Design of Peptidomimetic δ Opioid Receptor Antagonists Using the Message-Address Concept

P. S. Portoghese,*,† M. Sultana,† and A. E. Takemori‡

Department of Medicinal Chemistry, College of Pharmacy, and Department of Pharmacology, Medical School, University of Minnesota, Minneapolis, Minnesota 55455. *Received November 30, 1989*

Highly selective nonpeptide ligands with potent δ opioid receptor antagonist activity have been developed using the message-address concept. This approach envisaged the δ opioid receptor to contain two major recognition subsites; a message subsite which recognizes the pharmacophore, and an address subsite that is unique for the δ receptor type and confers selectivity. The message and address components of the δ-selective enkephalins were postulated to be Tyr[1] and Phe[4], respectively, with Gly[2]-Gly[3] functioning as a spacer. The message component of the target compounds in this study was derived from naltrexone and related structures. An indole system was fused to the C ring of naltrexone as a mimic of the address component. The benzene moiety of indole was viewed as the δ address component, mimicking the phenyl group of Phe[4], and the pyrrole portion was used as a rigid spacer. Members of the series (1-23) were evaluated for opioid antagonist activity on the guinea pig ileum (GPI) and mouse vas deferens (MVD) preparations. Naltrindole (NTI, 1) was the most potent member of the series, with K_e values of ~0.1 nM at δ receptors. The antagonism by NTI was ~220- and 350-fold greater at δ than at μ and κ opioid receptors. The binding of NTI and selected members of the series to guinea pig brain membranes was qualitatively consistent with their pharmacologic antagonist activity profiles in the MVD and GPI, but the K_i values were not in the same rank order. The selectivity of NTI arises mainly as a consequence of increased affinity at δ receptors. Thus, the K_e and K_i values of NTI were $^1/_{530}$ and $^1/_{90}$ that of the δ antagonist enkephalin analogue, ICI 174864. In contrast to NTI, ICI174864 derives its selectivity through greatly decreased recognition at μ and κ receptors. The implications of the high affinity and selectivity of NTI as a consequence of its conformational rigidity are discussed. It is suggested that any attempt to model a receptor-bound conformation of an opioid peptide should consider affinity and potency at multiple receptor sites rather than selectivity alone.

The presence of multiple opioid receptors is now firmly established. There are at least three major opioid receptor types that mediate a variety of central and peripheral physiologic effects.[1] One of these receptor types, which has been named δ, selectively recognizes the endogeneous enkephalins.[2] Although these receptors are widely distributed, their precise physiologic roles are not known. A key reason for this paucity of information has been the lack of potent and selective δ opioid receptor antagonists. The most widely employed opioid antagonists, naloxone[3] and naltrexone,[4] are not useful as tools to sort out the effects mediated by specific receptor types because they act at multiple opioid receptor populations. Although the enkephalin analogue $(allyl)_2$Tyr-Aib-Aib-Phe-Leu-OH (ICI174864)[5] is a δ-selective antagonist, its relatively low potency and peptidic nature limits its usefulness as a pharmacologic tool.

For these reasons we have investigated the possibility of designing nonpeptide δ opioid antagonists with the objective of obtaining high-affinity ligands that would not be subject to breakdown by peptidases and would be capable of penetrating the central nervous system (CNS) upon peripheral administration. In this paper we present a novel, successful approach to the design of peptidomimetic δ opioid antagonists (Table I) using the "message-address" concept.[6-8]

Design Rationale

Our rationale for the design of peptidomimetic δ-selective opioid antagonists was based on the message-address concept proposed by Schwyzer[6] to analyze structure-activity relationships of various peptide hormones. Accordingly, peptide hormones that are "sychnologically" organized contain a "message" sequence and "address" sequence of amino acid residues, each being proximal to one another in the peptide chain.[9] The message component is involved in signal transduction at the receptor, while the address provides additional binding affinity and is not essential to the transduction process. For a group of peptides associated with more than one receptor type, the message component is very similar or invariant, while the address segment is variable and a determinant of selectivity for a particular type of receptor.

As was pointed out by Chavkin and Goldstein,[10] the endogenous opioid peptides appear to conform to the message-address concept in that they contain a constant tetrapeptide sequence (Tyr-Gly-Gly-Phe) that can be viewed as the message, and a variable sequence that can operate as the address in conferring selectivity. This address sequence presumably is complementary to a receptor subsite that is unique for each opioid receptor type.

Another interpretation of the message-address concept, as applied to opioid peptides, is that the Tyr[1] residue comprises the message component, and the sequence starting with Phe[4] constitutes the address; in this context, Gly[2]-Gly[3] serves as a spacer.[8] This alternate view is consistent with the well-known structure-activity relationships of nonpeptide opioid ligands (e.g., morphine) that contain only one aromatic ring which presumably mimics the Tyr[1] residue.

In studies directed toward evaluating the message-address concept, we have reported[11] that a typically μ-se-

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‡ Department of Pharmacology.

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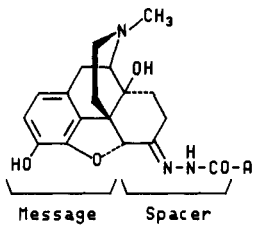


A (address)	Selectivity
Phe-Leu-OH	delta
Phe-Leu-Arg-Arg-Ile-OMe	kappa

Figure 1. The modulation of opioid receptor selectivity by attachment of a peptide address (A) to an opiate structure.

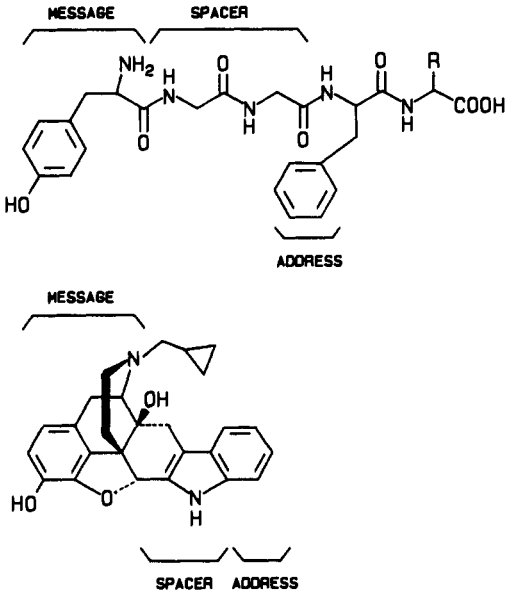


Figure 2. Comparison of the functional components of enkephalin (top) with those of naltrindole (bottom).

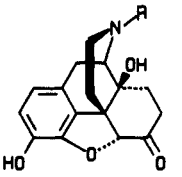
lective ligand such as oxymorphone can be transformed to a δ-selective ligand simply by attachment of a "δ address" (Phe-Leu) through a spacer to the C-6 position of the opiate (Figure 1). Similarly, a κ-selective ligand was synthesized by attachment of a "κ address" (Phe-Leu-Arg-Arg-IleOMe). These results suggested the feasibility of developing nonpeptide δ-selective opioid antagonists by the attachment of a nonpeptide moiety that would mimic a key recognition element of the δ address. Although the message-address concept was proposed for endogeneous agonists, it might serve as a useful model for the design of antagonists if such ligands also interact with both the message and address subsites of the receptor.

The design of such non-peptide δ opioid receptor antagonists was explored with a naltrexone-derived recognition unit ("message" component) that is joined to a benzene moiety which was envisaged to be the key δ address component that simulates Phe[4] of enkephalin. Naltrexone (**24**) was selected as the starting material because it offered the opportunity to synthesize ligands that contain a spacer fused to the C-ring of the morphinan structure. This was an important point in our design strategy, as the spacer would restrict the benzene moiety to a single conformation. Conformational rigidity was an important consideration because a rigid address moiety might confer greater δ selectivity by precluding possible conformational adaption of the ligand in the binding to other opioid receptor types. Since the receptor-bound conformation for the benzene ring of Phe[4] in the δ address is not known, an expeditious route to the target compounds seemed appropriate because we had no idea if the δ address mimic would be in the proper conformation to confer δ selectivity.

Our first candidate series that fulfilled the aforementioned criteria possesses the indole system. The pyrrole moiety in this aromatic system was viewed as part of a spacer to rigidly hold the benzene "address" component (Figure 2). Also, indole derivatives were considered reasonable targets because they are accessible in a single step through the Fischer indole synthesis.

Chemistry

The indole derivatives listed in Table I were obtained by reacting the appropriate phenylhydrazine hydrochloride with either naltrexone (**24**), naloxone (**25**), or oxymorphone (**26**) in acidic media. The products exhibited an H-5 NMR



24 $CH_2CH(CH_2)_2$

25 $CH_2CH{=}CH_2$

26 CH_3

absorption at δ 5.5–5.7, which is downfield from that of the starting ketones (δ 4.8) due to deshielding by the indole moiety. The indole protons (4′–7′) appeared in the range of δ 7.0–8.5. The indoles displayed UV maxima at 212 and 284 nm that are typical for this ring system.

The synthesis of 5′- and 7′-substituted indoles by the Fischer indole synthesis[12] were produced in generally moderate yields from the corresponding para- or ortho-substituted phenylhydrazines. The 1′-substituted indoles (**15**, **16**, **21**) were synthesized from methylphenylhydrazine or diphenylhydrazine. When (3-fluorophenyl)- and (3-methylphenyl)hydrazine were employed, they each afforded two regioisomers. The major regioisomers in both reactions were the 6′-substituted NTI analogues (**7**, **8**). The 4′- and 6′-fluoroindoles were obtained in a 1:4 ratio (NMR) with 6′-isomer **8** as the major product. The 6′-fluorine was coupled to H-5′ (J = 10.74 Hz) and to H-7′ (10.14 Hz) and exhibited long-range coupling to H-4′ (5.21 Hz). Only **8** was obtained in pure form by preparative HPLC. The mixture of 4′- and 6′-methyl regioisomers (ratio, 1:2.5) could not be separated by HPLC and were therefore tested as such. The NMR spectrum assigned to the 6′-isomer **7** exhibited two doublets (J = 8.02 Hz) at δ 7.23 and 6.78 corresponding to H-5′ and H-4′, respectively. A sharp singlet appeared at δ 7.12 due to H-7′. The 4′-methyl regioisomer showed a triplet (J = 8.0 Hz) at δ 6.94 for H-6′ and two doublets at δ 6.64 (J = 8.0 Hz) and 7.14 (J = 8.0 Hz) due to H-7′ and H-5′, respectively.

Molecular modification of the Fischer indole products afforded additional analogues. The 5′- and 7′-hydroxyl compounds (**6** and **14**) were derived from the corresponding methoxy derivatives (**5** and **12**) by treatment with BBr_3.

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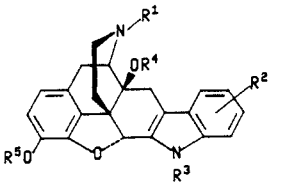
Table I. Physical Data for Naltrindole (NTI) Derivatives

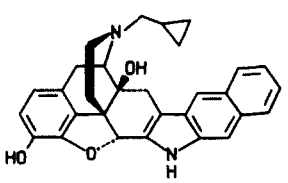


compd	R^1	R^2	R^3	R^4	R^5	R_f[a]	mp,[b] °C	% yield	reaction medium[c]	recryst solvent[d]	MS (M^+)	formula[e]
1 (NTI)	$CH_2CH(CH_2)_2$	H	H	H	H	0.47	270	71	A	H	415 (M^+ + 1)(CI)	$C_{26}H_{26}N_2O_3$·HCl
2	$CH_2CH(CH_2)_2$	5′-CH_3	H	H	H	0.38	260	70	B	I	428 (EI)	$C_{27}H_{28}N_2O_3$·HCl
3	$CH_2CH(CH_2)_2$	5′-F	H	H	H	0.40	275	75	C	H	432 (EI)	$C_{26}H_{25}N_2O_3F$· HCl·1.5H_2O
4	$CH_2CH(CH_2)_2$	5′-NO_2	H	H	H	0.32	285	55	B	H	459 (EI)	$C_{26}H_{25}N_3O_5$·HCl· 3.5H_2O
5	$CH_2CH(CH_2)_2$	5′-OCH_3	H	H	H	0.36	254	56	C	H	444 (EI)	$C_{27}H_{28}N_2O_4$·HCl· 1.5H_2O
6	$CH_2CH(CH_2)_2$	5′-OH	H	H	H	0.24	250	25	E	I	429 (M^+ − 1)(FAB)	$C_{26}H_{26}N_2O_4$[f]
7[g]	$CH_2CH(CH_2)_2$	6′-CH_3	H	H	H	0.50	260	55	A	H	428 (EI)	$C_{27}H_{28}N_2O_3$·HCl· H_2O
8	$CH_2CH(CH_2)_2$	6′-F	H	H	H	0.36	220	80	A	H	432 (EI)	$C_{26}H_{25}N_2O_3F$· HCl·1.5H_2O
9	$CH_2CH(CH_2)_2$	7′-Br	H	H	H	0.71	190	62	B	I	494 (EI)	$C_{26}H_{25}N_2O_3Br$[f]
10	$CH_2CH(CH_2)_2$	7′-CH_3	H	H	H	0.48	210	55	A	H	428 (EI)	$C_{27}H_{28}N_2O_3$·HCl· 2.5H_2O
11	$CH_2CH(CH_2)_2$	7′-F	H	H	H	0.43	230	50	A	H	432 (EI)	$C_{26}H_{25}N_2O_3F$· HCl·1.5H_2O[h]
12	$CH_2CH(CH_2)_2$	7′-OCH_3	H	H	H	0.82	250	63	C	I	444 (EI)	$C_{27}H_{28}N_2O_4$·HCl· 1.5H_2O
13	$CH_2CH(CH_2)_2$	7′-OC_2H_5	H	H	H	0.69	270	68	C	I	459 (M^+ + 1)(CI)	$C_{28}H_{30}N_2O_4$·HCl· 4.5H_2O
14	$CH_2CH(CH_2)_2$	7′-OH	H	H	H	0.16	275	72	E	I	429 (M^+ − 1)(FAB)	$C_{26}H_{26}N_2O_4$·HCl· 2H_2O
15	$CH_2CH(CH_2)_2$	H	CH_3	H	H	0.66	240	58	C	J	428 (EI)	$C_{27}H_{28}N_2O_3$·HCl· 2H_2O[i]
16	$CH_2CH(CH_2)_2$	H	C_6H_5	H	H	0.80	273	54	A	H	491 (M^+ + 1)(FAB)	$C_{30}H_{30}N_2O_3$·HCl[f]
17	$CH_2CH(CH_2)_2$	H	H	$COCH_3$	H	0.41	222	45	F	H	456 (EI)	$C_{28}H_{29}N_2O_4$·HCl· 5H_2O[j]
18	$CH_2CH(CH_2)_2$	H	H	H	CH_3	0.80	215	62	G	I	428 (EI)	$C_{27}H_{28}N_2O_3$· 1.5H_2O
19	$CH_2CH{=}CH_2$	H	H	H	H	0.48	260	65	A	I	400 (EI)	$C_{25}H_{24}N_2O_3$[f]
20 (OMI)	CH_3	H	H	H	H	0.39	230	60	A	H	374 (EI)	$C_{23}H_{22}N_2O_3$·HCl· 0.5H_2O
21	CH_3	H	CH_3	H	H	0.52	276	28	D	H	388 (EI)	$C_{24}H_{24}N_2O_3$[f]

[a] R_f was determined in $CHCl_3$/MeOH/NH_4OH, 9:1:0.5, v/v. [b] The compounds melted with decomposition at the temperature indicated. [c] A, MeOH/HCl (A gentle stream of HCl gas is bubbled through MeOH (50 mL) for 20 min; it cooled down and was used as a solvent for reaction); B, HCl (concentrated) CH_3COOH, 1:4, v/v; C, glacial acetic acid; D, MeOH HCl (concentrated), 20:1, v/v; E, derived from R^2 = OMe using BBr_3; F, derived from 1 by treating with acetic anhydride/pyridine followed by methanolysis (2:1, v/v); G, diazomethane treatment of 1. [d] H, EtOH/$CHCl_3$; I, EtOH; J, ethyl acetate. [e] Unless otherwise specified, compounds were within ±0.4% for CHN analysis. [f] Purity evaluated by chromatographic analysis. [g] A mixture of 6′ and 4′-regioisomers in a ratio of 2.5:1. [h] Anal. Calcd: C, 63.08. Found: C, 63.66. [i] Anal. Calcd: C, 64.85. Found: C, 64.09. [j] Anal. Calcd: C, 57.73. Found: C, 58.28.

14-Acetate ester **17** was prepared by treating NTI (**1**) with acetic anhydride in pyridine to afford the 3,14-diacetate intermediate, which was then converted to **17** by base-catalyzed methanolysis. Methyl ether **18** was prepared by treating a methanolic solution of NTI·HCl with excess diazomethane.

Benzindole compounds **22** and **23** were synthesized under conditions of the Fischer indole synthesis involving naltrexone and the corresponding β- and α-naphthyl-



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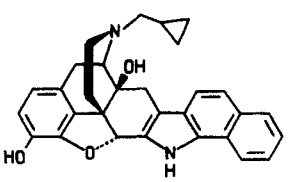

23

hydrazines. In the case of β-naphthylhydrazine, where two regioisomeric modes of cyclization are possible, only linear isomer **22** was detected in the reaction mixture. A possible explanation for this might be related to the hindered nature of the angular regioisomeric product. The linear structure of **22** was confirmed by NMR analysis.

Biological Results

Smooth Muscle Preparations. All target compounds were tested on the electrically stimulated guinea pig ileal longitudinal muscle[13] (GPI) and mouse vas deferens[2] (MVD) preparations as described previously[14] (Table II). Antagonists were incubated with the preparations 15 min prior to testing. Morphine (M), ethylketazocine (EK), and [D-Ala^2,D-Leu^5]enkephalin[15] (DADLE) were employed as μ-, κ-, and δ-selective agonists, respectively. Morphine and EK were employed in the GPI and DADLE was used in

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Table II. Opioid Antagonist Activity of NTI and Its Analogues

compd	DADLE[a] (δ)			M[b] (μ)			EK[c] (κ)			K_e selectivity ratio	
	IC_{50} ratio	concn[d]	K_e[e]	IC_{50} ratio	concn,[d]	K_e[e]	IC_{50} ratio	concn[d]	K_e[e]	μ/δ	κ/δ
1 (NTI)	152 ± 34	20	0.13	7.8 ± 1.6	200	29	5.4 ± 0.8	200	45	223	346
2	55.4 ± 10.5	200	3.7	1.6 ± 2.6	100	≥167	1.4 ± 0.3	100	≥250	≥45	≥68
3	104 ± 8	200	1.9	5.1 ± 1.0	200	49	6.3 ± 1.7	200	38	25	19
4	2.4 ± 0.3	200	146	1.9 ± 0.3	200	≥230	1.2 ± 0.1	200	≥870	≥1.6	≥5.6
5	53 ± 11.2	200	3.8	2.6 ± 1.0	100	≥63	9.2 ± 1.0	100	12	≥16	3.2
6	4.7 ± 1.2	100	27	6.4 ± 1.8	100	19	3.1 ± 1.0	100	48	0.7	1.8
7	30 ± 8	100	3.5	2.8 ± 0.6	100	55	1.7 ± 0.4	100	≥143	16	≥41
8	24 ± 6	100	4.4	1.8 ± 0.9	100	≥125	1.6 ± 0.4	100	≥167	≥28	≥38
9	85 ± 20.5	100	1.2	2.2 ± 0.8	100	83	1.7 ± 0.7	100	≥143	71	≥121
10	67 ± 4.9	100	1.5	7.1 ± 1.6	100	16	1.3 ± 0.4	100	≥333	11	≥221
11	287 ± 12	100	0.35	5.3 ± 0.7	100	23	1.3 ± 0.2	100	≥333	67	≥951
12	66 ± 0.6	100	1.5	3.0 ± 0.6	100	50	1.5 ± 0.29	100	≥185	32	≥120
13	178 ± 36	100	0.57	8.3 ± 0.6	100	13	0.67 ± 0.14	100		24	
14	239 ± 46	100	0.42	1.9 ± 0.5	100	≥111	1.4 ± 0.5	100	≥250	≥264	≥595
15	204 ± 44	200	0.99	9.9 ± 1.8	100	11	6.0 ± 1.0	100	20	11	20
16	33 ± 7.3	100	3.1	1.7 ± 0.3	100	≥149	6.7 ± 0.2	100	≥152	≥48	≥49
17	43 ± 2.9	100	2.4	1.4 ± 0.5	100	≥263	1.8 ± 0.25	100	≥127	≥111	≥54
18	1.5 ± 0.4	100	≥200	1.3 ± 0.7	100	≥333	0.6 ± 0.07	100	≥167		
19	93 ± 21	100	1.1	1.1 ± 0.09	100	≥714	2.2 ± 0.8	100	≥82	≥655	≥75
20 (OMI)	1.6 ± 0.5	5[f]	≥8.8	0.9 ± 0.3	100	≥111	0.6 ± 0.2	100	≥167		
21	1.2 ± 0.3	100	≥416	0.74 ± 0.03	100	≥135	0.50 ± 0.13	100	≥200		
22	4.7 ± 0.1	100	27	0.85 ± 0.06	100	≥118	0.91 ± 0.25	100	≥100	≥4	≥4
23	12.1 ± 2.7	100	9	1.2 ± 0.3	100	≥500	0.9 ± 0.4	100	≥111	≥55	≥12
ICI174864[g]	15.6 ± 2.4	1000	69	0.6 ± 0.1	1000	>1667	0.8 ± 0.1	1000	≥1250	≥24	≥18
naltrexone (24)	10.5 ± 2.3	300	32	98 ± 24	100	1.0	19.3 ± 5.9	100	5.5	0.04	0.17

[a] [D-Ala²,D-Leu⁵]enkephalin in the MVD. [b] Morphine in the GPI. [c] Ethylketazocine in the GPI. [d] Antagonist concentration expressed as nM. [e] K_e (nM) = [antagonist]/(IC_{50} ratio − 1), where the IC_{50} ratio is the IC_{50} of the agonist in the presence of antagonist divided by the control IC_{50} in the same preparation ($n \geq 3$). [f] The agonist effect of **20** precluded use of higher concentration. [g] (allyl)$_2$Tyr-Aib-Aib-Phe-Leu (see ref 5).

the MVD. The antagonist potencies are expressed as K_e values which were calculated from the equation K_e = [antagonist]/(IC_{50} ratio − 1), where the IC_{50} ratio represents the IC_{50} of the agonist in the presence of the antagonist divided by the control IC_{50} of the agonist.

Most of the members of the series were significantly more potent in antagonizing the δ-selective agonist DADLE. The most potent and selective member of the series was the unsubstituted indole naltrindole (**1**, NTI), with a K_e = 0.13 nM. It was noted that the NTI antagonist activity was washed out of the MVD preparation with difficulty. For example, when incubated with 20 nM NTI for 15 min, the initial IC_{50} ratio (152) was reduced to 18 after 30 washes. Washing the MVD 30 times with naltrexone solution (100 nM) was only slightly more effective (IC_{50} ratio = 11).

This nonequilibrium effect was independent of incubation time (10, 30, 60 min). The concentration–response relationship of the antagonism of the δ agonist [D-Pen²,D-Pen⁵]enkephalin[16] (DPDPE) by NTI is shown in Figure 3, and it can be noted that NTI afforded a parallel shift of the agonist response curve. Four of the ligands (**4**, **18**, **20**, **21**) behaved as very weak antagonists, having values of $K_e \geq 146$ nM for the antagonism of all three agonists. The remaining members of the series possessed K_e values at δ receptors between 1 and 27 nM and were more typically in the range of 1–10 nM. The only compound that approached the potency of NTI (**1**) was 7′-fluoro derivative **11**.

Except for oxymorphindole (**20**, OMI), members of the series possessed little or no agonist activity (≤20%) in the GPI or MVD. OMI (**20**) behaved as a partial agonist with an IC_{50} of 100 nM in the MVD preparation (Figure 4). Very weak agonist activity (10% maximal response at 1 μM) was noted in the GPI. The agonist activity of OMI was antagonized by NTI (Figure 4), but very feebly by naltrexone. The corresponding dimethyl derivative **21** was

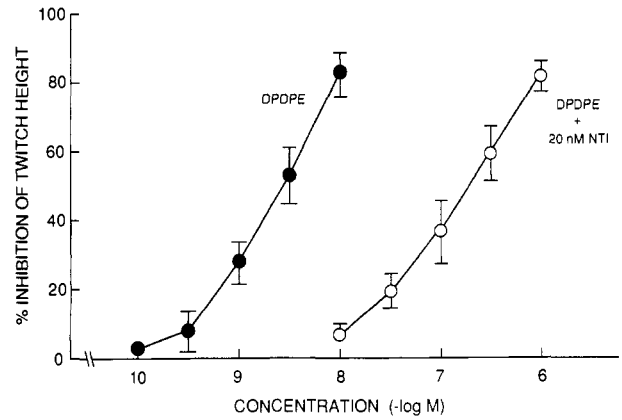


Figure 3. The displacement of the concentration–response curve of DPDPE by NTI in the MVD preparation.

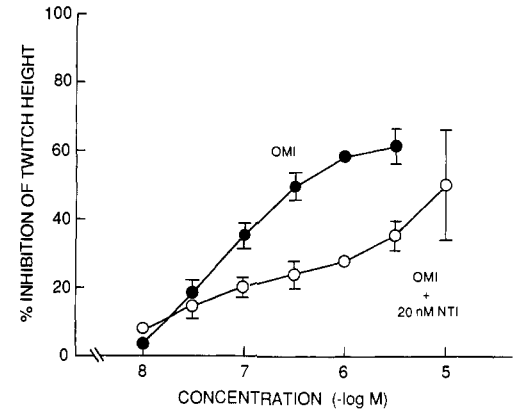


Figure 4. The agonist effect of oxymorphindole (OMI) in the absence and presence of NTI in the MVD preparation.

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

inactive as an agonist or as an antagonist.

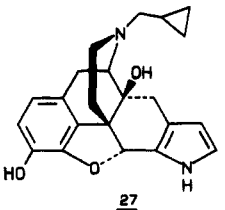
Binding

Opioid receptor affinities of selected compounds (Table III) were determined by displacement of radioligands from guinea pig brain membranes employing a modification of the method of Werling et al.[17] Binding to κ receptors was evaluated with [^{3}H]EK in the presence of 1 μM [D-Ala^2,MePhe^4,Gly-ol^6]-enkephalin[18] (DAMGO), to μ receptors with [^{3}H]DAMGO, and to δ receptors with [^{3}H]DADLE in the presence of 1 μM DAMGO. The binding data are expressed as K_i values. All of the indoles in Table III are δ-selective and qualitatively consistent with their pharmacologic profiles in the MVD and GPI. It is noteworthy that the 7′-F (**11**) and the 1′-Me (**15**) derivatives possess δ opioid receptor affinities that are nearly identical with that of NTI (**1**). The K_i selectivity ratios of these compounds are in fact substantially greater than that of NTI. Also, the partial agonist OMI (**20**), while possessing about $^1/_{20}$ affinity of NTI, possessed comparable selectivity.

Discussion

Naltrindole (**1**, NTI) and other indoles in the present series are the first reported nonpeptide ligands that possess potent, selective, δ opioid receptor antagonist activity. By way of comparison, the enkephalin analogue ICI174864[5] currently employed as a δ antagonist possessed a potency $^1/_{530}$ that of NTI. In terms of δ receptor binding, NTI has over a 1000-fold greater affinity than ICI174864. The fact that there are dramatic differences between the antagonist selectivity profiles of NTI and naltrexone illustrates the profound effect exerted by the indole moiety in this series. In this regard, NTI is 240 times more potent than naltrexone as an antagonist at δ receptors.

The rigidity of this indole system is a consequence of its fusion to ring C of the morphinan and this is viewed to be an important factor which contributes to the selectivity. The pyrrole moiety of the indole functions mainly as a rigid spacer to hold the benzene moiety, which is considered to be the relevant δ address component that mimics the Phe^4 phenyl group of enkephalin (Figure 2). Evidence for this spacer function was provided by compound **27**, which contains only the pyrrole moiety. We



27

have found[19] that **27** is a μ-selective ligand with substantially decreased δ opioid receptor antagonist activity relative to NTI.

It is noteworthy that the δ binding selectivity of NTI (**1**) and other potent antagonists in this series is related to their greatly increased affinity for δ opioid receptors (~1000-fold greater than that of naltrexone) and to decreased affinity for μ and κ sites. This suggests that the benzene moiety of the indole system confers selectivity by binding to a unique subsite (the address) on the δ opioid receptor while sterically hindering binding to other opioid receptor types.

The high antagonist potency and selectivity of NTI (**1**) for δ sites raises the possibility that this ligand may approximate a receptor-bound conformation of enkephalin if they are acting through a common binding site. As it is known[20] that replacement of a cyclopropyl (or allyl) group with methyl usually results in a change from antagonist to agonist activity in opiate structures, this possibility was explored with the oxymorphone-derived analogue oxymorphindole (**20**, OMI). Oxymorphone (**26**) is a potent μ-selective agonist and it was anticipated that its indole derivative **20** might be a δ-selective agonist if the conformational requirements of δ agonists and antagonists are similar.

On the MVD OMI (**20**) acted as a partial agonist (65% maximum response), and it was virtually inactive on the GPI (Figure 4). This was consistent with it acting as a δ-selective ligand. The fact that OMI was antagonized by NTI suggests that its agonist effect is mediated through the δ receptor system. However, it can be noted (Figure 4) that NTI causes a biphasic concentration–response relationship. Since NTI displayed avid binding to the δ opioid receptor system, it may be acting like an irreversible ligand in competing with OMI. Under such circumstances a decline in the concentration–response curve maximum would be expected due to the partial agonist character of OMI. The fact that the agonist concentration response curve of OMI is parallel with that of its control only in the μM and mM range suggests that these relatively high concentrations of OMI may afford a nonspecific "agonist" effect that is not antagonized by NTI.

The partial δ agonist character of OMI (**20**) raises the intriguing possibility that the receptor-bound conformations of agonist and antagonists differ.[21] The fact that the antagonist NTI (**1**) is bound more avidly (Table III) to δ receptors than OMI is consistent with the idea that the agonist state and antagonist state of the opioid receptor differ from one another. If the conformation of NTI is more complimentary to the antagonist state, then it would be expected that OMI which possesses a conformation identical with that of NTI, should possess considerably lower efficacy than peptidic δ agonists. Since this interpretation is valid only if the agonist effect of OMI and the antagonism of NTI are mediated through identical δ sites, additional studies are required to provide support to this possibility.

It appears that substitution at the 5′- or 6′-positions on the indole system leads to a decrease of δ opioid antagonist potency by at least 1 order of magnitude. Since this is usually not accompanied by a corresponding diminution of antagonist potency at μ and κ receptors, the result of such substitution is generally to decrease δ selectivity. At the 5′-position, the decreased potency change appears to be correlated with an increase in the size and polarity of the substituent. The 7′- and 1′-positions appear to have less of a detrimental effect on δ antagonist potency when substituted. This also is consistent with the greater δ antagonist potency of congener **23** relative to its regioisomer **22**. The fact that the benzindoles are not more potent or selective than NTI suggests that location of the benzene moiety at a greater distance from the opioid pharmacophore does not afford increased recognition at δ receptors.

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Table III. Opioid Receptor Binding of NTI Analogues

compd	K_i[a] (SE), nM			K_i selectivity ratio	
	δ	μ	κ	μ/δ	κ/δ
1 (NTI)	0.03 (0.001–0.17)	3.8 (0.6–22)	332 (267–413)	127	11066
3	0.9 (0.8–1.1)	1.7 (0.1–26)	15 (11–20)	2	17
9	0.14 (0.03–0.7)	29 (25–32)	333 (268–413)	205	2378
11	0.02 (0.002–0.25)	49 (16–607)	321 (259–399)	2450	16050
12	2.4 (0.3–17)	16 (1–221)	43 (25–72)	7	18
15	0.02 (0.003–1.1)	14 (4–47)	65 (44–97)	700	3250
16	0.6 (0.5–0.9)	28 (7–122)	746 (472–1183)	47	1243
18	40 (25–64)	550 (271–1117)	>1000	14	>25
19	1.1 (0.1–15)	104 (56–193)	321 (259–399)	95	292
20 (OMI)	0.7 (0.08–7)	468 (258–853)	4467 (1954–10190)	669	6381
ICI174864[b]	35 (19–65)	>1000	>1000	>29	>29
naltrexone	36 (15–89)	0.8 (0.3–2.3)	20 (15–27)	0.02	0.6
DPLPE[c]	11 (3–40)	570 (213–1528)	2218 (1033–4764)	52	202
DSLET[d]	14 (3–42)	59 (7–482)	>1000	4	>71

[a] Values are geometric means (95% CL) from three to five experiments. [b] Reference 5. [c] [D-Pen^2,L-Pen^5]enkephalin (Kazufumi, A.; Gee, K. W.; Mosberg, H. I.; Hruby, V. J.; Yamamura, H. I. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 2543). [d] [D-Ser^2,Leu^5,Thr^6]enkephalin (Gacel, G.; Fournie-Zaluski, M. C.; Roques, B. P. *FEBS Lett.* **1980**, *118*, 245).

The phenolic group is required for δ antagonist activity, as the NTI methyl ether **18** is inactive in blocking any of the opioid receptor types. The essential nature of the phenolic OH suggests that the message components of NTI and the opioid peptides bind to message recognition sites that are similar to one another.

Other modifications such as acetylation of the 14-hydroxy group (**17**) or replacement of the cyclopropylmethyl (**1**) with allyl (**19**) afforded reduction of δ antagonist potency with relatively minor reductions in selectivity. Thus, it is apparent that the 14-hydroxy group is not critical for selectivity. Moreover, the change from cyclopropyl to allyl afforded a decrease in δ antagonist potency similar to that observed for antagonists (e.g., **24** versus **25**) that are selective for other opioid receptor types.

The binding data (Table III) complement the pharmacologic studies (Table II) in establishing that OMI (**20**) and NTI (**1**) act by binding to sites on the δ opioid receptor system. In this regard OMI exhibited smilar binding selectivity relative to that of NTI. It is noteworthy that OMI possessed lower affinity than its antagonist NTI but 20–50-fold greater affinity than the δ selective peptides DPLPE, DSLET, and ICI74864. The lack of correlation of the agonist potency of OMI with binding of DPDLE and DSLET is probably related to its lower efficacy at δ receptors.

While receptor binding in every case qualitatively confirms the δ selectivity of ligands in this series, these data are not in the same rank order as the in vitro pharmacology. In fact, there are a number of congeners (e.g., **9** and **15**) that have approximately the same affinity for δ recognition sites as NTI (**1**) but are not as potent in the MVD preparation. The reason for this divergence is not known. One possible explanation is that the δ opioid receptor system in guinea pig brain membranes differ from that in the MVD preparation. Another possibility is that multiple recognition sites on the same δ opioid receptor system mediate antagonism. If this is the case, binding may not parallel antagonist potency if some of the sites occupied by naltrindole and its analogues are not common with those occupied by the radioligand.

Conclusions

Application of the message–address concept has led to the design and synthesis of peptidomimetic δ opioid antagonists with unprecedented antagonist potencies and affinities. The conformationally constrained benzene moiety of the indole system is viewed as the key δ address component that is responsible for the enhanced effects at δ receptors and the low binding and potency at μ and κ receptors. It is conceivable that the benzene moiety of NTI mimics the receptor-bound conformation of the Phe^4 phenyl group of enkephalin at a unique subsite on the address recognition locus of the δ receptor system. The implication is that the address recognition subsites on μ and κ opioid receptor systems possess different conformational requirements for the phenyl group of Phe^4. It therefore seems plausible that different conformational requirements of the address subsite for Phe^4 play an important contributory role in the selectivity of opioid peptides.

The nature of the selectivity of NTI (**1**) differs from that of the δ antagonist ICI174864 in that the binding of NTI is orders of magnitude greater than that of this peptide or any of the other established δ-selective ligands (Table III). The δ-selectivity of ICI174864 is therefore related to greatly decreased recognition by μ and κ receptors rather than increased affinity for δ receptors. Similarly, the δ agonist [D-Pen^2,D-Pen^5]enkephalin (DPDPE) is selective due to its low affinity at μ and κ sites rather than to improved fit at δ recognition sites. In this regard, the relationship between conformation and selectivity cannot be adequately addressed without a consideration of both the potencies and affinities of the ligands at the three opioid receptor types. Moreover, when one evaluates molecular parameters that might possibly contribute to selectivity, it should be emphasized that factors that decrease binding affinity at other opioid sites are as important as those that increase affinity at the target site. As a corollary, any attempts to model a receptor-bound conformation of an opioid peptide using conformationally restricted ligands[22] should take into account the affinity and potency of the molecular template at the target sites rather than considering selectivity alone.

Experimental Section

Melting points were determined in open capillary tubes with a Thomas-Hoover melting point apparatus and are uncorrected. Analyses were performed by M-H-W Laboratories, Phoenix, AZ. NMR spectra were recorded at ambient temperature on an IBM Bruker AC-300 spectrometer using DMSO-d_6 or $CDCl_3$ as solvent and Me_4Si as internal standard. Mass spectra were obtained on a Finnigan 4000, a AEIMS-30, or a VG7070EHF spectrometer. All TLC data were determined with Analtech silica gel (GHLF 21521). All reagents and solvents were reagent grade and used

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without subsequent purification. The phenylhydrazines used in the synthesis of the indoles were purchased from Aldrich Chemical Co. Naltrexone, naloxone, and oxymorphone were supplied by Mallinckrodt.

General Procedures. Fischer Indole Synthesis[23] (1–5, 7–13, 15, 16, 19–23). 17-(Cyclopropylmethyl)-6,7-dehydro-4,5α-epoxy-3,14-dihydroxy-6,7-2′,3′-indolomorphinan (1·HCl, Naltrindole, NTI). A mixture of naltrexone hydrochloride (**24**·HCl, 377 mg, 1.0 mmol) and phenylhydrazine hydrochloride (216 mg, 1.5 mmol) was dissolved in MeOH (20 mL) saturated with HCl. The resulting solution was refluxed under nitrogen for 5 h; at the end of this period the mixture was cooled and a solid precipitated. This precipitate was collected by filtration and was washed with cold EtOH (10 mL) followed by ether (10 mL). It was then crystallized from EtOH to afford 0.29 g (71%) of pure product 1·HCl: ^{1}H NMR (DMSO-d_6) δ 8.35 (1 H, s, NH), 7.25 and 7.40 (1 H each, d, H-4′ and 7′), 6.95 (1 H, t, H-5′), 7.11 (1 H, t, H-6′), 6.58 (2 H, dd, H-1, H-2), 5.75 (1 H, s, H-5); ^{13}C NMR (DMSO-d_6) δ 140.92 (C2′), 131.63 (C6), 127.55 (C3′), 122.91 (C6′), 119.41 (C5′), 119.20 (C4′), 111.9 (C7′), 110.87 (C7), 85.67 (C5); CIMS m/z 415 ($M^+ + 1$).

Methyl Ether Cleavage (6, 14). 17-(Cyclopropylmethyl)-6,7-dehydro-4,5α-epoxy-3,5′,14-trihydroxy-6,7-2′,3′-indolomorphinan (6). A stirred solution of **5** (free base, 444 mg, 1 mmol), in $CHCl_3$ (9 mL) was added dropwise to a solution of BBr_3 (0.48 mL, 5.1 mmol) in $CHCl_3$ (12 mL). The resulting solution was stirred for 2 h. The $CHCl_3$ then was removed in vacuo and the residue was made basic (pH 9) with saturated Na_2CO_3. Extraction with $CHCl_3$/2-propanol (3:1, 4 × 10 mL) gave **6** as a foam (107 mg, 25%): ^{1}H NMR (DMSO-d_6) δ 5.50 (s, 1 H, H-5), 6.6–7.1 (m, 4 H, 4′,5′,6′, and 7′).

17-(Cyclopropylmethyl)-6,7-dehydro-4,5α-epoxy-3-hydroxy-14-acetoxy-6,7-2′,3′-indolomorphinan Hydrochloride (17·HCl). A solution of 1·HCl (450 mg, 1 mmol) in acetic anhydride (20 mL) and pyridine (10 mL) was allowed to stand at room temperature for 24 h. The reaction mixture was concentrated in vacuo and the residue was dissolved in MeOH (10 mL) and saturated sodium bicarbonate (5 mL). After letting the solution stand for 2 h it was concentrated, dissolved in brine solution (10 mL), and extracted with $CHCl_3$ (2 × 10 mL). The combined organic phases were dried and concentrated to afford chromatographically pure solid, which was converted to hydrochloride salt (205 mg, 45%): ^{1}H NMR (DMSO-d_6) δ 7.35 (d, 1 H, H-4′), 7.31 (d, 1 H, H-7′), 7.09 (t, 1 H, H-6′), 6.94 (t, 1 H, H-5′), 6.78 (dd, 2 H, H-1 and 2), 5.77 (s, 1 H, H-5), 1.91 (s, 3 H, 14-OAc).

17-(Cyclopropylmethyl)-6,7-dehydro-4,5α-epoxy-3-methoxy-14-hydroxy-6,7-2′,3′-indolomorphinan Hydrochloride (18·HCl). To a solution of 1·HCl (450 mg, 1 mmol) in methanol (10 mL) was added a large excess of ethereal diazomethane. After the solution was stirred for 2 h at 23 °C the solvent was removed to afford the crude product, which was crystallized from ethanol to afford the monomethyl ether **18**·HCl (265 mg, 62%): ^{1}H NMR (DMSO-d_6) δ 8.02 (1 H, s, NH), 7.19 (d, 1 H, H-7′), 7.24 (d, 1 H, H-4′), 6.82 (1 H, t, H-6′), 6.79 (t, 1 H, H-5′), 6.54 (dd, 2 H, H-1 and 2), 5.61 (s, 1 H, H-5), 3.14 (s, 3 H, 3-OMe).

α- and β-Naphthylhydrazine. A stirred slurry of α- or β-naphthylamine (2 g, 0.01 mol) in concentrated hydrochloric acid (30 mL) was treated dropwise at −5 °C with sodium nitrite (0.89 g, 0.01 mol) in cold water (3 mL), care being taken not to allow the temperature to rise above 0 °C. After the addition of sodium nitrite the diazotization was continued for 0.75 h. The solution was quickly filtered and the filtrate was poured in a thin stream into a solution of stannous chloride dihydrate (14 g, 0.05 mol) in cold hydrochloric acid (60 mL). After 1 h the reaction mixture was filtered and washed with water (20 mL), followed with alcohol (30 mL) and finally with ether (100 mL). The crude product was dried in the desiccator and stored under nitrogen in the dark. **α-Naphthylhydrazine hydrochloride** (0.8 g, 41%): mp 119 °C (lit.[22] mp 116 °C); ^{1}H NMR (DMSO-d_6) δ 8.75 (b s, 1 H, NH), 8.10 (t, 1 H, ArH), 7.9 (t, 1 H, ArH), 7.55 (dd, 2 H, ArH), 7.45 (t, 1 H, ArH), 7.0 (d, 1 H, ArH); EIMS m/z 158 (M^+). **β-Naphthylhydrazine hydrochloride** (1.1 g, 57%): mp 123 °C; (lit.[22] mp 124 °C); ^{1}H NMR (DMSO-d_6) δ 8.2 (b s, 1 H, NH), 7.92 (d, 1 H, ArH), 7.40 (t, 1 H, ArH), 7.25 (t, 1 H, ArH), 7.20 (s, 1 H, ArH), 7.15 (d, 1 H, ArH); EIMS m/z 158 (M^+).

17-(Cyclopropylmethyl)-6,7-dehydro-4,5α-epoxy-3,14-dihydroxy-5′,6′-benz-6,7-2′,3′-indolomorphinan (22) was prepared under conditions identical with those of **23** using β-naphthylhydrazine (316 mg, 2 mmol). The free base was purified on a silica gel column to afford pure product (278 mg, 60%): mp 265 °C dec; R_f 0.52 ($CHCl_3$/MeOH/NH_4OH, 9:1:0.1, v/v); NMR (DMSO-d_6) δ 8.85 (b s, 1 H, NH), 8.20 (d, 1 H, $J \approx 8$ Hz, H-8′), 7.86 (d, 1 H, $J \approx 7.05$ Hz, H-11′), 7.62 (s, 2 H, H-4′ and 7′), 7.45 (t, 1 H, $J \approx 7$ Hz, H-9′), 7.35 (t, 1 H, $J \approx 7$ Hz, H-10′), 6.45 (dd, 2 H, H-1 and H-2), 5.60 (s, 1 H, H-5), 4.8 (b s, 1 H, 14-OH): EIMS m/z 464 M^+, 447 (M^+ − OH), 423 ($M^+ - C_3H_5$). The free base was converted to its hydrochloride and crystallized from EtOH to give white solid. Anal. ($C_{30}H_{28}N_2O_3$·HCl·$2H_2O$) C, H, N.

17-(Cyclopropylmethyl)-6,7-dehydro-4,5α-epoxy-3,14-dihydroxy-6′,7′-benz-6,7-2′,3′-indolomorphinan (23). A solution of **24**·HCl (377 mg, 1 mmol) and α-naphthylhydrazine (316 mg, 2 mmol) in glacial acetic acid (10 mL) was refluxed for 4 h. After addition of saturated sodium bicarbonate solution (70 mL) to the cooled reaction mixture, it was extracted with $CHCl_3$ (2 × 80 mL). The combined organic phases were concentrated to dryness to give a crude product that was purified on silica gel column ($CHCl_3$/MeOH/NH_4OH, 95:5:0.5, v/v) to afford pure product (301 mg, 65%): mp 275 °C dec; R_f 0.61 ($CHCl_3$/MeOH/NH_4OH, 9:1:0.1, v/v); ^{1}H NMR (DMSO-d_6) δ 12.01 (s, 1 H, ArOH), 9.05 (b s, 1 H, NH), 8.40 (d, 1 H, $J \approx 8$ Hz, H-4′), 7.82 (d, 1 H, $J \approx 8$ Hz, H-5′), 7.52 (d, 1 H, $J \approx 7.5$ Hz, H-11′), 7.48 (t, 1 H, H-10′), 7.39 (d, 1 H, $J \approx 8$ Hz, H-8′) 7.37 (t, 1 H, H-9′), 6.45 (dd, 2 H, H-1, H-2), 5.60 (s, 1 H, H-5); EIMS m/z 464 (M^+), 447 (M^+ − OH), 423 ($M^+ - C_3H_5$). The free base was converted to its hydrochloride salt and crystallized from $CHCl_3$ and MeOH to give an off-white solid. Anal. ($C_{30}H_{28}N_2O_3$·HCl·$2H_2O$) C, H, N.

Acknowledgment. This research was supported by the National Institute on Drug Abuse. We thank Michael Powers for conducting the smooth muscle assays and Joan Naeseth for the binding data.

Registry No. **1**, 111555-53-4; **1**·HCl, 111469-81-9; **2**, 111555-56-7; **2**·HCl, 111469-84-2; **3**, 111555-54-5; **3**·HCl, 111469-82-0; **4**, 122431-09-8; **4**·HCl, 126642-48-6; **5**, 111555-55-6; **5**·HCl, 111469-83-1; **6**, 122431-10-1; **7**, 122431-07-6; **7**·HCl, 126642-49-7; **7** (4′-isomer), 122431-13-4; **7** (4′-isomer)·HCl, 126642-55-5; **8**, 126642-56-6; **8**·HCl, 126580-39-0; **9**, 126580-40-3; **10**, 122431-14-5; **10**·HCl, 126642-50-0; **11**, 122431-12-3; **11**·HCl, 126642-51-1; **12**, 122431-15-6; **12**·HCl, 126642-52-2; **13**, 126642-57-7; **13**·HCl, 126580-41-4; **14**, 122431-16-7; **14**·HCl, 126642-53-3; **15**, 111555-57-8; **15**·HCl, 111469-85-3; **16**, 126642-58-8; **16**·HCl, 126580-42-5; **17**, 126642-59-9; **17**·HCl, 126580-43-6; **18**, 126580-44-7; **19**, 126580-45-8; **20**, 111469-88-6; **20**·HCl, 126642-54-4; **21**, 126580-46-9; **22**, 126642-60-2; **22**·HCl, 126580-47-0; **23**, 126642-61-3; **23**·HCl, 126580-48-1; **24**·HCl, 16676-29-2; **25**, 465-65-6; **26**, 76-41-5; $C_6H_5NHNH_2$·HCl, 59-88-1; 4-$CH_3C_6H_4NHNH_2$·HCl, 637-60-5; 4-$FC_6H_4NHNH_2$·HCl, 823-85-8; 4-$NO_2C_6H_4NHNH_2$·HCl, 636-99-7; 4-$CH_3OC_6H_4NHNH_2$·HCl, 19501-58-7; 3-$CH_3C_6H_4NHNH_2$·HCl, 637-04-7; 3-$FC_6H_4NHNH_2$·HCl, 2924-16-5; 2-$BrC_6H_4NHNH_2$·HCl, 50709-33-6; 2-$CH_3C_6H_4NHNH_2$·HCl, 635-26-7; 2-$FC_6H_4NHNH_2$·HCl, 2924-15-4; 2-$CH_3OC_6H_4NHNH_2$·HCl, 6971-45-5; 2-$C_2H_5OC_6H_4NHNH_2$·HCl, 126580-49-2; $C_6H_5N(CH_3)NH_2$·HCl, 39232-92-3; $(C_6H_5)_2NNH_2$·HCl, 530-47-2; α-naphthylamine, 134-32-7; β-naphthylamine, 91-59-8; α-naphthylhydrazine hydrochloride, 2243-56-3; β-naphthylhydrazine hydrochloride, 2243-58-5.

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