Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

Highly Selective Dopamine D_3 Receptor (D_3R) Antagonists and Partial Agonists Based on Eticlopride and the D_3R Crystal Structure: New Leads for Opioid Dependence Treatment

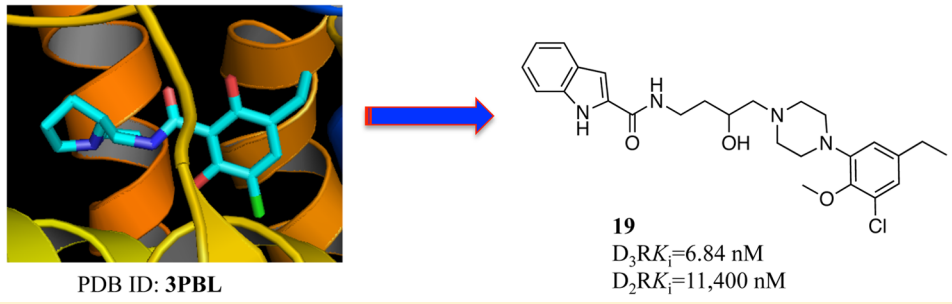
Vivek Kumar,[†] Alessandro Bonifazi,[†] Michael P. Ellenberger,[†] Thomas M. Keck,[†,‡] Elie Pommier,[†,§] Rana Rais,[§] Barbara S. Slusher,[§] Eliot Gardner,[†] Zhi-Bing You,[†] Zheng-Xiong Xi,[†] and Amy Hauck Newman*[,†]

[†]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, Department of Biomedical & Translational Sciences, College of Science and Mathematics, Rowan University, 201 Mullica Hill Road, Glassboro, New Jersey 08028, United States

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

Supporting Information



ABSTRACT: The recent and precipitous increase in opioid analgesic abuse and overdose has inspired investigation of the dopamine D_3 receptor (D_3R) as a target for therapeutic intervention. Metabolic instability or predicted toxicity has precluded successful translation of previously reported D_3R-selective antagonists to clinical use for cocaine abuse. Herein, we report a series of novel and D_3R crystal structure-guided 4-phenylpiperazines with exceptionally high D_3R affinities and/or selectivities with varying efficacies. Lead compound **19** was selected based on its in vitro profile: D_3R K_i = 6.84 nM, 1700-fold D_3R versus D_2R binding selectivity, and its metabolic stability in mouse microsomes. Compound **19** inhibited oxycodone-induced hyperlocomotion in mice and reduced oxycodone-induced locomotor sensitization. In addition, pretreatment with **19** also dose-dependently inhibited the acquisition of oxycodone-induced conditioned place preference (CPP) in rats. These findings support the D_3R as a target for opioid dependence treatment and compound **19** as a new lead molecule for development.

INTRODUCTION

Dopamine is a major neurotransmitter in the central nervous system responsible for many neurological processes, including emotion, cognition, reward, motivation, and fine motor control. Dopamine signaling is mediated by D_1-like (D_1 and D_5) and D_2-like (D_2, D_3 and D_4) receptor subtypes. Dopamine D_3 receptors (D_3R) have been implicated as potential pharmacotherapeutic targets for substance use disorders because of their restricted localization to limbic brain regions, effectiveness in animal models of drug abuse, and upregulation in the brains of cocaine addicts.[1] Inspired by early reports that the D_3R appeared to be involved in the reinforcing effects of cocaine and therefore a potential target for drug development,[2,3] an enormous effort has been made to develop D_3R selective antagonists or partial agonists targeting psychostimulant abuse.[4−7] However, the recent discovery that the investigational D_3R-selective antagonist, GSK598,809 (**1**, Figure 1), caused significant hypertension in dogs in the presence of cocaine, may preclude further development of these agents toward cocaine addiction.[8] Nevertheless, efficacy in both animal models of other substance use disorders, including heroin, alcohol, and nicotine support, continued efforts toward developing D_3R-selective antagonists and/or partial agonists that are metabolically stable and have appropriate drug-like properties for potential translation to specific drug abusing populations.[1,9,10]

The recent availability of the high-resolution structures of G-protein coupled receptors (GPCRs) has aided in structure-based drug design.[11,12] Specifically, the high-resolution crystal structure of D_3R can be used to guide the design of novel D_3R-

Received: June 7, 2016

DOI: 10.1021/acs.jmedchem.6b00860
J. Med. Chem. XXXX, XXX, XXX−XXX

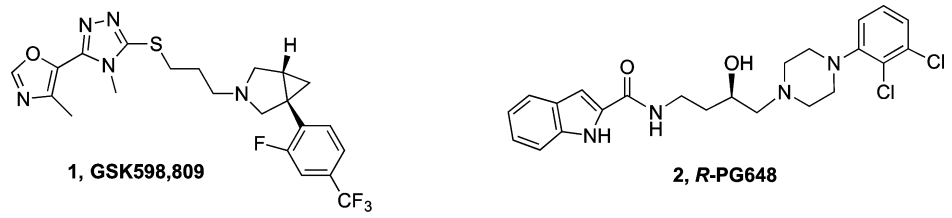


Figure 1. Representative D_3R antagonists.

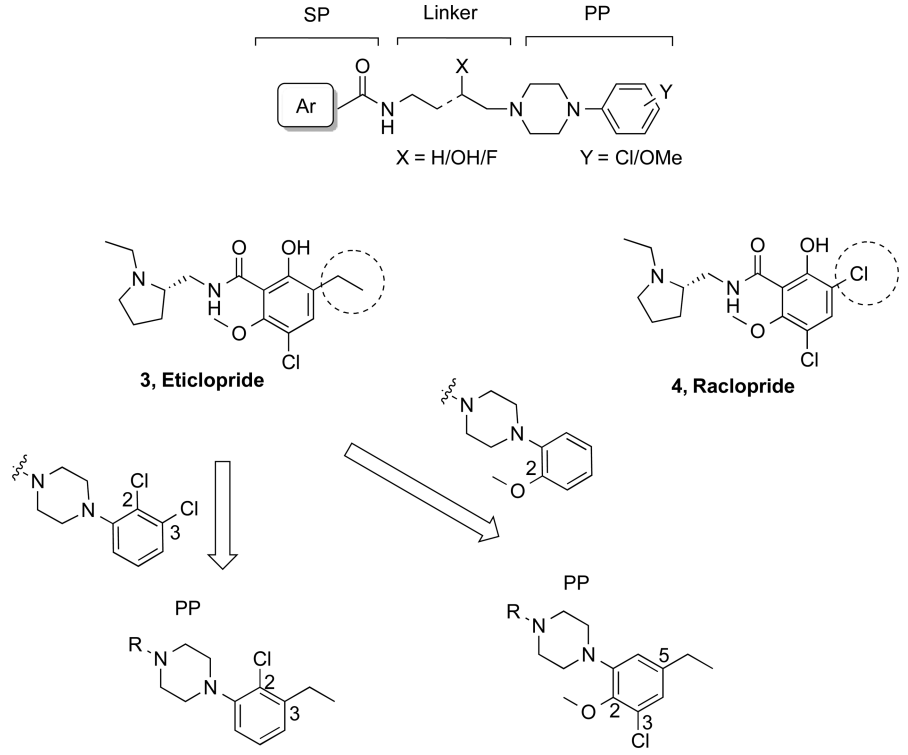


Figure 2. Design of the primary pharmacophore (PP).

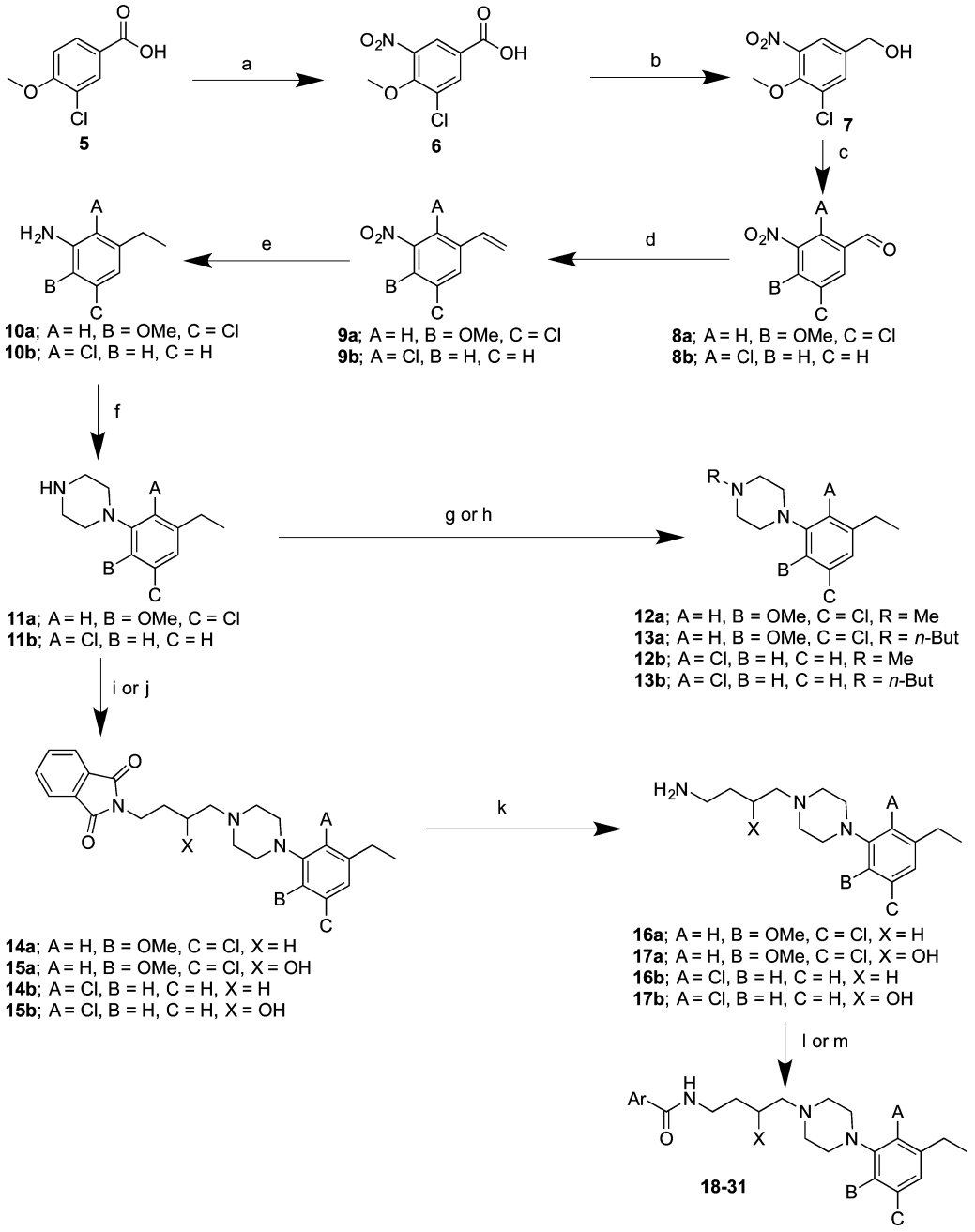
selective compounds with predicted efficacy.[11,13,14] In the crystal structure, eticlopride occupies the orthosteric binding site (OBS) defined by side chains from transmembrane helices II, III, IV, V, VI, and VII. Out of 18 eticlopride contact residues in the D_3R structure, 17 are identical in the D_2R and one is similar (valine 350 corresponds to isoleucine in D_2R).[13] Thus, the dopamine D_2-like receptors are largely homologous, with key differences allowing for exploitation of D_3R selectivity over D_2R.

Several classes of chemical structures have been explored in the search for highly D_3R selective ligands.[15,16] However, with the exception of **1**,[17] these molecules have only served as preclinical tools and none are available clinically. One challenge is that many of these preclinical tools lacked the appropriate ADME (absorption, distribution, metabolism, and excretion) properties to be deemed clinically useful.[18] Indeed, one of our lead compounds, *R*-PG648 (**2**, Figure 1), failed to clear the bar to clinical investigation due to metabolic instability and failure to show efficacy in nonhuman primates despite promise in rodent models of methamphetamine self-administration and relapse.[14,18,19] In addition, the preponderance of published studies have focused on developing these target molecules toward cocaine addiction, and yet a recent report suggests that toxic hypertension may result from treatment with a D_3R antagonist in the presence of cocaine (i.e., if the patient relapsed to cocaine taking), further dampening enthusiasm for developing these molecules for human psychostimulant abusers.[8]

Nevertheless, there remains a need to identify novel templates and better molecules to target the D_3R. First, there are several drugs of abuse (e.g., heroin) that do not produce hypertension and preclinical data support the efficacy of D_3R antagonists in attenuating their self-administration and relapse behaviors.[14,20] Second, structural determinants of affinity, selectivity, and efficacy at D_3R can be used for the highly homologous but functionally distinct D_2R, for which there are no highly selective agonists or antagonists.[21] Recent reports of D_2R bitopic ligands can trace their SAR to the D_3R crystal structure and molecular determinant studies done with D_3R-selective ligands and their synthons.[14] These reports highlight the importance of characterizing primary and secondary pharmacophores and the function of the respective OBS and secondary binding pocket (SBP) as well as potential allosteric sites that can have a significant impact on the pharmacology of these agents. Indeed, many new and exciting avenues of drug design pursuit have been aided by GPCR crystal structures and homology modeling.[22,23] Hence, the combination of small molecule SAR and clues from the D_3R crystal structure can produce structurally and pharmacologically unique molecules

DOI: 10.1021/acs.jmedchem.6b00860
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 1. Synthesis of Analogues Synthons 11−13 and Full-Length Analogues 18−31[a]



[a]Reagents and conditions: (a) fuming HNO_3, 0 °C to room temperature, 2 h; (b) $BH_3{\cdot}CS_2$, 24 h; (c) PCC, CH_2Cl_2, overnight; (d) $Ph_3P^+CH_3Br^-$, *n*-BuLi, THF; (e) 10% Pd/C, H_2, 50 psi, EtOH/EtOAc, 3 h; (f) bis(2-chloroethyl)amine·HCl, diethylene glycol monoethyl ether, 150 °C, 7 h; (g) HCHO, $NaBH(OAc)_3$, AcOH; (h) 1-bromobutane, K_2CO_3, acetone, reflux, 7 h; (i) *n*-bromobutyl phthalimide, K_2CO_3, acetone, reflux, 7 h; (j) 2-(2-oxiran-2-yl)ethyl)isoindoline-1,3-dione, IPA, reflux, overnight; (k) hydrazine, EtOH, reflux, overnight; (l) ArCOOH, CDI, THF/DMF, 0 °C to room temperature; (m) ethyl 4-ethyl-1*H*-imidazole-2-carboxylate, $(CH_3)_3Al$, CH_2Cl_2, room temperature.

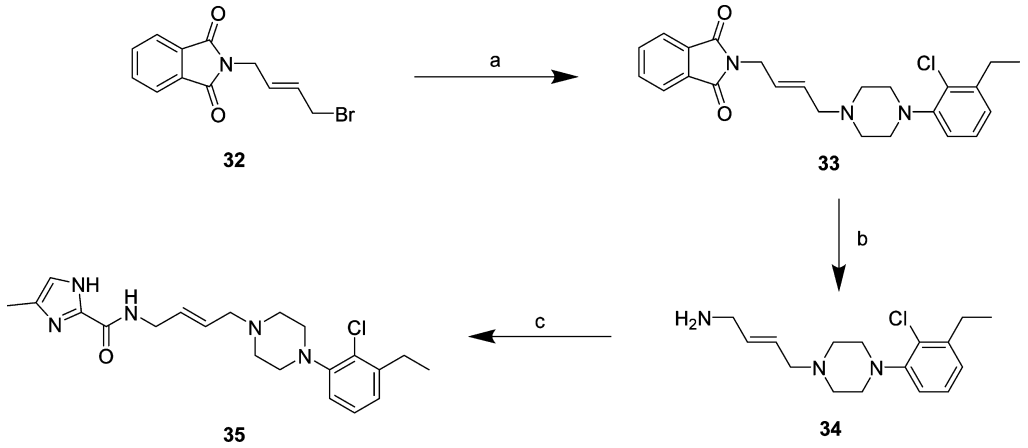
with D_3R or potentially D_2R selectivities and differing functional efficacies.[12]

Eticlopride (**3**, Figure 2) binds with subnanomolar affinity to both D_2R (K_i = 0.086 nM) and D_3R (K_i = 0.134 nM) but without selectivity (D_2R > D_3R; ratio = 0.64).[18] The high-resolution crystal structure of D_3R was solved with eticlopride and showed its primary pharmacophore (PP) to include the highly decorated 2-hydroxy, 3-ethyl, 5-chloro, 6-methoxy substituted benzamide function. Another commonly used D_2-like antagonist, raclopride[24] (**4**, Figure 2), is structurally similar to eticlopride but has a 3-chloro group instead of the 3-ethyl substituent. This simple substitution dramatically decreases its affinity at these receptor subtypes (D_3R K_i = 13.4 nM, D_2R K_i = 12.7 nM). Thus, the ethyl substitution, present in eticlopride, results in an increase of affinity for D_3R of ∼100 times and nearly 150 times at D_2R compared to raclopride.

When the D_3R-selective antagonist **2** was docked into the D_3R crystal structure, the 2,3-dichlorophenylpiperazine overlaid onto eticlopride and confirmed that this was the PP that binds to the OBS in the D_3R.[13,25] Subsequent synthon studies and SAR corroborated the molecular models and explained the roles of the OBS and SBP in binding affinities, selectivities, and

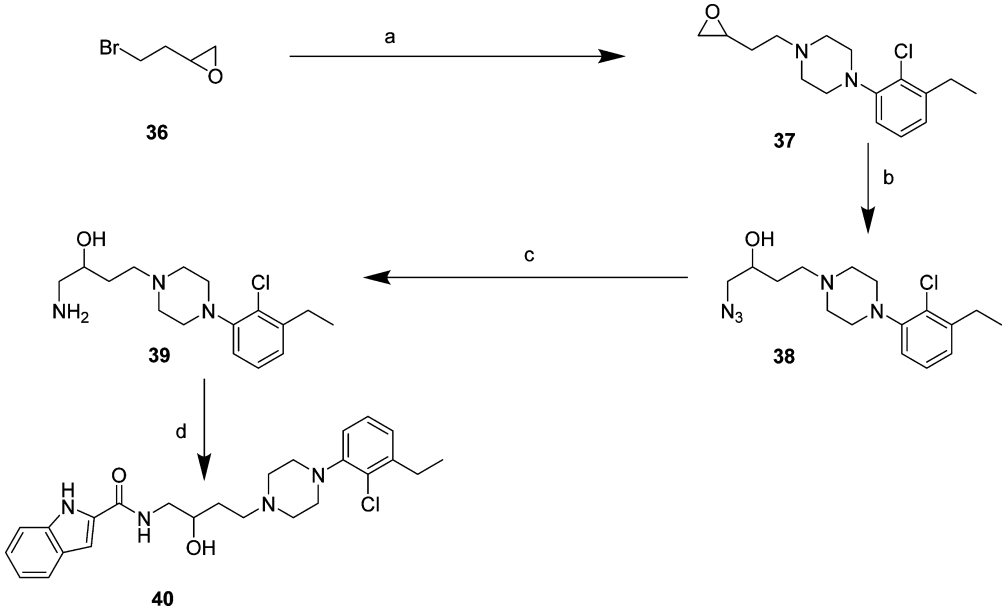
DOI: 10.1021/acs.jmedchem.6b00860
J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 2. Synthesis of 35[a]



[a]Reagents and conditions: (a) **11b**, K_2CO_3, acetone, reflux, overnight; (b) hydrazine, EtOH, reflux, overnight; (c) 4-methyl-1*H*-imidazole-2-carboxylic acid, CDI, THF, 0 °C to room temperature, overnight.

Scheme 3. Synthesis of 40[a]



[a]Reagents and conditions: (a) **11b**, K_2CO_3, acetone, reflux, overnight; (b) NaN_3, NH_4Cl, DMF, 100 °C, 6 h; (c) 10% Pd/C, H_2, EtOAc, 50 psi, 2 h; (d) indole-2-COOH, CDI, THF, 0 °C to room temperature.

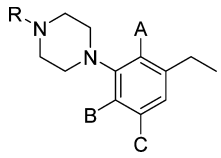
efficacies.[9,14] Herein, we reasoned that if the 2,3-dichloro-substituted phenylpiperazine was replaced with substituents borrowed from the eticlopride structure that the new templates might serve as PPs with potentially improved D_3R affinities, selectivities, and/or metabolic stability. Hence, we incorporated another privileged D_3R PP, the 2-methoxyphenylpiperazine, into our design to make the 3-chloro-5-ethyl-2-methoxyphenylpiperazine PP (Figure 2), which includes all but the 2-OH substituent from eticlopride and the 5-chloro, 6-methoxy substituent found in both eticlopride and raclopride. In addition, we designed a simpler hybrid PP, using the 2-Cl substituent from the 2,3-dichlorophenylpiperazine and the 3-ethyl group from eticlopride that gives rise to high affinity binding at D_2R and D_3R to give 2-chloro-3-ethylphenylpiperazine PP (Figure 2). Moreover, we explored the secondary pharmacophore with different heteroaryl amides and further investigated the 2-OH and 3-OH substituted butyl-linking chain. We had previously shown that although the 2-OH substituent retained high affinity binding at D_3R, the 3-OH gave higher D_3R selectivity.[26] Nevertheless, we wanted to determine if this remained the case when the PP was modified. After assessing in vitro binding and functional profiles, the most D_3R-selective compound in the series (**19**) was evaluated for metabolic stability in mouse microsomes. In addition, compound **19** was evaluated for its effects on locomotion in mice and its effects on increased locomotor activity induced by the prescription opioid analgesic, oxycodone, as well as oxycodone-induced conditioned place preference (CPP) in rats.

CHEMISTRY

The synthetic strategy used for the arylpiperazinebutyl carboxamide derivatives is shown in Scheme 1. Nitration of 3-chloro-4-methoxybenzoic acid (**5**) to **6** was followed by

DOI: 10.1021/acs.jmedchem.6b00860
J. Med. Chem. XXXX, XXX, XXX−XXX

Table 1. Human D_2-Like Receptor Binding Data for Synthons[a]



					$K_i \pm$ SEM (nM)					
compd	R	A	B	C	D_2R	D_3R	D_4R	D_2/D_3	D_4/D_3	D_4/D_2
11a	H	H	OMe	Cl	619 ± 97.6	446 ± 25.2	2300 ± 820	1.4	5.2	3.7
11b	H	Cl	H	H	139 ± 11.9	31.1 ± 0.462	316 ± 47.1	4.5	10	2.3
12a	Me	H	OMe	Cl	233 ± 22.4	179 ± 18.1	400 ± 83.4	1.3	2.2	1.7
12b	Me	Cl	H	H	58.1 ± 3.09	13.6 ± 0.488	89.9 ± 6.81	4.3	6.6	1.5
13a	*n*-But	H	OMe	Cl	29.8 ± 4.58	22.5 ± 4.86	322 ± 76.0	1.3	14	11
13b	*n*-But	Cl	H	H	2.93 ± 0.316	0.363 ± 0.052	65.1 ± 10.0	8.1	179	22

[a]Binding inhibition values determined using HEK 293 cells transfected with $hD_{2L}R$, hD_3R, or $hD_{4.4}$ and [^{3}H]*N*-methylspiperone radioligand as described.[59]

reduction to the alcohol **7**, using borane dimethyl sulfide complex, and oxidation to the aldehyde **8a**, in 84% yield.[27,28] Conversion of the aldehyde **8a** to the alkene **9a** was conducted under Wittig reaction conditions, and the nitro group was reduced to the aniline **10a** via catalytic hydrogenation.[29] Compound **10a** was cyclized to the *N*-aryl piperazine **11a**, and *N*-alkylation was carried out either through reductive amination to give **12a** or reacting with the appropriate alkyl halide under basic conditions to give **13a**.[30] Compound **14a** was generated by reacting the aryl piperazine **11a** with 4-bromobutyl phthalimide. The alcohol intermediate **15a** was synthesized via epoxide opening of 2-(2-oxiran-2-yl)ethyl)isoindoline-1,3-dione with **11a**, in 56% yield, using a previously reported procedure.[31,32] Deprotection of **14a** and **15a**, using hydrazine, gave the free amines **16a** and **17a**, respectively, which were reacted with the respective aryl carboxylic acid or ester to afford the 3-chloro-5-ethyl-2-methoxyphenylpiperazinebutylarylamide derivatives (**18**–**21**).[33,34] A similar strategy was used to synthesize the 2-chloro-3-ethylphenylpiperazinebutylarylamide derivatives (**22**–**31**) starting from **8b**.[35,36]

In Scheme 2, **11b** was *N*-alkylated with 4-bromobutenephthalimide (**32**)[37] to afford **33** in 98% yield. Deprotection using hydrazine gave the free amine **34**, which was reacted with 4-methyl-1*H*-imidazole-2-carboxylic acid in the presence of CDI (1,1′ carbonyldiimidazole) to give **35**.

In Scheme 3, **11b** was *N*-alkylated with 2-(2-bromoethyl)-oxirane (**36**)[38] to give epoxide **37** in 41% yield. The epoxide was regioselectively opened with sodium azide to **38** in 53% yield and reduced via catalytic hydrogenation to the amino-alcohol **39**, which was coupled with indole-2-carboxylic acid to give the 2-OH substituted analogue, **40**.[39,40] Note, all final compounds with hydroxylated linking chains are the racemic mixtures.

PHARMACOLOGICAL RESULTS AND DISCUSSION

To further dissect the contributions of individual pharmacophore components to D_3R selectivity and efficacy, we extended our previous SAR studies by modifying all three segments of the classical D_3R antagonist template (Figure 2). We first evaluated the binding affinities of this series of compounds by performing competition binding studies with [^{3}H]*N*-methylspiperone using membranes prepared from HEK293 cells stably expressing either the human D_2R, D_3R, or D_4R (Tables 1 and 2.) In addition, in Table 2, cLogP values and polar surface area (PSA) were calculated to provide measures of lipophilicity and predicted brain penetration, respectively, for all compounds.[41]

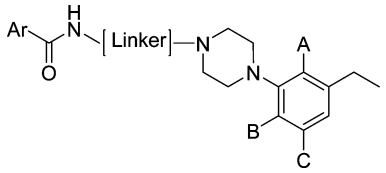
The privileged PPs, 2,3-dichlorophenylpiperazine and 2-methoxyphenylpiperazine, were replaced with the eticlopride-inspired synthons (**11a** and **11b**). These new PPs showed moderate binding affinities for both D_3R and D_2R receptors with relatively low binding affinities to D_4R and little or no selectivity, as shown in Table 1. As previously observed, when the alkyl linking chain was extended from the *N*-phenylpiperazine to *N*-methyl (**12a** and **12b**) and *N*-*n*-butyl analogues (**13a** and **13b**), binding affinities were improved for both D_3R and D_2R.[14,22,42] Of this set of synthons, compound **13b** demonstrated the highest D_3R affinity (K_i = 0.363 nM) and was the most D_3R selective (D_2R/D_3R = 8.1; D_4R/D_3R = 179).

Binding data for the full-length substituted ligands are shown in Table 2. The majority of analogues demonstrated binding affinities in the low to subnanomolar range for D_3R. In the 1-(2-chloro-3-ethylphenyl)piperazine PP based series (**22**–**31**), both **22** and **23** showed subnanomolar affinities for D_3R (K_i = 0.142 and 0.362 nM, respectively). Moreover, **23** was highly D_2R/D_3R selective (>400-fold). When the indole moiety of **22** and **23** was bioisoterically replaced with benzofuran, subnanomolar D_3R binding affinities were maintained for both **24** and **25**. The indole ring was also replaced with other heteroaryl ring systems, such as 2-(imidazo[1,2-*a*]pyridine) (**26** and **27**), 5-(6-methylimidazo[2,1-*b*]thiazole) (**28**), 2-(4-ethyl-1*H*-imidazole) (**29**), and 2-(4-methyl-1*H*-imidazole) (**30** and **31**). However, in most cases, although high D_3R binding affinities were retained, relative improvements in D_2R binding affinities reduced D_3R selectivity. No major change in the affinity and selectivity of **30** was observed when a *trans*-butenyl linker was introduced between the aryl amide and piperazine moiety to afford **35**.

In the more highly decorated 1-(3-chloro-5-ethyl-2-methoxyphenyl)piperazine-based series (**18**–**21**), the indole **18** showed a similar binding profile to its analogue **22** but with slightly higher D_3R selectivity (D_2R/D_3R = 82 versus 39). Compound **19** exhibited lower affinity to D_3R (K_i = 6.84 nM), but this analogue had the greatest D_3R versus D_2R binding selectivity (1700-fold) of all the 4-phenylpiperazines that we have synthesized and evaluated to date. The benzofuran derivative **20** showed reduced D_3R binding affinity (K_i = 3.19 nM) compared to **24** (K_i = 0.153 nM) but was similarly D_3R selective, whereas **21** (K_i = 36.1 nM) showed significantly

DOI: 10.1021/acs.jmedchem.6b00860
J. Med. Chem. XXXX, XXX, XXX−XXX

Table 2. Human D_2-Like Receptor Binding Data on Full-Length Analogues[a]



Compd	Series	Ar	A	B	C	Linker	cLogP[b]	PSA[c]	$D_{2L}R$	D_3R	$D_{4.4}R$	D_2R/D_3R
									K_i± S.E.M. (nM)			
18	a		H	OMe	Cl		5.53	56.84	27.8±6.71	0.341±0.031	1050±279	82
19	a		H	OMe	Cl		4.93	77.07	11400±3270	6.84±1.18	13900±1350	1700
20	a		H	OMe	Cl		5.50	54.04	137±9.00	3.19±0.268	714±293	43
21	a		H	OMe	Cl		4.91	74.27	1620±339	36.1±5.00	5290±319	45
22	b		Cl	H	H		5.76	47.61	5.50±0.805	0.142±0.025	334±113	39
23	b		Cl	H	H		5.17	67.84	151±25.4	0.362±0.047	5520±1660	417
24	b		Cl	H	H		5.74	44.81	6.34±0.959	0.153±0.005	356±64.4	41
25	b		Cl	H	H		5.14	65.04	164±32.4	0.985±0.105	2380±110	166
26	b		Cl	H	H		5.52	51.18	5.64±0.98	0.331±0.085	819±175	17
27	b		Cl	H	H		4.93	71.41	193±40.5	4.23±0.84	>10000	46
28	b		Cl	H	H		5.43	51.18	4.65±1.24	0.941±0.13	143±3.18	5
29	b		Cl	H	H		5.38	59.97	12.5±1.07	1.14±0.17	500±30.9	11
30	b		Cl	H	H		4.39	59.97	12.2±2.58	0.621±0.096	529±42.3	20
31	b		Cl	H	H		3.79	80.2	150±13.1	11.2±2.66	1960±344	13
35	b		Cl	H	H		4.31	59.97	16.1±1.2	1.28±0.22	503±157	13
40	b		Cl	H	H		5.05	67.84	6.83±0.53	0.200±0.018	305±70.5	34

[a]Binding inhibition values determined using HEK 293 cells transfected with $hD_{2L}R$, hD_3R, or $hD_{4.4}$ and [^{3}H]*N*-methylspiperone radioligand as described.[59] [b]Partition coefficients (clogP) were calculated using ChemDraw Professional Ultra 15.0. [c]Calculated according to ref 41.

reduced D_3R affinity compared to **25** (K_i = 0.985 nM) and was less D_3R selective. As expected, the introduction of a 3-OH group in the butyl linking chain of both series resulted in reduced binding affinities at D_2R and D_3R as well as somewhat

DOI: 10.1021/acs.jmedchem.6b00860
J. Med. Chem. XXXX, XXX, XXX−XXX

lower cLogP values, predicting decreased lipophilicity compared to corresponding parent aliphatic compounds. What was unexpected was the dramatic decrease in affinities in the "a" series as compared to the "b" series, when the 3-OH was introduced. In contrast, compared to all the 3-OH-butyl analogues, the 2-OH-butyl derivative **40** showed high D_3R affinity (K_i = 0.20 nM) but as previously observed for other 2-OH-substituted 4-phenylpiperazine analogues,[26] it exhibited lower D_3R selectivity, similar to compounds that had no OH substitution in the linking chain. None of the compounds demonstrated high binding affinity for D_4R. PSA values ranged from 45 to 80 with cLogP values in the range of 3.8−5.8, suggesting relatively high lipophilicity predictive of good blood−brain barrier penetration.

Functional Data and Off-Target Actions. D_3R efficacy was measured in a cell-based mitogenesis assay wherein a subset of compounds was tested for stimulation or inhibition of quinpirole-stimulated mitogenesis in CHO cells (Table 3.)

Table 3. Functional Data for Selected Compounds Using Stimulation or Inhibition of Quinpirole-Stimulated Mitogenesis in CHO Cells with Human Dopamine D_3R[a]

compd	agonist EC_{50} ± SEM (nM)	% stimulation	Antagonist IC_{50} ± SEM (nM)
11b	17.9 ± 0.48	64.7	>10000
12a	>9000	15.2	1840 ± 700
12b	710 ± 230	69.2	ND[b]
13a	>6600	17.2	930 ± 170
13b	590 ± 210	29.1	63 ± 11
18	>8300	5.8	330 ± 100
19	>8300	6.7	360 ± 100
20	>6600	4.9	420 ± 130
21	>7100	3.6	950 ± 350
22	4.70 ± 0.57	42.3	19.1 ± 3.5
23	2.58 ± 0.87	17.9	50.5 ± 7.2
24	410 ± 130	31.2	18.5 ± 6.3
25	196 ± 64	22.3	230 ± 27

[a]Data were obtained through the NIDA Addiction Treatment Discovery Program contract (ADA151001) with Oregon Health and Science University, using published methods.[21] [b]ND = not determined.

Interestingly, all of the synthons (**11**−**13**) demonstrated partial agonist profiles, with the 2-chloro-3-ethyl-phenylpiperazine "b" series being more efficacious than the 3-chloro-5-ethyl-2-methoxy-phenylpiperazine "a" series. This profile was repeated in the full-length molecules wherein the more highly decorated "a" series (more eticlopride-like) analogues (**18**−**21**) were all antagonists in the mitogenesis assay. As described previously, the 2-OCH_3-substituted 4-phenylpiperazine precludes the formation of H-bonds with transmembrane (TM) 5 serines (5.42 and 5.43) in contrast to the 3-Cl substituent in the 2,3-dichlorophenylpiperazine that positions these molecules in the OBS driving its transition to an active state. These residues are the same that form H-bonds with the catechol hydroxyl groups of dopamine and give rise to an agonist profile.[9,14] In contrast, the "b" series analogues **22**−**25** were all partial agonists at D_3R wherein there is no 3-Cl substituent but rather the PP is a 2-chloro-3-ethylphenylpiperazine. Molecular simulations studies are underway to address the molecular mechanism underlying the efficacy profiles of this series of compounds.

The same set of analogues that were evaluated in the D_3R mitogenesis assay was also tested for binding affinities at $5HT_{1A}$, $5HT_{2A}$, and $5HT_{2C}$ receptors (Table 4). In general, the "a" series of synthons demonstrated lower affinities at these 5HT receptor subtypes than the "b" series and this pattern was repeated in the full-length molecules (**18**−**21**) versus (**22**−**25**). Also, the "b" series synthons were functional agonists at $5HT_{1A}$ with moderately high potency, as were corresponding full-length molecules **22**, **24**, and **25**. All of the full-length analogues were selective for D_3R over the 5HT receptor subtypes, with compound **19** demonstrating the most selective profile in the series ($5HT_{1A}/D_3R$ = >1000; $5HT_{2A}/D_3R$ = 27; $5HT_{2C}/D_3R$ = 320). Hence this compound was selected for further evaluation. It is important to note that compound **19** does not bind to μ, δ, or κ opioid receptors at a concentration of 10 μM (data obtained through the NIDA Addiction Treatment Discovery Program contract (ADA151001) with Oregon Health and Science University) and thus there is no direct effect of this compound on the opioid receptor system.

Mouse Microsomal Metabolism Results. Compound **19** was tested for phase I metabolism following procedures previously described to predict the susceptibility to metabolism following in vivo administration.[43] Briefly, compound **19** was incubated in mouse liver microsomal incubations in the presence of NADPH and compound disappearance was measured over time using HPLC with tandem mass spectrometry (LC/MS/MS). As depicted in Figure 3 (see also Supporting Information, Table S2), compound **19** showed excellent metabolic stability with >80% remaining over 1 h. These data supported testing compound **19** in preclinical behavioral models of opioid dependence.

Locomotor Activity Studies in Mice with Compound 19 in the Absence and Presence of Oxycodone. Dopamine D_3R antagonists are effective in attenuating the self-administration of heroin, alcohol, or nicotine but not cocaine or methamphetamine under low fixed-ratio reinforcement schedules (e.g., FR1).[1,9] However, little is known as to whether D_3R antagonists are similarly effective in attenuating the addiction-related behaviors of prescription opioids. Opioid-induced locomotor sensitization and conditioned place preference (CPP) are commonly used animal models to evaluate pharmacological agents for their utility in the treatment of addiction.[44,45] Given the rapid increase in nonmedical use of prescription opioids such as oxycodone and hydrocodone, the development of new and effective medications for the treatment of opioid abuse and addiction is urgent.[46,47] Indeed, the recent rise in opioid overdose in the United States has been referred to as "epidemic".[48,49]

Oxycodone is the most commonly used prescription opioid analgesic. Like other opioid agonists such as morphine or heroin, acute (or single dose) administration of oxycodone produces a significant increase in locomotor activity in mice. Moreover, repeated administration of these opioid agonists produces a progressive increase in locomotion over time, i.e., locomotor sensitization.[50] In this study, we sought to (1) determine if compound **19** alone had any effect on basal locomotor activity, (2) if it would attenuate oxycodone-stimulated locomotor activity and sensitization, and (3) if this effect was long-lasting. The results of this evaluation are shown in Figure 4A−D. Figure 4A shows the overall locomotor effects of repeated, daily administration of oxycodone (4 mg/kg, ip) in the presence of vehicle (25% β-cyclodextrin) or one dose of compound **19** (5, 15 mg/kg, ip administered 15 min before

DOI: 10.1021/acs.jmedchem.6b00860
J. Med. Chem. XXXX, XXX, XXX−XXX

Table 4. Additional in Vitro Binding and Functional Data for Selected Compounds at $5HT_{1A}$, $5HT_{2A}$, and $5HT_{2C}$ Receptors[a]

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)	$5HT_{1A}$ [^{35}S]GTPγS binding	
				agonist EC_{50} ± SEM (nM)	% stimulation
11a	3560 ± 490	90.4 ± 4.7	22.4 ± 1.9	ND[b]	
11b	70.3 ± 4.2	7.00 ± 2.1	5.40 ± 1.6	77.0 ± 23	90.1
12a	4400 ± 1000	140 ± 14	17.2 ± 5.3	ND[b]	
12b	40.3 ± 8.9	3.22 ± 0.93	4.00 ± 1.3	27.7 ± 8.3	89
13a	2260 ± 490	470 ± 130	1000 ± 190	ND[b]	
13b	37.0 ± 8.9	53.0 ± 15	222 ± 59	48.0 ± 13	90.8
18	2330 ± 700	60.1 ± 9.5	144 ± 37	ND[b]	
19	>7600	188 ± 22	2190 ± 460	ND[b]	
20	560 ± 170	25.0 ± 6.6	55.0 ± 17	ND[b]	
21	2410 ± 830	42.9 ± 6.8	268 ± 34	ND[b]	
22	59 ± 13	5.22 ± 0.98	18.3 ± 2.2	149 ± 47	94.3
23	880 ± 160	28.1 ± 9.7	113 ± 33	ND[b]	
24	26.0 ± 8.1	2.41 ± 0.82	18.7 ± 4.5	31.0 ± 10	99.4
25	27.6 ± 5.3	3.20 ± 1.1	89.0 ± 22	118 ± 39	101.1

[a]Data were obtained through the NIDA Addiction Treatment Discovery Program contract (ADA151001) with Oregon Health and Science University. [b]ND = not determined. Functional assays for each receptor was not conducted if the K_i value for the binding assay was >250 nM for 5-HT receptors.

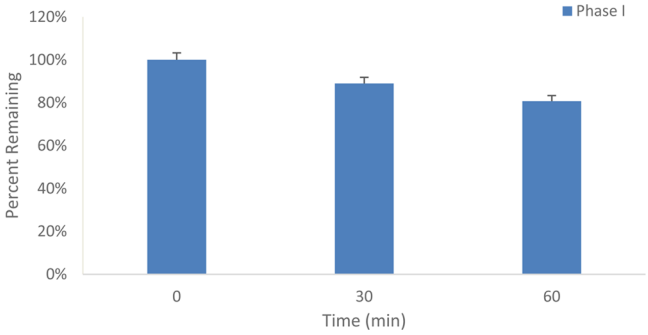


Figure 3. Mouse microsomal phase I metabolism data for compound **19**. Compound **19** was incubated in mouse liver microsomes with NADPH regenerating system, and compound disappearance was measured over time via LC/MS/MS. Compound **19** showed excellent metabolic stability substantiating its use in in vivo efficacy studies.

oxycodone injection). Compound **19** alone had no effect on basal locomotor activity in mice when tested on day 1 (Figure 4A,B). In contrast, oxycodone caused significant increases in locomotor activity (Figure 4A,C). Repeated oxycodone administration produced a progressive increase in locomotion (sensitization) over time (from day 2 to day 6 in the vehicle pretreatment group). Strikingly, pretreatment with compound **19** not only attenuated acute oxycodone-induced hyperactivity (Figure 4A, day 2) but also blocked the acquisition of repeated oxycodone-induced locomotor sensitization (Figure 4A, day 2-day 6) and the expression of oxycodone prime-induced locomotor sensitization after 2 days of withdrawal (Figure 4A, day 9). Two-way ANOVA for repeated measures over time (Figure 4A) revealed a statistically significant drug treatment main effect ($F_{2,21}$ = 12.24, p < 0.001), time main effect ($F_{6, 49}$ = 9.14, p < 0.001), and treatment × time interaction ($F_{12, 98}$ = 31.51, p < 0.001). Figure 4B–D shows the time courses of oxycodone-induced change in locomotor activity when tested on day 1 (Figure 4B: $F_{2,21}$ = 0.12, p = 0.88), day 2 (Figure 4C: $F_{2,21}$ = 5.22, p < 0.05) and day 9 (Figure 4D: $F_{2,21}$ = 5.95, p < 0.01), indicating that compound **19** pretreatment significantly attenuated oxycodone-induced increases in locomotion on each test day.

Effects of compound 19 on Oxycodone-induced CPP in Rats. Figure 5 shows the effects of compound **19** on oxycodone-induced CPP. Four days of oxycodone (e.g., Vehicle + oxycodone group) (3 mg/kg, ip) versus saline conditioning produced significant CPP compared to the (Vehicle + saline) control group of rats. Pretreatment with **19** (5, 15 mg/kg, ip, 15 min before each oxycodone injection) dose-dependently attenuated oxycodone-induced CPP (Figure 5). One-way ANOVA revealed a statistically significant treatment main effect ($F_{4,45}$ = 4.29, p < 0.01). Posthoc individual group comparisons indicated a significant reduction in oxycodone-induced CPP after 15 mg/kg **19** (p < 0.05) compared to (Vehicle + oxycodone) group. Compound **19** alone, at 15 mg/kg, did not produce CPP, suggesting a lack of reward by itself.

CONCLUSION

In the present study, we combined small molecule SAR with clues from the D_3R crystal structure to design two novel series of D_3R-selective antagonists and partial agonists. In the synthon study, PPs were synthesized including their alkyl homologated analogues, extending our previous findings to show that these compounds generally bind to both D_2R and D_3R but with varying affinities and modest, if any, D_3R selectivity, depending on the phenyl ring substitutions and the *N*-alkyl group. The 3-ethyl-2-chloro-substituted "b" series typically showed higher affinity binding at D_3R than the more highly substituted "a" series, with compound **13b** showing the highest D_3R affinity and selectivity. In addition, the "b" series synthons were far more efficacious in the D_3R mitogenesis functional assay, suggesting a novel interaction in the OBS that clearly dictates the efficacy of the full-length molecules in this series. In general, the full-length molecules based on both the new PPs, 3-chloro-5-ethyl-2-methoxyphenylpiperazine and 2-chloro-3-ethylphenylpiperazine, showed high D_3R binding affinities and selectivities over D_2R and D_4R. However, the 2-chloro-3-ethylphenylpiperazines had higher affinities for both D_2R and D_3R and as such were not as D_3R selective compared to their 3-chloro-5-ethyl-2-methoxyphenylpiperazine counterparts. Nevertheless, several of these analogues were quite D_3R

DOI: 10.1021/acs.jmedchem.6b00860
J. Med. Chem. XXXX, XXX, XXX−XXX

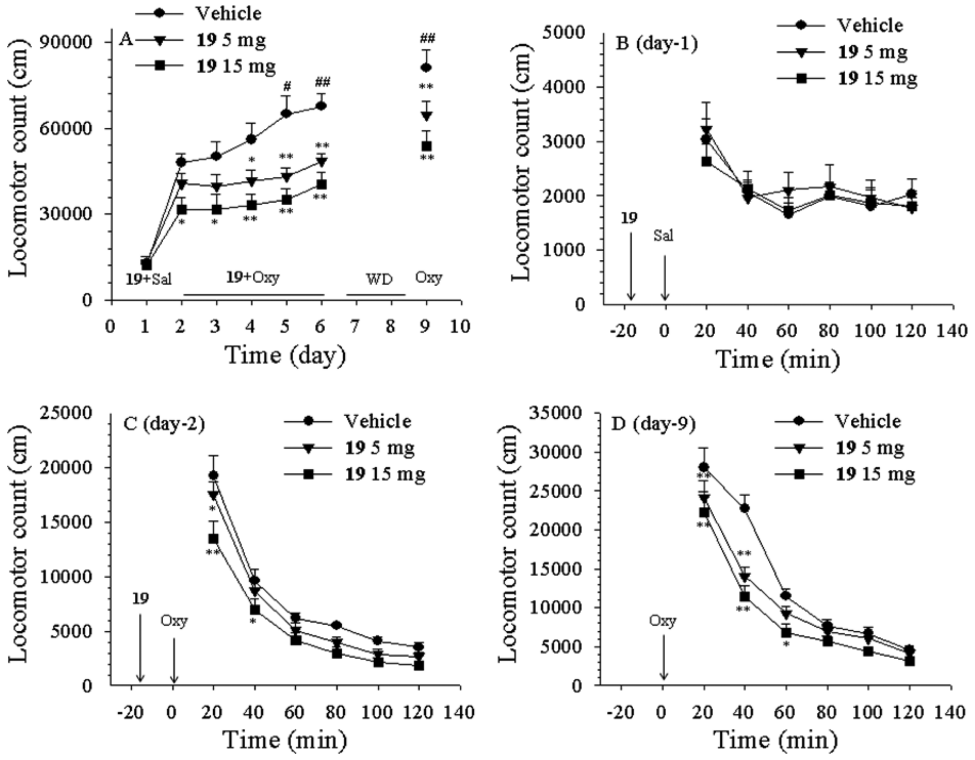


Figure 4. Effects of compound **19** (0, 5, 15 mg/kg, ip) on basal and oxycodone-induced increases in locomotor activity in mice. (A) Effects of oxycodone on locomotion over time (from day 2 to day 9) in the presence or absence of compound **19** treatment. On day 1, compound **19** alone (5, 15 mg/kg, 15 min before saline) was administered and had no effect on locomotion. From day 2 to day 6, each animal received one daily dose of **19** (vehicle, 5, 15 mg/kg, 15 min prior to oxycodone) and oxycodone (4 mg/kg) administration. In the vehicle treatment group, repeated daily administration of oxycodone produced a progressive increase in locomotion (i.e., locomotor sensitization) ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$, compared to the level of locomotor activity on day 2). This locomotor sensitization was dose-dependently blocked by **19** ($^{*}p < 0.05$, $^{**}p < 0.01$, compared to the vehicle control group at each time point labeled). After 5 days of the (vehicle/**19** + oxycodone) coadministration, animals underwent 2 days of withdrawal, followed by a 4 mg/kg oxycodone challenge injection (without **19** pretreatment) on day 9, indicating that oxycodone-induced locomotor sensitization was still present in the vehicle control group, which was blocked in the **19** pretreatment groups. (B) Time course of **19**-induced changes in locomotion (day 1), indicating that it has no effect on locomotor activity by itself. (C) Time course of oxycodone-induced changes in locomotion after the first injection of oxycodone (acute effect on day 2), indicating that **19** pretreatment dose-dependently inhibited oxycodone-induced hyperactivity (acute effect). (D) Time course of oxycodone priming-induced changes in locomotion in mice after 2 days of withdrawal from the last (vehicle/**19** + oxycodone) coadministration (day 9), indicating that repeated **19** pretreatment from day 2 to day 6 produced a long-lasting inhibition in oxycodone-induced increases in locomotion. $N = 8$ mice in each group.

selective (e.g., **23** and **25**) and given their partial agonist profile will be evaluated in vivo in due course.

The replacement of the indole secondary pharmacophore with other heteroaryl ring systems showed no improvement in D_3R selectivity in either series of molecules; on the basis of these and other SAR studies, the indole amide should be considered a privileged secondary pharmacophore for D_3R.[22] In addition, the indole-2-carboxamide scaffold occupies a secondary binding pocket between TM2 and TM7 within D_2R, giving rise to a negative allosteric modulator profile.[22] In the present study, no attempt to elucidate allosterism at D_3R was made, but it is intriguing to consider that depending on the PP, these molecules may show a bitopic profile. Identifying the structural determinants of these potentially bitopic agents will be of future interest.

The lead compound **19** was chosen in the present study based on its highly D_3R-selective profile in vitro. Indeed, this compound appears to be one of the most selective D_3R antagonist reported in the literature to date. This compound demonstrated exceptional metabolic stability in mouse microsomes that was significantly improved over our previous lead compound **2**.[18]

Locomotor sensitization is a commonly used animal model to determine the potential use of novel compounds for the treatment of substance use disorders.[51−54] In this model, compound **19** alone had no effect on basal locomotor activity. However, pretreatment with compound **19** significantly inhibited oxycodone-induced hyperlocomotion and reduced repeated oxycodone-induced locomotor sensitization in a dose-dependent manner, which provides in vivo evidence that the D_3R is a viable target for opioid use disorder medication development. To further determine the potential utility of compound **19** in treatment of prescription opioid abuse and dependence, we observed the effects of repeated **19** pretreatment for 4 consecutive days on the development of oxycodone-induced CPP. We found that compound **19** also dose-dependently blocked the acquisition or ability of oxycodone to establish CPP, suggesting that **19** pretreatment may prevent the development of oxycodone dependence. This is consistent with our recent finding that blockade of D_3Rs by another set of D_3R-selective partial agonists also inhibited intravenous heroin self-administration in wild-type mice but not in D_3R-knockout mice.[9] Given compound **19**'s exceptional D_3R-selectivity profile, the behavioral effects observed in the present study are likely mediated via blockade of central D_3Rs. This

DOI: 10.1021/acs.jmedchem.6b00860
J. Med. Chem. XXXX, XXX, XXX−XXX

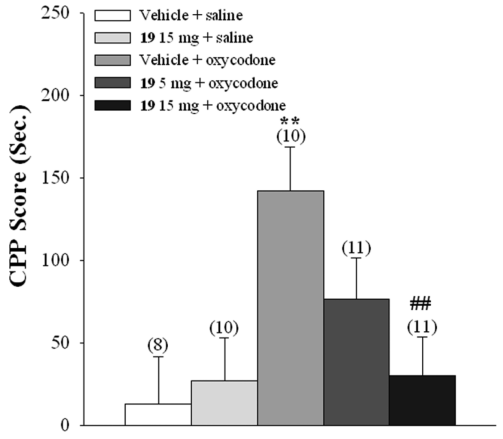


Figure 5. Effects of compound **19** on oxycodone-induced conditioned place preference. Oxycodone (3 mg/kg, ip) conditioning in the (Vehicle + oxycodone) produced a robust place preference to the oxycodone-paired compartment. Pretreatment with **19** (5, 15 mg/kg, ip, 15 min before oxycodone injection) dose-dependently blocked the acquisition of oxycodone-induced CPP. Compound **19** alone (15 mg/kg, ip) in the (**19** + saline) group did not produce significant place preference. ** $p < 0.01$, compared to the (Vehicle + vehicle) group; ## $p < 0.01$, compared to the (Vehicle + oxycodone) group. The number on each bar shows the animal number in each experimental group.

hypothesis is supported by recent findings that genetic deletion of D_3R in D_3-knockout mice blocked morphine-induced hyperactivity and repeated morphine-induced locomotor sensitization.[55]

Oxycodone is one of the most commonly used prescription opioids in clinical pain management. Nevertheless, a severe adverse effect that can arise with oxycodone's chronic use is its potential to produce dependence in patients. Epidemic-like increases in prescription opioid addiction and overdose in the U.S. and abroad strongly support consideration of novel mechanisms for treatment.[56−58] Of course, somatic symptoms associated with opioid overdose are unlikely to be modified by this class of drugs. These sometimes fatal consequences will require other medication strategies such as the opioid antagonist naloxone. Nevertheless, discovery of medications that might prevent relapse to drug seeking or, ideally, the development of dependence for patients that require long-term opioid therapy is of vital importance. The studies herein point to the D_3R as a potential therapeutic target and compound **19** as a new lead molecule for development.

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, Acros Organics, Maybridge, and Alfa Aesar. All amine final products were converted into the oxalate salt. Spectroscopic data and yields refer to the free base form of all 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 and polar surface area (PSA) values were calculated using ChemDraw Professional Ultra 15.0. Melting point determination was conducted using a Thomas−Hoover melting point apparatus and are uncorrected. On the basis of NMR and combustion data, all final compounds are ≥95% pure.

*3-Chloro-4-methoxy-5-nitrobenzoic Acid (**6**).* 3-Chloro-4-methoxybenzoic acid (**5**, 5.0 g, 26.8 mmol) was added in small portions to cold fuming HNO_3 (90%, 25 mL) at 0−5 °C. The reaction mixture was allowed to warm to 20 °C and stirred for additional 2 h. Cold water (50 mL) was added, and the precipitated product was filtered under the vacuum. The solid product was dissolved in $CHCl_3$, washed with brine solution and concentrated. The product was purified by flash chromatography using 8% MeOH/$CHCl_3$ as eluent to provide 4.87 g (79%) of product. ^{1}H NMR (400 MHz, CD_3OD) δ 8.27 (s, 1H), 8.22 (s, 1H), 4.02 (s, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 165.9, 153.9, 146.4, 136.3, 131.56, 125.9, 63.2. GC-MS (EI) m/z 231 (M^+).

*(3-Chloro-4-methoxy-5-nitrophenyl)methanol (**7**).* Borane dimethyl sulfide complex (10 M, 3.07 g, 40.4 mmol) was added dropwise to a solution of **6** (4.46 g, 19.3 mmol) in THF (60 mL) at 0−5 °C. The mixture was allowed to come to room temperature and stirred overnight. The reaction mixture was cooled to 0−5 °C and quenched carefully by dropwise addition of MeOH. The reaction mixture was evaporated (4.7 g) and used directly in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49−8.38 (m, 2H), 5.50 (s, 2H), 4.82 (m, 3H), 3.70 (bs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.9, 145.2, 137.9, 132.5, 130.6, 121.3, 63.1, 62.5. GC-MS (EI) m/z 217 (M^+).

*3-Chloro-4-methoxy-5-nitrobenzaldehyde (**8a**).*[28] Pyridinium chlorochromate (PCC, 9.31 g, 43.2 mmol) was added to a solution of **7** (4.70 g, 21.6 mmol) in CH_2Cl_2 (100 mL). The reaction mixture was stirred overnight and filtered through Celite. The product was purified by flash chromatography using 20% EtOAc/hexanes as eluent to provide 3.92 g (84%) of the product as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.94 (s, 1H), 8.19 (d, J = 2.0 Hz, 1H), 8.13 (d, J = 2.0 Hz, 1H), 4.10 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 187.8, 154.4, 145.7, 134.5, 132.2, 131.9, 124.8, 63.0. GC-MS (EI) m/z 215 (M^+).

*1-Chloro-2-methoxy-3-nitro-5-vinylbenzene (**9a**).* *N*-BuLi (12.0 mL, 19.2 mmol, 1.6 M in hexane) was added dropwise to a suspension of methyltriphenylphosponium bromide (7.20 g, 20.2 mmol) in dry THF (100 mL) at −78 °C under argon. The reaction mixture was allowed to warm to 0 °C and stirred for 1 h. The reaction mixture was again cooled to −78 °C, and the solution of **8a** (3.95 g, 18.3 mmol) in THF (50 mL) was added dropwise over 30 min. The reaction mixture was allowed to warm to 0 °C and stirred for 1 h. The reaction mixture was quenched with saturated NH_4Cl solution (50 mL), and the crude product was extracted with EtOAc (3 × 25 mL). The organic layer was combined, dried, concentrated, and purified using flash chromatography with 5% EtOAc/hexanes as eluent to provide 1.27 g (33%) of the product as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (s, 1H), 7.51 (s, 1H), 6.55−6.47 (m, 1H), 5.69 (d, J = 16 Hz, 1H), 5.32 (d, J = 10.8 Hz, 1H), 3.92 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.7, 145.2, 134.5, 133.1, 131.6, 130.5, 120.8, 117.2, 62.3. GC-MS (EI) m/z 213 (M^+).

*2-Chloro-1-nitro-3-vinylbenzene (**9b**).* The same procedure was used as described for **9a**, starting from **8b** to give the desired product in 70% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (dd, J = 8.0, 1.6 Hz, 1H), 7.64 (dd, J = 8.0, 1.6 Hz, 1H), 7.37 (t, J = 8.0 Hz, 1H), 7.12 (dd,

DOI: 10.1021/acs.jmedchem.6b00860
J. Med. Chem. XXXX, XXX, XXX−XXX

J = 17.2, 11.0 Hz, 1H), 5.81 (dd, J = 17.2, 0.8 Hz, 1H), 5.54 (dd, J = 10.8, 0.8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.6, 138.9, 132.1, 129.9, 127.2, 124.8, 123.9, 119.6.

*3-Chloro-5-ethyl-2-methoxyaniline (**10a**).* A mixture of **9a** (1.27 g, 5.94 mmol) and 10% Pd/C (0.20 g) in EtOH (30 mL) was stirred under an atmosphere of hydrogen (50 psi) at room temperature for 3 h. The reaction mixture was filtered through a Celite pad and evaporated under vacuum. The reaction mixture was sufficiently pure to be used for the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.60 (d, J = 2.0 Hz, 1H), 6.44 (d, J = 2.0 Hz, 1H), 3.96 (bs, 2H), 3.83 (s, 3H), 2.48 (q, J = 7.6 Hz, 2H), 1.19 (t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.3, 141.0, 140.9, 127.0, 118.3, 113.7, 59.4, 28.1, 15.2. GC-MS (EI) m/z 185 (M^+).

*2-Chloro-3-ethylaniline (**10b**).* The same procedure was used as described for **10a** using EtOAc as the solvent. The product was sufficiently pure to be used for the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.99 (t, J = 7.6 Hz, 1H), 6.66–6.62 (m, 2H), 4.04 (bs, 2H), 2.72 (q, J = 7.6 Hz, 2H), 1.22 (t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.1, 142.4, 126.9, 119.2, 118.9, 113.4, 27.1, 13.9.

*1-(3-Chloro-5-ethyl-2-methoxyphenyl)piperazine (**11a**).* The reaction mixture of **10a** (0.63 g, 3.39 mmol) and bis(2-chloroethyl)-amine hydrochloride (0.67 g, 3.73 mmol) in diethylene glycol monomethyl ether (2.0 mL) was heated at 150 °C for 7 h. The reaction mixture was allowed to come to room temperature and dissolved in MeOH (5 mL) followed by dilution with ether (250 mL). The salt was filtered, suspended in $CHCl_3$ (20 mL), and neutralized with 2N NaOH to pH 8–9. The organic layer was collected, concentrated, and purified by column chromatography using 2% MeOH/$CHCl_3$ as eluent to provide 0.36 g (56%) of solid product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.84 (d, J = 2 Hz, 1H), 6.61 (d, J = 2.4 Hz, 1H), 3.85 (s, 3H), 3.08–3.01 (m, 8H), 2.55 (q, J = 8 Hz, 2H), 1.20 (t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.8, 146.6, 140.9, 128.4, 122.4, 116.9, 59.2, 51.9, 46.8, 28.6, 15.6. GC-MS (EI) m/z 254.2 (M^+). The oxalate salt was precipitated from acetone; mp 190–191 °C. Anal. $C_{13}H_{19}ClN_2O{\cdot}C_2H_2O_4{\cdot}0.5H_2O$) C, H, N.

*1-(2-Chloro-3-ethylphenyl)piperazine (**11b**).* The same procedure was used as described for **11a**. The product was purified by flash chromatography using 2% MeOH/$CHCl_3$/ 0.1% NH_4OH as eluent to give the desired product in 46% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16–7.12 (m, 1H), 6.94–6.89 (m, 2H), 3.05–2.95 (m, 8H), 2.76 (q, J = 7.6 Hz, 2H), 1.21(t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.0, 143.2, 128.7, 126.9, 123.9, 118.0, 53.0, 46.3, 27.5, 14.1. The oxalate salt was precipitated from acetone; mp 166–167 °C. Anal. ($C_{12}H_{17}ClN_2{\cdot}1.5C_2H_2O_4{\cdot}0.1NH_4OH$) C, H, N.

*1-(3-Chloro-5-ethyl-2-methoxyphenyl)-4-methylpiperazine (**12a**).* Sodium triacetoxyborohydride (0.30 g, 1.2 mmol) and AcOH (0.1 mL) were added to a solution of **11a** (0.118 g, 0.46 mmol) and formaldehyde (0.042 g, 1.4 mmol) in 1,2-dichloroethane (10 mL) under an argon atmosphere. The reaction mixture was stirred overnight at room temperature and quenched with a 2N NaOH (10 mL) solution. The reaction mixture was extracted with EtOAc (3 × 15 mL). The organic layer was combined, dried, and concentrated. The crude product was purified by flash column chromatography using 15% acetone/$CHCl_3$ as eluent to afford 0.053 g (yield = 43%) of pure product as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.83 (d, J = 2 Hz, 1H), 6.61 (d, J = 2.4 Hz, 1H), 3.83 (s, 3H), 3.13 (s, 4H), 2.56 (s, 4H), 2.53 (q, J = 7.6 Hz, 2H), 2.33 (s, 3H), 1.19 (t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.5, 146.2, 140.9, 128.3, 122.3, 116.8, 59.1, 55.8, 50.3, 46.3, 28.6, 15.5. The oxalate salt was precipitated from acetone; mp 185–186 °C. Anal. ($C_{14}H_{21}ClN_2O{\cdot}1.5C_2H_2O_4{\cdot}H_2O$) C, H, N.

*1-(2-Chloro-3-ethylphenyl)-4-methylpiperazine (**12b**).* The same procedure was used as described for **12a**, employing **11b**. The product was purified by flash chromatography using 25% acetone/$CHCl_3$ as eluent to afford **12b** in 94% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 (d, J = 7.6 Hz, 1H), 6.94–6.91 (m, 2H), 3.06 (s, 4H), 2.76 (q, J = 7.6 Hz, 2H), 2.61 (s, 4H), 2.36 (s, 3H), 1.21(t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.7, 143.3, 128.8, 126.9, 124.1, 118.1, 55.5, 51.6, 46.2, 27.6, 14.2. The oxalate salt was precipitated from acetone; mp 158–159 °C. Anal. ($C_{13}H_{19}ClN_2{\cdot}1.5C_2H_2O_4{\cdot}0.5H_2O$) C, H, N.

*1-Butyl- 4-(3-chloro-5-ethyl-2-methoxyphenyl)piperazine (**13a**).* 1-Bromobutane (0.11 g, 0.78 mmol) was added to the reaction mixture of **11a** (0.10 g, 0.39 mmol) and K_2CO_3 (0.16 g, 1.2 mmol) in acetone (5 mL) and stirred at reflux for 17 h. The reaction mixture was filtered, concentrated, and purified using flash chromatography with 15% acetone/$CHCl_3$ as eluent to provide 0.10 g (81%) of product as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.82 (d, J = 2 Hz, 1H), 6.07 (d, J = 2 Hz, 1H), 3.82 (s, 3H), 3.13 (s, 4H), 2.61 (s, 4H), 2.58–2.50 (m, 2H), 2.40–2.36 (m, 2H), 1.52–1.47 (m, 2H), 1.37–1.31 (m, 2H), 1.19 (t, J = 7.2 Hz, 3H), 0.92 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.5, 146.3, 140.8, 128.3, 122.2, 116.7, 59.0, 58.7, 53.9, 50.4, 29.1, 28.6, 20.9, 15.5, 14.1. The oxalate salt was precipitated from acetone; mp 210–211 °C. Anal. ($C_{17}H_{27}ClN_2O{\cdot}C_2H_2O_4$) C, H, N.

*1-Butyl-4-(2-chloro-3-ethylphenyl)piperazine (**13b**).* The same procedure was used as described for **13a**, employing **11b**. The product was purified by flash chromatography using 20% EtOAc/hexanes as eluent to afford **13b** in 63% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.14 (d, J = 8 Hz, 1H), 6.93 (m, 2H), 3.06 (s, 4H), 2.72 (q, J = 7.6 Hz, 2H), 2.64 (s, 4H), 2.43–2.39 (m, 2H), 1.56–1.49 (m, 2H), 1.48–1.32 (m, 2H), 1.22 (t, J = 7.6 Hz, 3H), 0.94 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.8, 143.3, 128.8, 126.9, 123.9, 118.1, 58.7, 53.6, 51.7, 29.2, 27.6, 20.9, 14.2, 14.2. The *p*-toluenesulfonic acid salt was precipitated from ether; mp 145–146 °C. Anal. ($C_{16}H_{25}ClN_2{\cdot}C_7H_8O_3S{\cdot}0.25H_2O$) C, H, N.

*2-(4-(4-(3-Chloro-5-ethyl-2-methoxyphenyl)piperazin-1-yl)butyl)-isoindoline-1,3-dione (**14a**).* *N*-(4-Bromobutyl)phthalimide (0.38 g, 1.34 mmol) was added to a reaction mixture of **11a** (0.31 g, 1.22 mmol) and K_2CO_3 (0.51 g, 3.65 mmol) in acetone (15 mL) and stirred at reflux overnight. The crude product was filtered, concentrated, and purified by flash chromatography using 12% EtOAc/hexanes as eluent to provide 0.47 g (84%) of the product as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (dd, J = 5.6, 2.8 Hz, 2H), 7.57 (dd, J = 5.6, 3.2 Hz, 2H), 6.68 (d, J = 2.4 Hz, 1H), 6.49 (d, J = 2.0 Hz, 1H), 3.69 (s, 3H), 3.60 (t, J = 7.0 Hz, 2H), 3.01 (bs, 4H), 2.48 (bs, 4H), 2.40 (q, J = 7.6 Hz, 2H), 2.32 (t, J = 7.0 Hz, 2H), 1.62 (quintet, J = 7.6 Hz, 2H), 1.48–1.45 (m, 2H), 1.06 (t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.2, 146.3, 146.0, 140.6, 133.8, 132.0, 128.0, 123.0, 122.0, 116.6, 58.8, 57.9, 53.6, 50.0, 37.7, 28.3, 26.5, 23.9, 15.3.

*2-(4-(4-(2-Chloro-3-ethylphenyl)piperazin-1-yl)butyl)isoindoline-1,3-dione (**14b**).* The same procedure was used as described for **14a**, employing **11b**. The product was purified by flash chromatography using 20% EtOAc/hexanes as eluent to afford **14b** in 64% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85–7.82 (m, 2H), 7.71–7.69 (m, 2H), 7.15 (dt, J = 7.6, 2.4 Hz, 1H), 6.95–6.91 (m, 2H), 3.75 (dt, J = 6.4, 0.8 Hz, 2H), 3.06 (bs, 4H), 2.77 (dq, J = 7.6, 2.0 Hz, 2H), 2.64 (bs, 4H), 2.47 (t, J = 6.8 Hz, 2H), 1.80–1.75 (m, 2H), 1.63–1.59 (m, 2H), 1.27–1.21 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.9, 149.4, 142.8, 133.6, 131.9, 128.4, 126.6, 123.6, 122.8, 117.7, 57.8, 53.2, 51.3, 37.6, 27.2, 26.4, 24.0, 13.9.

*2-(4-(4-(3-Chloro-5-ethyl-2-methoxyphenyl)piperazin-1-yl)-3-hydroxybutyl)isoindoline-1,3-dione (**15a**).* A mixture of compounds **11a** (0.31 g, 1.21 mmol) and 2-(2-(oxiran-2-yl)ethyl)isoindoline-1,3-dione (0.26 g, 1.21 mmol) was stirred at reflux in 2-PrOH (20 mL) overnight. The reaction mixture was concentrated and purified by flash chromatography using 20% EtOAc/hexanes as eluent to provide 0.52 g (91%) of product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (dd, J = 5.2, 3.2 Hz, 2H), 7.71 (dd, J = 5.6, 2.8 Hz, 2H), 6.84 (d, J = 2.0 Hz, 1H), 6.60 (d, J = 2.0 Hz, 1H), 3.93–3.79 (m, 6H), 3.13 (bs, 4H), 2.81–2.79 (m, 2H), 2.60–2.58 (m, 2H), 2.55 (q, J = 8.0 Hz, 2H), 2.45–2.41 (m, 2H), 1.79 (q, J = 7.2 Hz, 2H), 1.19 (t, J = 8.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.5, 146.4, 145.9, 140.8, 133.9, 132.2, 128.2, 123.2, 122.4, 116.7, 64.5, 63.9, 59.1, 53.8, 50.3, 35.1, 33.6, 28.4, 15.4.

*2-(4-(4-(2-Chloro-3-ethylphenyl)piperazin-1-yl)-3-hydroxybutyl)-isoindoline-1,3-dione (**15b**).* The same procedure was used as described for **15a**, employing **11b**. The product was purified by flash chromatography using 30% EtOAc/hexanes as eluent to afford **15b** in 57.2% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (dd, J = 7.2, 5.6 Hz, 2H), 7.71 (dd, J = 5.6, 3.2 Hz, 2H), 7.15 (t, J = 8.0 Hz, 1H), 6.92 (dq,

DOI: 10.1021/acs.jmedchem.6b00860
J. Med. Chem. XXXX, XXX, XXX–XXX

J = 7.6, 1.6 Hz, 2H), 3.94−3.77 (m, 4H), 3.03 (bs, 4H), 2.84−2.81 (m, 2H), 2.76 (q, J = 7.6 Hz, 2H), 2.59 (m, 2H), 2.46−2.38 (m 2H), 1.79 (q, J = 7.0, 2H), 1.22 (t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.7, 151.7, 149.6, 143.4, 134.0, 132.4, 128.8, 127.0, 124.2, 123.4, 118.1, 64.6, 64.0, 51.8, 35.3, 33.8, 27.6, 14.2.

*4-(4-(3-Chloro-5-ethyl-2-methoxyphenyl)piperazin-1-yl)butan-1-amine (**16a**).* Hydrazine (0.097 g, 3.02 mmol) was added to a solution of compound **14a** (0.460 g, 1.00 mmol) in EtOH (15 mL) and stirred at reflux for 7 h. The solvent was evaporated, and the reaction mixture was diluted with 20% aq K_2CO_3 solution (15 mL) and extracted in $CHCl_3$ (2 × 15 mL). The organic layer was combined, dried, and concentrated to afford the product as a yellow oil, which was sufficiently pure to be used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.70 (d, J = 2.0 Hz, 1H), 6.49 (d, J = 2.0 Hz, 1H), 3.70 (s, 3H), 3.01 (bs, 4H), 2.59 (s, 2H), 2.47−2.38 (m, 6H), 2.27 (t, J = 7.6 Hz, 2H), 1.46−1.33 (m, 4H), 1.07 (t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.3, 146.1, 140.6, 128.0, 122.0, 116.5, 58.8, 58.5, 53.7, 50.2, 28.3, 24.2, 15.3.

*4-(4-(2-Chloro-3-ethylphenyl)piperazin-1-yl)butan-1-amine (**16b**).* The same procedure was used as described for **16a**, employing **14b** to afford **16b** in 85.1% yield. The product was sufficiently pure to be used for the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.99−6.95 (m, 1H), 6.77−6.74 (m, 2H), 2.89 (bs, 4H), 2.63−2.54 (m, 4H), 2.47 (bs, 4H), 2.27−2.23 (m, 2H), 1.43−1.28 (m, 4H), 1.08−1.04 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.3, 142.7, 128.3, 126.5, 123.5, 117.6, 58.2, 53.1, 51.2, 41.8, 31.5, 27.1, 24.0, 13.8.

*4-Amino-1-(4-(3-chloro-5-ethyl-2-methoxyphenyl)piperazin-1-yl)butan-2-ol (**17a**).* The same procedure was used as described for **16a**, employing **15a** to afford **16a** in 88.5% yield. The product was sufficiently pure to be used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.77 (d, J = 2.0 Hz, 1H),6.55 (d, J = 1.6 Hz, 1H), 3.85−3.81 (m, 1H), 3.76 (s, 3H), 3.06 (bs, 4H), 2.86 (m, 2H), 2.72−2.70 (m, 2H), 2.53−2.44 (m, 4H), 2.37−2.28 (m, 3H), 1.53−1.44 (m,2H), 1.13 (t, J = 8.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.3, 146.0, 140.7, 128.1, 122.2, 116.6, 66.2, 64.6, 58.9, 53.8, 50.3, 39.6, 37.7, 28.4, 15.4.

*4-Amino-1-(4-(2-chloro-3-ethylphenyl)piperazin-1-yl)butan-2-ol (**17b**).* The same procedure was used as described for **16a**, employing **15b** to afford **17b** in 89.6% yield. The product was sufficiently pure to be used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.03−7.0 (m, 1H), 6.82−6.77 (m, 2H), 3.80−3.71 (m, 1H), 2.92−2.61 (m, 10H), 2.49−2.24 (m, 7H), 1.46−1.40 (m, 2H), 1.10 (t, J = 7.6, 0.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.2, 142.8, 128.3, 126.6, 123.6, 117.7, 65.8, 64.4, 51.3, 39.3, 37.5, 27.1, 13.8.

General Amidation Procedure. CDI (1 equiv) was added to a solution of the carboxylic acid (1 equiv) in dry THF (10 mL/mmol) and stirred for 3 h at room temperature. The reaction mixture was cooled to 0 °C, and the amine (1 equiv) was added dropwise after diluting with dry THF (10 mL/mmol). The reaction mixture was allowed to come to room temperature and stirred overnight, concentrated, diluted with H_2O (20 mL), and extracted in $CHCl_3$ (3 × 10 mL). The organic layer was concentrated, and the product was purified by flash column chromatography to provide the desired amide product.

*N-(4-(4-(3-Chloro-5-ethyl-2-methoxyphenyl)piperazin-1-yl)-butyl)-1H-indole-2-carboxamide (**18**).* Compound **18** was prepared from indole-2-carboxylic acid and **16a** according to the general amidation procedure. The crude product was purified by flash chromatography using 25% acetone/$CHCl_3$ as eluent to give the desired product in 57.6% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.25 (s, 1H), 7.62 (d, J = 8.0 Hz, 1H), 7.45 (dd, J = 8.0, 0.8 Hz, 1H), 7.28−7.24 (m, 1H), 7.14−7.10 (m, 1H), 6.93 (m, 1H), 6.90 (s, 1H), 6.84 (d, J = 1.6 Hz, 1H), 6.58 (d, J = 1.6 Hz, 1H), 3.83 (s, 3H), 3.54 (q, J = 6.0 Hz, 2H), 3.14 (bs, 4H), 2.60 (bs, 4H), 2.53 (q, J = 7.6 Hz, 2H), 2.45 (d, J = 7.0 Hz, 2H), 1.73−1.64 (m, 4H), 1.19 (t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.0, 146.4, 146.0, 140.9, 136.6, 131.0, 128.2, 127.6, 124.3, 122.3, 121.8, 120.5, 116.7, 112.1, 102.3, 59.0, 58.0, 53.7, 50.1, 39.6, 28.4, 27.5, 24.2, 15.4. The oxalate salt was precipitated from acetone and crystallized in MeOH/ether; mp 220−221 °C. Anal. ($C_{26}H_{33}ClN_4O_2 \cdot C_2H_2O_4 \cdot 0.5H_2O$) C, H, N.

*N-(4-(4-(3-Chloro-5-ethyl-2-methoxyphenyl)piperazin-1-yl)-3-hydroxybutyl)-1H-indole-2-carboxamide (**19**).* Compound **19** was prepared from indole-2-carboxylic acid and **17a** according to the general amidation procedure. The crude product was purified by flash chromatography using 15% acetone/$CHCl_3$ as eluent to give the desired product in 22.2% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.90 (s, 1H), 7.63 (d, J = 8.0 Hz, 1H), 7.51−7.48 (m, 1H), 7.46 (dd, J = 8.0, 0.8 Hz, 1H), 7.28−7.24 (m, 1H), 7.14−7.10 (m, 1H), 6.87 (dd, J = 6.0, 2.0 Hz, 2H), 6.61 (d, J = 2.0 Hz, 1H), 3.96−3.84 (m, 2H), 3.84 (s, 3H), 3.57−3.51 (m, 1H), 3.15 (bs, 4H), 2.85−2.81 (m, 2H), 2.58−2.52 (m, 4H), 2.43 (d, J = 6.8 Hz, 2H), 1.86−1.82 (m, 1H), 1.67−1.62 (m, 1H), 1.21 (t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.8, 146.4, 146.0, 140.9, 136.4, 131.1, 128.3, 127.7, 124.2, 122.4, 121.8, 120.4, 116.7, 112.0, 102.0, 66.3, 63.8, 59.1, 53.7, 50.4, 38.0, 33.4, 28.5, 15.4. The oxalate salt was precipitated from acetone and crystallized in hot MeOH; mp 188−189 °C. Anal. ($C_{26}H_{33}ClN_4O_3 \cdot C_2H_2O_4 \cdot H_2O$) C, H, N.

*N-(4-(4-(3-Chloro-5-ethyl-2-methoxyphenyl)piperazin-1-yl)-butyl)benzofuran-2-carboxamide (**20**).* Compound **20** was prepared from benzofuran-2-carboxylic acid and **16a** according to the general amidation procedure. The crude product was purified by flash chromatography using 20% acetone/$CHCl_3$ as eluent to give the desired product in 76.5% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.63 (dd, J = 7.2, 1.2 Hz, 1H), 7.44−7.42 (m, 2H), 7.36 (dt, J = 7.0, 1.2 Hz, 1H), 7.28−7.24 (m, 1H), 7.13 (m, 1H), 6.82 (d, J = 2.0 Hz, 1H), 6.59 (d, J = 2.0 Hz, 1H), 3.82 (s, 3H), 3.51 (q, J = 6.4 Hz, 2H), 3.15 (bs, 4H), 2.61 (bs, 4H), 2.51 (q, J = 7.6 Hz, 2H), 2.45 (t, J = 7.0 Hz, 2H), 1.73−1.63 (m, 4H), 1.17 (t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.9, 154.7, 149.0, 146.4, 146.1, 140.8, 128.2, 127.7, 126.7, 123.7, 122.7, 122.2, 116.7, 111.6, 110.2, 59.0, 58.0, 53.8, 50.2, 39.2, 28.4, 27.5, 24.3, 15.4. The oxalate salt was precipitated from acetone and crystallized in MeOH/ether; mp 126−127 °C. Anal. ($C_{26}H_{32}ClN_3O_3 \cdot 2C_2H_2O_4 \cdot H_2O$) C, H, N.

*N-(4-(4-(3-Chloro-5-ethyl-2-methoxyphenyl)piperazin-1-yl)-3-hydroxybutyl)benzofuran-2-carboxamide (**21**).* Compound **21** was prepared from benzofuran-2-carboxylic acid and **17a** according to the general amidation procedure. The crude product was purified by flash chromatography using 20% acetone/$CHCl_3$ as eluent to give the desired product in 50.5% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (dd, J = 7.6, 0.8 Hz, 1H), 7.53 (m, 1H), 7.49 (dd, J = 8.4. 0.8 Hz, 1H), 7.44 (d, J = 0.8 Hz, 1H), 7.40−7.36 (m, 1H), 7.29−7.25 (m, 1H), 6.85 (d, J = 2.0 Hz, 1H), 6.61 (d, J = 2.0 Hz, 1H), 3.95−3.81 (m, 5H), 3.56−3.48 (m, 1H), 3.14 (bs, 4H), 2.84−2.81 (m, 2H), 2.62−2.51 (m, 4H), 2.46−2.42 (m, 2H), 1.87−1.80 (m, 1H), 1.68−1.61 (m, 1H), 1.20 (t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.0, 154.8, 149.0, 146.4, 146.0, 140.8, 128.3, 127.7, 126.7, 123.6, 122.6, 122.4, 116.7, 111.8, 110.1, 65.9, 63.8, 59.1, 53.7, 50.4, 37.3, 33.7, 28.5, 15.4. The oxalate salt was precipitated from acetone and crystallized in 2-PrOH/ether; mp 154−155 °C. Anal. ($C_{26}H_{32}ClN_3O_4 \cdot 1.5C_2H_2O_4 \cdot H_2O$) C, H, N.

*N-(4-(4-(2-Chloro-3-ethylphenyl)piperazin-1-yl)butyl)-1H-indole-2-carboxamide (**22**).* Compound **22** was prepared from indole-2-carboxylic acid and **16b** according to the general amidation procedure. The crude product was purified by flash chromatography using 30% acetone/$CHCl_3$ as eluent to give the desired product in 51.0% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.35 (s, 1H), 7.63 (d, J = 7.6 Hz, 1H), 7.47 (d, J = 8.8 Hz, 1H), 7.27 (dt, J = 8.0, 1.2 Hz, 1H), 7.13 (sextet, J = 4.0 Hz, 2H), 6.96−6.87 (m, 4H), 3.56 (dd, J = 12.8, 6.4 Hz, 2H), 3.06 (bs, 4H), 2.78 (q, J = 7.6 Hz, 2H), 2.65 (bs, 4H), 2.47 (t, J = 7.2 Hz, 2H), 1.72−1.64 (m, 4H), 1.23 (t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.1, 149.6, 143.3, 136.7, 131.1, 128.7, 127.6, 127.0, 124.3, 124.1, 121.9, 120.6, 118.1, 112.2, 102.2, 58.1 53.5, 51.5, 39.8, 27.7, 27.6, 24.5, 14.2. The oxalate salt was precipitated from acetone; mp 228−229 °C. Anal. ($C_{25}H_{31}ClN_4O \cdot C_2H_2O_4 \cdot 0.75H_2O$) C, H, N.

*N-(4-(4-(2-Chloro-3-ethylphenyl)piperazin-1-yl)-3-hydroxybutyl)-1H-indole-2-carboxamide (**23**).* Compound **23** was prepared from indole-2-carboxylic acid and **17b** according to the general amidation

DOI: 10.1021/acs.jmedchem.6b00860
J. Med. Chem. XXXX, XXX, XXX−XXX

procedure. The crude product was purified by flash chromatography using 30% acetone/$CHCl_3$ as eluent to give the desired product in 61.1% yield. ^{1}H NMR (400 MHz, DMSO) δ 11.52 (s, 1H), 8.43 (t, J = 5.6 Hz, 1H), 7.58 (d, J = 8.4 Hz, 1H), 7.40 (d, J = 8.4 Hz, 1H), 7.16 (quintet, J = 8 Hz, 2H), 7.07 (d, J = 1.6 Hz, 1H), 7.99 (m, 3H), 4.48 (d, J = 4.8 Hz, 1H), 3.73 (m, 1H), 3.45−3.33 (m, 2H), 2.91 (bs, 4H), 2.64 (q, J = 7.6 Hz, 2H), 2.58 (bs, 4H), 2.40−2.30 (m, 2H), 1.78 (m, 1H), 1.53 (m, 1H), 1.13 (t, J = 8 Hz, 3H). ^{13}C NMR (100 MHz, DMSO) δ 160.0, 149.4, 142.4, 136.3, 131.9, 127.6, 127.3, 127.1, 123.9, 123.1, 121.4, 119.6, 118.4, 112.2, 102.1, 65.4, 64.4, 53.6, 51.2, 36.1, 35.4, 26.8, 14.1. The oxalate salt was precipitated from acetone; mp 208−209 °C. Anal. ($C_{25}H_{31}ClN_4O_2\cdot C_2H_2O_4\cdot 0.75H_2O$) C, H, N.

*N-(4-(4-(2-Chloro-3-ethylphenyl)piperazin-1-yl)butyl)-benzofuran-2-carboxamide (**24**).* Compound **24** was prepared from benzofuran-2-carboxylic acid and **16b** according to the general amidation procedure. The crude product was purified by flash chromatography using 25% EtOAc/hexanes as eluent to give the desired product in 76.5% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65−7.63 (m, 1H), 7.46 (bs, 1H), 7.44 (d, J = 0.8 Hz, 1H), 7.38 (dt, J = 7.2, 1.2 Hz, 1H), 7.29−7.25 (m, 1H), 7.15−7.11 (m, 2H), 6.91 (dq, J = 7.2, 1.6 Hz, 2H), 3.53 (dd, J = 12.4, 6.4 Hz, 2H), 3.08 (bs, 4H), 2.77 (q, J = 7.6 Hz, 2H), 2.67 (bs, 4H), 2.48 (t, J = 7.0 Hz, 2H), 1.74−1.65 (m, 4H), 1.22 (t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.9, 154.7, 149.6, 149.0, 143.2, 128.7, 127.7, 126.9, 126.8, 124.0, 123.7, 122.7, 118.0, 111.7, 110.2, 58.0, 53.5, 51.5, 39.2, 27.6, 27.5, 24.4, 14.1. The oxalate salt was precipitated from acetone; mp 119−120 °C. Anal. ($C_{25}H_{30}ClN_3O_2\cdot C_2H_2O_4\cdot 0.5H_2O$) C, H, N.

*N-(4-(4-(2-Chloro-3-ethylphenyl)piperazin-1-yl)-3-hydroxybutyl)-benzofuran-2-carboxamide (**25**).* Compound **25** was prepared from benzofuran-2-carboxylic acid and **17b** according to the general amidation procedure. The crude product was purified by flash chromatography using 25% acetone/$CHCl_3$ as eluent to give the desired product in 52.1% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, J = 7.2 Hz, 1H), 7.58 (t, J = 5.0 Hz, 1H), 7.50−7.45 (m, 2H), 7.38 (dt, J = 7.2, 1.2 Hz, 1H), 7.28−7.24 (m, 1H), 7.15 (t, J = 7.6 Hz, 1H), 6.92 (dq, J = 8.0, 1.2 Hz, 2H), 3.94−3.84 (m, 3H), 3.55−3.50 (m, 1H), 3.06 (bs, 4H), 2.87−2.85 (m, 2H), 2.76 (q, J = 7.6 Hz, 2H), 2.62−2.61 (m, 2H), 2.48−02.43 (m, 2H), 1.85−1.80 (m, 1H), 1.66−1.63 (m, 1H), 1.22 (t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.0, 154.8, 149.4, 149.1, 143.3, 128.7, 127.7, 127.0, 126.7, 124.2, 123.6, 122.7, 118.0, 111.9, 110.1, 66.0, 63.9, 51.7, 37.5, 33.7, 27.5, 14.2. The oxalate salt was precipitated from ether; mp 147−148 °C. Anal. ($C_{25}H_{30}ClN_3O_3\cdot C_2H_2O_4\cdot H_2O$) C, H, N.

*N-(4-(4-(2-Chloro-3-ethylphenyl)piperazin-1-yl)butyl)imidazo-[1,2-a]pyridine-2-carboxamide (**26**).* Compound **26** was prepared from imidazo[1,2-*a*]pyridine-2-carboxylic acid and **16b** according to the general amidation procedure. The crude product was purified by flash chromatography using 3% MeOH/$CHCl_3$ as eluent to give the desired product in 78.4% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11−8.09 (m, 2H), 7.53−7.49 (m, 2H), 7.21−7.17 (m, 1H), 7.10 (t, J = 8.0 Hz, 1H), 6.89 (t, J = 7.2 Hz, 2H), 6.81−6.77 (m, 1H), 3.48 (q, J = 6.0 Hz, 2H), 3.03 (bs, 4H), 7.72 (q, J = 7.6 Hz, 2H), 2.62 (m, 4H), 2.45−2.42 (m, 2H), 1.65 (m, 4H), 1.18 (t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.7, 149.6, 144.4, 143.1, 140.0, 128.6, 126.9, 126.5, 126.0, 123.9, 118.0, 114.1, 113.3, 58.1, 53.4, 51.4, 39.0, 27.7, 27.4, 24.3, 14.1. The oxalate salt was precipitated from acetone; mp 144−145 °C. Anal. ($C_{24}H_{30}ClN_5O\cdot 2C_2H_2O_4\cdot 1.5H_2O$) C, H, N.

*N-(4-(4-(2-Chloro-3-ethylphenyl)piperazin-1-yl)-3-hydroxybutyl)-imidazo[1,2-a]pyridine-2-carboxamide (**27**).* Compound **27** was prepared from imidazo[1,2-*a*]pyridine-2-carboxylic acid and **17b** according to the general amidation procedure. The crude product was purified by flash chromatography using 2% MeOH/$CHCl_3$ as eluent to give the desired product in 46.3% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13−8.11 (m, 2H), 7.90 (bt, J = 6.0 Hz, 1H), 7.56 (dd, J = 7.2, 0.8 Hz, 1H), 7.23−7.19 (m, 1H), 7.14 (t, J = 8.0 Hz, 1H), 6.93 (dq, J = 7.6, 1.6 Hz, 2H), 6.82 (dt, J = 6.4, 1.2 Hz, 1H), 3.91−3.78 (m, 2H), 3.56−3.48 (m, 1H), 3.04 (bs, 4H), 2.86−2.83 (m, 2H), 2.75 (q, J = 7.6 Hz, 2H), 2.62−2.60 (m, 2H), 2.45−2.40 (m, 2H), 1.84−1.76 (m, 1H), 1.70−1.61 (m, 1H), 1.21 (t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.9, 149.5, 144.6, 143.2, 140.2, 128.7, 126.9, 126.4, 125.8, 124.0, 118.2, 118.0, 114.1, 113.3, 65.3, 63.9, 53.5, 51.6, 36.6, 34.4, 27.4, 14.1. The oxalate salt was precipitated from acetone; mp 137−138 °C. Anal. ($C_{24}H_{30}ClN_5O_2\cdot 2C_2H_2O_4\cdot H_2O\cdot 0.75CHCl_3$) C, H, N.

*N-(4-(4-(2-Chloro-3-ethylphenyl)piperazin-1-yl)butyl)-6-methylimidazo[2,1-b]thiazole-5-carboxamide (**28**).* Compound **28** was prepared from 6-methylimidazo[2,1-*b*]thiazole-5-carboxylic acid and **16b** according to the general amidation procedure. The crude product was purified by flash chromatography using 3% MeOH/$CHCl_3$ as eluent to give the desired product in 48.9% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (d, J = 4.0 Hz, 1H), 7.13−7.09 (m, 1H), 6.92−6.82 (m, 3H), 5.98 (m, 1H), 3.50−3.45 (m, 2H), 3.03 (bs, 4H), 2.73 (q, J = 7.6 Hz, 2H), 2.64 (bs, 4H), 2.58 (s, 3H), 2.48−2.45 (m, 2H), 1.66 (m, 4H), 1.19 (t, J = 8.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.7, 150.9, 149.4, 145.1, 143.2, 128.7, 126.9, 124.0, 121.4, 118.7, 117.9, 112.4, 58.1, 53.4, 51.3, 39.4, 27.9, 27.4, 24.2, 16.5, 14.1. The oxalate salt was precipitated from acetone; mp 113−114 °C. Anal. ($C_{23}H_{30}ClN_5OS\cdot 2C_2H_2O_4\cdot 2.5H_2O$) C, H, N.

*N-(4-(4-(2-Chloro-3-ethylphenyl)piperazin-1-yl)butyl)-4-ethyl-1H-imidazole-2-carboxamide (**29**).* A solution of trimethylaluminum (2 M in hexane) (0.18 mL, 3.54 mmol) was added dropwise to a solution of **16b** (0.105 g, 3.54 mmol) in CH_2Cl_2 (10 mL) under argon at room temperature. The reaction mixture was stirred at room temperature for 15 min, and ethyl 4-ethyl-1*H*-imidazole-2-carboxylate solution in CH_2Cl_2 (10 mL) was added dropwise and stirred for 6 h. The reaction mixture was quenched with 10% HCl (15 mL). The organic layer was extracted, dried over Na_2SO_4, concentrated, and purified using flash chromatography with 4% MeOH/$CHCl_3$ as eluent to provide 0.148 g (14.9%) of the product. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (bs, 1H), 7.13 (t, J = 7.6 Hz, 1H), 6.92 (t, J = 8.0 Hz, 2H), 6.82 (s, 1H), 3.46−3.43 (m, 2H), 3.06 (s, 4H), 2.75 (q, J = 7.6 Hz, 2H), 2.66 (m, 5H), 2.48−2.46 (m, 2H), 2.18−2.15 (m, 1H), 1.65 (s, 5H), 1.29−1.18 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.1, 149.5, 143.2, 140.0, 128.7, 126.9, 124.0, 118.0, 58.0, 53.4, 51.2, 39.1, 27.5, 27.4, 24.1, 14.1, 13.5. The oxalate salt was precipitated from acetone; mp 167−168 °C. Anal. ($C_{22}H_{32}ClN_5O\cdot 2.5C_2H_2O_4\cdot H_2O$) C, H, N.

*N-(4-(4-(2-Chloro-3-ethylphenyl)piperazin-1-yl)butyl)-4-methyl-1H-imidazole-2-carboxamide (**30**).* Compound **30** was prepared from 4-methyl-1*H*-imidazole-2-carboxylic acid and **16b** according to the general amidation procedure. The crude product was purified by flash chromatography using 3% MeOH/$CHCl_3$ as eluent to give the desired product in 65.2% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (bs, 1H), 7.13 (t, J = 8.0 Hz, 1H), 6.94−6.89 (m, 2H), 6.82 (s, 1H), 3.46 (q, J = 6.4 Hz, 2H), 3.06 (bs, 4H), 2.75 (q, J = 8.0 Hz, 2H), 2.64 (bs, 4H), 2.45 (t, J = 7.2 Hz, 2H), 2.28 (s, 3H), 1.68−1.61 (m, 4H), 1.20 (t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.3, 149.5, 143.2, 140.1, 128.6, 126.9, 124.0, 118.0, 58.1, 53.4, 51.4, 39.2, 27.5, 24.2, 14.1. The oxalate salt was precipitated from acetone; mp 175−176 °C. Anal. ($C_{21}H_{30}ClN_5O\cdot 2C_2H_2O_4\cdot H_2O$) C, H, N.

*N-(4-(4-(2-Chloro-3-ethylphenyl)piperazin-1-yl)-3-hydroxybutyl)-4-methyl-1H-imidazole-2-carboxamide (**31**).* Compound **31** was prepared from 4-methyl-1*H*-imidazole-2-carboxylic acid and **17b** according to the general amidation procedure. The crude product was purified by flash chromatography using 3% MeOH/$CHCl_3$ as eluent to give the desired product in 39.8% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.40 (bs, 1H), 8.11 (bs, 1H), 8.01 (s, 1H), 7.14 (t, J = 8.0 Hz, 1H), 6.95−6.88 (m, 2H), 6.83 (bs, 1H), 3.90−3.84 (m, 1H), 3.79−3.70 (m, 1H), 3.57−3.49 (m, 1H), 3.03 (bs, 4H), 2.83−2.81 (m, 2H), 2.75 (q, J = 8.0 Hz, 2H), 2.58 (bs, 2H), 2.42 (d, J = 6.6 Hz, 2H), 2.29 (bs, 3H), 1.81−1.73 (m, 1H), 1.68−1.59 (m, 1H), 1.20 (t, J = 8.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.6, 159.3, 149.4, 143.2, 140.0, 128.6, 126.9, 124.1, 118.0, 65.0, 63.9, 53.5, 51.6, 36.8, 34.1, 27.5, 14.1. The oxalate salt was precipitated from acetone; mp 130−131 °C. Anal. ($C_{21}H_{30}ClN_5O_2\cdot 2C_2H_2O_4\cdot 3H_2O$) C, H, N.

*(E)-2-(4-(4-(2-Chloro-3-ethylphenyl)piperazin-1-yl)but-2-en-1-yl)-isoindoline-1,3-dione (**33**).* Compound **32**[37] (0.592 g, 2.11 mmol) was added to the reaction mixture of **11b** (0.475 g, 2.11 mmol) and K_2CO_3 (1.458 g, 10.56 mmol) in acetone (25 mL) and stirred at reflux overnight. The reaction mixture was filtered, concentrated, and purified using flash chromatography with 15% acetone/$CHCl_3$ as

DOI: 10.1021/acs.jmedchem.6b00860
J. Med. Chem. XXXX, XXX, XXX−XXX

eluent to provide 0.880 g (98.2%) of product as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (dd, J = 5.2, 3.2 Hz, 2H), 7.53 (dd, J = 5.2, 3.2 Hz, 2H), 6.98 (t, J = 8.0 Hz, 1H), 6.76 (t, J = 6.4 Hz, 2H), 5.63 (d, J = 4.4 Hz, 1H), 5.61 (d, J = 4.4 Hz, 1H), 4.16 (d, J = 4.0 Hz, 2H), 2.90 (m, 6H), 2.59 (q, J = 8.0 Hz, 2H), 2.47 (bs, 4H), 1.06 (t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.6, 149.5, 142.9, 133.8, 132.0, 130.1, 128.5, 126.9, 126.8, 123.8, 123.1, 117.9, 60.1, 53.3, 51.4, 38.9, 27.4, 14.1.

*(E)-4-(4-(2-Chloro-3-ethylphenyl)piperazin-1-yl)but-2-en-1-amine (**34**).* The same procedure was used as described for **16a**. The product was isolated in 91.2% yield and was sufficiently pure to be used for the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.96 (t, J = 8.0 Hz, 1H), 6.75 (dd, J = 8.0. 1.6 Hz, 2H), 5.63−5.57 (m, 1H), 5.53−5.46 (m, 1H), 3.13 (d, J = 5.6 Hz, 2H), 2.88−2.87 (m, 6H), 2.59 (q, J = 7.6 Hz, 2H), 2.47 (bs, 4H), 1.16 (bs, 2H), 1.05 (t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.5, 142.9, 135.3, 128.5, 126.8, 126.0, 123.8, 117.9, 60.4, 53.2, 51.4, 43.7, 27.4, 14.1. GC-MS (EI) m/z 293.1 (M^+).

*(E)-N-(4-(4-(2-Chloro-3-ethylphenyl)piperazin-1-yl)but-2-en-1-yl)-4-methyl-1H-imidazole-2-carboxamide (**35**).* Compound **35** was prepared from 4-methyl-1*H*-imidazole-2-carboxylic acid and **34** according to the general amidation procedure. The crude product was purified by flash chromatography using 3% MeOH/$CHCl_3$ as eluent to give the desired product in 54.7% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.70−12.63 (m, 1H), 7.90 (bs, 1H), 7.13 (t, J = 8.0 Hz, 1H), 6.94−6.89 (m, 2H), 6.80 (s, 1H), 5.76 (d, J = 5.0, 1H), 5.73 (d, J = 5.0, 1H), 4.06 (t, J = 5.0, 2H), 3.05−3.04 (m, 6H), 2.75 (q, J = 7.6, 2H), 2.62 (bs, 4H), 2.31 (s, 2H), 2.24 (s, 1H), 1.20 (t, J = 8.0, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.1, 149.5, 143.2, 139.9, 129.3, 129.2, 128.7, 126.9, 124.0, 118.0, 60.3, 53.4, 51.4, 40.8, 27.5, 14.1. The oxalate salt was precipitated from acetone and crystallized in MeOH/ether; mp 166−167 °C. Anal. ($C_{21}H_{28}ClN_5O\cdot 2C_2H_2O_4\cdot 1.25H_2O$) C, H, N.

*1-(2-Chloro-3-ethylphenyl)-4-(2-(oxiran-2-yl)ethyl)piperazine (**37**).* 2-(2-Bromoethyl)oxirane (**36**, 0.269 g, 1.78 mmol) was added to a reaction mixture of **11b** (0.266 g, 1.18 mmol) and K_2CO_3 (0.491 g, 3.56 mmol) in acetone (20 mL) and stirred at reflux overnight. The crude product was filtered, concentrated, and purified by flash chromatography using 12% acetone/$CHCl_3$ as eluent to provide 0.145 g (41.4%) of the product as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.15 (t, J = 7.6 Hz, 1H), 6.95−6.91 (m, 2H), 3.06 (bs, 4H), 3.02−2.95 (m, 1H), 2.80−2.73 (m, 3H), 2.66 (bs, 4H), 2.62−2.55 (m, 2H), 2.53−2.51 (m, 1H), 1.87−1.79 (m, 1H), 1.76−1.67 (m, 1H), 1.22 (t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.6, 143.2, 128.7, 126.9, 124.0, 118.0, 55.0, 53.5, 51.6, 50.9, 47.1, 30.2, 27.5, 14.2. GC-MS (EI) m/z 294.1 (M^+).

*1-Azido-4-(4-(2-chloro-3-ethylphenyl)piperazin-1-yl)butan-2-ol (**38**).* A reaction mixture of **37** (0.170 g, 0.57 mmol), NaN_3 (0.056 g, 0.86 mmol), and NH_4Cl (0.062 g, 1.15 mmol) in DMF (5 mL) was heated at 100 °C for 6 h. The solvent was evaporated, and the reaction mixture was diluted with water (15 mL) and extracted in EtOAc (3 × 15 mL). The organic layer was combined, dried, concentrated, and purified by flash chromatography using 7% acetone/$CHCl_3$ as eluent to provide 0.103 g (52.9%) of the product as an oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17 (t, J = 7.6 Hz, 1H), 6.97 (dd, J = 7.6, 1.2 Hz, 1H), 6.91 (dd, J = 7.6, 1.2 Hz, 1H), 4.07−4.02 ((m, 1H), 3.28 (m, 2H), 3.07 (bs, 4H), 2.85−2.71 (m, 6H), 2.64−2.61 (m, 2H), 1.87−1.77 (m, 1H), 1.60−1.54 (m, 1H), 1.26−1.22 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.2, 143.3, 128.7, 127.0, 124.3, 118.1, 73.0, 57.3, 56.6, 53.4, 51.5, 28.5, 27.5, 14.1.

*1-Amino-4-(4-(2-chloro-3-ethylphenyl)piperazin-1-yl)butan-2-ol (**39**).* A mixture of compound **38** (0.123 g, 0.36 mmol) and 10% Pd/C (0.050 g) in EtOAc (10 mL) was stirred under an atmosphere of hydrogen (50 psi) at room temperature for 2 h. The reaction mixture was filtered through a Celite pad and evaporated under vacuum. The reaction mixture was sufficiently pure to be used for the next step without further purification. ^{1}H NMR (400 MHz, CD_3OD + $CDCl_3$) δ 7.07 (t, J = 7.6 Hz, 1H), 6.89 (dd, J = 13.6, 8.4 Hz, 2H), 4.89 (bs, 2H), 4.11 (bs, 1H), 3.33−3.17 (m, 10H), 3.04−2.99 (m, 1H), 2.64 (q, J = 7.2 Hz, 2H), 2.06−1.97 (m, 2H), 1.12 (t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.5, 143.3, 128.5, 127.1, 125.1, 118.3, 65.5, 54.2, 52.7, 48.7, 44.8, 28.8, 27.2, 13.8.

*N-(4-(4-(2-Chloro-3-ethylphenyl)piperazin-1-yl)-2-hydroxybutyl)-1H-indole-2-carboxamide (**40**).* Compound **40** was prepared from indole-2-carboxylic acid and **39** according to the general amidation procedure using DMF as solvent. The crude product was purified by flash chromatography using 2% MeOH/$CHCl_3$ as eluent to give the desired product in 16.4% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.73 (s, 1H), 7.63 (d, J = 7.6 Hz, 1H), 7.43 (dd, J = 8.4, 0.8 Hz, 1H), 7.28−7.23 (m, 1H), 7.16−7.09 (m, 2H), 6.95 (dd, J = 7.6, 1.2 Hz, 2H), 6.91 (d, J = 1.2 Hz, 1H), 6.86 (dd, J = 7.6, 1.6 Hz, 1H), 4.10−4.06 (m, 1H), 3.76−3.71 (m, 1H), 3.43−3.37 (m, 1H), 3.04 (bs, 4H), 2.93−2.81 (m, 2H), 2.79−2.69 (m, 4H), 2.62 (m, 2H), 1.82−1.78 (m, 1H), 1.62−1.57 (m, 1H), 1.21 (t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.9, 149.1, 143.3, 136.3, 130.8, 128.7, 127.7, 126.9, 124.3, 124.3, 121.9, 120.5, 118.0, 112.0 102.3, 72.6, 57.3, 53.4, 51.4, 45.3, 30.9, 28.6, 27.4, 14.0. The oxalate salt was precipitated from acetone; mp 225−226 °C. Anal. ($C_{25}H_{31}ClN_4O_2\cdot 1.5C_2H_2O_4\cdot 1.75H_2O$) C, H, N.

Radioligand Binding Assays. Binding at dopamine D_2-like receptors was determined using previously described methods.[59] Membranes were prepared from HEK293 cells expressing human $D_{2L}R$, D_3R, or $D_{4.4}R$, 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 1 mM or 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 100 μM to 100 pM or from 10 μM to 10 pM. Previously frozen membranes were diluted in fresh EBSS to a 100 μg/mL (for $hD_{2L}R$ or hD_3R) or 200 μg/mL ($hD_{4.4}R$) 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 of total protein for $hD_{2L}R$ or hD_3R, 20 μg of total protein for $hD_{4.4}R$), and 50 μL of [^{3}H]*N*-methylspiperone (0.4 nM final concentration; PerkinElmer). Nonspecific binding was determined using 10 μM butaclamol (Sigma-Aldrich, St. Louis, MO), and total binding was determined with 30% DMSO vehicle. All compound dilutions were tested in triplicate, and the reaction incubated for 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; these analyses were performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA). Reported K_i values were determined from at least three independent experiments.

DOI: 10.1021/acs.jmedchem.6b00860
J. Med. Chem. XXXX, XXX, XXX−XXX

Mouse Microsomal Stability Assay. The phase I metabolic stability assay for compound **19** was conducted in mouse liver microsomes as previously described with minor modifications.[43] 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 10 μM; 0.5 mg/mL microsomes). 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 sampled in triplicate.

Chromatographic analysis was performed using an Accela ultra high-performance system consisting of an analytical pump and an autosampler coupled with a 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 min, maintaining at 99% (2−2.5 min) and reequilibrating to 10% by 2.7 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 Supporting Information, Table S2.

Locomotor Activity Studies. Twenty-four male mice (22−25 g) with a C57BL/6J genetic background were purchased from the Charles River Laboratories (Raleigh, NC). After arrival, they were group housed in the animal facility under a reversed 12 h light−dark cycle (light on at 7:00 PM) with free access to food and water and allowed to acclimate to the new environment for 7 days prior to initiating the experiment. All procedures were in accordance with the "Principles of Laboratory Animal Care" outlined by National Institute of Health (NIH publication 86-23, 1996).

Locomotor activity tests were conducted in open-field chambers (43 × 43 × 30 cm^3) (Accuscan Instruments, Inc., Columbus, OH, USA). Before testing, the mice were habituated to the locomotor chamber (2 h/day for 3 consecutive days) and then were randomly divided into three dose groups (vehicle, 5, 15 mg/kg compound **19**). On habituation days, the animals were moved to the test room, acclimated there for 10 min, and then placed in their assigned locomotor chambers. On test day 1, each group of mice was pretreated with either vehicle (25% of 2-hydroxypropyl-β-cyclodextrin) or one dose of compound **19** (5, 15 mg/kg, ip) without an oxycodone injection. Then 15 min later, the animals were placed into the test chambers. Their locomotor activities were recorded every 20 min for 2 h using VersaMax version 3.0 software (Accuscan Instruments, Inc.). On test days 2−6, the three groups of mice first received vehicle or one dose of compound **19**, followed by an oxycodone injection 15 min after compound **19** pretreatment. After 72 h of withdrawal in the home cages in the animal facility, each animal was challenged with a 4 mg/kg oxycodone injection again (day 9) but without compound **19** pretreatment. The locomotor activities were recorded for 2 h/day after each oxycodone injection.

The locomotor behavioral data are expressed as means (±SEM). The traveled distance (cm) within 2 h (Figure 4A) or every 20 min (Figure 4B−D) was used to evaluate the locomotor effects of **19** and/or oxycodone in mice. Two-way ANOVA with repeated measures over time was used to evaluate the statistical significance of the changes in locomotor activity after compound **19** and/or oxycodone administration. Posthoc Fisher's least significant difference was used for multiple comparisons. $p < 0.05$ was considered statistically significant.

Oxycodone-Induced Conditioned Place Preference (CPP). Fifty male Long−Evans rats (275−300 g) were purchased from the Charles River Laboratories (Raleigh, NC) and housed in the animal facility at NIDA IRP under a reversed 12 h light−dark cycle (light on at 7:00 PM) with free access to food and water. All procedures were in accordance with the "Principles of Laboratory Animal Care" outlined by National Institute of Health (NIH publication 86-23, 1996).

CPP was tested in the place-conditioning apparatus (Med Associates, St Albans, VT) consisting of two side compartments (21 × 28 cm^2) and a central gray connecting area (21 × 12.5 cm^2); a sliding door separated each compartment from the connecting area. The two side compartments differed in wall color (black vs white), floor type (net vs grid), and illumination. The animals were then divided randomly into five treatment groups as shown in Figure 5 legend. Each rat was first exposed in the CPP apparatus for 15 min (preconditioning) and then followed by oxycodone CPP conditioning. Animals displayed significant bias in one compartment (defined by the time difference between two compartments ≥200 s) during preconditioning were excluded from the study. Oxycodone CPP procedures consisted of 2 days of oxycodone (3 mg/kg, ip) conditioning in one compartment and 2 days of saline conditioning in another compartment, alternatively (e.g., oxycodone−saline−oxycodone−saline). The compartments paired with oxycodone or saline were counterbalanced. On the conditioning days, each animal received saline or compound **19** (5, 15 mg/kg, ip) pretreatment 15 min prior to oxycodone injection. After oxycodone injection, animals were immediately confined in the assigned compartments for 40 min. To potentiate oxycodone CPP formation, animals received 4 days of oxycodone (3 mg/kg, ip, twice daily at 8:00 and 18:00) treatment at home cages.[60,61] In the (**19** + vehicle) group, saline was paired with one compartment and compound **19** (15 mg/kg) was paired with another compartment. In the (Vehicle + vehicle) group, saline was paired with each compartment. Then 24 h after the last saline or oxycodone conditioning injection, animals were placed in the same three-chamber CPP apparatus for 15 min and the time spent in each compartment was recorded. The CPP score was calculated by the time difference (seconds) that animal spent in drug-paired compartment versus saline-paired compartment.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.6b00860.

Elemental analysis results and additional metabolism data (PDF)

Molecular formula strings (CSV)

■ AUTHOR INFORMATION

Corresponding Author

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

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Support for this research was provided by the National Institute on Drug Abuse - Intramural Research Program, NIDA Addiction Treatment Discovery Program contract (ADA151001) with Oregon Health & Science University and Johns Hopkins Drug Discovery (P30MH075673 to BSS). We thank Dr. Anerbasha Shaik for help with the TOC Graphic.

■ 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; OBS, orthosteric binding site; SBP, secondary binding pocket; 5-HT, 5-hydroxytryptamine (serotonin); CMA, chloroform/methanol/ammonium hydroxide

■ REFERENCES

(1) Heidbreder, C. A.; Newman, A. H. Current perspectives on selective dopamine D_3 receptor antagonists as pharmacotherapeutics

DOI: 10.1021/acs.jmedchem.6b00860
J. Med. Chem. XXXX, XXX, XXX−XXX

for addictions and related disorders. *Ann. N. Y. Acad. Sci.* **2010**, *1187*, 4−34.
(2) Caine, S. B.; Koob, G. F. Modulation of cocaine self-administration in the rat through D-3 dopamine receptors. *Science* **1993**, *260*, 1814−1816.
(3) Pilla, M.; Perachon, S.; Sautel, F.; Garrido, F.; Mann, A.; Wermuth, C. G.; Schwartz, J. C.; Everitt, B. J.; Sokoloff, P. Selective inhibition of cocaine-seeking behaviour by a partial dopamine D3 receptor agonist. *Nature* **1999**, *400*, 371−375.
(4) Pich, E. M.; Collo, G. Pharmacological targeting of dopamine D3 receptors: Possible clinical applications of selective drugs. *Eur. Neuropsychopharmacol.* **2015**, *25*, 1437−1447.
(5) Le Foll, B.; Collo, G.; Rabiner, E. A.; Boileau, I.; Merlo Pich, E.; Sokoloff, P. Dopamine D3 receptor ligands for drug addiction treatment: update on recent findings. *Prog. Brain Res.* **2014**, *211*, 255−275.
(6) Paterson, N. E.; Vocci, F.; Sevak, R. J.; Wagreich, E.; London, E. D. Dopamine D3 receptors as a therapeutic target for methamphetamine dependence. *Am. J. Drug Alcohol Abuse* **2014**, *40*, 1−9.
(7) Newman, A. H.; Blaylock, B. L.; Nader, M. A.; Bergman, J.; Sibley, D. R.; Skolnick, P. Medication discovery for addiction: translating the dopamine D3 receptor hypothesis. *Biochem. Pharmacol.* **2012**, *84*, 882−890.
(8) Appel, N. M.; Li, S. H.; Holmes, T. H.; Acri, J. B. Dopamine D3 receptor antagonist (GSK598809) potentiates the hypertensive effects of cocaine in conscious, freely-moving dogs. *J. Pharmacol. Exp. Ther.* **2015**, *354*, 484−492.
(9) Boateng, C. A.; Bakare, O. M.; Zhan, J.; Banala, A. K.; Burzynski, C.; Pommier, E.; Keck, T. M.; Donthamsetti, P.; Javitch, J. A.; Rais, R.; Slusher, B. S.; Xi, Z.-X.; Newman, A. H. High affinity dopamine D_3 receptor (D_3R)-selective antagonists attenuate heroin self-administration in wildtype but not D_3R knockout mice. *J. Med. Chem.* **2015**, *58*, 6195−6213.
(10) Le Foll, B. L.; Collo, G.; Rabiner, E. A.; Boileau, I.; Pich, E. M.; Sokoloff, P. Dopamine D3 receptor ligands for drug addiction treatment: update on recent findings. *Prog. Brain Res.* **2014**, *211*, 255−275.
(11) Michino, M.; Beuming, T.; Donthamsetti, P.; Newman, A. H.; Javitch, J. A.; Shi, L. What can crystal structures of aminergic receptors tell us about designing subtype-selective ligands? *Pharmacol. Rev.* **2015**, *67*, 198−213.
(12) Keck, T. M.; Burzynski, C.; Shi, L.; Newman, A. H. Beyond small-molecule SAR: using the dopamine D3 receptor crystal structure to guide drug design. *Adv. Pharmacol.* **2014**, *69*, 267−300.
(13) Chien, E. Y. T.; Liu, W.; Zhao, Q.; Katritch, V.; Han, G. W.; Hanson, M. A.; Shi, L.; Newman, A. H.; Javitch, J. A.; Cherezov, V.; Stevens, R. C. Structure of the human dopamine D3 receptor in complex with a D2/D3 selective antagonist. *Science* **2010**, *330*, 1091−1095.
(14) Newman, A. H.; Beuming, T.; Banala, A. K.; Donthamsetti, P.; Pongetti, K.; LaBounty, A.; Levy, B.; Cao, J.; Michino, M.; Luedtke, R. R.; Javitch, J. A.; Shi, L. Molecular determinants of selectivity and efficacy at the dopamine D3 receptor. *J. Med. Chem.* **2012**, *55*, 6689−6899.
(15) Micheli, F. Recent advances in the development of dopamine D3 receptor antagonists: a medicinal chemistry perspective. *ChemMedChem* **2011**, *6*, 1152−1162.
(16) Micheli, F.; Heidbreder, C. Dopamine D3 receptor antagonists: a patent review (2007 - 2012). *Expert Opin. Ther. Pat.* **2013**, *23*, 363−381.
(17) Micheli, F.; Bonanomi, G.; Blaney, F. E.; Braggio, S.; Capelli, A. M.; Checchia, A.; Curcuruto, O.; Damiani, F.; Di Fabio, R.; Donati, D.; Gentile, G.; Gribble, A.; Hamprecht, D.; Tedesco, G.; Terreni, S.; Tarsi, L.; Lightfoot, A.; Stemp, G.; Macdonald, G.; Smith, A.; Pecoraro, M.; Petrone, M.; Perini, O.; Piner, J.; Rossi, T.; Worby, A.; Pilla, M.; Valerio, E.; Griffante, C.; Mugnaini, M.; Wood, M.; Scott, C.; Andreoli, M.; Lacroix, L.; Schwarz, A.; Gozzi, A.; Bifone, A.; Ashby, C. R., Jr.; Hagan, J. J.; Heidbreder, C. 1,2,4-Triazol-3-yl-thiopropyl-tetrahydrobenzazepines: a series of potent and selective dopamine D(3) receptor antagonists. *J. Med. Chem.* **2007**, *50*, 5076−5089.
(18) Keck, T. M.; John, W. S.; Czoty, P. W.; Nader, M. A.; Newman, A. H. Identifying medication targets for psychostimulant addiction: unraveling the dopamine D_3 receptor hypothesis. *J. Med. Chem.* **2015**, *58*, 5361−5380.
(19) Newman, A. H.; Grundt, P.; Cyriac, G.; Deschamps, J. R.; Taylor, M.; Kumar, R.; Ho, D.; Luedtke, R. R. *N*-(4-(4-(2,3-dichloro- or 2-methoxyphenyl)piperazin-1-yl)butyl)heterobiarylcarboxamides with functionalized linking chains as high affinity and enantioselective D3 receptor antagonists. *J. Med. Chem.* **2009**, *52*, 2559−2570.
(20) Galaj, E.; Manuszak, M.; Babic, S.; Ananthan, S.; Ranaldi, R. The selective dopamine D3 receptor antagonist, SR 21502, reduces cue-induced reinstatement of heroin seeking and heroin conditioned place preference in rats. *Drug Alcohol Depend.* **2015**, *156*, 228−233.
(21) Zou, M. F.; Keck, T. M.; Kumar, V.; Donthamsetti, P.; Michino, M.; Burzynski, C.; Schweppe, C.; Bonifazi, A.; Free, R. B.; Sibley, D. R.; Janowsky, A.; Shi, L.; Javitch, J. A.; Newman, A. H. Novel analogues of (*R*)-5-(methylamino)-5,6-dihydro-4*H*-imidazo[4,5,1-ij]-quinolin-2(1*H*)-one (sumanirole) provide clues to dopamine D2/D3 receptor agonist selectivity. *J. Med. Chem.* **2016**, *59*, 2973−2988.
(22) Mistry, S. N.; Shonberg, J.; Draper-Joyce, C. J.; Klein Herenbrink, C.; Michino, M.; Shi, L.; Christopoulos, A.; Capuano, B.; Scammells, P. J.; Lane, J. R. Discovery of a novel class of negative allosteric modulator of the dopamine D2 receptor through fragmentation of a bitopic ligand. *J. Med. Chem.* **2015**, *58*, 6819−6843.
(23) Carlsson, J.; Coleman, R. G.; Setola, V.; Irwin, J. J.; Fan, H.; Schlessinger, A.; Sali, A.; Roth, B. L.; Shoichet, B. K. Ligand discovery from a dopamine D3 receptor homology model and crystal structure. *Nat. Chem. Biol.* **2011**, *7*, 769−778.
(24) Hall, H.; Köhler, C.; Gawell, L.; Farde, L.; Sedvall, G. Raclopride, a new selective ligand for the dopamine-D2 receptors. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **1988**, *12*, 559−568.
(25) Ehrlich, K.; Gotz, A.; Bollinger, S.; Tschammer, N.; Bettinetti, L.; Harterich, S.; Hubner, H.; Lanig, H.; Gmeiner, P. Dopamine D_2, D_3, and D_4 selective phenylpiperazines as molecular probes to explore the origins of subtype specific receptor binding. *J. Med. Chem.* **2009**, *52*, 4923−4935.
(26) Grundt, P.; Prevatt, K. M.; Cao, J.; Taylor, J.; Floresca, C. Z.; Choi, J.-K.; Jenkins, B. G.; Luedtke, R. R.; Newman, A. H. Heterocyclic analogues of *N*-(4-(4-(2,3-dichlorophenyl)piperazin-1-yl)-butyl)-arylcarboxamides with functionalized linking chains as novel dopamine D_3 receptor ligands: potential substance abuse therapeutic agents. *J. Med. Chem.* **2007**, *50*, 4135−4146.
(27) Menzella, H. G.; Tran, T. T.; Carney, J. R.; Lau-Wee, J.; Galazzo, J.; Reeves, C. D.; Carreras, C.; Mukadam, S.; Eng, S.; Zhong, Z.; Timmermans, P. B.; Murli, S.; Ashley, G. W. Potent non-benzoquinone ansamycin heat shock protein 90 inhibitors from genetic engineering of Streptomyces hygroscopicus. *J. Med. Chem.* **2009**, *52*, 1518−1521.
(28) Mizuhara, T.; Kato, T.; Hirai, A.; Kurihara, H.; Shimada, Y.; Taniguchi, M.; Maeta, H.; Togami, H.; Shimura, K.; Matsuoka, M.; Okazaki, S.; Takeuchi, T.; Ohno, H.; Oishi, S.; Fujii, N. Structure-activity relationship study of phenylpyrazole derivatives as a novel class of anti-HIV agents. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 4557−4561.
(29) White, J. D.; Wang, G.; Quaranta, L. Studies on the synthesis of gymnodimine. Construction of the spiroimine portion via Diels-Alder cycloaddition. *Org. Lett.* **2003**, *5*, 4983−4986.
(30) Liu, K. G.; Robichaud, A. J. A general and convenient synthesis of *N*-aryl piperazines. *Tetrahedron Lett.* **2005**, *46*, 7921−7922.
(31) Newman, A. H.; Grundt, P.; Cyriac, G.; Deschamps, J. R.; Taylor, M.; Kumar, R.; Ho, D.; Luedtke, R. R. N-(4-(4-(2,3-Dichloro- or 2-methoxyphenyl)piperazin-1-yl)butyl)heterobiarylcarboxamides with functionalized linking chains as high affinity and enantioselective D3 receptor antagonists. *J. Med. Chem.* **2009**, *52*, 2559−2570.
(32) Kumar, V.; Banala, A. K.; Garcia, E. G.; Cao, J.; Keck, T. M.; Bonifazi, A.; Deschamps, J. R.; Newman, A. H. Chiral resolution and serendipitous fluorination reaction for the selective dopamine D3

DOI: 10.1021/acs.jmedchem.6b00860
J. Med. Chem. XXXX, XXX, XXX−XXX

receptor antagonist BAK2−66. *ACS Med. Chem. Lett.* **2014**, *5*, 647−651.
(33) Banala, A. K.; Levy, B. A.; Khatri, S. S.; Furman, C. A.; Roof, R. A.; Mishra, Y.; Griffin, S. A.; Sibley, D. R.; Luedtke, R. R.; Newman, A. H. N-(3-fluoro-4-(4-(2-methoxy or 2,3-dichlorophenyl)piperazine-1-yl)butyl)arylcarboxamides as selective dopamine D3 receptor ligands: critical role of the carboxamide linker for D3 receptor selectivity. *J. Med. Chem.* **2011**, *54*, 3581−3594.
(34) Basha, A.; Lipton, M.; Weinreb, S. M. A mild, general method for conversion of esters to amides. *Tetrahedron Lett.* **1977**, *18*, 4171−4172.
(35) Rovnyak, G.; Andersen, N.; Gougoutas, J.; Hedberg, A.; Kimball, S. D.; Malley, M.; Moreland, S.; Porubcan, M.; Pudzianowski, A. Studies directed toward ascertaining the active conformation of 1,4-dihydropyridine calcium entry blockers. *J. Med. Chem.* **1988**, *31*, 936−944.
(36) Sasaki, Y.; Murphy, W. A.; Heiman, M. L.; Lance, V. A.; Coy, D. H. Solid-phase synthesis and biological properties of psi [CH_2NH] pseudopeptide analogues of a highly potent somatostatin octapeptide. *J. Med. Chem.* **1987**, *30*, 1162−1166.
(37) Gagnon, H.; Beauchemin, S.; Kwiatkowska, A.; Couture, F.; D'Anjou, F.; Levesque, C.; Dufour, F.; Desbiens, A. R.; Vaillancourt, R.; Bernard, S.; Desjardins, R.; Malouin, F.; Dory, Y. L.; Day, R. Optimization of furin inhibitors to protect against the activation of influenza hemaglutinin H5 and Shiga toxin. *J. Med. Chem.* **2014**, *57*, 29−41.
(38) Cruickshank, P. A.; Fishman, M. Studies in alkylation. II. Reactions of epoxyalkyl bromides. *J. Org. Chem.* **1969**, *34*, 4060−4065.
(39) Grundt, P.; Prevatt, K. M.; Cao, J.; Taylor, M.; Floresca, C. Z.; Choi, J. K.; Jenkins, B. G.; Luedtke, R. R.; Newman, A. H. Heterocyclic analogues of *N*-(4-(4-(2,3-dichlorophenyl)piperazin-1-yl)butyl)-arylcarboxamides with functionalized linking chains as novel dopamine D3 receptor ligands: potential substance abuse therapeutic agents. *J. Med. Chem.* **2007**, *50*, 4135−4146.
(40) Duan, H.; Ning, M.; Zou, Q.; Ye, Y.; Feng, Y.; Zhang, L.; Leng, Y.; Shen, J. Discovery of intestinal targeted TGR5 agonists for the treatment of type 2 diabetes. *J. Med. Chem.* **2015**, *58*, 3315−3328.
(41) Ertl, P.; Rohde, B.; Selzer, P. Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties. *J. Med. Chem.* **2000**, *43*, 3714−3717.
(42) Furman, C. A.; Roof, R. A.; Moritz, A. E.; Miller, B. N.; Doyle, T. B.; Free, B.; Banala, A. K.; Paul, N. M.; Kumar, V.; Sibley, C. D.; Newman, A. H.; Sibley, D. R. Investigation of the binding and functional properties of extended length D_3 dopamine receptor-selective antagonists. *Eur. Neuropsychopharmacol.* **2015**, *25*, 1448−1461.
(43) Rais, R.; Thomas, A. G.; Wozniak, K.; Wu, Y.; Jaaro-Peled, H.; Sawa, A.; Strick, C. A.; Engle, S. J.; Brandon, N. J.; Rojas, C.; Slusher, B. S.; Tsukamoto, T. Pharmacokinetics of oral D-serine in D-amino acid oxidase knockout mice. *Drug Metab. Dispos.* **2012**, *40*, 2067−2073.
(44) Robinson, T. E.; Berridge, K. C. The incentive sensitization theory of addiction: some current issues. *Philos. Trans. R. Soc., B* **2008**, *363*, 3137−3146.
(45) Gardner, E. L. Use of animal models to develop antiaddiction medications. *Curr. Psychiatry Rep.* **2008**, *10*, 377−384.
(46) Compton, W. M.; Volkow, N. D. Major increases in opioid analgesic abuse in the United States: concerns and strategies. *Drug Alcohol Depend.* **2006**, *81*, 103−107.
(47) Compton, W. M.; Jones, C. M.; Baldwin, G. T. Relationship between nonmedical prescription-opioid use and heroin use. *N. Engl. J. Med.* **2016**, *374*, 154−163.
(48) Wilkerson, R. G.; Kim, H. K.; Windsor, T. A.; Mareiniss, D. P. The opioid epidemic in the United States. *Emerg. Med. Clin. North Am.* **2016**, *34*, e1−e23.
(49) Von Korff, M. R.; Franklin, G. Responding to America's iatrogenic epidemic of prescription opioid addiction and overdose. *Med. Care* **2016**, *54*, 426−429.
(50) Niikura, K.; Ho, A.; Kreek, M. J.; Zhang, Y. Oxycodone-induced conditioned place preference and sensitization of locomotor activity in adolescent and adult mice. *Pharmacol., Biochem. Behav.* **2013**, *110*, 112−116.
(51) Song, R.; Yang, R. F.; Wu, N.; Su, R. B.; Li, J.; Peng, X. Q.; Li, X.; Gaál, J.; Xi, Z. X.; Gardner, E. L. YQA14: a novel dopamine D3 receptor antagonist that inhibits cocaine self-administration in rats and mice, but not in D3 receptor-knockout mice. *Addict. Biol.* **2012**, *17*, 259−273.
(52) Sun, L.; Song, R.; Chen, Y.; Yang, R. F.; Wu, N.; Su, R. B.; Li, J. A selective D3 receptor antagonist YQA14 attenuates methamphetamine-induced behavioral sensitization and conditioned place preference in mice. *Acta Pharmacol. Sin.* **2016**, *37*, 157−165.
(53) Le Foll, B.; Diaz, J.; Sokoloff, P. Increased dopamine D3 receptor expression accompanying behavioral sensitization to nicotine in rats. *Synapse* **2003**, *47*, 176−183.
(54) Filip, M.; Papla, I.; Czepiel, K. Role of dopamine D3 receptors in controlling the expression of cocaine sensitization in rats. *Pol. J. Pharmacol.* **2002**, *54*, 687−691.
(55) Li, T.; Hou, Y.; Yan, C. X.; Chen, T.; Zhao, Y.; Li, S. B. Dopamine D3 receptor knock-out mice display deficits in locomotor sensitization after chronic morphine administration. *Neurosci. Lett.* **2010**, *485*, 256−260.
(56) Volkow, N. D.; McLellan, A. T. Opioid abuse in chronic pain-misconceptions and mitigation strategies. *N. Engl. J. Med.* **2016**, *374*, 1253−1263.
(57) Compton, W. M.; Volkow, N. D. Major increases in opioid analgesic abuse in the United States: concerns and strategies. *Drug Alcohol Depend.* **2006**, *81*, 103−107.
(58) Compton, W. M.; Jones, C. M.; Baldwin, G. T. Relationship between nonmedical prescription-opioid use and heroin use. *N. Engl. J. Med.* **2016**, *374*, 154−163.
(59) Chen, J.; Levant, B.; Jiang, C.; Keck, T. M.; Newman, A. H.; Wang, S. Tranylcypromine substituted cis-hydroxycyclobutylnaphthamides as potent and selective dopamine D(3) receptor antagonists. *J. Med. Chem.* **2014**, *57*, 4962−4968.
(60) Gaiardi, M.; Bartoletti, M.; Bacchi, A.; Gubellini, C.; Costa, M.; Babbini, M. Role of repeated exposure to morphine in determining its affective properties: place and taste conditioning studies in rats. *Psychopharmacology* **1991**, *103*, 183−186.
(61) Lett, B. T. Repeated exposures intensify rather than diminish the rewarding effects of amphetamine, morphine, and cocaine. *Psychopharmacology* **1989**, *98*, 357−362.

DOI: 10.1021/acs.jmedchem.6b00860
J. Med. Chem. XXXX, XXX, XXX−XXX