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High Affinity Dopamine D_3 Receptor (D_3R)-Selective Antagonists Attenuate Heroin Self-Administration in Wild-Type but not D_3R Knockout Mice

Comfort A. Boateng,[†,⊥] Oluyomi M. Bakare,[†,⊥] Jia Zhan,[†,#] Ashwini K. Banala,[†] Caitlin Burzynski,[†] Elie Pommier,[†] Thomas M. Keck,[†,‡] Prashant Donthamsetti,[∥] Jonathan A. Javitch,[∥] Rana Rais,[§] Barbara S. Slusher,[§] Zheng-Xiong Xi,[†] and Amy Hauck Newman*[,†]

[†]Molecular Targets and Medications Discovery Branch, National Institute on Drug Abuse— Intramural Research Program, National Institutes of Health, 333 Cassell Drive, Baltimore, Maryland 21224, United States

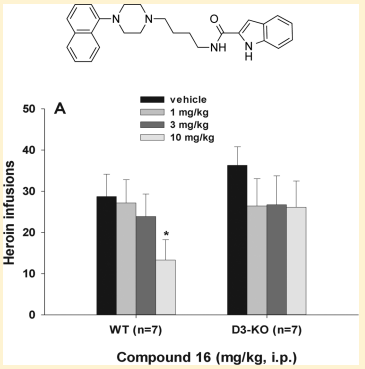
[‡]Department of Chemistry & Biochemistry, College of Science and Mathematics, Department of Biomedical & Translational Sciences, School of Biomedical Science & Health Professions, Rowan University, 201 Mullica Hill Road, Glassboro, New Jersey 08028, United States

[§]Department of Neurology, Brain Science Institute, The Johns Hopkins University School of Medicine, 855 North Wolfe Street, Baltimore, Maryland 21205, United States

[∥]Departments of Psychiatry and Pharmacology, Columbia University College of Physicians and Surgeons, and Division of Molecular Therapeutics, New York State Psychiatric Institute, New York, New York 10032, United States

Supporting Information

ABSTRACT: The dopamine D_3 receptor (D_3R) is a promising target for the development of pharmacotherapeutics to treat substance use disorders. Several D_3R-selective antagonists are effective in animal models of drug abuse, especially in models of relapse. Nevertheless, poor bioavailability, metabolic instability, and/or predicted toxicity have impeded success in translating these drug candidates to clinical use. Herein, we report a series of D_3R-selective 4-phenylpiperazines with improved metabolic stability. A subset of these compounds was evaluated for D_3R functional efficacy and off-target binding at selected 5-HT receptor subtypes, where significant overlap in SAR with D_3R has been observed. Several high affinity D_3R antagonists, including compounds **16** (K_i = 0.12 nM) and **32** (K_i = 0.35 nM), showed improved metabolic stability compared to the parent compound, PG648 (**6**). Notably, **16** and the classic D_3R antagonist SB277011A (**2**) were effective in reducing self-administration of heroin in wild-type but not D_3R knockout mice.



INTRODUCTION

The dopamine D_2-like family of receptors, comprising the D_2, D_3, and D_4 receptor subtypes (D_2R, D_3R, and D_4R, respectively), regulate physiological functions such as movement, emotion, and cognition. Numerous medications for the treatment of neuropsychiatric disorders, such as schizophrenia, target this important family of receptors.[1] The D_3R subtype is localized in key neurocircuits that underlie motivation and cognition, and in contrast to D_2R, do not appear to play a major role in movement.[2,3] Hence, the D_3R has been proposed as a promising target for development of psychostimulant addiction and relapse pharmacotherapy.[2,4−9] Additionally, seminal reports using [^{11}C]raclopride for PET imaging in both humans[10] and nonhuman primates[11] have demonstrated that overall D_2-like receptor availability in the basal ganglia is significantly reduced after chronic exposure to cocaine. Interestingly, the converse appears to be true for the D_3R subtype: recent human PET studies with the D_3R-preferential PET ligand [^{11}C]PHNO[12,13] confirmed earlier reports in post-mortem brains of cocaine overdose victims[14−16] that D_3R availability is actually increased upon chronic exposure to cocaine and methamphetamine.[17−19] Inspired by early preclinical studies using lead compounds BP897 (**1**)[20,21] and SB277011A (**2**),[22−24] intensive efforts toward discovering D_3R-selective antagonists and partial agonists as potential therapeutics for substance use disorders have been underway for nearly two decades.

Although early D_3R-selective partial agonists, such as **1**, and antagonists such as **2**, NGB2904 (**3**), and PG01037 (**4**), (Chart 1) have served as highly useful preclinical tools, none of these compounds have progressed to the clinic due to poor bioavailability and the advancement of newer generation analogues.[4−7,25−27] One example of a D_3R-selective compound tested in humans is GSK598,809 (**5**) (Chart 1), which was first reported in 2007.[25] Recently, [^{11}C]PHNO PET imaging studies demonstrated a correlation between this compound's D_3R occupancy and efficacy toward smoking cessation.[28,29] Although clinical studies have been conducted with **5** for

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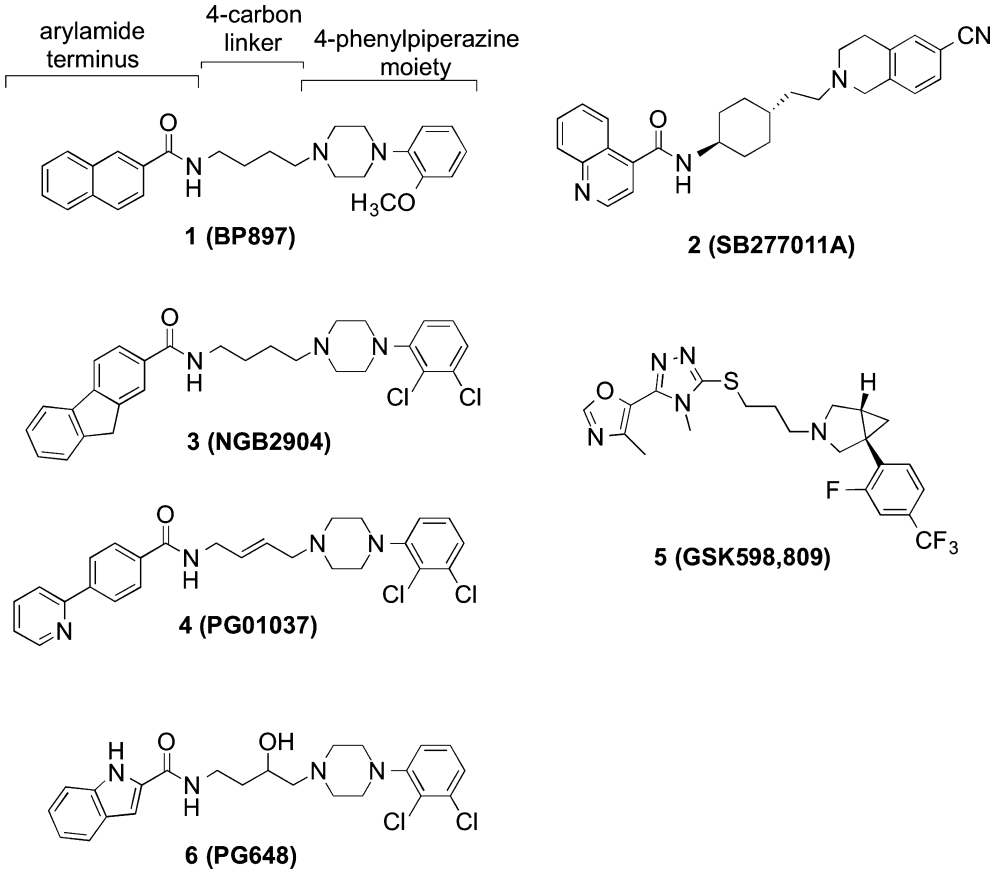
Chart 1. Selected Lead D_3R-Selective Antagonists and Partial Agonists



smoking cessation,[28−31] it is unlikely that this compound will be developed as a medication to treat other substance use disorders, as GlaxoSmithKline terminated this research and development program.

One of our lead compounds, *R*-PG648 (***R*-6**) binds with ~500-fold selectivity for D_3R (K_i = 0.53 nm) over D_2R (K_i = 295 nm) in vitro[26,27,32,33] and is an antagonist/weak partial agonist.[34] Using molecular simulations based on the D_3R X-ray crystal structure,[35] we demonstrated that the 4-phenylpiperazine moiety binds to the structurally similar D_2R/D_3R orthosteric binding site (OBS) in which dopamine and the D_2R/D_3R antagonist eticlopride bind, whereas the arylamide terminus binds in a secondary binding pocket (SBP) that is topographically divergent between D_2R and D_3R.[34−36] Despite early preclinical promise, ***R*-6** and its racemate (**6**) proved to be unsuitable for translation to clinical study.[33]

The development of D_3R antagonists with sufficient metabolic stability and efficacy as medications to treat substance use disorders remains a major challenge. Hence the purpose of the present study was to extend D_3R SAR by exploring modifications to all three segments (Chart 1) of the 4-phenylpiperazine class of molecules to identify ligands that demonstrate high D_3R binding affinity and subtype selectivity, with improved bioavailability and metabolic stability. Specifically, we replaced the 2,3-diCl-substitution of the 4-phenylpiperazine in lead compound **6** with a 2-OMe,3-Cl or a 2,3-naphthyl-substituent. Additionally, we explored bioisosteric replacement of the indolylamide of **6** with various heteroarylamides as well as further investigating the 3-hydroxy (3-OH) substituent in the 4-carbon linker. After assessing in vitro binding and functional efficacy profiles, and metabolic stability in mouse microsomes, two lead candidates were selected for behavioral evaluation in murine models of heroin self-administration and compared to the classic D_3R antagonist, **2**.

Although D_3R antagonists have largely been developed toward nicotine, cocaine, and methamphetamine abuse, they have shown efficacy in other preclinical models of drug and alcohol abuse.[37−39] For example, the D_3R antagonist **2** has been shown to block the acquisition and expression of heroin conditioned place preference in rats.[40] Nevertheless, to our knowledge, D_3R antagonists have not been evaluated for their effects on heroin self-administration. As D_3R antagonists are typically not effective in attenuating cocaine or methamphetamine self-administration under low fixed-ratio (FR) schedules (e.g., FR1, FR2),[41,42] we hypothesized that in the presence of these psychostimulants, high extracellular dopamine (DA) levels may compete at D_3R and thus render these D_3R-selective antagonists ineffective. Indeed, most reports show D_3R antagonists as being most effective in models of relapse when extracellular DA levels would be substantially lower than during active exposure to psychostimulants.[2,33] In comparison to psychostimulant drugs, heroin induces relatively moderate increases in extracellular DA within the accumbens during active drug-taking,[43−46] thus we posited that D_3R antagonists may be more effective in reducing heroin self-administration than psychostimulant use. If the data support our hypothesis, this would represent an alternative strategy to treating opioid abuse and addiction. Herein, we evaluate our lead compounds in both wild-type (WT) and D_3R knockout (KO) mice to not only assess effectiveness in attenuating heroin self-administration but also to determine the role of D_3R in these behaviors.

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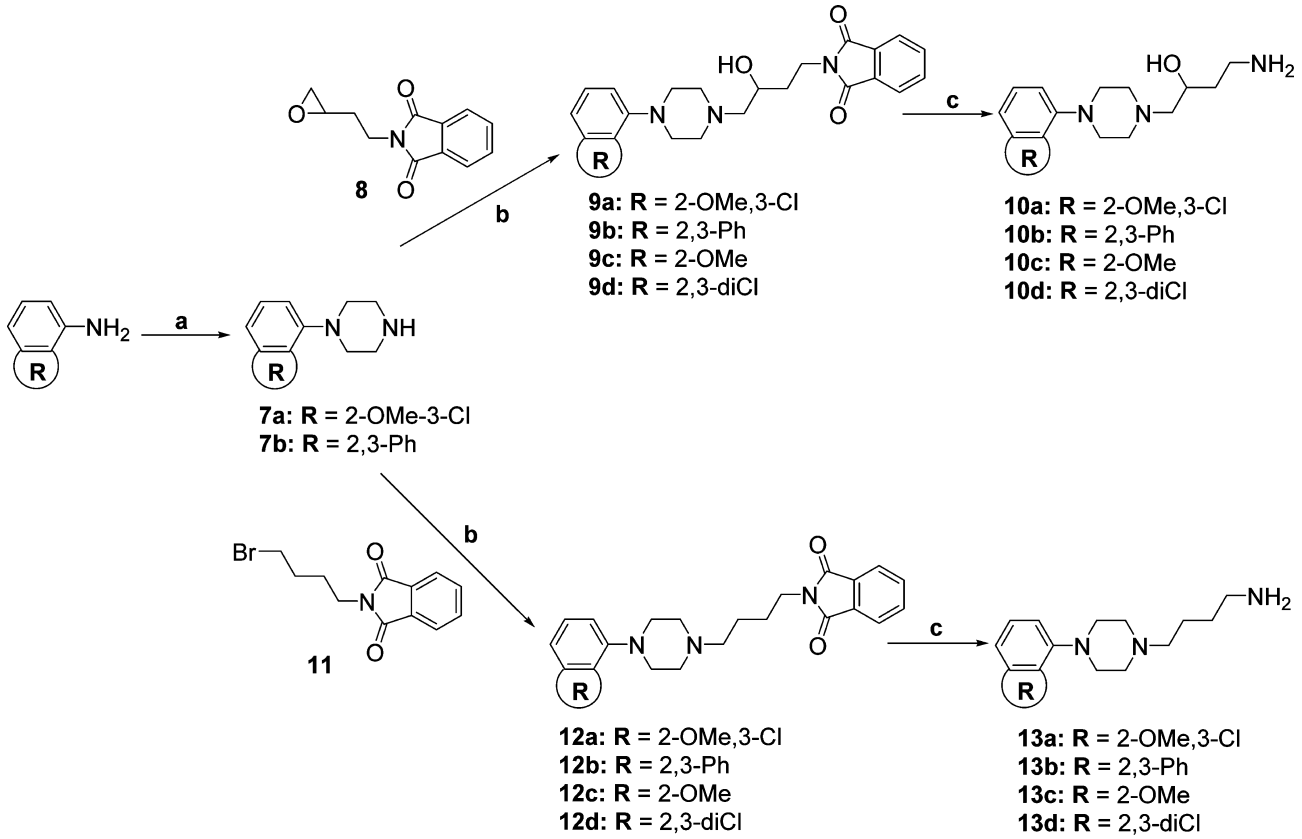
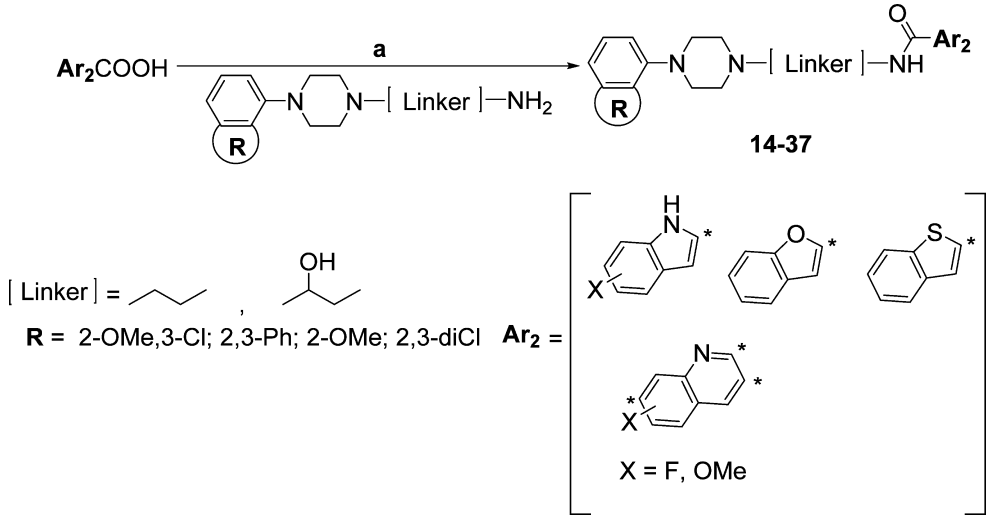
Scheme 1. Synthesis of 4-(4-Arylpiperazine)butylamine Intermediates[a]



[a]Reagents and conditions: (a) 3-chloro-2-methoxyaniline or naphthalen-1-amine, K_2CO_3, bis(2-chloroethyl)amine HCl, diglyme, reflux, 48 h; (b) appropriate 4-arylpiperazine, 2-propanol, reflux, overnight; (c) hydrazine (anhydrous), EtOH, reflux, 2−3 h.

Scheme 2. Synthesis of Target Compounds 14−37[a]



[a]Reagents and conditions: (a) (i) 1,1′-carbonyldiimidazole (CDI), THF, room temperature, 2 h; (ii) appropriate 4-arylpiperazine amine, THF, 0 °C to room temperature, overnight.

CHEMISTRY

The synthesis of 24 final compounds and their common intermediates are outlined in Schemes 1 and 2. In Scheme 1, **7a**[47] was synthesized using 3-chloro-2-methoxyaniline and bis(2-chloroethyl)amine HCl, in diglyme under reflux conditions. 1-(Naphthalene-1-yl)piperazine, **7b**, was synthesized as previously reported[48] via a nucleophilic substitution reaction with naphthalen-1-amine. The synthesis of the 4-(4-arylpiperazine)butylamine intermediates **10a−10d** and **13a−13d**, with or without a 3-OH substituent, respectively, was achieved starting with **7a**, **7b**, or commercially available 1-(2-methoxyphenyl)piperazine and 1-(2,3-dichlorophenyl)-piperazine.[49] Specifically, an epoxide ring-opening reaction of

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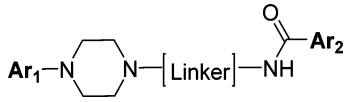
Table 1. Human Dopamine D_2-Like Receptor Binding Data in HEK Cells for Ligands with Varying Arylpiperazine and Arylamide Moieties[a]



Compd	Ar_1	[Linker]	Ar_2	cLogP	D_2R	D_3R	D_2R/D_3R
					K_i (nM) ± SEM		
6				4.9	746 ± 123	1.88 ± 0.112	397
14				4.5	53.0 ± 6.16	0.392 ± 0.0351	135
15				4.5	72.3 ± 8.30	0.907 ± 0.0478	80
16				5.1	12.9 ± 1.26	0.118 ± 0.0212	109
17				5.2	13.8 ± 2.09	0.173 ± 0.0786	80
18				5.1	14.8 ± 2.50	0.244 ± 0.0544	61
19				5.8	8.37 ± 0.792	0.128 ± 0.0311	65
20[50]				5.8	36.8± 9	0.28 ± 0.08	131
21				5.1	328 ± 47.7	1.8 ± 0.480	182

[a] K_i values determined by competitive inhibition of [^{3}H]*N*-methylspiperone binding in membranes harvested from HEK 293 cells stably expressing hD_2R or hD_3R.

8 with the corresponding 4-arylpiperazines under reflux conditions yielded the 3-OH-phthalimides **9a−9d**, which were subjected to phthalimide deprotection using hydrazine to give the 3-OH-substituted butylamine intermediates **10a−10d**. The synthesis of intermediate compounds **9c**, **9d**, **10c**, and **10d** was previously reported[49] using microwave conditions; however, the methods reported herein use conventional (oil bath) heating methods. To generate the 4-(4-arylpiperazine)-butylamine intermediates **13a−13d**, commercially available *N*-(4-bromobutyl)phthalimide **11** was reacted with the appropriate 4-arylpiperazine to give phthalimides **12a−12d**, which were then subjected to deprotection with hydrazine to give the desired 4-(4-arylpiperazine)butylamines **13a−13d**. While intermediates **12b−12d** and **13a−13d** have been previously reported,[34,50] our synthetic methods either differ completely from or improve upon these methods. The final ligands **14−37** (Scheme 2) featuring the arylpiperazine, four-carbon linker chain (with or without a 3-OH substituent), and arylamide moieties were synthesized via a general amidation procedure (method A),[50,51] in which various arylcarboxylic acids were coupled to corresponding primary amines **10** or **13** via an in situ *N*,*N*′-carbonyldiimidazole coupling reaction. All 3-OH-substituted compounds were racemic mixtures. As considerable enantioselectivity has so far not been demonstrated with either the 3-OH or 3-F substitution in this class of compounds,[26,27] no attempt to separate enantiomers was made.

PHARMACOLOGICAL RESULTS AND DISCUSSION

SAR at D_2R and D_3R. The objective of this study was to design metabolically stable ligands with high D_3R binding affinity and subtype selectivity. We extended our previous SAR studies by exploring modifications to all three segments of the lead compound **6** (Chart 1: arylamide moiety, 4-carbon linker, and 4-phenylpiperazine moiety) in an attempt to overcome bioavailabilty and metabolic shortcomings of previously reported preclinical candidates.[33]

We recently evaluated the binding affinities and selectivities at D_3R and D_2R of a library of synthons along with their full-length ligands.[34] Computational modeling studies demonstrated that the 3-Cl of the 2,3-diCl-phenylpiperazine moiety makes polar contacts with transmembrane domain 5 (TM5) serine (Ser) amino acid residues (particularly Ser193$^{5.43}$), which

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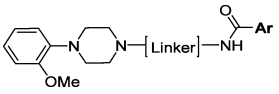
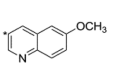
Table 2. Human Dopamine D_2-Like Receptor Binding Data in HEK Cells for *N*-(4-(4-(2-Methoxyphenyl)piperazin-1-yl)butyl)quinoline Carboxamide Ligands[a]



Compd	[Linker]	Ar	cLogP	D_2R	D_3R	D_2R/D_3R
				K_i (nM) ± SEM		
22			4.3	66.8 ± 12.5	3.46 ± 0.484	19
23			3.7	929 ± 176	97.9 ± 23.7	9.5
24			4.5	68.2 ± 21.1	2.51 ± 0.308	27
25			3.9	1,000 ± 62.4	53.1 ± 9.38	19
26			3.3	915 ± 106	33.8 ± 8.04	27
27			4.1	34.1 ± 9.36	2.14 ± 0.431	16
28			3.5	682 ± 85.9	44.8 ± 9.76	15
29			3.8	707 ± 127	52.9 ± 6.19	13
30			3.7	774 ± 215	69.9 ± 19.0	11
31			3.1	1,030 ± 193	37.7 ± 8.72	27

[a] K_i values determined by competitive inhibition of [^{3}H]*N*-methylspiperone binding in membranes harvested from HEK 293 cells stably expressing hD_2R or hD_3R.

correlates with observed partial agonist actions in vitro.[34] However, when the 2,3-diCl substituent was replaced with a 2-OMe, there were no polar contacts with TM5, which corresponds to the much weaker partial agonist effects observed with the 2-OMe-phenylpiperazine-substituted ligands.[34] In view of these data, we explored the effects of incorporating a hybrid 2-OMe,3-Cl-phenylpiperazine substituent as the primary pharmacophore. This 2-OMe,3-Cl- substitution pattern is present in the nonselective high affinity D_2R/D_3R receptor antagonist/inverse agonist, eticlopride. Thus, we hypothesized that this substitution would lead to an increase in affinity at D_3R compared to the 2,3-diCl-phenylpiperazine.

We also replaced the substituted phenyl ring on the piperazine with a naphthalene moiety, a bioisosteric replacement for the 2,3-diCl-phenyl ring, and predicted that this substituent might also result in compounds with high-affinity for D_3R. The naphthylpiperazine moiety is a well-described pharmacophore for serotonin receptors and specifically for 5-HT_{1A} agonists, potentially reducing D_3R selectivity of these analogues.[48,52] Notably, buspirone, a 5-HT_{1A} partial agonist that also antagonizes D_3R,[53] was recently reported to reduce cocaine and nicotine self-administration (alone or in combination) in nonhuman primates,[53−55] Although a recent clinical trial testing buspirone for cocaine dependence treatment proved unsuccessful,[56] developing novel D_3R antagonists/5-HT_{1A} partial agonists might also be an interesting combination for compounds with therapeutic potential.

To evaluate the binding affinities of this series of compounds, we performed competition binding studies with [^{3}H]*N*-methylspiperone using membranes prepared from HEK293 cells expressing either the human D_2R or D_3R (Tables 1−3.) In addition, cLogP values are provided as a relative measure of lipophilicity. Compounds **14**−**21** displayed moderate to high binding affinities for D_2R (K_i = 8−330 nM; Table 1). Moreover, all of these analogues, with the exception of **21** (K_i = 1.8 nM), displayed subnanomolar affinities at D_3R (K_i = 0.1−0.9 nM), demonstrating that the 2-OMe,3-Cl- and the 2,3-naphthyl-piperazines, as predicted, were well tolerated as the primary pharmacophores in this series. For example, the 2-OMe,3-Cl-phenylpiperazine (e.g., **14**; K_i = 0.39 nM) and the 2,3-naphthyl-piperazine (e.g., **16**; K_i = 0.12 nM) indolylamide analogues generally showed higher D_3R binding affinities compared to lead compound **6** (K_i = 1.9 nM), although neither was as D_3R selective. We also explored the bioisosteric

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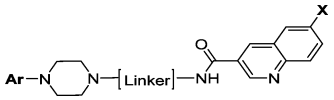
Table 3. Human Dopamine D_2-Like Receptor Binding Data in HEK Cells for Quinoline-3-carboxamide Ligands with Varying Arylpiperazines and 4-Carbon Linker Chains[a]



Compd	Ar	[Linker]	X	cLogP	D_2R	D_3R	D_2R/D_3R
					K_i (nM) ± SEM		
32			H	5.2	15.8 ± 4.13	0.351 ± 0.103	45
33			H	4.6	258 ± 8.72	3.63 ± 0.340	71
34			H	4.5	43.1 ± 7.07	1.99 ± 0.558	22
35			H	4.0	885 ± 136	31.6 ± 7.40	28
36			F	5.8	39.2 ± 4.70	0.570 ± 0.110	69
37			F	5.1	827 ± 212	8.05 ± 1.07	103

[a] K_i values determined by competitive inhibition of [^{3}H]*N*-methylspiperone binding in membranes harvested from HEK 293 cells stably expressing hD_2R or hD_3R.

replacement of the indole moiety (as the secondary pharmacophore) with benzofuran and benzothiophene moieties, substitutions that have previously demonstrated high D_3R affinities, in related series.[7,34,51,57,58]

As shown in Table 1, all these benzothienyl and benzofuranyl analogues displayed high D_3R affinities, with K_i values between 0.13 and 0.91 nM and good selectivity for D_3R over D_2R (61−80-fold). The presence of an electron-donating OMe group on the indole ring, in compound **17**, was well-tolerated and resulted in high affinity at D_3R and 80-fold selectivity for D_3R over D_2R. The presence of an electron-withdrawing F group on the indole ring in compound **20**[50] also resulted in high D_3R affinity (K_i = 0.43 nM), and D_3R selectivity was improved with the addition of a 3-OH in the linking chain (**21**). A small reduction in selectivity for D_3R over D_2R in comparison with **6** was observed with compound **21** due to a ∼2-fold improvement in D_2R affinity.

In summary, for the ligands reported in Table 1, compounds without the 3-OH substituent in the 4-carbon linker chain possessed higher cLogP values (i.e., were more lipophilic/hydrophobic) and displayed higher affinities at both D_2R and D_3R but lower selectivity for D_3R over D2R (e.g., compound **20** vs **21** in Table 1). Bioisosteric replacement of the indole moiety with other [5,6]-heteroaromatic moieties, such as benzofuran and benzothiophene, did not significantly influence D_3R binding affinity or selectivity.

To improve chemical and metabolic stability, we opted to further explore other heteroaromatic groups such as quinoline that does not have an acidic hydrogen or hydrogen-bond donor group like the N−H group of the indole. To this end, compounds **22**[59]**−31** were prepared that contained the 2-OMe-phenylpiperazine moiety along with either electron withdrawing or donating substituents on the quinoline moiety. We explored whether the position of the quinoline nitrogen (N), that is 2-quinolinyl (compounds **22−25**), 3-quinolinyl (compounds **26−30**), and 7-quinolinyl (compound **31**) positions, had any effect on binding affinity and/or selectivity. We also compared quinolinylamide analogues with or without a 3-OH substituent in the 4-carbon linker between the arylamide terminus and the 4-phenylpiperazine moiety.

Binding affinities for the 2-OMe-phenylpiperazine quinolinylamide analogues **22−31** are reported in Table 2. Compared to the indolylamide, benzofuran, and benzothiophene analogues (Table 1), the quinoline analogues generally displayed lower affinities at D_2R and D_3R as well as decreased selectivity for the D_3R over D_2R. Moreover, quinolinylamide analogues with a 3-OH substituent in the 4-carbon linker chain displayed reduced D_3R binding affinities and in contrast to previous reports, reduced D_3R-selectivity over D_2R as compared to the unsubstituted analogues.[26,49] Specifically, compounds **22**, **24**, and **27**, without a 3-OH substituent in the linker chain, displayed low nanomolar affinities at D_3R (K_i = 3.5, 2.5, and 2.1 nM, respectively) and showed equal or higher selectivity for D_3R compared to their corresponding 3-OH substituted analogues **23**, **25**, and **28** (K_i = 97.9, 53.1 and 44.8 nM, respectively). The position of the quinolinyl N did not appear to strongly influence binding affinity and/or selectivity at D_3R, although there appeared to be a slight preference for 3- and 7-quinolinyl positions over the 2-quinolinyl position (e.g., compounds **26** and **31** compared to **23**). While affinities at D_2R remained similarly low for compounds **23**, **26**, and **31**, there was a 3-fold decrease in affinity at D_3R and selectivity for D_3R over D_2R for compound **23** compared to compounds **26** and **31**. Hence, D_3R affinity and selectivity for the 2-, 3-, and 7-quinolinyl analogues follow the order 2- < 3- = 7-quinoline. Of note, within the 3-quinoline series (compounds **26−30**), the presence of an electron-withdrawing or donating substituent on the quinoline moiety did not substantially affect affinity or

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Table 4. Functional Data for Selected Compounds Using Stimulation or Inhibition of Quinpirole-Stimulated Mitogenesis in CHO Cells with Human Dopamine D_2R or D_3R[a]

	D_2R mitogenesis assay			D_3R mitogenesis assay		
compd	agonist EC_{50} ± SEM (nM)	% stimulation	antagonist IC_{50} ± SEM (nM)	agonist EC_{50} ± SEM (nM)	% stimulation	antagonist IC_{50} ± SEM (nM)
6	170 ± 61[b]	22[b]	4400 ± 60	>10000	ND[c]	30 ± 5.8[b]
16	76 ± 14	4.5	87 ± 20	140 ± 51	25	8.0 ± 1.9
20	>10000	ND[c]	564.40 ± 109	>10000[d]	ND[c]	52 ± 1.2[d]
26	ND[c]	ND[c]	ND[c]	0.95 ± 0.39	11	47 ± 11
32	170 ± 52	18	29 ± 8.7	20 ± 4.6	31	7.4 ± 2.5
33	200 ± 28	8.2	720 ± 190	13 ± 1.9	36	72 ± 12
34	>10000	<7	63 ± 13	16 ± 6.4	29	59 ± 24

[a]Data were obtained through the NIDA Addiction Treatment Discovery Program contract (ADA151001) with Oregon Health & Science University. [b]Previously published data.[26] [c]ND = not determined. Functional assays were not conducted if the K_i value for the binding assay was >500 nM. [d]Previously published data.[50]

Table 5. Additional in Vitro Binding and Functional Data for Selected Compounds at 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} Receptors[a]

				5-HT_{1A} [^{35}S]GTPγS binding	
compd	5-HT_{1A} [^{3}H]-8-OH-DPAT K_i ± SEM (nM)	5-HT_{2A} [^{125}I]DOI K_i ± SEM (nM)	5-HT_{2C} [^{125}I]DOI K_i ± SEM (nM)	agonist EC_{50} ± SEM (nM)	% stimulation
6	100 ± 24[b]	48 ± 13	86 ± 0.61	ND[c]	ND[c]
16	84 ± 7.1	55 ± 12	73 ± 16	250 ± 67	108
20	540 ± 48	>10000	990 ± 310	ND[c]	ND[c]
26	68 ± 9.8	350 ± 50	1,800 ± 130	340 ± 91	94
32	2.5 ± 0.35	0.33 ± 0.12	3.8 ± 1.4	9.0 ± 3.0	108
33	9.0 ± 3.2	2.4 ± 0.77	44 ± 5.0	17 ± 5.7	104
34	21 ± 5.4	1.7 ± 0.60	14 ± 2.6	43 ± 11	106

[a]Data were obtained through the NIDA Addiction Treatment Discovery Program contract (ADA151001) with Oregon Health & Science University. [b]Previously published data.[26] [c]ND = Not determined. Functional assays were not conducted if the K_i value for the binding assay was >250 nM.

selectivity at D_3R, although it appeared that electron-withdrawing substituents (e.g., F) induced less pronounced loss of affinity compared to electron-donating substituents (e.g., OMe). In general, with the 2-OMe-phenylpiperazine moiety, the quinolinylamide analogues were less potent and selective for D_3R over D_2R. On the basis of relative SAR within the quinoline−carboxamide analogues, we decided to explore the 3-quinoline moiety as a bioisosteric replacement for indole in the arylamide terminus in combination with the 2-OMe,3-Cl-phenylpiperazine, or 2,3-naphthyl-piperazine. In addition, we prepared the 2,3-diCl analogues for comparison.

The D_2R and D_3R binding affinities of ligands that incorporate the quinoline-3-carboxamide moiety with either 2-OMe,3-Cl-phenylpiperazine, 2,3-naphthyl-piperazine, or 2,3-diCl-phenylpiperazine (compounds **32**−**37**) are shown in Table 3. Comparison of these compounds and the quinoline-3-carboxamide analogues in Table 2 revealed improvement in binding affinities at the D_3R. While the 2-OMe,3-Cl-phenylpiperazine analogue **35** (K_i = 31.6 nM; D_2R/D_3R ratio = 28) is essentially equipotent at D_2R and D_3R as compound **26** (Table 2; K_i = 33.8 nM; D_2R/D_3R ratio = 27), the equivalent 2,3-naphthyl-piperazine analogue, **33**, displayed ∼10-fold increase in affinity (K_i = 3.63 nM) and ∼2-fold more D_3R selective (D_2R/D_3R ratio = 71) for D_3R over D_2R than **26**. Compound **32** had the highest D_3R affinity (K_i = 0.35 nM) of the 2,3-naphpthyl or 2-OMe,3-Cl-analogues. Moreover, the 2,3-diCl-phenylpiperazine analogues with a 6-F-quinoline (D_2R/D_3R ratio = 69 and 103 for **36** and **37**, respectively) were slightly more selective than the unsubstituted quinolines in this series. These data suggest that among the quinoline-3-carboxamides, both the 2,3-diCl-phenylpiperazine and the 2,3-naphthyl-piperazine are good replacements for the 2-OMe-phenyl-piperazine moiety. Addition of the 3-OH group in the linker in the 6-F-quinoline compound, **37**, showed somewhat lower affinity and selectivity as compared to its 5-F-indole analogue, **21**. Among the quinoline-3-carboxamide analogues, compound **32** with the 2,3-naphthyl-piperazine and **36** with 2,3-diCl-phenylpiperazine showed the highest D_3R affinity and their 3-OH-analogues, **33** and **37**, respectively, displayed highest D_3R-selectivity.

Functional Data and Serotonergic Actions. Six analogues were selected to compare with the parent compound **6** for their ability to antagonize quinpirole-induced activation of hD_2R or hD_3R in a cell-based mitogenesis assay (Table 4.) All of the analogues were moderately potent antagonists in the D_3R mitogenesis assay (IC_{50} = 7.4−72 nM), with compounds **16** and **32** being the most potent. Of note these compounds, as well as **26**, **33**, and **34**, showed weak partial agonist profiles at higher concentrations. In general, functional potencies of these analogues were lower than their binding affinities. Most of the analogues were more potent at D_3R than at D_2R in this assay, although selectivities varied.

These analogues were also tested for binding at 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors as well as for functional activity at 5-HT_{1A} (Table 5). Although all the analogues had affinity toward these 5-HT receptor subtypes, compounds **32**−**34**

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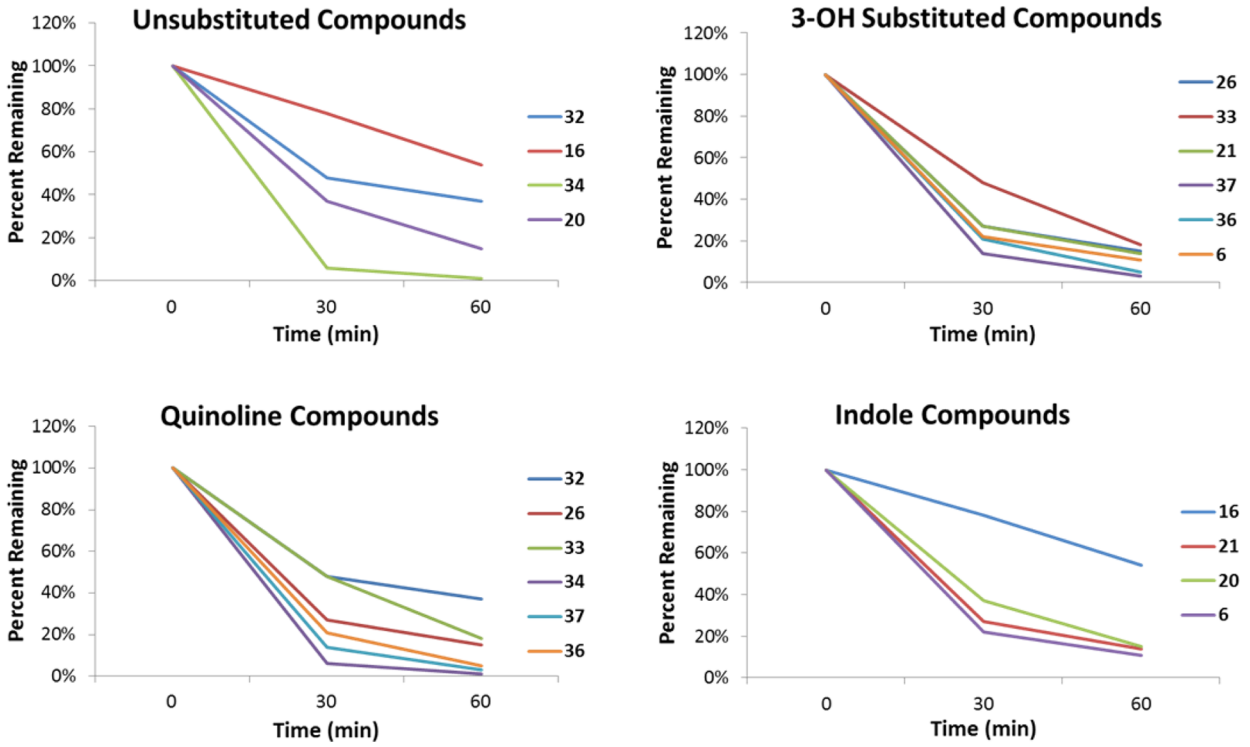

Figure 1. (a) Phase I metabolism data for all compounds with an unsubstituted linker chain. (b) Phase I metabolism data for all compounds with a 3-OH substituted linker chain. (c) Phase I metabolism data for all quinoline compounds. (d) Phase I metabolism data for all indole compounds.

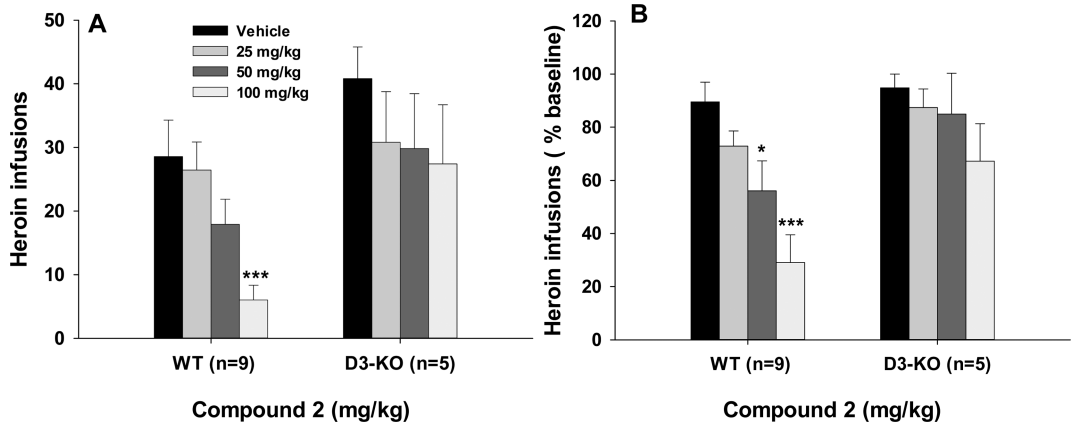

Figure 2. Effects of compound **2** on heroin self-administration in WT and D_3KO mice. All the data are expressed as mean ± SEM. (A) Total numbers of heroin infusions after injection of each dose of compound **2**. (B) Normalized data showing drug-induced % change in heroin self-administration over new basal levels immediately before each test day. One-way ANOVA with repeated measures over drug dose revealed a significant drug treatment main effect in WT mice ((A) $F_{3,24} = 10.93$, $p < 0.001$; (B) $F_{3,24} = 13.06$, $p < 0.001$) but not in D_3KO mice ((A) $F_{3,12} = 2.21$, $p > 0.05$; (B) $F_{3,12} = 1.46$, $p > 0.05$). *$p < 0.05$, ***$p < 0.001$, compared to vehicle.

exhibited the highest affinities, especially at 5-HT_{1A} and 5-HT_{2A}. All these analogues were full agonists at 5-HT_{1A} receptors, which was not surprising based on previously reported SAR for 5-HT_{1A}.[48,52] However, with the exception of **32**, all analogues were still significantly D_3R-selective. Interestingly, **32** showed relatively high affinity for all the 5-HT receptor subtypes tested, providing a unique profile among this series.

Microsomal Metabolism Results. Metabolic stability assays were conducted in mouse liver microsomes to predict in vivo stability after oral administration. The analogues were tested for phase I metabolism in mouse liver microsomal incubations in the presence of NADPH and were compared to parent compound **6** following procedures previously described.[60] Compound stability over a 1 h incubation is presented in Figure 1. In general, the compounds with the unsubstituted linker, regardless of the primary or secondary pharmacophores, were more metabolically stable (see Table S3 in Supporting Information). Of the quinoline analogues, **32** was the most stable, with calculated in vitro rate constant (k) of −0.017 and in vitro half-life ($t_{1/2}$) of 41.8 min, whereas **16** with the k value of −0.010 and in vitro $t_{1/2}$ of 67.5 min was the most stable of the indoles and significantly more stable than the parent compound, **6**, with k of −0.037 and $t_{1/2}$ of 18.8 min.

Heroin Self-Administration Studies in WT and D_3KO Mice. On the basis of their pharmacological profile and

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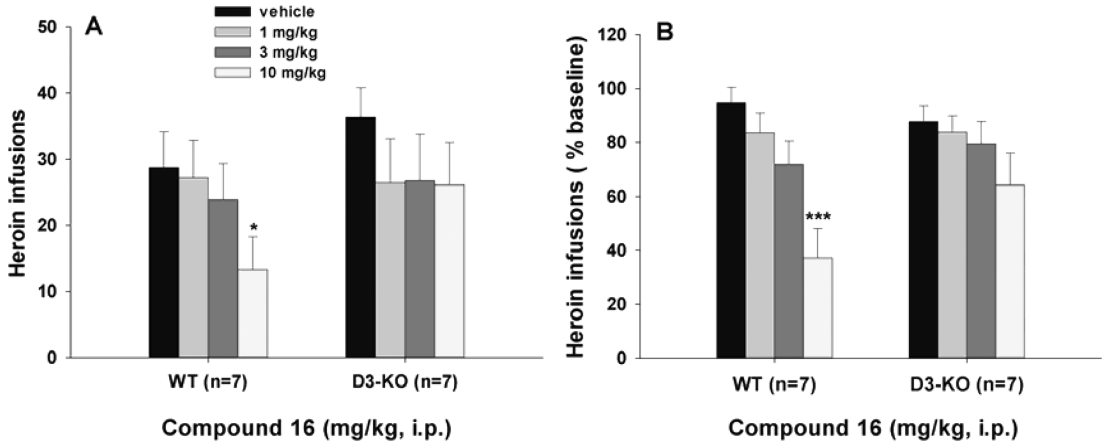

Figure 3. Effects of compound **16** on heroin self-administration in WT and D_3R-KO mice. All the data are expressed as mean ± SEM. (A) Total numbers of heroin infusions after injection of each dose of compound **16**. (B) Normalized data showing drug-induced % change in heroin self-administration over new baseline immediately before each test day. One-way ANOVA with repeated measures over drug dose revealed a statistically significant drug treatment main effect in WT mice ((A) $F_{3,18} = 2.13$, $p > 0.05$; (B) $F_{3,18} = 9.09$, $p < 0.001$) but not in D_3KO mice ((A) $F_{3,18} = 0.63$, $p > 0.05$; (B) D_3KO, $F_{3,18} = 1.78$, $p > 0.05$). We note that one-way ANOVA did not reveal a significant treatment main effect for the data shown in (A) WT mice. However, the direct two group comparison between the vehicle and 10 mg/kg **16** groups revealed a statistically significant reduction ((A) WT, paired t test, $q = 5.07$, $p < 0.05$). These may be related to the relatively smaller group size and/or the relatively variable basal levels of heroin self-administration in different subjects. Therefore, the renormalized data (% change over baseline) are provided ((B) in this figure; the same as in Figures 2B and 4B) in this study. *$p < 0.05$, ***$p < 0.001$, compared to vehicle.

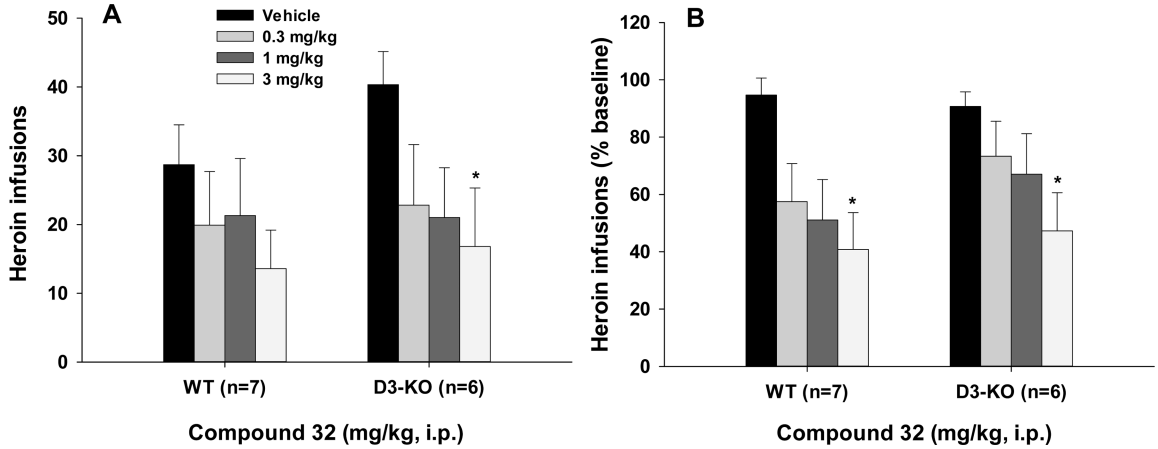

Figure 4. Effects of compound **32** on heroin self-administration in WT and D_3KO mice. All the data are expressed as mean ± SEM. (A) Total numbers of heroin infusions after injection of each dose of compound **32**. (B) Normalized data showing drug-induced % change in heroin self-administration over new baseline immediately before each test day. One-way ANOVA with repeated measures over drug dose revealed a statistically significant drug treatment main effect in WT mice ((A) $F_{3,18} = 0.83$, $p > 0.05$; (B) $F_{3,18} = 3.34$, $p < 0.05$) and D_3KO mice ((A) $F_{3,15} = 3.63$, $p < 0.05$; (B) $F_{3,15} = 4.39$, $p < 0.05$) *$p < 0.05$, compared to vehicle.

microsomal stability, compounds **16** and **32** were chosen for behavioral studies in WT and D_3KO mice. Of note, compound **16** was D_3R selective over the 5-HT receptor subtypes in contrast to compound **32**. Moreover, these two analogues were the most metabolically stable in this series. Mice were trained on an FR1 schedule of reinforcement, where every lever press results in an injection, initially with 0.1 mg/kg (iv) heroin, followed by 0.05 mg/kg and finally 0.025 mg/kg, to obtain more robust lever pressing behavior via a compensatory response to a reduced reinforcer (e.g., a lower dose of heroin). Both WT and D_3KO mice acquired heroin self-administration after 1−2 weeks of training. Overall, D_3KO mice display higher total number of heroin infusions taken over 3 h than WT mice although the difference was not statistically significant (Figures 2A, 3A, 4A). After stable heroin self-administration was achieved, we evaluated the effects of the classic D_3R antagonist **2**[22−24] in both WT and D_3KO mice. Figure 2A shows that pretreatment with **2** (25, 50, 100 mg/kg, i.p., 30 min prior to test) dose-dependently decreased the total number of infusions of heroin self-administered by the WT mice, with 100 mg/kg (ip) as the most effective dose.

Given the observation that the basal levels of heroin self-administration are variable in individual subjects, we further normalized drug-induced percent changes in self-administration over new basal levels immediately before each test day in each animal (Figure 2B) to more accurately reveal drug effects. We found that pretreatment with **2** produced a dose-dependent reduction in heroin self-administration in WT mice (Figure 2B). Of note, although the D_3KO mice self-administered heroin similarly to the WT mice, this behavior was not attenuated by **2**, suggesting that this D_3R antagonist reduces heroin self-administration through a D_3R-mediated mechanism. Likewise,

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in Figure 3, compound **16**, at the dose range of 1−10 mg/kg, also dose dependently attenuated heroin self-administration in the WT but not in the D_3KO mice. Of note, the effective dose of **16** was 5−10-fold lower than that of **2**, suggesting that **16** is more potent than **2** in this model. In our binding assays, compound **16** displayed ∼70-fold higher affinity for D_3R than **2** (K_i = 0.12 and 13.9 nM, respectively), which is consistent with its higher potency in vivo.

Compound **32** also dose dependently attenuated the rewarding effects of heroin, with 3 mg/kg being the most effective dose in both WT and D_3KO mice (Figure 4), reflecting the lack of D_3R selectivity of this compound. It is enticing to speculate that the 5-HT_{1A}, 5-HT_{2A}, and/or 5-HT_{2C} receptors may be playing a role in the behavioral effects of this drug as these serotonergic receptor subtypes have all been implicated in both drug reward and impulsivity.[61−64] However, additional experiments must be conducted in order to confirm this. Nevertheless, compound **32** effectively blocked self-administration of heroin more potently than either **2** or **16**, thus follow-up studies are underway.

CONCLUSION

In summary, we have synthesized a novel series of analogues of **6** wherein the 2,3-diCl-phenyl piperazine was replaced with a 2-OMe, a 2-OMe,3-Cl-, or a 2,3-naphthyl-substituent and a bioisosteric replacement of the indole moiety. In addition, we also explored analogues with either the unsubstituted or the 3-OH substituted 4-carbon linker between the arylpiperazine and aryl amide to investigate its effects on D_3R affinity, selectivity, efficacy, and metabolic stability. By varying the arylpiperazine substitution, we found several ligands that displayed high affinity and selectivity profiles at the D_3R versus D_2R. Overall, we identified indolylamide analogues, e.g., **16** (K_i = 0.12 nM), which displayed higher D_3R binding affinity compared to lead compound **6** (K_i = 1.9 nM) and >100-fold selectivity for D_3R over D_2R. Although in general, the 2-OMe-phenylpiperazine-quinolinylamides had lower affinities at D_3R, when substituted with the 2-OMe,3-Cl or the 2,3-naphthyl substituents, D_3R affinity was increased ∼10-fold. Also the 3-OH substituted analogues showed an increase in selectivity for D_3R over D_2R, as well as a decrease in lipophilicity, as described for other 4-phenylpiperazine-butylarylamides.[26,49]

On the basis of the D_2R/D_3R binding profiles, a subset of analogues was evaluated in a cell-based D_2R or D_3R functional assay. These analogues were potent antagonists in the D_3R mitogenesis assay (e.g., **16** and **32**; IC_{50} = 8.0 and 7.4 nM, respectively) but at higher concentrations demonstrated weak partial agonist profiles. They were also evaluated for activities at 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors. Among the selected ligands, in addition to its high binding affinity to D_3R (K_i = 0.35 nM), **32** displayed high affinity for the 5-HT receptor subtypes (K_i = 2.46 nM for 5-HT_{1A}, 0.33 nM for 5-HT_{2A}, 3.80 nM for 5-HT_{2C}) and was a potent 5-HT_{1A} agonist.

We then selected compounds based on their in vitro pharmacology profiles and evaluated them for metabolic stability. Analogues with or without the 3-OH substitution were compared; **16** and **32** were discovered to be the most metabolically stable in the series − 37% and 54%, respectively, remained after 60 min compared to the parent compound **6** (11% remaining after 60 min) in mouse microsomes. Of note, all the compounds with the 3-OH substituent were metabolically less stable.

D_3R antagonists have largely been developed as potential therapeutic agents for the treatment of nicotine or psychostimulant abuse. However, they are typically unable to block self-administration under a low FR schedule of reinforcement and are primarily effective in models of relapse.[2,4,33] We hypothesized that D_3R antagonists might be able to block self-administration of addictive drugs, like heroin, that more modestly stimulate dopamine release in the accumbens. Indeed, behavioral studies in WT and D_3KO mice that were trained to self-administer heroin demonstrated that D_3R-selective antagonists **2** and **16** significantly decreased drug self-administration in WT but not D_3KO mice, suggesting an effect that is mediated through D_3R. In addition, compound **16** was 5−10 times more potent than **2**, a classic D_3R antagonist, in its pharmacological effect in vivo. Compound **32** also attenuated heroin self-administration in the WT mice but appeared to be less selective for D_3R than either compounds **2** or **16**, as at the most effective dose of 3 mg/kg, self-administration behavior was reduced in both the WT and D_3KO mice. Its binding profile suggests that actions at the 5-HT receptor subtypes might also play a role in the behavioral actions of this compound. Additional behavioral assessment is naturally required, however, these are the first studies to show the effectiveness of D_3R antagonists in heroin self-administration and suggest an alternate medication strategy to methadone maintenance or buprenorphine, the current pharmacotherapeutic treatments for heroin addiction.

EXPERIMENTAL METHODS

Synthesis. 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, OChem Incorporation, Acros Organics, Maybridge, and Alfa Aesar. All amine final products were converted into either the oxalate or HCl salt. Spectroscopic data and yields refer to the free base form of compounds. Teledyne ISCO CombiFlash Rf or glass flash column chromatography were performed using silica gel (EMD Chemicals, Inc.; 230−400 mesh, 60 Å). ^{1}H and ^{13}C NMR spectra were acquired using a Varian Mercury Plus 400 spectrometer at 400 and 100 MHz, respectively. Chemical shifts are reported in parts-per-million (ppm) and referenced according to deuterated solvent for ^{1}H spectra ($CDCl_3$, 7.26, CD_3OD, 3.31, or DMSO-d_6, 2.50) and ^{13}C spectra ($CDCl_3$, 77.2, CD_3OD, 49.0, or 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 × 0.25 mm i.d. × 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 °C, respectively, and the oven temperature gradient used was as follows: the initial temperature (100 °C) was held for 3 min and then increased to 295 °C at 15 °C/min over 13 min and finally maintained at 295 °C for 10 min. Combustion analysis was performed by Atlantic Microlab, Inc. (Norcross, GA), and the results agree within ±0.4% of calculated values. cLogP values were calculated using ChemBioDraw Ultra 4.0. 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.

*1-(3-Chloro-2-methoxyphenyl)piperazine (**7a**).*[47] K_2CO_3 (3.31 g, 23.98 mmol) was added to a solution of 3-chloro-2-methoxyaniline (3.15 g, 19.98 mmol) and bis(2-chloroethyl)amine HCl (4.30 g, 23.98 mmol) in diglyme (20 mL). The resulting mixture was stirred at reflux

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for 48 h. The reaction mixture was cooled to room temperature, and diglyme was removed under vacuum. The crude mixture was diluted with water (100 mL) and EtOAc (150 mL) and then extracted with EtOAc (3 × 100 mL). The combined organic layer was dried over $MgSO_4$, filtered, and concentrated. The crude compound was purified by flash column chromatography (90% CMA) to give the pure compound **7a** (2.53 g, 56% yield). ^{1}H NMR ($CDCl_3$) δ 7.01 (dd, J = 8.0, 2.0 Hz, 1H), 6.95 (t, J = 8.0 Hz, 1H), 6.81 (dd, J = 8.0, 2.0 Hz, 1H), 3.88 (s, 3H), 3.10−3.03 (m, 8H), 1.89 (br s, 1H). ^{13}C NMR ($CDCl_3$) δ 148.9, 147.2, 128.9, 124.8, 123.5, 117.3, 59.2, 51.7, 46.6. GC-MS (EI) m/z 226 (M^+).

*1-(Naphthalen-1-yl)piperazine (**7b**).*[48] Compound **7b** was synthesized as described for **7a**, using naphthalen-1-amine (4.00 g, 27.94 mmol), bis(2-chloroethyl)amine HCl (5.98 g, 33.52 mmol), and K_2CO_3 (4.63 g, 33.52 mmol) in diglyme (25 mL). The product **7b** (3.40 g, 57% yield) was converted to the HCl salt in 2-propanol/acetone to form a gray solid; mp >250 °C (HCl salt). ^{1}H NMR ($CDCl_3$) δ 8.24−8.21 (m, 1H), 7.84−7.81 (m, 1H), 7.55 (d, J = 8.4 Hz, 1H), 7.51−7.44 (m, 2H), 7.41 (t, J = 8.4 Hz, 1H), 7.09 (dd, J = 7.6, 1.2 Hz, 1H), 3.16 (t, J = 4.8 Hz, 4H), 3.09 (br s, 4H), 1.99 (br s, 1H). ^{13}C NMR ($CDCl_3$) δ 149.75, 134.4, 128.6, 128.0, 125.5, 125.4, 124.9, 123.2, 123.1, 114.3, 54.1, 46.3. GC-MS (EI) m/z 212 (M^+).

*2-(2-(Oxiran-2-yl)ethyl)isoindoline-1,3-dione (**8**).* Phthalimide potassium salt (6.47 g, 34.9 mmol) was suspended in DMF (60 mL) under an argon atmosphere and stirred at room temperature for 10 min. A solution of 2-(2-bromoethyl)oxirane) (5.82 g, 38.50 mmol) in DMF (15 mL) was added dropwise, and the reaction mixture was left to stir at room temperature for 20 h. The white precipitate formed was filtered and the filtrate diluted with EtOAc (175 mL). The white precipitate collected was then dissolved in water and extracted with EtOAc (3 × 75 mL), and the extract was combined with the original diluted filtrate. The combined EtOAc extracts were consecutively washed with water (100 mL) and brine (100 mL), dried over Na_2SO_4, and filtered. The pure product, **8** (7.48 g, 98% yield), was obtained after solvent removal as an off-white solid; mp 84−86 °C. ^{1}H NMR ($CDCl_3$) δ 7.87−7.85 (m, 2H), 7.73−7.71 (m, 2H), 3.96−3.83 (m, 2H), 3.02−2.98 (m, 1H), 2.72 (t, J = 5.0 Hz, 1H), 2.45 (dd, J = 4.8, 2.4 Hz, 1H), 2.04−1.96 (m, 1H), 1.90−1.83 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 168.3, 133.9, 132.1, 123.3, 50.2, 46.4, 35.2, 31.6.

*2-(3-Hydroxy-4-(4-(3-chloro-2-methoxyphenyl)piperazin-1-yl)-butyl)isoindoline-1,3-dione (**9a**).* To a solution of **7a** (0.40 g, 1.77 mmol) in 2-propanol (7 mL) was added a solution of compound **8** (0.39 g, 1.77 mmol) in 2-propanol (15 mL). The reaction mixture was then stirred at reflux at 82 °C overnight under an argon atmosphere. After cooling to room temperature, the solvent was removed in vacuo, and the crude product was purified by flash column chromatography (50% EtOAC:hexanes) to give **9a** (450 mg, 57% yield). ^{1}H NMR ($CDCl_3$) δ 7.85−7.82 (m, 2H), 7.73−7.68 (m, 2H), 7.01−6.91 (m, 2H), 6.79−6.76 (dd, J = 8.4, 1.6 Hz, 1H), 3.95−3.84 (m, 2H), 3.83 (s, 3H), 3.81−3.74 (m, 1H), 3.10 (br s, 4H), 2.78 (br, 2H), 2.55 (br, 2H), 2.44−2.34 (m, 2H), 1.79 (q, J = 6.4, 2H). ^{13}C NMR ($CDCl_3$) δ 168.3, 148.7, 146.5, 133.9, 132.2, 128.7, 124.6, 123.3, 123.2, 117.1, 64.5, 63.9, 59.0, 53.7, 50.3, 35.1, 33.6.

*N-(3-Hydroxy-4-(4-(naphthalen-1-yl)piperazin-1-yl)butyl)-isoindoline-1,3-dione (**9b**).* Compound **9b** was synthesized as described for **9a** using **7b** (0.29 g, 1.38 mmol) and **8** (0.30 g, 1.38 mmol) in 2-propanol (8 mL). The pure product **9b** (400 mg, 68% yield) was isolated after purification by flash column chromatography (50% EtOAC:hexanes). ^{1}H NMR ($CDCl_3$) δ 8.18−8.15 (m, 1H), 7.86−7.84 (m, 2H), 7.83−7.80 (m, 1H), 7.73−7.69 (m, 2H), 7.55−7.53 (d, J = 8.0 Hz, 1H), 7.48−7.43 (m, 2H), 7.39 (t, J = 8.0 Hz, 1H), 7.07−7.05 (dd, J = 7.2, 1.2 Hz, 1H), 3.96−3.87 (m, 2H), 3.85−3.80 (m, 1H), 3.12 (br s, 4H), 2.92 (br s, 2H), 2.69 (br s, 2H), 2.53−2.41 (m, 2H), 1.82 (q, J = 6.4, 2H). ^{13}C NMR ($CDCl_3$) δ 168.5, 149.5, 134.7, 133.9, 132.2, 128.8, 128.4, 125.8, 125.8, 125.3, 123.54, 123.50, 123.2, 114.6, 64.6, 63.9, 53.0, 35.1, 33.7.

*2-(3-Hydroxy-4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-isoindoline-1,3-dione (**9c**).*[49] A solution of **8** (2.09 g, 9.62 mmol) in 2-propanol (5 mL) was added to a solution of 1-(2-methoxyphenyl)-piperazine (1.85 g, 9.62 mmol) in 2-propanol (40 mL). After refluxing the reaction mixture under an argon atmosphere overnight, the solvent was reduced in vacuo to give a clear, dark-orange solution. Ice-cold 2-propanol (30 mL) was added to the orange solution at room temperature, and the mixture was left to stir overnight at room temperature, resulting in precipitation of the desired product. Compound **9c** (2.87 g, 73% yield) was recovered after filtration as an off-white solid and washed with 2-propanol; mp 108−109 °C. ^{1}H NMR ($CDCl_3$) δ 7.86−7.84 (m, 2H), 7.72−7.70 (m, 2H), 7.02−6.98 (m, 1H), 6.93−6.91 (m, 2H), 6.86 (d, J = 8.0 Hz, 1H), 3.94−3.83 (m, 2H), 3.85 (s, 3H), 3.80−3.76 (m, 1H), 3.57 (br s, 1H), 3.07 (br s, 4H), 2.86−2.82 (m, 2H), 2.61−2.58 (m, 2H), 2.45−2.35 (m, 2H), 1.79 (q, J = 7.2, 2H). ^{13}C NMR ($CDCl_3$) δ 168.5, 152.2, 141.2, 133.9, 132.2, 123.2, 122.99, 120.99, 118.2, 111.2, 64.5, 63.9, 55.4, 53.4, 50.7, 35.2, 33.6.

*2-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)-3-hydroxybutyl)-isoindoline-1,3-dione (**9d**).*[49] Compound **9d** was synthesized as described for **9c** using 1-(2,3-dichlorophenyl)piperazine (900 mg, 3.89 mmol) and **8** (850 mg, 3.91 mmol) in 2-propanol (20 mL). The pure product, **9d** (1.37 g, 78% yield), was recovered as a white solid; mp 127.5−129 °C. ^{1}H NMR ($CDCl_3$) δ 7.88−7.83 (m, 2H), 7.74−7.69 (m, 2H), 7.17−7.12 (m, 2H), 6.94 (dd, J = 6.8, 2.8 Hz, 1H), 3.96−3.83 (m, 2H), 3.81−3.75 (m, 1H), 3.52 (br s, 1H), 3.04 (br s, 4H), 2.84−2.81 (m, 2H), 2.60−2.59 (m, 2H), 2.47−2.36 (m, 2H), 1.79 (q, J = 13.4 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 168.5, 151.1, 134.1, 133.9, 132.2, 127.5, 127.4, 124.7, 123.2, 118.6, 64.5, 63.8, 51.4, 35.1, 33.6.

*4-Amino-1-(4-(3-chloro-2-methoxyphenyl)piperazin-1-yl)butan-2-ol (**10a**).* Anhydrous hydrazine (0.16 mL, 5.07 mmol) was added to a suspension of **9a** (0.45 g, 1.01 mmol) in anhydrous ethanol (20 mL), and the mixture was stirred at reflux for 3 h under an argon atmosphere. Solvents were removed, and the recovered residue was partitioned between chloroform (50 mL) and a 20% aqueous K_2CO_3 (50 mL) solution. The layers were separated, and the organic layer was washed with water (50 mL) and brine (50 mL). The combined organic layer was dried over $MgSO_4$, filtered, and the solvent removed in vacuo to give **10a** (270 mg, 85% yield), which was used without further purification. ^{1}H NMR ($CDCl_3$) δ 7.01−6.92 (m, 2H), 6.80−6.77 (dd, J = 8, 2 Hz, 2H), 3.90−3.87 (m, 1H), 3.85 (s, 3H), 3.84−3.81 (m, 1H), 3.12 (br s, 5H), 3.07−2.93 (m, 2H), 2.78 (br m, 2H), 2.59 (br, 2H), 2.41−2.36 (m, 2H), 1.62−1.57 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 148.6, 146.6, 128.7, 124.6, 123.3, 117.1, 66.3, 64.5, 59.0, 58.9, 53.8, 50.3, 39.5, 37.2. GC-MS (EI) m/z 313 (M^+).

*4-Amino-1-(4-(4-(naphthalen-1-yl)piperazin-1-yl)butan-2-ol (**10b**).* Compound **10b** was prepared as described for **10a** using **9b** (0.35 g, 0.82 mmol) and anhydrous hydrazine (0.13 mL, 4.08 mmol) in anhydrous ethanol (20 mL). The resulting pure product **10b** (190 mg, 78% yield) was used without further purification. ^{1}H NMR ($CDCl_3$) δ 8.18−8.15 (m, 1H), 7.81−7.78 (m, 1H), 7.53 (d, J = 8.4 Hz, 1H), 7.48−7.42 (m, 2H), 7.40−7.35 (m, 1H), 7.09−7.03 (m, 1H), 3.92 (m, 1H), 3.11 (br s, 8H), 3.02−2.93 (m, 2H), 2.92 (br s, 2H), 2.73−2.64 (m, 2H), 2.45−2.42 (m, 1H), 1.67−1.55 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 149.3, 134.7, 128.8, 128.8, 128.4, 125.8, 125.8, 125.3, 123.5, 114.6, 77.2, 66.2, 64.5, 53.0, 39.4, 36.9. GC-MS (EI) m/z 299 (M^+).

*4-Amino-1-(4-(2-methoxyphenyl)piperazin-1-yl)butan-2-ol (**10c**).*[49] Compound **10c** was prepared as described for **10a** using **9c** (2.87 g, 7.01 mmol) and anhydrous hydrazine (0.33 mL, 10.51 mmol) in ethanol (35 mL). The pure product, **10c** (quantitative), was recovered as a yellow oil that solidified over time. ^{1}H NMR ($CDCl_3$) δ 7.02−6.98 (m, 1H), 6.95−6.90 (m, 2H), 6.86 (d, J = 8.0 Hz, 1H), 3.92−3.86 (m, 4H), 3.10 (br s, 4H), 3.00−2.90 (m, 2H), 2.87−2.83 (m, 2H), 2.63−2.61 (br m, 2H), 2.44−2.38 (m, 2H), 2.12 (br s, 3H), 1.62−1.51 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 152.3, 141.3, 123.0, 121.0, 118.2, 111.2, 66.1, 64.5, 55.4, 53.6, 50.8, 39.6, 37.6.

*4-Amino-1-(4-(2,3-dichlorophenyl)piperazin-1-yl)butan-2-ol (**10d**).*[49] Compound **10d** was synthesized as described for **10a** using **9d** (1.36 g, 3.03 mmol) and anhydrous hydrazine (0.145 mL, 4.62 mmol) in ethanol (16 mL). The pure product, **10d** (740 mg, 77% yield), was recovered as a viscous light-yellow oil. ^{1}H NMR ($CDCl_3$) δ 7.17−7.12 (m, 2H), 6.95 (dd, J = 6.8, 3.2 Hz, 1H), 3.93−3.87 (m, 2H), 3.07 (br s, 4H), 3.01−2.88 (m, 2H), 2.85−2.82 (m, 2H), 2.63−

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2.62 (m, 2H), 2.46−2.39 (m, 2H), 2.17 (br s, 2H), 1.62−1.50 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 151.2, 134.0, 127.5, 127.4, 124.6, 118.6, 66.3, 64.5, 53.4, 51.4, 39.7, 37.5.

*2-(4-(4-(3-Chloro-2-methoxyphenyl)piperazin-1-yl)butyl)-isoindoline-1,3-dione (**12a**).* K_2CO_3 (2.21 g, 16.00 mmol) was added to a solution of **7a** (904 mg, 4.00 mmol) and **11** 2-(4-bromobutyl)-isoindoline-1,3-dione (2.26 g, 8.00 mmol) in acetone (25 mL). The resulting solution was stirred at reflux overnight. After cooling the reaction mixture to room temperature, K_2CO_3 was removed via filtration and the filtrate was concentrated. The crude product was purified by flash column chromatography (50−70% EtOAC:Hexanes) to give pure **12a** (1.40 g, 82% yield). ^{1}H NMR ($CDCl_3$) δ 7.84 (dd, J = 5.6, 3.2 Hz, 2H), 7.71 (dd, J = 5.6, 3.6 Hz, 2H), 7.0−6.91 (m, 2H), 6.79 (dd, J = 7.6, 1.2 Hz, 1H), 3.85 (s, 1H), 3.73 (t, J = 7.2 Hz, 2H), 3.11 (br s, 4H), 2.58 (br s, 4H), 2.42 (t, J = 7.6 Hz, 2H), 1.78−1.70 (m, 2H), 1.61−1.55 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 168.6, 148.8, 146.8, 134.0, 132.3, 128.8, 124.7, 123.4, 123.3, 123.3, 117.2, 59.1, 58.2, 53.8, 50.3, 37.9, 26.7, 24.3.

*2-(4-(4-(Naphthalen-1-yl)piperazin-1-yl)butyl)isoindoline-1,3-dione (**12b**).*[65] Compound **11b** (3.00 g, 77% yield) was synthesized as described for **12a** using **7b** (2.01 g, 9.48 mmol), 2-(4-bromobutyl)-isoindoline-1,3-dione (5.35 g, 18.96 mmol), and K_2CO_3 (5.24 g, 37.92 mmol) in acetone (50 mL). ^{1}H NMR ($CDCl_3$) δ 8.20−8.17 (m, 1H), 7.86−7.82 (m, 3H), 7.72−7.70 (m, 2H), 7.53 (d, J = 8.0 Hz, 1H), 7.47−7.44 (m, 2H), 7.39 (t, J = 7.6 Hz, 1H), 7.07 (dd, J = 7.2, 1.2 Hz, 1H), 3.75 (t, J = 7.6 Hz, 2H), 3.13 (br s, 4H), 2.73 (br s, 4H), 2.51 (t, J = 7.6 Hz, 2H), 1.81−1.73 (m, 2H), 1.66−1.58 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 168.6, 149.8, 134.9, 134.1, 134.0, 132.3, 128.5, 126.0, 125.9, 125.4, 123.6, 123.5, 123.3, 114.8, 58.3, 53.9, 53.1, 38.0, 26.81, 24.4.

*2-(4-(4-(2-Methoxyphenyl)piperazin-1-yl)butyl)isoindoline-1,3-dione (**12c**).*[65] Compound **12c** was synthesized as described for **12a** using K_2CO_3 (19.64 g, 142.10 mmol), 1-(2-methoxyphenyl)piperazine HCl (8.11 g, 35.46 mmol), and 2-(4-bromobutyl)isoindoline-1,3-dione (10.01 g, 35.48 mmol) in acetonitrile (110 mL). The crude product was purified by column chromatography (10−90% EtOAc:hexanes) to give pure **12c** (2.74 g, 20% yield) as a thick-yellow oil. ^{1}H NMR ($CDCl_3$) δ 7.86−7.82 (m, 2H), 7.31−7.69 (m, 2H), 7.01−6.97 (m, 1H), 6.95−6.88 (m, 2H), 6.85 (dd, J = 7.8, 1.4 Hz, 1H), 3.85 (s, 3H), 3.73 (t, J = 7.0 Hz, 2H), 3.08 (br s, 4H), 2.64 (br s, 4H), 2.44 (t, J = 7.6 Hz, 2H), 1.78−1.70 (m, 2H), 1.62−1.54 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 168.4, 152.3, 141.4, 133.9, 132.2, 123.2, 122.8, 120.9, 118.2, 111.1, 58.1, 55.3, 53.4, 50.6, 37.9, 26.7, 24.2.

*2-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)butyl)isoindoline-1,3-dione (**12d**)..*[50,66] Compound **12d** was synthesized as described for **12a** using K_2CO_3 (2.50 g, 18.09 mmol), 1-(2,3-dichlorophenyl)-piperazine (1.03 g, 4.45 mmol), and 2-(4-bromobutyl)isoindoline-1,3-dione (2.51 g, 8.89 mmol) in acetone (31 mL). The crude product was purified by column chromatography (20−80% EtOAc:hexanes gradient) to give **12d** (1.60 g, 83% yield) as a white solid; mp 121−123 °C. ^{1}H NMR ($CDCl_3$) δ 7.86−7.82 (m, 2H), 7.73−7.69 (m, 2H), 7.15−7.11 (m, 2H), 6.94 (dd, J = 6.0, 3.2 Hz, 1H), 3.73 (t, J = 7.0 Hz, 2H), 3.05 (br s, 4H), 2.62 (br s, 4H), 2.45 (t, J = 7.6 Hz, 2H), 1.80−1.71 (m, 2H), 1.61−1.54 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 168.6, 151.4, 134.1, 134.0, 132.3, 127.6, 127.5, 124.6, 123.3, 118.7, 58.1, 53.4, 51.4, 37.99, 26.7, 24.4.

*4-(4-(3-Chloro-2-methoxyphenyl)piperazin-1-yl)butan-1-amine (**13a**).*[47] Anhydrous hydrazine (0.28 mL, 9.00 mmol) was added to a solution of **12a** (1.28 g, 3.00 mmol) in ethanol (30 mL). The solution was stirred at reflux for 3 h. The reaction mixture was cooled and concentrated. The crude reaction mixture was partitioned between $CHCl_3$ (50 mL) and 20% K_2CO_3 (20 mL) solution, and the organic layer was collected and dried over $MgSO_4$. The organic layer was filtered and concentrated to give **13a** (855 mg, 96% yield), which was used without further purification. ^{1}H NMR ($CDCl_3$) δ 7.01−6.92 (m, 2H), 6.81 (dd, J = 8.0, 1.2 Hz, 1H), 3.86 (s, 3H), 3.14 (br s, 4H), 2.73 (t, J = 6.8 Hz, 2H), 2.61 (br s, 4H), 2.41 (t, J = 7.2 Hz, 2H), 1.60−1.46 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 148.7, 146.7, 128.8, 124.7, 123.3, 117.2, 59.0, 58.6, 53.8, 50.3, 42.0, 31.6, 24.3.

*4-(4-(Naphthalen-1-yl)piperazin-1-yl)butan-1-amine (**13b**).*[67] Compound **13b** (1.88 g, 87% yield) was synthesized as described for **13a**, using **12b** (3.20 g, 7.75 mmol) and anhydrous hydrazine (0.73 mL, 23.25 mmol) in ethanol (50 mL). ^{1}H NMR ($CDCl_3$) δ 8.21−8.19 (m, 1H), 7.83−7.81 (m, 1H), 7.54 (d, J = 8.4 Hz, 1H), 7.47−7.43 (m, 2H), 7.40 (t, J = 8.0 Hz, 1H), 7.09 (d, J = 7.6 Hz, 2H), 3.15 (br s, 4H), 2.75 (t, J = 6.8 Hz, 4H), 2.49 (t, J = 7.6 Hz, 2H), 1.65−1.48 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 149.8, 134.8, 128.9, 128.5, 125.9, 125.8, 125.4, 123.7, 123.5, 117.7, 58.7, 53.9, 53.1, 42.3, 31.9, 24.5. GC-MS (EI) m/z 283 (M^+).

*4-(4-(2-Methoxyphenyl)piperazin-1-yl)butan-1-amine (**13c**).*[65] Compound **13c** was synthesized as described for **13a** using **12c** (2.62 g, 6.66 mmol) and anhydrous hydrazine (0.32 mL, 10.03 mmol) in ethanol (25 mL). The pure product, **13c** (850 mg, 48% yield), was obtained as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 7.02−6.91 (m, 3H), 6.86 (dd, J = 8.0, 1.2 Hz, 1H), 3.86 (s, 3H), 3.10 (br s, 4H), 2.73 (t, J = 6.8 Hz, 2H), 2.65 (br s, 4H), 2.42 (t, J = 7.6 Hz, 2H), 1.60−1.46 (m, 6H). ^{13}C NMR ($CDCl_3$) δ 152.3, 141.4, 122.9, 120.99, 118.2, 111.2, 58.6, 55.3, 53.5, 50.7, 42.2, 31.9, 24.3.

*4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)butan-1-amine (**13d**)..*[50,66] Compound **13d** was synthesized as described for **13a** using **12d** (1.46 g, 3.37 mmol) and anhydrous hydrazine (0.32 mL, 10.19 mmol) in ethanol (33 mL). The pure product, **13d** (1.01 g, 99% yield), was obtained as a light-yellow oil. ^{1}H NMR ($CDCl_3$) δ 7.16−7.11 (m, 2H), 6.96 (dd, J = 6.8, 3.2 Hz, 1H), 3.07 (br s, 4H), 2.73 (t, J = 6.6 Hz, 2H), 2.64 (br s, 4H), 2.43 (t, J = 7.6 Hz, 2H), 1.62−1.46 (m, 6H). ^{13}C NMR ($CDCl_3$) δ 151.3, 134.0, 127.5, 127.4, 124.5, 118.6, 58.5, 53.3, 51.3, 42.1, 31.7, 24.3.

General Amidation Procedure, Method A. 1,1′-Carbonyldiimidazole (1 equiv) was added to the solution of carboxylic acid (1 equiv) in THF (10 mL/mmol). The resulting mixture was stirred at room temperature for 2 h. The solution was cooled to 0 °C, and the amine substrate (1 equiv) in THF (3 mL/mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred overnight. The reaction mixture was concentrated, and the crude product was diluted with $CHCl_3$ (20 mL/mmol) and washed with saturated aqueous $NaHCO_3$ solution (2 × 10 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by flash column chromatography (EtOAc followed by 8% MeOH:$CHCl_3$), or as indicated.

*N-(4-(4-(3-Chloro-2-methoxyphenyl)piperazin-1-yl)butyl)-1H-indole-2-carboxamide (**14**).* Compound **14** was synthesized according to general method A, using **13a** (490 mg, 1.65 mmol) and commercially available 1*H*-indole-2-carboxylic acid (266 mg, 1.65 mmol). The pure product, **14** (450 mg, 62% yield), was converted to the HCl salt in 2-propanol/acetone; mp 235−236 °C (HCl salt). ^{1}H NMR (DMSO-d_6) δ 11.50 (br s, 1H), 8.42 (br s, 1H), 7.57 (d, J = 8.0 Hz, 1H), 7.39 (d, J = 8.4 Hz, 1H), 7.14 (t, J = 7.6 Hz, 1H), 7.08 (s, 1H), 7.01−6.98 (m, 3H), 6.87−6.85 (m, 1H), 3.74 (s, 3H), 3.29 (m, 2H), 3.01 (br s, 4H), 2.50 (br s, 4H), 2.34 (t, J = 7.2 Hz, 2H), 1.53 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 161.9, 148.8, 146.7, 136.5, 131.0, 128.8, 127.7, 124.8, 124.5, 123.4, 121.9, 120.7, 117.2, 112.2, 101.9, 59.1, 58.2, 53.9, 50.3, 39.7, 27.7, 24.5. Anal. ($C_{24}H_{29}ClN_4O_2$·HCl) C, H, N.

*N-(4-(4-(3-Chloro-2-methoxyphenyl)piperazin-1-yl)butyl)-benzofuran-2-carboxamide (**15**).* Compound **15** was synthesized according to general method A, using **13a** (420 mg, 1.41 mmol) and commercially available benzofuran-2-carboxylic acid (227 mg, 1.41 mmol). The pure product, **15** (385 mg, 62% yield), was converted to the oxalate salt in 2-propanol/acetone; mp 118−120 °C (oxalate salt). ^{1}H NMR ($CDCl_3$) δ 7.68−7.65 (m, 1H), 7.47−7.45 (m, 2H), 7.42−7.37 (m, 1H), 7.31−7.26 (m, 1H), 7.0 (dd, J = 8.0, 1.6 Hz, 2H), 6.94 (t, J = 8.0 Hz, 1H), 6.79 (dd, J = 8.0, 1.6 Hz, 1H), 3.86 (s, 3H), 3.53 (q, J = 6.0 Hz, 2H), 3.17 (br s, 4H), 2.64 (br s, 4H), 2.47 (t, J = 7.2 Hz, 2H), 1.75−1.65 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 159.0, 154.8, 149.1, 148.8, 146.7, 128.8, 127.8, 126.9, 124.7, 123.8, 123.3, 122.8, 117.2, 111.7, 110.4, 59.1, 58.1, 53.9, 50.3, 39.3, 27.7, 24.5. Anal. ($C_{24}H_{28}ClN_3O_3·C_2H_2O_4$·EtOAc) C, H, N.

*N-(4-(4-(Naphthalen-1-yl)piperazin-1-yl)butyl)-1H-indole-2-carboxamide (**16**).* Compound **16** was synthesized according to general method A, using **13b** (580 mg, 2.05 mmol) and 1*H*-indole-2-carboxylic acid (330 mg, 2.05 mmol). The pure product, **16** (524 mg, 60% yield), was converted to the HCl salt; mp >250 °C (HCl salt). ^{1}H

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NMR (DMSO-d_6) δ 11.52 (br s, 1H), 8.45 (t, J = 5.8 Hz, 1H), 8.08 (d, J = 10.0 Hz, 1H), 7.85 (d, J = 9.2 Hz, 1H), 7.57 (t, J = 8.8 Hz, 2H), 7.52−7.46 (m, 2H), 7.40 (t, J = 8.0 Hz, 2H), 7.15 (t, J = 7.6 Hz, 1H), 7.12−7.09 (m, 2H), 7.01 (t, J = 7.6 Hz, 1H), 3.39−3.32 (m, 2H), 3.01 (br s, 4H), 2.64 (br s, 4H), 2.43 (t, J = 6.8 Hz, 2H), 1.61−1.57 (m, 4H). ^{13}C NMR (DMSO-d_6) δ 161.0, 149.3, 136.3, 134.3, 131.9, 128.3, 128.1, 127.1, 126.0, 125.8, 125.3, 123.3, 123.1, 122.97, 121.4, 119.6, 114.5, 112.2, 102.2, 57.6, 53.2, 52.7, 38.7, 27.2, 23.8. Anal. ($C_{27}H_{34}N_4O\cdot HCl\cdot {}^3/_4H_2O$) C, H, N.

*5-Methoxy-N-(4-(4-(naphthalen-1-yl)piperazin-1-yl)butyl)-1H-indole-2-carboxamide (**17**).* Compound **17** was synthesized according to general method A, using **13b** (490 mg, 1.73 mmol) and commercially available 5-methoxy-1*H*-indole-2-carboxylic acid (330 mg, 1.73 mmol). The pure product, **17** (457 mg, 58% yield), was converted to the HCl salt; mp >250 °C (HCl salt). ^{1}H NMR ($CDCl_3$) δ 9.44 (s, 1H), 8.19 (d, J = 9.6 Hz, 1H), 7.82 (d, J = 9.2 Hz, 1H), 7.54 (d, J = 8.0 Hz, 1H), 7.49−7.43 (m, 2H), 7.38 (t, J = 7.8 Hz, 1H), 7.33 (d, J = 8.8 Hz, 1H), 7.06 (d, J = 6.0 Hz, 1H), 7.03 (d, J = 2.4 Hz, 1H), 6.96 (dd, J = 9.0, 2.4 Hz, 1H), 6.76 (d, J = 1.6 Hz, 1H), 6.56 (t, J = 5.4 Hz, 1H), 3.83 (s, 3H), 3.57−3.52 (m, 2H), 3.16 (br s, 4H), 2.75 (br s, 4H), 2.54 (t, J = 6.8 Hz, 2H), 1.77−1.69 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 161.9, 154.7, 149.6, 134.7, 131.6, 131.4, 128.9, 128.4, 127.99, 125.9, 125.8, 125.3, 123.55, 123.51, 115.7, 114.6, 112.8, 102.3, 101.4, 58.1, 55.7, 53.8, 52.9, 39.6, 27.6, 24.4. Anal. ($C_{28}H_{32}N_4O_2\cdot HCl\cdot {}^3/_2H_2O$) C, H, N.

*N-(4-(4-(Naphthalen-1-yl)piperazin-1-yl)butyl)benzofuran-2-carboxamide (**18**).* Compound **18** was synthesized according to general method A, using **13b** (500 mg, 1.77 mmol) and commercially available benzofuran-2-carboxylic acid (287 mg, 1.77 mmol). The pure product, **18** (491 mg, 65% yield), was converted to the oxalate salt in 2-propanol/acetone; mp 168−170 °C (oxalate salt). ^{1}H NMR ($CDCl_3$) δ 7.19 (d, J = 7.6 Hz, 1H), 7.84−7.81 (m, 1H), 7.67 (dd, J = 8.0, 1.2 Hz, 1H), 7.55 (d, J = 8.4 Hz, 1H), 7.48−7.44 (m, 4H), 7.42−7.36 (m, 2H), 7.32−7.26 (m, 1H), 7.09−7.05 (m, 2H), 7.56 (q, J = 6.0 Hz, 2H), 3.20 (br s, 4H), 2.83 (br s, 4H), 2.60 (t, J = 7.2 Hz, 2H), 1.75 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 159.1, 154.8, 149.5, 149.1, 134.8, 128.9, 128.5, 127.8, 126.9, 125.9, 125.9, 125.5, 123.8, 123.7, 123.6, 122.8, 114.9, 111.8, 110.4, 57.9, 53.6, 52.6, 39.2, 27.6, 24.0. Anal. ($C_{27}H_{29}N_3O_2\cdot C_2H_2O_4\cdot {}^1/_2H_2O$) C, H, N.

*N-(4-(4-(Naphthalen-1-yl)piperazin-1-yl)butyl)benzo[b]thiophene-2-carboxamide (**19**).* Compound **19** was synthesized according to by general method A, employing **13b** (540 mg, 1.91 mmol) and commercially available benzo[*b*]thiophene-2-carboxylic acid (340 mg, 1.91 mmol). The pure product, **19** (575 mg, 68% yield), was converted to the oxalate salt in 2-propanol/acetone; mp 183−185 °C (oxalate salt). ^{1}H NMR ($CDCl_3$) δ 8.18−8.15 (m, 1H), 7.87−7.81 (m, 4H), 7.55 (d, J = 8.4 Hz, 1H), 7.48−7.35 (m, 5H), 7.03 (dd, J = 7.2, 0.8 Hz, 1H), 6.86 (br s, 1H), 3.54 (q, 5.2 Hz, 2H), 3.18 (br s, 4H), 2.84 (br s, 4H), 2.63 (t, J = 7.2 Hz, 2H), 1.77−1.74 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 162.6, 149.4, 140.9, 139.3, 138.9, 134.9, 128.9, 128.6, 126.3, 125.9, 125.6, 125.2, 125.1, 125.0, 123.9, 123.5, 122.9, 114.9, 57.9, 53.6, 52.4, 39.9, 27.4, 23.9. Anal. ($C_{27}H_{29}N_3OS\cdot C_2H_2O_4\cdot {}^1/_2H_2O$) C, H, N.

*N-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)-3-hydroxybutyl)-5-fluoro-1H-indole-2-carboxamide (**21**).* Compound **21** was synthesized according to general method A employing **10d** (398 mg, 1.25 mmol) and commercially available 5-fluoroindole-2-carboxylic acid (222 mg, 1.24 mmol). The crude product was purified by column chromatography as described in general method A to give **21** (320 mg, 54% yield) as a light-brown solid; the pure product was converted to the oxalate salt in 2-propanol/acetone; mp 225−228 °C (oxalate salt). ^{1}H NMR (DMSO-d_6) δ 11.6 (s, 1H), 8.51 (t, J = 5.6 Hz, 1H), 7.42 (dd, J = 9.0, 4.6 Hz, 1H), 7.35 (dd, J = 9.8, 2.6 Hz, 1H), 7.29−7.23 (m, 2H), 7.10−6.99 (m, 3H), 4.67 (s, 1H), 3.46−3.30 (m, 3H), 2.94 (br s, 4H), 2.57 (br s, 4H), 2.39−2.30 (m, 2H), 1.80−1.72 (m, 1H), 1.57−1.48 (m, 1H). ^{13}C NMR (DMSO-d_6): δ 161.4, 157.6 ($^1J_{CF}$ = 231 Hz), 151.6, 133.8, 133.5, 133.0, 128.9, 127.6 ($^3J_{CF}$ = 10.6 Hz), 126.3, 124.7, 119.9, 113.9 ($^3J_{CF}$ = 9.9 Hz), 112.4 ($^2J_{CF}$ = 26.5 Hz), 106.0 ($^2J_{CF}$ = 22.8 Hz), 102.8 ($^4J_{CF}$ = 5.3 Hz), 65.9, 64.6, 53.7, 51.2, 36.5, 35.5. Anal. ($C_{23}H_{25}Cl_2FN_4O_2\cdot C_2H_2O_4$) C, H, N.

*N-(4-(4-(2-Methoxyphenyl)piperazin-1-yl)butyl)quinoline-2-carboxamide (**22**).*[59] Compound **22** was synthesized according to general method A using **13c** (762 mg, 2.89 mmol) and commercially available quinoline-2-carboxylic acid (500 mg, 2.89 mmol). The crude product was purified by column chromatography as described for general method A to give **22** (1.02 g, 84% yield) as a gummy dark-yellow solid; the pure product was converted to the oxalate salt in 2-propanol/acetone; mp 183−185 °C (oxalate salt). ^{1}H NMR ($CDCl_3$) δ 8.34 (br s, 1H), 8.31 (s, 2H), 8.11 (d, J = 8.4 Hz, 1H), 7.88 (dd, J = 8.0, 0.8 Hz, 1H), 7.76 (td, J = 8.6, 1.5 Hz, 1H), 7.62 (td, J = 8.2, 1.2 Hz, 1H), 7.01−6.97 (m, 1H), 6.95−6.83 (m, 2H), 6.85 (dd, J = 7.6, 1.2 Hz, 1H), 3.86 (s, 3H), 3.58 (q, J = 13.2 Hz, 2H), 3.11 (br s, 4H), 2.67 (br s, 4H), 2.49 (t, J = 7.2 Hz, 2H), 1.81−1.65 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 164.6, 152.4, 150.1, 146.6, 141.5, 137.6, 130.2, 129.8, 129.4, 127.9, 127.9, 122.9, 121.1, 119.0, 118.3, 111.3, 58.4, 55.5, 53.6, 50.8, 39.6, 27.9, 24.6; Anal. ($C_{25}H_{30}N_4O_2\cdot C_2H_2O_4$) C, H, N.

*N-(3-Hydroxy-4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-quinoline-2-carboxamide (**23**).* Compound **23** was synthesized according to general method A using **10c** (648 mg, 2.32 mmol) and commercially available quinoline-2-carboxylic acid (401 mg, 2.31 mmol). The crude product was recrystallized in hot 2-propanol, and **23** (761 mg, 76% yield) was isolated as a pure-yellow solid that was converted to the oxalate salt in 2-propanol/acetone; mp 151−153 °C (oxalate salt). ^{1}H NMR ($CDCl_3$) δ 8.73 (t, J = 5.4 Hz, 1H), 8.33−8.29 (m, 2H), 8.12 (dd, J = 8.4, 0.8 Hz, 1H), 7.88 (dd, J = 8.0, 1.6 Hz, 1H), 7.76 (td, J = 7.7, 1.7 Hz, 1H), 7.61 (td, J = 7.7, 1.1 Hz, 1H), 7.02−6.98 (m, 1H), 6.96−6.90 (m, 2H), 6.86 (d, J = 8.2 Hz, 1H), 3.96−3.83 (m, 6H), 3.66−3.58 (m, 1H), 3.10 (br s, 4H), 2.90−2.85 (br m, 2H), 2.64−2.62 (br m, 2H), 2.49−2.42 (m, 2H), 1.92−1.84 (m, 1H), 1.76−1.67 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 164.8, 152.3, 149.9, 146.6, 141.2, 137.4, 129.98, 129.8, 129.3, 127.8, 127.7, 123.0, 120.9, 118.9, 118.2, 111.2, 65.2, 64.0, 55.4, 53.5, 50.8, 37.1, 34.4. Anal. ($C_{25}H_{30}N_4O_3\cdot C_2H_2O_4$) C, H, N.

*6-Fluoro-N-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-quinoline-2-carboxamide (**24**).* Compound **24** was synthesized according to general method A using **13c** (691 mg, 2.62 mmol) and commercially available 6-fluoroquinoline-2-carboxylic acid (501 mg, 2.62 mmol). The crude product was purified by column chromatography as described for general method A to give **24** (326 mg, 28% yield) that was converted to the oxalate salt (beige-colored solid) in 2-propanol/acetone; mp 166−168 °C (sublimes; oxalate salt). ^{1}H NMR ($CDCl_3$) δ 8.33 (d, J = 8.4 Hz, 1H), 8.28−8.24 (m, 1H), 8.25 (d, J = 8.4 Hz, 1H), 8.11 (dd, J = 9.2, 5.2 Hz, 1H), 7.56−7.47 (m, 2H), 7.01−6.97 (m, 1H), 6.95−6.89 (m, 2H), 6.86 (dd, J = 7.8, 1.0 Hz, 1H), 3.86 (s, 3H), 3.58 (q, J = 13.0 Hz, 2H), 3.11 (br s, 4H), 2.68 (br s, 4H), 2.49 (t, J = 7.2 Hz, 2H), 1.80−1.65 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 164.2, 161.2 ($^1J_{CF}$ = 251 Hz), 152.3, 149.5, 143.5, 141.3, 136.8 ($^4J_{CF}$ = 5.2 Hz), 132.2 ($^3J_{CF}$ = 9.6 Hz), 130.0 ($^3J_{CF}$ = 10.4 Hz), 122.9, 120.9, 120.5 ($^2J_{CF}$ = 26.1 Hz), 119.7, 118.2, 111.2, 110.8 ($^2J_{CF}$ = 22.0 Hz), 58.3, 55.3, 53.5, 50.6, 39.5, 27.8, 24.4. Anal. ($C_{25}H_{29}FN_4O_2\cdot C_2H_2O_4$) C, H, N.

*6-Fluoro-N-(3-hydroxy-4-(4-(2-methoxyphenyl)piperazin-1-yl)-butyl)quinoline-2-carboxamide (**25**).* Compound **25** was synthesized according to general method A using **10c** (596 mg, 2.14 mmol) and commercially available 6-fluoroquinoline-2-carboxylic acid (407 mg, 2.14 mmol). The crude product was purified by column chromatography as described for general method A to give **25** (330 mg, 34% yield) as a gummy yellow anhydrous solid which was converted to the oxalate salt in 2-propanol/acetone; mp 115−118 °C (free base). ^{1}H NMR ($CDCl_3$) δ 8.69 (t, J = 5.6 Hz, 1H), 8.33 (dd, J = 8.4, 0.8 Hz, 1H), 8.25 (d, J = 8.4 Hz, 1H), 8.13 (dd, J = 9.2, 5.6 Hz, 1H), 7.56−7.47 (m, 2H), 7.03−6.98 (m, 1H), 6.97−6.90 (m, 2H), 6.86 (d, J = 8.2 Hz, 1H), 3.96−3.80 (m, 6H), 3.68−3.57 (m, 1H), 3.10 (br s, 4H), 2.90−2.86 (m, 2H), 2.64−2.62 (m, 2H), 2.49−2.42 (m, 2H), 1.91−1.83 (m, 1H), 1.75−1.66 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 164.5, 161.2 ($^1J_{CF}$ = 251 Hz), 152.3, 149.5 ($^4J_{CF}$ = 3.0 Hz), 143.6, 141.2, 136.8, 136.7, 132.5, 132.4, 130.0 ($^3J_{CF}$ = 10.3 Hz), 123.0, 121.0, 120.5 ($^2J_{CF}$ = 25.7 Hz), 119.7, 118.2, 111.2, 110.7 ($^2J_{CF}$ = 22.1 Hz), 65.4, 63.9, 55.4, 53.5, 50.8, 37.2, 34.3. Anal. ($C_{25}H_{29}FN_4O_3\cdot C_2H_2O_4\cdot {}^1/_2H_2O\cdot {}^1/_2C_3H_8O$) C, H, N.

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*N-(3-Hydroxy-4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-quinoline-3-carboxamide (**26**).* Compound **26** was synthesized according to general method A using **10c** (420 mg, 1.50 mmol) and commercially available quinoline-3-carboxylic acid (250 mg, 1.44 mmol). The crude product was purified by column chromatography as described for general method A, and **26** (460 mg, 73% yield) was obtained as a beige-colored solid from 2-propanol; mp 127−129 °C (free base). ^{1}H NMR ($CDCl_3$) δ 9.31 (sd, J = 2.0 Hz, 1H), 8.61 (sd, J = 2.0 Hz, 1H), 8.14 (d, J = 8.4 Hz, 1H), 7.92 (dd, J = 8.0, 1.2 Hz, 1H), 7.79 (td, J = 8.4, 1.4 Hz, 1H), 7.76 (br s, 1H), 7.60 (td, J = 8.2, 1.1 Hz, 1H), 7.04−6.99 (m, 1H), 6.95−6.93 (m, 2H), 6.87 (d, J = 8.2 Hz, 1H), 4.01−3.95 (m, 2H), 3.87 (s, 3H), 3.56−3.47 (m, 1H), 3.11 (br s, 4H), 2.93−2.88 (m, 2H), 2.64−2.62 (m, 2H), 2.50−2.42 (m, 2H), 1.92−1.84 (m, 1H), 1.72−1.62 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 165.3, 152.3, 149.2, 148.4, 141.1, 135.4, 131.0, 129.4, 128.8, 127.34, 127.27, 127.0, 123.1, 121.0, 118.2, 111.2, 66.9, 63.7, 55.4, 53.4, 50.8, 38.97, 32.9. Anal. ($C_{25}H_{30}N_4O_3$) C, H, N.

*6-Fluoro-N-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-quinoline-3-carboxamide (**27**).* Compound **27** was synthesized according to general method A using **13c** (427 mg, 1.62 mmol) and commercially available 6-fluoroquinoline-3-carboxylic acid (301 mg, 1.57 mmol). The crude product was purified by column chromatography as described for general method A to give **27** (410 mg, 60% yield) as a beige-colored solid; mp 125−127 °C (free base). ^{1}H NMR ($CDCl_3$) δ 9.21 (d, J = 2.4 Hz, 1H), 8.52 (d, J = 2.0 Hz, 1H), 8.15 (dd, J = 9.4, 5.4 Hz, 1H), 7.57 (td, J = 9.6, 2.1 Hz, 1H), 7.50 (dd, J = 8.6, 2.4 Hz, 1H), 7.32 (br s, 1H), 6.98 (td, J = 7.8, 1.7 Hz, 1H), 6.88−6.82 (m, 2H), 6.75 (dd, J = 8.0, 1.6 Hz, 1H), 3.84 (s, 3H), 3.56 (q, J = 11.8 Hz, 2H), 3.01 (br s, 4H), 2.66 (br s, 4H), 2.50 (t, J = 6.8 Hz, 2H), 1.84−1.68 (m, 4H). ^{13}C NMR ($CDCl_3$): δ 165.7, 160.9 ($^1J_{CF}$ = 250 Hz), 152.2, 147.7, 146.3, 140.96, 134.8 ($^4J_{CF}$ = 5.2 Hz), 132.0 ($^3J_{CF}$ = 8.9 Hz), 128.4, 127.7 ($^3J_{CF}$ = 10.3 Hz), 123.0, 121.4 ($^2J_{CF}$ = 25.8 Hz), 120.9, 118.0, 111.5 ($^2J_{CF}$ = 22.1 Hz), 111.1, 58.0, 55.3, 53.4, 50.4, 40.2, 27.4, 24.5. Anal. ($C_{25}H_{29}FN_4O_2\cdot{}^1/_2H_2O$) C, H, N.

*6-Fluoro-N-(3-hydroxy-4-(4-(2-methoxyphenyl)piperazin-1-yl)-butyl)quinoline-3-carboxamide (**28**).* Compound **28** was synthesized according to general method A using **10c** (450 mg, 1.61 mmol) and commercially available 6-fluoroquinoline-3-carboxylic acid (300 mg, 1.57 mmol). The crude product was purified by column chromatography as described for general method A to give **28** (520 mg, 73% yield) as a beige-colored solid; mp 161−161.5 °C (free base). ^{1}H NMR ($CDCl_3$) δ 9.26 (d, J = 2.4 Hz, 1H), 8.55 (sd, J = 2.0 Hz, 1H), 8.15 (dd, J = 9.0, 5.4 Hz, 1H), 7.78 (br s, 1H), 7.59−7.52 (m, 2H), 7.04−6.99 (m, 1H), 6.96−6.92 (m, 2H), 6.87 (d, J = 7.6 Hz, 1H), 4.03−3.94 (m, 2H), 3.87 (s, 3H), 3.55−3.47 (m, 1H), 3.11 (br s, 4H), 2.93−2.88 (m, 2H), 2.66−2.61 (m, 2H), 2.52−2.42 (m, 2H), 1.91−1.84 (m, 1H), 1.71−1.61 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 164.9, 160.8 ($^1J_{CF}$ = 250 Hz), 152.3, 147.8, 146.3, 141.0, 134.7 ($^4J_{CF}$ = 5.9 Hz), 131.9 ($^3J_{CF}$ = 8.9 Hz), 128.0, 127.8 ($^3J_{CF}$ = 10.4 Hz), 123.2, 121.3 ($^2J_{CF}$ = 26.6 Hz), 121.0, 118.2, 111.6 ($^2J_{CF}$ = 22.0 Hz), 111.2, 67.0, 63.6, 55.4, 53.4, 50.8, 39.1, 32.8. Anal. ($C_{25}H_{29}FN_4O_3$) C, H, N.

*N-(3-Hydroxy-4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-6-methylquinoline-3-carboxamide (**29**).* Compound **29** was synthesized according to general method A using **10c** (458 mg, 1.64 mmol) and commercially available 6-methylquinoline-3-carboxylic acid (301 mg, 1.61 mmol). The crude product was purified by column chromatography as described for general method A to give **29** (400 mg, 56% yield) as a yellow solid; mp 52−55 °C (free base). ^{1}H NMR ($CDCl_3$) δ 9.23 (d, J = 2.0 Hz, 1H), 8.52 (d, J = 2.0 Hz, 1H), 8.03 (d, J = 8.8 Hz, 1H), 7.71 (br s, 1H), 7.67 (s, 1H), 7.62 (dd, J = 8.6, 1.8 Hz, 1H), 7.04−6.99 (m, 1H), 6.96−6.93 (m, 2H), 6.87 (d, J = 8.2 Hz, 1H), 4.02−3.92 (m, 2H), 3.87 (s, 3H), 3.55−3.47 (m, 1H), 3.11 (br s, 4H), 2.93−2.88 (m, 2H), 2.66−2.61 (m, 2H), 2.56 (s, 3H), 2.50−2.42 (m, 2H), 1.91−1.84 (m, 1H), 1.71−1.61 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 165.5, 152.3, 147.9, 147.5, 141.1, 137.3, 134.8, 133.6, 129.0, 127.5, 127.2, 127.1, 123.1, 121.0, 118.2, 111.2, 66.9, 63.7, 55.4, 53.4, 50.8, 38.9, 32.9, 21.6. Anal. ($C_{26}H_{32}N_4O_3\cdot{}^5/_4H_2O$) C, H, N.

*N-(3-Hydroxy-4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-6-methoxyquinoline-3-carboxamide (**30**).* Compound **30** was synthesized according to general method A using **10c** (290 mg, 1.04 mmol) and commercially available 6-methoxyquinoline-3-carboxylic acid (211 mg, 1.04 mmol). The crude product was purified by column chromatography as described for general method A to give **30** (320 mg, 66% yield) as a gummy yellow solid; the pure product was converted to the oxalate salt in 2-propanol/acetone; mp 83 °C (sublimes at >97 °C; oxalate salt). ^{1}H NMR ($CDCl_3$) δ 9.13 (d, J = 2.0 Hz, 1H), 8.51 (d, J = 1.6 Hz, 1H), 8.02 (d, J = 9.2 Hz, 1H), 7.73 (br s, 1H), 7.43 (dd, J = 9.2, 2.8 Hz, 1H), 7.14 (d, J = 2.8 Hz, 1H), 7.04−6.99 (m, 1H), 6.95−6.92 (m, 2H), 6.87 (d, J = 7.8 Hz, 1H), 4.00−3.93 (m, 2H), 3.94 (s, 3H), 3.87 (s, 3H), 3.56−3.48 (m, 1H), 3.11 (br s, 4H), 2.93−2.88 (m, 2H), 2.66−2.61 (m, 2H), 2.51−2.42 (m, 2H), 1.91−1.84 (m, 1H), 1.72−1.62 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 165.6, 158.4, 152.4, 145.9, 145.6, 141.2, 134.4, 130.9, 128.4, 127.7, 124.1, 123.3, 121.2, 118.4, 111.4, 105.97, 67.0, 63.8, 55.8, 55.5, 53.5, 50.9, 39.0, 33.1. Anal. ($C_{26}H_{32}N_4O_4\cdot{}^3/_2C_2H_2O_4\cdot H_2O$) C, H, N.

*N-(3-Hydroxy-4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-quinoline-7-carboxamide (**31**).* Compound **31** was synthesized according to general method A using **10c** (420 mg, 1.50 mmol) and commercially available quinoline-7-carboxylic acid (250 mg, 1.44 mmol). The crude product was purified by column chromatography as described for general method A to give **31** (180 mg, 29% yield) as a light-yellow oil; the pure product was converted to the HCl salt in a $CHCl_3$/ether solvent mixture; mp >220 °C (decomposes; HCl salt). ^{1}H NMR ($CDCl_3$) δ 8.98 (s, 1H), 8.46 (s, 1H), 8.19 (d, J = 8.4 Hz, 1H), 8.07 (d, J = 8.0 Hz, 1H), 7.89 (d, J = 8.4 Hz, 1H), 7.65 (br s, 1H), 7.47−7.45 (m, 1H), 7.01−6.99 (m, 1H), 6.95−6.91 (m, 2H), 6.87 (d, J = 8.4 Hz, 1H), 3.99−3.94 (m, 2H), 3.87 (s, 3H), 3.54−3.49 (m, 1H), 3.11 (br s, 4H), 2.89 (br s, 2H), 2.62 (br s, 2H), 2.49−2.41 (m, 2H), 1.87−1.84 (m, 1H), 1.71−1.63 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 166.6, 152.3, 151.2, 147.7, 141.1, 135.9, 135.7, 129.7, 128.3, 127.7, 125.3, 123.1 122.2, 121.0, 118.3, 111.2, 66.8, 63.8, 55.4, 53.4, 50.8, 38.8, 33.1. Anal. ($C_{25}H_{30}N_4O_3\cdot 3HCl\cdot{}^5/_2H_2O$) C, H, N.

*N-(4-(4-(Naphthalen-1-yl)piperazin-1-yl)butyl)quinoline-3-carboxamide (**32**).* Compound **32** was synthesized according to general method A, using **13b** (250 mg, 0.88 mmol) and commercially available quinoline-3-carboxylic acid (153 mg, 0.88 mmol). The pure product, **32** (250 mg, 65% yield), was then converted to the oxalate salt in 2-propanol/acetone and isolated as a cream-colored solid; mp 178−179 °C (oxalate salt). ^{1}H NMR ($CDCl_3$) δ 9.28 (d, J = 2.4 Hz, 1H), 8.59 (d, J = 2.4 Hz, 1H), 8.16−8.13 (m, 2H), 7.90 (d, J = 8.4 Hz, 1H), 7.82−7.78 (m, 2H), 7.61 (t, J = 6.0 Hz, 1H), 7.52 (d, J = 8.4 Hz, 1H), 7.45−7.43 (m, 2H), 7.20 (s, 1H), 6.88 (dd, J = 7.2, 0.8 Hz, 1H) 3.59 (q, t, J = 6.4 Hz, 2H), 3.06 (br s, 4H), 2.75 (br s, 4H), 2.57 (t, J = 7.2 Hz, 2H), 1.82−1.73 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 165.6, 149.3, 149.2, 148.3, 135.5, 134.7, 131.9, 129.5, 128.8, 128.7, 128.4, 127.7, 127.5, 126.9, 125.8, 125.8, 125.3, 123.5, 123.43, 114.6, 58.1, 53.8, 52.7, 40.2, 27.5, 24.5. Anal. ($C_{28}H_{30}N_4O\cdot C_2H_2O_4\cdot{}^1/_4H_2O$) C, H, N.

*N-(3-Hydroxy-4-(4-(naphthalen-1-yl)piperazin-1-yl)butyl)-quinoline-3-carboxamide (**33**).* Compound **33** was synthesized according to general method A, using **10b** (150 mg, 0.50 mmol) and commercially available quinoline-3-carboxylic acid (87 mg, 0.50 mmol). The pure product, **33** (100 mg, 44% yield), was isolated as a brown solid; mp 90−92 °C (free base). ^{1}H NMR ($CDCl_3$) δ 9.32 (d, J = 2.4 Hz, 1H), 8.62 (d, J = 1.6 Hz, 1H), 8.19−8.13 (m, 2H), 7.92 (dd, J = 8.4, 1.2 Hz, 1H), 7.83−7.76 (m, 3H), 7.62−7.55 (m, 2H), 7.50−7.44 (m, 2H), 7.40 (t, J = 8.4 Hz, 1H), 7.08 (dd, J = 7.6, 1.2 Hz, 1H) 4.03−3.95 (m, 2H), 3.57−3.49 (m, 1H), 3.16 (br s, 4H), 2.98 (br s, 2H), 2.72−2.71 (m, 2H), 2.58−2.47 (m, 2H), 1.92−1.86 (m, 1H), 1.73−1.67 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 165.3, 149.3, 149.2, 148.4, 135.5, 134.8, 131.0, 129.4, 128.8, 128.7, 128.4, 127.33, 127.3, 127.0, 125.9, 125.8, 125.4, 123.7, 123.4, 114.7, 77.22, 66.9, 63.7, 59.0, 58.9, 38.8, 33.0. HRMS (MALDI-TOF) m/z 455.2443 $(M + H)^+$.

*N-(4-(4-(3-Chloro-2-methoxyphenyl)piperazin-1-yl)butyl)-quinoline-3-carboxamide (**34**).* Compound **34** was synthesized according to general method A, using **13a** (140 mg, 0.47 mmol) and commercially available quinoline-3-carboxylic acid (81 mg, 0.47 mmol). The pure product, **34** (120 mg, 56% yield), was then converted to the oxalate salt in 2-propanol/acetone and isolated as a cream-colored solid; mp 149−151 °C (oxalate salt). ^{1}H NMR ($CDCl_3$) δ 9.25 (d, J = 2.4 Hz, 1H), 8.57 (d, J = 1.6 Hz, 1H), 8.14

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(d, J = 8.4 Hz, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.82−7.77 (m, 1H), 7.62−7.58 (m, 1H), 7.11−7.10 (m, 1H), 6.98 (dd, J = 7.6, 1.6 Hz, 1H) 6.88 (t, J = 8.0 Hz, 1H), 6.63 (dd, J = 8.0, 1.2 Hz, 1H) 3.83 (s, 3H), 3.56 (q, J = 6.4 Hz, 2H), 3.06 (br s, 4H), 2.60 (br s, 4H), 2.48 (t, J = 6.8 Hz, 2H), 1.81−1.66 (m, 4H). ^{13}C NMR ($CDCl_3$) δ 165.9, 154.7, 149.2, 148.6, 148.3, 146.4, 135.5, 131.1, 129.4, 128.7, 127.6, 127.5, 126.9, 124.6, 123.3, 116.9, 58.9, 58.0, 53.7, 50.0, 40.2, 27.4, 24.5. Anal. ($C_{25}H_{29}ClN_4O_2 \cdot C_2H_2O_4 \cdot {}^3/_4H_2O$) C, H, N.

*N-(4-(4-(3-Chloro-2-methoxyphenyl)piperazin-1-yl)-3-hydroxybutyl)quinoline-3-carboxamide (**35**).* Compound **35** was synthesized according to general method A, using **10a** (250 mg, 0.79 mmol) and commercially available quinoline-3-carboxylic acid (138 mg, 0.79 mmol). The pure product, **35** (150 mg, 40% yield), was isolated as a sticky light-yellow solid (free base). ^{1}H NMR ($CDCl_3$) δ 9.29 (d, J = 2.4 Hz, 1H), 8.58 (d, J = 2.0 Hz, 1H), 8.09 (d, J = 8.0 Hz, 1H), 7.85 (d, J = 7.6 Hz, 2H), 7.76−7.72 (m, 1H), 7.57−7.53 (m, 1H), 6.99 (dd, J = 8.0, 1.6 Hz, 1H), 6.92 (t, J = 8.0 Hz, 1H), 6.76 (dd, J = 7.6, 1.6 Hz, 1H) 3.97−3.87 (m, 2H), 3.83 (s, 3H), 3.54−3.46 (m, 1H), 3.45 (s, 1H), 3.11 (br s, 4H), 2.84−2.80 (m, 2H), 2.58−2.56 (m, 2H), 2.45−2.42 (m, 2H), 1.86−1.80 (m, 1H), 1.69−1.60 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 165.4, 149.1, 148.7, 148.4, 146.4, 135.5, 135.4, 131.0, 129.3, 128.8, 127.3, 127.2, 126.9, 124.7, 123.4, 117.1, 66.7, 63.7, 59.0, 58.9, 53.6, 50.3, 38.7, 33.2. Anal. ($C_{25}H_{29}ClN_4O_3 \cdot H_2O$) C, H, N.

*N-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)butyl)-6-fluoroquinoline-3-carboxamide (**36**).* Compound **36** was synthesized according to general method A employing **13d** (340 mg, 1.12 mmol) and commercially available 6-fluoroquinoline-3-carboxylic acid (213 mg, 1.11 mmol). The crude product was purified by column chromatography as described in general method A and was then converted to the oxalate salt in 2-propanol/acetone to give **36** (280 mg, 53% yield) as a cream-colored solid; mp 234−236 °C (oxalate salt). ^{1}H NMR ($CDCl_3$) δ 9.20 (d, J = 2.0 Hz, 1H), 8.53 (d, J = 2.0 Hz, 1H), 8.15 (dd, J = 9.0, 5.4 Hz, 1H), 7.57 (td, J = 9.0, 2.9 Hz, 1H), 7.51 (dd, J = 8.6, 2.6 Hz, 1H), 7.16−7.13 (m, 2H), 7.08 (t, J = 8.0 Hz, 1H), 6.77 (dd, J = 7.8, 2.2 Hz, 1H), 3.57 (q, J = 12.2 Hz, 2H), 2.98 (br s, 4H), 2.63 (br s, 4H), 2.51 (t, J = 6.8 Hz, 2H), 1.80−1.75 (m, 2H), 1.74−1.69 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 165.6, 160.9 ($^1J_{CF}$ = 249 Hz), 151.0, 147.6 ($^4J_{CF}$ = 3.0 Hz), 146.3, 134.9 ($^3J_{CF}$ = 5.8 Hz), 134.0, 132.0 ($^3J_{CF}$ = 9.1 Hz), 128.4, 127.7, 127.5, 127.4, 124.7, 121.4 ($^2J_{CF}$ = 25.8 Hz), 118.5, 111.5 ($^2J_{CF}$ = 21.2 Hz), 58.0, 53.3, 51.1, 40.3, 27.4, 24.5. Anal. ($C_{24}H_{25}Cl_2FN_4O \cdot C_2H_2O_4$) C, H, N.

*N-(4-(4-(2,3-Dichlorophenyl)piperazin-1-yl)-3-hydroxybutyl)-6-fluoroquinoline-3-carboxamide (**37**).* Compound **37** was synthesized according to general method A employing **10d** (381 mg, 1.20 mmol) and commercially available 6-fluoroquinoline-3-carboxylic acid (226 mg, 1.18 mmol). The crude product was purified by column chromatography as described in general method A to give **37** (310 mg, 53% yield) as a cream-colored solid that was converted to the oxalate salt in a 2-propanol/acetone solvent mixture; mp 201−203 °C (oxalate salt). ^{1}H NMR ($CDCl_3$) δ 9.25 (d, J = 2.0 Hz, 1H), 8.56 (d, J = 1.6 Hz, 1H), 8.15 (dd, J = 9.2, 5.2 Hz, 1H), 7.76 (br s, 1H), 7.57 (dd, J = 8.4, 2.8 Hz, 1H), 7.53 (dd, J = 7.4, 2.6 Hz, 1H), 7.20−7.14 (m, 2H), 6.96 (dd, J = 6.6, 2.6 Hz, 1H), 4.03−3.94 (m, 3H), 3.55−3.47 (m, 1H), 3.09 (br s, 4H), 2.92−2.88 (m, 2H), 2.64−2.62 (m, 2H), 2.53−2.43 (m, 2H), 1.91−1.84 (m, 1H), 1.71−1.60 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 164.9, 160.8 ($^1J_{CF}$ = 24.8 Hz), 150.9, 147.7 ($^4J_{CF}$ = 3.0 Hz), 146.3, 134.8 ($^4J_{CF}$ = 6.1 Hz), 134.1, 131.9 ($^3J_{CF}$ = 9.1 Hz), 127.98, 127.8 ($^3J_{CF}$ = 10.6 Hz), 127.6, 127.5, 124.8, 121.3 ($^2J_{CF}$ = 25.8 Hz), 118.6, 111.6 ($^2J_{CF}$ = 22.0 Hz), 66.9, 63.6, 53.2, 51.4, 39.0, 32.9. Anal. ($C_{24}H_{25}Cl_2FN_4O_2 \cdot C_2H_2O_4 \cdot H_2O$) C, H, N.

Radioligand Binding Assays. Binding at dopamine D_2-like receptors was determined using previously described methods.[68] Membranes were prepared from HEK293 cells expressing human D_2R, D_3R, or D_4R, 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 nonessential amino acids, 1× 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% CO_2. Upon reaching 80−90% confluence, cells were harvested using premixed Earle's Balanced Salt Solution (EBSS) with 5 μM 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 of hypotonic lysis buffer (5 mM $MgCl_2 \cdot 6H_2O$, 5 mM Tris, pH 7.4 at 4 °C) and centrifuged at 20000 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% H_2O 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 hD_2R or hD_3R) or 200 μg/mL (hD_4R) stock for binding. Radioligand competition experiments were conducted in glass tubes containing 300 μL of fresh EBSS buffer with 0.2 mM sodium metabisulfite, 50 μL of diluted test compound, 100 μL of membranes (10 μg total protein for hD_2R or hD_3R, 20 μg total protein for hD_4R), and 50 μL of [^{3}H]*N*-methylspiperone (0.4 nM final concentration; PerkinElmer). Non-specific 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 1 h at room temperature. The reaction was terminated by filtration through Whatman GF/B filters, presoaked for 1 h 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. Then 3 mL of CytoScint liquid scintillation cocktail (MP Biomedicals, Solon, OH) was added and vials were counted using a PerkinElmer Tri-Carb 2910 TR liquid scintillation counter (Waltham, MA). IC_{50} values for each compound were determined from dose−response curves, and K_i values were calculated using the Cheng−Prusoff equation;[69] these analyses were performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA). Reported K_i values were determined from least three independent experiments.

Mouse Microsomal Stability Assay. Phase I metabolic stability assays were conducted in mouse liver microsomes as previously described[60] with minor modifications. In brief, the reaction was carried out with 100 mM potassium phosphate buffer, pH 7.4, in the presence of NADPH regenerating system, (compound final concentration was 1 μM; and 0.2 mg/mL microsomes). Negative controls without cofactors were assessed to determine the non-CYP mediated metabolism. Positive controls for phase I metabolism (testosterone) were also evaluated. Compound disappearance was monitored over time using a liquid chromatography and tandem mass spectrometry (LC/MS/MS) method. All reactions were performed in triplicate.

Chromatographic analysis was performed using an Accela ultra high-performance system consisting of an analytical pump and an autosampler coupled with TSQ Vantage mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA). Separation of the analyte from potentially interfering material was achieved at ambient temperature using Agilent Eclipse Plus column (100 mm × 2.1 mm i.d.) packed with a 1.8 μm C18 stationary phase. The mobile phase used was composed of 0.1% formic acid in acetonitrile and 0.1% formic acid in H_2O with gradient elution, starting with 10% (organic) linearly increasing to 99% up to 2.5 min, maintaining at 99% (2.5−3.5 min), and reequlibrating to 10% by 4.5 min. The total run time for each analyte was 4.5 min. The mass transitions used for compounds for LC/MS/MS analysis are given in Table S1 (Supporting Information).

The calculations of in vitro half-lives were performed as described previously.[70] Briefly, for the determination of in vitro half-life ($t_{1/2}$), the analyte/IS peak area ratios were converted to percentage drug remaining, using the T = 0 area ratio values as 100%. The slope of the linear regression from log percentage remaining versus incubation time

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relationships ($-k$) was used in the conversion to in vitro $t_{1/2}$, using the equation $t_{1/2} = -0.693/k$.

Heroin Self-Administration. *Animals.* Male WT and D_3KO mice with a C57BL/6J genetic background were bred at the National Institute on Drug Abuse from three $D_3^{\pm}$ breeding pairs purchased from Jackson Laboratory. This strain of D_3KO mice expresses a truncated D_3R, including the extracellular N-terminal, the first intracellular loop, and part of the second intracellular loop (a total of 148 residues) but lacks downstream sequences from the second intracellular loop (from residue 149) (Song et al., 2011). Genotyping was performed in our laboratory in accordance with a protocol of Charles River Laboratories. All mice used in the present experiments were matched for age (8−14 wk) and weight (25−35 g). They were housed individually in a climate-controlled animal colony room on a reversed light−dark cycle (lights on at 7:00 PM, lights off at 7:00 AM) with free access to food and water. All experimental procedures were conducted in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals and were approved by the National Institute on Drug Abuse's Animal Care and Use Committee.

Heroin Self-Administration. Intravenous catheterization surgery and the self-administration procedures were performed as described previously (Xi et al., 2011; Song et al., 2012). Briefly, WT or D_3KO mice were initially trained for oral sucrose (5% solution) self-administration for 2−3 days to learn operant lever pressing for sucrose reward. After lever pressing behavior was achieved, animals were prepared for iv catheterization surgery. After 5−7 days of recovery from surgery, each mouse was placed into a test chamber (Med Associates, St. Albans, VT) and allowed to lever-press for iv heroin, beginning with 0.1 mg/kg/infusion for a week, and then the heroin dose was lowered to 0.05 and 0.025 mg/kg/infusion in the following weeks. Each heroin dose was maintained for 5−7 days until stable self-administration was achieved. A progressively decreased heroin dose regimen was chosen based on the fact that total numbers of heroin infusions at higher doses (0.1 mg/kg/infusion) are usually very low (~20). Lowering the heroin dose results in a significant increase in the numbers of heroin infusions or active lever presses by a compensatory mechanism that significantly increases "system sensitivity" to detect the effects of experimental drugs on heroin self-administration and prevent potential bottom effects. Each lever press led to a delivery of 0.015 mL of the drug solution over 4.2 s under an FR1 reinforcement schedule. Each session lasted 3 h or until the animal received the maximally allowed 50 heroin infusions to prevent drug overdose. Daily drug self-administration continued until stable day-to-day operant behavior was established with a steady behavioral response pattern for at least three consecutive days.

Effects of the Test Compounds on Heroin Self-Administration. Animals were divided into two groups to observe the effects of **16** (WT, $n = 7$; D_3KO, $n = 7$) and **32** (WT, $n = 7$; D_3KO, $n = 6$) on heroin self-administration, respectively. After the completion of the tests with **16** and **32**, the animals with stable heroin self-administration (WT, $n = 9$; D_3KO, $n = 5$) were used again to observed the effects of **2** on heroin self-administration. On the test day, each dose of **2** (0, 25, 50, 100 mg/kg), **16** (0, 1, 3, or 10 mg/kg, ip), or **32** (0, 0.3, 1, or 3 mg/kg) was given 30 min prior to daily heroin self-administration. After each test, animals continued until heroin self-administration recovered to the basal levels before each drug test. The time intervals between tests were 3−5 days. The sequence of tests for each dose was counterbalanced.

■ ASSOCIATED CONTENT

Supporting Information

Elemental analysis results, selected reaction monitoring transitions of D_3R-selective ligands, HRMS data for compound **33**, and metabolism data (PDF). Molecular formula strings (CSV). The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b00776.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: (443)-740-2887. Fax: (443)-740-2111. E-mail: anewman@intra.nida.nih.gov.

Present Address

[#]For J.Z.: Department of Anesthesiology, Zhongnan Hospital, Wuhan University Wuhan, Hubei 430071, China

Author Contributions

[⊥]C.A.B. and O.M.B. contributed equally.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

DA, dopamine; SAR, structure activity relationship; TM, transmembrane; D_2R, dopamine D_2 receptor; D_3R, dopamine D_3 receptor; D_4R, dopamine D_4 receptor; PET, positron emission tomography; PHNO, (+)-4-propyl-9-hydronaphthoxazine; WT, wildtype; KO, knockout; FR, fixed ratio; OBS, orthosteric binding site; SBP, secondary binding pocket; 5-HT, 5-hydroxytryptamine (serotonin); CMA, chloroform/methanol/ammonium hydroxide

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