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The Discovery and Development of the *N*-Substituted *trans*-3,4-Dimethyl-4-(3'-hydroxyphenyl)piperidine Class of Pure Opioid Receptor Antagonists

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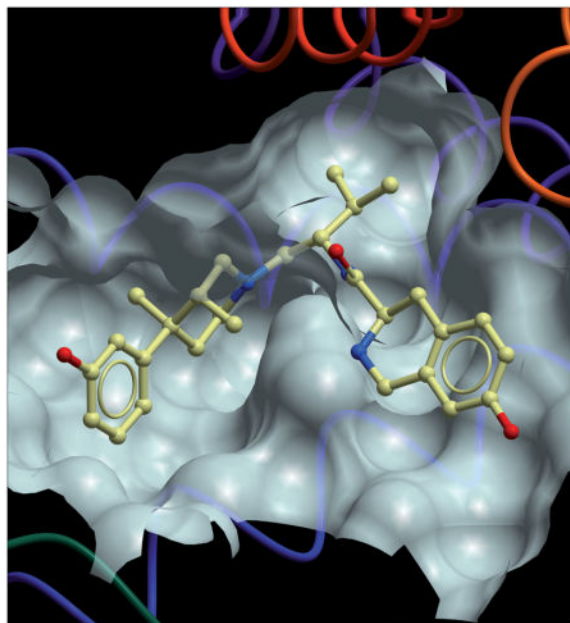
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Abstract

N-Substituted *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidines are a class of pure opioid receptor antagonists with a novel pharmacophore. This opioid receptor antagonist pharmacophore was used as a lead structure to design and develop several interesting and useful opioid receptor antagonists. In this review we describe: 1) early SAR studies that led to the discovery of LY255582 and analogues that are nonselective opioid receptor antagonists developed for the treatment of obesity; 2) the discovery and commercialization of LY246736 (alvimopan; ENTEREG®), a peripherally selective opioid receptor antagonist that accelerates the time to upper and lower GI recovery following surgeries that include partial bowel resection with primary anastomosis; and 3) the discovery and development of the potent and selective κ opioid receptor antagonist JD_{Tic} and analogues as potential pharmacotherapies for treating depression, anxiety, and substance abuse (nicotine, alcohol, and cocaine). In addition, the use of JD_{Tic} for obtaining the X-ray structure of the human κ opioid receptor is discussed.

Graphical Abstract

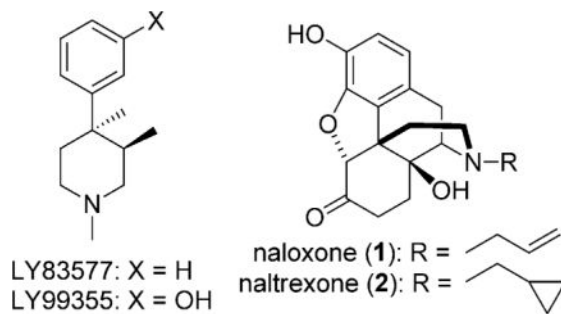


Keywords

alvimopan; drug design; JDTic; medicinal chemistry; opioid antagonists

Introduction

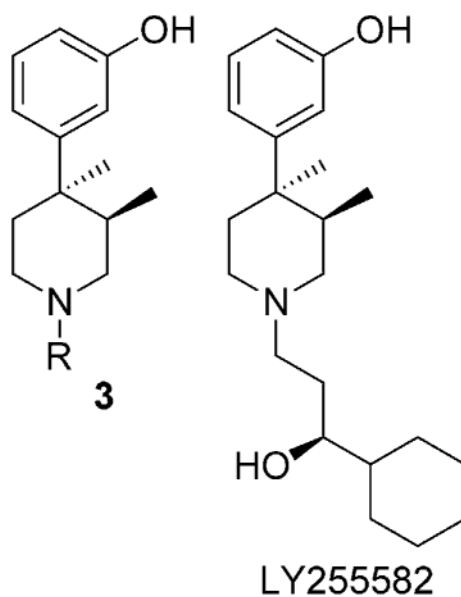
In the late 1970s Zimmerman and co-workers reported *N*-methyl-*trans*-3,4-dimethyl-4-phenylpiperidine (LY83577) to be an opioid receptor pure antagonist, the potency of which could be significantly increased by attachment of a phenolic group to the aromatic ring to give the *N*-methyl-*trans*-3,4-di-methyl-4-(3-hydroxyphenyl)piperidine (LY99335).^[1–3] LY99335^{–1} (s.c.) in the gave antagonist AD₅₀ values of 25 and 34 mg kg^{–1} rat-tail heat analgesic and mouse-writhing analgesic tests, respectively; it showed no agonist activity in either of these tests, and was therefore deemed a pure antagonist.^[1] This discovery was surprising at the time, because opioid ligands with an *N*-methyl substituent had always been opioid receptor agonists. In general, at this time all opioid receptor antagonists or even partial agonists consistently had *N*-allyl- or *N*-cyclopropylmethyl substituents.^[4]



Prior to the discovery of LY99335, naloxone (**1**) and naltrexone (**2**) were the only pharmacologically pure opioid receptor antagonists (i.e., antagonists devoid of any opioid agonist effects) that had been well characterized.^[5] Because LY99335 has a novel pharmacophore, it has been used as a lead compound for the discovery and development of a number of novel, pure opioid receptor antagonists. Herein we review the discovery and development of several of these compounds.

Early Studies and Discovery of LY255582

The synthesis and evaluation of a large number of racemic N-substituted *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidines **3** (for which R = various N substituents) further documented the pure opioid receptor antagonist nature of the LY99335 class of compounds.^[6,7] Opioid receptor affinity and selectivity were determined by using radiolabeled opioid receptor ligands to displace opioid receptor type selective radioligands from receptor sites in membrane preparations from animal brain tissue. Table 1 lists the receptor binding data at the μ and κ receptors, opioid antagonist activity in the mouse-writhing and rat-diuresis assays, and food-inhibition feeding activity in the obese Zucker rat model for a few of the more potent compounds.



The study showed that all compounds tested were pure opioid receptor antagonists regardless of the N substituent. The N substituent only affected opioid receptor binding affinity and opioid receptor antagonist potency. Optimal potency was observed if the N substituent was a phenyl, thiophene, or cyclohexyl group attached to the nitrogen via a three-atom spacer (compounds **3a–d**). The addition of a hydroxy group α to the phenyl, thiophene, or cyclohexyl groups or a keto group α to the cyclohexyl group improved potency (**3e–h**).

A few compounds were separated into their *trans*-(3*R*,4*R*)- and *trans*-(3*S*,4*S*)-dimethyl-4-(3-hydroxyphenyl)piperidine isomers.^[7] The results for isomers of **3c** and **3h** are listed in Table

2 in comparison with the results for naloxone and naltrexone. In general, the *3R,4R* isomer was more potent than the *3S,4S* isomer in the receptor binding test, the opioid antagonist test, and the anorectant test. (*3R,4R*)-3,4-Dimethyl-1-[(3*S*)-3-hydroxy-3-cyclohexylpropyl-4-(3-hydroxyphenyl)piperidine (LY255582) emerged as having the best activity profile, both in decreasing food consumption and as an opioid antagonist. X-ray crystallography studies confirmed that LY255582 has the *3R,4R,3'S* configuration, in which 3' refers to the chiral center in the N substituent.^[7] The X-ray structure showed the piperidine ring of LY255582 to be in a chair conformation, and the 4-(3-hydroxyphenyl) ring to assume an equatorial orientation. This information, along with results of studies using a variety of di- and trimethyl-substituted 4-(3-hydroxyphenyl)piperidines combined with data from NMR studies, suggested that a 3-hydroxyphenyl equatorial piperidine chair conformation mediates the opioid antagonist properties of the *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine class of opioid antagonists.^[8, 9]

Researchers at Adolor showed that *trans*-3,4-dimethyl-4-(3-carboxamidophenyl)piperidine (**4b**) derived from *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine (**4a**) displayed high affinity for cloned human μ opioid receptor and good μ selectivity relative to the κ and δ receptors (Table 3).^[10] In addition, **4b** displayed potent μ antagonist activity in a [³⁵S]GTP γ S assay (IC₅₀ = 1.9 nM), similar to that of **4a**.^[10] Thus, the carboxamide moiety in **4b** is a good bioisostere of the phenolic group present in **4a**.

Although LY255582 showed exceptional potency and durability in decreasing food intake and body weight gain in obese Zucker rats relative to naltrexone,^[11, 12] its bioavailability was <1 % in both rat and dog, primarily due to extensive first-pass metabolism.^[13] Because the Eli Lilly research team suspected the problem was with the 3-hydroxyphenyl group, they synthesized and evaluated a number of LY255582 analogues modified at the 3-hydroxyphenyl group with the hope that an analogue would be identified that retained the favorable properties of LY255582 while lacking its drawbacks.^[14] To this end, the compounds listed in Table 4 were synthesized and evaluated for binding affinity at cloned μ , κ , and δ opioid receptors expressed in Chinese hamster ovary (CHO) cells using [³H]diprenorphine (μ and κ assays) or [³H]bremazocine (δ receptor assay). Functional antagonist potency (K_b) was also determined by using a [³⁵S]GTP γ S binding assay. With the exception of **5h**, **5i**, and **5l**, all the LY255582 analogues had considerably weaker radioligand binding affinity and particularly [³⁵S]GTP γ S binding affinity than LY255582. From a structure–activity relationship (SAR) standpoint, this study showed that removal of the hydroxy group to give **5c**, or moving it to other positions on the phenyl ring (compounds **5a,b**) significantly decreased binding affinity (K_i) and antagonist efficacy (K_b) at all three opioid receptors. Replacement of the 3-hydroxy group with amino (**5d**), urea (**5e**), acetylamido (**5f**), or methanesulfonylamido (**5g**) groups decreases binding affinity and antagonist efficacy relative to LY255582, but not to as large a degree as removing or changing the position of the 3-hydroxy group. Replacing the 3-hydroxy with a carbomethoxy (**5j**) or carboxylic acid (**5k**) group caused a large decrease in binding affinity and antagonist efficacy.

Compound **5l**, with respective K_i values of 0.2, 12.7, and 6.4 nM at the μ , κ , and δ opioid receptors, had the best opioid binding affinity of all the compounds tested (Table 4). As **5l**

had K_b values of 0.1, 1.9, and 2.8 nM compared with K_b values of 0.04, 0.3, and 1.2 nM at the μ , κ , and δ for LY255582, it was selected for further development. Compound **51** had ED₅₀ values of 0.024 mg kg⁻¹ s.c. and 0.015 mg kg⁻¹ p.o., compared with 0.017 mg kg⁻¹ s.c. and 0.26 mg kg⁻¹ p.o. for LY255582 in the mice tail-flick assay. The oral bioavailability (% *F*) of **51** in rats was 32 % relative to 2.5 % for LY255582.^[14] The Eli Lilly group also showed that administration of **51** at 3 mg kg⁻¹ p.o. resulted in a significant decrease in the cumulative amounts of food consumed over periods of 1 and 2 h. In contrast, LY255582 administered at 3 mg kg⁻¹ p.o. was inactive over the same time period. The results from these studies showed that **51** had much greater potential as a drug to treat obesity than LY255582.^[14]

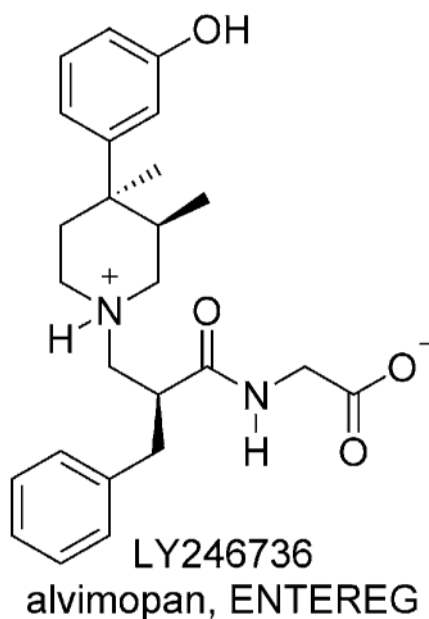
Given the favorable properties of **51**, it is surprising that the compound was not developed. This could have been due to the fact that the Eli Lilly researchers had discovered a new class of nonselective opioid receptor antagonists that were considered more favorable for development.^[15, 16]

Discovery of LY246736 (alvimopan; ENTEREG®)

The administration of narcotics results in significant inhibitory effects on gastrointestinal (GI) function, including motility, secretion, absorption, and blood flow.^[17] This occurs through activation of μ opioid receptors in the enteric nervous system, the same receptor type that is activated in the central nervous system (CNS) to provide analgesia. It had been hypothesized that a peripherally selective μ opioid receptor antagonist, when co-administered with a narcotic, may significantly decrease or eliminate opioid-induced GI dysfunction while maintaining pain relief and avoiding opioid withdrawal syndrome. The hypothesis was supported, in part, from preclinical animal and human clinical studies with naloxone. Because naloxone readily traverses the blood–brain barrier, it is unsuitable as an adjunct to narcotic therapy. As a result, Zimmerman and coworkers explored the development of peripherally selective *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine opioid antagonists for the treatment of GI motility disorders.^[18, 19]

The 4-(3-hydroxyphenyl)-3,4-dimethylpiperidine scaffold was derivatized with functional groups spanning a range of sizes, polarity, and charge to achieve peripheral μ opioid receptor antagonism while minimizing CNS drug exposure. In vitro receptor binding and tissue preparations were used to determine in vitro μ receptor affinity and functional antagonism. Phenotypic whole-animal screening played a key role in establishing relative peripheral drug action and prioritizing molecules for further characterization. Central opioid activity was assessed by determining the AD₅₀ for molecules to reverse a fully efficacious analgesic dose of morphine in the mouse-writhing assay. A specially designed assay was developed to assess peripheral opioid activity by determining the ED₅₀ value required to precipitate diarrhea in morphine-dependent mice. The calculated AD₅₀/ED₅₀ ratio provided an index of peripheral selectivity; the higher the ratio, the greater the relative peripheral action of the molecule.^[18] Several hundred compounds were synthesized and evaluated in these assays. The in vitro μ K_i , in vivo AD₅₀, and ED₅₀ and peripheral index (ratio) for selected analogues **6 a–h** are presented in Table 5. Naloxone (AD₅₀ = 0.08 mg kg⁻¹ s.c.; ED₅₀ = 0.05 mg kg⁻¹ s.c.) possessed a peripheral index of 1.6, indicating little discrimination between CNS and

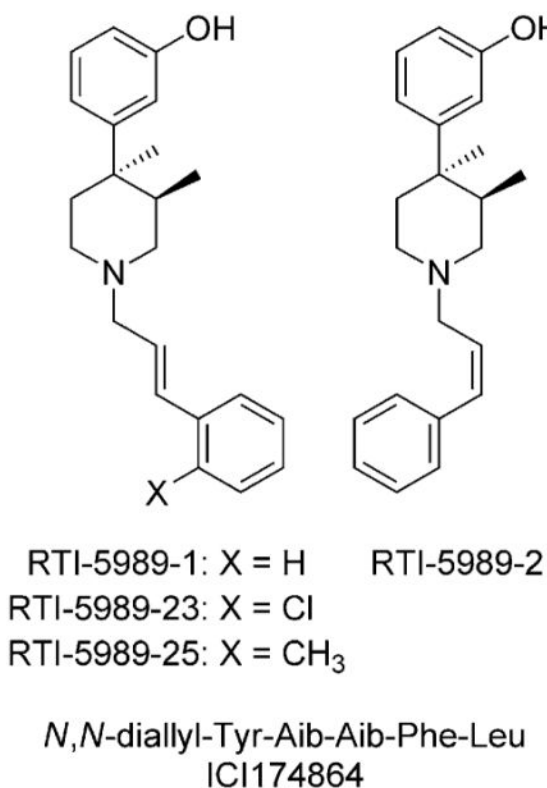
peripheral action. In contrast, compound **6 c** (a mixture of eight stereoisomers: $AD_{50} = 40 \text{ mg kg}^{-1} \text{ s.c.}$; $ED_{50} = 0.15 \text{ mg kg}^{-1} \text{ s.c.}$) had an index of ~ 260 , indicating a high degree of separation between central and peripheral action. The peripheral index for compound **6 g** (a mixture of eight stereoisomers) was >1400 . Separation of its isomers yielded the $3R,4R,2'S$ antipode **6h**, LY246736 (alvimopan): $AD_{50} = 9 \text{ mg kg}^{-1} \text{ s.c.}$; $ED_{50} 0.04 \text{ mg kg}^{-1} \text{ s.c.}$; peripheral index = 225. Alvimopan's peripheral index was confirmed through additional pharmacological testing, including reversal of morphine analgesia in mouse hot-plate ($ID_{50} = 6.0 \text{ mg kg}^{-1} \text{ s.c.}$) versus the mouse small intestinal transit assay ($ID_{50} = 0.03 \text{ mg kg}^{-1} \text{ s.c.}$).^[19] The high degree of peripheral action is believed to be due, in part, to its zwitterionic character. In vitro binding studies showed alvimopan to be a potent, relatively nonselective opioid antagonist with K_i values of 0.77, 4.4, and 40 nM for the μ , δ , and κ opioid receptors, respectively,^[19] displaying >100 -fold selectivity over other aminergic G-protein-coupled receptors. In the isolated guinea pig ileum (μ and κ) and mouse vas deferens (δ), alvimopan showed pA_2 values of 9.7, 7.8, and 8.7 for the μ , κ , and δ receptors, respectively. As a pure antagonist, alvimopan displayed no intrinsic activity in human μ and δ , and guinea pig κ , relative to full agonists. In 2008, alvimopan was approved by the US Food and Drug Administration (FDA) as the marketed product ENTEREG® for short-term (up to seven days) in-hospital use to accelerate return of GI function following bowel resection surgery. In 2013, following submission of a supplemental new drug application (NDA) in 2012, the FDA approved an expanded indication for ENTEREG® including any surgery that involves a partial bowel resection with primary anastomosis.^[20] Human pharmacokinetics, pharmacodynamic and clinical trial data are reviewed elsewhere.^[21]



More Recent SAR and Conformational Studies

To gain additional insight into the importance of the conformational flexibility of the N substituent of N-substituted *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidines responsible for their opioid receptor antagonist potency and selectivity, the opioid receptor binding

affinity and [35 S]GTP γ S functional antagonist efficacy of compounds RTI-5989-1, RTI-5989-23, and RTI-5989-25, which have more conformationally rigid N sub-stituents than previously studied compounds, were compared with those of LY255582 and naltrexone.^[22] RTI-5989-1, RTI-5989-23, and RTI-5989-25, bearing the *trans*-4'-aryl-2'-butenyl N substituent, displayed binding and selectivity patterns for the μ opioid receptor over the δ and κ receptors similar to those of LY255582 (Table 6). With a K_i value of 0.32 nM, LY255582, was found to be the most potent at the μ receptor. RTI-5989-1 had a K_i value of 0.74 nM at the μ receptor and K_i values of 322 and 122 nM at the δ and κ receptors, making it the most μ -selective analogue. In the [35 S]GTP γ S functional assay, LY255582, RTI-5989-1, RTI-5989-23, and RTI-5989-25 did not stimulate GTP binding at concentrations up to 10 μ M, and are therefore, as expected, pure opioid receptor antagonists (Table 7). With $K_e = 0.013$ nM at the μ opioid receptor, RTI-5989-25 is slightly more potent than LY255582 ($K_e = 0.021$ nM). Both compounds are much more potent than naltrexone ($K_e = 0.93$ nM at μ). LY255582 and RTI-5989-25 have very similar K_e values at the δ and κ receptors (Table 7).



In general, these N-substituted *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine analogues were more potent as inhibitors of agonist-stimulated [35 S]GTP γ S binding than as inhibitors of radioligand binding. Figure 1 shows the ratio of K_i values in the binding assay to the K_e values in the [35 S]GTP γ S assay for RTI-5989-25 and for naltrexone. The most pronounced differences were observed with the δ receptors, for which RTI-5989-25 was more than 400-fold more potent in the [35 S]GTP γ S assay than the inhibition of radioligand binding assay. For comparison, naltrex-one showed little variation from near unity (Figure 1). Because the

[³⁵S]GTPγS assay was not available to the Eli Lilly group at the time they developed the N-substituted *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperi-class of opioid antagonists, they were unaware of this unusual property difference with these compounds and naltrexone.

It is now widely accepted that GPCRs can exist in equilibrium between an active (R*) and an inactive (R) state.^[23, 24] Even in the absence of agonist, these receptors can maintain a conformation that can activate G protein and thus display constitutive activity. Compounds that preferentially stabilize the inactive (R) form of the receptor abolish this agonist-independent activity and are termed inverse agonists.

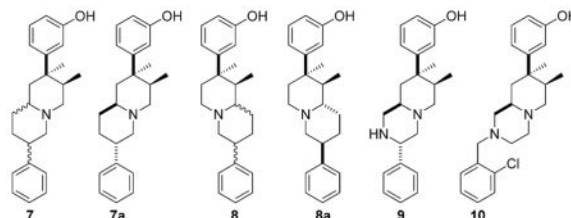
RTI-5989-23 and RTI-5989-25 were shown to be a new class of potent δ opioid inverse agonists^[25] (Table 8). RTI-5989-25 was 27-fold more potent as an inverse agonist at the δ opioid receptor than reference standard ICI174864 (ICI; *N,N*-diallyl-Tyr-Aib-Aib-Leu). The change in surface receptors elicited by the inverse agonists RTI-5989-25 and ICI was compared with the effects of neutral antagonists naltrindole (NTD) and H-Tyr-Tic-Phe-Phe-OH (TIPP) and several other compounds (Figure 2). RTI-5989-23, RTI-5989-25, and ICI caused a significant up-regulation of surface receptors, the neutral antagonists TIPP and NTD caused no changes in surface receptor numbers, and etorphine, fentanyl, morphine, and buprenorphine all caused a decrease in surface receptors.^[25] This was the first demonstration that ligand treatment is able to increase δ opioid cell-surface receptor population.

RTI-5989-25 was also an inverse agonist in HEK293 FLAG-μ cells.^[26] RTI-5989-25 was shown to have a higher affinity (0.011 nM) for the receptor in basal state (no Na⁺) and 0.062 nM for the activated receptor (R*G; high Na⁺/GTP), whereas the neutral antagonist naltrexone showed very little difference (Table 9). RTI-5989-25 also increased μ opioid receptor expression in HEK293 FLAG-μ cells^[26] by 41 %, relative to 11 % for the peptide H-(D-Phe)-Cys-Tyr-(D-Trp)-Arg-Thr-Pen-Thr-NH₂ (CTAP; Figure 3).

After learning that the N-substituted *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidines displayed inverse agonist activity, Eli Lilly scientists showed the in vitro inverse agonist efficacy and affinities in the presence of Na⁺ with their *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine antagonist correlated with data obtained in their obese rat studies.^[27] They had previously found no other in vitro/in vivo data correlation.

Because the relative potency increase was greater for the δ and κ receptors, the compounds did not show μ selectivity in the [³⁵S]GTPγS test. Comparison of the μ, δ, and κ binding affinity *K_i* values of 0.74, 322, and 122 nM, respectively, for RTI-5989-1 with those for the *cis* isomer RTI-5989-2 of 11.4, 931, and 298 nM suggest that the conformational orientation of N substituents is important for binding potency (Table 6). These findings suggest that the high binding affinity, selectivity, and antagonist potency of analogues with *N*-phenylpropyl or *N*-cyclohexylpropyl (**3b**, R = C₆H₅(CH₂)₃ and **3a**, R = C₆H₁₁(CH₂)₃, respectively; Table 1) are achieved through a conformation wherein the constricting chains of the N substituents are extended away from the piperidine nitrogen atom with the appended ring system rotated out of plane relative to the connecting-chain atoms. This conformation is quite similar to that observed in the solid state for LY255582, as determined by single-crystal X-ray analysis.^[17]

To provide information concerning the antagonist bioactive conformation of *N*-phenylpropyl-*trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine (**4a**), researchers at Adolor synthesized each of the four stereoisomers of general structures **7** and **8**. They compared their potencies in blocking the binding of the nonselective opioid antagonist [³H]diprenorphine to cloned human μ , κ , and δ opioid receptors and in inhibiting agonist (loperamide)-stimulated [³⁵S]GTP γ S binding to membranes containing the cloned μ opioid receptor.^[28]



Of the eight constrained **4 a** analogues studied, only **7 a** and **8 a**, with respective K_i values of 0.62 and 0.90 nM, showed μ binding affinity similar to the μ affinity of **4 a** (K_i = 1.8 nM; Table 10).^[29] Compound **7 a** also had an IC_{50} value of 0.54 in the [³⁵S]GTP γ S functional assay at the μ receptor. Surprisingly, **8 a** showed no antagonist activity in the μ receptor functional assay. The compound was a full agonist, with EC_{50} = 53 nM (Table 10). A modeling study showed low-energy conformations of **4a** and **7 a** to be very similar to each other.^[28, 29] These structures are quite similar to the X-ray structure of LY255582.

Adolor researchers also synthesized a series of octahydro-1*H*-pyrido[1,2-*a*]pyrazine derivatives and evaluated them for their opioid receptor binding and functional activity.^[30] Compound **9**, which is a bioisostere of **7a** with the methylene group at position 6 of **7 a** replaced with an NH function, had K_i values of 3.6, 18, and 89 nM at the μ , κ , and δ opioid receptors, respectively, and K_e = 1.1 nM at the μ receptor in the [³⁵S]GTP γ S functional test (Table 10).^[30] The most potent compound was obtained by adding an *N*-3-chlorobenzyl group to **9** to yield **10**.^[28] Compound **10** had K_i values of 0.47, 16, and 57 nM at the μ , κ , and δ opioid receptors, respectively, and K_e = 1.8 nM at the μ receptor in the [³⁵S]GTP γ S functional assay.^[30]

Discovery of JDTC

Even though the Eli Lilly group synthesized and evaluated hundreds of *N*-substituted *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)-piperidines for their opioid receptor properties, no selective antagonist for the κ opioid receptor was identified. As a strategy for obtaining a selective κ opioid receptor antagonist, general structure **11** was subjected to two iterations of synthesis of libraries and opioid receptor property evaluation.^[31, 32] The study led to the identification of JDTC as a potent and selective κ opioid receptor antagonist. Table 11 lists the results from studies by three different laboratories showing JDTC to be a more potent and selective κ opioid receptor antagonist than nor-BNI in [³⁵S]GTP γ S binding assays. In the studies, JDTC and nor-BNI were evaluated for their ability to antagonize opioid receptor agonist stimulated binding in a [³⁵S]GTP γ S assay using cloned human μ , δ , and κ receptors (Table 11). In one study, conducted under the NIDA Opioid Treatment Discovery Program

(OTDP), JDTic had a K_e value of 0.01 nM at the κ receptor and was 341- and 7930-fold selective for the κ receptor relative to the μ and δ opioid receptors, respectively (Table 11). In a study conducted at RTI, JDTic was found to have $K_e = 0.02$ nM and was 1255- and 3830-fold selective for the κ receptor relative to the μ and δ opioid receptors, respectively (Table 11).^[33] For comparison, nor-BNI had $K_e = 0.04$ and 0.05 nM in the OTDP and RTI studies and was 475- and 115-fold (OTDP study) and 520- and 580-fold (RTI study) selective for the κ relative to the μ and δ opioid receptors, respectively.^[31, 33] In a study conducted at Lilly Research Laboratories, JDTic had a K_e value of 0.098 nM and was 67- and 1718-fold selective for the κ relative to the μ and δ opioid receptors, respectively.^[34] In comparison, nor-BNI had $K_e = 0.80$ nM and 41- and 18-fold selectivity for the κ relative to the μ and δ opioid receptors, respectively (Table 11).

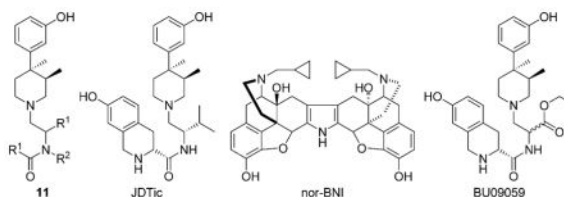


Table 11 also lists the results from a study conducted by the NIDA Intramural Research Program (IRP), which showed that JDTic to have a K_e value of 0.02 nM compared with 0.038 nM for nor-BNI at the κ receptor. JDTic was 108- and >15 000-fold selective for the κ relative to the μ and δ opioid receptors, respectively, and nor-BNI was 440- and 268-fold selective in the same regard. JDTic was more potent as an antagonist of [35 S]GTP γ S binding at the κ receptor in all four assays. Both compounds were very selective for the κ relative to the μ and δ receptors in all tests.

AstraZeneca Pharmaceuticals also reported a comparison of the opioid receptor antagonist properties of JDTic and nor-BNI in a [35 S]GTP γ S binding assay. However, they only reported IC₅₀ values, and the data are not directly comparable with those of the other four studies.^[35]

JDTic and nor-BNI have also been tested in three different opioid receptor binding assays, and the results are listed in Table 12. In a comparison of the opioid receptor binding affinities for JDTic and nor-BNI using rat brain for the μ and δ receptors and guinea pig brain for the κ receptor, with [3 H]DAMGO, [3 H]DADLE, and [3 H]U69,593 as the respective radioligands for μ , δ , and κ receptors, the K_i values for JDTic are 3.73, 301, and 0.32 nM at μ , δ , and κ receptors compared with 65, 86, and 1.09 nM for nor-BNI.^[31] In a separate study using human cloned μ , δ , and κ opioid receptors and using [3 H]DAMGO, [3 H]DPDPE, and [3 H]U69,593 as radioligands, JDTic had K_i values of 0.96, 29.6, and 0.41 nM compared with 21, 5.7, and 0.2 nM for nor-BNI.^[31] In a third study, which also used human cloned μ , δ , and κ opioid receptors, but with [3 H]diprenorphine as the radioligand for all three receptors, JDTic showed K_i values of 11.5, 188, and 0.059 nM relative to 32.4, 6.56, and 0.15 nM for nor-BNI.^[34] Results from these three studies show that JDTic has a higher affinity for the κ receptor than nor-BNI in the brain tissue test, nor-BNI has greater affinity than JDTic in one of the cloned opioid receptor tests, and JDTic had higher affinity than nor-BNI in the other study using cloned human receptors. nor-BNI is more selective for

κ relative to the μ opioid receptor in all three of the radioligand binding tests. In contrast, JDTic is more selective for the κ receptor than the δ receptor in all three tests.

The following discussion makes it apparent that results from various animal behavioral pharmacology studies correlate better with data from [35 S]GTP γ S studies than they do with those of the radioligand binding studies. JDTic is a potent κ receptor antagonist of selective κ opioid agonist-induced antinociception in mouse, rat, and monkey assays and rat diuresis studies following administration of the selective κ opioid receptor agonist U50,488 for the rat and monkey studies and enadoline for the mouse studies. JDTic has ED₅₀ values of 4.1 (s.c.) and 27.3 mg kg⁻¹ (p.o.) for its ability to antagonize an ED₅₀ dose of the selective κ opioid receptor agonist enadoline in the mouse tail-flick test.^[36] In contrast, JDTic, at doses of 1, 3, 10, 30, and 100 mg kg⁻¹ (s.c.), was without effect when given 24 h before an ED₈₀ dose of the μ opioid receptor selective agonist sufentanil.^[36] A time course study of JDTic (p.o. administration) in combination with enadoline showed significant antagonist activity at 24 h, 7 d, and 28 d relative to the 2 h effect. In a more recent study, JDTic administered i.p. in mice showed significant antagonism of U50,488 up to 21 d in a warm-water tail-withdrawal test.^[37] In another more recent study, JDTic (10 mg kg⁻¹ s.c.) at a 2 h pretreatment time-point blocked U50,488-induced antinociception, but had no effect on morphine-induced antinociception in a rat tail-flick test.^[38]

JDTic was also evaluated for its κ opioid receptor antagonist effect in the squirrel monkey shock titration antinociception test.^[36] A cumulative dose–effect curve for the κ -selective agonist U50,488, alone and in combination with JDTic, was obtained in three monkeys (Table 13). When administered alone, U50,488 increased median shock levels dose-dependently, with peak increases occurring between 5.6 and 10 mg kg⁻¹. JDTic (1.0 mg kg⁻¹) was administered intramuscularly (i.m.), and the U50,488 dose–effect curve was re-determined 2 h and 3, 7, and 10 d later. The results from the U50,488/JDTic combinations are presented in Figure 4. JDTic shifted the U50,488 dose–effect curve to the right, with shifts apparent up to 7 and 10 d following administration of JDTic. ED₅₀ values for U50,488 antinociception are listed in Table 13.

JDTic was compared with nor-BNI for its ability to antagonize U50,488-induced diuresis. During the first 5 h immediately following the administration of JDTic +U50,488 (week 0), JDTic significantly decreased the polyuria at 3, 17, and 100 mg kg⁻¹ (s.c.) with AD₅₀ = 2.81 mg kg⁻¹ (see Table 14 and Figure 5 A).^[36] The antagonism was still present one week after administration, with all doses (0.3, 1, 3, 17 and 100 mg kg⁻¹ (s.c.)) significantly decreasing polyuria, ED₅₀ = 0.41 mg kg⁻¹. The antagonism remained at 2 and 3 weeks, with AD₅₀ values of 1.64 and 73 mg kg⁻¹, respectively. Thus, similar to the antinociception study in mice, JDTic also has a long duration of action in this rat diuresis study.

The antagonism of U50,488-induced diuresis by nor-BNI was significant at week 0 with 1 and 3 mg kg⁻¹ (s.c.) doses and at weeks 1 and 2 at 3 mg kg⁻¹ (s.c.).^[36] Due to the restricted range of doses in the study, no AD₅₀ values were determined (Figure 5 B). JDTic at 0.3 and 3 mg kg⁻¹ (s.c.) produced greater antagonism than nor-BNI at the same mg kg⁻¹ (s.c.) doses.

Depressive disorders are reported to be the most common co-morbid conditions among individuals with cocaine abuse.^[39] In addition, cocaine abusers with depressive symptoms may be especially vulnerable to relapse.^[40, 41] Because commonly used antidepressants have not proven very useful in treating cocaine abuse, compounds with antidepressant activity that operates through a new mechanism are of particular interest. As central administration of the κ opioid receptor nor-BNI was reported to have antidepressant-like effects in the Porsolt forced swim test (FST) in rats,^[42] an FST study was conducted in which JD_{Tic} was compared with nor-BNI and the common antidepressant desipramine.^[43] The results from the study are given in Table 15. The top and bottom of Table 15 show results obtained during the initial FST and retest one week later, respectively. Scores for immobility, swimming, and climbing for a 60 min period are listed in the table. Values in boldface are scores that differ significantly from those of vehicle. Desipramine changed immobility with all doses less than vehicle, swimming with 5.6 mg kg⁻¹ (i.p.) greater than vehicle, and climbing, with all doses greater than vehicle. nor-BNI changed immobility with 1 and 10 mg kg⁻¹ (s.c.) less than vehicle and swimming, with 1 and 10 mg kg⁻¹ (s.c.) greater than vehicle but not climbing. JD_{Tic} changed immobility with the three lower doses less than vehicle, swimming with the three lower doses greater than vehicle, and climbing, although no dose differed from vehicle. At 10 mg kg⁻¹, JD_{Tic} did not differ from vehicle on any of the three measures, an indication that the dose was beyond the behaviorally relevant range.

Results during FST retest are listed at the bottom of Table 15. Desipramine did not differ significantly from vehicle on any measure. nor-BNI did not differ significantly from vehicle on any measure either; however, there was a trend toward decreased immobility and increased swimming. JD_{Tic} changed immobility with the two highest doses less than vehicle, swimming with 1 mg kg⁻¹ (s.c.) greater than vehicle, and climbing with 10 mg kg⁻¹ greater than vehicle.

Because depression and anxiety are often co-morbid in humans, and as selective serotonin re-uptake inhibitor (SSRI) antidepressants are anxiolytic, it was of interest to study JD_{Tic} in animal models of anxiety. Thus, JD_{Tic} was tested to determine if it would affect unlearned fear (anxiety) in the elevated plus maze (EPM) and open field (OF) tests and learned fear in the fear-potentiated startle (FPS) test.^[44] Similar to known anti-anxiety drugs, JD_{Tic} affected behavior in the EPM test in a dose-dependent manner. Rats spent more time in the open arms and made more entries into the open arms of the maze after treatment with 10 mg kg⁻¹ (Figure 6 A). JD_{Tic} did not affect closed-arm entries or maze crosses. The effects of JD_{Tic} on behavior in the EPM are qualitatively similar to those observed with the benzodiazepine drug chlordiazepoxide; they contrast with the effects of the antidepressant fluoxetine, which in this paradigm at 10 mg kg⁻¹ i.p., decreased the percentage time in open arms and open-arm entries (though not significantly). nor-BNI showed a profile almost identical to that of JD_{Tic} when evaluated in the EPM test. When evaluated in the OF test 72 h after i.p. JD_{Tic} administration, JD_{Tic} did not affect locomotor activity at any dose, regardless of whether the data were analyzed as 5 min time bins or total activity over the entire 1 h test sessions, nor did it affect the distance rats traveled in the interior of the OF.^[44]

Five and seven days after the EPM and locomotor tests, the rats were trained [to a condition stimulus (CS)] and tested in the FPS paradigm.^[44] Animals that have learned to associate

light, which is a CS with the foot-shock, typically exhibit a greater startle response (sound) in the presence of the light relative to the dark due to increased fear elicited by the CS. Treatment with JD_{Tic} before FPS training affected the expression of the conditioned response. Percentage of FPS = [(startle in the presence of light) – (startle in the dark)] / (startle in the dark) 0 100. At a dose of 10 mg kg⁻¹, JD_{Tic} decreased FPS (Figure 6 B). As was the case in the EPM, nor-BNI also decreased conditioned fear in the FPS paradigm.

Because stress has often been implicated in relapse to cocaine abuse, JD_{Tic} was tested for its ability to block foot shock-induced stress reinstatement of extinguished responding previously reinforced by cocaine in rats (a cocaine relapse model).^[43] The results are summarized in Figure 7, which plots the number of lever presses seeking cocaine against the oral dose of JD_{Tic}. The dotted horizontal lines indicate the range of the average number of active lever presses emitted across groups of rats during the last extinction sessions immediately preceding the last session. Responding was not significantly affected by the 3 mg kg⁻¹ dose of JD_{Tic} relative to the vehicle group. However, the 10 and 30 mg kg⁻¹ doses of JD_{Tic} significantly decreased levels of reinstatement responding relative to levels of the vehicle-treated group: 39 and 22.75 responses, respectively, relative to 64.75 active responses for vehicle pre-treatment group. The AD₅₀ was 19.95 mg kg⁻¹.

JD_{Tic} was also evaluated for its ability to block cocaine-induced reinstatement in rats.^[43] This test is thought to be a model of a slip back to cocaine-taking relapses in humans. As JD_{Tic} did not prevent reinstatement of cocaine seeking in this model, it is selective for stress-induced reinstatement of cocaine seeking.

JD_{Tic} was evaluated for its ability to block nicotine physical (somatic signs and hyperalgesia) and affective (anxiety-related behavior and conditioned place aversion [CPA]) nicotine withdrawal signs in mice.^[45] Anxiety-related behavior (affective), somatic signs, and hyperalgesia (physical) were measured in mice following 18–24 h withdrawal from chronic nicotine and treatment with either JD_{Tic} or its vehicle. The results showed that nicotine withdrawal alone significantly increased anxiety-related behavior in the plus maze, increased expression of somatic withdrawal signs, and decreased response latencies in the hot-plate test (Figure 8). Eighteen-hour pretreatment with JD_{Tic} (8 mg kg⁻¹ s.c.) significantly blocked all of these nicotine withdrawal signs. JD_{Tic}-treated mice exhibited a loss of anxiety-related behavior, attenuation of somatic signs, and an increased latency on the hot plate. The doses of JD_{Tic} used in this assessment did not significantly affect behavioral responses in saline-infused mice in any withdrawal test and did not precipitate significant nicotine withdrawal signs in nicotine-dependent mice at 1, 8, or 18 h after administration.

A place-conditioning procedure was used to measure effects of JD_{Tic} on expression of a CPA associated with nicotine withdrawal.^[45] Mice receiving chronic infusions of nicotine or saline via a minipump were exposed to conditioning sessions with mecamylamine (a nicotinic acetylcholine receptor antagonist) or its vehicle, and JD_{Tic} was administered 18 h prior to testing. Figure 9 shows that mecamylamine treatment alone (3.5 mg kg⁻¹ s.c.) resulted in a significant CPA in chronic nicotine-exposed mice pretreated with vehicle. Pretreatment with JD_{Tic} (16 mg kg⁻¹ s.c.) 18 h prior to the test day blocked expression of

the mecamlamine-induced CPA. The doses of JDTC used did not produce significant responses in saline-infused mice. In summary, JDTC attenuated the expression of both the physical (somatic signs, hyperalgesia) and affective (anxiety-related behavior, CPA) signs of nicotine-induced withdrawal.

JDTC was evaluated for its effect on alcohol abuse using several different animal models. Pretreatment with JDTC decreased alcohol-withdrawal-induced anxiety (hangover anxiety) using an elevated plus maze test, decreased cue-induced rein-statement of alcohol seeking, but had no effect on stress-induced reinstatement of alcohol seeking in male Wistar rats.^[38] In another study, pretreatment with JDTC was effective at decreasing alcohol seeking and alcohol relapse in P-rats using the Pavlovian spontaneous recovery (PSR) and alcohol deprivation effect (ADE) models, respectively.^[46] In contrast to the self-administration study in male Wistar rats, JDTC did not decrease alcohol maintenance responding in P-rats.^[46] In 2012, JDTC completed pre-IND safety assessment and was advanced into phase I human clinical trials. The results of clinical evaluation have not yet been published.

JDTC analogues and pharmacology

Early SAR studies revealed that the potent and selective κ opioid receptor antagonist activity resulted from: 1) the isoquinoline amino group and the 7-hydroxy group held in a rigid orientation by the 1,2,3,4-tetrahydroisoquinoline structure in its 3*R* attachment to the amide carboxyl; 2) an *S* configuration of the 2-methylpropyl group in the spacer between the piperidine ring and the D-hydroxy-Tic acyl group; and 3) the lack of a substituent on the amide nitrogen atom.^[31, 32, 47]

Several JDTC analogues have since been developed that have sub-nanomolar K_e values for the κ opioid receptor and are >100-fold selective for the κ opioid receptor relative to the μ and δ opioid receptors (Table 16).^[33, 48] Methylation of the phenols in the 7-hydroxy-D-Tic or the 4-(3-hydroxyphenyl) portions to give RTI-5989-212 and RTI-5989-241, respectively, changing the isopropyl substituent adjacent to the amide nitrogen to a *sec*-butyl group to give RTI-5989-194, and adding a methyl group adjacent to the carbonyl group or to the isoquinoline nitrogen to give RTI-5989-240 and RTI-5989-97, respectively, all result in compounds with high potency and selectivity for the κ opioid receptor. Moreover, adding a second methyl substituent to RTI-5989-241 to give RTI-5989-251, replacement of the isopropyl group in RTI-5989-241 with a *sec*-butyl group, and replacement of the hydroxy group in the 4-(3-hydroxyphenyl) portion of JDTC with a carboxamide group led to antagonists with high potency and selectivity for the κ opioid receptor.

RTI-5989-194, RTI-5989-212, and RTI-5989-230 were evaluated for their ability to block U50,488-induced diuresis, and RTI-5989-194 was also evaluated for its ability to prevent foot-shock-induced reinstatement of cocaine self-administration.^[49] RTI-5989-194 attenuated U50,488-induced diuresis through s.c., i.p., or p.o. administration, and similar to JDTC effectiveness, increased one week following administration. RTI-5989-230 blocked U50,488-induced diuresis at 24 h and 8 d at doses of 1, 10, and 30 mg kg⁻¹ i.p.; 15 d at doses of 10 and 30 mg kg⁻¹ i.p.; and 22 and 29 d at 30 mg kg⁻¹ i.p. However, unlike JDTC and RTI-5989-194, it was ineffective at oral doses of 3, 10, and 30 mg kg⁻¹. RTI-5989-212

was ineffective in blocking U50,488-induced diuresis when administered at 0.3, 1, 3, or 10 mg kg⁻¹ s.c. or 30 mg kg⁻¹ i.p. Although RTI-5989-194 was not as effective as JDTic, it did decrease foot-shock-induced cocaine reinstatement of self-administration to the level of responding on the last day of extension.

In another study, JDTic, RTI-5989-97, RTI-5989-194, RTI-5989-212, RTI-5989-240, and RTI-5989-241 were tested for their ability to antagonize U50,488-induced antinociception in C57BL/6 wild-type mice over a 28 day period using a warm-water tail-withdrawal assay,^[37] and JDTic and all five analogues antagonized the U50,488-induced antinociception. JDTic as well as RTI-5989-97, RTI-5989-194, and RTI-5989-241 had long durations of action (>7 d), and RTI-5989-212 and RTI-5989-240 had durations of action <1 d. Interestingly, JDTic and the three analogues that had long durations of action in the warm-water tail-withdrawal assay stimulated JNK, whereas the two short-acting analogues, RTI-5989-212 and RTI-5989-240, failed to activate JNK.^[37]

A very recent paper reported in vitro and in vivo studies of BU09059 (mixture of diastereomers), an analogue of JDTic, which differs from JDTic by having an ester function in place of the isopropyl group in JDTic.^[50] Using [³H]diprenorphine as the radioligand along with cloned κ , μ , and δ receptors, the compound had K_i values of 1.72, 26.5, and 1060 nM at the κ , μ , and δ receptors, respectively. In an in vitro efficacy determination using an isolated guinea pig tissue and mouse vas deferens assays, BU09059 had a K_B value of 2.39 nM at the κ receptors and had no significant effects on the E_{\max} values of DAMGO (μ) and DPDPE (δ). BU09059 blocked U50,488-induced antinociception in a warm-water tail-withdrawal assay 1 h post-injection in mice (i.p. administration). The maximal effect was at 24 h; however, some activity remained 7 and 14 d post-injection.

X-ray Crystallography

One of the holy grails of opioid receptor research has been to obtain X-ray structures of the κ , μ , and δ opioid receptor subtypes. In 2012 the crystal structure of the human κ opioid receptor in complex with JDTic was determined at 2.9 Å resolution.^[51] The crystal structure showed a tight fit of JDTic forming ionic, polar, and hydrophobic interactions with the receptor. Figure 10 shows a few of the more important interactions. The protonated amines of both the piperidine and isoquinoline moieties of JDTic form salt bridges with the Asp138 side chain. This strong ionic interaction with the two amino groups holds JDTic in the inverted V shape as shown in Figure 10. This conformation was also found in the X-ray structure of JDTic.^[51] The isopropyl group of JDTic reaches deep into the orthosteric pocket to form a hydrophobic interaction with the Trp287 side chain. Even though the crystal structure does not show receptor interactions for either of the phenolic groups, the structure does show several structured water molecules (shown as red spheres in Figure 10) that could mediate their polar interactions. The binding pocket is partially capped by the ELC.2 hairpin and the conserved disulfide shown at the bottom left of Figure 10. In addition to the X-ray structure of JDTic bound to the κ opioid receptor in the inactive state, the X-ray structures for the μ , δ , and ORL-1 receptors bound to inhibitors of these receptors in the inactive state were reported.^[52–54] These X-ray structures provide a highly useful tool to better understand

opioid ligand receptor interactions and, thus, valuable information for the design of new κ , μ , δ , and ORL-1 opioid receptor antagonists and agonists.

Summary

This review celebrates the discovery and rich history of the N-substituted *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine class of pure opioid antagonists. Zimmerman's publication of the pharmacophore in 1978 spawned three decades of research in academic and pharmaceutical laboratories investigating the pharmacophore as a lead structure. The antagonist activity of the class is a consequence of the equatorial orientation of the 4-aryl ring, mandated by the diaxially disposed *trans*-3,4-dimethyl groups. This fact is supported by numerous studies using flexible and constrained analogues and is corroborated by the recently published X-ray crystal structure of JD_{Tic} in complex with the κ opioid receptor. Early SAR studies revealed the importance of the 3-hydroxyphenyl substitution, as found in LY255528, for receptor affinity and antagonist potency. This substitution was carried forward into all future analogue designs. Derivatization of the piperidine ring nitrogen atom permitted "fine tuning" of the pharmacophore's physicochemical and other drug-like properties, culminating in the discovery and development of ENTEREG®, a peripheral μ antagonist for the treatment of post-operative ileus and most recently, the clinical candidate, JD_{Tic}, a selective, centrally acting potent and selective κ opioid receptor antagonist. The observation that both N-substituted *trans*-3,4-dimethyl-4-(3-hydroxy-phenyl)piperidines, JD_{Tic} and RTI-5989-194, prevented foot-shock-induced reinstatement of cocaine seeking in rats suggests the implication of the dynorphine- κ opioid receptor system in cocaine relapse.

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Biographies



F. Ivy Carroll received his BS degree in chemistry from Auburn University (Alabama, USA) and his PhD in chemistry from the University of North Carolina. He joined RTI in 1961 as a Research Chemist and rose steadily to the position of Vice President of the Chemistry and Life Sciences Group, 1996–2001. Dr. Carroll also served as Director of the Center for Organic and Medicinal Chemistry from 1975 to 2007. He is presently a Distinguished Fellow for Medicinal Chemistry. Dr. Carroll has varied research interests, but since 1990, a major thrust of his research efforts has involved the development of pharmacotherapies for substance abuse (cocaine, nicotine, methamphetamine, opioids, and ethanol) and other CNS disorders. Dr. Carroll has published 475 peer-reviewed publications, 34 book chapters, and 36 patents.



Roland E. Dolle is currently Senior Director of Medicinal Chemistry at Cubist. After earning his PhD in organic chemistry from the University of Pennsylvania in 1985, Dr. Dolle began his career in drug discovery at Smith-Kline Beckman (now GSK). He has since held research positions of increasing responsibility at several bio-pharmaceutical companies. His research teams have advanced 18 drug candidates into preclinical and clinical development. Dr. Dolle has published 245 abstracts/articles and is an inventor on 70 issued US patents. Dr. Dolle's interest in opioid chemistry and pharmacology started in 2000 at Adolor Corporation, where he directed discovery research in the design and synthesis of novel μ , κ , and δ opioid receptor agonists and antagonists as medicines for pain management.

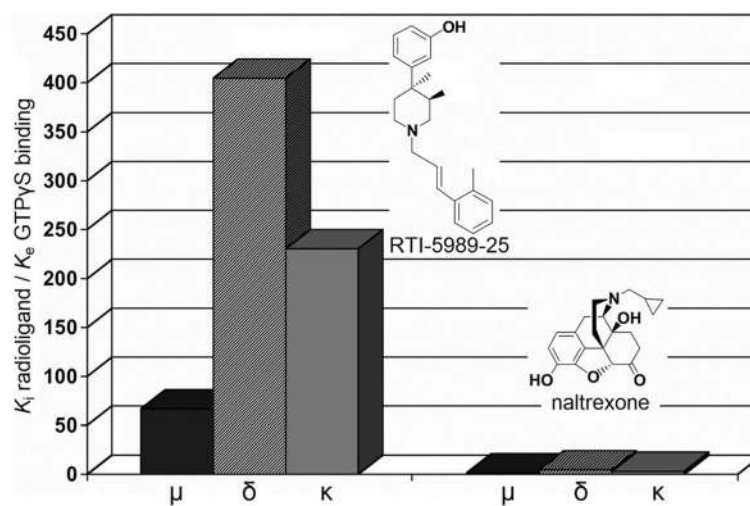


Figure 1.
Comparison of radioligand binding and functional assays for RTI-5989-25 and naltrexone.

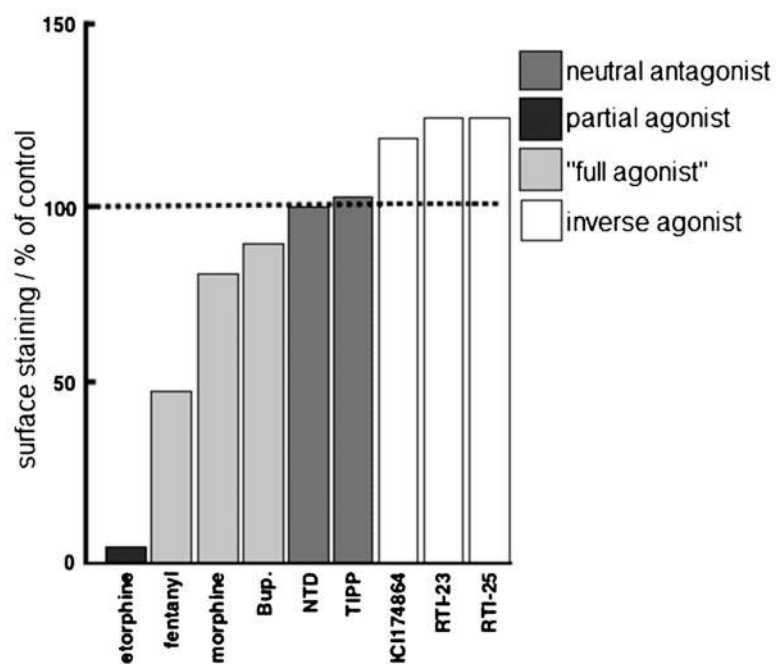


Figure 2.
Change in surface receptors after 24 h treatment of HEK293 DOR cells with RTI-5989-23, RTI-5989-25, and several other opioids.

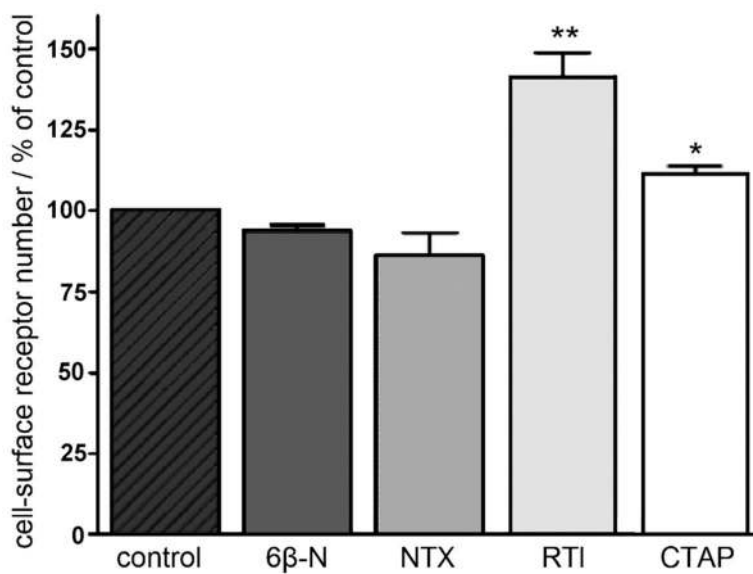


Figure 3.

Cell-surface receptor levels in HEK293 FLAG-μ cells treated with 6β-naltrexol (6β-N), naltrexone (NTX), RTI-5989-25 (RTI), or CTAP. (Modified from Ref. [26]; reprinted with permission, Copyright 2009, *British Journal of Pharmacology*.)

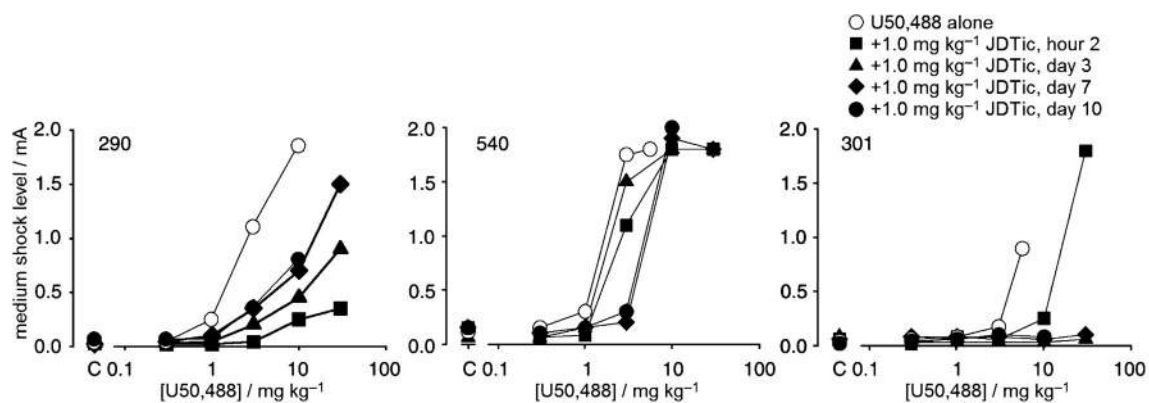


Figure 4.

Effects of intramuscular U50,488 alone and for U50,488 antinociception in combination with 1.0 mg kg⁻¹ intramuscular JDtic on median shock level in three individual monkeys. (Data taken from Ref. [36]; reprinted with permission, Copyright 2004, *European Journal of Pharmacology*.)

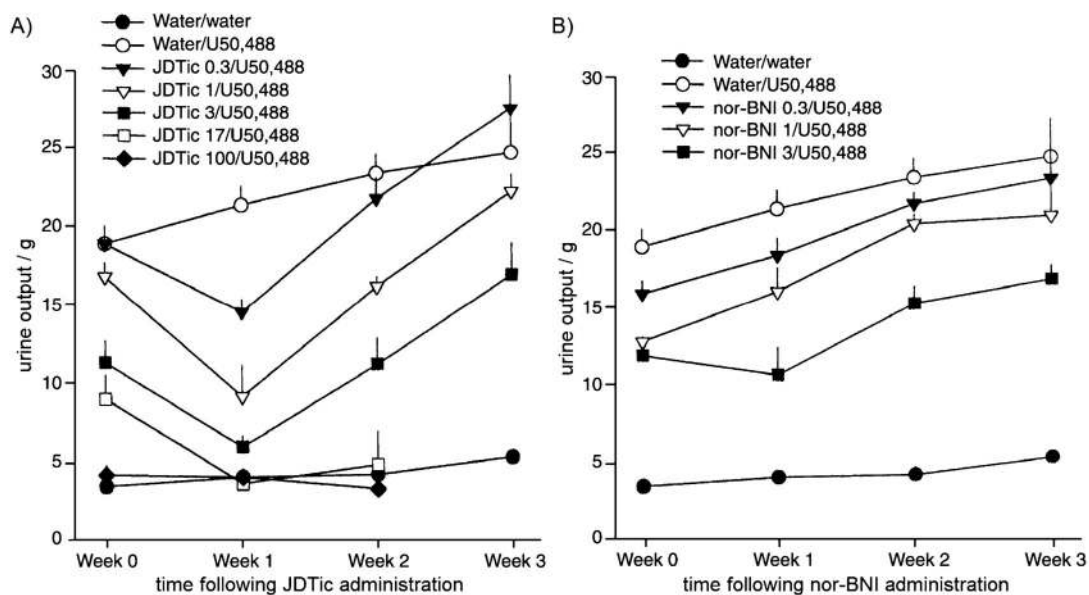


Figure 5.

Antagonism of U50,488-induced urine output by various doses (mg kg^{-1}) of A) JDtic and B) nor-BNI. JDtic and nor-BNI were only administered once at Week 0. (Data taken from Ref. [36]; reprinted with permission, Copyright 2004, *European Journal of Pharmacology*.)

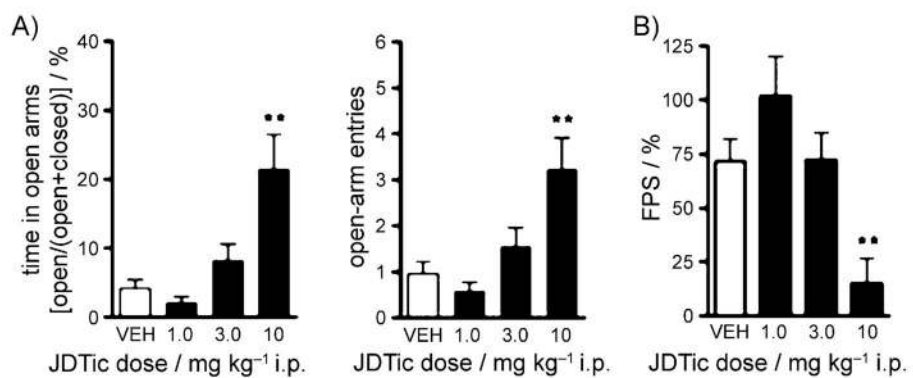


Figure 6. Effect of JDtic on A) elevated plus maze behavior and B) fear-potentiated startle (FPS); VEH =vehicle. (Data taken from Ref. [44]; reprinted with permission, Copyright 2007, *Journal of Pharmacology and Experimental Therapeutics*.)

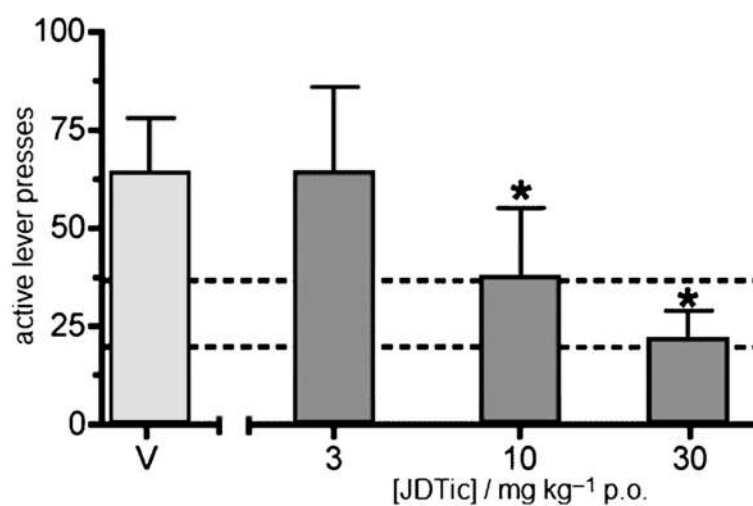


Figure 7.
Effect of JDtic on stress-induced reinstatement of cocaine-reinforced lever pressing. V
=vehicle.

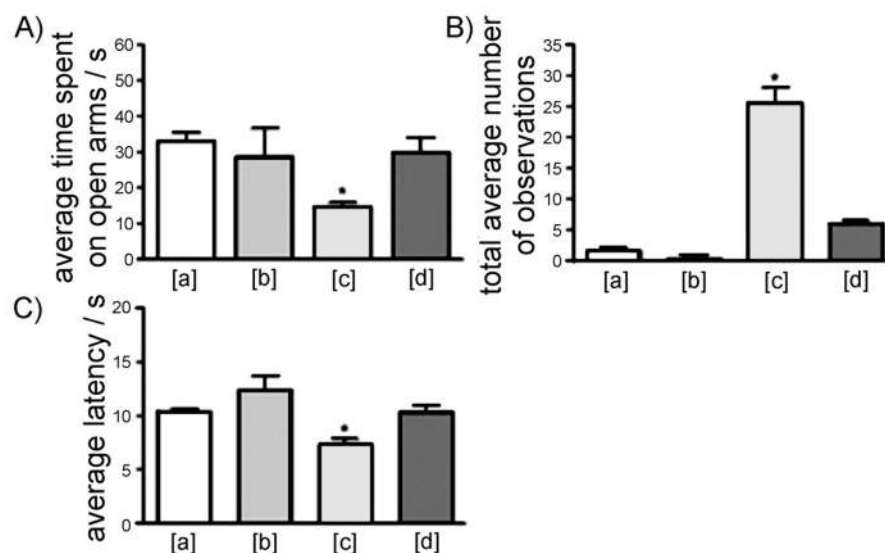


Figure 8.

Physical and somatic nicotine withdrawal is blocked by pretreatment with JD_TTic. Mice chronically infused with nicotine for seven days ($36 \text{ mg kg}^{-1} \text{ d}^{-1}$) were withdrawn from nicotine for 18–24 h. A significant A) anxiety-related response, B) increase in somatic withdrawal signs, and C) hyperalgesia response were observed in nicotine-withdrawn mice. Treatments: [a] saline MP-vehicle, [b] saline MP-JD_TTic 8 mg kg^{-1} , [c] nicotine MP-vehicle, [d] nicotine MP-JD_TTic 8 mg kg^{-1} ; * $p < 0.05$ versus saline and JD_TTic control groups, and versus nicotine–JD_TTic group; MP = minipump. (Data taken from Ref. [45]; reprinted with permission, Copyright 2010, *Psychopharmacology*.)

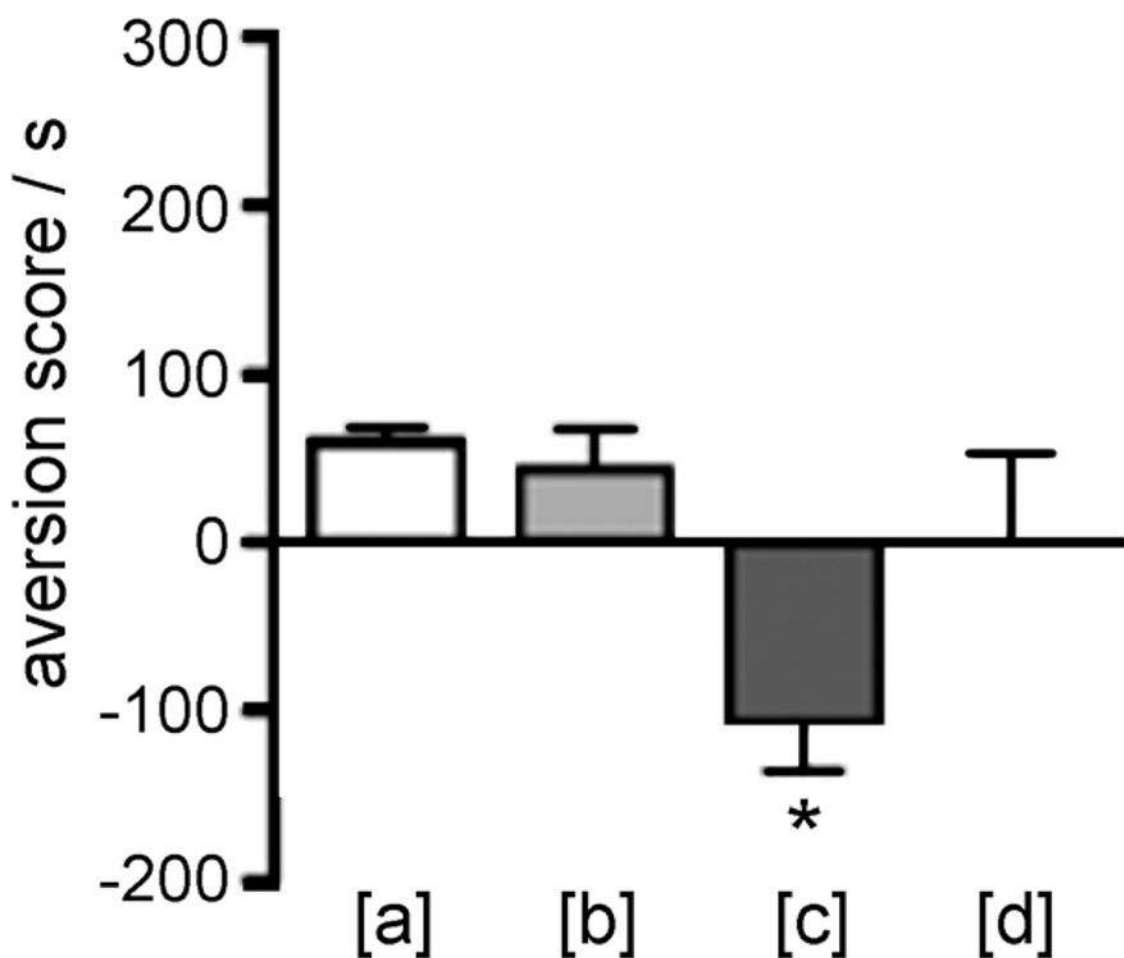


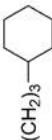
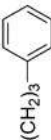
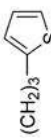

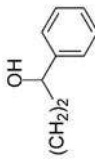
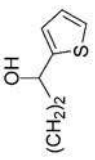
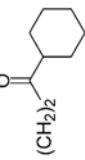
Figure 9. Nicotine withdrawal conditioned place aversion is blocked by pre-treatment with JDTic. Expression of aversion was blocked by an 18 h pre-treatment with JDTic (16 mg kg⁻¹, s.c.). Treatments: [a] saline-saline, [b] saline-JDTic 16 mg kg⁻¹, [c] nicotine-mec (3.5 mg kg⁻¹)-vehicle, [d] nicotine-mec-JDTic 16 mg kg⁻¹; **p* < 0.05 versus saline groups and nicotine-JDTic; mec =mecamylamine. (Data taken from Ref. [45]; reprinted with permission, Copyright 2010, *Psychopharmacology*.)

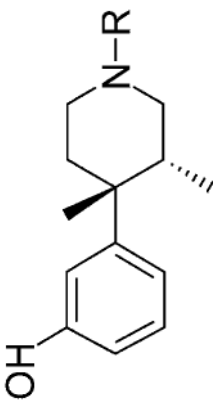
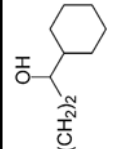


Figure 10.
Binding of JDTic in the human κ opioid receptor crystal structure.

Table 1

In vitro and in vivo pharmacological properties of **3 a–h**.

Compd	R	Receptor Binding:		Opioid Antagonist Activity: AD ₅₀ [mg kg ⁻¹ s.c.]			Feeding Inhibition:
		<i>K</i> _i [nM]	Mouse Writhing Assay ^[a]	Rat Diuresis ^[b]	ED ₅₀		
		μ, [³ H]Nal	κ, [³ H]EKC	μ, morphine	κ, U50,488	κ, bremazocine	[mg kg ⁻¹ s.c.] ^[c]
3a		0.49	2.3	0.35	0.19	1.0	0.12
3b		0.69	15	2.4	5.3	1.1	1.3
3c		0.56	6.1	0.22	0.30	1.0	0.07
3d		0.26	10	0.12	0.24	1.9	0.06
3e ^[d]		1.0	12.5	0.05	0.92	2.5	0.50
3f ^[d]		0.5	11.7	0.07	0.14	1.4	0.07
3g		0.34	3.9	0.12	0.26	–	0.13

<div>  </div>		Receptor Binding:		Opioid Antagonist Activity: AD ₅₀ [mg kg ⁻¹ s.c.]		Feeding Inhibition:	
		K _i [nM]	Mouse Writhing Assay ^[a]	Rat Diuresis ^[b]	ED ₅₀		
Compd	R	μ, [³ H]Nal	κ, [³ H]EKC	μ, morphine	κ, U50,488	κ, bremazocine	[mg kg ⁻¹ s.c.] ^[c]
3a ^[d]		0.29	4.8	0.07	0.14	0.49	0.05

^[a] Dose required for 50 % reduction in analgesic response to either morphine (1.25 mg kg⁻¹ s.c.) or U50,488 (2.5 mg kg⁻¹ s.c.).

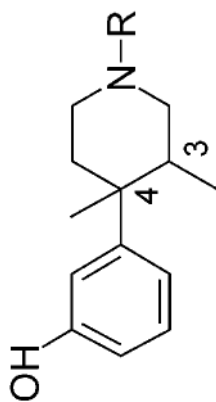


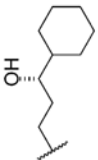
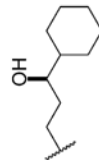
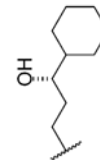
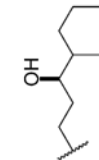
^[b] Dose required to decrease 5 h bremazocine (0.08 mg kg⁻¹ s.c.)-induced urination by 50 %.

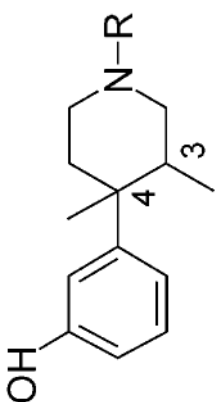
^[c] Dose required to decrease food consumption by 20 %.

^[d] Mixture of 3,4,3'-stereoisomers. Data taken from Ref. [6].

Table 2

Comparison of in vitro and in vivo pharmacological properties of various isomers.

Receptor Binding:			Opioid Antagonist Activity: AD ₅₀ [mg kg ⁻¹ s.c.]			Feeding Inhibition:			
Compd	R	K _i [nM]	Mouse Writhing Assay ^[a]			Rat Diuresis ^[b]			
			μ, [³ H]Nal	κ, [³ H]EKC	δ, [³ H]DADL	μ, morphine	κ, U50,488	κ, brenazocine	[mg kg ⁻¹ s.c.] ^[c]
	(CH ₂) ₃ - 	0.2	3.29	10.27	0.03	0.25	1.18		0.11
	(CH ₂) ₃ - 	1.8	12.5	–	0.24	0.65	1.66		0.4
[(+)-3 <i>R</i> ,4 <i>R</i> ,3'- <i>S</i>]- 3 h LY255582		0.41	2.0	5.2	0.015	0.05	0.38		0.04
[(+)-3 <i>R</i> ,4 <i>R</i> ,3'- <i>R</i>]- 3 h		2.4	11.4	–	0.03	0.36	1.1		0.16
[(-)-3 <i>S</i> ,4 <i>S</i> ,3'- <i>S</i>]- 3 h		1.4	6.5	–	0.13	0.24	1.6		0.11
[(-)-3 <i>S</i> ,4 <i>S</i> ,3'- <i>R</i>]- 3 h		2.2	14.3	–	0.52	0.52	5.2		>1.25 ^[d]
naloxone		6.3	66	32	0.08	1.1	3.5		1.2 ^[e]

		Receptor Binding:		Opioid Antagonist Activity: AD ₅₀ [mg kg ⁻¹ s.c.]		Feeding Inhibition:		
Compd	R	μ, [³ H]Nal	κ, [³ H]EKC	δ, [³ H]DADL	μ, morphine	κ, U50,488	κ, bremazocine	[mg kg ⁻¹ s.c.][<i>c</i>]
naltrexone		0.56	6.0	6.0	0.05	0.06	2.5	1.7[<i>f</i>]

[*a*] Dose required for 50 % reduction in analgesic response to either morphine (1.25 mg kg⁻¹ s.c.) or U50,488 (2.5 mg kg⁻¹ s.c.).

[*b*] Dose required to decrease 5 h bremazocine (0.08 mg kg⁻¹ s.c.)-induced urination by 50 %.

[*c*] Dose required to decrease food consumption by 20 %.

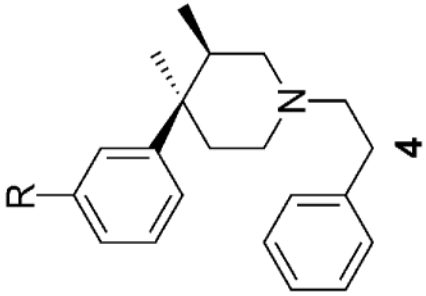
[*d*] Effect on feeding was minimal and not dose-related.

[*e*] Measured over a 2 h time period due to short duration of action.

[*f*] The maximum effect achieved was ~20 % and was not dose-related. Data taken from Ref. [6].

Opioid receptor (μ , κ , and δ) binding data and in vitro antagonist activity (μ) of *N*-phenethyl-*trans*-3,4-dimethyl-4-(3-substituted phenyl)piperidines.

Table 3

						
Compd	R	K_i [nM] ^[a] or inhib. [%] ^[c]	μ	IC ₅₀ [nM] ^[b]	K_i [nM] ^[a] or inhib. [%] ^[c]	δ
naloxone		3.7	7.3	9.2		33
4a	OH	1.9	2.0	17		33
4b	CONH ₂	4.7	1.9	95		72

^[a]Compound potencies were determined by testing the ability of each compound, at a range of concentrations, to inhibit binding of the nonselective opioid antagonist, [³H]diprenorphine, to cloned human μ , κ , and δ opioid receptors, expressed in separate cell lines; K_i values are geometric means computed from at least three separate determinations.

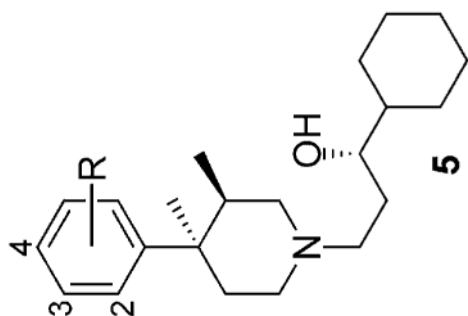
^[b]Antagonist potencies were assessed by the ability to inhibit agonist (loperamide)-stimulated [³⁵S]GTP- γ S binding to membranes containing the cloned human μ opioid receptor.

^[c]Percent inhibition of [³H]diprenorphine binding to the cloned human μ , κ , and δ opioid receptors using a competitor concentration of 10 μ M. Data taken from Ref. [10].

Opioid receptor binding data (K_i) and [^{35}S]GTP γ S antagonist activity (K_b) of LY255582 and **5 a–l**.

Table 4

Compd	R	K_i [nM]			K_b [nM]		
		μ	κ	δ	μ	κ	δ
LY255582	3-OH	0.1	4.7	4.8	0.04	0.3	1.2
5a	2-OH	410	1340	6993[a]	40.5	559.1	>2038
5b	4-OH	30.4	224.3	364.0[a]	1.3	92.2	122.4
5c	H	7.7	749	169	1.6	40.6	47.1
5d	3-NH ₂	1.9	55.2	44.9	0.7	15.7	24.7
5e	3-NHCONHCH ₃	1.8	695	51.3	0.3	9.8	14.3
5f	3-NHCOCH ₃	2.4	133	64.9	0.4	16.4	25.8
5g	3-NHSO ₂ CH ₃	9.4	192	251	nd	nd	nd
5h	3-NHCO ₂ CH ₃	1.0	10.6	14.2	0.1	0.6	1.9
5i	3-NHCO ₂ Et	1.2	12.3	24.6	0.1	0.7	3.2
5j	3-CO ₂ CH ₃	43.3	2326	1635	34.6	984	1242
5k	3-CO ₂ H	143	3015	3507	nd	nd	nd

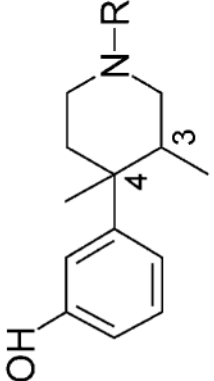
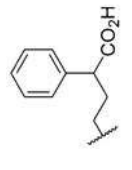
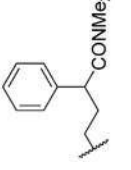
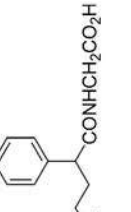
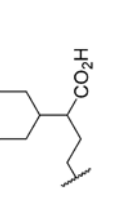


Compd	R	K_i [nM]				K_b [nM]			
		μ	κ	δ	μ	κ	μ	κ	δ
5l	3-CONH ₂	0.2	12.7	6.4	0.1	1.9			2.8

[a] K_i values were measured by using [³H]diprenorphine instead of [³H]bremazocine; nd: not determined. Data taken from Ref. [14].

Table 5

Comparison of pharmacological properties of peripheral-acting μ opioid receptor antagonists including ENTEREG®.

Compd	R	K_i (μ) [nM]	AD ₅₀ [mg kg ⁻¹ s.c.] [a]	ED ₅₀ [mg kg ⁻¹ s.c.] [b]	AD ₅₀ /ED ₅₀ [c]
6a: 3 <i>RS</i> ,4 <i>RS</i>		2.01	0.64	0.12	5.3
6b: 3 <i>RS</i> ,4 <i>RS</i>		1.17	0.54	0.06	9
6c: 3 <i>RS</i> ,4 <i>RS</i>		1.89	40	0.15	267
6d: 3 <i>RS</i> ,4 <i>RS</i>		0.66	1.50	0.02	75
6e: 3 <i>RS</i> ,4 <i>RS</i>		0.91	15.3	0.30	51

Compd	R	K_i (μ) [nM]	AD ₅₀ [mg kg ⁻¹ s.c.] ^[a]	ED ₅₀ [mg kg ⁻¹ s.c.] ^[b]	AD ₅₀ /ED ₅₀ ^[c]
6f : 3 <i>RS</i> ,4 <i>RS</i>		1.65	3.90	0.17	23
6g : 3 <i>RS</i> ,4 <i>RS</i>		0.89	>40	0.028	>1428
6h : 3 <i>R</i> ,4 <i>R</i> ^[d]		0.77	9.00	0.04	225
naloxone		3.7	0.08	0.05	1.6

^[a] Antagonism of morphine analgesia: dose calculated for 50 % decrease in the analgesic response to morphine (1.25 mg kg⁻¹ s.c.) in the writhing assay.

^[b] Precipitation of diarrhea in morphine-dependent mice: dose calculated to produce diarrhea in 50 % of the mice treated.

^[c] Index of relative peripheral activity. Data taken from Refs. [18] and [19].

^[d] Also known as LY246582; alvimopan; ENTEREG®.

Table 6

Radioligand binding results from assays with guinea pig brain membranes.

Compd	$K_i \pm SD$ [nM] ^[a]		
	[³ H]DAMGO	[³ H]DADLE	[³ H]U69,593
LY255582	0.32	198	28.0
RTI-5989-23	1.12	168	35.8
RTI-5989-25	0.86	142	38.9
RTI-5989-1	0.74	322	122
RTI-5989-2	11.4	931	298
naltrexone	1.39	94.9	4.71

^[a]Data taken from Ref. [22]

Table 7

Comparison of inhibition of [³⁵S]GTPγS binding in guinea pig caudate stimulated by selective opioid receptor agonists by N-substituted *trans*-3,4-dimethyl-(3-hydroxyphenyl)piperidine analogues.

Compd	<i>K_e</i> [nM] ^[a]		
	μ (DAMGO)	δ (SNC-80)	κ (U69,593)
naltrexone	0.930	19.3	2.06
LY255582	0.021	0.312	0.330
RTI-5989-1	0.039	1.48	1.04
RTI-5989-23	0.026	1.07	0.567
RTI-5989-25	0.013	0.355	0.170

^[a]Data taken from Ref. [22].

Table 8

Comparison of potencies and efficacies of RTI-5989-25 and RTI-5989-23 with ICI174864 inverse agonists in [³⁵S]GTPγS binding assays in HEK293 DOR cells.^[a]

Compd	IC ₅₀ [nM]	Stimulation [³⁵ S]GTPγS Binding/Control [%]
ICI174864	176	75
RTI-5989-23	8.2	65
RTI-5989-25	6.6	66

^[a]Data taken from Ref. [25].

Table 9Opioid antagonist affinities for μ opioid receptor in C6 glioma cells.

Compd	Competitive Binding	
	Tris +Na ⁺ , GTP γ S	K_i [nM] ^[a] (p <i>K_i</i>)
naltrexone	0.38 \pm 0.08 (9.42)	0.46 \pm 0.21 (9.33)
RTI-5989-25	0.062 \pm 0.024 (10.21)	0.011 \pm 0.005 [*] (10.96)

^[a] K_i values were determined by competitive displacement of [³H]diprenorphine (0.2 nM) binding in 50 mM Tris buffer (pH 7.4) in the presence and absence of 100 mM NaCl and 10 μ M GTP γ S;

^{*} p < 0.05 compared with Tris buffer. Data taken from Ref. [26].

Table 10

Opioid receptor (μ , κ , and δ) binding data and in vitro antagonist activity (μ) of **4 a**, **7 a**, **8 a**, **9**, and **10**.

Compd	IC ₅₀ [nM] ^[a]		K _i [nM] ^[b]	
	μ	μ	κ	δ
4a	1.1	1.8	17	33
7a	0.54	0.62	9.0	31
8a	— ^[c]	0.90	65	2.1
9	1.1	3.6	18	89
10	1.8	0.47	16	57

^[a] Antagonist potencies were assessed by the ability to inhibit agonist (loperamide)-stimulated [³⁵S]GTP γ S binding to membranes containing the cloned human μ opioid receptor.

^[b] Compound potencies were determined by testing the ability of each compound, at a range of concentrations, to inhibit binding of the nonselective opioid antagonist, [³H]diprenorphine, to cloned human μ , κ , and δ opioid receptors, expressed in separate cell lines; K_i values are geometric means and 95 % confidence intervals computed from at least three separate determinations.

^[c] Compound **8** is an agonist: EC₅₀ (μ) = 53 nM, 95 % C.I. = 31–93. Data taken from Ref. [28].

Table 11

Comparison of inhibition of opioid agonist-stimulated [³⁵S]GTPγS binding against human and guinea pig opioid receptors for JDTic and nor-BNI.

Compd	μ (DAMGO)	δ (DPDPE)	K _e [nM]	κ (SNC-80)	κ (U69,593)	μ/κ	δ/κ
JDTic ^[a,c]	3.41	79.3		0.01		341	7930
JDTic ^[a,d]	25.1	76.4		0.02		1255	3830
JDTic ^[a,e]	6.58	168		0.098		67	1718
JDTic ^[b,f]	2.16	–	>300	0.02		108	>15 000
nor-BNI ^[a,c]	19	4.4		0.04		475	115
nor-BNI ^[a,d]	26	29		0.05		520	580
nor-BNI ^[a,e]	32.9	14.1		0.80		41	18
nor-BNI ^[b,f]	16.7	–	10.2	0.038		440	268

^[a]Cloned human μ, δ, and κ opioid receptors.

^[b]Guinea pig.

^[c]Data provided by NIDA from the OTDP and taken from Ref. [31].

^[d]Data obtained from studies conducted at RT1 and taken from Ref. [33].

^[e]Studies for these data (K_b values) were conducted at Lilly Research Laboratories; taken from Ref. [34].

^[f]Data provided by NIDA Intramural Research Program (IRP) and taken from Ref. [31].

Table 12
Comparison of radioligand binding results at the μ , δ , and κ opioid receptors for JDTic and nor-BNI.

Compd	K_i [nM]		μ/κ	
	μ	δ	μ	δ/κ
JDTic ^[a]	3.73	301	0.32	12
nor-BNI ^[a]	65	86	1.09	60
JDTic ^[b]	0.96	29.6	0.41	2.3
nor-BNI ^[b]	21	5.7	0.2	105
JDTic ^[c]	11.5	188	0.059	194
nor-BNI ^[c]	32.4	6.56	0.15	216
				44

^[a] Rat brain receptors were used for the μ and δ receptors, and guinea pig brains were used for the κ receptor; [³H]DAMGO, [³H]DADLE, and [³H]U69,593 were used for the μ , δ , and κ receptors, respectively; data taken from Ref. [31].

^[b] Human cloned μ , δ , and κ receptors were used; [³H]DAMGO, [³H]DPDPE, and [³H]U69,593 were used for the μ , δ , and κ receptors, respectively; data taken from Ref. [31].

^[c] Human cloned receptors were used for the μ , δ , and κ receptors; [³H]diprenorphine was used for all three receptors; data taken from Ref. [34].

ED₅₀ values for U50,488 antinociception when administered alone and in combination with JD₁Tic in squirrel monkey opioid antagonist assays.

Table 13

Monkey #	ED ₅₀ [mg kg ⁻¹ i.m.]/[a]				
	alone, at 15 min	2 h post-JD ₁ Tic	3 d post-JD ₁ Tic	7 d post-JD ₁ Tic	10 d post-JD ₁ Tic
290	2.61	>30	>30	14.89	16.63
540	1.62	2.53	1.90	5.17	4.72
301	11.42	16.78	>30	>30	>30

[a] JD₁Tic administered at 1.0 mg kg⁻¹ i.m.; data taken from Ref. [36].

Table 14

AD₅₀ values to block U50,488 antagonism by JD_{Tic} in rat diuresis assays.

Week	AD ₅₀ [mg kg ⁻¹] ^[a]
0	2.81
1	0.41
2	1.64
3	>3

^[a] 5 h data, taken from Ref. [36].

Table 15

Effect of desipramine, nor-BNI, and JD_{Tic} on mean scores in rat FST.^[a]

Test	Vehicle	Desipramine	nor-BNI			JD _{Tic}		
Dose [mg kg ⁻¹]:	-	5.6	10	17	0.3	1	3	10
<i>Main test</i>								
Immobile	40	22	23	20	34	27	31	34
Swim	11	20	13	14	18	22	17	16
Climb	9	18	24	26	8	12	12	11
<i>Retest one week later with no injections</i>								
Immobile	51	43	43	47	45	38	46	35
Swim	2	4	4	5	6	6	3	4
Climb	7	13	14	9	9	17	12	22

^[a]Results following the FST during the initial (top) and retest (bottom) conditions that followed one week after drug administration. Results are shown for vehicle, desipramine, nor-BNI, and JD_{Tic} treatment groups for scores obtained for immobility time, swimming time, and climbing time. Boldface values indicate statistically significant difference. Data taken from Ref. [43].

Table 16

Inhibition of agonist stimulated [³⁵S]GTPγS binding by compounds in cloned human MOR, DOR, and KOR.

RTI-5989- [a]	K _e [nM]									
	R ¹	R ²	R ³	R ⁴	R ⁵	μ (DAMGO)	δ (DPDPE)	κ (U69,593)	μ/κ	δ/κ
JDTC	H	OH	H	H	H	3.41	79.3	0.02	341	7930
212[a]	CH ₃	OH	H	H	H	51	118	0.06	850	1966
241[a]	H	OCH ₃	H	H	H	24	21.2	0.037	649	573
194[a]	H	OH	CH ₃	H	H	3	24	0.03	100	800
240[a]	H	OH	H	CH ₃	H	3.6	854	0.03	120	28500
97	H	OH	H	H	CH ₃	210	491	0.16	1313	3070
251[a]	H	OCH ₃	H	CH ₃	H	123	2200	0.26	473	8500
255[a]	H	OCH ₃	CH ₃	CH ₃	H	59.7	2100	0.52	114	4040
230[b]	H	CONH ₂	H	H	H	21	480	0.12	175	400

[a] Data taken from Ref. [33].

^{b/}Data taken from Ref. [48].

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