Identification of the First *trans*-(3*R*,4*R*)-Dimethyl-4-(3-hydroxyphenyl)piperidine Derivative To Possess Highly Potent and Selective Opioid κ Receptor Antagonist Activity

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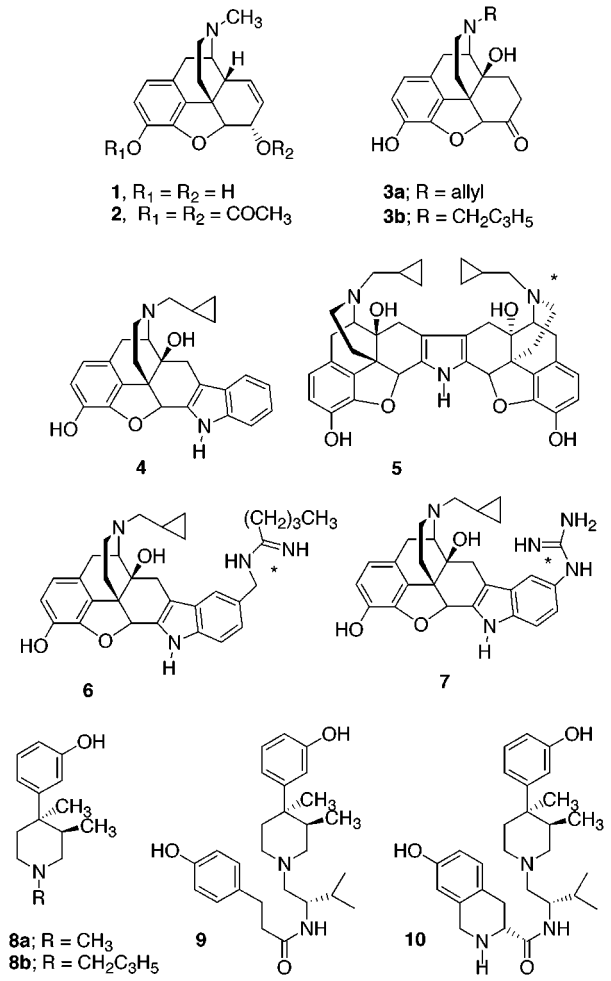
Abstract: A structurally novel opioid κ receptor selective ligand has been identified. This compound, (3*R*)-7-hydroxy-*N*-((1*S*)-1-{[(3*R*,4*R*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl)-1,2,3,4-tetrahydro-3-isoquinolinecarboxamide (JDTic, **10**) demonstrated high affinity for the κ receptor in the binding assay (κ K_i = 0.3 nM) and highly potent and selective κ antagonism in the [^{35}S]GTP-γ-S assay using cloned opioid receptors (κ K_i = 0.006 nM, μ/κ ratio = 570, δ/κ ratio > 16600).

Introduction. Compounds that exert their actions via the opioid receptor system have been studied intensely for many years in an effort to eliminate the unwanted side effects produced by the most frequently used or abused opiates morphine (**1**) and heroin (**2**). Among the many side effects produced by these compounds, addiction, tolerance, and respiratory depression are of greatest concern. While many agonists have been identified for the opioid receptor system, very few antagonists have appeared, and of these, naltrexone (**3a**) and naloxone (**3b**) have found the greatest application and have received the most attention particularly as it relates to the development of receptor subtype selective antagonists.[1] By applying the message-address concept of Schwyzer[2] to naltrexone (**3a**), Portoghese has developed receptor subtype selective antagonists for both the δ and the κ opioid receptors. In the case of 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 potent and δ selective antagonist naltrindole (NTI, **4**).[3]

The κ selectivity of the antagonist nor-binaltorphimine (nor-BNI, **5**)[4] has also been explained according to the message-address concept. In this case, it is the N-17′ nitrogen (marked by an asterisk in Chart 1) that is responsible for conferring selectivity by interacting with an acidic residue specific to the κ receptor. This assertion has been supported by a variety of different experiments, but perhaps the most notable example was the demonstration that addition of a basic 5′-methyleneamidino group to NTI (**4**) resulted in a remarkable change of receptor preference from δ to κ for the ligand 5′-[(N2-butylamidino)methyl]naltrindole (**6**).[5] Further refinements of this concept lead to the discovery of **7**, C5′-guanidinylnaltrindole (GNTI), which displays even greater potency and selectivity for the κ receptor compared with its predecessors.[6]

Chart 1



From this brief review it is clear that the oxymorphone framework of naltrexone (**3b**) has provided a reliable scaffold upon which to build receptor subtype selective antagonists. We have studied modifications of the *trans*-(3,4)-dimethyl-4-(3-hydroxyphenyl)piperidine class of opioid antagonist such as **8a**,**b** as an alternate approach to developing potent and selective κ opioid antagonists.[7−10] This class of compounds has long been considered novel relative to the classical antagonists such as **3a**,**b** because unlike the oxymorphone-based compounds, the opioid antagonist activity in this class of compounds (e.g., **8a**,**b**) is not mediated by the structure of the N-substituent. Thus, while only N-allyl and N-cyclopropylmethyl substituents exhibit potent pure antagonist activity in the oxymorphone series

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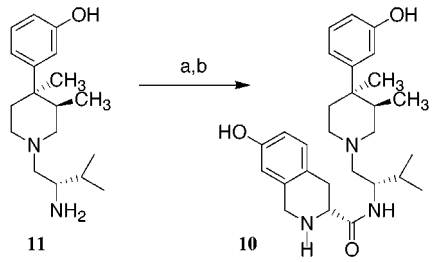
Scheme 1[a]



11 10

[a] Reagents: (a) Boc-D-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, BOP, TEA, THF; (b) TFA, CH_2Cl_2.

(**3a**,**b**), all N-substituted derivatives of *trans*-(3,4)-dimethyl-4-(3-hydroxyphenyl)piperidine, thus far reported, show antagonist activity.[7,8]

Recently we reported the discovery of **9** as the first *trans*-(3,4)-dimethyl-4-(3-hydroxyphenyl)piperidine analogue[11] to display a preference for the opioid κ receptor. Obtained from the screening of libraries biased for opioid antagonist activity through incorporation of *trans*-(3,4)-dimethyl-4-(3-hydroxyphenyl)piperidine into each ligand, compound **9** (RTI-5989-29) was found to be κ selective in binding but not in functional assays.[11] We now report the discovery of (3*R*)-7-hydroxy-*N*-((1*S*)-1-{[(3*R*,4*R*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl)-1,2,3,4-tetrahydro-3-isoquinolinecarboxamide (JDTic, **10**) which behaves in a manner opposite to that observed for **9**. Thus, compound **10** shows minimal μ versus κ selectivity in binding assays but excellent selectivity and κ antagonist potency in functional assays. A comparison of the behaviors of compound **9**, its methylenamino-bridged analogue JDTic (**10**), and the prototypical κ antagonist nor-BNI (**5**) as determined in biological assays is provided herein.

Chemistry. The synthesis of the novel antagonist **10** was accomplished by coupling the previously reported amine 3-[1-(2*S*-amino-3-methylbutyl)-3*R*,4*R*-dimethyl-4-piperidinyl]phenol (**11**)[11] with Boc-D-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid using benzotriazol-1-yl-oxy-tris-(dimethylamino)phosphonium hexafluorophosphate (BOP reagent) in THF followed by removal of the Boc-protecting group with trifluoroacetic acid (TFA) in dry methylene chloride as shown in Scheme 1.

Biological. The binding affinities of the novel κ antagonist **10**, the lead compound **9**, and the standard κ antagonist **5** for the μ, δ, and κ opioid receptors were determined using competitive binding assays following previously reported procedures (Table 1).[12] In the binding assay, μ and δ receptors were obtained from rat brain while κ receptors were obtained from guinea pig brain. Measures of antagonism were obtained by monitoring the test compound's ability to inhibit stimulation of [^{35}S]GTP-γ-S binding produced by the selective agonists (D-Ala^2,MePhe^4,Gly-ol^5)enkephalin (DAMGO, μ receptor), (+)-4-[(αR)-α-(2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide (SNC-80, δ receptor), and 5α,7α,8β-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]benzeneacetamide (U69,593, κ receptor) in guinea pig caudate (Table 2).[13] Compounds **10** and nor-BNI (**5**) were further studied in cloned human (δ, κ) and rat (μ) receptors using the same selective agonists mentioned above with the exception that 2-(3,4-dichlorophenyl)-*N*-methyl-*N*-[(1*R*,2*R*)-2-(1-pyrrolidinyl)cyclohexyl]acetamide [(−)-U50,488] was used as the selective κ agonist (Table 3).

Table 1. Radioligand Binding Results at the μ, δ, and κ Opioid Receptors for Compound **9**, the Standard κ Antagonist nor-BNI (**5**), and the Novel κ Antagonist **10**

	K_i (nM ± SD)				
compd	μ [^{3}H]DAMGO[a]	δ [^{3}H]DADLE[b]	κ [^{3}H]U69,593[c]	μ/κ	δ/κ
5, nor-BNI	65.06 ± 5.6	86 ± 7.3	1.09 ± 0.14	60	79
9	171 ± 15	>3400	3.84 ± 0.26	45	>885
10	3.73 ± 0.17	301 ± 50	0.32 ± 0.05	12	940

[a] [^{3}H]DAMGO [(D-Ala^2,MePhe^4,Gly-ol^5)enkephalin]. Tritiated ligand selective for μ opioid receptor. [b] [^{3}H]DADLE [(D-Ala^2,D-Leu^5)enkephalin]. Tritiated ligand selective for δ opioid receptor. [c] [^{3}H]U69,593 {[^{3}H](5α,7α,8β)-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]benzeneacetamide}. Tritiated ligand selective for κ opioid receptor.

Table 2. Antagonist Activity of nor-BNI (**5**), RTI5989-29 (**9**), and Compound **10** on Agonist Stimulated [^{35}S]GTP-γ-S Binding in Guinea Pig Caudate Membranes

	apparent functional K_i (nM ± SD)				
compd	μ DAMGO[a]	δ SNC-80[b]	κ U69,593[c]	μ/κ	δ/κ
5, nor-BNI[d]	16.7 ± 1.5	10.2 ± 1.0	0.038 ± 0.005	439	268
9[d]	7.25 ± 0.52	450 ± 74.1	4.7 ± 0.56	1.5	96
10	2.16 ± 0.75	>300	0.02 ± 0.002	108	>15000

[a] Apparent functional K_i (vs 10 μM DAMGO, an agonist selective for μ opioid receptor). [b] Apparent functional K_i (vs 10 μM SNC-80, an agonist selective for δ opioid receptor). [c] Apparent functional K_i (vs 10 μM U69,593, an agonist selective for κ opioid receptor). The apparent functional K_i is calculated by the Cheng−Prusoff equation where $K_i = IC_{50}/(1 + [L]/ED_{50})$, and the experimentally determined ED_{50} values are as follows: DAMGO, 592 nM; SNC-80, 317 nM; U69,593, 684 nM. [d] Data taken from ref 11.

Table 3. Antagonist Activity of nor-BNI (**5**) and Compound **10** on Agonist Stimulated [^{35}S]GTP-γ-S Binding in Cloned Opioid Receptors

	apparent functional K_i (nM ± SD)				
compd	μ DAMGO[a]	δ SNC-80[b]	κ U50,488[c]	μ/κ	δ/κ
5, nor-BNI	15.8 ± 5.7	12.1 ± 3.1	0.07 ± 0.03	225	172
10	3.42 ± 0.83	>100	0.006 ± 0.001	570	>16600

[a] Apparent functional K_i (vs 1 μM DAMGO, an agonist selective for μ opioid receptor). [b] Apparent functional K_i (vs 200 nM SNC-80, an agonist selective for δ opioid receptor). [c] Apparent functional K_i (vs 2 μM U50,488, an agonist selective for κ opioid receptor). The apparent functional K_i is calculated as illustrated in Table 2 where the experimentally determined ED_{50} values are as follows: DAMGO, 59 nM; SNC-80, 2 nM; U50,488, 24 nM.

Results and Discussion. Comparison of the data for compounds **9** and **10** (Table 1) reveals that **9** with a μ/κ and δ/κ ratio of 45 and >885, respectively, is superior to **10** which possesses ratios of 12 and 940. Indeed, compound **9** possesses a μ/κ K_i ratio comparable to the standard antagonist **5** and a far superior δ/κ K_i ratio and is thus selective for the κ opioid receptor over either the μ or the δ receptor. In contrast, JDTic (**10**) shows very little κ versus μ selectivity in this assay relative to the analogue **9**. The principal reason for this is the 46-fold greater affinity of **10** for the μ receptor relative to compound **9**. The difference in affinity at this site overshadows the 10-fold improvement that **10** shows for the κ receptor relative to **9**. The overall result is that **10** possesses low κ versus μ selectivity in the binding assay.

In the functional assay using guinea pig membranes (Table 2), the standard antagonist nor-BNI (**5**) with a K_i value of 0.038 nM shows a 28-fold increase in its κ receptor K_i value relative to that seen in the binding assay. At the μ and δ receptors, however, the K_i values for nor-BNI of 16.7 nM and 10.2 nM represent only 4- and 8.5-fold increases, respectively. Overall this translates into a significant increase in μ versus κ and δ versus κ selectivity in this assay relative to that observed in the binding assay.

Compound **9** also shows significant changes in behavior between these two assays, but these do not parallel those found for nor-BNI (**5**). Instead, the *trans*-(3,4)-dimethyl-4-(3-hydroxyphenyl)piperidine (**9**) shows no μ versus κ selectivity compared with its 45-fold selectivity seen in the binding assay; an effect driven primarily by its 25-fold increase in μ receptor K_i. Overall, compound **9** is not selective in this functional assay and does not compare favorably with the standard κ antagonist nor-BNI (**5**).

In stark contrast, the novel ligand JDTic (**10**), like nor-BNI (**5**), shows a 16-fold improvement in its κ receptor K_i value in the functional assay relative to the binding assay (0.02 nM versus 0.3 nM). Since the K_i values for **10** in the μ and δ assays do not increase substantially, the shift to higher potency for **10** in the κ receptor functional assay results in greater than 100-fold μ versus κ selectivity and a remarkable >15000-fold selectivity for the δ versus κ receptor.

In the [^{35}S]GTP-γ-S functional assay using cloned opioid receptors (Table 3), compound **10** with a K_i value of 0.006 nM demonstrates a 3.4-fold increase in κ antagonist potency relative to the functional assay utilizing guinea pig membranes. This represents a 53-fold overall improvement in the κ receptor K_i value compared with the binding assay, and as before there is little shift in K_i values for either the μ or δ receptors. This effectively boosts the selectivities of **10** to 570 and >16000-fold for μ versus κ and δ versus κ, respectively. In this assay, the novel antagonist **10** is observed to be an order of magnitude more potent and considerably more selective than the prototypical antagonist nor-BNI (**5**), which shows μ versus κ and δ versus κ selectivities of 225- and 172-fold, respectively.

Conclusions. The discovery of the potent and selective κ opioid receptor antagonist (3*R*)-7-hydroxy-*N*-((1*S*)-1-{[(3*R*,4*R*)-4-(3-hydroxyphenyl)-3,4-dimethyl-1-piperidinyl]methyl}-2-methylpropyl)-1,2,3,4-tetrahydro-3-isoquinolinecarboxamide (JDTic, **10**) represents a significant advancement in the development of the *trans*-(3,4)-dimethyl-4-(3-hydroxyphenyl)piperidine class of opioid antagonist. However, it also raises many questions. Of particular interest is the nature of the relationship between compound **10** and the oxymorphone-based κ antagonists such as **5** or **6**. For example, do both classes of antagonist utilize the same recognition loci within the κ receptor to express antagonist activity and selectivity? Also, what physical event in the κ receptor/ligand interaction gives rise to the conundrum whereby some κ antagonists show an inverse relationship between selectivity in binding and selectivity in functional potency?[11,14,15] Additionally, the presence of the two independent tyrosyl mimicking moieties within the structure of JDTic (**10**) is intriguing as this tandem arrangement raises the possibility that two independent tyrosine binding subsites may exist within the κ receptor.

While providing answers to these questions lies beyond the scope of this communication, the establishment of a new structural class of compounds exhibiting potent and selective κ antagonist activity holds the promise to expand fundamentally our understanding of the κ receptor. Such information in turn could provide new opportunities for the development of treatments for substance abuse. Further studies focused on addressing the questions and possibilities related above are currently underway and will be reported shortly.

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Supporting Information Available: Data for compound **10** includes (1) HPLC trace, (2) ^{1}H NMR spectra, and (3) electrospray mass spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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