Brief Articles

Importance of Phenolic Address Groups in Opioid Kappa Receptor Selective Antagonists

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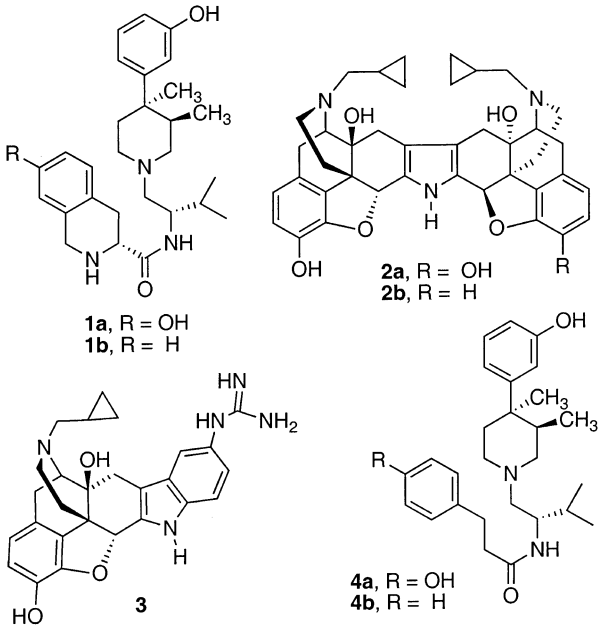
In vitro characterization and comparison of JDTic, its dehydroxy analogue and *nor*-BNI, and its dehydroxy analogue demonstrates that the N-substituted 3,4-dimethyl-(3-hydroxyphenyl)-piperidine-derived antagonist, JDTic, relies more heavily on its phenol address group for affinity and antagonist activity relative to the corresponding naltrexone derived antagonists, *nor*-BNI. The structural flexibility of the former class of compound relative to the latter is postulated to underlie the difference.

Introduction

It has been established that at least three distinct opioid receptor subtypes, μ, δ, and κ, are responsible for modulation of a diverse array of biological events ranging from nociception to immune regulation.[1] Fundamental to the study of this complex receptor system has been the identification of both agonists and antagonists displaying a high degree of receptor subtype selectivity.[2] Along these lines, we recently disclosed that (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, **1a**) is a potent and selective antagonist for the opioid κ receptor—the first nonopiate-derived compound to possess such attributes.[3]

We recently showed that compound **1a**, which possesses two basic amines and two phenol groups (one each in both the address and message groups), requires both sets of these groups to express its potent κ opioid potency and selectivity in the functional [^{35}S]GTPγS binding assay using cells expressing cloned opioid receptors.[3] While an amino function in the address groups have been shown to be fundamental to κ selective antagonists, the observation that a phenol group could contribute was without precedent.[4] In fact, this observation is not consistent with the precedents previously established by oxymorphone-based κ antagonists, most notably the prototypical κ selective antagonist *nor*-binaltorphimine (*nor*-BNI, **2a**).[5] Like JDTic (**1a**), *nor*-BNI possesses two basic amine groups and two phenol groups, both of which might also be envisioned to interact with independent recognition domains. However, it has been shown that *nor*-BNI (**2a**) can be structurally simplified to give a more potent and selective κ antagonist guanidinenaltrindole (GNTI, **3**), a compound that retains two basic amine groups but only one phenol.[6] Clearly, the additional phenol is not required for κ antagonist activity in **3**. In contrast, the second phenol group in the κ antagonist, **1a** and **4a**, is critical to their κ potency as evidenced by the fact that the desphenol analogues, **1b** and **4b**, have weak κ potency and lack κ selectivity.[3,7] In this communication we compared the dephenolic analogues, **1b**, **4b**, **2b**, with analogues **1a**, **4a**, and **2a**, all of which have phenolic groups in both the message and address fragments, using radioligand binding and functional [^{35}S]GTPγS binding assays.



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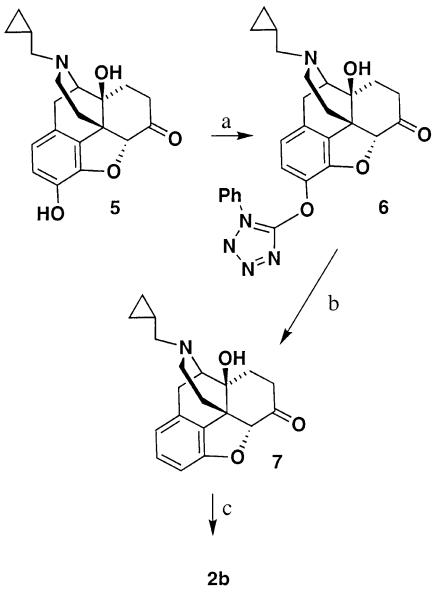
Scheme 1[a]



Reagents: (a) 5-chloro-1-phenyl-1*H*-tetrazole, DMF, K_2CO_3; (b) 10% Pd/C, H_2, HOAc; (c) naltrexone·HCl, *N*-aminosuccinamide·HCl, DMF.

Chemistry

The syntheses of JDTic (**1a**), its dehydroxy analogue (**1b**), and the phenylpiperidines **4a**,**b** have been reported previously.[3,7] The synthesis of the dehydroxy analogue of *nor*-BNI (**2b**) was accomplished using the reported synthesis for *nor*-BNI starting from equal parts naltrexone (**5**) and dehydroxynaltrexone (**7**) (Scheme 1).[8] Intermediate **7** was prepared from naltrexone (**5**) by deoxygenating via the two-step method that began with the preparation of the tetrazole intermediate **6** followed by reduction with hydrogen and palladium on carbon.[8] The dehydroxynaltrexone (**7**) thus obtained was then coupled to naltrexone (**5**) using *N*-aminosuccinimide in DMF to give *nor*-BNI (**2a**) and dehydroxy-BNI (**2b**) in the expected 1:2 ratio. The dehydroxy *nor*-BNI was separated and characterized by electrospray mass spectrum and elemental analyses.

Biological Results

The binding affinities of the test compounds for the μ, δ, and κ opioid receptors were determined using competitive binding assays following previously reported procedures.[9] Membranes possessing μ and δ opioid receptors were prepared from rat brain. κ opioid receptors were assayed using membranes prepared from guinea pig caudate. Measures of functional antagonism were obtained by monitoring the ability of a test compound to inhibit stimulation of [^{35}S]GTPγS 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 membranes (Table 2).

Results and Discussion

The radioligand binding results obtained for the test compounds are provided in Table 1. Comparison of the κ binding data for compound **1a** and its dehydroxy analogue **1b** reveals that loss of the hydroxyl group shows a 6.5-fold loss of κ affinity, whereas the dehydroxy analogue **4b** suffers a dramatic loss of κ affinity compared to **4a** going from 3.8 to over 446 nM, a 2 orders of magnitude shift. The data from the oxymorphone compounds **2a** and **2b** on the other hand illustrates that the loss of the phenol address group has little affect on κ affinity. Of the three sets, compound **4a**, which lacks a basic amino group, relies most heavily upon the second phenol group for κ affinity.

In the μ receptor assay, the dehydroxy compound **1b** demonstrates a nearly 210-fold loss of affinity relative to **1a**, whereas the loss of hydroxyl from **4a** is of little consequence since this compound displayed little affinity for this receptor in either case. The dehydroxy analogue of *nor*-BNI on the other hand (**2b**) showed an unexpected improvement in μ affinity. Comparison of the data for *nor*-BNI (**2a**) to that of its dehydroxy derivative (**2b**) revealed that while the κ opioid receptor affinity of compound **2b** shows little change, the μ affinity for **2b** was roughly 10-fold greater than that of its precursor **2a** which results in an order of magnitude loss of κ/μ selectivity relative to **2a**. Indeed, compared with **2a**, the dehydroxy analogue **2b** is not selective for the κ receptor relative to the μ receptor. Clearly, in the binding assay the phenol hydroxyl group produces a pronounced effect on μ affinity and as a consequence overall ligand selectivity for both **1a** and **2a**.

In the δ receptor assay, the results observed for **1a** and **1b** and **2a** and **2b** roughly parallel the behavior

Table 1. Radioligand Binding Results at the μ, δ, and κ Opioid Receptors Using Opioid Receptors from Brain Tissue (μ and δ of Rat and κ of Guinea Pig)

	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
1a[d]	3.73 ± 0.17	301 ± 50	0.32 ± 0.05	12	940
1b[d]	775 ± 75	>4900	2.1 ± 0.17	369	>2333
2a[d]	65 ± 5.6	86 ± 7.3	1.09 ± 0.14	60	79
2b	8.3 ± 0.4	110.8 ± 10.7	1.33 ± 0.24	6	83
4a[d]	171 ± 15	>3400	3.84 ± 0.26	45	>885
4b	946 ± 76	>5051	446 ± 553	2	>11

[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] using 100 nM DAMGO to block binding to the μ 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. [d] Data taken from ref 7.

Table 2. Inhibition by Antagonists of [^{35}S]GTPγS Binding in Guinea Pig Caudate Stimulated by the Opioid Receptor Subtype-Selective Agonists, DAMGO (μ), SNC-80 (δ), and U69,593 (κ)

	apparent functional K_i (nM ± SD)				
compd	μ DAMGO[a]	δ SNC-80[b]	κ U69,593[c]	μ/κ	δ/κ
1a[d]	2.16 ± 0.75	>300	0.02 ± 0.002	108	>15 000
1b[d]	68.6 ± 6.5	213 ± 24	11.5 ±1.0	6	18
2a[d]	16.7 ± 1.5	10.2 ± 1.0	0.038 ± 0.005	439	268
2b	5.55 ± 1.00	>300 nM	0.13 ± 0.01	43	>2307
4a[d]	7.25 ± 0.52	450 ± 74.1	4.7 ± 0.56	1.5	96

[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. [d] Data taken from ref 3.

observed in the κ receptor assay with little affect on affinity or selectivity resulting from this functional group change.

In the functional assays (Table 2), compound **1a** is observed to be a highly potent κ antagonist with good μ/κ (>100-fold) and excellent δ/κ selectivity (>15 000-fold). The dehydroxy derivative **1b** on the other hand possesses neither potent κ antagonist activity *nor* κ selectivity. The driving force behind this dramatic change in behavior is the 575-fold loss of antagonist potency for **1b** relative to **1a** in the κ receptor assay. The antagonist potency for compound **1b** is also significantly lower in the μ assay (32-fold) but shows a small improvement in the δ receptor assay. Overall it is clear that the hydroxyl group in the Tic residue is important not only for κ receptor recognition but also to κ antagonist potency and ultimately to its selectivity versus the remaining opioid receptors. This is in line with the observations made in the binding assay, where compound **4a** showed good affinity relative to **4b,** which showed very low affinity without the phenol address group.

Compound **2b**, the dehydroxy analogue of *nor*-BNI (**2a**), shows an unexpected 3.5-fold loss of κ antagonist potency compared with *nor*-BNI (**2a**). In line with the increase in μ affinity observed in Table 1, the μ antagonist potency of compound **2b** is also slightly increased (3-fold). In the δ receptor assay, however, the antagonist potency observed for **2b** is not in line with the observations made in binding, as the dehydroxy analogue **2b** does not antagonize the selective δ agonist SNC-80 at the range of concentrations examined. Overall then, the loss of one of the hydroxyl groups in *nor*-BNI (**2a**) is manifested by a negative impact on κ selectivity relative to μ (43- versus 439-fold) and a concomitant dramatic enhancement of κ selectivity relative to δ (>2307- versus 268-fold). Taken together, the data shows that pendant phenol hydroxyl groups can produce profound effects on opioid receptor potency and on the resulting receptor subtype selectivity. Furthermore, while the observation clearly holds for both of the two known classes of κ selective antagonist, this pronounced effect has until now been unappreciated.

In some part, the lack of previous observation of this behavior lies in the fact that earlier studies with *nor*-BNI seeking to understand the role of the phenol groups relied upon the monomethylated derivative as the probe.[10] It has long been established that several classes of opioid compounds rely upon a hydrogen bond with their phenol group for observed activity.[11] In these cases, methylation of the phenol leads to reduced affinity for the target receptor. In the case of *nor*-BNI, however, methylation of the phenol group showed only a small effect on radioligand binding affinity leading to the conclusion that *nor*-BNI does not rely upon this hydroxyl group for its activity. In light of the findings with the dehydroxy derivative in this study, it may be concluded that while a hydrogen bond may not affect activity, the oxygen atom alone is sufficient to impart the κ selective antagonist activity observed with *nor*-BNI. Additionally, these findings suggest that a similar study with the monomethylated derivative of JDTic might provide valuable information concerning the role of its oxygen in regards to overall antagonist activity.

Viewed collectively, the data obtained in this study reveals the important effect on κ opioid potency and selectivity imparted by phenol address groups, but it also clearly supports the notion that precedents established for oxymorphone-based κ antagonists such as *nor*-BNI (**2a**) do not necessarily apply to phenylpiperidine-based antagonists such as JDTic (**1a**). The significant change in κ potency observed for **1b** compared with the small change observed for **2b** is certainly the most compelling evidence. However, the opposite sense of change observed in the μ receptor assays for these same compounds, wherein the oxymorphone-based compound **2b** showed increasing affinity and potency for this receptor while the phenylpiperidine compound **1b** demonstrated clear losses of the same potency, is also worthy of note. At the heart of this divergent behavior, a consideration of the differences in structure comes first to mind. For example, Portoghese has demonstrated in several instances that the powerful directing effect of the address amino group in oxymorphone-based κ antagonists relies heavily upon the rigidity of the molecular scaffold.[12] Clearly *nor*-BNI (**2a**), dehydroxy *nor*-BNI (**2b**), and GNTI (**3**) possess very rigid molecular scaffolds that serve to hold their pendant address groups in orientations that maximize interaction with κ specific recognition sites. Whether or not JDTic (**1a**) interacts with residues similar to those utilized by *nor*-BNI remains to be seen; nevertheless, it is clear by inspection that the scaffold of JDTic is more flexible compared to **2a**. Having demonstrated that **1a** is far more reliant upon its pendant phenol hydroxyl group for activity compared with **2a**, it is not unreasonable to suggest that JDTic may require multiple interactions with the receptor in order to overcome the flexibility inherent in its scaffold as compared to **2a** or **3**. In other words, an additional hydroxyl interaction could compensate for the rigid scaffold found in the oxymorphone-based compounds such as **2a**. Alternatively, JDTic and *nor*-BNI could be interacting with different address subsites within the κ receptor. Which of these factors contributes most to the divergent behavior observed herein remains to be determined.

Conclusions

We have shown that in addition to basic amino groups, phenolic hydroxyl groups play an important role in the selectivity and potency observed in both the phenylpiperidine-based κ antagonist JDTic (**1a**) and the prototypical oxymorphone-based antagonist *nor*-BNI (**2a**).

Experimental Section

^{1}H NMR were determined on a Bruker WM-250 or a Bruker 300 spectrometer using tetramethylsilane as an internal standard. Mass spectral data was obtained using a Finnegan LCQ electrospray mass spectrometer in positive ion mode at atmospheric pressure. Silica gel 60 (230−400 mesh) was used for all column chromatography. All reactions were followed by thin-layer chromatography using Whatman silica gel 60 TLC plates and were visualized by UV or by charring using 5% phosphomolybdic acid in ethanol. All solvents were reagent grade. Tetrahydrofuran and diethyl ether were dried over sodium benzophenone ketyl and distilled prior to use. Methylene chloride and chloroform were distilled from calcium hydride if used as reaction solvents. HCl in dry ethyl ether was purchased from Aldrich Chemical Co. and used while fresh before discoloration.

[^{3}H]DAMGO, DAMGO, and [^{3}H][D-Ala^2,D-Leu^5]enkephalin were obtained via the Research Technology Branch, NIDA, and were prepared by Multiple Peptide Systems (San Diego, CA). [^{3}H]U69,593 and [^{35}S]GTPγS were obtained from DuPont New England Nuclear (Boston, MA). U69,593 was obtained from Research Biochemicals International (Natick, MA). Levallorphan was a generous gift from Kenner C. Rice, Ph.D., NIDDK, NIH (Bethesda, MD). GTPγS and GDP were obtained from Sigma Chemical Company (St. Louis, MO).

Dehydroxy-*nor*-BNI (2b). A mixture of naltrexone (0.294 g, 0.643 mmol), dehydroxynaltrexone (**7**) hydrochloride (0.284 g, 0.643 mmol), and *N*-aminosuccinimide (0.096 g, 0.643 mmol) in anhydrous DMF (25 mL) was heated to 90 °C under N_2 for 24 h. The crude reaction mixture was diluted with 10% sodium bicarbonate (pH 9) and extracted into EtOAc. The combined organics were washed several times with H_2O, dried over Na_2SO_4, and evaporated under reduced pressure. The resulting solid was subjected to flash chromatography on silica gel using a gradient from $CHCl_3/CH_3OH/NH_4OH$, 95:4:1 to 85:14:1, as eluent to afford dehydroxy-*nor*-BNI (**2b**, 0.106 g, 21%). An analytical sample was prepared by recrystallization from CH_3OH/Et_2O: mp 221 °C dec; ^{1}H NMR ($CDCl_3$) δ 0.10−0.25 (m, 4H), 0.45−0.60 (m, 4H), 0.75−0.85 (m, 2H), 1.50−1.80 (m, 4H), 2.15−2.45 (m, 10H), 2.60−2.80 (m, 4H), 3.00−3.25 (m, 4H), 5.46 (s, 1H), 5.53 (s, 1H), 5.58−6.67 (m, 4H), 6.98−7.03 (t, J = 7.5 Hz, 1H), 8.40 (s, 1H). Anal. ($C_{40}H_{43}N_3O_5$) C, H, N.

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Supporting Information Available: Elemental analysis and mass spectral data for **2b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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