Role of Spacer and Address Components in Peptidomimetic δ Opioid Receptor Antagonists Related to Naltrindole

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A series of heterocyclic analogues **2–5** related to naltrindole (1) (NTI) and 6-arylnaltrexone derivatives **6–8** were synthesized in order to determine the role of the spacer and the address moieties in conferring δ opioid receptor antagonist activity. The benzofuran (NTB), quinoxaline, and quinoline analogues (**2**, **3**, and **4**, respectively) were δ-selective opioid antagonists in vitro and bound selectively to δ receptors. The tetrahydroindole derivative **5**, while δ-selective, was considerably less potent than its indole analogue **13**. The data for **2–4** indicate that heterocycles other than pyrrole can serve as a spacer for the δ address moiety. Moreover, the lower δ antagonist potency of **5** illustrates the importance of the aromatic address component. Molecular dynamics simulations of enkephalin using a zipper binding model are consistent with a δ address subsite that may accommodate the benzene moiety of NTI or the Phe^4 phenyl group of leucine enkephalin. The considerably lower δ opioid receptor antagonist potencies of the 6-aryl derivatives **6–8** are consistent with the conformational mobility of the aryl group and its location in the molecule.

The existence of multiple populations of opioid receptors is now generally recognized and they have been classified into at least three major types (δ, κ, μ).[1] This multiplicity has created a need for highly selective antagonists for identifying the receptor types that are activated by endogenous opioid peptides.

Recently, we reported on a series of highly selective, nonpeptide δ-opioid receptor antagonists.[2] The most potent and selective member of this series, naltrindole (1) (NTI) (Chart I), has become a valuable pharmacologic tool in opioid research. The rationale for designing this series was based on the "message–address" concept for the recognition of peptide hormones by receptors.[3,4] Here we report on studies that were conducted to further investigate the structure–activity relationship of the naltrindole series in an effort to delineate the role of the address and spacer elements of NTI in conferring δ selectivity.

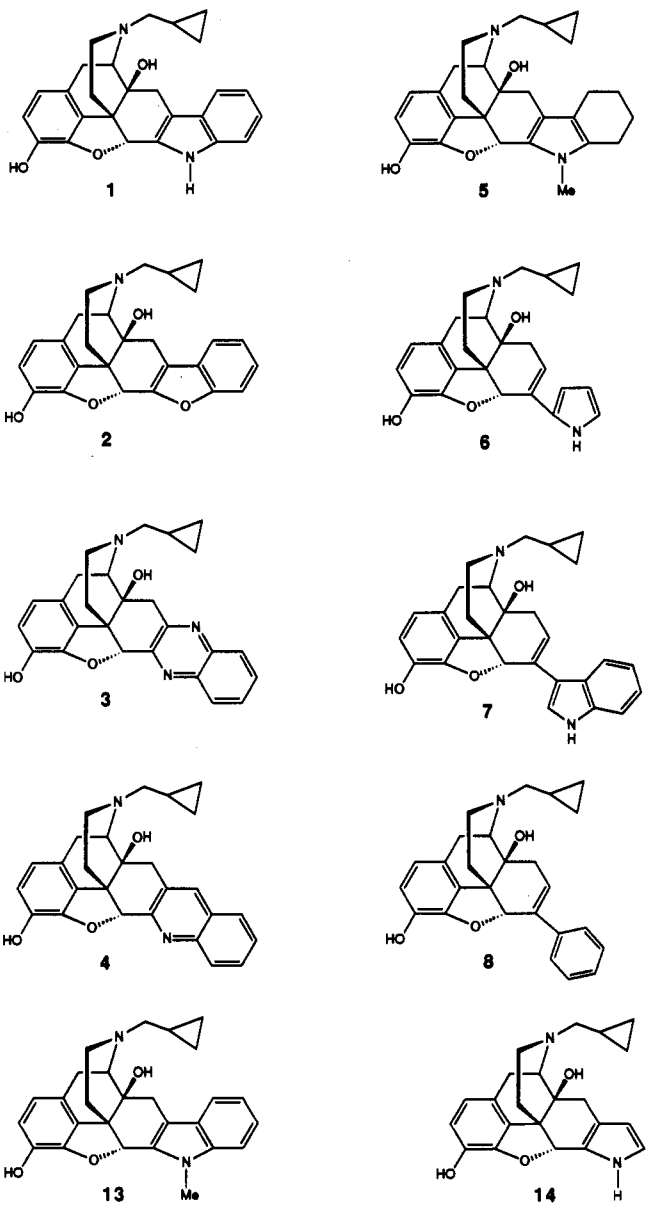
Rationale

The opioid peptides appear to conform to the message–address concept, in that their information content can be divided into two parts.[4,5] The message sequence comprises the portion of the peptide that is responsible for triggering a receptor transduction process that leads to a specific biological effect; the address component consists of a unique sequence of amino acid residues that augment receptor binding without contributing to the transduction process. In accordance with this model, each opioid receptor type contains a unique address subsite next to the message recognition site.

Our rationale for applying this concept to the design of 1 was based on the assumption that both opioid agonists and antagonists interact with the same message and address subsites of the receptor.[2] This model considered the tyramine moiety that is common to NTI and the opioid peptides, to be recognized by the message subsite on the δ receptor. The Phe^4 residue of the δ-selective opioid peptide, enkephalin, was envisaged to be a key part of the δ address.[2] This Phe^4 phenyl group was viewed to be simulated by the conformationally immobilized benzene moiety of the indole system in (1).

In an effort to determine whether the address and spacer components of the indole system reside with the benzene and pyrrole moieties, respectively, or with some other component, we have investigated a number of heterocyclic NTI analogues **2–5**. Also, several 6-aryl-substituted naltrexone derivatives **6–8** were synthesized to evaluate the effect of mobile aryl groups on selectivity. These compounds were investigated because the 7-aryl derivatives were not easily accessible.

Chart I



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(1) Paterson, S. J.; Robson, L. E.; Kosterlitz, H. W. In *The Peptides*; Academic Press: New York, 1984; Vol. 6, p 147.

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Scheme I

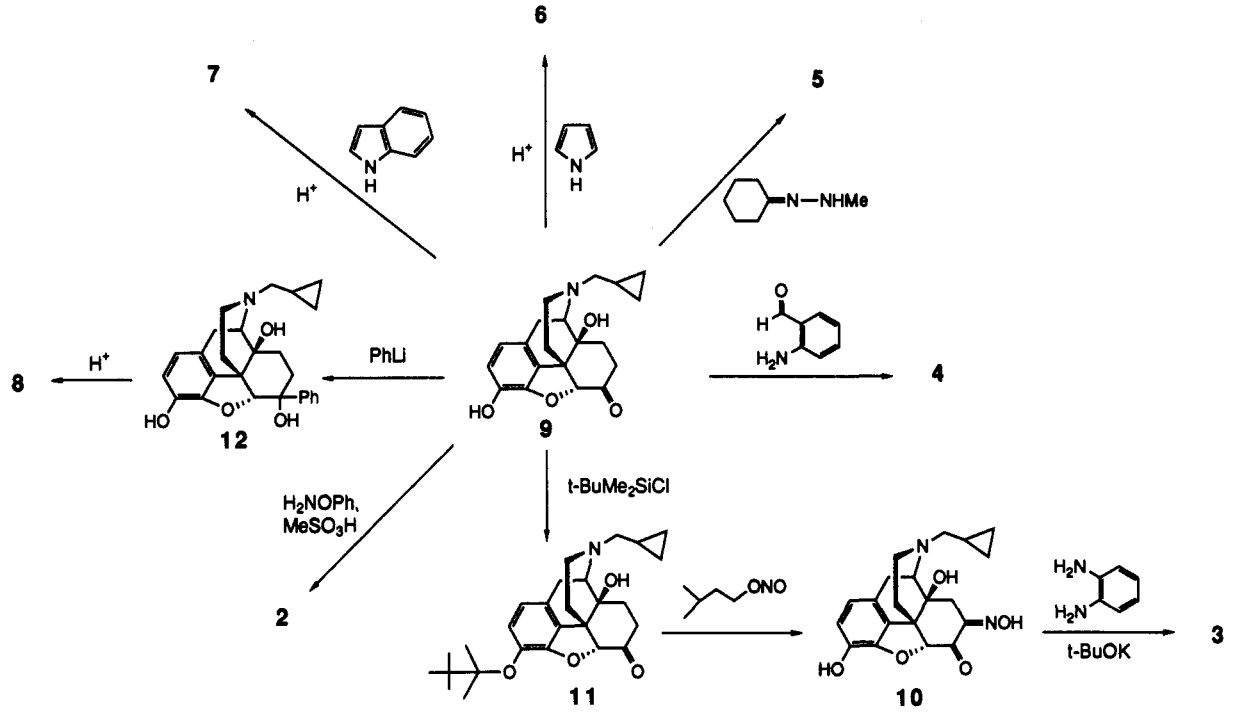

Table I. Antagonist Potency of Naltrindole Congeners and Related Compounds in the MVD and GPI

	conc, nM			DADLE (δ)[a]		M (μ)[b]		EK (κ)[b]		K_e selectivity ratio	
comp	δ	μ	κ	IC_{50} ratio	K_e	IC_{50} ratio	K_e	IC_{50} ratio	K_e	μ/δ	κ/δ
1	20	200	200	152 ± 34 (4)	0.13	7.8 ± 1.6 (3)	29	5.4 ± 0.8 (5)	46	223	345
2	20[c]	200	200	75 ± 2.3 (3)	0.27	8.5 ± 1.8 (3)	27	5.2 ± 0.7 (3)	48	100	178
3	200	200	200	104 ± 23 (9)	1.9	37 ± 7.5 (3)	5.5	21 ± 4.5 (6)	10	3	5
4	100	100	100	54 ± 13 (8)	1.9	9.9 ± 0.5 (3)	11	7.5 ± 1.2 (3)	15.4	6	8
5	100	1000	1000	12 ± 2.5 (5)	9.2	7.3 ± 0.2 (6)	158	4.9 ± 0.7 (3)	254	17	28
6	100	20	20	13 ± 3.2 (4)	8.2	*d*	–	89 ± 4.6 (3)	0.23	–	0.03
7	200	200	200	41 ± 11 (7)	5.1	189 ± 29 (3)	1.1	243 ± 46 (3)	0.83	0.21	0.16
8	100	100	100	8.4 ± 2.1 (3)	13.5	148 ± 14 (3)	0.68	59 ± 23 (3)	1.7	0.05	0.1
13[e]	200	100	100	204 ± 44 (8)	0.99	9.9 ± 1.8 (3)	11	6.0 ± 1.0 (3)	20	11	20
naltrexone (9)	300	100	100	10.5 ± 2.3 (4)	32	98 ± 24 (4)	1.0	19.3 ± 5.9 (4)	5.5	0.03	0.17

[a] [D-Ala^2,D-Leu^5]enkephalin in the MVD preparation. [b] Morphine (M) or ethylketazocine (EK) in the GPI preparation. [c] At 200 nM, K_e = 1.1 nM (n = 5). [d] Apparent noncompetitive inhibition; maximum agonist effect of morphine (30 μM) was 56.9 ± 4.1% (4). [e] Reference 2a.

Chemistry

The benzofuran analogue **2** (NTB) was of interest because it is isosteric with **1**. It was synthesized from naltrexone hydrochloride (**9**·HCl) and *O*-phenylhydroxylamine hydrochloride in the presence of methansulfonic acid (Scheme I).

The quinoxaline and quinoline analogues (**3** and **4**, respectively) were considered as target compounds because the 6-membered heterocyclic spacer holds the benzene moiety in an orientation which differs from that of **1** or the benzofuran **2**.

The quinoxaline **3** was prepared by condensing 1,2-phenylenediamine with 7-oximinoaltrexone (**10**). The oximino intermediate **10** employed in this synthesis was obtained by treatment of 3-(*tert*-butyldimethylsilyl)naltrexone[6] (**11**) with isoamyl nitrite and potassium *tert*-butoxide. The quinoline analogue **4** was obtained by reacting a mixture of **9**·HCl with 2-aminobenzaldehyde.

The tetrahydroindole analogue **5** was synthesized in order to determine if a benzene moiety is necessary for high δ selectivity. Our reason for selecting the *N*-methyl analogue **5** as a target rather than tetrahydro-NTI was based on synthetic expediency, as our efforts to prepare the latter were unsuccessful. The synthesis of **5** was accomplished in low yield by conducting a Piloty-type[7] pyrrole synthesis involving **9** (as its 6-methylimine derivative generated in situ) and cyclohexanone and methylhydrazone.

Several 6-aryl compounds (**6**–**8**) were synthesized in an effort to evaluate the effect of a conformationally mobile aromatic group on δ selectivity. Ideally, such groups should be attached to the 7-position, but we were unable to prepare such compounds. The 6-pyrrolyl and 6-indolyl derivatives (**6** and **7**, respectively) were obtained by acid-catalyzed condensation of **9** with pyrrole or indole. The

(2) (a) Portoghese, P. S.; Sultana, M.; Takemori, A. E. *J. Med. Chem.* **1990**, *33*, 1714. (b) Portoghese, P. S.; Sultana, M.; Nagase, H.; Takemori, A. E. *J. Med. Chem.* **1988**, *31*, 281. (c) Portoghese, P. S.; Sultana, M.; Takemori, A. E. *Eur. J. Pharmacol.* **1988**, *146*, 185.
(3) Schwyzer, R. *Ann. N.Y. Acad. Sci.* **1977**, *297*, 3.
(4) Portoghese, P. S. *Trends Pharmacol. Sci.* **1989**, *10*, 230.
(5) Chavkin, C.; Goldstein, A. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 6543.
(6) Nagase, H.; Abe, A.; Portoghese, P. J. *J. Org. Chem.* **1989**, *54*, 4120.
(7) Piloty, O. *Ber. Dtsch. Chem. Ges.* **1910**, *43*, 489.

6-phenyl derivative **8** was prepared by reacting **9** with phenyllithium to afford the phenylcarbinol intermediate **12** which was dehydrated with HCl.

Pharmacology

Target compounds **2–8** were evaluated on the electrically stimulated guinea pig ileal longitudinal muscle (GPI) and mouse vas deferens (MVD) preparations as described previously (Table I).[8] The ligands were incubated with the preparations 15 min prior to testing with the standard agonists, morphine (M), ethylketazocine (EK), and [D-Ala^2,D-Leu^5]enkephalin[9] (DADLE). These agonists are selective for μ, κ, and δ receptors, respectively. Morphine and EK were tested on the GPI and DADLE on the MVD. The agonist potencies in the presence and absence of the target compound were determined on the same preparation and were expressed as IC_{50} values. The IC_{50} ratio, which represents the IC_{50} in the presence of antagonist divided by the control IC_{50} value, was employed to calculate the antagonist potency expressed as K_e = [antagonist]/(IC_{50} ratio −1).

All of the target compounds were effective antagonists of DADLE with K_e values ranging between 1–14 nM. The most potent (K_e = 1–2 nM) members of this group were the C-ring-fused heterocyclic analogues **2–4** that contain a benzene moiety. It is noteworthy that the antagonist potency of tetrahydroindole compound **5** was about one-ninth that of its corresponding indole **13**. By comparison, naltrexone (**9**) was considerably less potent at δ opioid receptors.

Binding

Opioid receptor binding experiments using guinea pig brain membranes were carried out on selected compounds by displacement of selective radioligands by using a modification of the procedure of Werling et al.[10] Binding to κ sites was evaluated with [^{3}H]EK in the presence of 1 μM [D-Ala^2,MePhe^4,Gly-ol^6]enkephalin[11] (DAMGO), a μ-selective ligand; to δ receptors, with [^{3}H]DADLE in the presence of 1 μM DAMGO; and to μ receptors, with [^{3}H]DAMGO. The selectivities of all of the binding data in Table II agree qualitatively with the pharmacologic results. However, the rank orders of affinities and antagonist potencies differed.

Molecular Dynamics Simulations of Leucine-enkephalin. The simulations were performed by using AMBER[12] 3.0 Rev A (UCSF) with the United Atom force field[13] and displayed with MD Display.[14] The starting conformation was arbitrarily chosen such that the two aromatic rings of Leu-enkephalin almost overlapped the corresponding rings of NTI. This constrained structure was then briefly energy minimized with MM2 to remove any high energy interactions of the Gly residues. Constant-temperature molecular dynamics at 300 K was conducted with the Tyr^1 residue fixed in an effort to mimic a zipper type mechanism[15] for binding of the peptide to the δ site. The peptide showed a range of motion around this starting conformation, but even after 300 ps of dynamics, the molecule had not strayed far from this original bent backbone configuration (Figure 1).

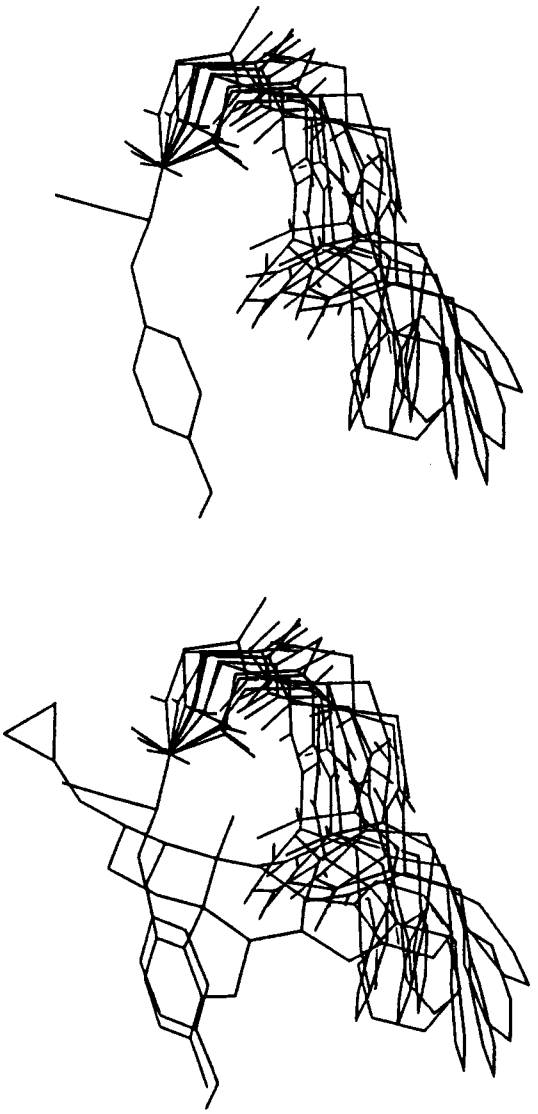

Figure 1. The conformations (at 0.5-ps intervals) of leucine-enkephalin derived from molecular dynamics simulations (300 K) during a 5-ps period in which the tyramine moiety of Tyr^1 has been fixed in a conformation identical with that of NTI 1 (upper illustration). Superposition of the tyramine moiety of 1 with that of enkephalin (lower) illustrates that there is overlap of conformational space occupied by the Phe^4 phenyl group and the indolic benzene moiety.

The temperature was raised to 800 K in 100 K steps, running 5 ps at each temperature for equilibration. After maintaining the Leu-enkephalin at 800 K for 50 ps, the Leu-enkephalin was decreased to 300 K at a rate of 50 K per 5 ps to permit equilibration. This simulated annealing process was conducted twice, starting from two different extended conformations found at 800 K. The fact that the annealed peptide backbone conformation resembled the original to a remarkable degree each time, suggests that this may be close to a global minimum. While this agrees with experimental and theoretical data,[16] multiple an-

(8) Portoghese, P. S.; Takemori, A. E. *Life Sci.* **1985**, *36*, 801.
(9) Fournie-Zaluski, M.-C.; Gacel, G.; Maigret, B.; Premilat, S.; Roques, B. P. *Mol. Pharmacol.* **1981**, *20*, 484.
(10) Werling, L. L.; Zarr, G. D.; Brown, S. R.; Cox, B. M. *J. Pharmacol. Exp. Ther.* **1985**, *233*, 722.
(11) Handa, B. K.; Lane, A. C.; Lord, J. A. H.; Morgan, B. A.; Rance, M. J.; Smith, C. F. C. *Eur. J. Pharmacol.* **1981**, *70*, 531.
(12) Singh, U. C.; Brown, F. K.; Bash, P. A.; Kollman, P. A. *J. Am. Chem. Soc.* **1987**, *109*, 1607.
(13) Weiner, S. J.; Kollman, P. A.; Case, A.; Singh, U. C.; Ghio, C.; Alagona, G.; Profeta, S., Jr.; Weiner, P. *J. Am. Chem. Soc.* **1984**, *106*, 765.
(14) Quantum Chemistry Program Exchange, Creative Arts Building 181, Indiana University, Bloomington, IN 47405.
(15) Burgen, A. S. V.; Roberts, G. C. K.; Feeney, J. *Nature* **1975**, *253*, 753.

Table II. Opioid Receptor Binding of NTI Analogues

compd	$K_i \times 10^{10}$ [a]			K_i selectivity ratio	
	μ	κ	δ	μ/δ	κ/δ
1[b]	38 (6–223)	3327 (2679–4130)	0.31 (0.01–6.9)	123	10732
2	188 (75–470)	1524 (929–2506)	0.13 (0.001–13.2)	1446	11723
3	73 (34–160)	1905 (991–3664)	8.4 (0.9–82)	9	227
5	168 (106–264)	2032 (1574–2624)	0.54 (0.04–7.1)	311	3762
7	6.3 (1.4–29)	62 (34–114)	9.9 (2.4–41)	0.6	6
13	137 (40–469)	650 (436–973)	0.18 (0.003–11.5)	761	3611
naltrexone[b] (9)	7.6 (2.5–22.9)	198 (147–269)	364 (150–8851)	0.02	0.54

[a] Geometric mean (95% confidence interval). [b] Binding data from ref 2a.

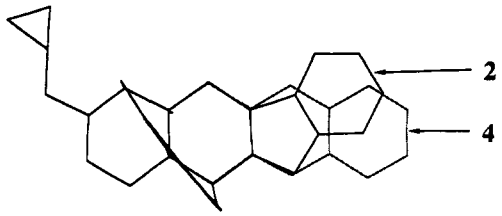

Figure 2. Superposition of 1 upon its quinoxaline analogue 3. Note the difference between the position of the benzene moieties.

nealing runs should be done to confirm these preliminary results.

Discussion

In the present study we have replaced the indole system of naltrindole (1) with other heterocycles in an effort to evaluate the structural requirements for the spacer and address components responsible for high δ opioid receptor antagonist potency and selectivity. The fact that isosteric replacement of the indole system with benzofuran afforded an antagonist **2** with relatively high potency and good selectivity at δ receptors indicates that the pyrrole portion of the indole is not essential. Data reported earlier for the μ-selective pyrrole analogue[17] **14** suggested that the benzene moiety of the indole system is the critical address component for high potency and selectivity at δ receptors.

We had proposed that the pyrrole moiety of 1 functions mainly as a spacer to hold the critical benzene moiety in a fixed conformation.[2] That other heterocycles also can serve as spacers is demonstrated with the quinoxaline and quinoline analogues (**3** and **4**, respectively). These ligands are δ-selective, but their δ antagonist potencies are less than that of **2**. The lower selectivity of **3** and **4** arises from a combination of their lower antagonist potency at δ receptors and increased potency at μ and κ receptors. The fact that the spacers in these ligands are 6-membered heterocycles, places the benzene moiety in a different orientation with respect to the pharmacophore (message component) as illustrated in Figure 2. This lowers the antagonist potency at δ receptors with a concomitant increase at μ and κ receptors. A consequence of these dual effects is the diminution of δ selectivity.

Significantly, δ opioid receptor binding affinity of **2** is approximately twice that of **1** (Table II). These data reflect a rank order opposite that was observed in the MVD preparation, where NTI is twice as potent as NTB (Table I). We have reported[2a] a similar lack of correlation within the indole series, and have suggested that binding may not parallel antagonist potency if some of the sites bound by the radioligand are not in common with those involved in δ antagonism. This appears to be supported by recent studies, utilizing NTB as a pharmacologic antagonist in mice, which have suggested the existence of δ opioid receptor subtypes.[18]

In order to establish that the requirement of the address component is more consistent with an aromatic group rather than a saturated moiety, we synthesized the tetrahydroindole **5**. The opioid antagonist profile of this ligand was compared with that of *N*-methyl-NTI (**13**) which was reported[2a] to be a potent δ antagonist. The fact that **13** is ~9× more potent and has 3× greater affinity than **5** is consistent with the role of the benzene moiety as a mimic of the phenyl group of Phe[4] in enkephalin. In this connection, it has been reported[19] that enkephalin analogues that contain a hexahydro-Phe[4] are less potent as agonists on the MVD preparation.

The fact that the tetrahydroindole **5** is apparently as δ selective as its indole counterpart **13** despite its lower antagonist potency illustrates an important point concerning the design of selective ligands. Namely, that concomitant decrease of potency at the δ receptor and other opioid receptor types that are not targets can still afford highly selective ligands. Examples of this are illustrated with the δ-selective agonist, Tyr-c-(D-Pen-Gly-Phe-D-Pen)[20] (DPDPE), and the δ-selective antagonist, $(allyl)_2$-Tyr-Aib-Aib-Phe-Leu-OH[21] (ICI174864). Although DPDPE is less potent than DADLE at δ receptors, it is considerably more selective as a consequence of its low affinity for μ and κ sites. Similarly, ICI174864 has about the same affinity as naltrexone for δ receptors, but it is highly δ selective by virtue of its very low affinity for other sites.

Molecular dynamics simulations support the idea that Phe[4] of enkephalin serves an address function. The simulations of leucine enkephalin were carried out with the tyramine moiety of Tyr[1] immobilized in the same conformation as in the opiate structure, with the remainder of the peptide unrestrained (Figure 1). This approach was taken in an effort to mimic a zipper-type[14] mechanism for

(16) (a) Renugopalakrishnan, V.; Rapaka, R. S.; Bhargava, H. N. In *Opioid Peptides*; Szekely, J., Ramabadran, K., Eds.; CRC Press: Boca Raton, FL, 1990; Vol. 4 (Biochemistry and Applied Physiology), p 53–114. (b) Belleney, J.; Gacel, G.; Fournié-Zaluski, M. C.; Malgret, B.; Roques, B. P. *Biochemistry* **1989**, *28*, 7392. (c) Kawai, H.; Kikuchi, T.; Okamato, Y. *Protein Engineering* **1989**, *3*, 85. (d) Ishida, T.; Yoneda, S.; Doi, M.; Inoue, M.; Kitamura, K. *Biochem. J.* **1988**, *255*, 621. (e) Yoneda, S.; Kitamura, K.; Doi, M.; Inoue, M.; Ishida, T. *FEBS Lett.* **1988**, *239*, 271. (f) Schiller, P. In *The Peptides*; Udenfriend, S., Meienhofer, J., Eds.; Academic Press: New York, 1984; Vol. 6, p 219.

(17) Portoghese, P. S.; Nagase, H.; Lipkowski, A.; Larson, D. L.; Takemori, A. E. *J. Med. Chem.* **1988**, *31*, 836.

(18) Sofuoglu, M.; Portoghese, P. S.; Takemori, A. E. *J. Pharmacol. Exp. Ther.*, in press.

(19) Audigier, Y.; Mazarguil, H.; Gout, R.; Cros, J. *Eur. J. Pharmacol.* **1980**, *63*, 35.

(20) Mosberg, H. I.; Hurst, R.; Hruby, V. J.; Gee, K.; Yamamura, H. I.; Galligan, J. J.; Burks, T. I. *Proc. Nat. Acad. Sci. U.S.A.* **1983**, *80*, 5871.

(21) Cotton, R.; Giles, M. G.; Miller, L.; Shaw, J. S.; Timms, D. *Eur. J. Pharmacol.* **1984**, *97*, 331.

binding of the peptide to the δ site. Thus, we envisaged leucine-enkephalin to undergo nucleation of Tyr[1] at the message subsite followed by binding of the Phe[4] residue with a δ address subsite. In this model, Gly[2]-Gly[3] functions both as a spacer to link the message to the address, and as a conformational determinant that contributes to the bend in the peptide chain.

The results of these simulations showed that the conformational space occupied by the phenyl group of Phe[4] was restricted to the region of the indolic benzene moiety of NTI 1 (Figure 1). This might permit binding of Phe[4] to a δ address subsite in the locus of the indolic benzene moiety. However, it is unlikely that the Phe[4] phenyl group would conformationally adapt to an orientation identical with that of the indolic benzene moiety because complete superposition of both rings was not observed during the simulation. The stability of the bent leucine-enkephalin backbone, as judged from our molecular dynamics simulations involving the simulated annealing, is consistent with recently reported conformational studies of δ-selective enkephalin-related peptides by NMR and energy calculations.[16]

Finally, the considerably lower δ opioid receptor antagonist potency and absence of δ selectivity of the 6-arylnaltrexone derivatives **6–8** are consistent with the conformational mobility of these aryl groups and the fact that their location with respect to the pharmacophore differs from that in **1**.

Conclusions

This study has confirmed that the putative δ address component of **1** is the indolic benzene moiety, and that it confers selectivity by the simultaneous operation of two different mechanisms. The first involves an increase in binding affinity as a consequence of the interaction of the indolic benzene moiety with an address subsite on the δ receptor. The second mechanism includes the steric interference of this δ address component with the address subsites of other receptor types. Either of these mechanisms is sufficient to confer selectivity. Ideally, it is most desirable to introduce a group that accomplishes both of these effects so that both high potency and high selectivity are obtained in the same molecule.

Experimental Section

Melting points were determined in open capillary tubes with a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ, and are within ±0.4% of the theoretical values. IR spectra were obtained on a Perkin-Elmer 281 infrared spectrophotometer. NMR spectra were recorded at ambient temperature on IBM-Bruker AC-300 with Me_2SO-d_6 and $CDCl_3$ as solvents and Me_4Si as internal standard. Mass spectra were obtained on a VG70,70EF instrument. All TLC data were determined with Analtech, Inc. Silica gel GHF 21521 and the eluents $CHCl_3$–MeOH–NH_4OH or *n*-BuOH–AcOH–H_2O are denoted by CMA and BAW, respectively. Unless otherwise stated, all reagents and solvents were reagent grade and used without subsequent purification.

17-(Cyclopropylmethyl)-6,7-didehydro-3,14β-dihydroxy-4,5α-epoxy-6,7-2′,3′-benzo[*b*]furanomorphinan Methanesulfonate (2·$MeSO_3H$). A solution of naltrexone hydrochloride (100 mg, 0.26 mmol), *O*-phenylhydroxylamine hydrochloride (80 mg, 0.55 mmol), and methanesulfonic acid (0.1 mL, 1.04 mmol) in EtOH (5 mL) was stirred under reflux for 18 h. After cooling, the resulting precipitate was collected, washed with EtOH (2×), and dried at 110 °C in vacuo to afford 2·$MeSO_3H$ (88 mg, 80%): mp 250 °C dec; R_f 0.70, (BAW, 2:1:1). Data for free base: R_f 0.58 (CMA, 19:1:0.1); IR (liq film, cm^{-1}) 3012, 2926, 2835, 1640, 1616; ^1H NMR ($CDCl_3$, δ) 0.15 (2 H, m), 0.55 (2 H, m), 0.90 (1 H, m), 1.83 (1 H, m), 2.11–2.55 (4 H, m), 2.63 (1 H, d, J = 16.0 Hz), 2.73–2.85 (3 H, m), 3.16 (1 H, d, J = 18.5 Hz), 3.38 (1 H, d, J = 6.4 Hz), 5.65 (1 H, s), 6.55 (1 H, d, J = 8.1 Hz), 6.75 (1 H, d, J = 8.1 Hz), 7.18 (1 H, m), 7.25 (1 H, m), 7.38 (1 H, d, J = 7.7 Hz), 7.43 (1 H, d, J = 8.1 Hz); EIMS (m/e) 415 (M^+). Anal. ($C_{28}H_{25}O_4N·CH_3SO_3H·H_2O$) C, H, N, S.

17-(Cyclopropylmethyl)-6,7-didehydro-3,14β-dihydroxy-4,5α-epoxy-6,7-2′,3′-quinoxalinomorphinan (3). A solution of 7-oxyiminonaltrexone (**10**) (160 mg, 0.39 mmol) and 1,2-phenylenediamine (42 mg, 0.39 mmol) in DMF (1 mL) was stirred at 100 °C for 19 h. The solvent was removed in vacuo, and methanol, saturated sodium bicarbonate solution, and chloroform were added to the mixture. The resulting mixture was filtered and the filtrate was extracted with chloroform (3×). The combined organic phases were washed with brine, dried, and concentrated to give an oil which was purified on a sephadex column [LH-20, MeOH] to afford **3** (100 mg, 50%): R_f 0.27 (CMA, 19:1:0.1); ^1H NMR ($CDCl_3$, δ) 0.19 (2 H, m), 0.59 (2 H, m), 0.91 (1 H, m), 1.90 (1 H, d, J = 8.9 Hz), 2.31–2.55 (4 H, m), 2.68–2.86 (2 H, m), 3.02–3.16 (2 H, m), 3.22 (1 H, d, J = 18.6 Hz), 3.37 (1 H, d, J = 6.3 Hz), 5.72 (1 H, s), 6.63 (1 H, d, J = 8.1 Hz), 6.79 (1 H, d, J = 8.1 Hz), 7.71 (2 H, m), 7.99 (1 H, m), 8.12 (1 H, m); FABMS 428 (M^+ + 1). Data for **3**·HCl: R_f 0.66 (BAW, 2:1:1). Anal. ($C_{26}H_{25}O_3N_3·HCl·2.5H_2O$) C, N, Cl. Calcd: H, 6.14. Found: H, 5.63.

17-(Cyclopropylmethyl)-6,7-didehydro-3,14β-dihydroxy-4,5α-epoxy-6,7-2′,3′-quinolinomorphinan (4). To a solution of naltrexone hydrochloride (200 mg, 0.52 mmol) in ethanol (5 mL) were added 2-aminobenzaldehyde (180 mg, 1.50 mmol) and methanesulfonic acid (0.07 mL, 0.73 mmol). The mixture was stirred under reflux for 14 h. Ethyl acetate and saturated sodium bicarbonate solution were added to the mixture. The mixture was filtered and the filtrate was extracted with EtOAc (3×). The combined organic phases were washed with brine, dried, and concentrated to give a crude product. Methanol was added to the product to afford a precipitate which was collected and washed with MeOH to give pure **4** (200 mg, 88.6%): mp 168–170 °C; R_f 0.28 (CMA, 19:1:0.1); IR (KBr, cm^{-1}) 2918, 2825, 1637, 1616; ^1H NMR ($CDCl_3$, δ) 0.20 (2 H, m), 0.60 (2 H, m), 0.90 (1 H, m), 1.83 (1 H, m), 2.35–2.52 (4 H, m), 2.65–2.91 (4 H, m), 3.20 (1 H, d, J = 18.6 Hz), 2.72 (1 H, dd, J = 18.6 Hz, 12.0 Hz), 3.35 (1 H, d, J = 6.4 Hz), 5.68 (1 H, s), 6.58 (1 H, d, J = 8.1 Hz), 6.68 (1 H, d, J = 8.1 Hz), 7.45 (1 H, m), 7.61 (1 H, m), 7.68 (1 H, d, J = 6.0 Hz), 7.80 (1 H, s), 8.05 (1 H, d, J = 6.0 Hz); EIMS 426 (M^+). Anal. ($C_{27}H_{26}O_3N_2$) C, H, N. Data for **4**·HCl: mp >280 °C dec; R_f 0.66 (BAW, 2/1/1). Anal. ($C_{27}H_{26}O_3N_2·HCl·H_2O$) C, H, N, Cl.

17-(Cyclopropylmethyl)-6,7-didehydro-3,14β-dihydroxy-4,5β-epoxy-6,7-2′,3′-(*N*-methyl-4′,5′,6′,7′-tetrahydroindolo)morphinan (5). Cyclohexanone (212 μL, 2.05 mmol) in acetic acid (0.5 mL) was stirred with methylhydrazine (109 μL, 2.05 mmol) for 5 min on a steam bath and this was added to naltrexone base derived from 771.4 mg (2.044 mmol) of its hydrochloride. After heating on a steam bath for 3 min, methylamine hydrochloride was added to the mixture and the heating continued for a period of 40 min. The acetic acid was removed in vacuo, the oily residue was basified with ammonium hydroxide, extracted (EtOAc), and dried ($MgSO_4$), and the solvent was removed in vacuo. The residue was chromatographed first on silica gel (MeOH–$CHCl_3$–NH_4OH, 1:19:0.2), secondly on alumina (EtOAc–hexane–MeOH–NH_4OH, 11.8:10.8:8:1:0.23), and finally on silica gel (EtOAc–hexane–MeOH–NH_4OH, 11.8:10.8:1:0.23) to afford a solid which was dissolved in hot $CHCl_3$ and 4 volumes of CCl_4 and allowed to cool in an ice bath for 2 h. The crystalline product **5** (44 mg, 5%), R_f = 0.55 (EtOAc–hexane–MeOH–NH_4OH, 8.7:7.7:1:0.2), mp > 230 °C dec, was collected by filtration: ^1H NMR ($CDCl_3$) δ 0.1–0.2 (m, 2 H, H_{20} and H_{21}), 0.5–0.6 (m, 2 H, H_{20} and H_{21}), 0.8–1.0 (m, 1 H, H_{19}), 1.54–1.82 (m, 5 H), 2.1–2.54 (m, 10 H), 2.65–2.78 (m, 2 H), 3.075 (d, J = 18.45 Hz, 1 H, H_{10}), 3.277 (d, J = 6.12 Hz, 1 H, H_9), 3.413 (s, 3 H, *N*-methyl), 5.619 (s, 1 H, H_5), 6.476 (d, J = 8.06 Hz, 1 H, H_1), 6.561 (d, J = 8.06 Hz, 1 H, H_2); ^{13}C NMR ($CDCl_3$) δ 3.787, 4.089 (C_{20} and C_{21}), 9.382 (C_{19}), 21.144 ($C_{5'}$), 21.781 ($C_{6'}$), 23.126, 23.211, and 23.359 (C_{10}, $C_{4'}$, and $C_{7'}$), 29.097 (C_{15}), 29.702 (N–Me), 31.820 (C_8), 43.773 (C_{16}), 47.956 (C_{13}), 59.479 (C_{18}), 62.339 (C_9), 73.466 (C_{14}), 86.034 (C_5), 114.468 ($C_{3'}$), 116.014 ($C_{7'}$), 116.537 (C_2), 118.217 (C_1), 121.263 ($C_{2'}$), 125.227 (C_{11}), 130.314 and 131.069 (C_{12} and C_6), 138.983 (C_4), 143.149 (C_3); IR (KBr) 3416, 1638, 1623, 1506 cm^{-1}; mass spectrum, m/e 432.240 (M^+, EI, calcd for $C_{27}H_{32}N_2O_3$, 432.241), 433.245 (M

+ H^+, CI, calcd for $C_{27}H_{33}N_2O_3$, 433.249). Anal. ($C_{27}H_{32}N_3O_3$) C, H, N.

17-(Cyclopropylmethyl)-6,7-didehydro-3,14β-dihydroxy-4,5α-epoxy-6-(2′-pyrrolyl)morphinan (6). To a stirred solution of naltrexone hydrochloride (300 mg, 0.79 mmol) in DMF (6 mL) were added pyrrole (0.15 mL, 2.31 mmol) and methanesulfonic acid (0.3 mL, 3.13 mmol). The resulting solution was stirred at 100 °C for 14 h. Chloroform and saturated sodium bicarbonate solution were added to the solution and the mixture was extracted with $CHCl_3$ (3×). The combined organic phases were washed with brine, dried, and concentrated to give a crude product which was crystallized from methanol to afford pure **6** (248 mg, 80%): mp 254–256 °C; R_f 0.25 (CMA, 19:1:0.1); ^1H NMR (DMSO-d_6, δ) 0.08, (2 H, m), 0.50 (2 H, m), 0.85 (1 H, m), 1.50 (1 H, m), 1.94–2.73 (8 H, m), 3.05 (1 H, d, J = 18.5 Hz), 3.15 (1 H, d, J = 5.0 Hz), 5.18 (1 H, s), 6.02 (2 H, br, s), 6.21 (1 H, br, s), 6.45 (1 H, d, J = 8.0 Hz), 6.55 (1 H, d, J = 8.0 Hz), 6.75 (1 H, br, s); EIMS (m/e) 390 (M^+). Data for **6**·HCl: mp > 300 °C dec; R_f 0.67 (BAW, 2:1:1). Anal. ($C_{24}H_{26}O_3N$·HCl·0.5H_2O) C, H, N. Cl.

17-(Cyclopropylmethyl)-6,7-didehydro-3,14β-dihydroxy-4,5α-epoxy-6-(3′-indolyl)morphinan (7). To a stirred solution of naltrexone hydrochloride (300 mg, 0.79 mmol), in DMF (6 mL) were added indole (300 mg, 2.56 mmol) and methanesulfonic acid (0.3 mL, 4.38 mmol). The resulting solution was stirred at 100 °C for 14 h. Methanol, chloroform, and saturated sodium bicarbonate solution were added, and the mixture was filtered. The filtrate was extracted with $CHCl_3$ (3×) and the combined organic phases were washed with brine, dried, and concentrated to give a crude product. The product was chromatographed on sephadex column (LH-20, MeOH) to afford pure **7** (290 mg, 83%): R_f 0.14 (CMA, 19:1:0.1); IR (liquid film, cm^{-1}) 3290, 3020, 2930, 1610; ^1H NMR ($CDCl_3$, δ) 0.15 (2 H, m), 0.54 (2 H, m), 0.90 (1 H, m), 1.70 (1 H, d, J = 9.4 Hz), 2.13–2.45 (6 H, m), 2.58–2.77 (2 H, m), 3.10 (1 H, d, J = 18.4 Hz), 3.21 (1 H, d, J = 6.4 Hz), 5.38 (1 H, s), 6.33 (1 H, dd, J = 5.6 Hz, 5.4 Hz), 6.56 (1 H, d, J = 6.8 Hz), 6.67 (1 H, d, J = 6.8 Hz), 7.10 (1 H, t, J = 7.5), 7.15 (1 H, t, J = 7.5 Hz), 7.28 (1 H, d, J = 7.5 Hz), 7.40 (1 H, s), 7.70 (1 H, d, J = 7.5 Hz), 8.10 (1 H, br, s); CIMS 441 (M^+ + 1). Data for **7**·HCl: mp > 205 dec; R_f 0.72 (BAW, 2/1/1). Anal. ($C_{28}H_{28}O_3N_2$·HCl·1.5H_2O) C, N, H: calcd, 6.40, found, 5.90.

17-(Cyclopropylmethyl)-6,7-didehydro-3,14β-dihydroxy-4,5a-epoxy-6-phenylmorphinan (8). Compound **12** (31 mg, 0.074 mmol) was dissolved in concentrated HCl (5 mL). After 20 min, the solution was concentrated in vacuo and the residue was taken up in saturated aqueous $NaHCO_3$ (5 mL). The suspension was extracted with EtOAc (3 × 10 mL), the combined extracts were washed with brine, and dried (Na_2SO_4), and the solvent was removed in vacuo to yield **8** (26 mg, 88%, mp 217–221 °C dec): ^1H NMR δ 0.08–0.16 (m, 2 H), 0.50–0.58 (m, 2 H), 0.75–0.91 (m, 1 H), 1.65–1.75 (m, 1 H), 2.09–2.45 (m, 7 H), 2.54–2.73 (m, 2 H), 3.06 (d, 1 H, J = 19.6 Hz), 3.18 (d, 1 H, J = 6.8 Hz), 5.51 (s, 1 H), 6.19 (dd, 1 H, J = 2.5, 5.9 Hz), 6.53 (d, 1 H, J = 7.4 Hz), 6.66 (d, 1 H, J = 7.4 Hz), 7.19–7.35 (m, 3 H), 7.45–7.51 (m, 2 H); mass spectrum, (70 eV) m/z 401.1996 (M^+) calcd for $C_{26}H_{27}NO_3$, 401.1990. Anal. ($C_{26}H_{27}NO_3$) C, H, N.

7-Oximinonaltrexone (10). To a stirred solution of 3-(*tert*-butyldimethylsilyl)naltrexone[6] (11) (1 g, 2.2 mmol) in *tert*-butyl alcohol (25 mL) were added to isoamyl nitrite (0.4 mL, 3.06 mmol) and potassium *tert*-butoxide (600 mg, 5.31 mmol) in a water bath. The mixture was stirred at 24 °C for 40 min. Water was added, and the resulting mixture was stirred at 24 °C for 30 min and adjusted to pH 7 with acetic acid (0.3 mL) in a water bath. The mixture was extracted with $CHCl_3$ (4×) and the combined organic phases were dried and concentrated to give a solid which was purified by Sephadex column (LH-20, MeOH) to afford **10** (550 mg, 67.9%). Crystallization afforded a pure **10**: mp > 220 °C dec; IR (KBr, cm^{-1}) 3370, 3050, 2920, 2830, 1705, 1635, 1615, 1500, 1440, 825, 807, 705; ^1H NMR ($CDCl_3$, δ) 0.12 (2 H, m), 0.50 (2 H, m), 0.89 (1 H, m), 1.49 (1 H, d, J = 11.6 Hz), 2.03 (1 H, d, J = 18.2 Hz), 2.18 (1 H, m), 2.35 (3 H, m), 2.65 (2 H, m), 2.83 (1 H, d, J = 18.2 Hz), 3.07 (1 H, d, J = 18.7 Hz), 3.22 (1 H, d, J = 6.1 Hz), 5.38 (1 H, s), 6.55 (1 H, d, J = 8.2 Hz), 6.62 (1 H, d, J = 8.2 Hz), 9.28 (br, s); CI mass 371 (M^+ + 1). Anal. ($C_{20}H_2O_5N_2$) C, H, N.

17-(Cyclopropylmethyl)-4,5α-epoxy-6-phenyl-3,6,14β-trihydroxymorphinan (12). Phenyllithium (2.0 M in 7:3 Et_2O–cyclohexane, 8 mL, 16 mmol) was slowly added to a stirred solution of naltrexone (400 mg, 1.17 mmol) in dry Et_2O (15 mL) and THF (15 mL) at 0 °C under nitrogen. The resulting suspension was allowed to warm to room temperature, stirred for 16 h, and quenched by the addition of aqueous NH_4Cl solution (20% w/v, 10 mL). The mixture was evaporated to about 10 mL, and extracted with EtOAc (3 × 20 mL). The combined extracts were washed with brine, dried (Na_2SO_4), and evaporated to an oil. The oil was crystallized from $CHCl_3$ (1 mL) to afford **12** (156 mg, 32%, mp 213–215 °C). Chromatography of the mother liquor yielded an additional 133 mg (total yield 59%), along with recovered naltrexone (124 mg): ^1H NMR δ 0.09–0.19 (m, 2 H), 0.50–0.59 (m, 2 H), 0.79–0.91 (m, 1 H), 1.35–1.51 (m, 2 H), 1.73–1.82 (m, 2 H), 2.17 (ddd, 1 H, J = 3.7, 12.8, 12.8 Hz), 2.25–2.50 (m, 4 H), 2.57–2.70 (m, 2 H), 3.05 (d, 1 H, J = 18.9 Hz), 3.13 (d, 1 H, J = 6.7 Hz), 4.92 (s, 1 H), 6.58 (d, 1 H, J = 7.4 Hz), 6.70 (d, 1 H, J = 7.4 Hz), 7.21–7.29 (m, 1 H), 7.31–7.40 (m, 2 H), 7.49–7.56 (m, 2 H); mass spectrum, (70 eV) m/z 419.2080 (M^+); calcd for $C_{26}H_{29}NO_4$, 419.2096.

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Registry No. 1, 111555-53-4; **2**, 111555-58-9; **2**·$MeSO_3H$, 122517-78-6; **3**, 122431-17-8; **4**, 122431-18-9; **5**, 133042-75-8; **6**, 133042-76-9; **7**, 133042-77-0; **8**, 133042-78-1; **9**, 16590-41-3; **9**·HCl, 16676-29-2; **10**, 122431-19-0; 11, 96453-52-0; **12**, 133042-79-2; **13**, 111555-57-8; PhLi, 591-51-5; H_2NOPh, 4846-21-3; 1,2-phenylenediamine, 95-54-5; 2-aminobenzaldehyde, 529-23-7; cyclohexanone, 108-94-1; methylhydrazine, 60-34-4; methylhydrazine methylhydrazone, 1567-83-5; pyrrole, 109-97-7; indole, 120-72-9; amyl nitrite, 110-46-3.