Journal of Medicinal Chemistry Article

J. Med. Chem. **2009,** *52,* 5685–5702 **5685**
DOI: 10.1021/jm900773n

Spirocyclic Delta Opioid Receptor Agonists for the Treatment of Pain: Discovery of *N*,*N*-Diethyl-3-hydroxy-4-(spiro[chromene-2,4′-piperidine]-4-yl) Benzamide (ADL5747)

Bertrand Le Bourdonnec,*[,†] Rolf T. Windh,[‡] Lara K. Leister,[†] Q. Jean Zhou,[†] Christopher W. Ajello,[†] Minghua Gu,[†,⊥] Guo-Hua Chu,[†] Paul A. Tuthill,[†] William M. Barker,[†] Michael Koblish,[‡] Daniel D. Wiant,[‡] Thomas M. Graczyk,[‡] Serge Belanger,[‡] Joel A. Cassel,[‡] Marina S. Feschenko,[‡] Bernice L. Brogdon,[§] Steven A. Smith,[§,#] Michael J. Derelanko,[∥] Steve Kutz,[∥] Patrick J. Little,[‡] Robert N. DeHaven,[‡] Diane L. DeHaven-Hudkins,[‡] and Roland E. Dolle[†]

[†]*Departments of Chemistry and* [‡]*Pharmacology and* [§]*DMPK and* [∥]*Preclinical Safety Assessment, Adolor Corporation, 700 Pennsylvania Drive, Exton, Pennsylvania 19341.* [⊥]*GlaxoSmithKline R&D, PA.* [#]*UCB Pharma SA, Belgium.*

Received June 1, 2009

Selective, nonpeptidic δ opioid receptor agonists have been the subject of great interest as potential novel analgesic agents. The discoveries of BW373U86 (**1**) and SNC80 (**2**) contributed to the rapid expansion of research in this field. However, poor drug-like properties and low therapeutic indices have prevented clinical evaluation of these agents. Doses of **1** and **2** similar to those required for analgesic activity produce convulsions in rodents and nonhuman primates. Recently, we described a novel series of potent, selective, and orally bioavailable delta opioid receptor agonists. The lead derivative, ADL5859 (**4**), is currently in phase II proof-of-concept studies for the management of pain. Further structure activity relationship exploration has led to the discovery of ADL5747 (**36**), which is approximately 50-fold more potent than **4** in an animal model of inflammatory pain. On the basis of its favorable efficacy, safety, and pharmacokinetic profile, **36** was selected as a clinical candidate for the treatment of pain.

Introduction

The biological effects of endogenous opioid peptides are mediated through three classes of naloxone-sensitive opioid receptors: mu (μ), kappa (κ), and delta (δ). All three opioid receptors are localized throughout the central nervous system (CNS[a]) and periphery, and all are associated with pain relief in animal models.[1] Whereas μ opioid receptor agonists such as morphine still serve as the gold standards for managing a wide range of pain conditions, their pronounced side effects, including sedation, respiratory depression, and constipation, limit their utility. The δ opioid receptor (DOR) is an attractive target for the development of new drugs to control pain. Delta opioid receptors in rodents and primates are localized in cerebral cortex, striatum, amygdala, brainstem nuclei associated with pain processing, spinal cord, and dorsal root ganglia.[2−11] The localization pattern of DORs is consistent with a role in pain regulation and may also support the proposed roles for DORs in modulation of affective disorders and striatal neurodegeneration. Delta opioid receptor agonists are active in animal models of inflammatory[12−14] and neuropathic pain,[14−16] and efficacy in visceral pain models has also been reported.[17−19] Efficacy of DOR agonists in inflammatory pain models is more similar to that of nonsteroidal anti-inflammatory drugs (NSAIDs) than of morphine in that DOR agonists reverse the hyperalgesia associated with inflammation but do not inhibit acute pain. Aside from pain, DOR agonists also have potential application in several therapeutic indications, including affective disorder,[20,21] organ protection,[22] and neurodegenerative diseases.[23,24] Delta opioid agonists may possess potential clinical benefits compared with the μ opioid agonists currently used for pain relief, including reduced respiratory depression,[25,26] constipation,[14,27] physical dependence,[28−30] and abuse liability.[26,31]

Delta opioid receptor research received a major boost when **1**[32] (Chart 1), a prototypic small molecule nonpeptidic DOR agonist, was disclosed by Burroughs Wellcome in 1993. Subsequent DOR agonist research centered around the structure−activity relationships (SAR) of this compound series,[33−35] and led to the discovery of **2**,[36] the optically pure enantiomer of the methyl ether analogue of **1**. Receptor binding and in vitro bioassays have revealed a high degree of selectivity for δ over μ and κ opioid receptors for this compound.[36,37]

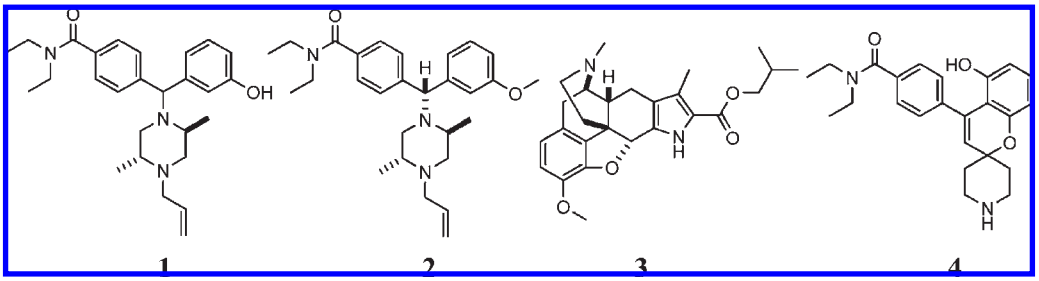
However, further development of **1** and **2** has not been pursued because they both elicit convulsions in animals at doses similar to those required for analgesic activity.[38] The DOR agonist induced convulsions consist of a single mild, brief (10−15 s) convulsive event, usually occurring within minutes of drug administration, and are followed by a brief period of catalepsy before the animals return to apparently normal behavior.[38] Sensitivity to the convulsive effects of these agonists in mice varies with genetic strain,[39]

*To whom correspondence should be addressed. Phone: 1-484-595-1061. Fax: 1-484-595-1551. E-mail: blebourdonnec@adolor.com.

[a] Abbreviations: SAR, structure−activity relationship; CYP, cytochrome P450; DDI, drug−drug interaction; hERG, human ether-a-go-go related gene; FCA, Freund's Complete Adjuvant; AUC, area under the curve; CNS: central nervous system; EEG: electroencephalogram; MEST, maximum electroshock seizure threshold; DMPK, drug metabolism and pharmacokinetic; NSAID: nonsteroidal anti-inflammatory drug; TBTU, *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate; DOR: δ opioid receptor; SEM: standard error of the mean; CHO, Chinese hamster ovary; MAMC, 7-methoxy-4-aminomethyl-coumarin; HAMC, 7-hydroxy-4-aminomethyl-coumarin; PPT, paw pressure thresholds; HEK, human embryonic kidney.

Published on Web 08/20/2009

Chart 1. Structures of **1**−**4**

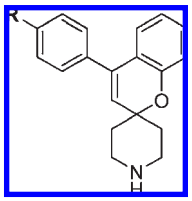


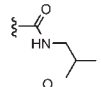
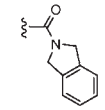
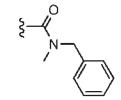
and convulsions have also been reported in rats[40] and nonhuman primates,[26,41,42] often in the same dose range as analgesia. The convulsions induced by **1** and **2** can be blocked with DOR antagonists in all species tested,[26,39−42] and the absence of these convulsions in mice lacking the DOR clearly demonstrates the requirement for DOR activation in this effect.[39] However, continued interest in DOR agonist research has been encouraged by the knowledge that, in contrast to compounds **1**, **2**, and structurally related analogues, DOR peptide agonists do not generally produce convulsions in rodents.[43−45] Recent studies with DOR agonists of different chemical classes also demonstrate that not all small molecule DOR agonists produce the behavioral activation and convulsions that are characteristic of **1** and **2**. SB-235863 (**3**)[14] does not produce seizures at doses up to 70 mg/kg po and fails to decrease maximum electroshock seizure threshold (MEST) or potentiate metrazol-induced seizures.[14] Therefore, it appears that whereas the δ receptor is required for behavioral activation and induction of convulsions by compounds **1**, **2**, and structurally related analogues, activation of the DOR does not necessarily result in these behaviors. The reasons for differences in behavioral effects among DOR agonists are unclear. We reported previously the discovery of ADL5859 (**4**), a potent and highly selective full agonist at the DOR that is structurally distinct from other chemical classes of δ agonists.[46] Importantly, **4** does not produce compound **2**-like behaviors, including convulsions, increased locomotor activity, or stereotypic activity, at oral doses up to 1000 mg/kg. This agent is in phase II clinical trials as an analgesic. Herein, we describe our continued efforts in this area and report the discovery of ADL5747 (**36**), a small molecule DOR agonist currently undergoing phase I safety evaluation in normal healthy volunteers.

Chemistry

The examples listed in Tables 1−4 were prepared according to Schemes 1−7. The synthesis of compounds **5**[46] and **18**−**21** is outlined in Scheme 1. Condensation of the ketone derivatives **37a**−**e** with 2-hydroxyacetophenone in refluxing methanol in the presence of pyrrolidine provided the spirocyclic ketones **38a**−**e**, which were converted to the corresponding enol triflate derivatives **39a**−**e** using *N*-phenylbis(trifluoromethanesulfonamide) as triflating agent. Suzuki type coupling of the enol triflate derivatives **39a**−**e** with 4-(*N*,*N*-diethylaminocarbonyl)phenylboronic acid in ethylene glycol dimethyl ether in the presence of tetrakis(triphenylphosphine)palladium(0), lithium chloride, and an aqueous solution of sodium carbonate provided compounds **21** and **40a**−**d**. The *N*-Boc derivatives **40a**−**d** were then converted to the target compounds **5**, and **18**−**20**, respectively, under acidic conditions. The synthesis of compounds **6**−**16** is described in Scheme 2. Condensation of the enol triflate **39a** with 4-(methoxycarbonyl)phenylboronic acid under standard Suzuki coupling conditions provided the methyl ester **41**, which was hydrolyzed to the corresponding carboxylic acid derivative **42** under basic conditions. Coupling of **42** with various amines using *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) as coupling agent afforded the primary, secondary, and tertiary carboxamide derivatives **43a**−**k**. Treatment of the *N*-Boc derivatives **43a**−**k** with anhydrous hydrochloric acid in methanol provided the final compounds **6**−**16**. The 3,4-dihydroisoquinolin-1(2*H*)-one derivative **17**, constrained analogue of **5**, was prepared according to Scheme 3. Alkylation of 6-methoxy-3,4-dihydroisoquinolin-1(2*H*)-one **44**[35] with ethyl iodide in tetrahydrofuran in the presence of sodium hydride afforded the methyl ether **45**, which was converted to the phenolic derivative **46** by treatment with boron tribromide. Condensation of **46** with trifluoromethanesulfonic anhydride in dichloromethane in the presence of pyridine gave the triflate derivative **47**. Conversion of the enol triflate **39a** to the boronate derivative **48** was achieved in good yield in *N*,*N*-dimethylformamide in the presence of bis(pinacolato)diborane, dichloro[1,1′-bis(diphenylphosphino)ferrocene]palladium(II) dichloromethane adduct, and potassium acetate. Coupling of **48** with the triflate derivative **47** under Suzuki coupling conditions afforded the *N*-Boc derivative **49**, which was converted to the final compound **17** under acidic conditions. The preparation of compounds **22**−**24** is described in Scheme 4. The spiro[chromene-2,4′-piperidine] derivative **5** was converted to the spiro-[chroman-2,4′-piperidine] analogue **22** (racemic mixture) by hydrogenation in methanol in the presence of Pearlman's catalyst. The enantiomers **23** and **24**, derived from **22**, were separated by chiral HPLC. Condensation of **23** with (1*S*)-(+)-10-camphorsulfonyl chloride (used as chiral derivatization agent) in dichloromethane in the presence of triethylamine provided the chiral sulfonamide derivative **50**. The absolute configuration of **50** was determined by X-ray crystallography (Chart 2),[47] thereby establishing, by inference, the absolute configuration of enantiomers **23** and **24**. Compounds **25**−**27** and **29**−**35** were prepared according to Scheme 5. The aryl bromides **52a**−**d**,**f**−**j** were obtained by coupling of their corresponding carboxylic acid derivatives with *N*,*N*-diethylamine using TBTU as coupling agent. Treatment of 5-bromo-2-iodopyrimidine **53** with *n*-butyllithium in hexane/toluene followed by addition of carbon dioxide provided 5-bromopyrimidine-2-carboxylic acid (**54**). Treatment of **54** with oxalyl chloride in dichloromethane gave the corresponding acyl chloride derivative, which reacted with diethylamine in tetrahydrofuran to yield the amide **52e**. Coupling of the aryl bromides **52a**−**j** with the boronate derivative **48** in ethylene glycol dimethyl ether in the presence of tetrakis(triphenylphosphine)palladium(0), lithium chloride, and an aqueous

Table 1. In Vitro Profile of Compounds **5**−**17** at Opioid Receptors (δ, μ, and κ), hERG, and CYP2D6

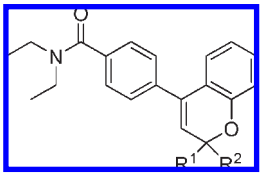


compd	R	K_i (δ) (nM)[a]	EC_{50} (δ) (nM)[a]	K_i (μ) (nM) or % inh. @ 10 μM[a]	K_i (κ) (nM) or % inh. @ 10 μM[a]	IC_{50} (hERG) (nM)[b]	IC_{50} (CYP2D6) (nM)[c]
5		1.8 (1.1-2.8)	19 (11-32)	32 ± 5%	29 ± 5%	7900	4300
6		5.6 (3.7-8.5)	90 (51-160)	51 ± 3%	800 (590-1100)	11989	89
7		17 (4.0-76)	110 (12-1100)	2700 (1500-4700)	560 (130-2400)	11589	440
8		18 (12-26)	110 (76-170)	41 ± 7%	1,600	>100000	1200
9		13 (7.6-21)	450 (290-690)	42 ± 5%	26 ± 5%	3190	670
10		1.2 (0.62-2.3)	47 (9.8-230)	33 ± 4%	28 ± 1%	6809	4100
11		10 (3.5-29)	81 (33-190)	40 ± 4%	21 ± 9%	23070	2800
12		27 (0.28-2600)	200 (48-800)	37 ± 6%	41 ± 3%	10566	4700
13		15 (8.7-27)	100 (43-240)	35 ± 8%	25 ± 4%	3027	4400
14		50 (4.5-570)	240 (230-260)	22 ± 4%	20 ± 2%	54256	7600
15		0.53 (0.0062-45)	45 (16-120)	450 (370-550)	890 (180-4500)	3501	1800
16		18 (1.6-130)	100 (46-220)	1800 (1200-2800)	60±1%	1571	1000
17[d]		8.1 (3.8-17)	190 (130-270)	48 ± 6%	42 ± 2%	13500	2800

[a] See detailed pharmacological methods in the Experimental Section. [b] Inhibition of hERG channel currents in voltage-clamped HEK293 cells stably expressing hERG potassium channels; IC_{50} values are geometric means from at least three separate determinations. [c] Fluorescence-based assay; see detailed methods in the Experimental Section. [d] See structure in Scheme 3.

solution of sodium carbonate yielded the *N*-Boc protected spirocyclic piperidine derivatives **51a**−**j**, which were converted to the final compounds **25**−**27** and **29**−**35**, respectively, under acidic conditions. The synthesis of compound **28** is outlined in Scheme 6. Coupling of the carboxylic acid **55** with *N*,*N*-diethylamine in the presence of TBTU afforded the bromide **56**, which was converted to the boronate **57** in *N*,*N*-dimethylformamide in the presence of bis(pinacolato)-diborane, potassium acetate, and 1,1′-bis(diphenylphosphino)-ferrocene palladium(II) chloride complex with dichloromethane. Suzuki coupling of the enol triflate **39a** with **57** in ethylene glycol dimethyl ether in the presence of palladium on carbon, lithium chloride, and an aqueous solution of sodium carbonate afforded compound **58**, which was converted to the final product (compound **28**) under acidic conditions. The medicinal chemistry route used to prepare compound **36** is shown in Scheme 7. Compound **60** was prepared in two steps from carboxylic acid **59**, i.e., esterification of **59** using acetyl chloride in methanol, followed by conversion of the resulting phenolic intermediate to the methoxymethyl (MOM) ether derivative **60** using chloro(methoxy)methane as alkylating agent. Hydrolysis of ester **60** under basic conditions gave the carboxylic acid **61**, which was coupled with *N*,*N*-diethylamine in the presence of TBTU to afford the corresponding carboxamide derivative **62**. Suzuki coupling of the boronate **48** with **62** in ethylene glycol dimethyl ether in the presence of palladium on

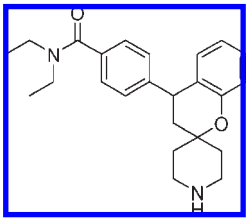
Table 2. In Vitro Profile of Compounds **18**−**21** at Opioid Receptors (δ, μ, and κ), hERG, and CYP2D6



compd	R	K_i (δ) (nM)[a]	EC_{50} (δ) (nM)[a]	%inh. @ 10 μM (μ)[a]	%inh. @ 10 μM (κ)[a]	IC_{50} (hERG) (nM)[b]	IC_{50} (CYP2D6) (nM)[c]
18		1.8 (0.34-3.8)	20 (10-41)	41 ± 4%	46 ± 2%	11784	2600
19		18 (7.4-46)	28 (4.3-180)	13 ± 4%	31 ± 9%	6429	2400
20		56 (18-170)	480 (350-660)	8.1 ± 5.8%	19 ± 8%	12653	2200
21		1400 (490-4100)	nd[d]	28 ± 9%	27 ± 7%	nd[d]	nd[d]

[a] See detailed pharmacological methods in the Experimental Section. [b] Inhibition of hERG channel currents in voltage-clamped HEK293 cells stably expressing hERG potassium channels; IC_{50} values are geometric means from at least three separate determinations. [c] Fluorescence-based assay; see detailed methods in the Experimental Section. [d] nd: not determined.

Table 3. In Vitro Profile of Compounds **22**−**24** at Opioid Receptors (δ, μ, and κ), hERG, and CYP2D6



compd	configuration	K_i (δ) (nM)[a]	EC_{50} (δ) (nM)[a]	%inh at 10 μM (μ)[a]	%inh at 10 μM (κ)[a]	IC_{50} (hERG) (nM)[b]	IC_{50} (CYP2D6) (nM)[c]
22	(±)	1.6 (1.0−2.6)	21 (11−42)	19 ± 6%	20 ± 2%	5094	6200
23	*R*-(−)	0.93 (0.57−1.5)	16 (10−25)	33 ± 7%	25 ± 3%	2543	7100
24	*S*-(+)	29 (18−47)	420 (310−560)	18 ± 4%	24 ± 5%	18471	3100

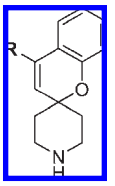
[a] See detailed pharmacological methods in the Experimental Section. [b] Inhibition of hERG channel currents in voltage-clamped HEK293 cells stably expressing hERG potassium channels; IC_{50} values are geometric means from at least three separate determinations. [c] Fluorescence-based assay; see detailed methods in the Experimental Section.

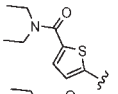
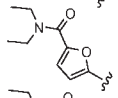
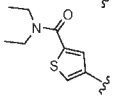
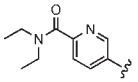
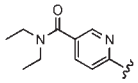
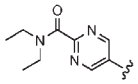
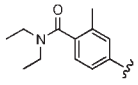
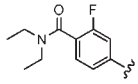
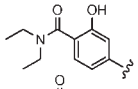
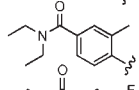
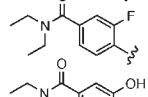
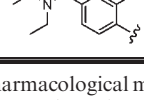
carbon, lithium chloride, and an aqueous solution of sodium carbonate under microwave irradiation afforded compound **63**, which was converted to **36** under acidic conditions.

Results and Discussion

Compound **5**, the lead molecule in this series, was previously identified as a potent DOR agonist, displaying excellent selectivity (>1000-fold) for δ over μ and κ opioid receptors.[46] Off-target profiling of **5** revealed that this compound weakly inhibited the human ether-a-go-go-related gene (hERG) potassium channel (IC_{50} = 7900 nM).[46] Blocking the hERG K^+ channel could raise serious cardiovascular issues. The hERG channel is responsible for the I_{Kr} current, a critical component in ventricular repolarization. Its blockade can lead to increased QT_c interval and potentially ventricular arrhythmias.[48] Additional studies also demonstrated that compound **5** moderately inhibited the activity of the drug metabolizing enzyme cytochrome P450 2D6 (CYP2D6) in vitro (IC_{50} = 4300 nM). CYP2D6 is a polymorphic member of the P450 superfamily, which is absent in 5−9% of the Caucasian population, resulting in a deficiency in drug oxidation known as debrisoquine/sparteine polymorphism.[49] A number of drugs have been clinically implicated in major drug−drug interactions (DDI) via CYP2D6 inhibition.[50−52] As a result, it is prudent to minimize potential issues related to CYP2D6-mediated DDIs at an early step of the drug discovery process. The objective of the lead optimization program was to identify orally active, potent, and selective DOR agonists [K_i (δ) < 10 nM; K_i (μ), K_i (κ) > 1000 × K_i (δ)] displaying reduced hERG and CYP2D6 inhibitory activities (IC_{50} > 10000 nM) when compared to **5**. The derivatives **5**−**36** were tested for their affinities toward the cloned human δ, μ, and κ opioid receptors as measured by their abilities to displace specific [^{3}H]diprenorphine binding. Selected compounds were also evaluated for their in vitro functional

Table 4. In Vitro Profile of Compounds **25**−**36** at Opioid Receptors (δ, μ, and κ), hERG, and CYP2D6

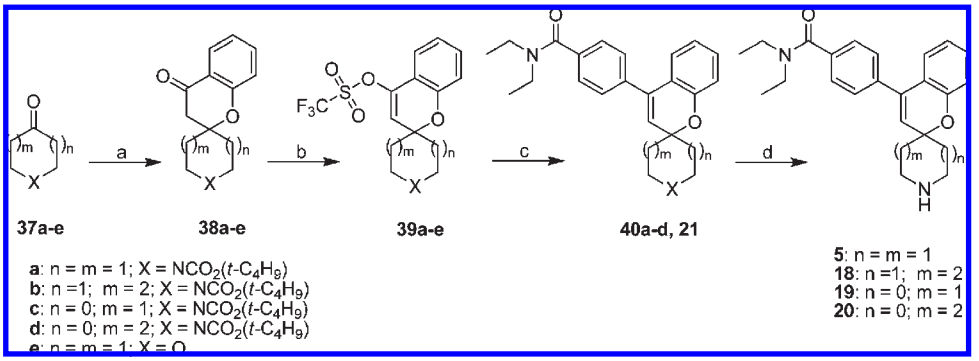


compd	R	K_i (δ) (nM)[a]	EC_{50} (δ) (nM)[a]	K_i (μ) (nM) or % inh. @ 10μM[a]	K_i (κ) (nM) or % inh. @10 μM[a]	IC_{50} (hERG) (nM)[b]	IC_{50} (CYP2D6) (nM)[c]
25		1.5 (1.0-2.2)	69 (34-140)	39 ± 7%	29 ± 4%	1261	2100
26		20 (1.9-220)	980 (320-3000)	3400 (690-16000)	41 ± 3%	5132	640
27		6.9 (4.0-12)	370 (130-1100)	220 (4.1-11000)	34 ± 8%	3463	5700
28		2.5 (1.8-3.4)	150 (46-490)	8.8 ± 7.3%	20 ± 8%	44113	30000
29		11 (1.4-93)	270 (180-420)	43 ± 26%	5.2 ± 3.1%	71666	33000
30		4.6 (1.5-14)	92 (65-130)	25 ± 2%	3.4 ± 1.7%	>100000	21000
31		3.3 (2.4-4.6)	310 (87-1100)	40 ± 5%	35 ± 6%	4485	9500
32		1.2 (0.69-2.0)	66 (35-130)	51 ± 7%	42 ± 6%	3794	5300
33		0.69 (0.46-1.0)	26 (12-55)	49 ± 2%	49 ± 2%	21079	9500
34		2.4 (1.9-3.0)	360 (220-580)	3400 (1800-6300)	390 (41-3700)	4243	4600
35		13 (8.8-18)	980 (48-2000)	46 ± 4%	36 ± 8%	418	7500
36		2.7 (2.1-3.4)	94 (76-120)	18 ± 4%	25 ± 2%	56700	43000

[a] See detailed pharmacological methods in the Experimental Section. [b] Inhibition of hERG channel currents in voltage-clamped HEK293 cells stably expressing hERG potassium channels; IC_{50} values are geometric means from at least three separate determinations. [c] Fluorescence-based assay; see detailed methods in the Experimental Section.

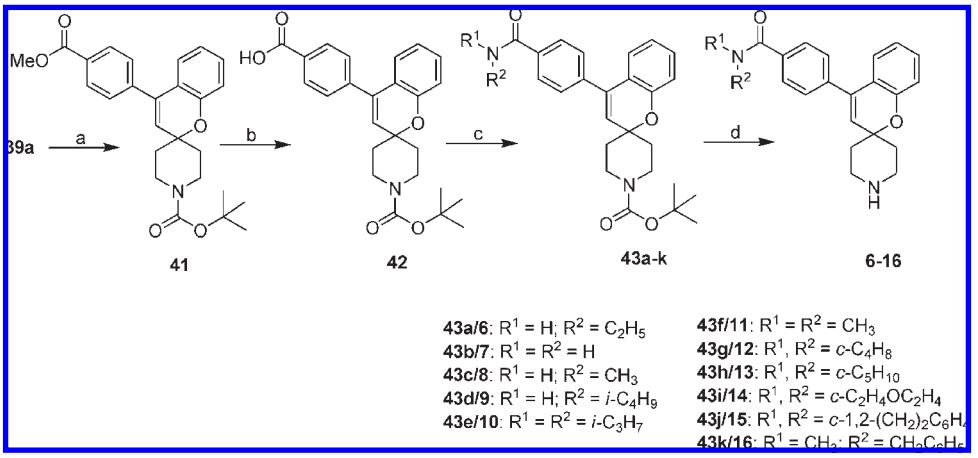
agonist potencies to stimulate binding of guanosine 5′-*O*-(3-[^{35}S]thio)triphosphate ([^{35}S]GTPγS) to DOR-containing cell membrane preparations. All compounds tested in this [^{35}S]GTPγS functional assay behaved as full agonists at the DORs, with maximal stimulation similar to the reference DOR agonist **1**. The compounds displaying potent affinity at the DOR were also evaluated for their inhibitory activity toward CYP2D6 and the hERG K^+ channel. The *N*,*N*-diethylcarboxamide moiety of **5** was previously shown to be a key element for potent affinity at the DOR.[46] On the basis of this result, the SAR at the amide position of **5** was explored further. As indicated in Table 1, conversion of the *N*,*N*-diethylbenzamide group of **5** to an *N*-ethylbenzamide moiety (compound **6**) resulted in only a slight decrease (3-fold) in DOR binding affinity. However, this subtle structural modification led to a 47-fold increase in the CYP2D6 inhibitory activity. Replacement of the two ethyl groups of **5** with hydrogen atoms (compound **7**) was accompanied by a ~10-fold reduction in the binding affinity toward DOR. Similarly, the methyl and isobutyl carboxamide derivatives **8** and **9** displayed significantly weaker DOR binding affinity than **5**. The *N*,*N*-dimethylcarboxamide derivative **11**, despite its reduced DOR affinity when compared to **5**, displayed an improved hERG profile (IC_{50} = 23070 nM). However, the CYP2D6 inhibitory profile of **11** was still less than desirable (IC_{50} = 2800 nM). Cyclization of the ethyl substituent of **5** into pyrrolidino-, piperidino-, or morpholino-groups (compounds **12**−**14**, respectively) resulted in reduced binding affinities at the DOR. Comparison of the DOR affinity of **5** (K_i = 1.8 nM) and its constrained analogue **17**

Scheme 1. Synthesis of **5**, **18**−**21**[a]



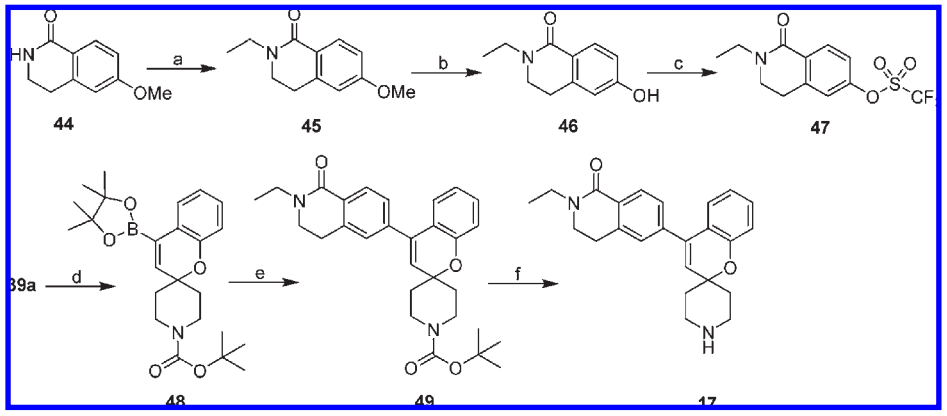
[a] Reagents and conditions: (a) 2-hydroxyacetophenone, pyrrolidine, MeOH; (b) $C_6H_5N(SO_2CF_3)_2$, LiHMDS, THF; (c) 4-(*N*,*N*-diethylaminocarbonyl)phenylboronic acid, $Pd[P(C_6H_5)_3)_4$, LiCl, aq Na_2CO_3, DME; (d) anhyd HCl, $(C_2H_5)_2O$, CH_2Cl_2.

Scheme 2. Synthesis of **6**−**16**[a]



[a] Reagents and conditions: (a) 4-(methoxycarbonyl)phenylboronic acid, $Pd[P(C_6H_5)_3]_4$, LiCl, aq Na_2CO_3, DME; (b) LiOH, THF, H_2O; (c) R^1R^2NH, TBTU, iPr_2EtN, CH_3CN; (d) anhyd HCl, $(C_2H_5)_2O$, MeOH.

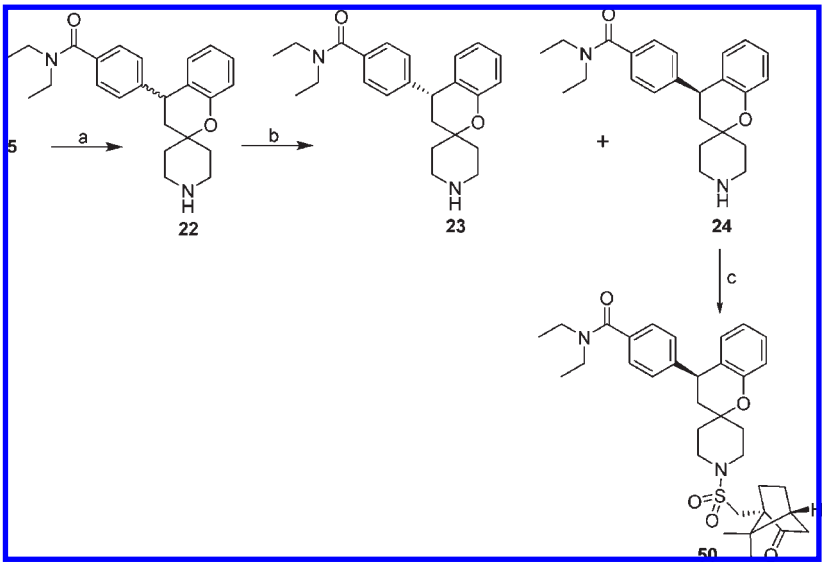
Scheme 3. Synthesis of **17**[a]



[a] Reagents and conditions: (a) NaH, C_2H_5I, THF; (b) BBr_3, CH_2Cl_2; (c) $(CF_3SO_2)O$, pyridine, CH_2Cl_2; (d) bis(pinacolato)diborane, Pd-(Cl_2)dppf·CH_2Cl_2, KOAc, CH_2Cl_2, DMF; (e) **47**, $Pd(Cl_2)$dppf·CH_2Cl_2, KOAc, DMF; (f) anhyd HCl, $(C_2H_5)_2O$, CH_2Cl_2.

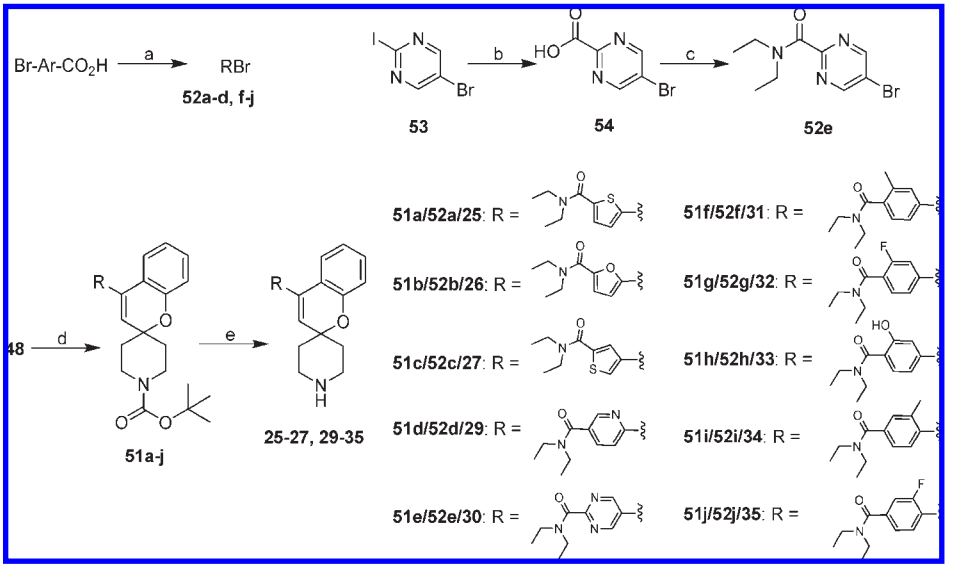
(K_i = 8.1 nM) also indicated that the free rotation of the amide bond is necessary for optimal binding affinity at the DOR. Among the series of *N*,*N*-disubstituted derivatives, the isoindolin-2-yl carboxamide **15** showed the highest affinity at the DOR (K_i = 0.53 nM). However, compound **15** did not meet in vitro advancement criteria for hERG and CYP2D6 inhibitory activity (hERG: IC_{50} = 3501 nM; CYP2D6: IC_{50} = 1800 nM). We next investigated the SAR at the piperidine moiety of **5**. As shown in Table 2, the azepane derivative **18** bound to the DOR with the same affinity as **5**. However, despite its improved hERG profile when compared to **5**, compound **18** still suffered from moderate inhibitory activity at CYP2D6 (IC_{50} = 2600 nM). Replacement of the 4,4′-disubstituted piperidine template of **5** with a 3,3′-disubstituted pyrrolidine (compound **19**) or a 3,3′-disubstituted piperidine (compound **20**) moieties resulted in a significant decrease (10-fold and 30-fold, respectively) in the binding affinity toward the DOR. We previously reported that the NH functionality of **5**,

Scheme 4. Synthesis of **22**−**24**[a]



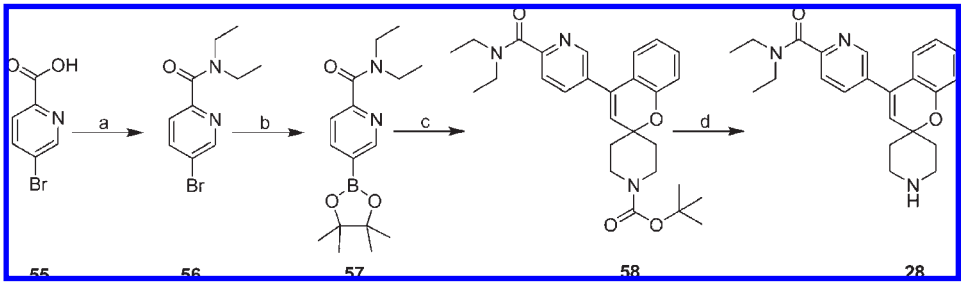
[a] Reagents and conditions: (a) $Pd(OH)_2$, H_2, MeOH; (b) chiral separation; (c) (1*S*,4*R*)-7,7-dimethly1−2-oxobicyclo[2,2,1]heptan-1-yl)methanesulfonyl chloride, Et_3N_3, CH_2Cl_2.

Scheme 5. Synthesis of **25**−**27** and **29**−**35**[a]



[a] Reagents and conditions: (a) Et_2NH, TBTU, iPr_2EtN, CH_3CN; (b) (i) nBuLi, CO_2, hexane, toluene, (ii) AcOH; (c) (i) ClCOCOCl, CH_2Cl_2, (ii) Et_2-NH, THF; (d) RBr (**52a**−**j**), $Pd[P(C_6H_5)_3]_4$, LiCl, Na_2CO_3, DME; (e) anhyd HCl, $(C_2H_5)_2O$, CH_2Cl_2.

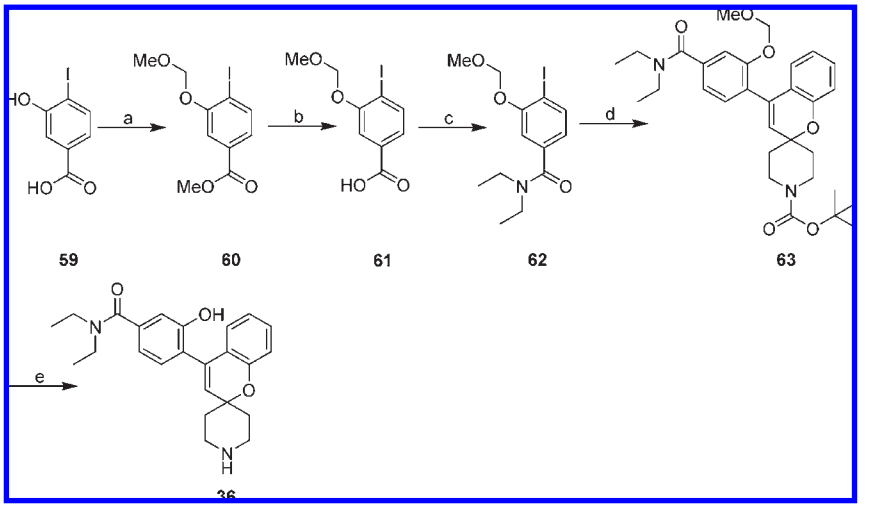
Scheme 6. Synthesis of **28**[a]



[a] Reagents and conditions: (a) (i) ClCOCOCl, CH_2Cl_2, (ii) Et_2NH, Et_3N, CH_2Cl_2; (b) bis(pinacolato)diborane, $Pd(Cl_2)$dppf·CH_2Cl_2, KOAc, DMF; (c) **39a**, 10% Pd/C, LiCl, Na_2CO_3, DME; (d) anhyd HCl, dioxane, CH_2Cl_2

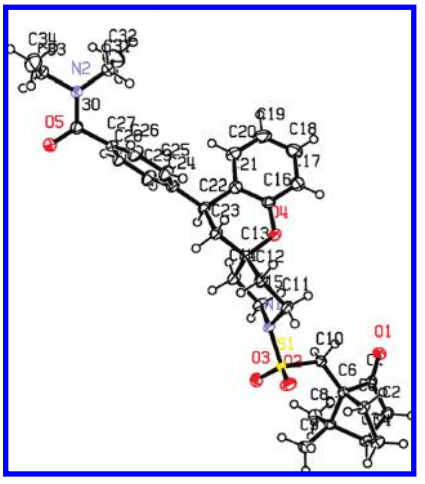
protonated at physiological pH, is of crucial importance for optimal affinity at the DOR.[46] This positively charged nitrogen is presumably involved in electrostatic interaction with an anionic residue of the DOR. On the basis of this hypothesis,

Scheme 7. Synthesis of **36**[a]



[a] Reagents and conditions: (a) (i) MeOH, CH_3COCl, (ii) CH_3OCH_2Cl, iPr_2EtN, CH_2Cl_2; (b) LiOH, acetone, THF, H_2O; (c) Et_2NH, iPr_2EtN, TBTU, CH_3CN; (d) **48**, 10% Pd/C, LiCl, Na_2CO_3, DME; (e) anhyd HCl, dioxane, MeOH.

Chart 2. X-ray Structure of **50** Showing Labeling of the Non-hydrogen Atoms



it was not too surprising to note that the replacement of the piperidine nitrogen of **5** with an oxygen atom, as in **21**, led to a dramatic decrease (770-fold) in the DOR binding affinity. As shown in Table 3, the spiro[chroman-2,4′-piperidine] derivative **22**, saturated analogue of **5**, displayed potent affinity and agonist activity at the DOR (K_i = 1.6 nM; EC_{50} = 21 nM). Because **22** is racemic, the synthesis of enantiomers **23** and **24** was undertaken to see if additional potency at the DOR and selectivity versus hERG and CYP2D6 were to be gained. As indicated in Table 3, the *R*-isomer **23** was superior to the S-isomer **24** in terms of binding affinity and agonist potency at the DOR. Unfortunately, the *R*-isomer **23** was also a more potent hERG inhibitor than the *S*-isomer **24** and the racemic **22**. On the basis of its unfavorable hERG profile, compound **23** was not profiled further. We then studied the effect of replacing the phenyl group of the *N*,*N*-diethylbenzamide moiety of **5**, with various heterocyclic systems (compounds **25**−**30**, Table 4). In particular, the medicinal chemistry strategy consisted in reducing the overall lipophilicity of the spirocyclic derivatives in an attempt to decrease simultaneously hERG and CYP2D6 inhibitory activities while maintaining good affinity and selectivity for the DOR. As indicated in Table 4, the thiophene derivative **25** was identified as a potent and selective DOR agonist. However, **25** displayed increased hERG and CYP2D6 inhibitory activities when compared to **5**. Replacement of the thiophene group of **25** with a furan moiety (compound **26**) led to a 13-fold decrease in the binding affinity toward the DOR. Substitution of the pendant phenyl ring of **5** with a pyridine template (compound **28**) resulted in a significant reduction (5- to 7-fold) in the hERG and CYP2D6 inhibitory activities, attributed to a decrease in the overall lipophilicity (**5**: clogP = 3.15; **28**: clogP = 2.05). Importantly, **28** retained potent affinity (K_i = 2.5 nM) and high selectivity for the DOR. The pyridine derivative **29**, a regioisomeric analogue of **28**, also displayed weak inhibitory activity at hERG and CYP2D6 while maintaining good DOR affinity and selectivity. The favorable in vitro profile of compound **30** also demonstrated that the *N*,*N*-diethylpyrimidine-2-carboxamide was an effective replacement of the *N*,*N*-diethylbenzamide group. The effect of substitution of the pendant phenyl ring of **5** was also studied (compounds **31**−**36**). As illustrated in Table 4, substitution of the phenyl ring of the *N*,*N*-diethylbenzamide moiety of **5** with a polar hydroxyl group (compounds **33** and **36**) was beneficial to decrease the hERG and CYP2D6 inhibitory activities while maintaining potent affinity at the DOR and good selectivity for δ versus μ and κ. As anticipated, replacement of the hydroxyl group of **33** and **36** with more lipophilic methyl or fluorine substituent (compounds **31**, **32**, **34**, **35**) led to a significant increase in the hERG inhibitory activity. Having thoroughly investigated the in vitro SAR of this spirocyclic class of DOR agonists, several compounds were evaluated for analgesic action in vivo. In particular, compounds **28** and **36** were tested in the rat Freund's Complete Adjuvant (FCA) mechanical hyperalgesia assay used as a model of inflammatory pain.[53,54] At the screening dose of 3 mg/kg po, compound **28** produced only 11% reversal of hyperalgesia in the inflamed paw (the paw pressure threshold of the inflamed paw returned to that of the uninflamed paw). In contrast, under the same assay conditions, compound **36** when dosed orally at 3 mg/kg produced 148% reversal of hyperalgesia in the inflamed paw. The reason for the difference of in vivo efficacy of compounds **28** and **36** is unknown. The oral potency of **36** in the FCA assay (ED_{50} = 0.03 mg/kg) was greater than that of **4** (ED_{50} = 1.4 mg/kg)[46] (Figure 1). The level of

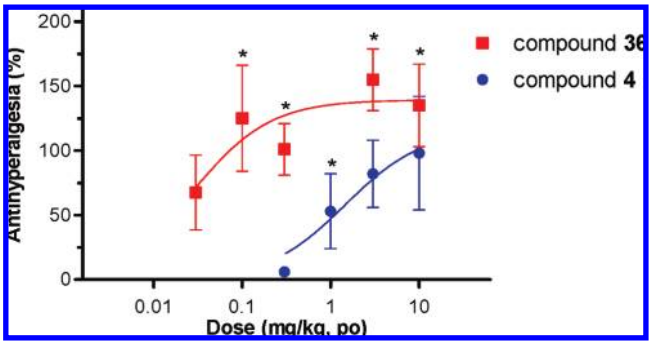


Figure 1. Dose response effect of the antihyperalgesic activity produced by **4** and **36** in the rat FCA assay. Values on the graph represent the mean and standard error of the mean (SEM) using $n = 8-24$ (*: significantly different from appropriate vehicle group, $p < 0.05$).

antihyperalgesia observed following 3 mg/kg po of **36** was similar to that produced by 3 mg/kg po of **4** and 30 mg/kg sc of the nonselective cyclooxygenase inhibitor indomethacin (Figure 2). The antihyperalgesic efficacy of **36** was less than that of morphine (Figure 2), which increased paw pressure thresholds in both the inflamed and the uninflamed paw. Additional studies showed that the antihyperalgesia produced by 3 mg/kg po of **36** was prevented by sc pretreatment with 0.3 mg/kg of the DOR antagonist naltrindole (Figure 3), thus demonstrating that the antihyperalgesic activity of **36** is mediated through activation of DORs.

Compound **36** was also evaluated across a broad in vitro selectivity panel that included adrenergic, muscarinic, and nicotinic cholinergic, dopaminergic, peptidergic, and serotonergic receptors, various ion channels, and enzymes. These studies demonstrated that **36** was a selective DOR agonist as it did not inhibit ligand binding to over 100 nonopioid receptors, channels, and enzymes at a concentration of 10 μM.

The pharmacokinetics of **36** after iv and po administration to male rats are summarized in Table 5. Similar to the rat pharmacokinetics of **4**, **36** is a high clearance compound with a large volume of distribution. The elimination of **36** was moderate with a half-life of 5.3 h after a single iv dose of 2.5 mg/kg. Following iv administration, the systemic clearance of **36** approximated rat hepatic blood flow, i.e., 3.3 L/h/kg.[55] After a 10 mg/kg po dose, maximum plasma concentrations were reached within 1.3 h postdose. The oral bioavailability of **36** in rat was 37.6%. Table 5 also summarizes the pharmacokinetics of **36** in male beagle dogs after single iv and po doses of 1 and 3 mg/kg, respectively.

Unlike the moderate half-life elimination of **4** (5.1 h), the half-life of **36** in dog was long, 12.2 h, after a 1 mg/kg iv dose. After a single 3 mg/kg oral dose (solution gavage) of **36**, maximum plasma concentrations were reached between 15 and 30 min postdose and ranged from 808 to 900 ng/mL. The volume of distribution, 5.1 L/kg, was greater than total body water (0.6 L/kg in dog),[55] indicating tissue binding. Compared with hepatic blood flow, approximately 1.9 L/h/kg in dog,[55] the systemic plasma clearance was low, i.e., 0.38 L/h/kg. The oral bioavailability of **36** in dog was 54.5%.

The plasma protein binding of **36** was species-dependent, with the least degree of binding in human plasma. The overall mean free fraction in rat, dog, and human plasma was 21.5%, 28.9%, and 36.5%, respectively. Compound **36** was moderately metabolized by rat and dog hepatic microsomes, with

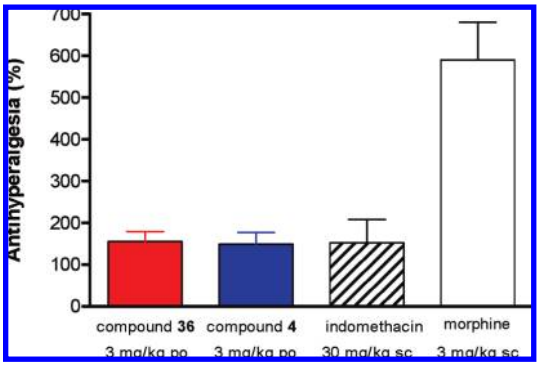


Figure 2. Antihyperalgesia produced by **4**, **36**, indomethacin, and morphine in the rat FCA assay. Values on the chart represent the mean and standard error of the mean (SEM) using $n = 8-11$.

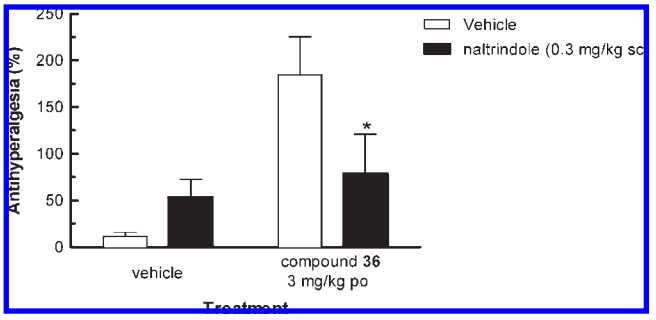


Figure 3. Antagonism by naltrindole of the antihyperalgesia produced by 3 mg/kg po of compound **36** in the rat FCA assay. Values on the graph represent the mean and standard error of the mean (SEM) using $n = 15-16$ (*: significantly different from vehicle−**36** treatment group, $p < 0.05$).

Table 5. Pharmacokinetics of **36** in Male Sprague−Dawley Rats and Male Beagle Dogs after iv and po Administration[a]

	iv		po	
pharmacokinetic species	rat	dog	rat	dog
dose (mg/kg)	2.5	1.0	10	3.0
CLs (L/h/kg)	3.3 ± 0.5	0.38 ± 0.06		
V_{dss} (L/kg)	18.1 ± 3.7	5.1 ± 0.8		
$t_{1/2}$ (h)[b]	5.3 ± 0.1	12.2 ± 0.4	4.9 ± 0.2	7.7 ± 0.8
$AUC_{0-\infty}$ (ng·h/mL)	780 ± 125	2,637 ± 426	1152 ± 84	4203 ± 346
T_{max} (h)			1.3 ± 0.6	0.4 ± 0.1
C_{max} (ng/mL)			246 ± 55	852 ± 46
F (%)			37.6 ± 2.7	54.5 ± 13.0

[a] Values represent the mean ± standard deviation of 3 animals. [b] Expressed as harmonic mean.

59.7% and 63.9% remaining after a 30 min incubation, respectively. Little metabolism was observed in human liver microsomes (94.5% remaining after 30 min). Compound **36** (1 μM) was metabolized in vitro by human P450 isozymes CYP3A4, CYP2C19, and CYP2D6, with 22.5, 78.4, and 63.2% remaining after a 1 h incubation, respectively. Compound **36** showed very little inhibitory activity toward drug-metabolizing CYP enzymes (CYP1A2: 0% inhibition at 10 μM; CYP2C19: 12.8% inhibition at 10 μM; CYP2C9: 11.4% inhibition at 10 μM; CYP2D6: 12.5% inhibition at 10 μM; CYP3A4: 18% inhibition at 10 μM). Additional experiments were conducted to study the disposition of **36** in plasma and brain microdialysates of freely moving rats after

oral dosing. The concentrations of **36** were determined in plasma of rats for approximately 6 h following a 30 mg/kg po dose of **36**. The corresponding concentrations in the brain were determined by analysis of microdialysates from probes placed into the raphe magnus. The area under the curve (AUC) of compound **36** (total concentration) in plasma was 4611 ng·h/mL. On the basis of the plasma protein binding of **36** determined in vitro (see above), the AUC of compound **36** (free concentration) in plasma was calculated to be 990 ng·h/mL. The AUC of **36** in the raphe magnus was 105 ng·h/mL, which indicates that **36** was able to penetrate the CNS. The levels of **36** in the CNS were approximately 10% of the corresponding free concentration of drug in plasma.

Compound **36** was also evaluated in vitro for the potential to inhibit four other major cardiac ion channels related to potassium, sodium, and calcium current generation [I_{to}(rKv4.3), hKvLQT1/h min K, hHNa, and $I_{Ca,L}$]. The studies were conducted on human embryonic kidney (HEK) cells transfected with cDNAs for the ion channels of interest. Compound **36** showed relatively little inhibition of these ion channel currents. At 100 μM, the following average inhibition was observed: hKvLQT1/h min K, 30.5%; hHNa, 10.2%; $I_{Ca,L}$, 29.5%; I_{to} (rKv4.3), 10.3%. A bacterial mutagenicity assay (Ames study) conducted with **36** indicated that this compound was not likely to be mutagenic. A chromosomal aberration study was also performed in human peripheral blood lymphocytes. In this study, it was concluded that **36** was negative for the induction of structural and numerical chromosome aberrations. To determine whether compound **36** produced sedation or behavioral activation in rats, the effect of oral administration of 30, 100, or 300 mg/kg po of **36** on the spontaneous locomotor activity of rats was assessed in locomotor activity chambers. No significant effect on horizontal or vertical activity was observed after any dose (data not shown). In contrast, sc administration of 1 mg/kg of **2** significantly increased horizontal and vertical locomotor activity. The effects of **36** on EEG were investigated following iv administration. In a telemetered rat preparation, **36** failed to produce seizures or preseizure waveforms following bolus administration of 10 and 30 mg/kg. No overt side effects of compound **36** were identified. These data highlight a vastly improved CNS safety margin for compound **36** compared with **2**.

Conclusion

In summary, further SAR exploration in this novel series of DOR agonists led to the discovery of **36**, a potent and selective DOR agonist displaying a preclinical profile appropriate for clinical evaluation. Increasing the polarity of the lead compound **5** was beneficial to further decrease the hERG and CYP2D6 liabilities while keeping potent affinity and selectivity for the DOR. Compound **36** demonstrated robust and potent activity in a rodent assay of inflammatory pain following oral administration. Indeed, **36** was approximately 50-fold more potent than **4** in the rat FCA assay of inflammatory pain after oral dosing. Moreover, preclinical studies suggested that **36** shared the wide safety margin observed previously for **4**. Compound **36** has been advanced to phase I safety evaluation conducted in normal healthy volunteers.

Experimental Section

1. Chemistry. General. All chemicals were reagent grade and used without further purification. Thin-layer chromatography (TLC) was performed on silica gel 6F glass-backed plates (250 μm) from Analtech and visualized by UV 254 irradiation and iodine. Flash chromatography was conducted using the ISCO CombiFlash with RediSep silica gel cartridges (4 g, 12 g, 40 g, 120 g). Chromatographic elution solvent systems are reported as volume:volume ratios. All ^{1}H NMR spectra were recorded at ambient temperature on a Bruker 400 MHz spectrometer. They are reported in ppm on the δ scale from TMS. LC-MS data were obtained using a Thermo-Finnigan Surveyor HPLC and a Thermo-Finnigan AQA MS using either positive or negative electrospray ionization. Program (positive): solvent A, 10 mM ammonium acetate, pH 4.5, 1% acetonitrile; solvent B, acetonitrile; column: Michrom Bioresources Magic C18 Macro Bullet, detector (PDA λ = 220−300 nm; gradient: 96%A−100%B in 3.2 min, hold 100%B for 0.4 min). Program (negative): solvent A, 1 mM ammonium acetate, pH 4.5, 1% acetonitrile; solvent B, acetonitrile; column, Michrom Bioresources Magic C18 Macro Bullet, detector (PDA λ = 220−300 nm; gradient: 96%A−100%B in 3.2 min, hold 100% B for 0.4 min). Elemental analyses were performed by Atlantic Microlabs, Norcross, GA and are within ±0.4% of theoretical values.

***N*,*N*-Diethyl-4-(spiro[chromene-2,4′-piperidine]-4-yl)benzamide (5).** A 2 N solution of hydrochloric acid in diethyl ether (34.6 mL, 69.24 mmol, 5.5 equiv) was added dropwise to a cooled (0 °C) solution of **40a** (6.00 g, 12.59 mmol, 1.0 equiv) in anhydrous dichloromethane (70 mL). The mixture was warmed to room temperature, and stirring was continued for an additional 10 h. Diethyl ether (100 mL) was added to the solution, and the resulting precipitate was collected by filtration and washed with diethyl ether. Yield: 99%. ^{1}H NMR (DMSO-d_6) δ 9.06 (m, 2H), 7.43 (s, 4H), 7.27 (t, 1H), 7.00 (m, 3H), 5.95 (s, 1H), 3.45 (m, 2H), 3.23 (m, 6H), 2.00 (m, 4H), 1.12 (m, 6H). LCMS (ESI): m/z 377.4 (M + H$^+$). HPLC purity: 100%. Anal. ($C_{24}H_{28}N_2O_2\cdot$1HCl) C, H, N.

***N*-Ethyl-4-(spiro[chromene-2,4′-piperidine]-4-yl)benzamide (6).** Compound **6** was synthesized in a similar manner to **7** using ethylamine in step c and **43a** in step d. ^{1}H NMR (DMSO-d_6) δ 8.50 (m, 1H), 7.90 (d, 2H), 7.40 (d, 2H), 7.20 (m, 1H), 6.90 (m, 3H), 5.85 (s, 1H), 3.30 (m, 2H), 2.90 (m, 2H), 2.70 (m, 2H), 1.85−1.70 (m, 4H), 1.10 (t, 3H). LCMS (ESI): m/z 349.2 (M + H$^+$). HPLC purity: 99.8%. Anal. ($C_{22}H_{24}N_2O_2\cdot$1HCl$\cdot^1/_4H_2O$) C, H, N.

4-(Spiro[chromene-2,4′-piperidine]-4-yl)benzamide (7). A 2 N solution of hydrochloric acid in diethyl ether (0.12 mL, 0.24 mmol, 5.5 equiv) was added dropwise to a cooled (0 °C) solution of **43b** (18 mg, 0.04 mmol, 1.0 equiv) in anhydrous methanol (5 mL). The mixture was stirred overnight at room temperature and then concentrated under reduced pressure. The crude product was triturated with ethyl acetate. The resulting precipitate was collected by filtration. Yield: 70%. ^{1}H NMR (DMSO-d_6) δ 8.99 (m, 2H), 8.06 (m, 1H), 7.95 (m, 2H), 7.46 (m, 3H), 7.27 (m, 1H), 7.06 (m, 1H), 6.96 (m, 2H), 5.95 (s, 1H), 3.24 (m, 4H), 2.08 (m, 4H). LCMS (ESI): m/z 321.1 (M + H$^+$). HPLC purity: 95.6%.

***N*-Methyl-4-(spiro[chromene-2,4′-piperidine]-4-yl)benzamide (8).** Compound **8** was synthesized in a similar manner to **7** using methylamine in step c and **43c** in step d. (DMSO-d_6) δ 9.05 (m, 2H), 8.55 (m, 1H), 7.92 (m, 2H), 7.41 (m, 2H), 7.26 (m, 1H), 7.06 (m, 1H), 6.95 (m, 2H), 5.95 (s, 1H), 3.20 (m, 4H), 2.81 (m, 3H), 2.08 (m, 4H). LCMS (ESI): m/z 335.2 (M + H$^+$). HPLC purity: 96.4%.

***N*-Isobutyl-4-(spiro[chromene-2,4′-piperidine]-4-yl)benzamide (9).** Compound **9** was synthesized in a similar manner to **7** using isobutylamine in step c and **43d** in step d. ^{1}H NMR ($CDCl_3$) δ 9.75 (brs, 1H), 9.31 (brs, 1H), 7.81 (d, 2H), 7.39 (d, 2H), 7.21 (m, 1H), 6.98 (m, 2H), 6.90 (m, 1H), 6.25 (m, 1H), 5.56 (s, 1H), 3.46 (m, 2H), 3.33 (m, 4H), 2.30 (m, 2H), 2.12 (m, 2H), 1.94 (m, 1H), 1.04 (d, 6H). LCMS (ESI): m/z 377.2 (M + H$^+$). HPLC purity: 99.5%.

***N,N*-Diisopropyl-4-(spiro[chromene-2,4′-piperidine]-4-yl)benzamide (10).** Compound **10** was synthesized in a similar manner to **7** using *N,N*-diisopropylamine in step c and **43e** in step d. ^{1}H NMR (DMSO-d_6) δ 8.98 (m, 2H), 7.39 (dd, 4H), 7.24 (m, 1H), 6.95 (m, 3H), 5.91 (s, 1H), 3.66 (brs, 2H), 3.22 (m, 4H), 2.10 (m, 4H), 1.30 (m, 12H). LCMS (ESI): m/z 405.3 (M + H$^+$). HPLC purity: 99%. Anal. ($C_{26}H_{32}N_2O_2 \cdot 1HCl \cdot {}^1/_2H_2O$) C, H, N.

***N,N*-Dimethyl-4-(spiro[chromene-2,4′-piperidine]-4-yl)benzamide (11).** Compound **11** was synthesized in a similar manner to **7** using *N,N*-dimethylamine in step c and **43f** in step d. ^{1}H NMR (DMSO-d_6) δ 9.08 (m, 2H), 7.42 (m, 4H), 7.24 (m, 1H), 7.00 (m, 3H), 5.91 (s, 1H), 3.25 (m, 4H), 2.96 (m, 6H), 2.07 (m, 4H). LCMS (ESI): m/z 349.1 (M + H$^+$). HPLC purity: 98.3%.

Pyrrolidin-1-yl(4-(spiro[chromene-2,4′-piperidine]-4-yl)phenyl)methanone (12). Compound **12** was synthesized in a similar manner to **7** using pyrrolidine in step c and **43g** in step d. ^{1}H NMR (DMSO-d_6) δ 8.91 (m, 2H), 7.58 (d, 2H), 7.41 (d, 2H), 7.25 (m, 1H), 7.00 (m, 3H), 5.92 (s, 1H), 3.49 (m, 2H), 3.41 (m, 2H), 3.24 (m, 4H), 2.09 (m, 2H), 2.00 (m, 2H), 1.84 (m, 4H). LCMS (ESI): m/z 375.1 (M + H$^+$). HPLC purity: 95.3%.

Piperidin-1-yl(4-(spiro[chromene-2,4′-piperidine]-4-yl)phenyl)methanone (13). Compound **13** was synthesized in a similar manner to **7** using piperidine in step c and **43h** in step d. ^{1}H NMR (DMSO-d_6) δ 8.90 (m, 2H), 7.44 (m, 4H), 7.26 (m, 1H), 7.00 (m, 3H), 5.91 (s, 1H), 3.59 (m, 2H), 3.21 (m, 6H), 2.09 (m, 2H), 1.99 (m, 2H), 1.55 (m, 6H). LCMS (ESI): m/z 389.1 (M + H$^+$). HPLC purity: 96.9%.

Morpholino(4-(spiro[chromene-2,4′-piperidine]-4-yl)phenyl)methanone (14). Compound **14** was synthesized in a similar manner to **7** using morpholine in step c and **43i** in step d. ^{1}H NMR (DMSO-d_6) δ 8.91 (m, 2H), 7.46 (m, 4H), 7.26 (m, 1H), 7.01 (m, 3H), 5.94 (s, 1H), 3.61 (m, 6H), 3.35 (m, 2H), 3.21 (m, 4H), 2.09 (m, 2H), 1.98 (m, 2H). LCMS (ESI): m/z 391.1 (M+ H$^+$). HPLC purity: 98.0%.

Isoindolin-2-yl(4-(spiro[chromene-2,4′-piperidine]-4-yl)phenyl)methanone (15). Compound **15** was synthesized in a similar manner to **7** using isoindoline in step c and **43j** in step d. ^{1}H NMR (DMSO-d_6) δ 8.90 (m, 2H), 7.70 (d, 2H), 7.50 (d, 2H), 7.40 (m, 1H), 7.30 (m, 4H), 7.00 (m, 3H), 5.95 (s, 1H), 4.90 (s, 2H), 4.80 (s, 2H), 3.30 (brm, 4H), 2.05 (m, 4H). LCMS (ESI): m/z 423.1 (M + H$^+$). HPLC purity: 96.1%. Anal. ($C_{28}H_{26}N_2O_2 \cdot 1HCl \cdot 1H_2O$) C, H, N.

***N*-Benzyl-*N*-methyl-4-(spiro[chromene-2,4′-piperidine]-4-yl)-benzamide (16).** Compound **16** was synthesized in a similar manner to **7** using *N*-methyl-1-phenylmethanamine in step c and **43k** in step d. ^{1}H NMR (DMSO-d_6) δ 8.88 (m, 2H), 7.40 (brm, 10H), 7.00 (m, 3H), 5.94 (s, 1H), 4.70 (m, 1H), 4.52 (m, 1H), 3.21 (m, 4H), 2.88 (m, 3H), 2.02 (m, 4H). LCMS (ESI): m/z 425.2 (M + H$^+$). HPLC purity: 95.5%. Anal. ($C_{28}H_{28}N_2O_2 \cdot 1HCl \cdot {}^3/_5H_2O$) C, H, N.

2-Ethyl-6-(spiro[chromene-2,4′-piperidine]-4-yl)-3,4-dihydroisoquinolin-1(2*H*)-one (17). To a solution of **49** (0.150 g, 0.316 mmol, 1.0 equiv) in anhydrous methylene chloride (5 mL) at 0 °C under a nitrogen atmosphere was added a 1 N solution of anhydrous hydrochloric acid in diethyl ether (1.26 mL, 1.26 mmol, 4.0 equiv). The reaction was warmed to room temperature and stirred for 4 days at room temperature. Diethyl ether was added (5 mL), and the resulting precipitate was collected by filtration. Yield: 27%. ^{1}H NMR (DMSO-d_6) δ 8.80 (brs, 2H), 7.92 (d, 1H), 7.29 (m, 3H), 7.05 (d, 1H), 6.97 (m, 2H), 5.94 (s, 1H), 3.54 (m, 4H), 3.23 (brm, 4H), 3.00 (t, 2H), 2.08 (brm, 2H), 1.97 (brm, 2H), 1.13 (t, 3H). LCMS (ESI): m/z 375.3 (M + H$^+$). HPLC purity: 99.4%. Anal. ($C_{24}H_{26}N_2O_2 \cdot 1HCl \cdot 1H_2O$) C, H, N.

***N,N*-Diethyl-4-(spiro[azepane-4,2′-chromene]-4′-yl)benzamide (18).** Compound **18** was synthesized in a similar manner to **5** using **37b** in step a, **38b** in step b, **39b** in step c, and **40b** in step d. ^{1}H NMR ($CDCl_3$) δ 9.76 (m, 2H), 7.41 (m, 2H), 7.36 (m, 2H), 7.20 (m, 1H), 7.00 (dd, 1H), 6.97 (dd, 1H), 6.88 (m, 1H), 5.63 (s, 1H), 3.68−3.23 (m, 8H), 2.50−2.23 (m, 4H), 2.02−1.82 (m, 2H), 1.35−1.07 (m, 6H). LCMS (ESI): m/z 391.2 (M+H$^+$). HPLC purity: 99%. Anal. ($C_{25}H_{30}N_2O_2 \cdot 1HCl$) C, H, N.

***N,N*-Diethyl-4-(spiro[chromene-2,3′-pyrrolidine]-4-yl)benzamide (19).** Compound **19** was synthesized in a similar manner to **5** using **37c** in step a, **38c** in step b, **39c** in step c, and **40c** in step d. ^{1}H NMR ($CDCl_3$) δ 10.20 (m, 2H), 7.40 (m, 4H), 7.22 (m, 1H), 7.04 (m, 2H), 6.91 (m, 1H), 5.66 (s, 1H), 3.85−3.50 (m, 5H), 3.31 (m, 3H), 2.60 (m, 1H), 2.13 (m, 1H), 1.27 (m, 3H), 1.16 (m, 3H). LCMS (ESI): m/z 363.2 (M + H$^+$). HPLC purity: 99%.

***N,N*-Diethyl-4-(spiro[chromene-2,3′-piperidine]-4-yl)benzamide (20).** Compound **20** was synthesized in a similar manner to **5** using **37d** in step a, **38d** in step b, **39d** in step c, and **40d** in step d. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.33 (m, 1H), 9.21 (m, 1H), 7.39 (m, 5H), 7.21 (m, 1H), 6.98 (m, 1H), 6.87 (m, 1H), 5.50 (s, 1H), 3.55 (m, 4H), 3.34 (m, 2H), 2.93 (m, 2H), 2.44 (m, 1H), 2.33 (m, 1H), 1.83 (m, 1H), 1.70 (m, 1H), 1.26 (m, 3H), 1.16 (m, 3H). LCMS (ESI): m/z 377.0 (M + H$^+$). HPLC purity: 99%.

***N,N*-Diethyl-4-(2′,3′,5′,6′-tetrahydrospiro[chromene-2,4′-pyran]-4-yl)benzamide (21).** Compound **21** was synthesized in a similar manner to **40a** using **37e** in step a, **38e** in step b, and **39e** in step c. ^{1}H NMR (DMSO-d_6) δ 7.42 (d, 2H), 7.38 (d, 2H), 7.19 (m, 1H), 6.97 (m, 2H), 6.86 (m, 1H), 5.62 (s, 1H), 3.96 (m, 2H), 3.79 (m, 2H), 3.57 (brs, 2H), 3.32 (brs, 2H), 2.03 (d, 2H), 1.84 (m, 2H), 1.21 (brd, 6H). LCMS (ESI): m/z 378.2 (M + H$^+$). HPLC purity: 97.2%.

***N,N*-Diethyl-4-(spiro[chroman-2,4′-piperidine]-4-yl)benzamide (22).** A solution of **5** (0.66 g, 1.75 mmol, 1.0 equiv) in anhydrous methanol (13 mL) was hydrogenated at atmospheric pressure in the presence of palladium hydroxide (0.120 g, 0.09 mmol, 0.05 equiv) for 10 h. The mixture was then filtered through celite. The filtrate was concentrated and was hydrogenated at atmospheric pressure in the presence of palladium hydroxide (0.120 g) for an additional 10 h. The mixture was filtered through celite, and the filtrate was concentrated to dryness under reduced pressure. To a cold (0 °C) solution of the resulting oil in anhydrous dichloromethane was added dropwise a 2 N solution of anhydrous hydrochloric acid in diethyl ether (5 mL). The mixture was then stirred for 1 h at room temperature and concentrated under reduced pressure. Diethyl ether was added. The resulting precipitate was collected by filtration and washed with diethyl ether and ethyl acetate. Yield: 63%. ^{1}H NMR (DMSO-d_6) δ 9.15 (m, 2H), 7.30 (m, 4H), 7.10 (m, 1H), 6.90 (m, 1H), 6.75 (m, 1H), 6.60 (m, 1H), 4.20 (m, 1H), 3.40 (m, 3H), 3.20 (m, 4H), 3.00 (m, 1H), 2.15 (m, 1H), 1.95 (m, 5H), 1.05 (m, 6H). LCMS (ESI): m/z 379.1 (M + H$^+$). HPLC purity: 99.3%. Anal. ($C_{24}H_{30}N_2O_2 \cdot 1HCl \cdot {}^3/_4H_2O$) C, H, N.

(*S*)-*N,N*-Diethyl-4-(spiro[chroman-2,4′-piperidine]-4-yl)benzamide (23). The enantiomers derived from **22** (racemic mixture) (10 g, 24.10 mmol, 1.0 equiv) were separated by chiral HPLC:

Column: Chiralpak AD-H, 4.6 mm × 250 mm, 5 μ, Chiral Technologies PN no. 19325; column temperature: room temperature; detection: UV photo diode array, 200−300 nm, extract at 275 nm; injection volume: 40 μL of 2 mg/mL sample in EtOH/MeOH (80:20); flow: 1 mL/min; mobile phase: 85% solution A, 15% solution B; solution A: 0.1% diisopropylethylamine in hexane (HPLC grade); solution B: 80% ethanol, 20% methanol (both HPLC grade); run time: 25 min.; HPLC: Waters Alliance 2695 (system dwell volume is ∼350 μL); detector: Waters 996 (resolution: 4.8 nm, scan rate: 1 Hz). Yield: 40%. ^{1}H NMR (DMSO-d_6) δ 9.12 (m, 2H), 7.28 (m, 4H), 7.14 (m, 1H), 6.90 (d, 1H), 6.79 (m, 1H), 6.63 (d, 1H), 4.25 (m, 1H), 3.44 (m, 3H), 3.24 (m, 4H), 2.96 (m, 1H), 2.18 (m, 1H), 1.97 (m, 5H), 1.10 (m, 6H). LCMS (ESI): m/z 379.4 (M + H$^+$). HPLC purity: 99.1%. Anal. ($C_{24}H_{30}N_2O_2 \cdot 1HCl \cdot {}^1/_4H_2O$) C, H, N. Chiral HPLC method: t_R = 11.914 min. (*ee* = 100%). $[\alpha]_D^{25} = -63.59$ (c. 0.01, MeOH).

(*R*)-*N,N*-Diethyl-4-(spiro[chroman-2,4′-piperidine]-4-yl)benzamide (24). The enantiomers derived from **22** (racemic mixture) (10 g, 24.10 mmol, 1.0 equiv) were separated by chiral HPLC:

Column: Chiralpak AD-H, 4.6 mm × 250 mm, 5 μ, Chiral Technologies PN no. 19325; column temperature: room temperature; detection: UV photo diode array, 200−300 nm, extract at 275 nm; injection volume: 40 μL of 2 mg/mL sample in EtOH/MeOH (80:20); flow: 1 mL/min; mobile phase: 85% solution A, 15% solution B. Solution A: 0.1% diisopropylethylamine in hexane (HPLC grade); solution B: 80% ethanol, 20% methanol (both HPLC grade); run time: 25 min. HPLC: Waters Alliance 2695 (system dwell volume is ∼350 μL); detector: Waters 996 (resolution: 4.8 nm, scan rate: 1 Hz). Yield: 40%. ^{1}H NMR (DMSO-d_6) δ 9.10 (m, 2H), 7.28 (m, 4H), 7.14 (m, 1H), 6.90 (d, 1H), 6.80 (m, 1H), 6.63 (d, 1H), 4.25 (m, 1H), 3.42 (m, 3H), 3.24 (m, 4H), 2.97 (m, 1H), 2.20 (m, 1H), 1.97 (m, 5H), 1.10 (m, 6H). LCMS (ESI): m/z 379.4 (M + H$^+$). HPLC purity: 99.3%. Anal. ($C_{24}H_{30}N_2O_2 \cdot 1HCl \cdot {}^1/_4H_2O$) C, H, N. Chiral HPLC method: t_R = 8.64 min. (ee = 97%). $[\alpha]_D^{25}$ = +58.40 (c. 0.01, MeOH).

***N,N*-Diethyl-5-(spiro[chromene-2,4′-piperidine]-4-yl)thiophene-2-carboxamide (25).** Compound **25** was synthesized in a similar manner to **33** with the following exceptions: step a did not require heating. Step d used **52a** and the reaction was refluxed overnight; the crude product was washed with water instead of a 0.5 N aqueous solution of hydrochloric acid. Step e went to completion without being resubjected to anhydrous hydrochloric acid and the final product was isolated as the hydrochloride salt. ^{1}H NMR (DMSO-d_6) δ 9.07 (brs, 2H), 7.41 (d, 1H), 7.37 (d, 1H), 7.31 (t, 1H), 7.22 (d, 1H), 7.07 (d, 1H), 7.02 (t, 1H), 6.12 (s, 1H), 3.50 (brm, 4H), 3.21 (brm, 4H0, 2.03 (brm, 4H), 1.18 (brt, 6H). LCMS (ESI): m/z 383.3 (M + H$^+$). HPLC purity: 99%. Anal. ($C_{22}H_{26}N_2O_2S \cdot 1HCl$) C, H, N.

***N,N*-Diethyl-5-(spiro[chromene-2,4′-piperidine]-4-yl)furan-2-carboxamide (26).** Compound **26** was synthesized in a similar manner to **33** with the following exceptions: Step a did not require heating. Step d used **52b**, and the reaction was refluxed overnight; the crude product was washed with water instead of a 0.5 N aqueous solution of hydrochloric acid. Step e went to completion without being resubjected to anhydrous hydrochloric acid, and the product was isolated as the hydrochloride salt. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.05 (brs, 2H), 7.52 (d, 1H), 7.32 (t, 1H), 7.07 (brm, 3H), 6.91 (d, 1H), 6.26 (s, 1H), 3.50 (brs, 4H), 3.20 (brm, 4H), 2.05 (brm, 4H), 1.17 (brs, 6H). LCMS (ESI): m/z 367.3 (M + H$^+$). HPLC purity: 99%.

***N,N*-Diethyl-4-(spiro[chromene-2,4′-piperidine]-4-yl)thiophene-2-carboxamide (27).** Compound **27** was synthesized in a similar manner to **33** with the following exceptions: Step a did not require heating. Step d used **52c** and the reaction was refluxed overnight; the crude product was washed with water instead of a 0.5 N aqueous solution of hydrochloric acid. Step e went to completion without being resubjected to anhydrous hydrochloric acid, and the product was isolated as the hydrochloride salt. ^{1}H NMR (DMSO-d_6) δ 9.01 (brs, 2H), 7.80 (s, 1H), 7.41 (s, 1H), 7.27 (t, 1H), 7.19 (d, 1H), 7.04 (d, 1H), 6.99 (t, 1H), 6.04 (s, 1H), 3.48 (brm, 4H), 3.21 (brm, 4H), 2.02 (brm, 4H), 1.16 (brt, 6H). LCMS (ESI): m/z 383.4 (M + H$^+$). HPLC purity: 98.9%.

***N,N*-Diethyl-5-(spiro[chromene-2,4′-piperidine]-4-yl)picolinamide (28).** To a cold (0 °C) solution of **58** (2 g, 4.18 mmol, 1.0 equiv) in anhydrous dichloromethane (20 mL) was slowly added a 4.0 M solution of hydrogen chloride in dioxane (5.2 mL, 20.8 mmol, 5.0 equiv). The reaction mixture was stirred at room temperature for 10 h and then concentrated under reduced pressure. The resulting foamy solids were soaked in diethyl ether to give the fine powders, which were collected by filtration and washed sequentially with ethyl acetate and diethyl ether. Yield: 95%. ^{1}H NMR (DMSO-d_6) δ 8.99 (m, 2H), 8.60 (d, 1H), 7.90 (dd, 1H), 7.61 (d, 1H), 7.29 (m, 1H), 7.06 (d, 1H), 6.98 (m, 2H), 6.09 (s, 1H), 3.47 (q, 2H), 3.35−3.13 (m, 6H), 2.06 (m, 4H), 1.17 (t, 3H), 1.11 (t, 3H). LCMS (ESI): m/z 378.4 (M + H$^+$). HPLC purity: 99%. Anal. ($C_{23}H_{27}N_3O_2 \cdot 2HCl \cdot {}^1/_2H_2O$) C, H, N.

***N,N*-Diethyl-6-(spiro[chromene-2,4′-piperidine]-4-yl)nicotinamide (29).** Compound **29** was synthesized in a similar manner to **33** with the following exceptions: Step a did not require heating. Step d used **52d** and the reaction was refluxed overnight; the crude product was washed with water instead of a 0.5 N aqueous solution of hydrochloric acid. Step e went to completion without being resubjected to anhydrous hydrochloric acid and the product was isolated as the hydrochloride salt. ^{1}H NMR (DMSO-d_6) δ 8.94 (brm, 2H), 8.64 (s, 1H), 7.92 (dd, 1H), 7.65 (d, 1H), 7.29 (m, 2H), 7.05 (d, 1H), 6.96 (t, 1H), 6.22 (s, 1H), 3.48 (m, 2H), 3.24 (brm, 6H), 2.05 (brm, 4H), 1.14 (brd, 6H). LCMS (ESI): m/z 378.4 (M + H$^+$). HPLC purity: 99%. Anal. ($C_{23}H_{27}N_3O_2 \cdot 1HCl \cdot {}^4/_3H_2O$) C, H, N.

***N,N*-Diethyl-5-(spiro[chromene-2,4′-piperidine]-4-yl)pyrimidine-2-carboxamide (30).** Compound **30** was synthesized in a similar manner to **33** with the following exceptions: Step a was replaced with steps b and c. Step d used **52e** and the reaction was refluxed overnight; the crude product was washed with water instead of a 0.5 N aqueous solution of hydrochloric acid. Step e went to completion without being resubjected to anhydrous hydrochloric acid, and the product was isolated as the hydrochloride salt. ^{1}H NMR (DMSO-d_6) δ 8.81 (m, 2H), 7.18 (m, 1H), 6.92 (m, 2H), 6.85 (m, 1H), 6.06 (s, 0.8H), 6.04 (s, 0.2H), 3.41 (q, 2H), 3.06 (q, 2H), 2.86 (m, 2H), 2.76 (m, 2H), 1.73 (brm, 4H), 1.10 (t, 3H), 1.00 (t, 3H). LCMS (ESI): m/z 379.3 (M + H$^+$). HPLC purity: 99.1%.

***N,N*-Diethyl-2-methyl-4-(spiro[chromene-2,4′-piperidine]-4-yl)-benzamide (31).** Compound **31** was synthesized in a similar manner to **33** with the following exceptions: Step a did not require heating. Step d used **52f** and the reaction was refluxed overnight; the crude product was washed with water instead of a 0.5 N aqueous solution of hydrochloric acid. Step e went to completion without being resubjected to anhydrous hydrochloric acid and the product was isolated as the hydrochloride salt. ^{1}H NMR ($CDCl_3$) δ 9.76 (brs, 1H), 9.63 (brs, 1H), 7.20 (m, 4H), 7.05 (dd, 1H), 6.93 (m, 2H), 5.60 (s, 1H), 3.76 (brs, 2H), 3.42 (brm, 4H), 3.18 (q, 2H), 2.32 (s, 3H), 2.21 (brm, 4H), 1.28 (t, 3H), 1.08 (t, 3H). LCMS (ESI): m/z 391.0 (M + H$^+$). HPLC purity: 100%. Anal. ($C_{25}H_{30}N_2O_2 \cdot 1HCl$) C, H, N.

***N,N*-Diethyl-2-fluoro-4-(spiro[chromene-2,4′-piperidine]-4-yl)-benzamide (32).** Compound **32** was synthesized in a similar manner to **33** with the following exceptions: Step a did not require heating. Step d used **52g** and the reaction was refluxed overnight; the crude product was washed with water instead of a 0.5 N aqueous solution of hydrochloric acid. Step e went to completion without being resubjected to anhydrous hydrochloric acid and the product was isolated as the hydrochloride salt. ^{1}H NMR (DMSO-d_6) δ 8.85 (brs, 2H), 7.43 (t, 1H), 7.35 (d, 1H), 7.27 (m, 2H), 7.04 (m, 2H), 6.97 (m, 1H), 6.03 (s, 1H), 3.48 (q, 2H), 3.22 (brm, 6H), 2.04 (brm, 4H), 1.16 (t, 3H), 1.04 (t, 3H). LCMS (ESI): m/z 395.0 (M + H+). HPLC purity: 100%. Anal. ($C_{24}H_{27}FN_2O_2 \cdot 1HCl \cdot {}^1/_4H_2O$) C, H, N.

***N,N*-Diethyl-2-hydroxy-4-(spiro[chromene-2,4′-piperidine]-4-yl)benzamide (33).** To a solution of **51h** (0.20 g, 0.406 mmol, 1.0 equiv) in methylene chloride (2 mL) was added a 1 N solution of anhydrous hydrochloric acid in diethyl ether (10 mL, 10 mmol, 25 equiv). The mixture was stirred at room temperature for 16 h. The mixture was concentrated under reduced pressure and treated with diethyl ether. The resulting precipitate was collected by filtration. By LC/MS some starting material remained, so the precipitate was treated with an excess of a 4 N solution of anhydrous hydrochloric acid in dioxane. This mixture was stirred at room temperature for 16 h. The mixture was concentrated under reduced pressure and the crude product was purified by column chromatography (eluent: methylene chloride/methanol mixtures of increasing polarity). Yield: 66%. ^{1}H NMR (DMSO-d_6) δ 9.91 (brs, 1H), 9.08 (brs, 2H), 7.26 (m, 1H), 7.13 (d, 1H), 7.04 (m, 2H), 6.95 (m, 1H), 6.84 (m, 2H), 5.87 (s, 1H), 3.66 (brs, 4H), 3.20 (brm, 4H), 2.05 (brm, 4H), 1.08 (brd, 6H). LCMS (ESI): m/z 393.4 (M + H$^+$). HPLC purity: 99%. Anal. ($C_{24}H_{28}N_2O_3 \cdot 1HCl \cdot {}^3/_2H_2O$) C, H, N.

***N,N*-Diethyl-3-methyl-4-(spiro[chromene-2,4′-piperidine]-4-yl)-benzamide (34).** Compound **34** was synthesized in a similar manner to **33** with the following exceptions: Step a did not require heating. Step d used **52i** and the reaction was refluxed overnight;

the crude product was washed with water instead of a 0.5 N aqueous solution of hydrochloric acid. Step e went to completion without being resubjected to anhydrous hydrochloric acid and the product was isolated as the hydrochloride salt. ^{1}H NMR ($CDCl_3$) δ 9.78 (brs, 1H), 9.62 (brs, 1H), 7.22 (m, 3H), 7.13 (d, 1H), 6.92 (d, 1H), 6.84 (t, 1H), 6.63 (dd, 1H), 5.48 (s, 1H), 3.42 (brm, 8H), 2.36 (brm, 2H), 2.21 (m, 2H), 2.13 (s, 3H), 1.21 (brd, 6H). LCMS (ESI): m/z 391.0 ($M + H^+$). HPLC purity: 100%. Anal. ($C_{25}H_{30}N_2O_2 \cdot 1HCl$) C, H, N.

***N,N*-Diethyl-3-fluoro-4-(spiro[chromene-2,4′-piperidine]-4-yl)-benzamide (35).** Compound **35** was synthesized in a similar manner to **33** with the following exceptions: Step a did not require heating. Step d used **52j** and the reaction was refluxed overnight; the crude product was washed with water instead of a 0.5 N aqueous solution of hydrochloric acid. Step e went to completion without being resubjected to anhydrous hydrochloric acid and the product was isolated as the hydrochloride salt. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.92 (brs, 2H), 7.29 (m, 3H), 7.13 (s, 1H), 7.05 (d, 1H), 6.98 (m, 2H), 6.01 (s, 1H), 3.43 (brm, 2H), 3.23 (brm, 6H), 2.04 (brm, 4H), 1.10 (brd, 6H). LCMS (ESI): m/z 395.0 ($M + H^+$). HPLC purity: 99%. Anal. ($C_{24}H_{27}FN_2O_2 \cdot 1HCl \cdot {}^1/_4H_2O$) C, H, N.

***N,N*-Diethyl-3-hydroxy-4-(spiro[chromene-2,4′-piperidine]-4-yl)benzamide (36).** To a solution of **63** (0.647 g, 1.21 mmol, 1 equiv) in methanol (3 mL) was added an excess of a 4 N solution of anhydrous hydrochloric acid in dioxane (20 mL). The mixture was stirred at room temperature for 16 h. The mixture was concentrated under reduced pressure and treated with a mixture of methylene chloride (15 mL) and ethyl acetate (25 mL). The resulting precipitate was collected by filtration and dried under vacuum. Yield: 77%. ^{1}H NMR (DMSO-d_6) δ 9.75 (s, 1H), 8.84 (brm, 2H), 7.16 (m, 2H), 6.96 (d, 1H), 6.84 (m, 3H), 6.72 (d, 1H), 5.78 (s, 1H), 3.42 (brs, 2H), 3.22 (brs, 6H), 2.10 (brm, 2H), 1.96 (brm, 2H), 1.12 (brs, 6H). LCMS (ESI): m/z 393.3 ($M + H^+$). HPLC purity: 99.3%. Anal. ($C_{24}H_{28}N_2O_3 \cdot 1HCl \cdot {}^1/_4H_2O$) C, H, N.

***tert*-Butyl 4-Oxospiro[chroman-2,4′-piperidine]-1′-carboxylate (38a).** Pyrrolidine (42 mL, 0.5 mol, 2.0 equiv) was added dropwise at room temperature to a solution of **37a** (49.8 g, 0.249 mol, 1.0 equiv) and 2-hydroxyacetophenone (34 g, 0.249 mol, 1.0 equiv) in anhydrous methanol (400 mL). The solution was refluxed overnight and then concentrated under reduced pressure. Diethyl ether (500 mL) was added. The organic mixture was washed with a 1 N aqueous solution of hydrochloric acid, a 1 N aqueous solution of sodium hydroxide, brine, and dried over sodium sulfate. Hexane (300 mL) was added to the mixture. The resulting precipitate was collected by filtration, washed with hexane, and used for the next step without further purification. Yield: 72%. ^{1}H NMR ($CDCl_3$) δ 7.86 (d, 1H), 7.50 (t, 1H), 7.00 (m, 2H), 3.87 (m, 2H), 3.22 (m, 2H), 2.72 (s, 2H), 2.05 (d, 2H), 1.61 (m, 2H), 1.46 (s, 9H). LCMS (ESI): m/z 318.0 ($M + H^+$).

***tert*-Butyl 4-(Trifluoromethylsulfonyloxy)spiro[chromene-2,4′-piperidine]-1′-carboxylate (39a).** To a solution of **38a** (25 g, 0.078 mol, 1.0 equiv) in tetrahydrofuran (250 mL) at −78 °C under nitrogen was added dropwise a 1 N solution of lithium bis-(trimethylsilyl)amide in tetrahydrofuran (94.5 mL, 0.095 mol, 1.2 equiv). The mixture was stirred for 1 h at −78 °C. A solution of *N*-phenylbis(trifluoromethanesulfonamide) (33.8 g, 0.095 mol, 1.2 equiv) in tetrahydrofuran (150 mL) was added dropwise. The mixture was warmed slowly to room temperature, and stirring was continued for a further 12 h. The mixture was then poured into ice water, and the two phases were separated. The organic phase was washed with a 1 N aqueous solution of hydrochloric acid, a 1 N aqueous solution of sodium hydroxide, brine, and dried over sodium sulfate. The crude product was purified by column chromatography (eluent: hexane/ethyl acetate mixtures of increasing polarity). Yield: 70%. ^{1}H NMR (DMSO-d_6) δ 7.45−7.20 (m, 2H), 7.00 (m, 2H), 6.15 (s, 1H), 3.70 (m, 2H), 3.20 (m, 2H), 1.90 (m, 2H), 1.75 (m, 2H), 1.40 (s, 9H). LCMS (ESI): m/z 450.1 ($M + H^+$).

***tert*-Butyl 4-(4-(Diethylcarbamoyl)phenyl)spiro[chromene-2,4′-piperidine]-1′-carboxylate (40a).** To a solution of **39a** (15 g, 33.37 mmol, 1.0 equiv) in dimethoxyethane (100 mL) was added sequentially a 2 N aqueous solution of sodium carbonate (50.06 mL, 100.12 mmol, 3.0 equiv), lithium chloride (4.24 g, 100.12 mmol, 3.0 equiv), 4-(*N*,*N*-diethylaminocarbonyl)phenylboronic acid (8.12 g, 36.71 mmol, 1.1 equiv), and tetrakis-(triphenylphosphine)palladium(0) (0.77 g, 0.67 mmol, 0.02 equiv). The mixture was refluxed for 10 h under nitrogen. The mixture was then cooled to room temperature, and water (250 mL) was added. The mixture was extracted with ethyl acetate. The organic layer was further washed with brine and dried over sodium sulfate. The crude product was purified by column chromatography (eluent: hexane/ethyl acetate mixtures of increasing polarity). Yield: 73%. ^{1}H NMR ($CDCl_3$) δ 7.35 (m, 4H), 7.15 (t, 1H), 7.00−6.80 (m, 3H), 5.55 (s, 1H), 3.85 (m, 2H), 3.55 (m, 2H), 3.30 (m, 4H), 2.00 (m, 2H), 1.65 (m, 2H), 1.40 (s, 9H); 1.20 (m, 6H). LCMS (ESI): m/z 477.2 ($M + H^+$).

***tert*-Butyl 4-(4-(Methoxycarbonyl)phenyl)spiro[chromene-2,4′-piperidine]-1′-carboxylate (41).** To a solution of **39a** (7.80 g, 17.35 mmol, 1.0 equiv) in dimethoxyethane (75 mL) was added sequentially a 2 N aqueous solution of sodium carbonate (26.03 mL, 52.06 mmol, 3.0 equiv), lithium chloride (2.21 g, 52.06 mmol, 3.0 equiv), 4-(methoxycarbonyl)phenylboronic acid (3.44 g, 19.09 mmol, 1.1 equiv), and tetrakis(triphenylphosphine)palladium(0) (0.40 g, 0.35 mmol, 0.02 equiv). The mixture was refluxed overnight under nitrogen. The mixture was then cooled to room temperature, and water (250 mL) was added. The mixture was extracted with ethyl acetate. The organic layer was further washed with brine and dried over sodium sulfate. The crude product was purified by column chromatography (eluent: hexane/ethyl acetate mixtures of increasing polarity). Yield: 64%. ^{1}H NMR (DMSO-d_6) δ 8.02 (d, 2H), 7.49 (d, 2H), 7.23 (m, 1H), 6.99 (d, 1H), 6.92 (m, 2H), 5.92 (s, 1H), 3.88 (s, 3H), 3.70 (m, 2H), 3.27 (m, 2H), 1.89 (m, 2H), 1.71 (m, 2H), 1.42 (s, 9H). LCMS (ESI): m/z 436.0 ($M + H^+$).

4-(1′-(*tert*-Butoxycarbonyl)spiro[chromene-2,4′-piperidine]-4-yl)benzoic Acid (42). A solution of **41** (4.71 g, 10.81 mmol, 1.0 equiv) in tetrahydrofuran (30 mL) at 0 °C under nitrogen was added dropwise to a solution of lithium hydroxide monohydrate (0.54 g, 12.98 mmol, 1.2 equiv) in water (30 mL). The mixture was stirred overnight at room temperature. The mixture was then concentrated under reduced pressure. Water was added to the residue. The mixture was then acidified to pH 2 using concentrated hydrochloric acid. The resulting precipitate was collected by filtration, and the crude product was used for the next step without further purification. Yield: 98%. ^{1}H NMR (DMSO-d_6) δ 13.03 (brs, 1H), 8.01 (d, 2H), 7.47 (d, 2H), 7.23 (m, 1H), 6.98 (d, 1H), 6.92 (m, 2H), 5.91 (s, 1H), 3.70 (m, 2H), 3.28 (m, 2H), 1.86 (m, 2H), 1.72 (m, 2H), 1.42 (s, 9H). LCMS (ESI): m/z 420.1 ($M - H^-$).

***tert*-Butyl 4-(4-Carbamoylphenyl)spiro[chromene-2,4′-piperidine]-1′-carboxylate (43b).** *O*-Benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (150.8 mg, 0.47 mmol, 1.1 equiv) was added to a cooled (0 °C) solution of **42** (180.0 mg, 0.43 mmol, 1.0 equiv), ammonium chloride (50.3 mg, 0.94 mmol, 2.2 equiv), and *N*,*N*-diisopropylethylamine (0.25 mL, 0.94 mmol, 2.2 equiv) in acetonitrile (5 mL). The solution was stirred overnight at room temperature and then concentrated under reduced pressure. Ethyl acetate (10 mL) and a saturated aqueous solution of sodium bicarbonate (10 mL) were added to the crude product, and the mixture was stirred for 20 min at room temperature. The phases were separated and the organic layer was separated, washed with a saturated aqueous solution of sodium bicarbonate, brine, dried over sodium sulfate, and filtered. The organics were concentrated under reduced pressure, and the crude product was purified by column chromatography (eluent: hexane/ethyl acetate mixtures of increasing polarity). Yield: 10%. LCMS (ESI): m/z 421.2 ($M+H^+$).

2-Ethyl-6-methoxy-3,4-dihydroisoquinolin-1(2*H*)-one (45). To a suspension of NaH (0.81 g, 33.86 mmol, 6.0 equiv) in

tetrahydrofuran (30 mL) under a nitrogen atmosphere was added dropwise a solution of **44** (1.0 g, 5.64 mmol, 1.0 equiv) in tetrahydrofuran (15 mL). To this mixture was added dropwise ethyliodide (2.28 mL, 28.22 mmol, 5.0 equiv), and stirring was continued for 16 h at room temperature. A thick precipitate formed; therefore, additional amounts of tetrahydrofuran (15 mL) and ethyliodide (1.0 mL, 12.4 mmol, 2.2 equiv) were added and stirring was continued for an additional 24 h at room temperature. The reaction was quenched by addition of a 1 N aqueous solution of hydrochloric acid followed by ethyl acetate and water. The layers were separated. The organic phase was dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (eluent: hexane/ethyl acetate mixtures of increasing polarity). Yield: 83%. ^{1}H NMR ($CDCl_3$) δ 8.03 (d, 1H), 6.84 (dd, 1H), 6.65 (d, 1H), 3.84 (s, 3H), 3.61 (q, 2H), 3.53 (t, 2H), 2.95 (t, 2H), 1.21 (t, 3H). LCMS (ESI): m/z 206.1 ($M+H^+$).

2-Ethyl-6-hydroxy-3,4-dihydroisoquinolin-1(2*H*)-one (46). To a solution of **45** (0.96 g, 4.68 mmol, 1.0 equiv) in anhydrous methylene chloride (30 mL) at −78 °C under a nitrogen atmosphere was added dropwise a 1 N solution of boron tribromide in methylene chloride (9.35 mL, 9.35 mmol, 2.0 equiv). The reaction was warmed to room temperature, and stirring was continued for 16 h at room temperature. The mixture was cooled in an ice bath, quenched with methanol (10 mL), and concentrated under reduced pressure. The crude mixture was dissolved in ethyl acetate, and the solution was washed with a 1 N aqueous solution of hydrochloric acid and brine. The organic phase was dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude solid was triturated in ethyl acetate/hexane (1:1). The precipitate was collected by filtration. Yield: 74%. ^{1}H NMR ($CDCl_3$) δ 7.89 (d, 1H), 6.82 (dd, 1H), 6.68 (d, 1H), 3.63 (q, 2H), 3.54 (t, 2H), 2.91 (t, 2H), 1.22 (t, 3H). LCMS (ESI): m/z 192.1 ($M + H^+$).

2-Ethyl-1-oxo-1,2,3,4-tetrahydroisoquinolin-6-yl Trifluoromethanesulfonate (47). To a solution of **46** (0.38 g, 1.99 mmol, 1.0 equiv) and pyridine (0.32 mL, 3.98 mmol, 2.0 equiv) in methylene chloride (10 mL) at 0 °C under a nitrogen atmosphere was added trifluoromethanesulfonic anhydride (0.40 mL, 2.38 mmol, 1.2 equiv). The reaction was warmed to room temperature, and stirring was continued for 2 h at room temperature. Methylene chloride was added to the mixture, which was washed with a 1 N aqueous solution of hydrochloric acid and with a 1 N aqueous solution of sodium hydroxide. The organic phase was dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (eluent: hexane/ethyl acetate, 1:1). Yield: 45%.

^{1}H NMR ($CDCl_3$) δ 8.18 (d, 1H), 7.23 (dd, 1H), 7.11 (d, 1H), 3.62 (m, 4H), 3.04 (t, 2H), 1.23 (t, 3H). $CDCl_3$) δ 7.89 (d, 1H), 6.82 (dd, 1H), 6.68 (d, 1H), 3.63 (q, 2H), 3.54 (t, 2H), 2.91 (t, 2H), 1.22 (t, 3H). LCMS (ESI): m/z 324.1 ($M + H^+$).

***tert*-Butyl 4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)spiro-[chromene-2,4′-piperidine]-1′-carboxylate (48).** To a solution of bis(pinacolato)diboron (14.7 g, 57.8 mmol, 2.0 equiv) in *N*,*N*-dimethylformamide (200 mL) at room temperature under a nitrogen atmosphere was added 1,1′-bis(diphenylphosphino)ferrocene palladium(II) chloride complex with dichloromethane (710 mg, 0.867 mmol, 0.03 equiv) followed by addition of potassium acetate (8.58 g, 86.7 mmol, 3.0 equiv.) The mixture was heated to 80 °C, followed by dropwise addition of a solution of **39a** (13.0 g, 28.9 mmol, 1.0 equiv) in *N*,*N*-dimethylformamide (100 mL). After the addition was complete, the reaction mixture was heated at 80 °C for an additional 16 h. The solvent was evaporated under vacuum, and the residue was added to a 1 N aqueous solution of hydrochloric acid. The aqueous residue was extracted with ethyl acetate. The organic extracts were washed with brine, dried over sodium sulfate, filtered, and concentrated under reduced pressure to afford a brown semisolid. The crude product was purified by column chromatography (eluent: hexane/ethyl acetate mixtures of increasing polarity). Yield: 96%. ^{1}H NMR ($CDCl_3$) δ 7.71 (d, 1H), 7.11 (t, 1H), 6.90 (t, 1H), 6.83 (d, 1H), 6.28 (s, 1H), 3.84 (brs, 2H), 3.27 (brm, 2H), 1.96 (d, 2H), 1.60 (m, 2H), 1.34 (s, 9H), 1.26 (s, 12H). LCMS (ESI): m/z 428.0 ($M + H^+$).

***tert*-Butyl 4-(2-Ethyl-1-oxo-1,2,3,4-tetrahydroisoquinolin-6-yl)-spiro[chromene-2,4′-piperidine]-1′-carboxylate (49).** To a solution of **47** (0.100 g, 0.309 mmol, 1.0 equiv) in *N*,*N*-dimethylformamide (5 mL) under a nitrogen atmosphere was added **48** (0.145 g, 0.340 mmol, 1.1 equiv), potassium acetate (0.091 g, 0.928 mmol, 3.0 equiv) and 1,1′-bis(diphenylphosphino)ferrocene palladium(II)/dichloromethane complex (0.005 g, 0.006 mmol, 0.02 equiv). The reaction was stirred at 65 °C for 16 h. The mixture was cooled to room temperature. Water was added, and the mixture was extracted with ethyl acetate. The organic phase was dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (eluent: hexane/ethyl acetate mixtures of increasing polarity). Yield: 45%. ^{1}H NMR ($CDCl_3$) δ 8.11 (d, 1H), 7.31 (dd, 1H), 7.19 (m, 1H), 7.15 (s, 1H), 6.96 (m, 2H), 6.86 (m, 1H), 5.58 (s, 1H), 3.86 (brm, 2H), 3.65 (q, 2H), 3.59 (t, 2H), 3.34 (m, 2H), 3.01 (t, 2H), 2.05 (m, 2H), 1.67 (m, 2H), 1.48 (s, 9H), 1.26 (t, 3H). LCMS (ESI): m/z 475.3 ($M + H^+$).

4-((*S*)-1′-(((1*S*,4*R*)-7,7-Dimethyl-2-oxobicyclo[2.2.1]heptan-1-yl)-methylsulfonyl)spiro[chroman-2,4′-piperidine]-4-yl)-*N*,*N*-diethylbenzamide (50). ((1*S*,4*R*)-7,7-Dimethyl-2-oxobicyclo[2.2.1]heptan-1-yl)methanesulfonyl chloride (0.45 g, 1.78 mmol, 1.1 equiv) was added at 0 °C to a solution of **24** (0.67 g, 1.61 mmol, 1 equiv) and triethylamine (0.74 mL, 5.33 mmol, 3.3 equiv) in dichloromethane (6 mL). The reaction was warmed to room temperature and stirred overnight at room temperature. The mixture was washed with a saturated aqueous solution of sodium bicarbonate and brine, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (eluent: hexane/ethyl acetate mixtures of increasing polarity). Yield: 64%. ^{1}H NMR (DMSO-d_6) δ 7.30 (m, 4H), 7.11 (t, 1H), 6.90 (d, 1H), 6.77 (t, 1H), 6.61 (d, 1H), 4.23 (m, 1H), 3.39 (brm, 9H), 2.93 (d, 1H), 2.37 (m, 2H), 2.24 (m, 1H), 2.06 (m, 2H), 1.93 (m, 6H), 1.53 (m, 1H), 1.41 (m, 1H), 1.10 (m, 6H), 1.03 (s, 3H), 0.83 (s, 3H). LCMS (ESI): m/z 593.4 ($M + H^+$). Anal. ($C_{33}H_{44}N_2O_5S\cdot{}^1/_4H_2O$) C, H, N.

***tert*-Butyl 4-(4-(Diethylcarbamoyl)-3-hydroxyphenyl)spiro[chromene-2,4′-piperidine]-1′-carboxylate (51h).** To a solution of **52h** (0.30 g, 1.11 mmol, 1.0 equiv) in dimethoxyethane (10 mL) was added sequentially a 2 N aqueous solution of sodium carbonate (1.66 mL, 3.32 mmol, 3.0 equiv), lithium chloride (0.141 g, 3.32 mmol, 3.0 equiv), **48** (0.57 g, 1.33 mmol, 1.2 equiv), and tetrakis-(triphenylphosphine)palladium(0) (0.128 g, 0.11 mmol, 0.1 equiv). The reaction was conducted under microwave conditions (A: 25 to 170 °C for 10 min; B: 170 °C for 10 min). The crude mixture was dissolved in ethyl acetate. The mixture was washed with a 0.5 N aqueous solution of hydrochloric acid, brine, and dried over magnesium sulfate. The mixture was filtered and the filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography (eluent: hexane/ethyl acetate mixtures of increasing polarity). Yield: 37%. ^{1}H NMR ($CDCl_3$) δ 9.94 (s, 1H), 7.29 (d, 1H), 7.18 (m, 1H), 7.06 (dd, 1H), 7.00 (d, 1H), 6.94 (d, 1H), 6.85 (m, 2H), 5.59 (s, 1H), 3.85 (brs, 2H), 3.55 (q, 4H), 3.34 (brs, 2H), 2.04 (brm, 2H), 1.66 (m, 2H), 1.48 (s, 9H), 1.30 (t, 6H). LCMS (ESI): m/z 493.2 ($M + H^+$).

5-Bromo-*N*,*N*-diethylpyrimidine-2-carboxamide (52e). To a solution of **54** (0.055 g, 0.27 mmol, 1.0 equiv) in methylene chloride (5 mL) was added oxalyl chloride (0.050 mL, 0.58 mmol, 2.1 equiv). The mixture was refluxed for 1 h and concentrated under reduced pressure to give the crude acyl chloride. To a solution of the crude acyl chloride (0.060 g, 0.27 mmol, 1.0 equiv) in tetrahydrofuran (2.5 mL) was added *N*,*N*-diethylamine (0.11 mL, 1.06 mmol, 4.0 equiv). The mixture was stirred for 16 h and then diluted with ethyl acetate. The organic mixture was washed with water, with a saturated aqueous solution of sodium bicarbonate, a 1 N aqueous solution of

hydrochloric acid, and brine. The organic mixture was dried over sodium sulfate, filtered, concentrated under reduced pressure, and the crude product was used for the next step without further purification. Note: the product was isolated with a 17% impurity corresponding to *N*,*N*-diethyl-2-iodopyrimidine-5-carboxamide. Yield: 86%. ^{1}H NMR ($CDCl_3$) δ 8.82 (s, 2H), 3.56 (q, 2H), 3.20 (q, 2H), 1.28 (t, 3H), 1.18 (t, 3H).

4-Bromo-*N*,*N*-diethyl-2-hydroxybenzamide (52h). To a mixture of diethylamine (0.85 g, 11.58 mmol, 2.5 equiv), *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) (1.93 g, 6.02 mmol, 1.3 equiv) and *N*,*N*-diisopropylethylamine (1.25 g, 9.72 mmol, 2.1 equiv) in acetonitrile (50 mL) at 0 °C was added dropwise a solution of 4-bromo-2-hydroxybenzoic acid (1.0 g, 4.63 mmol, 1.0 equiv) in acetonitrile (10 mL). The mixture was warmed to room temperature and stirred for 48 h at room temperature. An additional portion of TBTU (1.04 g, 3.24 mmol, 0.7 equiv) was added to the mixture, which was heated at 60 °C for 5 h. The mixture was concentrated under reduced pressure, and the residue was dissolved in ethyl acetate. The mixture was washed with water and brine, dried over magnesium sulfate, and filtered. The solution was concentrated under reduced pressure. The crude product was purified by column chromatography (eluent: hexane/ethyl acetate mixtures of increasing polarity). Yield: 63%. ^{1}H NMR ($CDCl_3$) δ 10.08 (s, 1H), 7.17 (d, 1H), 7.12 (d, 1H), 6.98 (dd, 1H), 3.50 (q, 4H), 1.27 (t, 6H). LCMS (ESI): m/z 270.1 ($M - H^-$).

5-Bromopyrimidine-2-carboxylic Acid (54). To a mixture of a 2.5 M solution of *n*-butyl lithium in hexanes (0.84 mL, 2.1 mmol, 1.05 equiv) and toluene (4 mL) at −78 °C was added a solution of **53** (0.57 g, 2.0 mmol, 1.0 equiv) in toluene (2 mL). The reaction was stirred for 1 h at −78 °C. The reaction was quenched with freshly crushed dry ice. The mixture was warmed slowly to room temperature and was stirred for 2 h at room temperature. The mixture was concentrated under reduced pressure, and the resulting solid was treated with acetic acid. The solid was collected by filtration, dried under vacuum, and used for the next step without further purification. Yield: 62%. ^{1}H NMR (CD_3OD) δ 8.90 (s, 2H).

5-Bromo-*N*,*N*-diethylpicolinamide (56). To a suspension of **55** (808 mg, 3.01 mmol, 1.0 equiv) in dry dichloromethane (5 mL) was added oxalyl chloride (0.34 mL, 3.96 mmol, 1.3 equiv) followed by 2 drops of *N*,*N*-dimethylformamide. The reaction mixture was heated under reflux for 1 h. After cooling to room temperature, the mixture was concentrated under reduced pressure to provide the crude acyl chloride. To a suspension of the crude acyl chloride (as of 3.01 mmol, 1.0 equiv) in dry tetrahydrofuran (5 mL) was added *N*,*N*-diethylamine (1.56 mL, 15.08 mmol, 5.0 equiv) dropwise. The reaction mixture was stirred at room temperature for 2 h. Ethyl acetate (20 mL) was added, and the mixture was washed with water (20 mL), saturated aqueous sodium bicarbonate (30 mL), 1 N aqueous hydrochloric acid (20 mL), and brine. The organics were dried over sodium sulfate, filtered, and concentrated under reduced pressure to give a red−brown crystalline solid. Yield: 88% over two steps. ^{1}H NMR ($CDCl_3$) δ 8.64 (d, 1H), 7.91 (dd, 1H), 7.53 (d, 1H), 3.56 (q, 2H), 3.39 (q, 2H), 1.27 (t, 3H), 1.17 (t, 3H). LCMS (ESI): m/z 257.2 ($M + H^+$).

***N*,*N*-Diethyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)picolinamide (57).** To a solution of bis(pinacolato)diboron (2.18 g, 8.6 mmol, 1.2 equiv) in *N*,*N*-dimethylformamide (10 mL) at 0 °C was added potassium acetate (2.3 g, 23.4 mmol, 3.0 equiv), 1,1′-bis(diphenylphosphino)ferrocene palladium(II) chloride complex with dichloromethane (171 mg, 0.23 mmol, 0.03 equiv). The reaction mixture was heated at 80 °C at which point a solution of **56** (2.0 g, 7.8 mmol, 1.0 equiv) in *N*,*N*-dimethylformamide (10 mL) was added dropwise. The reaction mixture was stirred at 80 °C for an additional 10 h. Ethyl acetate (75 mL) and water (50 mL) were added, and the two phases were separated. The organic phase was washed with brine (50 mL), dried over sodium sulfate, filtered, and concentrated under reduced pressure to give a dark-brown oil, which solidified to needles. The crude product was triturated with hexane. The resulting solid was collected by filtration. Yield: 52%. ^{1}H NMR ($CDCl_3$) δ 8.92 (d, 1H), 8.14 (dd, 1H), 7.53 (d, 1H), 3.55 (q, 2H), 3.32 (q, 2H), 1.36 (s, 12H), 1.27 (t, 3H), 1.12 (t, 3H).

***tert*-Butyl 4-(6-(Diethylcarbamoyl)pyridin-3-yl)spiro[chromene-2,4′-piperidine]-1′-carboxylate (58).** To a solution of **39a** (1.48 g, 3.29 mmol, 1.0 equiv) in dimethoxyethane (20 mL) under nitrogen was added sequentially a 2 N aqueous solution of sodium carbonate (4.94 mL, 9.87 mmol, 3.0 equiv), lithium chloride (0.42 g, 9.87 mmol, 3.0 equiv), palladium (70 mg, 10 wt. % (dry basis) on activated carbon, 0.033 mmol, 0.01 equiv), and **57** (1.0 g, 3.29 mmol, 1.0 equiv). The mixture was heated under reflux for 10 h. Dichloromethane (200 mL) was added to dilute the reaction mixture, and palladium(0) on carbon was filtered off on a celite pad. The filtrate was washed with brine, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (eluent: hexane/ethyl acetate mixtures of increasing polarity). Yield: 76%. ^{1}H NMR ($CDCl_3$) δ 8.56 (dd, 1H), 7.75 (dd, 1H), 7.64 (dd, 1H), 7.22 (m, 1H), 6.99−6.85 (m, 3H), 5.62 (s, 1H), 3.88 (m, 2H), 3.59 (q, 2H), 3.45 (q, 2H), 3.34 (m, 2H), 2.06 (m, 2H), 1.69 (m, 2H), 1.48 (s, 9H), 1.29 (t, 3H), 1.20 (t, 3H). LCMS (ESI): m/z 478.0 ($M + H^+$).

Methyl 4-Iodo-3-(methoxymethoxy)benzoate (60). To an acidic methanolic solution, which was prepared by dropwise addition of acetyl chloride (25 mL) to anhydrous methanol (500 mL), was added **59** (171.61 g, 0.65 mol). The mixture was heated to reflux for 30 h. The reaction mixture was allowed to cool to room temperature and was concentrated under reduced pressure. The residue was diluted in ethyl acetate (750 mL), washed with a saturated sodium bicarbonate solution (750 mL), water (750 mL), brine (750 mL), and dried over sodium sulfate. The solution was filtered, and the filtrate was concentrated under reduced pressure. The crude product was dried under vacuum. Yield: 94%. ^{1}H NMR ($CDCl_3$) δ 7.74 (d, 1H), 7.64 (d, 1H), 7.31 (dd, 1H), 5.80 (s, 1H), 3.90 (s, 3H). To a solution of the crude ester (169.4 g, 0.61 mol, 1.0 equiv) and *N*,*N*-diisopropylethylamine (318.8 mL, 1.83 mol, 3.0 equiv) in methylene chloride (750 mL) at room temperature under nitrogen was added dropwise chloromethyl methyl ether (136.7 mL, 1.80 mol, 2.95 equiv). The mixture was heated under reflux for 22 h and then allowed to cool to room temperature. The mixture was concentrated to half the volume under reduced pressure and washed with a 1 N hydrochloric acid solution (2 × 1 L) and brine (1 L). The organic layer was dried over sodium sulfate, filtered, and the filtrate was concentrated under reduced pressure. The crude product was used for the next step without further purification. Yield: 95%. ^{1}H NMR ($CDCl_3$) δ 7.83 (d, 1H), 7.65 (d, 1H), 7.39 (dd, 1H), 5.30 (s, 2H), 3.90 (s, 3H), 3.52 (s, 3H).

4-Iodo-3-(methoxymethoxy)benzoic Acid (61). A solution of **60** (186.66 g, 0.58 mol, 1.0 equiv) in acetone (400 mL) was added to a solution of lithium hydroxide monohydrate (97.32 g, 2.32 mol, 4.0 equiv) in a 1:1 tetrahydrofuran/water solution (900 mL). The mixture was stirred at room temperature for 16 h. The mixture was reduced to half of its volume under reduced pressure and acidified with a 6 N aqueous solution of hydrochloric acid (∼300 mL). The crude mixture was extracted with ethyl acetate (750 mL). The organic layer was washed with brine, dried over sodium sulfate, and filtered. The filtrate was concentrated under reduced pressure to afford the acid **61** as a light-yellow crystalline solid, which was used for the next step without further purification. Yield: 99%. ^{1}H NMR ($CDCl_3$) δ 7.89 (d, 1H), 7.73 (d, 1H), 7.47 (dd, 1H), 5.30 (s, 2H), 3.50 (s, 3H).

***N*,*N*-Diethyl-4-iodo-3-(methoxymethoxy)benzamide (62).** To a mixture of **61** (104.74 g, 0.34 mol, 1.0 equiv) and TBTU (125.0 g, 0.39 mol, 1.15 equiv) in acetonitrile (3.75 L) at 0 °C was added diethylamine (40.5 mL, 0.39 mol, 1.15 equiv) and *N*,*N*-diisopropylethylamine (125.4 mL, 0.72 mol, 2.1 equiv). The mixture was warmed to room temperature, stirred for 18 h at room temperature, and concentrated under reduced pressure. The crude mixture

was dissolved in ethyl acetate (1 L). The mixture was washed with a saturated aqueous solution of sodium bicarbonate (800 mL), dried over sodium sulfate, and filtered. The filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography (first eluting with a 20% ethyl acetate/hexane mixture then 40% ethyl acetate/hexane). Yield: 97%. ^{1}H NMR ($CDCl_3$) δ 7.80 (d, 1H), 7.08 (d, 1H), 6.75 (dd, 1H), 5.23 (s, 2H), 3.50 (s, 3H), 3.62−3.07 (brm, 4H), 1.33−1.02 (brm, 6H).

***tert*-Butyl 4-(4-(Diethylcarbamoyl)-2-(methoxymethoxy)phenyl)-spiro[chromene-2,4′-piperidine]-1′-carboxylate (63).** To a solution of **62** (1.02 g, 2.82 mmol, 1.0 equiv) in dimethoxyethane (DME) (20 mL) was added sequentially a 2-N aqueous solution of sodium carbonate (4.23 mL, 8.46 mmol, 3.0 equiv), lithium chloride (0.359 g, 8.46 mmol, 3.0 equiv), **48** (1.44 g, 3.38 mmol, 1.2 equiv), and palladium on carbon (10%, 50% water) (0.038 g, 0.007 mmol, 0.0025 equiv). The coupling reaction was conducted under microwave conditions (A: 25−170 °C for 10 min; B: 170 °C for 7 min). The mixture was dissolved in ethyl acetate, washed with water, and dried over sodium sulfate. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography (eluent: hexane/ethyl acetate mixtures of increasing polarity). Yield: 50%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (m, 2H), 7.13 (m, 1H), 7.06 (dd, 1H), 6.90 (d, 1H), 6.76 (m, 2H), 5.53 (s, 1H), 5.04 (s, 2H), 3.87 (brs, 2H), 3.55 (brs, 2H), 3.34 (brs, 4H), 3.30 (s, 3H), 2.08 (brm, 2H), 1.67 (brm, 2H), 1.48 (s, 9H), 1.24 (brm, 6H). LCMS (ESI): m/z 537.3 ($M + H^+$).

2. Biological Methods. Radioligand Binding Assays. Membrane preparations from Chinese hamster ovary (CHO) cells stably expressing human δ, μ, or κ opioid receptors were prepared as described previously.[56] The assay buffer used is composed of 50 mM tris(hydroxymethyl) aminomethane HCl, pH 7.8, 1.0 mM ethylene glycol bis(β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA free acid), 5.0 mM $MgCl_2$, 10 mg/L leupeptin, 10 mg/L pepstatin A, 200 mg/L bacitracin, and 0.5 mg/L aprotinin. After dilution in assay buffer and homogenization in a Polytron homogenizer (Brinkmann, Westbury, NY) for 30 s at a setting of 1, membrane proteins (10−80 μg) in 250 μL of assay buffer were added to mixtures containing test compound and [^{3}H]diprenorphine (0.5−1.0 nM, 25000−50000 dpm) in 250 μL of assay buffer in 96-well deep-well polystyrene titer plates (Beckman) and incubated at room temperature for 60 min. Reactions were terminated by vacuum filtration with a Brandel MPXR-96T harvester through GF/B filters that had been pretreated with a solution of 0.5% polyethylenimine and 0.1% bovine serum albumin for at least 1 h. The filters were washed four times with 1.0 mL each of ice-cold 50 mM Tris-HCl, pH 7.8, and 30 μL of Microscint-20 (PerkinElmer Life and Analytical Sciences, Shelton, CT) was added to each filter. Radioactivity on the filters was determined by scintillation spectrometry in a PerkinElmer TopCount. [^{3}H]Diprenorphine with a specific activity of 50 Ci/mmol was purchased from PerkinElmer Life and Analytical Sciences, Inc. (Shelton, CT). The K_D values for [^{3}H]diprenorphine binding were 0.33 nM for the κ and μ opioid receptors and 0.26 nM for the DOR. Receptor expression levels, determined as B_{max} values from Scatchard analyses, were 4400, 4700, and 2100 fmol/mg of protein for the κ, μ, and DORs, respectively. Preliminary experiments were performed to show that no specific binding was lost during the wash of the filters, that binding achieved equilibrium within the incubation time and remained at equilibrium for at least an additional 60 min, and that binding was linear with regard to protein concentration. Nonspecific binding, determined in the presence of 10 μM unlabeled naloxone, was less than 10% of total binding. Protein was quantified by the method of Bradford.[57] The data from competition experiments were fit by nonlinear regression analysis using GraphPad Prism 4.02 for Windows (GraphPad Software, San Diego, CA) using the four-parameter equation for one-site competition, and K_i values were subsequently calculated from EC_{50} values by the Cheng−Prusoff equation.

Receptor-Mediated [^{35}S]GTPγS Binding. Receptor-mediated [^{35}S]GTPγS binding was performed by modifications of the methods of Selley and collaborators[58] and Traynor and Nahorski.[59] Assays were carried out in 96-well FlashPlates (PerkinElmer Life and Analytical Sciences, Shelton, CT). Membranes prepared from CHO cells expressing the human DOR (50−100 μg of protein) were added to assay mixtures containing agonist, approximately 100000 dpm (100 pM) [^{35}S]GTPγS, 3.0 μM GDP, 75 mM NaCl, 15 mM $MgCl_2$, 1.0 mM EGTA, 1.1 mM dithiothreitol, 10 mg/L leupeptin, 10 mg/L pepstatin A, 200 mg/L bacitracin, and 0.5 mg/L aprotinin in 50 mM Tris-HCl buffer, pH 7.8. After incubation at room temperature for 1 h, the plates were sealed and centrifuged at 800*g* in a swinging bucket rotor for 5 min and bound radioactivity was determined with a TopCount microplate scintillation counter (PerkinElmer). Agonist potencies were assessed by measuring stimulation of [^{35}S]GTPγS binding by a series of concentrations of agonist, using **1** as a reference agonist. The concentration to give half-maximal stimulation (EC_{50}) was determined by nonlinear regression using GraphPad Prism 4.02 for Windows (GraphPad Software, San Diego, CA).

Fluorescence-Based CYP2D6 Inhibition Assay. CYP2D6 activity was measured in microsomes containing human recombinant CYP2D6 (BD Biosciences) using 7-methoxy-4-aminomethyl-coumarin (MAMC) as substrate.[60] Conversion of MAMC to 7-hydroxy-4-aminomethyl-coumarin (HAMC) was measured using a PerkinElmer Fusion with a 390 nm excitation filter and a 460 nm emission filter. IC_{50} values are geometric means computed from at least three separate determinations.

Freund's Complete Adjuvant (FCA)-Induced Mechanical Hyperalgesia in Rats. For the assay, the methods of DeHaven-Hudkins and collaborators were used to determine mechanical hyperalgesia in rats 24 h after intraplantar administration of 150 μL Freund's Complete Adjuvant (FCA).[53] To determine paw pressure thresholds (PPTs), the rats were lightly restrained in a gauze wrap and pressure was applied to the dorsal surface of the inflamed and uninflamed paw with a conical piston using a pressure analgesia apparatus (Stoelting Instruments, Wood Dale, IL). The PPT was defined as the amount of force (in grams) required to elicit an escape response using a cutoff value of 250 g. PPTs were determined before and at specified times after drug treatment. The antihyperalgesia value was calculated for each rat using the following formula: % AH = 100-(posttreatment $PPT_{(inflamed)}$ − baseline $PPT_{(inflamed)}$)/(baseline $PPT_{(uninflamed)}$ − baseline $PPT_{(inflamed)}$). The dose required to produce a half-maximal effect (ED_{50}) was determined by nonlinear regression using GraphPad Prism 4.02 for Windows (GraphPad Software, San Diego, CA), which was also used for statistical analyses. The level of significance was set at $p < 0.05$. To determine the ED_{50} value for the dose−response experiments, a mean % AH was determined for each dose and the data were analyzed using nonlinear regression analysis of a fitted sigmoidal dose−response curve. The minimum value was set at 0, and the maximum value was constrained at a value that did not exceed the mean % AH ± the standard deviation of the mean for the highest value. One-way analysis of variance (ANOVA) with Tukey's posthoc test was used to determine group differences in the dose−response and time course experiments. Two-way ANOVA with Bonferroni's posthoc test was used to determine differences due to pretreatment and treatment in the antagonism experiment. The level of significance was set at $p < 0.05$. GraphPad Prism 4.02 for Windows (GraphPad Software, San Diego, CA) was used for curve fitting and for all statistical analyses.

Acknowledgment. This research was sponsored by Adolor Corporation. All authors are currently or were employees of Adolor Corporation at the time this work was conducted. We thank Glenn P. A. Yap from the University of Delaware

(Department of Chemistry and Biochemistry) for providing crystallographic data of compound **50**. We also thank Pfizer Inc. for their careful review of the manuscript. Administrative assistance was provided by UBC Scientific Solutions and funded by Adolor Corp. and Pfizer Inc.

Supporting Information Available: Table of crystallographic data for compound **50**. Table of elemental analyses for compounds **5**−**36**. This material is available free of charge via the Internet at http://pubs.acs.org.

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