Journal of Medicinal Chemistry

ACS AuthorChoice

Article

pubs.acs.org/jmc

Terms of Use

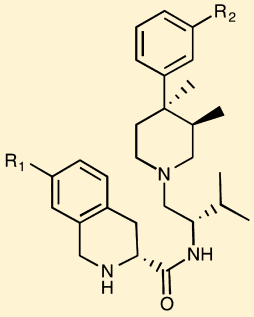
Design, Synthesis, and Biological Evaluation of (3*R*)-1,2,3,4-Tetrahydro-7-hydroxy-*N*-[(1*S*)-1-[[(3*R*,4*R*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl]-2-methylpropyl]-3-isoquinolinecarboxamide (JDTic) Analogues: In Vitro Pharmacology and ADME Profile

Chad M. Kormos, Moses G. Gichinga, Rangan Maitra, Scott P. Runyon, James B. Thomas, Lawrence E. Brieaddy, S. Wayne Mascarella, Hernán A. Navarro, and F. Ivy Carroll*

Research Triangle Institute, 3040 Cornwallis Road, P.O. Box 12194, Research Triangle Park, North Carolina 27709-6679, United States

Supporting Information

ABSTRACT: JDTic analogues **4**–**15** which have the hydroxyl groups replaced with other groups were synthesized and their in vitro efficacy at the μ, δ, and κ opioid receptors determined and compared to JDTic using [^{35}S]GTPγS assays. Compounds **4**, **5**, **6**, **13**, **14**, and **15** had K_e = 0.024, 0.01, 0.039, 0.02, 0.11, and 0.041 nM compared to the K_e = 0.02 nM for JDTic at the κ receptor and were highly selective for the κ receptor relative to the μ and δ opioid receptors. Unexpectedly, replacement of the 3-hydroxyl substituent of the 4-(3-hydroxyphenyl) group of JDTic with a H, F, or Cl substituent leads to potent and selective KOR antagonists. In vitro studies to determine various ADME properties combined with calculated TPSA, clogP, and logBB values suggests that the potent and selective κ opioid receptors **4**, **5**, **13**, and **14** deserve consideration for further development toward potential drugs for CNS disorders.



INTRODUCTION

The opioid receptors (μ, δ, κ, and the opioid-like receptor ORL-1) belong to the super family of G-protein coupled receptors (GPCRs) that possess seven helical trans-membrane spanning domains in their architecture.[1] The majority of research efforts focused upon this group of proteins has been directed toward the μ receptor because it mediates the analgesic actions of opiates such as morphine (Chart 1).[2] Over the years, however, it has become increasingly clear that the entire family of opioid proteins are actively involved in a host of important physiological processes.[2]

Studies with selective κ opioid receptor antagonists have shown that this system is intimately involved in brain processes that relate to stress, fear, and anxiety as well as reward-seeking behavior.[3,4] Studies have shown that (3*R*)-1,2,3,4-tetrahydro-7-hydroxy-*N*-[(1*S*)-1-[[(3*R*,4*R*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl]-2-methylpropyl]-3-isoquinolinecarboxamide (JDTic) and nor-binaltorphimine (nor-BNI), another κ opioid selective antagonist, dose-dependently reduce fear and stress-induced responses in multiple behavioral paradigms with rodents (immobility in the forced-swim assay,[5,6] reduction of exploratory behavior in the elevated plus maze, and fear-potentiated startle).[7] Further, selective κ opioid receptor antagonists have been shown to reduce stress-induced reinstatement of cocaine self-administration in rats[5] to block the stress-induced potentiation of cocaine place preference conditioning,[8−10] to decrease dependence-induced ethanol self-administration,[11] to attenuate the expression of both the physical (somatic signs hyperalgesia) and effective (anxiety-related behavior conditional place aversion) signs of nicotine-induced withdrawal in mice, to diminish deprivation-induced eating in rats,[12] and to prevent prepulse inhibition mediated by U50,488.[13] These observations regarding the behavioral consequences of receptor blockade in several animal tests suggest that κ opioid receptor antagonists could be useful for treating anxiety, depression, schizophrenia, addiction, and eating disorders.

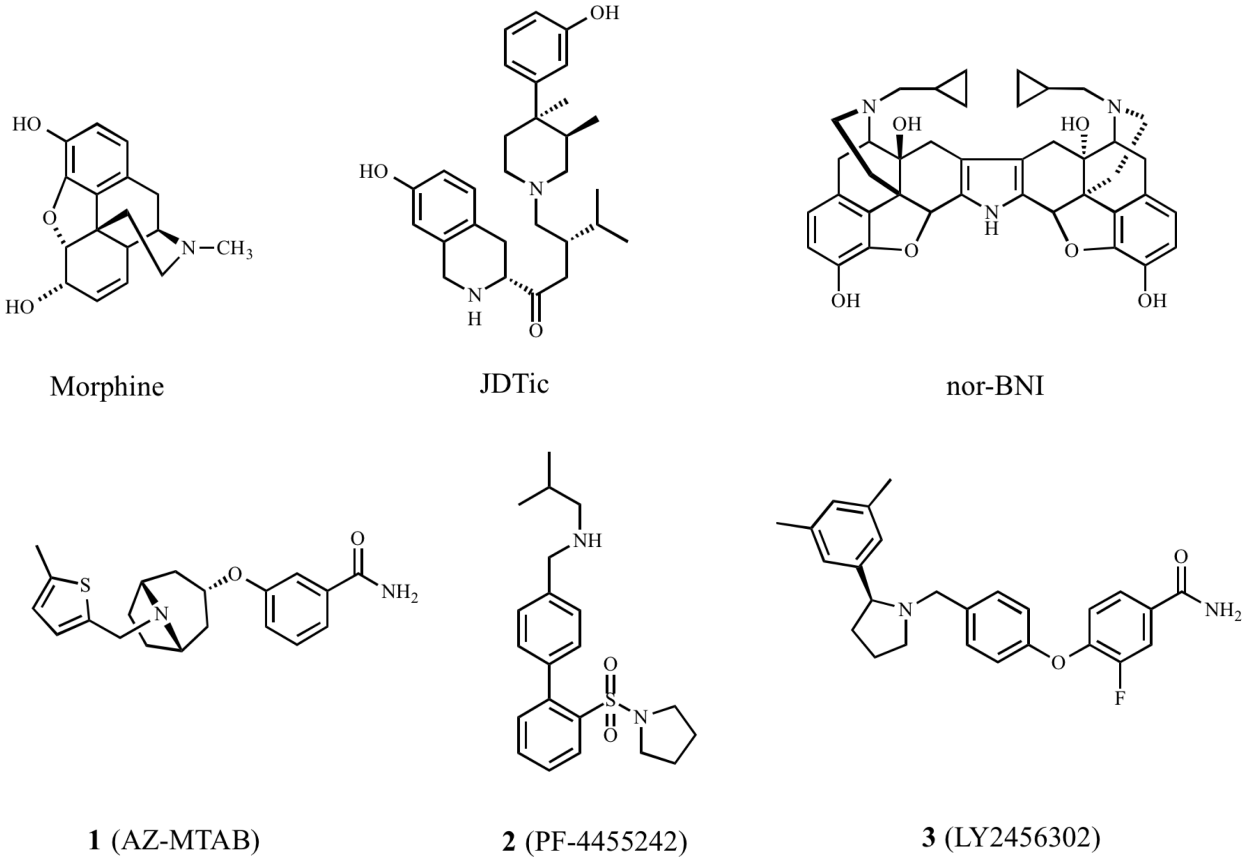
Compounds **1** (AZ-MTAB),[14,15] **2** (PF-4455242),[16,17] and **3** (LY2456302)[18−20] have been reported as newer selective κ opioid receptor antagonists (Chart 1). See also ref 3 for a review of these studies. These newer κ opioid receptor antagonists show activity in various animal models similar to those reported for norBNI and JDTic. In addition, JDTic and compounds **2** and **3** have undergone phase 1 and/or phase 2 studies directed toward various CNS disorders.[21−25]

No drugs for the treatment of cocaine and methamphetamine abuse, however, are currently available.[3] Further, nicotine replacement therapy (NRT), bupropion, and varenicline are used to treat nicotine addiction, but no more than 25% of patients respond to these treatments.[26] Naltrexone is used to treat alcoholism but has limited efficacy.[27] A number of antidepressants are on the market, but many patients do not

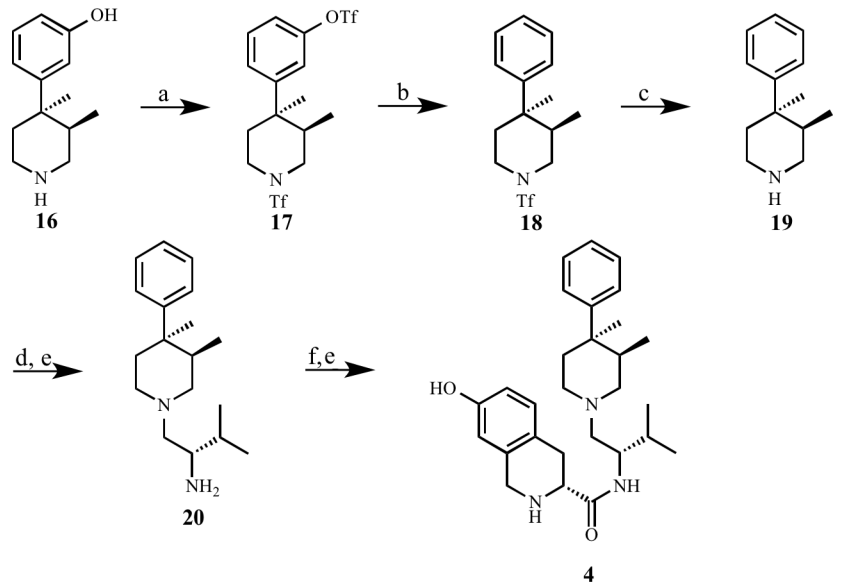
Received: May 28, 2014
Published: August 18, 2014

   dx.doi.org/10.1021/jm5008177 | *J. Med. Chem.* 2014, 57, 7367−7381

Chart 1. Structures of Morphine, JDTic, nor-BNI, AZ-MTAB (1), PF-4455242 (2), and LY2456302 (3)



Scheme 1[a]

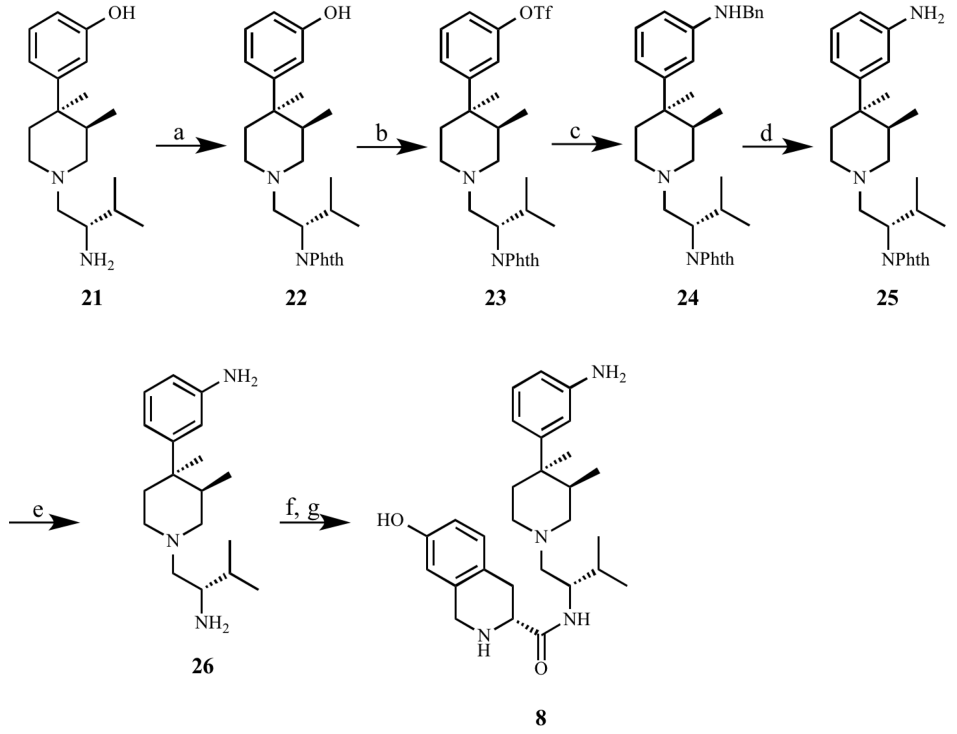


[a]Reagents: (a) Tf_2O, DIPEA, CH_2Cl_2, −78 °C; (b) $PdCl_2(PPh_3)_2$, HCO_2H, NBu_3, DMF, 80 °C; (c) LAH, toluene/THF; (d) Boc-L-valinal, $NaCNBH_3$, CF_3CH_2OH; (e) TFA, CH_2Cl_2; (f) Boc-7-hydroxy-D-Tic, HBTU, NEt_3, CH_2Cl_2.

respond to any of them.[28] In addition, all of these therapeutic agents have undesirable side effects. Accordingly, κ opioid antagonists remain of high interest. In this study, we report the synthesis and in vitro efficacy as determined by [^{35}S]GTPγS assay of JDTic analogues **4**−**15** (see Table 2 for structures). These compounds have the hydroxyl group on the 4-(3-hydroxylphenyl) or 7-hydroxy-tetrahydroisoquinoline parts of JDTic replaced with other functional groups. A comparison of their in vitro efficacy properties to those of JDTic show that several of the analogues were potent and selective κ opioid receptor antagonists. Preclinical ADME studies show that some of the antagonists have better drug-like properties than JDTic.

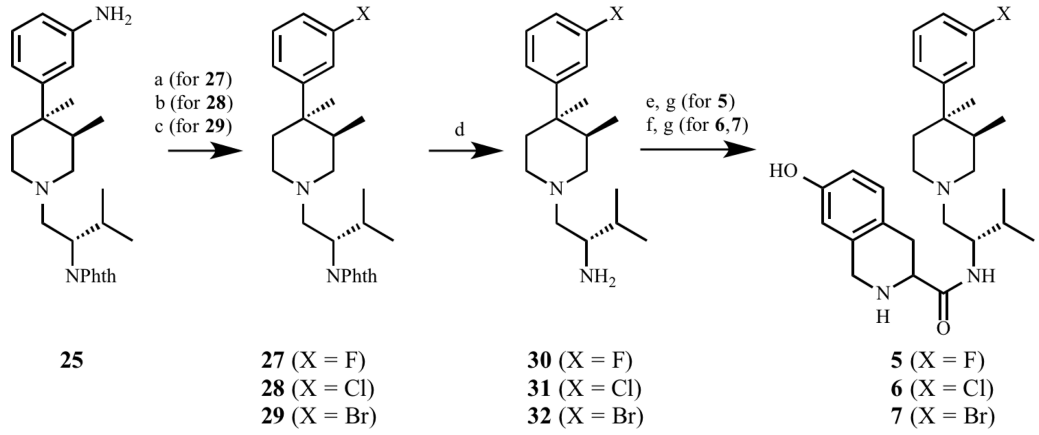
dx.doi.org/10.1021/jm5008177 | J. Med. Chem. 2014, 57, 7367−7381

Scheme 2[a]



[a]Reagents: (a) phthalic anhydride, $CHCl_3$; (b) $PhN(Tf)_2$, NEt_3, CH_2Cl_2; (c) $BnNH_2$, JohnPhos, $Pd(OAc)_2$, tKBuO, tol; (d) $Pd(OH)_2/C$, H_2, EtOH; (e) HCl, dioxane; (f) EDC·HCl, NEt_3, Boc-7-hydroxy-D-Tic; (g) HCl, MeOH.

Scheme 3[a]



[a]Reagents: (a) $BF_3·OEt_2$, $C_5H_{11}ONO$, CH_2Cl_2, then heat, neat; (b) $NaNO_2$, HCl, then CuCl; (c) $NaNO_2$, HBr; then CuBr; (d) hydrazine, EtOH; (e) HBTU, CH_3CN, NEt_3, Boc-7-hydroxy-D-Tic; (f) EDC·HCl, NEt_3, Boc-7-hydroxy-D-Tic, CH_2Cl_2, HOBt; (g) HCl.

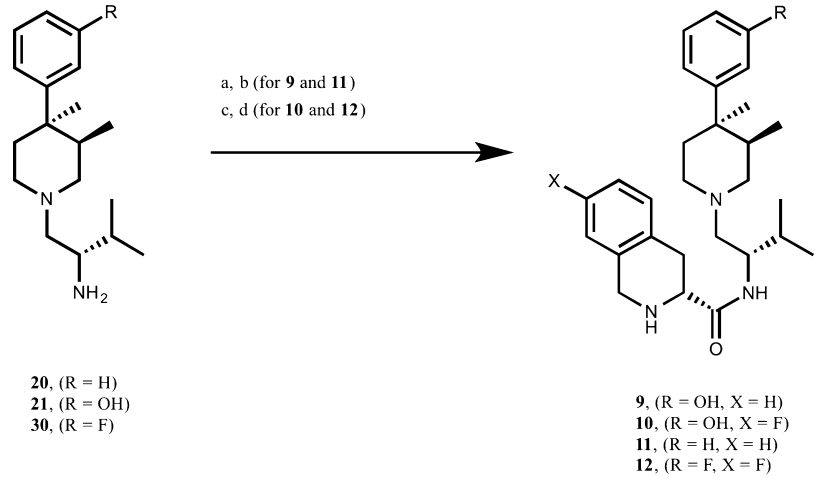
CHEMISTRY

The synthesis of **4** is outlined in Scheme 1. Bis-triflate **17** was prepared by treating **16** with an excess of triflic anhydride at −78 °C. Subjection of **17** to palladium-catalyzed transfer hydrogenation in DMF at 80 °C afforded intermediate **18**. Reduction of **18** with lithium aluminum hydride in toluene and tetrahydrofuran mixture cleaved the triflamide to give (3*R*,4*R*)-3,4-dimethyl-4-phenylpiperidine (**19**). Reductive amination of **19** with Boc-L-valinal, prepared according to the procedure reported by Skiles et al.,[29] followed by *t*-butoxycarbonyl (Boc) deprotection with trifluoroacetic acid in dichloromethane afforded **20**. Coupling of **20** with (3*R*)-2-(*tert*-butoxycarbonyl)-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Boc-7-hydroxy-D-Tic) using *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) in dichloromethane followed by Boc deprotection with trifluoroacetic acid in dichloromethane afforded **4**.

Scheme 2 shows the synthesis of **8** via intermediate **25**, which was also used to synthesize **5**, **6**, and **7** (Scheme 3). Compound **21**[30] was treated with phthalic anhydride to form the phthalimide **22**. Treatment of **22** with *N*-phenyl(bis-trifluoromethanesulfonimide) afforded the triflate **23**. Palladium-catalyzed coupling of **23** with benzylamine yielded **24**. Subjection of **24** to transfer hydrogenation afforded aniline **25**. Deprotection of the phthalimide with aqueous hydrogen chloride in dioxane afforded amine **26**. Amide coupling of **26** with Boc-7-hydroxy-D-Tic,

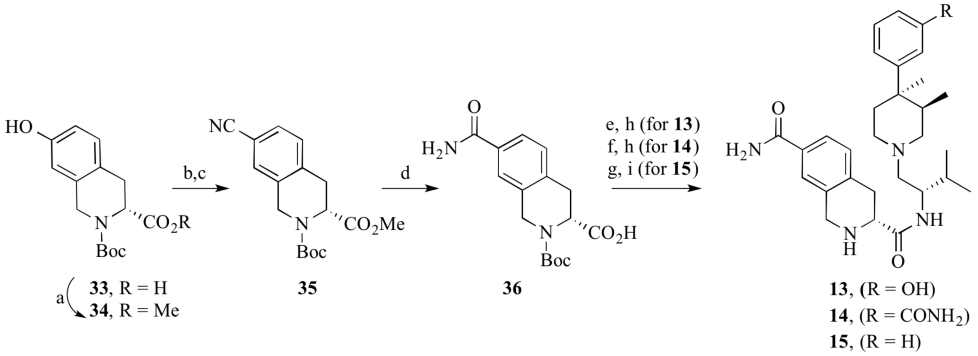
dx.doi.org/10.1021/jm5008177 | J. Med. Chem. 2014, 57, 7367−7381

Scheme 4[a]



[a]Reagent: (a) Boc-D-Tic, HBTU, NEt_3, CH_2Cl_2; (b) TFA, CH_2Cl_2; (c) Boc-7-fluoro-D-Tic, EDC·HCl, NEt_3, CH_2Cl_2; (d) MeOH, HCl.

Scheme 5[a]



[a]Reagents: (a) $TMSCHN_2$, CH_3OH/toluene; (b) Tf_2O, CH_2Cl_2, NEt_3; (c) $Pd(PPh_3)_4$, $Zn(CN)_2$, DMF; (d) aq LiOH, dioxane; then 30% H_2O_2; (e) **21**, HBTU, NEt_3; (f) 3-{(3*R*,4*R*)-1-[(2*S*)-2-amino-3-methylbutyl]-3,4-dimethylpiperidin-4-yl}benzamide, HBTU, NEt_3; (g) **20**, EDC·HCl, DIPEA; (h) TFA, CH_2Cl_2; (i) HCl, MeOH.

followed by removal of the Boc protecting group with hydrochloric acid in aqueous methanol, afforded the desired final product **8**.

The compounds **5**, **6**, and **7** where the phenolic group of the 4-(3-hydroxyphenyl) group in JDTic has been replaced by a fluoro, chloro, and bromo substituent, respectively, were prepared as described in Scheme 3. The diazotization of **25** followed by Schiemann fluorination afforded **27**. Alternatively, Sandmeyer halogenation of the diazo intermediate afforded the chloro and bromo intermediates **28** and **29**. Subsequent deprotection of the phthaloyl protected amines present in **27**, **28**, and **29** using hydrazine in ethanol afforded **30**, **31**, and **32**, respectively. These intermediates were coupled with Boc-7-hydroxy-D-Tic using HBTU in acetonitrile or 1-ethyl-3-(3-(dimethylamino)propyl)-carbodiimide hydrochloride (EDC·HCl) and a catalytic amount of *N*-hydroxybenzotriazole (HOBt) in dichloromethane followed by treatment with hydrogen chloride to afford **5**, **6**, and **7**.

The synthesis of **9**–**12** is illustrated in Scheme 4. Coupling of amines **20** and **21**[30] with commercially available Boc-(3*R*)-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Boc-D-Tic), followed by treatment with trifluoroacetic acid in dichloromethane, gave **11** and **9**, respectively. Coupling of **21** and **30** with (3*R*)-2-[*tert*-butoxy)carbonyl]-7-fluoro-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (7-fluoro-Boc-D-Tic), followed by Boc deprotection using aqueous methanolic hydrogen chloride, afforded **10** and **12**, respectively.

The synthesis of **13**, **14**, and **15** is illustrated in Scheme 5. The methyl ester of Boc-7-hydroxy-D-Tic (**33**) prepared using trimethylsilyldiazomethane in methanol and toluene was converted to the intermediate aryl triflate with triflic anhydride,[31] which was transformed to the benzonitrile (**35**) via palladium-catalyzed cyanation.[32] Careful hydrolysis of the methyl ester using lithium hydroxide in aqueous dioxane, followed by addition of hydrogen peroxide to the cooled solution, resulted in a very rapid hydrolysis of the benzonitrile to the benzamide **36**. The appropriate amine (**21**, 3-{(3*R*,4*R*)-1-[(2*S*)-2-amino-3-methylbutyl]-3,4-dimethylpiperidin-4-yl}benzamide,[33] or **20**) could then be coupled with **36** using HBTU or EDC·HCl to afford the intermediate amides which yielded the desired compounds **13**, **14**, and **15** upon deprotection of the Boc group with trifluoroacetic acid in dichloromethane or hydrochloric acid in aqueous methanol.

Pharmacology. Because [^{35}S]GTPγS binding strongly correlates with animal behavior studies of previously reported κ antagonists, measures of opioid receptor antagonism and specificity for the compounds in the study were obtained by monitoring the ability of selected test compounds to inhibit stimulation of [^{35}S]GTPγS binding produced by the selective agonists (D-Ala,[2] MePhe,[4] Gly-ol[5])encephalin (DAMGO, μ

dx.doi.org/10.1021/jm5008177 | J. Med. Chem. 2014, 57, 7367–7381

Chart 2. Structures of LY83577, LY99355, LY255582, and Compounds 37 and 41−45

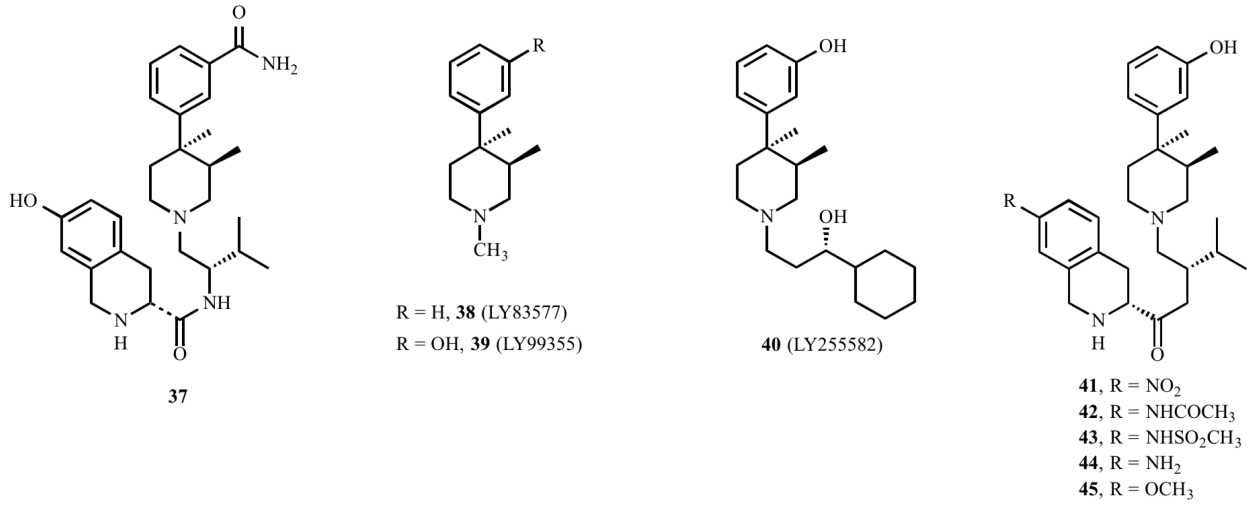
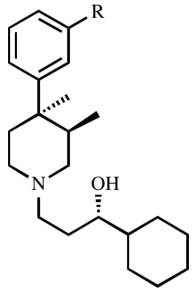


Table 1. Opioid Receptor Binding Data (K_i) and [^{35}S]GTPγS Antagonist Activity (K_b) of LY255582 and Deoxy-LY255582



		K_i (nM)			K_b (nM)		
compd	R	μ	κ	δ	μ	κ	δ
LY255582[a]	OH	0.1	4.7	4.8	0.04	0.3	1.2
deoxy-LY255582[a]	H	7.7	749	169	1.6	40.6	47.1

[a]Data taken from ref 42.

receptor) cyclo[D-Pen^2,D-Pen^5]encephalin (DPDPE, δ) and 5,7,8-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]benzeneacetamide (U69,593, κ) in cloned human receptors using previously reported methods.[34] K_e values were calculated as previously reported.[34]

In Vitro ADME Studies. Several in vitro studies were conducted to characterize κ opioid receptor antagonists **4**, **5**, **13**, and **14** and compared to the results from JDTic and previously reported **37**[33] (Chart 2), which like compounds **13** and **14** has its phenol groups replaced by a carboxamido group. An in vitro model using MDCK-MDR1 cells was used to predict brain penetration. Plasma and S9 stability of each compound was determined using procedures similar to those previously reported.[35,36] Compounds that interact with the human ether-a-go-go gene hERG product (which is a potassium channel) are cardiotoxic. Thus, the affinity of synthesized κ opioid receptor antagonists toward the hERG channel was determined. The interaction of these test compounds with the hERG channel was analyzed using a radioligand displacement assay based on a protocol developed by Chiu et al.[37] For these studies, [^{3}H]astemizole was used as the high-affinity hERG radioligand (K_i ∼ 20 nM). See Experimental Section for details.

Solubility of the compounds was determined using a kinetic 96-well plate assay essentially as described by Zhu et al.[38] See Experimental Section for details.

The parallel artificial membrane permeability assay (PAMPA) was used to predict oral absorption in a 96-well format as has been described previously and detailed in the Experimental Section.

RESULTS AND DISCUSSION

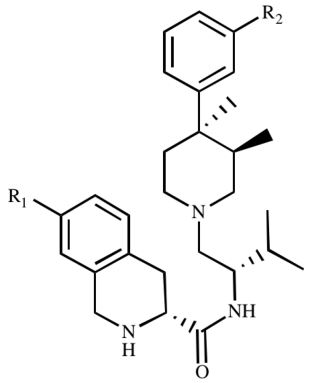
In the late 1970s, Zimmerman and co-workers reported that *N*-methyl-*trans*-4-phenylpiperidine **38** (LY83577) (Chart 2) was an opioid receptor pure antagonist whose potency was significantly increased by adding a phenolic group to the aromatic ring to give the *N*-methyl-*trans*-3,4-dimethyl-4-(3-hydroxyphenyl)-piperidine **39** (LY99335) (Chart 2).[39−41]

In a study directed toward determining if the 3-hydroxy group present on the aromatic ring in **40** (LY255582) was required for potent antagonist efficacy, it was found that removal of the 3-hydroxy group led to 40-, 135-, and 39-fold reduction in the K_b value at the μ, δ, and κ receptors relative to **40** using [^{35}S]GTPγS assays (Table 1).[42] In addition, replacement of the 3-hydroxy group in **40** with 10 other functional groups all led to much reduced in vitro antagonist efficacy relative to **40**, suggesting that the phenolic group was essential for the high potency of **40**.[42]

In the present studies, we demonstrate that **4**, which has the hydroxyl group of the 4-(3-hydroxyphenyl) group in JDTic replaced by a hydrogen (Table 2), is a potent and selective κ opioid receptor antagonist. Compound **4** has K_e values of 8.9,

dx.doi.org/10.1021/jm5008177 | J. Med. Chem. 2014, 57, 7367−7381

Table 2. Inhibition of Agonist-Stimulated [^{35}S]GTPγS Binding in Cloned Human μ, δ, and κ Opioid Receptors



			K_e (nM)				
compd	R_1	R_2	μ, DAMGO[a]	δ, DPDPE[a]	κ, U69,593[a]	μ/κ	δ/κ
JDTic	OH	OH	25 ± 4	74 ± 2	0.02 ± 0.01	1250	3700
4	OH	H	8.9 ± 3	442 ± 130	0.024 ± 0.01	370	18400
5	OH	F	14.8 ± 5	249 ± 44	0.01 ± 0.004	1480	24900
6	OH	Cl	6.81 ± 2.6	685 ± 99	0.039 ± 0.001	175	17600
7	OH	Br	6.56 ± 0.44	594 ± 160	0.268 ± 0.01	25	2200
8	OH	NH_2	10.6 ± 3.6	1899 ± 657	0.25 ± 0.09	42	7600
9	H	OH	16 ± 5	158 ± 49	4.3 ± 2	3.7	37
10	F	OH	7.7 ± 0.9	*b*	2.20 ± 0.47	3.5	
11	H	H	724 ± 146	>3 μM	16 ± 7	45	>188
12	F	F	360 ± 63	*b*	2.22 ± 0.47	162	
13	$CONH_2$	OH	7.09 ± 2.58	131 ± 23	0.02 ± 0.005	355	6550
14	$CONH_2$	$CONH_2$	25.3 ± 7.89	517 ± 52	0.11 ± 0.02	230	4700
15	$CONH_2$	H	6.70 ± 2.1	111 ± 29	0.041 ± 0.006	163	2700
37[c]	OH	$CONH_2$	21 ± 3	478 ± 75	0.12 ± 0.03	175	4000

[a]The data represents the mean (SE) from at least three independent experiments. [b]These compounds are weak inverse agonists at the δ opioid receptor. [c]Data taken from ref 33.

442, and 0.024 nM at the μ, δ, and κ receptors, respectively, compared to K_e values of 25, 74, and 0.02 nM for JDTic. Compound **4** with 370- and 18400-fold selectivity for the κ receptor relative to the μ and δ receptors was more selective than JDTic for the δ receptor but a little less selective than JDTic for the μ receptor. Both compounds, however, are highly selective for the κ receptor relative to both the μ and δ receptors. This discovery is in contrast to previously reported structural activity studies of **40** as well as other compounds in this class of compounds.

Compound **5**, which has a fluoro group in place of the hydrogen present in **4** or the hydroxyl group in JDTic, with K_e values of 14.8, 249, and 0.01 nM at the μ, δ, and κ receptors, respectively, and 1480- and 24900-fold κ selectivity relative to the μ and δ receptors, is both a more potent and more selective κ opioid receptor antagonist than JDTic or **4**. Compounds **6** and **7** have a chloro and bromo group, respectively, in place of the hydroxyl group in JDTic. Compound **6** has a K_e = 0.039 nM at the κ receptor, with 175- and 17600-fold selectivity for the κ relative to the μ and δ receptors and thus has good κ potency and selectivity. Compound **7**, which has the larger bromo group in place of the hydroxyl in JDTic, has a weaker K_e value of 0.268 nM at the κ receptor and only a 25-fold selectivity for the κ receptor relative to the μ receptor. Replacement of the hydroxyl group in JDTic with the amino electron donating amino group to give **8** results in a decrease in potency at the κ receptor (K_e = 0.25 nM) and increased potency at the μ receptor (K_e = 10.6 nM). This results in only 42-fold selectivity for the κ relative to the μ receptor. Compound **8** has 7600-fold selectivity for the κ relative to the δ receptor and thus is more selective than JDTic for the κ relative to the δ receptor.

In a previous report, we compared the opioid receptor antagonist efficacy of compounds **41**−**45** (Chart 2) using the same conditions as that used in this study.[33] The nitro (**41**), acetylamino (**42**), methanesulfonylamino (**43**), and amino (**44**) analogues were 320-, 70-, 200-, and 10-times less potent as a κ antagonist than JDTic and were not as selective for the κ receptor relative to the μ and δ receptors as JDTic. The methoxy (**45**) analogue was only 3-fold less potent than JDTic as a κ antagonist. Compound **45** was selective for the κ receptor relative to the μ and δ but was not as selective as JDTic.

The critical importance of a methoxy or hydroxyl group in the tetrahydroisoquinoline carboxamide (Tic) part of JDTic is further shown by the results with **9** and **10**, where the Tic hydroxyl group in JDTic is replaced by a hydrogen or fluoro substituent, respectively. Compound **9** has K_e values of 16, 158, and 4.3 nM at the μ, δ, and κ receptors, respectively, with only 3.7- and 37-fold selectivity for the κ relative to the μ and δ receptors. Thus, **9** is much less potent and selective as a κ opioid antagonist than JDTic or **4** (compound **9** was previously characterized as the free base but was not evaluated for opioid antagonst efficacy under conditions used in this study).[43] Compound **10** with K_e values of 7.7 and 2.20 nM at the μ and κ receptors, respectively, is also much less potent and selective as a κ opioid receptor antagonist. Surprisingly, **10** behaved as a weak inverse agonist at the δ receptor.

dx.doi.org/10.1021/jm5008177 | J. Med. Chem. 2014, 57, 7367−7381

Compound **11** can be viewed as a compound having both hydroxyl groups in JDTic replaced by a hydrogen or by replacement of the Tic hydroxyl group in **4** with a hydrogen. Viewing the change either way again shows the importance of the Tic hydroxyl group to the high κ potency and selectivity. Compound **11** with a K_e value of 16 nM at the κ receptor has low potency for this receptor.

Compound **12** can be viewed as a compound having both hydroxyl groups in JDTic replaced by fluoro groups or by replacement of the Tic hydroxyl group in **5** with a fluoro group. Regardless of how **12** is viewed, its low κ potency (K_e = 2.22 nM compared to 0.02 nM for JDTic) shows the importance of Tic hydroxyl to the κ potency and selectivity of JDTic, **4**, and **5**. Similar to **10**, **12** also behaved as a weak inverse agonist in the δ opioid receptor assay. Of all the JDTic analogues synthesized and evaluated herein, these are the only two compounds that are inverse agonists in the δ receptor assay.

One interesting finding from the X-ray crystallographic structure of the human κ opioid receptor is that the interaction of ligand hydroxyls with the receptor is mediated by intervening structured water molecules (Figure 1a, receptor pocket waters indicated by blue−green spheres).[44] As illustrated in the two-dimensional KOR-JDTic interaction diagram of the 7-hydroxy-D-Tic, hydroxyl participates in hydrogen bonds with two structured water molecules which in turn interact with residues Lys227 and Tyr139 (Figure 1b). This binding arrangement suggested that replacing the ligand hydroxyls with a substituent, which could replace the structured water in the X-ray structure, both in location and hydrogen bonding capacity, would result in direct ligand-to-receptor hydrogen bonding interactions. Removing the dependency on water molecules in the receptor pocket might result in enhanced or altered properties. Three of the compounds, **13**, **14**, and **15**, were prepared to test this hypothesis by replacing one or both of the JDTic hydroxyls with carboxamide substituents. The feasibility of this bioisosteric equivalence of a hydroxyl−water pair with a carboxamide group was tested by computational docking studies of compound **13** (in which the 7-hydroxyl of 7-hydroxy-D-Tic is replaced by a carboxamide). As anticipated, the overall binding pose of compound **13** is identical to that observed for JDTic, with the **13** carboxamide group directly providing a hydrogen-bond interaction with Lys227 (Figure 2a). The two-dimensional KOR-**13** interaction diagram of the docking result (Figure 2b) illustrates that **13** carboxamide interaction with Lys227 may not require an intervening water molecule.

In this study, we found that **13** had a K_e = 0.02 nM and thus was as potent a κ opioid receptor antagonist as JDTic (Table 2). Compound **14**, which has both phenolic groups in JDTic replaced by a carboxamide group, has a K_e = 0.11 nM at the κ opioid receptor. All three compounds are highly selective for the κ relative to the μ and δ receptors. Compound **15** can be viewed as an analogue of **4**, where the hydroxyl group in the Tic portion of **7** has been replaced by a carboxamide group. This compound with a K_e = 0.041 nM at the κ receptor and 163- and 2700-fold selectivity for the κ receptor relative to the μ and δ receptors, respectively, is slightly less κ potent and selective than compound **4**. In previous studies, we reported that **37**, which has a carboxamide group replacing the hydroxyl of the 4-(3-hydroxyphenyl) group, has a K_e = 0.12 nM at the κ receptor, which was only 6 times less potent than JDTic as a κ opioid receptor (KOR) antagonist (Table 2).[33]

Calculated physiochemical properties such as topological polar surface area (TPSA), lipophilicity (clogP), and derived

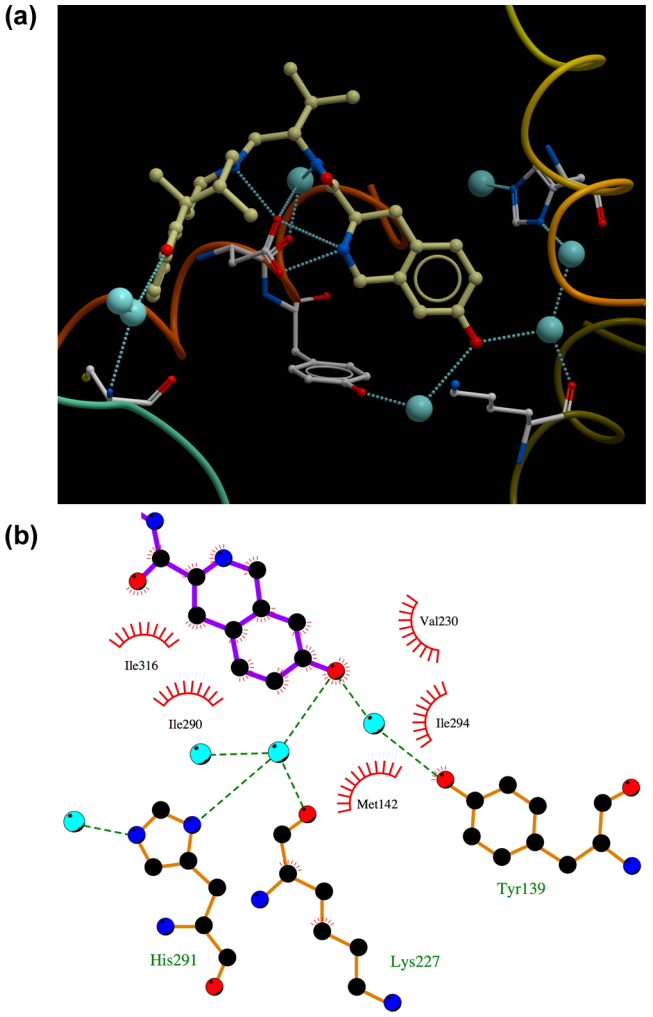


Figure 1. (a) Three-dimensional view of the hydrogen-bonding interactions between JDTic and the KOR (PDB 4DJH). (b) Two-dimensional diagram of the hydrogen-bond and hydrophobic interactions between the Tic moiety of JDTic and the KOR (PDB 4DJH). (Water molecules are rendered as blue green spheres.) Hydrogen bonds are indicated with green, dashed lines. The origin of hydrophobic interactions are indicated by the direction of red line segments around the receptor residues and ligand atoms.

values such as logBB are useful indicators of a compound's potential to penetrate the brain. These molecular descriptors were calculated for JDTic, previously reported **37**,[33] as well as **4**, **5**, **13**, and **14** (Table 3). In general, CNS drugs have clogP in the range 2−4,[45] TPSA less than 76 Å^2,[46] and logBB greater than −1.[47] The lead compound, JDTic, which proceeded through phase 1 clinical studies, has a TPSA = 84.83, which is larger than 76 Å^2. Compounds **13**, **14**, and **37**, with TPSA values of 107.69, 130.55, and 107.69, respectively, are also above the 76 Å^2. Compounds **4** and **5** both have TPSA values of 64.6, which is less than 76 Å^2. JDTic and all of the analogues except **5** had clogP values of less than 4. Even **5** had a clogP = 4.15, just above the recommended threshold. JDTic, **4**, and **5** have logBB values of −0.57, −0.23, and −0.19 and thus are greater than −1, predicting good brain penetration. Compounds **13**, **14**, and **37** with logBB values of −0.98, −1.39, and −1.02, are predicted to have poorer brain penetration.

Compounds that interact with the human ether-a-go-go gene (hERG) product, which is a potassium channel, can produce QT

dx.doi.org/10.1021/jm5008177 | J. Med. Chem. 2014, 57, 7367−7381

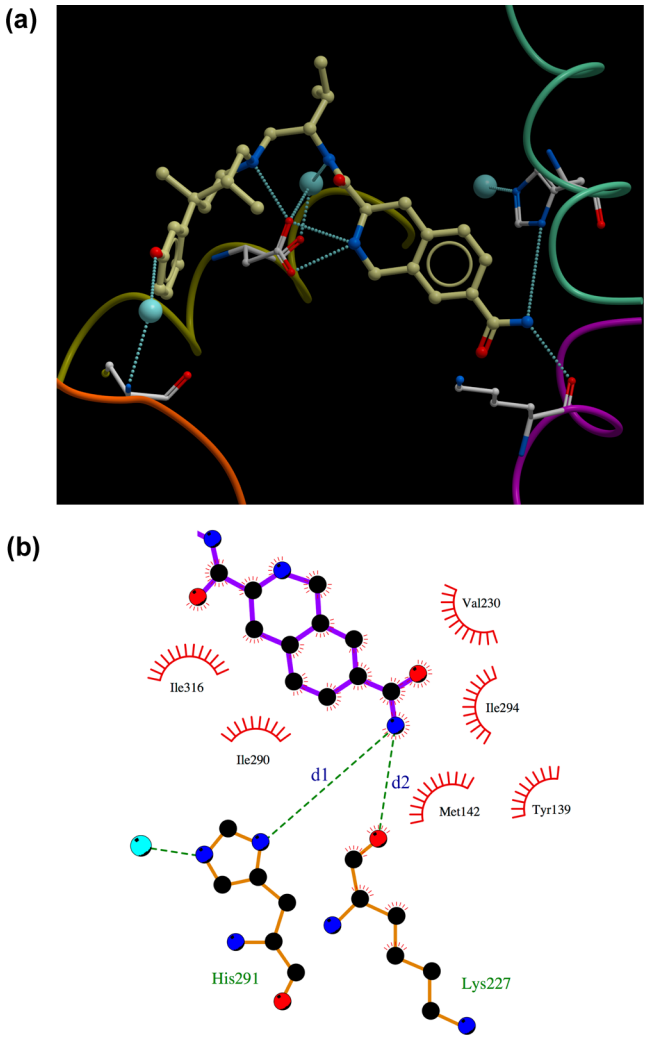


Figure 2. (a) Three-dimensional view of the hydrogen-bonding interactions between compound **13** and the KOR (docking calculation). (b) Two-dimensional diagram of the hydrogen-bond and hydrophobic interactions between the tetrahydroisoquinoline-7-carboxamide group of compound **13** and the KOR (docking calculation). Hydrogen bonds are indicated with green, dashed lines. The origin of hydrophobic interactions are indicated by the direction of red line segments around the receptor residues and ligand atoms. The HIS291 nitrogen-to-carboxamide nitrogen distance (d1) is 3.89 Å and the LYS227 carbonyl oxygen-to-carboxamide nitrogen distance (d2) is 2.83 Å.

Table 3. Calculated Physiochemical Properties

compd	TPSA (Å^2)	cLogP	LogBB
JDTic	84.83	3.60	−0.57
4	64.60	3.89	−0.23
5	64.60	4.15	−0.19
13	107.69	3.10	−0.98
14	130.55	2.64	−1.39
37	107.69	2.45	−1.02

prolongation and cardiotoxic effects. Compounds **13**, **14**, and **37** have K_i values of >10 μM (Table 4). Compounds **4** and **5** have K_i values of 7.05 and 6.25 μM, similar to the 8.82 μM value for JDTic (Table 4). Compounds that have >5% permeability in the MDCK assay, >50% stability in the plasma and S9 stability assay, >25% transported in the PAMPA assay, and >20 μM solubility are considered desirable. All of the JDTic analogues except previously reported **37** have >5% permeability in the MDCK assay. Compounds **4**, **13**, and **14**, with 27, 17, and 14%, were more permeable than JDTic, which had 11% permeability (Table 4). All of the compounds showed >20 μM solubility at pH 3, and all compounds were more soluble than JDTic, which was considered to be highly soluble. Compounds **13**, **14**, and **37** all also had >20% solubility at pH 7.4. Compounds **4** and **5** had values of 11 and 10 μM, which are similar to the 11 μM for JDTic. JDTic and the synthesized analogues showed good stability in both the plasma and S9 stability assays. JDTic, **4**, **13**, and **14** had >25% transported at both pH 5.5 and 7.4 in the PAMPA assay. On the basis of the MDCK, solubility, plasma and S9 stability, and PAMPA results combined with the calculated TPSA, clogP, and logBB data, **4** appears to have the best overall profile. However, the K_i = 7.05 μM in the hERG could be of concern. Compounds **13** and **14** show very favorable MDCK, solubility, plasma and S9 stability, and PAMPA results as well as K_i values of >10 μM for binding in the hERG assay. However, the calculated TPSA and logBB suggest that brain penetration could be a concern.

Previously reported **37** was evaluated for its ability to block κ agonist U50,488-induced diuresis at 3−30 mg/kg ig and 1−30 mg/kg ip in rats.[48] Compound **37** blocked the U50,488-induced diuresis at 24 h and 8 days at 1, 10, and 30 mg/kg, 15 days at 10 and 30 mg/kg, and 22 and 29 days at 30 mg/kg following ip administration. Compound **37** was ineffective in blocking the U50,488-induced diuresis when given ig. Because κ opioid receptor agonist-induced diuresis is mediated by the central nervous system,[49−51] the high potency of **37** in this assay after ip administration suggest that the high TPSA valve (107.69) and logBB (−1.02) are misleading for this JDTic analogue having an aromatic carboxamido substituent. Similarly, the high TPSA and logBB values for **13** and **14**, both of which have aromatic carboxamido substituents, could also be misleading. The lack of efficacy after oral (ig) administration of **37** could be due to its poor PAMPA values (1.2 and 3.2% at pH 7.4 and 5.5, respectively) and its poor MDCK value (2%). Compounds **13** and **14** have very satisfactory PAMPA and MDCK values.

CONCLUSION

In summary, these studies provide the unexpected finding that replacement of the 3-hydroxyl substituent of the 4-(3-hydroxphenol) group of JDTic with either a hydrogen, fluoro, or chloro group leads to κ opioid receptor antagonists that are as highly potent and selective as JDTic. This finding is in contrast to what would have been predicted based on structure−activity relationship studies of other N-substituted 3,4-dimethyl-4-(3-hydroxyphenyl)piperidines such as **40** (LY255582),[42] as well as much of the SAR studies reported for opioid ligands in general.

The high κ opioid receptor potency and selectivity relative to the μ and δ opioid receptors of **4**, **5**, and **14** combined with their favorable hERG, MDCK, PAMPA, solubility, and plasma and S9 stability in vitro preclinical studies and calculated TPSA, clogP, and logBB values suggest that the compounds should be considered for further development as potential drugs for treating depression, anxiety, schizophrenia, and addiction (cocaine, nicotine, methamphetamine, alcohol, and eating disorders).

EXPERIMENTAL SECTION

Melting points were determined using a MEL-TEMP II capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra were obtained on a Varian

dx.doi.org/10.1021/jm5008177 | J. Med. Chem. 2014, 57, 7367−7381

Table 4. In Vitro ADME Data

			solubility (μM)				PAMPA[a]	
compd	hERG (K_i, μM)	MDCK[a] (%)	pH 7.4	pH 3	plasma stability (% of parent)	S9 stability (% of parent)	pH 7.4	pH 5.5
JDTic	8.820	11	11	34	97	76	26.8	57.9
4	7.05	27	11	42	67.0	82.0	91.4	67.3
5	6.25	6	10	47	49.6	77.5	19.6	3
13	>10	17	38	77	84.7	91.5	28.3	39.1
14	>10	14	81	100	55.3	90.9	32.2	36.4
37	>10	2	54	36	85	82	1.2	3.2

[a]Percent transported from the optical to basal side.

Avance DPX-500 MHz NMR spectrometer or a Bruker Unity Inova 300 MHz NMR spectrometer. Chemical shifts are reported in parts per million (ppm) with reference to internal solvent. Mass spectra (MS) were conducted on a PerkinElmer Sciex AP1 150 EX mass spectrometer equipped with ESI (turbospray) source. Elemental analyses were performed by Atlantic Microlab Inc., Atlanta, GA. The purity of the compounds (>95%) was established by elemental analysis. Optical rotations were measured on an AutoPol III polarimeter, purchased from Rudolf Research. Analytical thin-layer chromatography (TLC) was carried out using EMD silica gel 60 F_{254} TLC plates. TLC visualization was achieved with a UV lamp or in an iodine chamber. Flash column chromatography was done on a CombiFlash Companion system using ISCO prepacked silica gel columns or using EM Science silica gel 60A (230−400 mesh). Solvent system: CMA80 80:18:2 $CHCl_3$:MeOH:concd NH_4OH. Unless otherwise stated, reagent-grade chemicals were obtained from commercial sources and were used without further purification. All moisture- and air-sensitive reactions and reagent transfers were carried out under dry nitrogen.

(3*R*)-*N*-[(1*S*)-1-{[(3*R*,4*R*)-3,4-Dimethyl-4-phenylpiperidin-1-yl]methyl}-2-methylpropyl]-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (4) Dihydrochloride. The amine **20** (137 mg, 0.50 mmol), Boc-7-hydroxy-D-Tic (161 mg, 0.55 mmol), HBTU (208 mg, 0.55 mmol), and NEt_3 (280 μL, 2.2 mmol) were stirred in CH_2Cl_2 (11 mL) for 12 h. The concentrated residue was subjected to chromatography on silica gel using a step gradient up to 50% CMA80 in CH_2Cl_2 to afford the Boc-protected intermediate. This Boc-protected compound was stirred in TFA/CH_2Cl_2 (1:1, 10 mL) for 12 h, concentrated, and the residue subjected to chromatography on silica gel (12 g) using a gradient up to 50% CMA80 in CH_2Cl_2 as the eluent to afford the free base of **4**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.08−7.34 (m, 6H), 6.83−6.95 (m, 1H), 6.58−6.68 (m, 1H), 6.43−6.54 (m, 1H), 3.95−4.12 (m, 1H), 3.82 (s, 2H), 3.33−3.49 (m, 1H), 2.95−3.15 (m, 1H), 2.44−2.91 (m, 5H), 2.20−2.43 (m, 2H), 1.84−2.03 (m, 2H), 1.60 (d, J = 13.0 Hz, 1H), 1.29 (s, 3H), 0.82−0.98 (m, 6H), 0.64−0.79 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.3, 154.9, 136.6, 130.2, 128.1, 125.7, 125.7, 125.4, 125.2, 114.2, 112.3, 59.6, 57.1, 55.5, 51.2, 50.6, 47.9, 38.8, 38.4, 30.9, 30.7, 30.4, 27.7, 19.1, 17.8, 16.3. MS (ESI) m/z 450.7 $(M + H)^+$. The free base was converted to 67.7 mg (24%) of the dihydrochloride salt (over two steps) as a white powder: mp 195−199 °C, $[\alpha]^{25}_D$ +101 (c 0.17, CH_3OH). Anal. ($C_{28}H_{41}Cl_2N_3O_2\cdot 2H_2O$) C, H, N.

(3*R*)-*N*-[(1*S*)-1-{[(3*R*,4*R*)-4-(3-Fluorophenyl)-3,4-dimethylpiperidin-1-yl] methyl}-2-methylpropyl]-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (5) Dihydrochloride. The amine **30** (146 mg, 0.50 mmol) was combined with Boc-7-hydroxy-D-Tic (150 mg, 0.54 mmol) and HBTU (200 mg, 0.53 mmol) and dissolved in CH_3CN (10 mL) before NEt_3 (0.2 mL, 1.4 mmol) was added. The concentrated residue was subjected to chromatography on silica gel using a gradient of EtOAc in hexanes to afford an oil (225 mg) which was dissolved in CH_3OH (3 mL) and 6 N HCl (3 mL) and stirred 12 h. The concentrated residue was dissolved in dilute NH_4OH and extracted with CH_2Cl_2. The combined organics were dried (Na_2SO_4) and concentrated. The residue was subjected to chromatography on silica gel using EtOAc then a gradient of CMA80 in CH_2Cl_2 as the eluent to afford the free base of **5**. ^{1}H NMR ($CDCl_3$) δ 7.17−7.26 (m, 1H), 7.11 (d, J = 9.0 Hz, 1H), 7.01 (d, J = 8.1 Hz, 1H), 6.78−6.97 (m, 3H), 6.65 (d, J = 8.3 Hz, 1H), 6.51 (s, 1H), 4.03 (dd, J = 4.9, 9.8 Hz, 1H), 3.87 (s, 2H), 3.45 (dd, J = 4.7,10.9 Hz, 1H), 3.07 (dd, J = 4.5, 16.0 Hz, 1H), 2.54−2.80 (m, 3H), 2.38−2.52 (m, 3H), 2.14−2.37 (m, 2H), 1.84−2.01 (m, 2H), 1.54 (d, J = 12.6 Hz, 1H), 1.22−1.33 (m, 4H), 0.92 (dd, J = 7.0, 8.9 Hz, 6H), 0.68 (d, J = 6.8 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.2, 163.0 (d, J = 244 Hz), 155.1, 153.1 (d, J = 6.5 Hz), 136.7, 130.1, 129.4 (d, J = 8.4 Hz), 125.2, 121.3 (d, J = 2.3 Hz), 114.2, 112.8 (d, J = 21.8 Hz), 112.3, 112.1 (d, J = 19.5 Hz), 59.5, 57.1, 55.4, 51.1, 50.8, 47.9, 38.8, 30.8, 30.5, 30.4, 27.5, 19.2, 17.7, 16.2. MS (ESI) m/z 468.1 $(M + H)^+$. The free base was converted to the dihydrochloride salt (100 mg, 34% over two steps) as a white powder. ^{19}F NMR (282 MHz, DMSO-d_6) δ −112.97; mp 219−223 °C (fusion); $[\alpha]^{25}_D$ = +174 (c 0.4, CH_3OH). Anal. ($C_{28}H_{40}Cl_2FN_3O_2\cdot 3H_2O$) C, H, N.

(3*R*)-*N*-[(1*S*)-1-{[(3*R*,4*R*)-4-(3-Chlorophenyl)-3,4-dimethylpiperidin-1-yl]methyl}-2-methylpropyl]-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (6) Dihydrochloride. To a solution of **31** (56 mg, 0.18 mmol) and Boc-7-hydroxy-D-Tic (59 mg, 0.20 mmol) in CH_2Cl_2 (10 mL) was added EDC·HCl (77 mg, 0.40 mmol), HOBt (3 mg, 0.02 mmol), and NEt_3 (115 μL, 0.82 mmol). The reaction mixture was stirred at ambient temperature for 12 h then was diluted with CH_2Cl_2 (20 mL) and washed with aq $NaHCO_3$ (10 mL). The organic layer was dried (Na_2SO_4) and evaporated. The residue was subjected to chromatography on silica gel using a gradient up to 50% CMA80 in $CHCl_3$ to afford the 76 mg (72%) of the Boc-protected intermediate as a white solid. The intermediate was dissolved in acetonitrile (5 mL) and treated with HCl in dioxane (4.0 M, 0.33 mL). The reaction stirred for 12 h then was concentrated. The resulting residue was subjected to chromatography on silica using a gradient up to 50% CMA80 in $CHCl_3$ as the eluent to afford 35 mg (48%) of the free base **6** as white solid. ^{1}H NMR (CD_3OD) δ 7.13−7.45 (m, 4H), 7.01 (d, J = 8.1 Hz, 1H), 6.68 (d, J = 7.5 Hz, 1H), 6.58 (br s, 1H), 4.93 (br s, 2H), 4.04−4.43 (m, 3H), 3.96 (br s, 1H), 3.23 (br s, 1H), 3.07−3.19 (m, 3H), 3.02 (br s, 2H), 2.53 (br s, 1H), 2.30 (br s, 1H), 1.90 (br s, 1H), 1.66−1.86 (m, 1H), 1.41 (s, 3H), 0.99 (br s, 6H), 0.64−0.88 (m, 3H). ^{13}C NMR (CD_3OD) δ 175.3, 162.1, 155.3, 139.7, 135.1, 133.6, 131.4, 130.6, 128.9, 125.7, 120.9, 117.6, 66.3, 61.4, 58.8, 56.9, 55.0, 49.4, 42.8, 42.3, 36.0, 33.8, 32.4, 30.8, 23.7, 22.2, 19.6. MS (ESI) m/z 484.2 $(M + H)^+$. The product was converted to the dihydrochloride salt: $[\alpha]^{25}_D$ = +92.0° (c 0.52, MeOH). Anal. ($C_{28}H_{40}Cl_3N_3O_2\cdot 2.5H_2O$) C, H, N).

(3*R*)-*N*-[(1*S*)-1-{[(3*R*,4*R*)-4-(3-Bromophenyl)-3,4-dimethylpiperidin-1-yl]methyl}-2-methylpropyl]-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (7) Dihydrochloride. To a solution of **32** (94 mg, 0.26 mmol) and Boc-7-hydroxy-D-Tic (89 mg, 0.30 mmol) in CH_2Cl_2 (10 mL) was added EDC·HCl (116 mg, 0.60 mmol), HOBt (5 mg, 0.03 mmol), and triethylamine (173 μL, 1.24 mmol). The reaction mixture was stirred at ambient temperature for 12 h. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with aq $NaHCO_3$ (10 mL). The organic layer was dried (Na_2SO_4) and concentrated to a residue which was subjected to chromatography on silica gel using a gradient up to 50% CMA80 in $CHCl_3$ as the eluent to afford 79 mg (46%) of the Boc-protected intermediate as a white solid. ^{1}H NMR ($CDCl_3$) δ 7.13−7.27 (m, 2H), 7.09 (d, J = 4.90 Hz, 2H), 6.91 (d, J = 8.3 Hz, 1H), 6.50−6.75 (m, 1H), 6.45 (br s, 1H), 4.38−4.54 (m, 1H), 4.34 (br s, 1H), 3.78 (br s, 1H), 3.11 (dd, J = 3.0, 15.1 Hz, 1H), 2.90 (dd, J = 5.8, 15.3 Hz, 1H), 2.50 (br s, 1H), 2.27−2.42 (m, 1H), 2.24 (br s, 1H), 1.93−2.14 (m, 3H), 1.59−1.93 (m, 2H), 1.43 (s, 9H), 1.35 (br s, 1H), 1.14 (s, 3H), 0.78 (d, J = 6.6 Hz, 3H), 0.69 (d, J = 6.8 Hz, 3H), 0.52 (d, J = 6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 171.3, 155.7, 152.9, 134.0,

dx.doi.org/10.1021/jm5008177 | J. Med. Chem. 2014, 57, 7367−7381

129.7, 129.3, 128.8, 128.4, 124.4, 122.5, 114.8, 112.9, 81.6, 59.9, 56.1, 51.3, 50.0, 44.8, 38.8, 38.7, 30.5, 29.8, 28.4, 27.4, 19.2, 16.9, 16.0. MS (ESI) m/z 628.6 $(M + H)^+$. The intermediate was dissolved in acetonitrile (5 mL) and treated with HCl in dioxane (4.0 M, 0.3 mL) and stirred for 12 h. Concentration of the reaction mixture afforded a white solid which was chromatographed on silica using a gradient up to 50% CMA80 in $CHCl_3$ to afford 56 mg (88%) of **7** free base as white solid. ^{1}H NMR ($CDCl_3$) δ 7.14−7.27 (m, 2H), 6.95−7.14 (m, 2H), 6.84 (d, J = 8.3 Hz, 1H), 6.48−6.66 (m, 1H), 6.43 (d, J = 2.1 Hz, 1H), 3.94−4.23 (m, 1H), 3.80 (br s, 2H), 3.38 (dd, J = 4.7, 10.7 Hz, 1H), 3.00 (dd, J = 4.2, 16.3 Hz, 1H), 2.70 (br s, 1H), 2.57 (dd, J = 11.0, 14.9 Hz, 2H), 2.43 (br s, 2H), 2.27 (d, J = 8.10 Hz, 1H), 2.17 (br s, 1H), 1.98 (s, 1H), 1.87 (br s, 2H), 1.49 (d, J = 12.2 Hz, 1H), 0.97−1.28 (m, 3H), 0.70−0.91 (m, 6H), 0.62 (d, J = 6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 173.1, 154.5, 152.5, 136.8, 130.2, 129.7, 128.9, 128.5, 125.6, 124.4, 122.6, 114.0, 112.2, 59.4, 57.0, 55.2, 51.1, 50.6, 47.9, 38.7, 38.6, 30.6, 30.3, 27.5, 19.2, 17.8, 16.2. MS (ESI) m/z 528.6 $(M + H)^+$. The free base was converted to the dihydrochloride salt: $[\alpha]^{25}_D$ = +98.0 (c 0.61, MeOH). Anal. ($C_{28}H_{40}BrCl_2N_3O_2 \cdot 0.5H_2O$) C, H, N.

(3*R*)-*N*-[(1*S*)-1-{[(3*R*,4*R*)-4-(3-Aminophenyl)-3,4-dimethylpiperidin-1-yl]methyl}-2-methylpropyl]-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (8) Trihydrochloride. To a solution **26** (125 mg, 0.43 mmol) in CH_2Cl_2 (10 mL) was added Boc-7-hydroxy-D-Tic (125 mg, 0.43 mmol), HOBt (10 mg, 0.1 mmol), and EDC·HCl (191 mg, 1.0 mmol), followed by the addition of diisopropylethylamine (0.15 mL, 0.86 mmol). The resulting solution was stirred at room temperature for 12 h then washed with saturated aqueous $NaHCO_3$ (5 mL). The aqueous layer was extracted once with EtOAc (15 mL). The combined organic layers were washed with brine (5 mL), dried (Na_2SO_4), and concentrated. The resulting residue was purified by chromatography on silica gel using a gradient up to 50% CMA80 in CH_2Cl_2 as the eluent. The product containing fractions were combined and concentrated to afford 174 mg (72%) of the Boc-protected intermediate. The intermediate was then dissolved in CH_3OH (10 mL) to which aq HCl (6 N, 10 mL) was added. The resulting solution was stirred 12 h then concentrated. The resulting residue was subjected to chromatography on silica gel using a gradient up to 50% CMA80 in CH_2Cl_2 as the eluent to afford **8** free base. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.99−7.18 (m, 2H), 6.85 (d, J = 8.3 Hz, 1H), 6.41−6.72 (m, 5H), 4.02 (dt, J = 4.6, 9.1 Hz, 1H), 3.82 (s, 2H), 3.38−3.48 (m, 2H), 3.02 (dd, J = 4.7, 16.2 Hz, 1H), 2.51−2.79 (m, 3H), 2.36−2.51 (m, 3H), 2.10−2.35 (m, 2H), 1.79−2.00 (m, 2H), 1.50 (d, J = 12.6 Hz, 1H), 1.16−1.31 (m, 3H), 0.83−0.98 (m, 6H), 0.66 (d, J = 6.8 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.1, 154.9, 151.3, 145.8, 136.4, 130.0, 128.8, 124.9, 116.4, 114.2, 112.9, 112.4, 112.2, 59.4, 56.8, 55.3, 51.2, 50.7, 47.6, 38.7, 38.2, 30.7, 30.5, 30.1, 27.3, 19.0, 17.7, 16.2. The free base was converted to the trihydrochloride salt, affording 132.4 mg (50% over two steps) of a white powder. MS (ESI) m/z 465.5 $(M + H)^+$, mp 241−243 °C (fusion), $[\alpha]^{25}_D$ +98.7 (c 0.38, CH_3OH). Anal. ($C_{28}H_{43}Cl_3N_4O_2 \cdot 2.5H_2O$) C, H, N.

(3*R*)-*N*-[(1*S*)-1-{[(3*R*,4*R*)-4-(3-Hydroxyphenyl)-3,4-dimethylpiperidin-1-yl]methyl}-2-methylpropyl]-7-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (9) Dihydrochloride. The amine **21** (145 mg, 0.50 mmol), Boc-D-Tic (152 mg, 0.55 mmol), HBTU (208 mg, 0.55 mmol), and NEt_3 (280 μL, 2.2 mmol) were stirred in CH_2Cl_2 (11 mL) for 12 h. The concentrated residue was subjected to chromatography on silica gel using a step gradient up to 50% CMA80 in CH_2Cl_2 as the eluent to afford the Boc-protected intermediate. This Boc-protected compound was stirred in TFA/CH_2Cl_2 (1:1, 10 mL) for 12 h, concentrated, and the residue subjected to chromatography on silica gel (12 g) using a gradient up to 50% CMA80 in CH_2Cl_2 as the eluent to afford the free base of **9**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.96−7.21 (m, 5H), 6.73−6.82 (m, 2H), 6.59−6.67 (m, 1H), 4.07 (dt, J = 4.9, 9.3 Hz, 1H), 4.00 (s, 2H), 3.56 (dd, J = 5.1, 10.7 Hz, 1H), 3.20 (dd, J = 5.0, 16.7 Hz, 1H), 2.70−2.83 (m, 2H), 2.64 (d, J = 10.4 Hz, 1H), 2.29−2.49 (m, 4H), 2.20 (dt, J = 4.3, 12.4 Hz, 1H), 1.80−2.01 (m, 2H), 1.54 (d, J = 13.0 Hz, 1H), 1.23−1.30 (m, 3H), 0.93 (dd, J = 6.8, 9.0 Hz, 6H), 0.67 (d, J = 6.8 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.9, 155.9, 152.1, 135.8, 134.4, 129.3, 129.1, 126.5, 126.1, 125.5, 117.7, 113.1, 112.4, 59.6, 56.7, 55.1, 51.1, 50.6, 47.8, 38.9, 38.5, 31.1, 30.6, 27.5, 19.2, 17.8, 16.2. MS (ESI) m/z 450.5 $(M + H)^+$. The free base was converted to the dihydrochloride salt (50.3 mg, 18% over two steps) as a white powder: mp 197−200 °C (fusion), $[\alpha]^{25}_D$ = +108° (c 0.10, CH_3OH). Anal. ($C_{28}H_{41}Cl_2N_3O_2 \cdot 2H_2O$) C, H, N.

(3*R*)-7-Fluoro-*N*-[(1*S*)-1-{[(3*R*,4*R*)-4-(3-Hydroxyphenyl)-3,4-dimethylpiperidine-1-yl]methyl}-2-methylpropyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (10) Dihydrochloride. Amine **21** (145 mg, 0.50 mmol) and acid 7-fluoro-Boc-D-Tic (162 mg, 0.55 mmol) were combined in CH_2Cl_2 (10 mL) and treated with EDC·HCl (191 mg, 1.0 mmol) then NEt_3 (0.35 mL, 2.5 mmol). After 12 h, the concentrated residue was subjected to chromatography on silica gel using a gradient up to 60% CMA80 in CH_2Cl_2 as the eluent. The product containing fractions were concentrated then treated with MeOH (5 mL) and aq HCl (6 N, 5 mL). After 1 h, the concentrated residue was subjected to chromatography on silica gel using a gradient up to 75% CMA80 in CH_2Cl_2 as the eluent to afford **10** free base. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.06−7.22 (m, 2H), 7.01 (dd, J = 5.8, 8.3 Hz, 1H), 6.61−6.86 (m, 5H), 4.09 (tt, J = 4.7, 9.4 Hz, 1H), 3.95 (s, 2H), 3.52 (dd, J = 4.9, 10.6 Hz, 1H), 3.14 (dd, J = 4.8, 16.5 Hz, 1H), 2.61−2.82 (m, 3H), 2.27−2.55 (m, 4H), 2.11−2.27 (m, 1H), 1.80−1.98 (m, 2H), 1.52 (d, J = 12.8 Hz, 1H), 1.25 (s, 3H), 0.92 (t, J = 7.7 Hz, 6H), 0.66 (d, J = 6.8 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.9, 161.2 (d, J = 245 Hz), 156.4, 151.9, 137.4 (d, J = 6.5 Hz), 130.7 (d, J = 7.8 Hz), 129.7 (d, J = 2.9 Hz), 129.1, 117.3, 113.6 (d, J = 21.2 Hz), 113.1, 112.6, 112.1 (d, J = 21.1 Hz), 59.6, 56.7, 55.1, 51.3, 50.6, 47.6, 38.8, 38.4, 30.8, 30.7, 30.3, 27.5, 19.2, 17.8, 16.3. ^{19}F NMR (282 MHz, $CDCl_3$) δ −116.49. MS (ESI) m/z 468.5 $(M + H)^+$. The free base was converted to the dihydrochloride salt (90.7 mg, 15% over two steps) as a white powder: mp 202−206 °C (fusion); $[\alpha]^{25}_D$ = +93 (c 0.1, CH_3OH). Anal. ($C_{28}H_{40}Cl_2FN_3O_2 \cdot 2.5H_2O$) C, H, N.

(3*R*)-*N*-[(1*S*)-1-{[(3*R*,4*R*)-3,4-Dimethyl-4-phenylpiperidin-1-yl]methyl}-2-methylpropyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (11) Dihydrochloride. The amine **20** (137 mg, 0.50 mmol), Boc-D-Tic (152 mg, 0.55 mmol), HBTU (208 mg, 0.55 mmol), and NEt_3 (280 μL, 2.2 mmol) were stirred in CH_2Cl_2 (11 mL) for 12 h. The concentrated residue was subjected to chromatography on silica gel using a step gradient up to 50% CMA80 in CH_2Cl_2 as the eluent to afford the Boc-protected intermediate. This Boc-protected compound was stirred in TFA/CH_2Cl_2 (1:1, 10 mL) for 12 h, concentrated, and the residue subjected to chromatography on silica gel (12 g) using a gradient up to 50% CMA80 in CH_2Cl_2 as the eluent to afford the free base of **11**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20−7.34 (m, 4H), 7.06−7.19 (m, 3H), 6.96−7.05 (m, 1H), 4.03−4.10 (m, 1H), 4.00 (s, 2H), 3.53 (dd, J = 4.9, 10.9 Hz, 1H), 3.13−3.25 (m, 1H), 2.88 (d, J = 15.1 Hz, 1H), 2.67−2.84 (m, 3H), 2.58 (d, J = 10.4 Hz, 3H), 2.26−2.48 (m, 3H), 2.00−2.09 (m, 1H), 1.85−2.01 (m, 1H), 1.56−1.69 (m, 1H), 1.31 (s, 3H), 0.88−0.99 (m, 6H), 0.70 (d, J = 7.0 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.9, 135.9, 134.4, 129.3, 128.2, 126.4, 126.1, 125.6, 125.5, 59.2, 56.8, 55.1, 51.2, 50.3. 47.9, 38.6, 38.3, 31.2, 30.7, 27.5, 19.2, 17.9, 16.1. MS (ESI) m/z 434.5 $(m + H)^+$. The free base was converted to the dihydrochloride salt (21.9 mg, 8% over two steps) as a white powder: mp 162−165 °C (fusion), $[\alpha]^{25}_D$ = +96° (c 0.10, CH_3OH). Anal. ($C_{28}H_{41}Cl_2N_3O \cdot 2H_2O$) C, H, N.

(3*R*)-7-Fluoro-*N*-[(1*S*)-1-{[(3*R*,4*R*)-4-(3-Fluorophenyl)-3,4-dimethylpiperidin-1-yl]methyl}-2-methylpropyl]-1,2,3,4-tetrahydroisoquinoline-3-carboxamide (12) Dihydrochloride. Amine **30** (24.2 mg, 0.083 mmol) and acid 7-fluoro-Boc-D-Tic (55.7 mg, 0.19 mmol) were combined in CH_2Cl_2 (8 mL) and treated with EDC·HCl (40 mg, 0.2 mmol) then NEt_3 (0.10 mL, 0.72 mmol). After 12 h, the concentrated residue was subjected to chromatography on silica gel using a gradient up to 50% CMA80 in CH_2Cl_2. The product containing fractions were concentrated then treated with MeOH (5 mL) and aq HCl (5 mL). After 1 h, the concentrated residue was subjected to chromatography on silica gel using a gradient up to 50% EtOAc in hexanes with 1% NH_3 (prepared by adding 1% concd NH_4OH by volume as the eluent and drying over Na_2SO_4) as the eluent to afford **12** free base. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19−7.29 (m, 1H), 6.98−7.14 (m, 3H), 6.93 (td, J = 2.1, 11.3 Hz, 1H), 6.84 (tt, J = 2.8, 8.3 Hz, 2H), 6.73 (dd, J = 2.6, 9.0 Hz, 1H), 3.94−4.10 (m, 3H), 3.51 (dd, J = 4.9, 10.6 Hz, 1H), 3.15 (dd, J = 5.0, 16.5 Hz, 1H), 2.58−2.85 (m, 3H), 2.06−2.54

dx.doi.org/10.1021/jm5008177 | J. Med. Chem. 2014, 57, 7367−7381

(m, 5H), 1.85−2.02 (m, 2H), 1.56 (dd, J = 1.2, 12.9 Hz, 1H), 1.28 (s, 3H), 0.88−0.99 (m, 6H), 0.66 (d, J = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.5, 163.2 (d, J = 244.4 Hz), 161.3 (d, J = 244.7 Hz), 153.3 (d, J = 6.4 Hz), 137.7 (d, J = 6.5 Hz), 130.8 (d, J = 7.8 Hz), 130.0 (d, J = 2.9 Hz), 129.6 (d, J = 8.2 Hz), 121.3 (d, J = 2.6 Hz), 113.7 (d, J = 21.2), 112.8 (d, J = 21.4 Hz), 112.3 (d, J = 21.1 Hz), 112.2 (d, J = 21.1 Hz), 59.5, 56.7, 55.2, 51.2, 50.6, 47.7, 38.7, 38.7, 30.6, 30.5, 30.4, 27.4, 19.2, 17.8, 16.2. ^{19}F NMR (282 MHz, $CDCl_3$) δ −113.5, −116.6. MS (ESI) m/z 470.9 $(M + H)^+$. The free base was converted to the dihydrochloride salt (21.9 mg, 46% over two steps) as a white powder: mp 152−156 °C (fusion), $[\alpha]^{25}_D$ = +115 (c 0.10, CH_3OH). Anal. ($C_{28}H_{39}Cl_2F_2N_3O\cdot 2H_2O$) C, H, N.

(3*R*)-*N*^{3}-[(1*S*)-1-{[(3*R*,4*R*)-4-(3-Hydroxyphenyl)-3,4-dimethylpiperidin-1-yl]methyl}-2-methylpropyl]-1,2,3,4-tetrahydroisoquinoline-3,7-dicarboxamide (13) Dihydrochloride. The amine **21** (153 mg, 0.53 mmol) was added to a solution of the acid **36** (170 mg, 0.53 mmol), HBTU (209 mg, 0.55 mmol), and NEt_3 (0.24 mL, 1.7 mmol) in CH_3CN (40 mL). After 12 h, the residue obtained on concentration was subjected to chromatography on silica gel using a gradient up to 70% CMA80 in CH_2Cl_2 as the eluent. The product containing fractions were dissolved in CH_2Cl_2 (5 mL) and TFA (5 mL) and stirred 12 h. The residue obtained on concentration was subjected to chromatography on silica gel using a gradient of CMA80 in CH_2Cl_2 as the eluent. The product containing fractions were concentrated and subjected to chromatography on C18-reverse phase using a gradient from 40 to 60% aq CH_3CN with 0.1% TFA. The product containing fractions were concentrated and again subjected to chromatography on silica gel using a gradient of CMA80 in CH_2Cl_2 to afford **13** free base. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (s, 1H), 7.45 (d, J = 7.9 Hz, 1H), 7.19 (d, J = 9.5 Hz, 1H), 7.13−7.01 (m, 2H), 6.74−6.59 (m, 3H), 6.49 (bs, 1H), 6.02 (bs, 1H), 4.12−3.86 (m, 3H), 3.64 (t, J = 6.7 Hz, 1H), 3.14−2.94 (m, 2H), 2.73−2.54 (m, 2H), 2.50−2.22 (m, 4H), 2.12−1.98 (m, 1H), 1.90−1.70 (m, 2H), 1.44 (d, J = 12.5 Hz, 1H), 1.21 (s, 3H), 0.97−0.87 (m, 6H), 0.38 (d, J = 6.8 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.4, 170.0, 156.5, 152.1, 138.8, 136.2, 130.8, 129.7, 129.3, 125.4, 125.1, 117.6, 113.2, 112.7, 60.6, 55.6, 54.9, 51.9, 50.9, 46.2, 38.8, 38.5, 31.1, 30.7, 30.2, 27.3, 19.6, 18.1, 16.1. MS (ESI) m/z 493.7 $(M + H)^+$. The free base was converted to 41.9 mg of the dihydrochloride salt (13%) as a white powder: mp 195−200 °C (fusion), $[\alpha]^{25}_D$ +103° (c 1.00, CH_3OH). Anal. ($C_{29}H_{42}Cl_2N_4O_3\cdot 3H_2O$) C, H, N.

(3*R*)-*N*^{3}-[(1*S*)-1-{[(3*R*,4*R*)-4-(3-Carbamoylphenyl)-3,4-dimethylpiperidin-1-yl]methyl}-2-methylpropyl]-1,2,3,4-tetrahydroisoquinoline-3,7-dicarboxamide (14) Dihydrochloride. The amine 3-{(3*R*,4*R*)-1-[(2*S*)-2-amino-3-methylbutyl]-3,4-dimethylpiperidin-4-yl}benzamide[33] (24 mg, 0.08 mmol) was added to a solution of the acid **36** (25 mg, 0.08 mmol), HBTU (30 mg, 0.08 mmol), and NEt_3 (30 μL, 0.2 mmol) in CH_2Cl_2 (10 mL). After 12 h, the concentrated residue was subjected to chromatography on silica gel using a gradient up to 70% CMA80 in CH_2Cl_2 as the eluent. The product containing fractions were dissolved in CH_2Cl_2 (5 mL) and TFA (5 mL) and stirred 12 h. The concentrated residue was subjected to chromatography on silica gel using a gradient of CMA80 in CH_2Cl_2 to afford **14** free base. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72 (s, 1H), 7.54 (dd, J = 6.59, 14.69 Hz, 3H), 7.29−7.46 (m, 2H), 7.01−7.22 (m, 2H), 5.90−6.78 (m, 4H), 3.99 (br s, 3H), 3.59 (d, J = 2.83 Hz, 1H), 3.12 (d, J = 13.37 Hz, 1H), 2.80−3.00 (m, 1H), 2.72 (d, J = 9.80 Hz, 1H), 2.59 (d, J = 10.93 Hz, 1H), 2.12−2.51 (m, 5H), 1.89 (dd, J = 6.97, 12.24 Hz, 3H), 1.58 (d, J = 12.06 Hz, 1H), 1.19−1.33 (m, 3H), 0.92 (t, J = 7.72 Hz, 6H), 0.53 (d, J = 6.78 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.2, 172.1, 170.2, 169.4, 151.1, 138.8, 136.2, 133.2, 131.1, 129.4, 128.3, 125.1, 125.0, 124.7, 124.1, 59.9, 55.9, 55.0, 51.3, 50.8, 46.9, 38.7, 38.5, 30.7, 30.7, 30.6, 27.4, 19.2, 17.9, 16.2. MS (ESI) m/z 521.0 $(M + H)^+$. The free base was converted to 20.4 mg (39%) of the dihydrochloride salt as a pale-yellow powder: mp 210−215 °C (fusion), $[\alpha]^{25}_D$ +101° (c 0.50, CH_3OH). Anal. ($C_{30}H_{43}Cl_2N_5O_3\cdot 3.25H_2O$) C, H, N.

(3*R*)-*N*^{3}-[(1*S*)-1-{[(3*R*,4*R*)-3,4-Dimethyl-4-phenylpiperidin-1-yl]methyl}-2-methylpropyl]-1,2,3,4-tetrahydroisoquinoline-3,7-dicarboxamide (15) Dihydrochloride. To a solution of **20** (100 mg, 0.3 mmol) in CH_2Cl_2 (10 mL) was added **36** (100 mg, 0.3 mmol), HOBt (10 mg, 0.1 mmol), and EDC·HCl (75 mg, 0.4 mmol), followed by the addition of diisopropylethylamine (0.26 mL, 1.5 mmol). The resulting cloudy solution remained cloudy upon the addition of NMP (0.1 mL). After 12 h, the mixture was washed with saturated aqueous $NaHCO_3$ (10 mL). The aqueous layer was extracted with CH_2Cl_2:THF (2:1, 20 mL × 2). The combined organic layers were washed with brine (5 mL), dried (Na_2SO_4), and concentrated. The resulting residue was purified by chromatography on silica gel using a gradient up to 40% CMA80 in CH_2Cl_2 as the eluent. The product containing fractions were combined and concentrated to afford 134 mg of the Boc-protected intermediate. The intermediate was then dissolved in CH_3OH (10 mL) to which aq HCl (6 N, 10 mL) was added. The resulting solution was stirred 1 h and concentrated. The resulting residue was subjected to chromatography on silica gel using a gradient up to 75% CMA80 in CH_2Cl_2 to afford **15** free base. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38−7.51 (m, 2H), 6.95−7.28 (m, 9H), 5.93−6.34 (m, 2H), 3.84−4.04 (m, 2H), 3.46 (dd, J = 5.0, 10.3 Hz, 1H), 3.11 (dd, J = 4.8, 17.1 Hz, 1H), 2.62−2.83 (m, 2H), 2.55 (d, J = 10.7 Hz, 1H), 2.30−2.46 (m, 3H), 2.08−2.29 (m, 2H), 1.75−1.99 (m, 2H), 1.51 (d, J = 12.2 Hz, 1H), 1.13−1.28 (m, 3H), 0.74−0.94 (m, 6H), 0.56 (d, J = 6.8 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.2, 169.3, 150.1, 138.8, 136.2, 131.1, 129.4, 128.0, 125.4, 125.3, 125.0, 59.5, 56.3, 55.2, 51.3, 50.6, 47.4, 38.6, 38.4, 31.0, 30.5, 27.5, 19.1, 17.7, 16.3. MS (ESI) m/z 477.5 $(M + H)^+$. The free base was converted to the dihydrochloride salt, which was sonicated in EtOAc. The solvent was decanted and the solids dried under nitrogen to afford 43 mg (24% over two steps) as a white powder: mp 218−222 °C (fusion), $[\alpha]^{25}_D$ +105 (c 0.195, CH_3OH). Anal. ($C_{29}H_{42}Cl_2N_4O_2\cdot 2.75H_2O$) C, H, N.

3-[(3*R*,4*R*)-3,4-Dimethyl-1-(trifluoromethane)-sulfonylpiperidin-4-yl]phenyl trifluoromethanesulfonate (17). The title compound was prepared by the addition of trifluoromethanesulfonic anhydride (3.4 mL, 20 mmol) to 3-[(3*R*,4*R*)-3−4-dimethylpiperidin-4-yl]phenol (**16**) (1.0 g, 4.9 mmol) and diisopropylethylamine (5.1 mL, 29 mmol) in CH_2Cl_2 (30 mL) at −78 °C. The solution was allowed to warm to room temperature, quenched with a brine wash, and concentrated. The resulting residue was dissolved in diethyl ether. The ether layer was washed with 1 M HCl, aq $NaHCO_3$, then brine. After drying (Na_2SO_4), concentration afforded **17** in quantitative yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41−7.48 (m, 1H), 7.24−7.32 (m, 1H), 7.11−7.19 (m, 2H), 4.01 (d, J = 13.2 Hz, 1H), 3.54−3.71 (m, 2H), 3.31−3.45 (m, 1H), 2.36 (dt, J = 5.0, 13.1 Hz, 1H), 2.06−2.20 (m, 1H), 1.74 (d, J = 13.6 Hz, 1H), 1.42 (s, 3H), 0.75 (d, J = 7.0 Hz, 3H). This material was used without further purification.

(3*R*,4*R*)-3,4-Dimethyl-4-phenyl-1-(trifluoromethane)-sulfonylpiperidine (18). A solution of the triflate **17** (2.3 g, 4.9 mmol) in DMF (10 mL) was treated with NBu_3 (3.5 mL, 15 mmol), $PdCl_2(PPh_3)$ (170 mg, 0.25 mmol), and formic acid (0.4 mL, 11 mmol). The solution was heated to 80 °C for 5 h, then concentrated and purified by rapid elution of the product through silica gel using 20% EtOAc in hexanes as eluent to afford 1.43 g (90%) of **18**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30−7.39 (m, 2H), 7.20−7.28 (m, 3H), 3.98 (d, J = 13.0 Hz, 1H), 3.62 (bs, 2H), 3.30−3.46 (m, 1H), 2.38 (dt, J = 5.1, 13.1 Hz, 1H), 2.08−2.22 (m, 1H), 1.68−1.79 (m, 1H), 1.40 (s, 3H), 0.75 (d, J = 7.0 Hz, 3H). This material was used without further purification.

(3*R*,4*R*)-3,4-Dimethyl-4-phenylpiperidine (19). A sample of triflamide **18** (520 mg, 1.6 mmol) was dissolved in toluene (10 mL) and THF (5 mL) and treated with $LiAlH_4$ (320 mg, 8.3 mmol) and heated with a microwave to 150 °C in a sealed tube for 10 min. The cooled solution was diluted with ether, chilled in an ice bath, and quenched with the sequential addition of water (0.3 mL), 15% NaOH (0.3 mL), then water (0.6 mL). The resulting suspension was filtered through Celite and concentrated to afford 226 mg of an oil. The ^{1}H NMR suggested **19** contained about 15% unreacted starting material **18**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.08−7.38 (m, 5H), 3.26 (dd, J = 3.3, 13.1 Hz, 1H), 2.91−3.07 (m, 2H), 2.67−2.78 (m, 1H), 2.07−2.23 (m, 1H), 1.83−2.00 (m, 2H), 1.49−1.62 (m, 1H), 1.39 (s, 3H), 0.71 (d, J = 7.2 Hz, 3H). The material was used without further purification.

(2*S*)-1-[(3*R*,4*R*)-3,4-Dimethyl-4phenylpiperidin-1-y1]-3-methylbutan-2-amine (20). The amine (**19**) (226 mg, ∼0.92 mmol) was combined with Boc-L-valinal (355 mg, 1.8 mmol) in trifluoroethanol (5 mL) and treated with $Na(CN)BH_3$ (3 mL, 1 M in THF). After 1 h,

dx.doi.org/10.1021/jm5008177 | J. Med. Chem. 2014, 57, 7367−7381

the solution was concentrated and subjected to chromatography on silica gel using a gradient of EtOAc in hexanes as the eluent to afford 367 mg (61% over two steps from **18**) of the Boc-protected product. The Boc-protected compound was stirred in 1:1 CH_2Cl_2:TFA overnight. The concentrated residue was subjected to chromatography on silica gel eluting with a gradient of CMA80 in CH_2Cl_2 as eluent to afford **20** in quantitative yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.23−7.36 (m, 4H), 7.12−7.21 (m, 1H), 2.12−2.81 (m, 7H), 2.02 (d, J = 6.8 Hz, 1H), 1.45−1.79 (m, 5H), 1.32 (s, 3H), 0.85−0.97 (m, 6H), 0.69−0.79 (m, 3H). This material was used without further purification.

2-[(1*S*)-1-{[3*R*,4*R*)-4-(3-Hydroxyphenyl)-3,4-dimethylpiperidin-1-yl]methyl}-2-methylpropyl]-1*H*-isoindole-1,3(2*H*)-dione (22). A solution of 3-{(3*R*,4*R*)-1-[(2*S*)-2-amino-3-methylbutyl]-3,4-dimethylpiperidin-4-yl}phenol (**21**) (6.84 g, 23.6 mmol) in $CHCl_3$ (230 mL) was refluxed with phthalic anhydride (4.5 g, 30 mmol) for 24 h. The cooled solution was washed with aq $NaHCO_3$ then concentrated. The residue was subjected to chromatography on silica gel using a gradient of CMA80 in CH_2Cl_2 as the eluent to afford 5.80 g (58%) of **22**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (d, J = 2.3 Hz, 2H), 7.63 (d, J = 2.6 Hz, 2H), 7.08 (t, J = 7.8 Hz, 1H), 6.70 (d, J = 8.1 Hz, 1H), 6.64 (bs, 1H), 6.58 (d, J = 7.9 Hz, 1H), 4.00−4.12 (m, 1H), 3.26 (5, J = 12.0 Hz, 1H), 2.63 (d, J = 10.0 Hz, 2H), 2.23−2.57 (m, 4H), 1.92−2.09 (m, 1H), 1.82 (d, J = 6.2 Hz, 1H), 1.42 (d, J = 12.6 Hz, 1H), 1.23 (s, 3H), 1.05 (d, J = 6.8 Hz, 3H), 0.88 (d, J = 6.6 Hz, 3H), 0.29 (d, J = 6.8 Hz, 3H). MS (ESI) m/z 421.7 $(M + H)^+$. This material was used without further purification.

3-{(3*R*,4*R*)-1-[(2*S*)-2-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-3-methylbutyl]-3,4-dimethylpiperidin-4yl}phenyl Trifuoromethanesulfonate (23). The phthalimide-protected **22** (5.80 g, 13.8 mmol) was dissolved in CH_2Cl_2 (150 mL) containing triethylamine (2.8 mL, 20 mmol) and *N*-phenyl-bis(trifluoromethanesulfonimide) (5.4 g, 15 mmol). After 12 h, the solution was washed with aq $NaHCO_3$, dried (Na_2SO_4), and concentrated. The residue was subjected to chromatography on silica gel using a gradient 0−20% EtOAc in hexanes as the eluent to afford 4.81 g (63%) of triflate **23**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (bs, 2H), 7.61−7.70 (m, 2H), 7.24−7.35 (m, 1H), 7.16 (d, J = 7.9 Hz, 1H), 6.97−7.05 (m, 2H), 3.98−4.13 (m, 1H), 3.26 (t, J = 12.2 Hz, 1H), 2.59−2.76 (m, 2H), 2.24−2.57 (m, 4H), 2.02 (dt, J = 4.7, 12.4 Hz, 1H), 1.84 (d, J = 5.8 Hz, 1H), 1.25 (s, 3H), 1.05 (d, J = 6.6 Hz, 3H), 0.89 (d, J = 6.8 Hz, 3H), 0.26 (d, J = 7.0 Hz, 3H). This material was used without further purification.

2-[(1*S*-1-({(3*R*,4*R*)-4-[3-(Benzylamino)phenyl]-3,4-dimethylpiperidin-1-yl}methyl-2-methylpropyl]-1*H*-isoindole-1,3(2*H*)-dione (24). The triflate **23** (1.59 g, 2.9 mmol), benzylamine (0.46 mL, 4.2 mmol), potassium *tert*-butoxide (550 mg, 4.9 mmol), $Pd(OAc)_2$ (7.7 mg, 0.035 mmol), and (2-biphenyl)di-*tert*-butylphosphine (21 mg, 0.07 mmol) were combined and degassed in toluene (3.5 mL) then stirred overnight at room temperature. The mixture formed a gel which was dissolved with CH_2Cl_2. The organic layer was washed with aq NH_4Cl, dried (Na_2SO_4), and concentrated. The residue was subjected to chromatography on silica gel using a gradient of EtOAc in hexanes as the eluent to afford 352 mg (24%) of **24**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, J = 2.6 Hz, 2H), 7.64 (dd, J = 3.0, 5.3 Hz, 2H), 7.20−7.37 (m, 5H), 7.03 (t, J = 7.8 Hz, 1H), 6.52 (d, J = 7.7 Hz, 1 H), 6.35−6.46 (m, 2H), 4.25 (s, 2H), 3.98−4.10 (m, 1H), 3.17−3.29 (m, 1H), 2.55−2.70 (m, 2H), 2.23−2.55 (m, 5H), 1.98 (dt, J = 4.5, 12.6 Hz, 1H), 1.79 (d, J = 6.8 Hz, 1H), 1.39 (d, J = 13.0 Hz, 1H), 1.20 (s, 3H), 1.04 (d, J = 6.6 Hz, 3H), 0.89 (d, J = 6.8 Hz, 3H), 0.28 (d, J = 7.0 Hz, 3H). This material was used without further purification.

2-[(1*S*-1-({(3*R*,4*R*)-4-(3-(Aminophenyl)-3,4-dimethylpiperidin-1-yl]methyl}-2-methylpropyl]-1*H*-isoindole-1,3(2*H*)-dione (25). The benzyl aniline **24** (353 mg, 0.69 mmol) was dissolved in EtOH (100 mL) with 20% $Pd(OH)_2$/C (0.2 g) and shaken under hydrogen (45 psi) overnight. The filtered concentrate was subjected to chromatography on silica gel using 20% EtOAc in hexanes as the eluent to recover 202 mg of starting material, followed by 50% EtOAc in hexanes with 1% NH_3 as the eluent to afford 55 mg (19%) of the desired aniline **25**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, J = 2.6 Hz, 2H), 7.57−7.68 (m, 2H), 7.00 (t, J = 7.8 Hz, 1H), 6.56 (d, J = 8.1 Hz, 1 H), 6.39−6.50 (m, 2H), 4.06 (ddd, J = 4.8, 9.8, 11.6 Hz, 1H), 3.24 (t, J = 12.1 Hz, 1H), 2.56−2.70 (m, 2H), 2.21−2.54 (m, 4H), 1.92−2.03 (m, 1H), 1.75−1.87 (m, 1H), 1.40 (dd, J = 1.2, 12.9 Hz, 1H), 1.21 (s, 3H), 1.05 (d, J = 6.6 Hz, 3H), 0.85−0.94 (m, 3H), 0.29 (d, J = 67.0 Hz, 3H). This material was used without further purification.

3-{(3*R*,4*R*)-1-[(2*S*)-2-Amino-3-methylbutyl]-3,4-dimethylpiperidin-4-yl}aniline (26). A solution of **25** (1.34 g, 3.2 mmol) in dioxane (6 mL) and HCl (6 M, 6 mL) was stirred at reflux for 18 h. The mixture was concentrated then partitioned between aq $NaHCO_3$ and EtOAc. The aqueous layer was adjusted to pH 10. The organic layer was separated, dried (Na_2SO_4), and concentrated. The resulting residue was subjected to chromatography on silica gel with 50% EtOAc in CMA80 as the eluent (R_f 0.5) to afford 0.41 g (44%) of **26** as a light-orange oil. ^{1}H NMR ($CDCl_3$) δ 7.10 (t, J = 7.8 Hz, 1H), 6.67−6.73 (m, 1H), 6.62 (t, J = 2.0 Hz, 1H), 6.51 (ddd, J = 0.9, 2.3, 7.8 Hz, 1H), 3.61 (br s, 1H), 2.57−2.78 (m, 2H), 2.51 (dt, J = 2.6, 11.8 Hz, 1H), 2.27−2.43 (m, 3H), 2.25 (d, J = 3.6 Hz, 1H), 2.13−2.22 (m, 1H), 1.89−2.03 (m, 2H), 1.68 (br s, 2H), 1.45−1.59 (m, 2H), 1.27−1.31 (m, 3H), 0.92 (d, J = 7.0 Hz, 6H), 0.78 (d, J = 7.0 Hz, 3H). MS (ESI) m/z 290.3 $(M + H)^+$. This material was used without further purification.

2-[(1*S*)-1-{[(3*R*,4*R*)-4-(3-Fluorophenyl)-3,4-dimethylpiperidin-1-yl]methyl}-2-methylpropyl]-1*H*-isoindole-1,3(2*H*)-dione (27). The aniline **25** (55 mg, 0.13 mmol) was dissolved in CH_2Cl_2 (1 mL) and treated with $BF_3{\cdot}OEt_2$ (32 μL, 0.26 mmol) then isoamyl nitrite (26 μL, 0.20 mmol). After stirring 15 min, the solution was cooled and diethyl ether was added. The resulting crystalline solids were collected by filtration, dried, and heated neat. The resulting residue was subjected to chromatography on silica gel eluting with EtOAc as the eluent to afford 36 mg (66%) of **27**. ^{1}H NMR ($CDCl_3$) δ 7.79−7.90 (m, 2H), 7.68−7.79 (m, 2H), 7.11−7.34 (m, 2H), 6.80−7.04 (m, 3H), 4.14−4.26 (m, 1H), 4.00 (t, J = 11.9 Hz, 1H), 3.24−3.78 (m, 4H), 2.55 (br s, 2H), 2.18−2.37 (m, 2H), 1.41 (s, 3H), 1.06−1.17 (m, 3H), 0.77−0.89 (m, 3H), 0.52−0.74 (m, 3H). This material was used without further purification.

2-[(1*S*)-1-{[(3*R*,4*R*)-4-(3-Chlorophenyl)-3,4-dimethylpiperidin-1-yl]methyl}-2-methylpropyl]-1*H*-isoindole-1,3(2*H*)-dione (28). A solution of **25** (340 mg, 0.81 mmol) in aq HCl (37%, 5 mL) was cooled to −5 °C and stirred for 10 min. A solution of $NaNO_2$ (63 mg, 0.89 mmol) in water (1.5 mL) was added dropwise to the reaction which then was stirred for 1 h. A cold solution of copper(I) chloride (92 mg, 0.93 mmol) in water (1.5 mL) was then added dropwise. The reaction mixture was stirred for 30 min and allowed to warm to rt then was heated to 65 °C for 3 h. The resulting suspension was poured in to a mixture of concd NH_4OH (20 mL) and ice (6 g). The resulting solution with was extracted with ethyl acetate (2 × 40 mL). The combined organic layers were dried (Na_2SO_4) and evaporated to a residue, which was subjected to chromatography on silica gel using a gradient up to 50% EtOAc in hexanes as the eluent to afford 100 mg (28%) of **28** as an oil that solidified to a white solid upon standing. ^{1}H NMR ($CDCl_3$) δ 7.77 (br s, 2H), 7.65 (br s, 2H), 6.95−7.21 (m, 4H), 4.06 (m, 1H), 3.26 (t, J = 12.1 Hz, 1H), 2.58−2.79 (m, 2H), 2.18−2.56 (m, 3H), 1.93−2.11 (m, 1H), 1.76−1.91 (m, 1H), 1.37−1.50 (m, 1H), 1.24 (s, 3H), 1.06 (d, J = 6.8 Hz, 3H), 0.90 (d, J = 6.8 Hz, 3H), 0.28 (d, J = 6.9 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 169.1, 152.8, 134.0, 133.6, 132.0, 129.2, 125.8, 125.3, 123.7, 122.8, 57.2, 55.0, 54.1, 51.4, 38.6, 38.5, 30.4, 29.9, 27.2, 20.4, 20.3, 15.4. MS (ESI) m/z 439.4 $(M + H)^+$. This material was used withut further purification.

2-[(1*S*)-1-{[(3*R*,4*R*)-4-(3-Bromophenyl)-3,4-dimethylpiperidin-1-yl]methyl}-2-methylpropyl]-1*H*-isoindole-1,3(2*H*)-dione (29). A solution of **25** (250 mg, 0.6 mmol in aq HBr (48%, 0.5 mL) and water (1 mL) was cooled to −5 °C and stirred for 10 min. A solution of $NaNO_2$ (46 mg, 0.65 mmol) in water (1 mL) was added dropwise, and the reaction mixture stirred for 2 h. Urea was added to the reaction mixture to consume any excess nitrous acid. Copper(I) bromide (103 mg, 0.72 mmol), aq HBr (48%, 0.2 mL), and water (0.5 mL) were added consecutively to the reaction mixture. After stirring for 1 h, the reaction mixture was heated to 65 °C for 30 min. The resulting suspension was poured in to a mixture of concd NH_4OH (20 mL) and ice (6 g). The resulting solution was extracted with ethyl acetate (2 × 40 mL). The combined organic layers were dried (Na_2SO_4) and evaporated to a residue, which was subjected to chromatography on silica gel using a gradient up to 50% EtOAc in hexanes as the eluent to afford 110 mg (38%) of **29** as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 7.77 (br s, 2H), 7.65

dx.doi.org/10.1021/jm5008177 | J. Med. Chem. 2014, 57, 7367−7381

(dd, J = 2.9, 5.2 Hz, 2H), 7.18−7.26 (m, 2H), 6.94−7.18 (m, 2H), 3.94−4.21 (m, 1H), 3.26 (t, J = 12.1 Hz, 1H), 2.58−2.78 (m, 2H), 2.20−2.57 (m, 4H), 1.94−2.03 (m, 1H), 1.77−1.93 (m, 1H), 1.37−1.55 (m, 1H), 1.23 (s, 3H), 1.06 (d, J = 6.6 Hz, 3H), 0.91 (d, J = 6.8 Hz, 3H), 0.28 (d, 3H, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 169.1, 153.1, 133.6, 132.0, 129.6, 128.7, 128.3, 128.0, 125.4, 125.1, 124.2, 122.8, 122.5, 57.2, 55.0, 54.1, 51.4, 38.7, 38.4, 30.3, 29.9, 27.3, 20.4, 20.3, 15.5. This material was used without further purification.

(2*S*)-1-[(3*R*,4*R*)-4-(3-Fluorophenyl)-3,4-dimethylpiperidin-1-yl]-3-methylbutan-2-amine (30). A solution of **27** (36 mg, 0.085 mmol) was heated at reflux in EtOH (10 mL) and hydrazine (1 mL) overnight. The concentrated residue was dissolved in EtOAc then washed with aq $NaHCO_3$. The organic layer was dried (Na_2SO_4) and concentrated to afford 24 mg (96%) of the amine **30**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.12−7.26 (m, 1H), 6.98 (d, J = 8.1 Hz, 1H), 6.90 (dd, J = 1.9, 11.3 Hz, 1H), 6.79 (dt, J = 2.1, 8.3 Hz, 1H), 2.07−2.77 (m, 8H), 1.83−1.96 (m, 1H), 1.42−1.58 (m, 2H), 1.08−1.31 (m, 5H), 0.74−0.95 (m, 6H), 0.60−0.74 (m, 3H). This material was used without further purification.

(2*S*)-1-[(3*R*,4*R*)-4-(3-Chlorophenyl)-3,4-dimethylpiperidin-1-yl]-3-methylbutan-2-amine (31). To a solution of **28** (100 mg, 0.23 mmol) in ethanol (15 mL) was added hydrazine monohydrate (115 mg, 2.3 mmol). The reaction mixture was stirred at reflux under nitrogen for 12 h and then concentrated to obtain a white solid that was dissolved in aq $NaHCO_3$ (10 mL) and extracted with EtOAc (2 × 20 mL). The combined organic layers were dried (Na_2SO_4) and concentrated to afford 56 mg (79%) of **31** as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.94−7.18 (m, 4H), 2.50−2.73 (m, 3H), 2.45 (m, 1H), 2.04−2.38 (m, 3H), 1.79−2.02 (m, 2H), 1.37−1.56 (m, 2H), 1.23 (m, 3H), 0.85 (d, J = 6.8 Hz, 6H), 0.69 (d, J = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.7, 133.1, 128.3, 124.9, 124.5, 122.8, 61.7, 53.5, 51.9, 51.0, 37.9, 37.6, 31.1, 29.6, 26.4, 18.3, 17.2, 15.2. MS (ESI) m/z 309.4 $(M + H)^+$. This material was used without further purification.

(2*S*)-1-[(3*R*,4*R*)-4-(3-Bromophenyl)-3,4-dimethylpiperidin-1-yl]-3-methylbutan-2-amine (32). To a solution of **29** (110 mg, 0.23 mmol) in ethanol (15 mL) was added hydrazine monohydrate (115 mg, 2.3 mmol). The reaction mixture was stirred at reflux under nitrogen for 12 h and then concentrated to obtain a white solid that was dissolved in aq $NaHCO_3$ (10 mL) and extracted with EtOAc (2 × 20 mL). The combined organic layers were dried (Na_2SO_4) and concentrated to obtain 94 mg (99%) of **32** as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33 (s, 1H), 7.17−7.28 (m, 1H), 7.03−7.17 (m, 2H), 2.51−2.71 (m, 3H), 2.10−2.49 (m, 4H), 1.82−2.00 (m, 1H), 1.38−1.65 (m, 3H), 1.23 (m, 3H), 0.85 (d, J = 6.8 Hz, 6H), 0.56−0.76 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.1, 128.7, 127.8, 127.4, 127.1, 124.5, 124.3, 123.2, 121.6, 61.8, 53.5, 51.9, 51.0, 37.9, 37.6, 31.2, 29.6, 26.4, 18.3, 17.2, 15.3. MS (ESI) m/z 353.4 $(M + H)^+$. This material was used without further purification.

Methyl (3*R*)-2-(*tert*-Butoxycarbonyl)-7-cyano-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (35). A solution of Boc-7-hydroxy-D-Tic (1.47 g, 5 mmol) in toluene (35 mL) and CH_3OH (10 mL) was treated with a solution of $TMSCHN_2$ in ether (2.0 M, 2.5 mL) until a slight yellow persisted. The excess reagent was quenched with acetic acid then the solution was concentrated. The residue was dissolved in CH_2Cl_2 (50 mL) and NEt_3 (0.9 mL, 6.5 mmol) and treated with Tf_2O (0.85 mL, 5.0 mmol) at 0 °C. The reaction was allowed to warm to room temperature and concentrated to a residue and subjected to a plug of silica gel, eluting with 20% EtOAc in hexanes as the eluent. The fractions containing product were concentrated and dissolved in DMF (6 mL) with $Zn(CN)_2$ (1.0 g, 8.5 mmol). The mixture was degassed and kept under nitrogen as $Pd(PPh_3)_4$ (200 mg, 0.2 mmol) was added. The mixture was heated to 100 °C for 4 h, cooled, then partitioned between EtOAc and aq $NaHCO_3$. The organic layer was dried (Na_2SO_4), concentrated, and subjected to chromatography on silica gel using 20% EtOAc in hexanes to afford 1.37 g of **35** (86% over 3 steps). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39−7.52 (m, 2H), 7.22−7.31 (m, 1H), 4.68−4.81 (m, 1H), 4.42−4.60 (m, 1H), 3.64 (d, J = 4.9 Hz, 3H), 3.30 (d, J = 2.1 Hz, 1H), 3.22 (d, J = 5.8 Hz, 2H), 1.50 (d, J = 19 Hz, 9H). This material was used without further purification.

(3*R*)-2-(*tert*-Butoxycarbonyl)-7-carbamoyl-1,2,3,4-tetrahydroisoquinoline-3-carboxylic Acid (36). A sample of **35** (320 mg, 1.0 mmol) was dissolved in dioxane (2 mL) and THF (1 mL) then treated with aq LiOH (1 M, 3 mL) overnight. The resulting solution was cooled in an ice bath and treated cautiously with H_2O_2 (30%, 1 mL). After warming, the reaction mixture was acidified with HCl (2 M) and diluted with water. The resulting solids were separated by filtration and dried to afford 230 mg of **36** (72%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.72 (s, 1H), 7.90 (br s, 1H), 7.61−7.74 (m, 1H), 7.21−7.37 (m, 1H), 4.89 (br s, 1H), 4.68 (s, 1H), 4.55−4.64 (m, 1H), 4.52 (d, J = 5.7 Hz, 1H), 4.36−4.48 (m, 1H), 3.06−3.26 (m, 2H), 1.33−1.53 (m, 9H). This material was used without further purification.

hERG Assay. Preparations of membranes overexpressing human hERG were purchased from PerkinElmer. The binding assays were performed for 60 min using 4 μg hERG expressing membranes, ∼3 nM [^{3}H]Astemizole, and various concentrations of the test agent in a binding buffer (10 mM HEPES, pH 7.4, 130 mM NaCl, 5 mM KCl, 0.8 mM $MgCl_2$, 1 mM NaEDTA, 10 mM glucose, 0.1% BSA). Binding was terminated by rapid filtration onto GF/B fiber filtermats, presoaked in 0.3% polyethylenimine, followed by rapid washing 6 times (2 mL) with ice-cold solution containing 25 mM Tris-HCl, pH 7.4, 130 mM NaCl, 5 mM KCl, 0.8 mM $MgCl_2$, 0.05 mM $CaCl_2$, and 0.1% BSA using a Brandel harvester. Filters were dried and counted after addition of a scintillant. Data were analyzed using nonlinear regression (GraphPad Prism), and K_i values were determined as described before.[52] All experiments were performed at least twice in duplicate, and data reported are mean values.

Solubility Determination. For these experiments, 10 mM DMSO stocks of compounds were directly diluted into 10 mM phosphate buffer at pH 7.4 or 3 and shaken for 90 min at room temperature. The final concentration of DMSO was 1%. After the incubation, samples were filtered through a 0.4 μm filterplate (Millipore). Filtrates were carefully collected. Analysis of compounds was performed by LC/MS using previously available methods and concentrations determined. Data are reported as mean values from three determinations.

PAMPA Assay. A commercially available PAMPA assay system was used (BD Gentest Precoated PAMPA System). Assays were performed in duplicate at 10 μM final concentration at pH 7.4 and 5.5 as has been described previously in PBS buffer.[38] The donor plate was on top and receiver plate on the bottom. Samples were incubated for 4 h and then collected carefully from each plate. Quantification was performed using LC/MS.

Docking Studies and Calculation. The ligand preparation, receptor preparation, and docking calculations were conducted under our previously reported methods.[34] The two-dimensional interaction diagrams were generated using LigPlot+.[53]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Elemental analysis data for compounds **4**−**15**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 919 541-6679. Fax: 919 541-8868. E-mail: fic@rti.org.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was supported by the National Institute on Drug Abuse grants DA09045 and DA021002. We thank Dr. Ann Decker, Tiffany Langston, Keith Warner, and Rodney Snyder for conducting the in vitro testing and in vitro preclinical studies.

ABBREVIATIONS USED

GPCRs, G-protein-coupled receptors; SAR, structure−activity relationship; [^{35}S]GTPγS, sulfur-35 guanosine-5′-*O*-(3-thio)-triphosphate; DAMGO, [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin; DPDPE, [D-Pen^2,D-Pen^5]enkephalin; U69,593, (5α,7α,8β)-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro-[4,5]dec-8-yl]-benzeneacetamide; CHO, Chinese hamster ovary; GDP, guanosine diphosphate; HBTU, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; EDC, 1-ethyl-3(dimethylaminopropyl)carbodimide

REFERENCES

(1) Dhawan, B. N.; Cesselin, F.; Raghubir, R.; Reisine, T.; Bradley, P. B.; Portoghese, P. S.; Hamon, M. International Union of Pharmacology. XII. Classification of opioid receptors. *Pharmacol. Rev.* **1996**, *48*, 567−592.

(2) Aldrich, J. V.; Vigil-Cruz, S. C. Narcotic Analgesics. In *Burger's Medicinal Chemistry and Drug Discovery*, 6th ed.; Abraham, D. J., Ed.; John Wiley & Sons: New York, 2003; Vol. *6*, Chapter 7, pp 329−481.

(3) Carroll, F. I.; Carlezon, J.; William, A. Development of Kappa Opioid Receptor Antagonists. *J. Med. Chem.* **2013**, *56*, 2178−2195.

(4) Carroll, F. I.; Dolle, R. E. The discovery and development of the N-substituted *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine class of pure opioid receptor antagonists. *ChemMedChem* **2014**, *9*, 1638−1654.

(5) Beardsley, P. M.; Howard, J. L.; Shelton, K. L.; Carroll, F. I. Differential effects of the novel kappa opioid receptor antagonist, JDTic, on reinstatement of cocaine-seeking induced by footshock stressors vs cocaine primes and its antidepressant-like effects in rats. *Psychopharmacology (Berlin, Ger.)* **2005**, *183*, 118−126.

(6) Mague, S. D.; Pliakas, A. M.; Todtenkopf, M. S.; Tomasiewicz, H. C.; Zhang, Y.; Stevens, W. C., Jr.; Jones, R. M.; Portoghese, P. S.; Carlezon, W. A., Jr. Antidepressant-like effects of kappa-opioid receptor antagonists in the forced swim test in rats. *J. Pharmacol. Exp. Ther.* **2003**, *305*, 323−330.

(7) Knoll, A. T.; Meloni, E. G.; Thomas, J. B.; Carroll, F. I.; Carlezon, W. A., Jr. Anxiolytic-like effects of κ-opioid receptor antagonists in models of unlearned and learned fear in rats. *J. Pharmacol. Exp. Ther.* **2007**, *323*, 838−845.

(8) McLaughlin, J. P.; Marton-Popovici, M.; Chavkin, C. Kappa opioid receptor antagonism and prodynorphin gene disruption block stress-induced behavioral responses. *J. Neurosci.* **2003**, *23*, 5674−5683.

(9) Redila, V. A.; Chavkin, C. Stress-induced reinstatement of cocaine seeking is mediated by the kappa opioid system. *Psychopharmacology (Berlin, Ger.)* **2008**, *200*, 59−70.

(10) Carey, A. N.; Borozny, K.; Aldrich, J. V.; McLaughlin, J. P. Reinstatement of cocaine place-conditioning prevented by the peptide kappa-opioid receptor antagonist arodyn. *Eur. J. Pharmacol.* **2007**, *569*, 84−89.

(11) Walker, B. M.; Koob, G. F. Pharmacological evidence for a motivational role of κ-opioid systems in ethanol dependence. *Neuropsychopharmacology* **2007**, *33*, 643−652.

(12) Bodnar, R. J.; Glass, M. J.; Ragnauth, A.; Cooper, M. L. General, mu and kappa opioid antagonists in the nucleus accumbens alter food intake under deprivation, glucoprivic and palatable conditions. *Brain Res.* **1995**, *700*, 205−212.

(13) Bortolato, M.; Aru, G. N.; Frau, R.; Orru, M.; Fa, M.; Manunta, M.; Puddu, M.; Mereu, G.; Gessa, G. L. Kappa opioid receptor activation disrupts prepulse inhibition of the acoustic startle in rats. *Biol. Psychiatry* **2005**, *57*, 1550−1558.

(14) Brugel, T. A.; Smith, R. W.; Balestra, M.; Becker, C.; Daniels, T.; Hoerter, T. N.; Koether, G. M.; Throner, S. R.; Panko, L. M.; Folmer, J. J.; Cacciola, J.; Hunter, A. M.; Liu, R.; Edwards, P. D.; Brown, D. G.; Gordon, J.; Ledonne, N. C.; Pietras, M.; Schroeder, P.; Sygowski, L. A.; Hirata, L. T.; Zacco, A.; Peters, M. F. Discovery of 8-azabicyclo[3.2.1]-octan-3-yloxy-benzamides as selective antagonists of the kappa opioid receptor. Part 1. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5847−5852.

(15) Peters, M. F.; Zacco, A.; Gordon, J.; Maciag, C. M.; Litwin, L. C.; Thompson, C.; Schroeder, P.; Sygowski, L. A.; Piser, T. M.; Brugel, T. A. Identification of short-acting kappa-opioid receptor antagonists with anxiolytic-like activity. *Eur. J. Pharmacol.* **2011**, *661*, 27−34.

(16) Grimwood, S.; Lu, Y.; Schmidt, A. W.; Vanase-Frawley, M. A.; Sawant-Basak, A.; Miller, E.; McLean, S.; Freeman, J.; Wong, S.; McLaughlin, J. P.; Verhoest, P. R. Pharmacological characterization of 2-methyl-*N*-((2′-(pyrrolidin-1-ylsulfonyl)biphenyl-4-yl)methyl)propan-1-amine (PF-04455242), a high-affinity antagonist selective for kappa-opioid receptors. *J. Pharmacol. Exp. Ther.* **2011**, *339*, 555−566.

(17) Verhoest, P. R.; Sawant Basak, A.; Parikh, V.; Hayward, M.; Kauffman, G. W.; Paradis, V.; McHardy, S. F.; McLean, S.; Grimwood, S.; Schmidt, A. W.; Vanase-Frawley, M.; Freeman, J.; Van Deusen, J.; Cox, L.; Wong, D.; Liras, S. Design and Discovery of a Selective Small Molecule Kappa Opioid Antagonist (2-Methyl-*N*-((2′-(pyrrolidin-1-ylsulfonyl)biphenyl-4-yl)methyl)propan-1-amine, PF-4455242. *J. Med. Chem.* **2011**, *54*, 5868−5877.

(18) Buezo, N. D.; McKinzie, D. L.; Mitch, C. H.; Pedregal-Tercero, C. Kappa Selective Opioid Receptor Antagonist. US Patent 8,173,695 B2, 2012.

(19) Rorick-Kehn, L. M.; Witkin, J. M.; Statnick, M. A.; Eberle, E. L.; McKinzie, J. H.; Kahl, S. D.; Forster, B. M.; Wong, C. J.; Li, X.; Crile, R. S.; Shaw, D. B.; Sahr, A. E.; Adams, B. L.; Quimby, S. J.; Diaz, N.; Jimenez, A.; Pedregal, C.; Mitch, C. H.; Knopp, K. L.; Anderson, W. H.; Cramer, J. W.; McKinzie, D. L. LY2456302 is a novel, potent, orally-bioavailable small molecule kappa-selective antagonist with activity in animal models predictive of efficacy in mood and addictive disorders. *Neuropharmacology* **2014**, *77*, 131−144.

(20) Mitch, C. H.; Quimby, S. J.; Diaz, N.; Pedregal, C.; de la Torre, M. G.; Jimenez, A.; Shi, Q.; Canada, E. J.; Kahl, S. D.; Statnick, M. A.; McKinzie, D. L.; Benesh, D. R.; Rash, K. S.; Barth, V. N. Discovery of aminobenzyloxyarylamides as kappa opioid receptor selective antagonists: application to preclinical development of a kappa opioid receptor antagonist receptor occupancy tracer. *J. Med. Chem.* **2011**, *54*, 8000−8012.

(21) RTI International. First in Humans Study of JDTic. . In *clinicaltrials.gov* National Library of Medicine: Bethesda, MD, 2012; http://clinicaltrials.gov/ct2/show/record/NCT01431586 (accessed July 2, 2014).

(22) Lowe, S. L.; Wong, C. J.; Witcher, J.; Gonzales, C. R.; Dickinson, G. L.; Bell, R. L.; Rorick-Kehn, L.; Weller, M.; Stoltz, R. R.; Royalty, J.; Tauscher-Wisniewski, S. Safety, Tolerability, and Pharmacokinetic Evaluation of Single- and Multiple-Ascending Doses of a Novel Kappa Opioid Receptor Antagonist LY2456302 and Drug Interaction With Ethanol in Healthy Subjects. *J. Clin. Pharmacol.* **2014**, *54*, 968−978.

(23) Pfizer. ICMJE A Study of Kappa Opioid Receptor Occupancy of PF-04455242, Using PET (Positron Emission Tomography). In *clinicaltrials.gov*; National Library of Medicine: Bethesda, MD, 2009; [cited 2014 July 2] http://clinicaltrials.gov/ct2/show/record/NCT00939887 (accessed July 2, 2014).

(24) Eli Lilly and Company. A Study of Brain Receptor Occupancy in Healthy Subjects. In *clinicaltrials.gov*; National Library of Medicine: Bethesda, MD, 2011; http://clinicaltrials.gov/ct2/show/record/NCT01232439 (accessed July 2, 2014).

(25) Ehrich, E. W. 1.3. New Clinical Research in Opioid Modulation Indicates Novel Utility in Treating Resistant Depression. *Neuropsychopharmacology* **2012**, *38*, S1.

(26) *Smoking and Tobacco Use-Fact Sheet: Quitting Smoking (updated November 2011)*; Centers for Disease Control and Prevention: Atlanta, GA, 2011; http://www.cdc.gov/tobacco/data_statistics/fact_sheets/cessation/quitting/index.htm (accessed July 1, 2014).

(27) Soyka, M.; Rosner, S. Opioid antagonists for pharmacological treatment of alcohol dependence—a critical review. *Curr. Drug Abuse Rev.* **2008**, *1*, 280−291.

(28) Lutz, P. E.; Kieffer, B. L. Opioid receptors: distinct roles in mood disorders. *Trends Neurosci.* **2013**, *36*, 195−206.

(29) Skiles, J. W.; Miao, C.; Sorcek, R.; Jacober, S.; Mui, P. W.; Chow, G.; Weldon, S. M.; Possanza, G.; Skoog, M.; Keirns, J.; Letts, G.; Rosenthal, A. S. Inhibition of human leukocyte elastase by N-substituted

peptides containing α,α-difluorostatone residues at P1. *J. Med. Chem.* **1992**, *35*, 4795−4808.
(30) Thomas, J. B.; Fall, M. J.; Cooper, J. B.; Rothman, R. B.; Mascarella, S. W.; Xu, H.; Partilla, J. S.; Dersch, C. M.; McCullough, K. B.; Cantrell, B. E.; Zimmerman, D. M.; Carroll, F. I. Identification of an opioid κ receptor subtype-selective N-substituent for (+)-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine. *J. Med. Chem.* **1998**, *41*, 5188−5197.
(31) Pagé, D.; McClory, A.; Mischki, T.; Schmidt, R.; Butterworth, J.; St-Onge, S.; Labarre, M.; Payza, K.; Brown, W. Novel Dmt-Tic dipeptide analogues as selective delta-opioid receptor antagonists. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 167−170.
(32) Selnick, H. G.; Smith, G. R.; Tebben, A. J. An improved procedure for the cyanation of aryl triflates: a convenient synthesis of 6-cyano-1,2,3,4-tetrahydroisoquinoline. *Synth. Commun.* **1995**, *25*, 3255−3261.
(33) Cai, T. B.; Zou, Z.; Thomas, J. B.; Brieaddy, L.; Navarro, H. A.; Carroll, F. I. Synthesis and in vitro opioid receptor functional antagonism of analogues of the selective kappa opioid receptor antagonist (3*R*)-7-hydroxy-*N*-((1*S*)-1-{[(3*R*,4*R*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl)-1,2,3,4-tetrahydro-3-isoquinolinecarboxamide (JDTic). *J. Med. Chem.* **2008**, *51*, 1849−1860.
(34) Kormos, C. M.; Jin, C.; Cueva, J. P.; Runyon, S. P.; Thomas, J. B.; Brieaddy, L. E.; Mascarella, S. W.; Navarro, H. A.; Gilmour, B. P.; Carroll, F. I. Discovery of *N*-{4-[(3-Hydroxyphenyl)-3-methylpiperazin-1-yl]methyl-2-methylpropyl}-4-phenoxybenzamide Analogues as Selective Kappa Opioid Receptor Antagonists. *J. Med. Chem.* **2013**, *56*, 4551−4367.
(35) Fulp, A.; Bortoff, K.; Seltzman, H.; Zhang, Y.; Mathews, J.; Snyder, R.; Fennell, T.; Maitra, R. Design and synthesis of cannabinoid receptor 1 antagonists for peripheral selectivity. *J. Med. Chem.* **2012**, *55*, 2820−2834.
(36) Fulp, A.; Bortoff, K.; Zhang, Y.; Seltzman, H.; Mathews, J.; Snyder, R.; Fennell, T.; Maitra, R. Diphenyl purine derivatives as peripherally selective cannabinoid receptor 1 antagonists. *J. Med. Chem.* **2012**, *55*, 10022−10032.
(37) Chiu, P. J.; Marcoe, K. F.; Bounds, S. E.; Lin, C. H.; Feng, J. J.; Lin, A.; Cheng, F. C.; Crumb, W. J.; Mitchell, R. Validation of a [^{3}H]astemizole binding assay in HEK293 cells expressing HERG K^+ channels. *J. Pharmacol. Sci.* **2004**, *95*, 311−319.
(38) Zhu, C.; Jiang, L.; Chen, T. M.; Hwang, K. K. A comparative study of artificial membrane permeability assay for high throughput profiling of drug absorption potential. *Eur. J. Med. Chem.* **2002**, *37*, 399−407.
(39) Zimmerman, D. M.; Nickander, R. In 39th Annual Scientific Meeting of the committee on Problems of Drug Dependence, 1977, pp 252−261.
(40) Zimmerman, D. M.; Nickander, R.; Horng, J. S.; Wong, D. T. New structural concepts for narcotic antagonists defined in a 4-phenylpiperidine series. *Nature* **1978**, *275*, 332−334.
(41) Zimmerman, D. M.; Smits, S.; Nickander, R. Further investigation of novel 3-methyl-4-phenylpiperidine narcotic antagonists. In *Proceedings of the 40th Annual Scientific Meeting of the Committee on Problems of Drug Dependence*; National Institute on Drug Abuse: Rockville, MD, 1978; pp 237−247.
(42) Diaz, N.; Benvenga, M.; Emmerson, P.; Favors, R.; Mangold, M.; McKinzie, J.; Patel, N.; Peters, S.; Quimby, S.; Shannon, H.; Siegel, M.; Statnick, M.; Thomas, E.; Woodland, J.; Surface, P.; Mitch, C. SAR and biological evaluation of novel *trans*-3,4-dimethyl-4-arylpiperidine derivatives as opioid antagonists. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3844−3848.
(43) Thomas, J. B.; Atkinson, R. N.; Vinson, N. A.; Catanzaro, J. L.; Perretta, C. L.; Fix, S. E.; Mascarella, S. W.; Rothman, R. B.; Xu, H.; Dersch, C. M.; Cantrell, B. E.; Zimmerman, D. M.; Carroll, F. I. Identification of (3*R*)-7-hydroxy-*N*-((1*S*)-1-[[(3*R*,4*R*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl]-2-methylpropyl)-1,2,3,4-tetrahydro-3-isoquinolinecarboxamide as a novel potent and selective opioid kappa receptor antagonist. *J. Med. Chem.* **2003**, *46*, 3127−3137.
(44) Wu, H.; Wacker, D.; Mileni, M.; Katritch, V.; Han, G. W.; Vardy, E.; Liu, W.; Thompson, A. A.; Huang, X. P.; Carroll, F. I.; Mascarella, S. W.; Westkaemper, R. B.; Mosier, P. D.; Roth, B. L.; Cherezov, V.; Stevens, R. C. Structure of the human kappa-opioid receptor in complex with JDTic. *Nature* **2012**, *485*, 327−332.
(45) Summerfeld, S. G.; Read, K.; Begley, D. J.; Obradovic, T.; Hidalgo, I. J.; Coggon, S.; Lewis, A. V.; Porter, R. A.; Jeffrey, P. Central nervous system drug disposition: the relationship between in situ brain permeability and brain free fraction. *J. Pharmacol. Exp. Ther.* **2007**, *322*, 205−213.
(46) Ghose, A. K.; Herbertz, T.; Hudkins, R. L.; Dorsey, B. D.; Mallamo, J. P. Knowledge-based, central nervous system (CNS) lead selection and lead optimization for CNS drug discovery. *ACS Chem. Neurosci.* **2012**, *3*, 50−68.
(47) Clark, D. E. Rapid calculation of polar molecular surface area and its application to the prediction of transport phenomena. 2. Prediction of blood−brain barrier penetration. *J. Pharm. Sci.* **1999**, *88*, 815−821.
(48) Beardsley, P. M.; Pollard, G. T.; Howard, J. L.; Carroll, F. I. Effectiveness of analogs of the kappa opioid receptor antagonist (3*R*)-7-hydroxy-*N*-((1*S*)-1-{[(3*R*,4*R*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl)-1,2,3,4-tetrahydro-3-isoquinolinecarboxami de (JDTic) to reduce U50,488-induced diuresis and stress-induced cocaine reinstatement in rats. *Psychopharmacology (Berlin, Ger.)* **2010**, *210*, 189−198.
(49) Brooks, D. P.; Giardina, G.; Gellai, M.; Dondio, G.; Edwards, R. M.; Petrone, G.; DePalma, P. D.; Sbacchi, M.; Jugus, M.; Misiano, P. Opiate receptors within the blood−brain barrier mediate kappa agonist-induced water diuresis. *J. Pharmacol. Exp. Ther.* **1993**, *266*, 164−171.
(50) Cabral, A. M.; Varner, K. J.; Kapusta, D. R. Renal excretory responses produced by central administration of opioid agonists in ketamine and xylazine-anesthetized rats. *J. Pharmacol. Exp. Ther.* **1997**, *282*, 609−616.
(51) Ko, M. C.; Willmont, K. J.; Lee, H.; Flory, G. S.; Woods, J. H. Ultra-long antagonism of kappa opioid agonist-induced diuresis by intracisternal nor-binaltorphimine in monkeys. *Brain Res.* **2003**, *982*, 38−44.
(52) Cheng, Y.-C.; Prusoff, W. H. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which cause 50% inhibition (I_{50}) of an enzyme reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.
(53) Laskowski, R. A.; Swindells, M. B. LigPlot+: multiple ligand−protein interaction diagrams for drug discovery. *J. Chem. Inf. Model.* **2011**, *51*, 2778−86.