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Letters

Discovery of the First N-Substituted 4β-Methyl-5-(3-hydroxyphenyl)morphan To Possess Highly Potent and Selective Opioid δ Receptor Antagonist Activity

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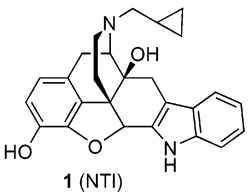
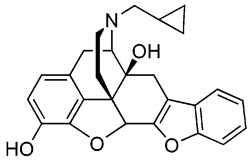
Abstract: A structurally novel opioid δ receptor selective antagonist has been identified. This compound, (+)-5-(3-hydroxyphenyl)-4-methyl-2-(3-phenylpropyl)-2-azabicyclo[3.3.1]-non-7-yl-(1-phenyl-1-cyclopentane)carboxamide [(+)-KF4, (+)-**4**], showed a K_e value of 0.15 nM in the [^{35}S]GTPγS functional assay. (+)-KF4 is also a δ inverse agonist with an IC_{50} value of 1.8 nM. To our knowledge, this is the first potent and selective δ opioid receptor antagonist from the 5-phenylmorphan class of opioids.

It is now well established that opioid receptors belong to the superfamily of G-protein-coupled receptors (GPCRs). Distinct cDNAs encoding the μ, δ, and κ receptors have been cloned, and studies with opioid receptor knockout mice have clarified the role each receptor type plays in mediating effects of morphine.[1,2] Since the discovery of the three distinct opioid receptors, researchers studying the underlying mechanisms of opioid activity have sought highly potent and receptor subtype selective agonists and antagonists.[3] In recent years, the δ opioid receptor has received considerable attention. Numerous studies have been directed toward the development of δ agonists as potentially new analgesics with reduced side effects relative to μ opioid agonists.[4−7] In addition, studies have also suggested that δ opioid antagonists are involved in a number of biological processes.[8] For example, δ opioid antagonists might be useful in the treatment of L-DOPA-induced dyskinesia in Parkinson's disease,[9] alcohol abuse,[10] used as potential antitussive drugs,[11] and in the regulation of tumor cell growth.[12−14]

Very few pure opioid receptor antagonists selective for the δ subtype have been reported. By applying the message−address concept of Schwyzer[15] to naltrexone, Portoghese developed receptor subtype selective antagonists for the δ receptor. Attachment of a properly aligned phenyl ring (δ address) to naltrexone (the opioid message) to act as a mimic for Phe^4 of the enkephalins provided the δ selective antagonists naltrindole (NTI, **1**), naltriben (NTB, **2**), and benzylidenenaltrexone (BNTX, **3**). Even though NTI and NTB have proven highly



1 (NTI)



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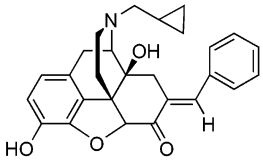

3 (BNTX)

useful for the characterization of the δ opioid receptor, Takemori et al. reported that these compounds showed some agonist effects.[16,17] More recently, McLamore et al. reported that modification of the N-substituent of NTI resulted in major changes in affinity, selectivity, and potency of this class of compounds.[18] A few δ opioid antagonists have been developed from other classes of opioids.[19]

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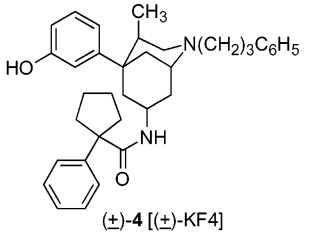
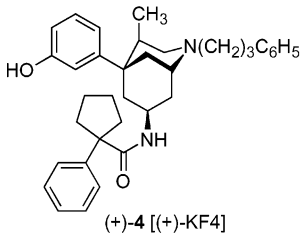
In this paper, we describe the synthesis of (±)-, (+)-, and (−)-5-(3-hydroxyphenyl)-4-methyl-2-(3-phenylpropyl)-2-azabicyclo[3.3.1]non-7-yl-(1-phenyl-1-cyclopentane)carboxamide (**4**, KF4) and report that the (+)-isomer is a potent and selective δ opioid antagonist, which also shows inverse agonist activity.

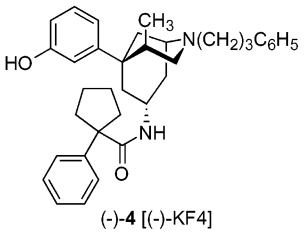
Chemistry. The coupling of (±)-, (+)-, and (−)-5-(3-hydroxyphenyl)-4-methyl-2-(3-phenylpropyl)-2-azabicyclo-[3.3.1]nonan-7-amine [(±)-, (+)-, and (−)-**5**][20] with 1-phenyl-1-cyclopentanecarboxylic acid, using benzotriazol-1-yloxy-tris-(dimethylamino)phosphonium hexafluorophosphate (BOP reagent) in THF, provided the desired (±)-, (+)-, and (−)-5-(3-hydroxy)phenyl-4-methyl-2-(3-phenylpropyl)-2-azabicyclo[3.3.1]non-7-yl-(1-phenyl-1-cyclopentane)carboxamides (**4**). For simplification only the synthesis of (+)-**4** is shown in Scheme 1.



(±)-**4** [(±)-KF4]



(+)-**4** [(+)-KF4]



(-)-**4** [(-)-KF4]

Results. Measures of functional antagonism and selectivity of (+)-, (−)-, and (±)-**4** along with standard compounds **1** (NTI) and ICI 174,864 [$(C_3H_5)_2$Tyr-Aib-Aib-Phe-Leu] were obtained by monitoring the ability of test compounds to inhibit stimulated [^{35}S]GTPγS binding produced by the selective agonists DAMGO (μ), DPDPE (δ), or U69,593 (κ) using cloned human opioid receptors expressed in CHO cells. Agonist dose−response curves were run in the presence or absence of a single concentration of test compound. The K_e values were calculated using the formula: $K_e = [L]/\{(A'/A)-1\}$, where [L] is the concentration of antagonist and A′ and A the agonist EC_{50} value in the presence or absence of antagonist, respectively. The results are listed in Table 1. Compound (+)-**4** with a K_e value of 0.15 nM is at least as potent as NTI (K_e = 0.21 nM) and was 28 and 3.7 times more potent than the (−)- and (±)-isomers that possessed K_e values of 4.24 and 0.55 nM, respectively. Compound (+)-**4** possessed K_e values of 8.7 and 17.9 nM at the μ and κ opioid receptors compared to K_e values of 33 and 16.1 nM for NTI. Compound (+)-**4** with μ/δ and κ/μ ratios of 58 and 119, respectively, is less selective than NTI at the μ receptor (μ/δ = 157) and slightly more selective at the κ receptor (κ/δ = 77).

Scheme 1

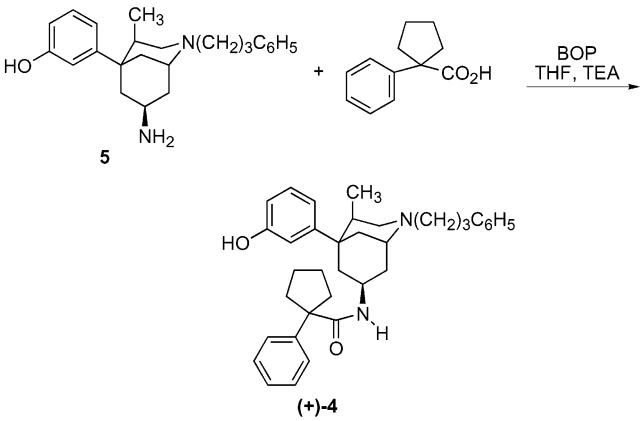


Table 1. Inhibition of Agonist Stimulated [^{35}S]GTPγS Binding by Compounds in Cloned Human μ, δ, and κ Opioid Receptors[a]

compd	μ, DAMGO K_e (nM)	δ, DPDPE K_e (nM)	κ, U69,593 K_e (nM)	μ/δ	κ/δ
(±)-**4**	12.2 ± 3.7	0.55 ± 0.22	3.52 ± 0.46	22	6.4
(+)-**4**	8.7 ± 2.7	0.15 ± 0.03	17.9 ± 6.3	58	119
(−)-**4**	40.3 ± 2.9	4.24 ± 0.17	5.51 ± 0.38	9.5	1.3
NTI, **1**	33 ± 23	0.21 ± 0.06	16.1 ± 5.3	157	77
ICI 174,864	42 ± 15.9	7.85 ± 3.3	339 ± 179	5.4	43

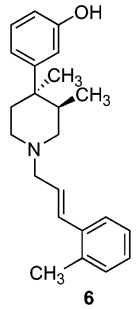
[a] The data represent the mean ± SE from at least three independent experiments. The final GDP assay concentration was 10 μM.

Table 2. Inverse Agonist Potencies and Efficacies of Compounds at the Cloned Human δ Receptor[a]

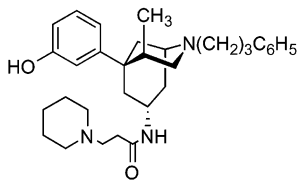
compd	IC_{50}	% of basal binding
(±)-**4**	2.4 ± 0.8	76 ± 5
(+)-**4**	1.8 ± 0.6	70 ± 10
(−)-**4**	15.2 ± 4.2	78 ± 5
ICI 174,864	83 ± 35	75 ± 4
6 (RTI-5989-25)	2.8 ± 0.8	76 ± 12
PTX		61 ± 7

[a] The data represent the mean ± SE from at least three independent experiments. The final GDP assay concentration was 1 μM.

The potencies and efficacies of (±)-, (+)-, and (−)-**4** as inverse agonists were determined in the [^{35}S]GTPγS binding assay and compared to the standard inverse agonist ICI 174,864 and compound **6** (RTI-5989−25), a previously reported inverse agonist from our laboratory (Table 2).[21] Similar to ICI 174,864 and RTI-5989−25, (±)-, (+)-, and (−)-**4** were all inverse agonists. The (+)-isomer with an IC_{50} value of 1.8 nM was the most potent, being 46 times more potent than ICI 174,864. PTX (pertussis toxin, 100 ng/mL) caused greater reductions in basal [^{35}S]GTPγS binding than any of the test compounds indicating a population of G_i proteins not linked to the δ receptor. No agonist activity was observed at the δ opioid receptor with (±)-, (+)-, and (−)-**4** (maximum assay concentration 31.6 μM) and none of the compounds displayed intrinsic activity at either the μ or κ receptors.



6



(-)-**7** [(-)-KAA1]

The δ receptor binding affinities of (±)-, (+)-, and (−)-**4** along with the reference compounds NTI and ICI 174,

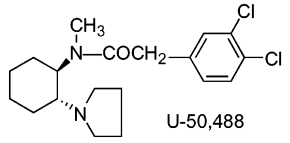
Table 3. [^{3}H]Cl-DPDPE Competition Binding Assays Using Human Cloned δ Receptors[a]

compd	$K_i \pm$ SE, nM [^{3}H]Cl-DPDPE
(±)-**4**	2.1 ± 0.5
(+)-**4**	0.96 ± 0.20
(−)-**4**	9.3 ± 1.8
NTI, **1**	0.07 ± 0.022
ICI 174,864	19.1 ± 3.0

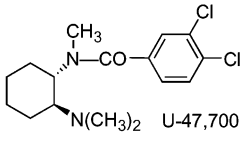
[a] The data represent the mean ± SE from at least three independent experiments. [^{3}H]Cl-DPDPE (K_d = 2.1 nM) binding was carried out in [^{35}S]GTPγS binding assay buffer containing 10 μM GDP.

864 for the δ opioid receptor are listed in Table 3. The (+)-isomer with a K_i value of 0.96 nM was 10 and two times more potent than the (−)- and (±)-isomers of **4** that possessed K_i values of 9.3 nM and 2.0 nM, respectively. The affinity of (+)-**4** was about an order or magnitude lower than that of reference compound NTI (K_i = 0.07 nM). However, (±)-, (+)-, and (−)-**4** all possessed higher affinity for the δ opioid receptor than the reference compound ICI 174,864 (K_i = 19.1 nM).

Discussion. We recently reported that the addition of a 4β-methyl substituent to N-substituted-5-(3-hydroxyphenyl)morphans resulted in compounds that showed pure opioid antagonist activity.[20] Importantly, we showed that the addition of a 7α-3-(1-piperidinyl)-propanamido group to the (+)- and (−)-*N*-phenylpropyl-4β-methyl-5-(3-hydroxyphenyl)morphan core structures resulted in (1*S*,4*R*,5*R*,7*S*)-(+)-**7** [(+)-KAA1] and (1*R*,4*S*,5*S*,7*R*)-(−)-**7** [(−)-KAA1] isomers which were selective antagonists for the κ opioid receptor.[20] (−)-KAA1 with a K_e value of 4.3 nM was eight times more potent than (+)-KAA1 which possessed a K_e value of 36 nM.[20] In the present study we found that the addition of a 7α-1-phenyl-1-cyclopentanecarboxamido group to the core (+)- and (−)-*N*-phenylpropyl-4β-methyl-5-(3-hydroxyphenyl)morphan resulted in (1*S*,4*R*,5*R*,7*S*)-(+)-**4** [(+)-KF4] and (1*R*,4*S*,5*S*,7*R*)-(−)-**4** [(−)-KF4] which were potent and selective antagonists for the δ opioid receptor. These results can be rationalized using the message−address concept that Portoghese used to explain the δ selective properties of NTI.[22] In this case the *N*-phenylpropyl-4β-methyl-5-(3-hydroxyphenyl)morphan acts as the opioid "message" fragment with the 7α-1-phenyl-1-cyclopentanecarboxamido providing the phenyl ring for the δ address moiety. Surprisingly and in contrast to the κ selective antagonists (+)- and (−)-KAA1, (+)-KF4 was more potent than (−)-KF4. Apparently, the (+)-isomer places the phenyl ring of the 7α-1-phenyl-1-cyclopentanecarboxamido group in a more favorable "address" location. It is interesting to note that Szmuszkovicz found that the κ agonist U-50,488, which possesses an (*S*,*S*)-configuration, was much more potent than its enantiomer, whereas the structurally similar μ agonist U-47,700 possessed an (*R*,*R*)-configuration.[23]



U-50,488



U-47,700

Constitutive activity has become a well-described characteristic of many GPCRs. One of the first GPCRs to be described as having constitutive activity is the $G_{i/o}$-coupled δ opioid receptor.[24,25] Subsequently, several laboratories including our own have also reported constitutive activity for the δ opioid receptor system.[21,26−30] Compounds that show negative intrinsic activity are referred to as inverse agonist. In contrast to NTI, which is a neutral antagonist,[21] we have shown that the δ opioid selective antagonists (±)-, (+)-, and (−)-**4** are also inverse agonists. The potency of (+)-**4** is 46 times greater than the standard inverse agonist ICI 174,864. Its potency is also slightly greater than the nonselective opioid δ inverse agonist **6** (RTI-5989−25). δ-Selective inverse agonists may have use as tools to study the part that constitutive activity plays in disease states.[31] On the basis of reports in the literature, the δ receptor has been connected to mood disorders as well as pain; thus, (+)-**4** or other analogues might prove useful for treatment of pain or as new antidepressants.[32] Coadministration of δ receptor antagonists, such as NTI, with morphine is well-known to attenuate the development of morphine tolerance and dependence. It is interesting to speculate that inverse δ antagonists would be even more effective, since they would reduce constitutive δ receptor activity.[33] Surprisingly, even though (+)-**4** is slightly more potent than NTI in the [^{35}S]GTPγS functional assay, it was much less potent than NTI in the radioligand binding assay.

Conclusion. Using the "message−address" concept, we developed (+)-5-(3-hydroxyphenyl)-4-methyl-2-(3-propylphenyl)-2-azabicyclo[3.3.1]non-7-yl-(1-phenyl-1-cyclopentane)carboxamide (+)-[**4**] as a potent and selective δ opioid receptor antagonist from the [7-amino-4-methyl-2-(3-phenylpropyl)-2-azabicyclo[3.3.1]non-5-yl]phenol core message component (**5**). The phenyl group in the phenyl-1-cyclopentanecarboxoyl moiety served as the "address". To our knowledge this is the first potent and selective δ opioid antagonist reported from the 5-phenylmorphan class of opioids.

In a previous study, we reported the development of (1*R*,4*S*,5*S*,7*R*)-(−)-**7** as the first potent κ opioid selective antagonist derived from the 5-phenylmorphan class of opioid. It is particularly interesting to note that the more potent κ opioid receptor ligand (−)-**7** is derived from (1*R*,4*S*,5*S*,7*R*)-(−)-**5**, whereas the more potent δ opioid receptor ligand (+)-**4** is derived from (1*S*,4*R*,5*R*,7*S*)-(+)-**5**. Thus, the "message" component from (−)-**7** and (+)-**4** is enantiomeric in nature. The selective δ opioid receptor antagonist (+)-**4** is also a potent inverse agonist.

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Supporting Information Available: Data for compounds (+)- and (−)-**4** include (1) HPLC trace, (2) ^{1}H NMR spectra, and (3) APCI mass spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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