** and **35**.* Wild-type and β-arrestin-2 knockout mice were treated with vehicle or 2 mg/kg compounds **19** or **35** (ip) and were immediately placed into an open field. Thirty minutes later, the animals were administered 6 mg/kg PCP (ip) and were immediately returned to the open field for 90 min. Horizontal activity, measured as distance traveled, was recorded over 5 min segments for the duration of testing. RMANOVA for the first 30 min of testing (baseline) revealed a significance within subject effects of time [$F(5300) = 38.344$, $p < 0.001$]; the time by genotype, time by treatment, or the time by treatment by genotype interactions were not significant (Figure 2).

A second analysis was run to analyze the locomotor activity of the mice following PCP treatment over the entire 90 min following injection. A RMANOVA noted a significant within subject effects of time [$F(171020) = 64.520$, $p < 0.001$] and significant time by genotype [$F(171020) = 1.592$, $p < 0.012$] and time by treatment interactions [$F(341020) = 2.316$, $p < 0.001$]. Because these analyses indicated significant genotype and treatment effects, the RMANOVA was run within each treatment condition as a function of genotype. For the PCP-treated mice, no genotype differences were discerned across time (Figure 2). For mice treated with **19**, there was a significant within subjects effect of time [$F(17340) = 26.012$, $p < 0.001$] and time by genotype interaction [$F(17340) = 1.901$, $p < 0.017$]. The Bonferroni test reported that **19** suppressed PCP-induced locomotion in the WT relative to the β-arrestin 2 knockout mice at 50, 55, 60, 65, 75, and 105 min (ps <0.042). For mice treated with **35**, there was a significant within subject effect of time [$F(17272) = 14.194$, $p < 0.001$] and time by genotype interaction [$F(17272) = 1.729$, $p < 0.038$]. The Bonferroni test found that 35 suppressed PCP-induced locomotion in the WT relative to the β-arrestin 2 knockout mice at 90 min ($p < 0.050$). Hence, these data show that at 2 mg/kg **19** is more efficacious than **35** in reducing PCP-stimulated locomotion in wild-type mice; neither compound affected PCP-induced activity in the β-arrestin-2 knockout mice.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Radioligand binding affinities of compounds **19** and **35** at select dopamine and serotonin receptors. ^{1}H and ^{13}C NMR spectra of compounds **35** and **37**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*For J.J.: phone, 919-843-8459; fax, 919-843-8465; E-mail, jianjin@unc.edu. For B.L.R.: phone, 919-966-7535; fax, 919-843-5788; E-mail, bryan_roth@med.unc.edu.

Author Contributions

⊥These authors contributed equally to this work.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the NIH (U19MH082441S1 and U19MH082441) for financial support and Dr. Weihe Zhang for critical reading of synthetic procedures and characterization data.

■ ABBREVIATIONS USED

GPCR, G protein-coupled receptor; SFSR, structure−functional selectivity relationships; D_2R, dopamine D_2 receptor; cAMP, cyclic adenosine monophosphate; PK, pharmacokinetic; CNS, central nervous system; SAR, structure−activity relationship; LHS, left-hand side; RHS, right-hand side; ip, intraperitoneal

■ REFERENCES

(1) Furchgott, R. F. The use of haloalkylamines in the differentiation of receptors and in the determination of dissociation constants of agonist-receptor complexes. In *Advances in Drug Research*; Harper, N.; Simmonds, A., Eds.; Academic Press: New York, 1966; Vol. 3, pp 21−55.

(2) Roth, B. L.; Chuang, D. M. Multiple mechanisms of serotonergic signal transduction. *Life Sci.* **1987**, *41*, 1051−1064.

(3) Berg, K. A.; Maayani, S.; Goldfarb, J.; Scaramellini, C.; Leff, P.; Clarke, W. P. Effector pathway-dependent relative efficacy at serotonin type 2A and 2C receptors: evidence for agonist-directed trafficking of receptor stimulus. *Mol. Pharmacol.* **1998**, *54*, 94−104.

(4) Parrish, J.; Braden, M.; Gundy, E.; Nichols, D. E. Differential phospholipase C activation by phenylalkylamine serotonin 5-HT2A receptor agonists. *J. Neurochem.* **2005**, *95*, 1575−1584.

(5) Ghosh, D.; Snyder, S. E.; Watts, V. J.; Mailman, R. B.; Nichols, D. E. 9-Dihydroxy-2,3,7,11b-tetrahydro-1*H*-naph[1,2,3-*de*]isoquinoline: a potent full dopamine D1 agonist containing a rigid beta-phenyldopamine pharmacophore. *J. Med. Chem.* **1996**, *39*, 549−555.

(6) Kilts, J. D.; Connery, H. S.; Arrington, E. G.; Lewis, M. M.; Lawler, C. P.; Oxford, G. S.; O'Malley, K. L.; Todd, R. D.; Blake, B. L.; Nichols, D. E.; Mailman, R. B. Functional selectivity of dopamine receptor agonists. II. Actions of dihydrexidine in D2L receptor-transfected MN9D cells and pituitary lactotrophs. *J. Pharmacol. Exp. Ther.* **2002**, *301*, 1179−1189.

(7) Mottola, D. M.; Kilts, J. D.; Lewis, M. M.; Connery, H. S.; Walker, Q. D.; Jones, S. R.; Booth, R. G.; Hyslop, D. K.; Piercey, M.; Wightman, R. M.; Lawler, C. P.; Nichols, D. E.; Mailman, R. B. Functional selectivity of dopamine receptor agonists. I. Selective activation of postsynaptic dopamine D2 receptors linked to adenylate cyclase. *J. Pharmacol. Exp. Ther.* **2002**, *301*, 1166−1178.

(8) Beaulieu, J. M.; Tirotta, E.; Sotnikova, T. D.; Masri, B.; Salahpour, A.; Gainetdinov, R. R.; Borrelli, E.; Caron, M. G. Regulation of Akt signaling by D2 and D3 dopamine receptors in vivo. *J. Neurosci.* **2007**, *27*, 881−885.

(9) Beaulieu, J. M.; Sotnikova, T. D.; Marion, S.; Lefkowitz, R. J.; Gainetdinov, R. R.; Caron, M. G. An Akt/beta-arrestin 2/PP2A signaling complex mediates dopaminergic neurotransmission and behavior. *Cell* **2005**, *122*, 261−273.

(10) Chidiac, P.; Hebert, T.; Valiquette, M.; Dennis, M.; Bouvier, M. Inverse agonist activity of beta-adrenergic antagonists. *Mol. Pharmacol.* **1993**, *45*, 490−499.

(11) Barker, E. L.; Westphal, R. S.; Schmidt, D.; Sanders-Bush, E. Constitutively active 5-hydroxytryptamine2C receptors reveal novel inverse agonist activity of receptor ligands. *J. Biol. Chem.* **1994**, *269*, 11687−11690.

(12) Rauser, L.; Savage, J. E.; Meltzer, H. Y.; Roth, B. L. Inverse agonist actions of typical and atypical antipsychotic drugs at the human 5-hydroxytryptamine(2C) receptor. *J. Pharmacol. Exp. Ther.* **2001**, *299*, 83−89.

(13) Gilliland, S. L.; Alper, R. H. Characterization of dopaminergic compounds at hD2short, hD4.2 and hD4.7 receptors in agonist-stimulated [35S]GTPgammaS binding assays. *Naunyn Schmiedebergs Arch. Pharmacol.* **2000**, *361*, 498−504.

(14) Burstein, E. S.; Ma, J.; Wong, S.; Gao, Y.; Pham, E.; Knapp, A. E.; Nash, N. R.; Olsson, R.; Davis, R. E.; Hacksell, U.; Weiner, D. M.; Brann, M. R. Intrinsic efficacy of antipsychotics at human D2, D3, and D4 dopamine receptors: identification of the clozapine metabolite *N*-desmethylclozapine as a D2/D3 partial agonist. *J. Pharmacol. Exp. Ther.* **2005**, *315*, 1278−1287.

(15) Peroutka, S. J.; Snyder, S. H. Long-term antidepressant treatment decreases spiroperidol-labeled serotonin receptor binding. *Science* **1980**, *210*, 88−90.

(16) Berry, S.; Shah, M.; Khan, N.; Roth, B. Rapid agonist-induced internalization of the 5-hydroxytryptamine2A receptor occurs via the endosome pathway in vitro. *Mol. Pharmacol.* **1996**, *50*, 306−313.

(17) Willins, D. L.; Alsayegh, L.; Berry, S. A.; Backstrom, J. R.; Sanders-Bush, E.; Friedman, L.; Khan, N.; Roth, B. L. Serotonergic antagonist effects on trafficking of serotonin 5-HT2A receptors in vitro and in vivo. *Ann. N.Y. Acad. Sci.* **1998**, *861*, 121−127.

(18) Willins, D.; Berry, S.; Alsayegh, L.; Backstrom, J.; Sanders-Bush, E.; Roth, B. Clozapine and other 5-hydroxytryptamine2A receptor antagonists alter the subcellular distribution of 5-hydroxytryptamine2A receptors in vitro and in vivo. *Neuroscience* **1999**, *91*, 599−606.

(19) Urban, J. D.; Clarke, W. P.; von Zastrow, M.; Nichols, D. E.; Kobilka, B.; Weinstein, H.; Javitch, J. A.; Roth, B. L.; Christopoulos, A.; Sexton, P. M.; Miller, K. J.; Spedding, M.; Mailman, R. B. Functional selectivity and classical concepts of quantitative pharmacology. *J. Pharmacol. Exp. Ther.* **2007**, *320*, 1−13.

(20) Mailman, R. B. GPCR functional selectivity has therapeutic impact. *Trends Pharmacol. Sci.* **2007**, *28*, 390−396.

(21) Luttrell, L. M.; Ferguson, S. S.; Daaka, Y.; Miller, W. E.; Maudsley, S.; Della Rocca, G. J.; Lin, F.; Kawakatsu, H.; Owada, K.; Luttrell, D. K.; Caron, M. G.; Lefkowitz, R. J. Beta-arrestin-dependent

formation of beta2 adrenergic receptor–Src protein kinase complexes. *Science* **1999**, *283*, 655–661.
(22) Lefkowitz, R. J.; Shenoy, S. K. Transduction of receptor signals by beta-arrestins. *Science* **2005**, *308*, 512–517.
(23) Abbas, A.; Roth, B. L. Arresting serotonin. *Proc. Natl. Acad. Sci. U.S.A* **2008**, *105*, 831–832.
(24) Urban, J. D.; Vargas, G. A.; von Zastrow, M.; Mailman, R. B. Aripiprazole has functionally selective actions at dopamine D2 receptor-mediated signaling pathways. *Neuropsychopharmacology* **2007**, *32*, 67–77.
(25) Violin, J. D.; Lefkowitz, R. J. [beta]-Arrestin-biased ligands at seven-transmembrane receptors. *Trends Pharmacol. Sci.* **2007**, *28*, 416–422.
(26) Gesty-Palmer, D.; Flannery, P.; Yuan, L.; Corsino, L.; Spurney, R.; Lefkowitz, R. J.; Luttrell, L. M. A beta-arrestin-biased agonist of the parathyroid hormone receptor (PTH1R) promotes bone formation independent of G protein activation. *Sci. Transl. Med.* **2009**, *1*, 1ra1.
(27) Butini, S.; Gemma, S.; Campiani, G.; Franceschini, S.; Trotta, F.; Borriello, M.; Ceres, N.; Ros, S.; Coccone, S. S.; Bernetti, M.; De Angelis, M.; Brindisi, M.; Nacci, V.; Fiorini, I.; Novellino, E.; Cagnotto, A.; Mennini, T.; Sandager-Nielsen, K.; Andreasen, J. T.; Scheel-Kruger, J.; Mikkelsen, J. D.; Fattorusso, C. Discovery of a new class of potential multifunctional atypical antipsychotic agents targeting dopamine D3 and serotonin 5-HT1A and 5-HT2A receptors: design, synthesis, and effects on behavior. *J. Med. Chem.* **2009**, *52*, 151–169.
(28) Allen, J. A.; Yost, J. M.; Setola, V.; Chen, X.; Sassano, M. F.; Chen, M.; Peterson, S.; Yadav, P. N.; Huang, X.-P.; Feng, B.; Jensen, N. H.; Che, X.; Bai, X.; Frye, S. V.; Wetsel, W. C.; Caron, M. G.; Javitch, J. A.; Roth, B. L.; Jin, J. Discovery of Beta-Arrestin-Biased Dopamine D2 Ligands for Probing Signal Transduction Pathways Essential for Antipsychotic Efficacy. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 18488–18493.
(29) Lawler, C. P.; Prioleau, C.; Lewis, M. M.; Mak, C.; Jiang, D.; Schetz, J. A.; Gonzalez, A. M.; Sibley, D. R.; Mailman, R. B. Interactions of the novel antipsychotic aripiprazole (OPC-14597) with dopamine and serotonin receptor subtypes. *Neuropsychopharmacology* **1999**, *20*, 612–627.
(30) Mailman, R. B.; Murthy, V. Third generation antipsychotic drugs: partial agonism or receptor functional selectivity? *Curr. Pharm. Des.* **2010**, *16*, 488–501.
(31) Mallikaarjun, S.; Salazar, D. E.; Bramer, S. L. Pharmacokinetics, Tolerability, and Safety of Aripiprazole following Multiple Oral Dosing in Normal Healthy Volunteers. *J. Clin. Pharmacol.* **2004**, *44*, 179–187.
(32) Johnson, D. S.; Choi, C.; Fay, L. K.; Favor, D. A.; Repine, J. T.; White, A. D.; Akunne, H. C.; Fitzgerald, L.; Nicholls, K.; Snyder, B. J.; Whetzel, S. Z.; Zhang, L.; Serpa, K. A. Discovery of PF-00217830: Aryl piperazine napthyridinones as D2 partial agonists for schizophrenia and bipolar disorder. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2621–2625.
(33) Oshiro, Y.; Sato, S.; Kurahashi, N.; Tanaka, T.; Kikuchi, T.; Tottori, K.; Uwahodo, Y.; Nishi, T. Novel antipsychotic agents with dopamine autoreceptor agonist properties: synthesis and pharmacology of 7-[4-(4-phenyl-1-piperazinyl)butoxy]-3,4-dihydro-2(1*H*)-quinolinone derivatives. *J. Med. Chem.* **1998**, *41*, 658–667.
(34) Vangveravong, S.; Zhang, Z.; Taylor, M.; Bearden, M.; Xu, J.; Cui, J.; Wang, W.; Luedtke, R. R.; Mach, R. H. Synthesis and characterization of selective dopamine D(2) receptor ligands using aripiprazole as the lead compound. *Bioorg. Med. Chem.* **2011**, *19*, 3502–3511.
(35) Martin, G. E.; Elgin, R. J.; Mathiasen, J. R.; Davis, C. B.; Kesslick, J. M.; Baldy, W. J.; Shank, R. P.; Distefano, D. L.; Fedde, C. L.; Scott, M. K. Activity of Aromatic Substituted Phenylpiperazines Lacking Affinity for Dopamine Binding Sites in a Preclinical Test of Antipsychotic Efficacy. *J. Med. Chem.* **1989**, *32*, 1052–1056.
(36) Elworthy, T. R.; Ford, A. P. D. W.; Bantle, G. W.; Morgans, D. J.; Ozer, R. S.; Palmer, W. S.; Repke, D. B.; Romero, M.; Sandoval, L.; Sjogren, E. B.; Talamas, F. X.; Vazquez, A.; Wu, H.; Arredondo, N. F.; Blue, D. R.; DeSousa, A.; Gross, L. M.; Kava, M. S.; Lesnick, J. D.; Vimont, R. L.; Williams, T. J.; Zhu, Q. M.; Pfister, J. R.; Clarke, D. E. *N*-Arylpiperazinyl-*N*′-propylamino derivatives of heteroaryl amides as functional uroselective alpha(1)-adrenoceptor antagonists. *J. Med. Chem.* **1997**, *40*, 2674–2687.
(37) Fu, X. T.; Lei; Yuan, M.; Shi, J. Design, synthesis and vasorelaxant activity of *R*,*S*-1-(substituted phenyl)-4-[3-(naphtha-1-yl-oxy)-2-hydroxypropyl]-piperazine derivatives. *Yaoxue Xuebao* **2007**, *42*, 735–740.
(38) Tsuruta, K.; Frey, E. A.; Grewe, C. W.; Cote, T. E.; Eskay, R. L.; Kebabian, J. W. Evidence that LY-141865 specifically stimulates the D-2 dopamine receptor. *Nature* **1981**, *292*, 463–465.
(39) Banno, K.; Fujioka, T.; Kikuchi, T.; Oshiro, Y.; Hiyama, T.; Nakagawa, K. Studies on 2(1*H*)-quinolinone derivatives as neuroleptic agents I. Synthesis and biological activities of (4-phenyl-1-piperazinyl)-propoxy-2(1*H*)-quinolinone derivatives. *Chem. Pharm. Bull.* **1988**, *36*, 4377–4388.
(40) Kiss, B.; Horvath, A.; Nemethy, Z.; Schmidt, E.; Laszlovszky, I.; Bugovics, G.; Fazekas, K.; Hornok, K.; Orosz, S.; Gyertyan, I.; Agai-Csongor, E.; Domany, G.; Tihanyi, K.; Adham, N.; Szombathelyi, Z. Cariprazine (RGH-188), a dopamine D(3) receptor-preferring, D(3)/D(2) dopamine receptor antagonist–partial agonist antipsychotic candidate: in vitro and neurochemical profile. *J. Pharmacol. Exp. Ther.* **2010**, *333*, 328–340.
(41) van Wijngaarden, I.; Kruse, C. G.; van der Heyden, J. A.; Tulp, M. T. 2-Phenylpyrroles as conformationally restricted benzamide analogues. A new class of potential antipsychotics. 2. *J. Med. Chem.* **1988**, *31*, 1934–1940.
(42) van Wijngaarden, I.; Kruse, C. G.; van Hes, R.; van der Heyden, J. A.; Tulp, M. T. 2-Phenylpyrroles as conformationally restricted benzamide analogues. A new class of potential antipsychotics. 1. *J. Med. Chem.* **1987**, *30*, 2099–2104.
(43) Bohn, L. M.; Lefkowitz, R. J.; Gainetdinov, R. R.; Peppel, K.; Caron, M. G.; Lin, F. T. Enhanced morphine analgesia in mice lacking beta-arrestin 2. *Science* **1999**, *286*, 2495–2498.
(44) Nordquist, R. E.; Risterucci, C.; Moreau, J. L.; von Kienlin, M.; Kunnecke, B.; Maco, M.; Freichel, C.; Riemer, C.; Spooren, W. Effects of aripiprazole/OPC-14597 on motor activity, pharmacological models of psychosis, and brain activity in rats. *Neuropharmacology* **2008**, *54*, 405–416.
(45) Gottlieb, H. E.; Kotlyar, V.; Nudelman, A. NMR chemical shifts of common laboratory solvents as trace impurities. *J. Org. Chem.* **1997**, *62*, 7512–7515.
(46) Barnea, G.; Strapps, W.; Herrada, G.; Berman, Y.; Ong, J.; Kloss, B.; Axel, R.; Lee, K. J. The genetic design of signaling cascades to record receptor activation. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 64–69.