The Role of the Side Chain in Determining Relative δ- and κ-Affinity in C5′-Substituted Analogues of Naltrindole

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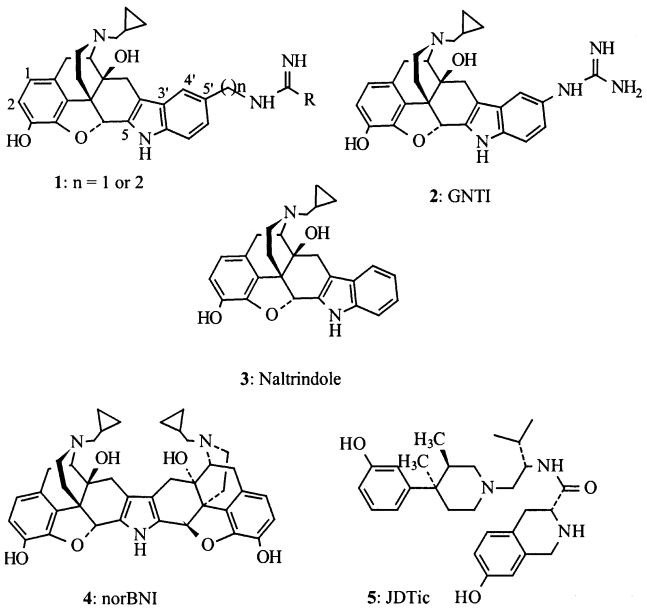
The role of the side chain in 5′-substituted analogues of naltrindole has been further explored with the synthesis of series of amides, amidines, and ureas. Amidines (**8**, **13**) had greatest selectivity for the κ receptor, as predicted from consideration of the message-address concept. It was also found that an appropriately located carbonyl group, in ureas (**10**) and amides (**7**), led to retention of affinity and antagonist potency at the δ receptor.

Introduction

The precise role of the kappa (κ) opioid receptor has yet to be well established. However, interactions at this receptor are known to create a number of physiological effects, including analgesia,[1] prevention of neurodegeneration in stroke and cerebral ischemia models,[2] and protection against epileptic convulsions.[2,3] There is also significant interest in the role of κ-agonists in cocaine abuse, and in particular the findings that κ-agonists can block many of cocaine's behavioral effects.[4] While having pharmacological effects of their own, κ-antagonists have mostly been used as pharmacological tools to help define the role of the κ-opioid system.

In recent years a number of κ-opioid antagonists have been discovered, greatly extending our understanding of the requirements for selective binding to this receptor. The majority of these ligands (e.g., **1** and **2**)[5−7] are related to the δ-antagonist naltrindole (**3**),[8] as is the most well-known and studied κ-antagonist, norBNI (**4**).[9] An exception is the recently reported 3-hydroxyphenylpiperidine, JDTic (**5**).[10] κ-Selectivity in the ligands related to naltrindole is reportedly derived from the presence of a basic, or cationic, group in the side chain.[11] At the κ-receptor this group is believed to interact with Glu297, present at the top of transmembrane region 6, while it is thought that negative interactions could occur between the side chain and residues corresponding to Glu297 in the μ and δ receptors.[12] These are Lys303 (μ) and Trp284 (δ) and may reduce binding affinity to the μ- and δ-receptors through electrostatic repulsion (Lys303) and steric hindrance (Trp284). Whatever the exact binding interactions, it is accepted that an appropriately located basic or cationic group is both required, and sufficient, for κ-selectivity. In the series of amidines reported by Olmsted et al.[5] it is interesting to note that selectivity for the κ-receptor is also related to the length of alkyl chain side chain attached to the amidine group. Thus the *n*-butyl analogue is more selective than its *n*-propyl or ethyl congeners. We were interested in exploring the role of lipophilic binding in determining κ-selectivity in naltrindole derivatives having both basic and nonbasic side chains and to this end synthesized series of amidines, amides, and ureas. We here report the synthesis and preliminary pharmacological characterization of these ligands and suggest that while the presence of a basic group within the side chain normally results in κ-selectivity, an appropriately located carbonyl group can lead to retention of substantial δ-antagonist activity and loss of selectivity.



Synthesis. The amidines (**8**) were prepared as reported previously for **8d**.[6] Thus amine (**6**)[6,11] was treated with the appropriate ethyl alkylimidate hydrochloride, itself prepared from the alkylnitrile by treatment with HCl in dry ethanol. The products were purified by preperative TLC on silica gel plates. Yields ranged from 15 to 24% with a major side product for **8b**, **8c**, and **8d** being the equivalent amide (**7a**−**c**: 20−30%) (Scheme 1). The formation of amides in the reaction appeared to be due to hydrolysis of the alkylimidate. The ureas (**10**) were obtained by treating amine (**6**) with the appropriate isocyanate (Scheme 1).[16] This led mainly to the desired ureas, but also gave approximately 10% of the urethane derivatives (**9**). Base-promoted hydrolysis to

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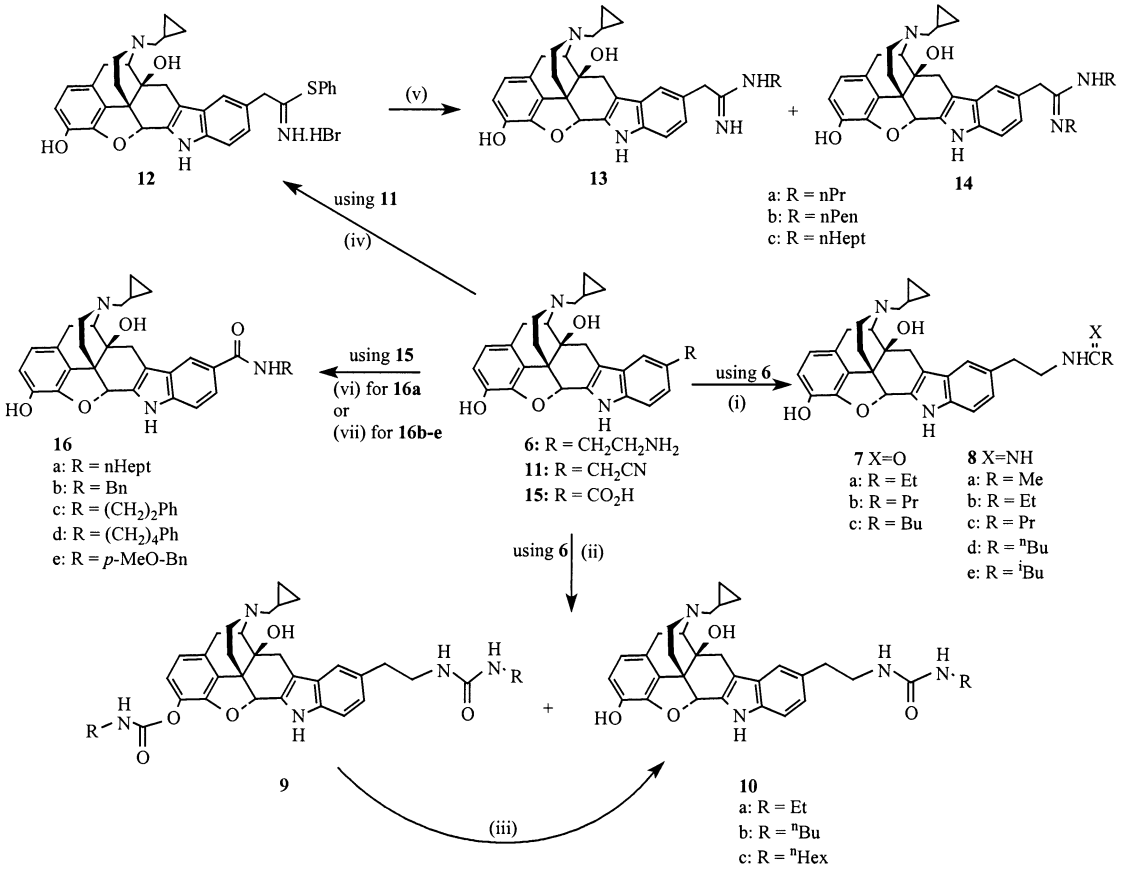
Scheme 1[a]



[a] (i) RC(=NH·HCl)OEt, EtOH; (ii) RNCO, CH_2Cl_2/MeOH (10:1), rt, 5 h; (iii): K_2CO_3 (5 equiv), MeOH/H_2O (9:1), rt, 12 h; (iv): PhSH, HBr, MeOH; (v) H_2NR (vi) BOP, NEt_3, CH_2CH_2, rt, 24 h; (vii) EDCl, DMAP, NEt_3, RNH_2, CH_2Cl_2, rt, 12 h.

10 with potassium carbonate gave an overall yield of approximately 50%. Preparation of the reverse amidines (**13**) utilized the thiophenyl imidic ester (**12**) prepared by treating nitrile (**11**)[6,11] with thiophenol in methanol under acidic conditions (Scheme 1).[13] Addition of the appropriate amine to crude **12** gave the required amidines that were purified by preparative thin-layer chromatography, in 20−30% yield. Also isolated were disubstituted amidines (**14**) in 5−10% and unreacted nitrile (10%). Amide (**16a**) was prepared from the known acid (**15**)[20] by BOP promoted coupling[14] with heptylamine in 83% yield (Scheme 1). Under the same conditions the benzyl and arylalkylamines (**9b**−**e**) gave <10% yield. Improved, although still low, yields of ~20% were obtained in these cases with the use of EDCI/DMAP.[15]

Results and Discussion

Opioid antagonist activity was determined by stimulation of [^{35}S]GTPγS in cloned human opioid receptors transfected into Chinese hamster ovary (CHO) cells (Table 1).[17,18] None of the compounds stimulated [^{35}S]GTPγS binding for any type of opioid receptor but were found to be antagonists of the selective agonists, DAMGO (μ), Cl-DPDPE (δ), and U69,593 (κ) (Table 1). The ligands were also evaluated in binding assays in CHO cells transfected with cloned human opioid receptors (Table 2).[17] The displaced radioligands were [^{3}H]-DAMGO (μ), [^{3}H]-Cl-DPDPE (δ), and [^{3}H]-U69,593 (κ). Binding affinity data for **8d** has previously been reported.[6] Differences were noted between the binding and functional assay data for some of the compounds (e.g., **8d** and **13a**). Previously an inverse relationship between selectivity in binding and selectivity in functional potency has been noted for related compounds.[10]

Table 1. Antagonist Activities in [^{35}S] GTPγS Assays in Human Recombinant Receptors in CHO Cells

	K_e (nM) ± SEM					
compd	μ-CHO membranes DAMGO	δ-CHO membranes DPDPE	κ-CHO membranes U69,593	*n*	μ/κ	δ/κ
7a	4.94 ± 1.08	0.38 ± 0.05	0.48 ± 0.27	4	10	1
7b	3.17 ± 0.34	0.30 ± 0.10	0.35 ± 0.23	4	9	1
7c	3.37 ± 1.22	0.23 ± 0.04	0.46 ± 0.14	5	7	0.5
8a	3.78 ± 0.68	1.79 ± 0.69	0.21 ± 0.04	6	18	9
8b	4.70 ± 1.34	1.77 ± 0.25	0.24 ± 0.03	4	20	7
8c	4.21 ± 1.61	1.89 ± 0.33	0.18 ± 0.06	4	23	11
8d	5.33 ± 0.63	3.31 ± 0.54	0.17 ± 0.05	5	31	20
8e	14.73 ± 0.83	5.23 ± 0.13	0.32 ± 0.02	5	46	16
10a	1.60 ± 0.15	0.65 ± 0.02	2.47 ± 0.20	4	0.6	0.3
10b	1.63 ± 0.12	0.53 ± 0.08	1.52 ± 0.16	4	1	0.3
10c	1.79 ± 0.32	1.04 ± 0.18	1.71 ± 0.16	4	1	0.6
13a	3.19 ± 0.25	4.41 ± 0.79	0.05 ± 0.004	4	64	88
13b	5.61 ± 0.28	3.83 ± 0.40	0.21 ± 0.03	5	27	18
13c	2.04 ± 0.62	5.83 ± 0.42	0.37 ± 0.06	5	6	16
16a	6.86 ± 0.93	6.95 ± 0.86	0.29 ± 0.08	5	24	24
16b	4.40 ± 0.74	2.99 ± 0.22	0.73 ± 0.04	5	6	4
16c	2.70 ± 0.31	1.21 ± 0.05	0.17 ± 0.03	6	16	7
16d	2.78 ± 0.21	5.15 ± 0.25	0.26 ± 0.02	5	10	20
16e	0.94 ± 0.12	6.20 ± 0.49	0.28 ± 0.04	8	3	22
4, norBNI	18.9 ± 1.8	4.42 ± 0.38	0.04 ± 0.004		484	113

It is apparent from previous reports by, in particular, Portoghese's group,[11] that affinities for the three opioid

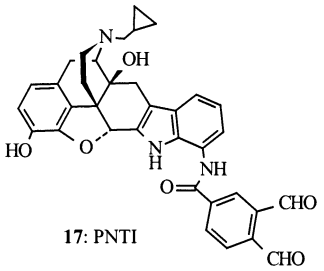
Table 2. Receptor Binding to Recombinant Human Opioid Receptors Transfected into CHO Cells

	K_i (nM) ± SEM					
compd	μ [^{3}H]-DAMGO	δ [^{3}H]-DPDPE	κ [^{3}H]U69,593	n^a	μ/κ	δ/κ
7a	22.97 ± 11.10	3.12 ± 0.50	1.57 ± 0.80	2	15	2
7b	30.97 ± 0.10	3.70 ± 0.34	0.85 ± 0.40	2	36	4
7c	31.59 ± 0.59	7.67 ± 0.64	0.68 ± 0.30	2	46	11
8a	22.32 ± 2.27	21.38 ± 1.32	0.29 ± 0.10	2	77	74
8b	26.35 ± 0.46	21.76 ± 1.50	0.28 ± 0.10	2	94	78
8c	40.79 ± 6.32	27.56 ± 2.11	0.25 ± 0.10	2	163	110
8d[b]	219.16 ± 84.47	38.22 ± 4.91	0.30 ± 0.20	2	730	127
8e	47.40 ± 7.07	20.10 ± 4.29	1.39 ± 0.14	2	34	14
10a	37.64 ± 14.41	2.43 ± 0.46	12.32 ± 1.29	2	3	0.2
10b	4.80 ± 0.91	2.60 ± 0.54	6.33 ± 0.40	2	0.8	0.4
10c	13.58 ± 4.06	2.25 ± 0.16	8.13 ± 2.67	2	2	0.3
13a	13.48 ± 0.54	5.29 ± 0.27	1.60 ± 0.28	2	8	3
13b	25.29 ± 4.06	17.02 ± 7.19	1.44 ± 0.04	2	18	12
13c	56.62 ± 7.69	7.33 ± 1.13	5.61 ± 0.37	2	10	1
16a	61.91 ± 6.03	70.15 ± 33.93	21.89 ± 7.11	2	3	3
16b	43.95 ± 8.27	11.19 ± 2.86	10.33 ± 0.66	2	4	1
16c	23.53 ± 7.32	3.53 ± 1.24	2.21 ± 0.35	2	11	2
16d	37.05 ± 9.45	59.45 ± 4.82	6.18 ± 0.47	2	6	10
16e	4.64 ± 0.58	7.41 ± 0.59	2.11 ± 1.04	2	2	4
4, norBNI	21.0 ± 5.0	5.7 ± 0.9	0.20 ± 0.05	2	105	28

[a] All values are the average of two experiments, each carried out in triplicate. [b] Data from ref 6.

receptors do not show large changes on alteration of the side chain group in C5′-substituted analogues of naltrindole. Changes in antagonist activity in vitro appear to be quite subtle, and this is also found in the current study. Nearly all the compounds synthesized had subnanomolar K_e's for the κ-receptor and low nanomolar K_e's at the μ and δ-receptors. However, the effect of the small changes observed on antagonist selectivity were significant. Thus selectivities in the GTPγS assay ranged from 64-fold and 88-fold selectivity for κ over μ and δ receptors (**13a**) to 2−4-fold selectivity for δ over μ and κ receptors (**10a**). For amidines (**8**), lengthening the R group caused some reduction in antagonist potency at μ and δ-receptors, with no change at κ, resulting in increased κ-selectivity. An identical effect was observed in the binding assays, with **8d** proving the most κ-selective compound in this assay.

For the reverse-amidines (**13**) an increase in length of chain had little effect on μ or δ-antagonist activity in the GTPγS assay, but slightly lowered κ-antagonist activity and hence selectivity. In this assay **13a** was, in fact, the most potent and selective κ-antagonist tested within these series. Amides (**7a**−**c**, **16a**−**e**) were also found to be potent antagonists of the κ-opioid receptor, again having K_e's in the subnanomolar range. At the μ receptor, the amides typically had low nanomolar Ke's, resulting in selectivity of 1 order of magnitude for κ. At neither the κ nor μ receptors was there any consistent SAR with the length of side chain, location of the amide bond, or type of side chain (aliphatic versus aromatic) having any significant influence on the antagonist activity of these ligands. It was at the δ-receptor that the greatest interest was found. Amides **7a**−**c** possessed subnanomolar antagonist potency for the δ-receptor, resulting in no κ/δ selectivity, whereas amides **16a**−**e** had low nanomolar K_e's at δ, meaning they were somewhat κ selective. The higher δ-antagonist potency of amides (**7**) compared to amides (**16**) was somewhat surprising, especially when comparing **16a** to **7c**. **16a** has a C5′-side chain of 9 atoms in total length, only one longer than in **7c**, yet **16a** is a κ-selective antagonist while **7c** has slightly higher affinity for the δ-receptor than for κ. This suggested that location of the amide bond in the side chain could play a significant role in determining δ-affinity. It was hypothesized that the carbonyl of **7** might be hydrogen bonding to a residue on the δ-receptor, resulting in the retention of affinity. With a series of ureas (**10**) it was possible to test this hypothesis. The urea moiety is basic, and thus should be detrimental to δ-affinity, but also contains a carbonyl group in the same location as amides **7**, which if the hypothesis was correct, would enhance δ-affinity. The ureas did in fact retain δ-antagonist potency and affinity with $K_{e\delta}$'s of 0.5−1 nM. Particularly striking is the comparison of the ureas with the amidines (**8**). For example, urea **10a** and amidine **8c** have the same overall length of side chain and thus any change in profile must be due to the nature of the basic group. In the GTPγS functional assay **8c** is 23-fold and 11-fold selective for κ over μ- and δ-receptors, respectively, urea **10a** is slightly (2−4-fold) selective for the δ-receptor over μ and κ. In binding, this change in selectivity was even more pronounced with **8c** being >100-fold selective for κ- over μ- and δ-receptors, while **10a** was 5-fold and 15-fold selective for δ- over κ- and μ-receptors, respectively. Thus replacement of the amidine moiety by the urea group results in a significant shift in selectivity from κ to δ. When considered in conjunction with the data for amides **7** and **16** this appears to confirm that the carbonyl group is a key binding motif in retaining δ-activity. It is interesting to note that the δ-selective fluorogenic affinity label PNTI (**17**) has been postulated



17: PNTI

to interact with Lys214.[19] Similarly, preliminary molecular modeling studies (data unpublished) suggest the possibility of a hydrogen bond between the carbonyl of the urea moiety and Lys214. Thus, rather than the side chain having a negative interaction with Trp284, this positive interaction could lead to the retention in affinity observed experimentally.

Conclusions

The influence of side chain length and lipophilicity appears to vary between series. Thus an increase in chain length was detrimental to the selectivity of amidines (**13a**−**c**) yet beneficial for **8a**−**d**. While it is clear that in most circumstances the presence of a basic side chain, attached to C5′ of the naltrindole nulcleus, leads to κ-selective antagonists, it is now apparent that an appropriately positioned carbonyl group can negate this effect and result in retention of affinity for the δ-receptor. Furthermore it is suggested that this effect may be the result of hydrogen bonding between the carbonyl group and Lys214 of the δ-receptor.

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Supporting Information Available: Procedures for the synthesis of all compounds, including spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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