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Synthesis and opioid activities of some naltrexone oxime ethers

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Summary — A series of alkyl, cycloalkyl, aryl, and aralkyl ethers of naltrexone oxime was prepared. The compounds were examined in binding assays for μ, δ and κ opioid receptor affinity. In addition, the naltrexone oxime ethers were studied in animal models that measure opioid agonist and antagonist activity. These studies led to the discovery of several compounds, notably phenethyl **3e** and phenylpropyl **3f** ethers of naltrexone, which have a 10-fold increase in potency at the κ opioid receptor with potent μ and κ agonist properties *in vivo*.

naltrexone / oxime ether / opioid receptor / analgesia / receptor binding / kappa

Introduction

Derivation of new compounds in which the pain relieving properties of narcotic opioid analgesics are separated from their addictive and psychotomimetic effects remains a major objective of opioid research. Added impetus for this research was provided by the unequivocal pharmacological, neurophysiological, and behavioral definition of 3 primary subtypes of opioid receptors, *ie* μ, δ, and κ receptors [1–3]. Each of the subpopulations of opioid receptors is associated with the production of analgesia; however, the side effects mediated by the 3 subclasses are different [4]. Thus, subtype-selective agonists and antagonists offer the promise of freedom from side effects associated with non-selective opioids. In particular, selective κ agonists may be effective analgesics that lack the side-effect liability, *eg*, respiratory depression, constipation, and physical dependence, which are associated with stimulation of μ receptors [5–8].

In a search for novel, subtype-selective opioid analgesics with decreased side effects, a series of naltrexone oxime ethers was prepared. The selection of this series for study was based on earlier observations that the C-6 carbonyl group in 14-hydroxydihydromorphinones is readily modified and changes at this position do not adversely affect the potency of the derivatives [9, 10]. The oxime ethers may also serve as probes of a hydrophobic bonding region complementary to the C-6 position, which is suggested by studies with dimeric azines [11] and phenylhydrazones [12]. The naltrexone oxime ethers described in this report were examined for μ, δ, and κ opioid receptor affinity. In addition, the compounds were examined for opioid agonist and antagonist activity in animal models. In this report are described the synthesis of a series of naltrexone oxime ethers [13–15] and the results of their examination in receptor binding and pharmacological studies which led to the discovery of several more potent derivatives of naltrexone with μ and κ opioid agonist properties *in vivo*.

Chemistry

Condensation of naltrexone **1** with hydroxylamine (**2**, R = H) and its methyl ether (**2**, R = CH_3) to give naltrexone oxime **3a** and its *O*-methyl ether **3b** has been described previously [10]. Similar oximination of **1** with *O*-alkyl and *O*-aryloxyamines **2c–r** under mildly acidic conditions as outlined in scheme 1 provided a series of naltrexone oxime ethers **3c–s** (table I). Kolb and Gober [16] have extensively studied naltrexazones for possible *syn*- and *anti*-isomerism using ^{13}C-NMR. An analogous study of **3b** (**2**, R = CH_3) showed a single sharp peak at 156.745 ppm, suggesting the exclusive presence of the *anti*-isomer. Therefore, the sterically more crowded naltrexone oximes **3c–s** are expected to be the pure *anti*-isomers.

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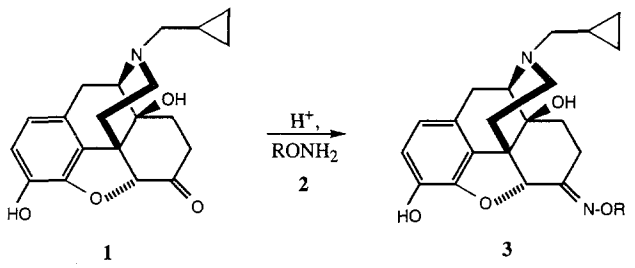


Scheme 1.

Several of the substituted oxyamines **2b–d** and **i** are available from commercial sources. Two general procedures were employed to obtain other the *O*-substituted oxyamines required for synthesis of **3e–h, j–o**. *O*-Alkyloxyamines **2e–h** were obtained (scheme 2, *Method A*) by reaction of the appropriate alcohol with *N*-hydroxyphthalimide in the presence of betaine formed from triphenylphosphine and diethyl azodicarboxylate (DEAD) followed by hydrazinolysis of the resulting *N*-alkoxyphthalimide *via* a previously described route [17]. *O*-Aryloxyamines **2j–o** were derived by amination of the appropriate phenols with *O*-mesitylenesulfonyloxyamine [18] according to general *Method B* [19] (scheme 2).

Other *O*-substituted oxyamines were obtained as detailed in the *Experimental protocols*. Amination by *Method B* (scheme 2) of 4-phthalimidomethylphenol, obtained from 4-cyanophenol by stepwise triisopropylsilyl protection of the phenolic hydroxyl, reduction of the nitrile, reaction of the amine with phthalic anhydride, and deprotection of the phenol, gave *O*-(4-phthalimidomethylphenyl)oxyamine **2p**. Hydrazinolysis of **2p** produced the aminomethyl derivative **2q**. *O*-(4-Amidinophenyl)oxylamine **2r** was obtained from *O*-(4-cyanophenyl)oxylamine **2o**, *via* ammonolysis of an intermediate imino ether. *O*-(1-Adamantyl)oxyamine **2s** was prepared by hydrazinolysis of *N*-(1-adamantyloxy)phthalimide which resulted from the reaction of 1-bromoadamantane with *N*-hydroxyphthalimide in the presence of silver tetrafluoroborate [20].

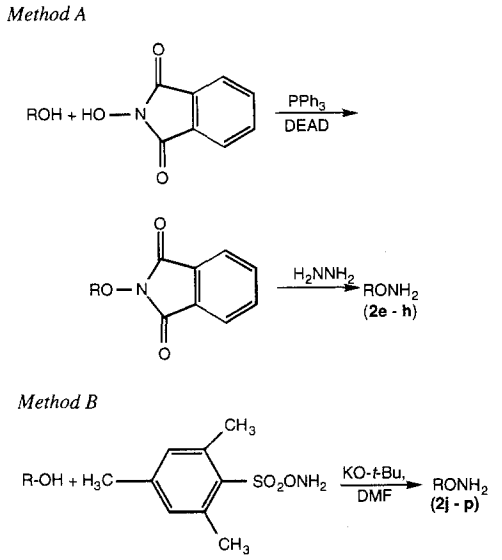
Results and discussion

Affinities for μ and δ opioid receptors were measured by the ability of the test compounds to inhibit [^{3}H]-D-

Table I. Analytical and physical data for naltrexone oxime ethers **3**.

Compound	*R*	*Yield (%)*[a]	*mp (°C)*	*Formula*	*Recrystallization solvent*	*Anal*[b]
3a	H	51	235–236	$C_{20}H_{24}N_2O_4$	EtOH/Et_2O	C, H, N
3b	CH_3	50	172–173	$C_{21}H_{26}N_2O_4$	EtOH/Et_2O	C, H, N
3c	*t*-Bu	74	> 305 (dec)	$C_{24}H_{32}N_2O_4$·HCl	EtOH/Et_2O	C, H, N, Cl
3d	$PhCH_2$	50	280–282	$C_{27}H_{30}N_2O_4$·HCl	EtOH/Et_2O	C, H, N, Cl
3e	$Ph(CH_2)_2$	40	259–260	$C_{28}H_{32}N_2O_4$·HCl·0.25H_2O	EtOH/Et_2O	C, H, N, Cl
3f	$Ph(CH_2)_3$	66	250–251	$C_{29}H_{34}N_2O_4$·HCl·0.5H_2O	EtOH/Et_2O	C, H, N, Cl
3g	*c*-C_5H_9	88	290–291	$C_{25}H_{32}N_2O_4$·HCl	EtOH/Et_2O	C, H, N, Cl
3h	*c*-C_6H_{11}	80	296–297	$C_{26}H_{34}N_2O_4$·HCl·0.5H_2O	EtOH/Et_2O	C, H, N, Cl
3i	Ph	50	220–222	$C_{26}H_{28}N_2O_4$·HCl	EtOH/Et_2O	C, H, N, Cl
3j	3,4-$(CH_3)_2$Ph	37	230–232	$C_{28}H_{32}N_2O_4$·HCl·0.5H_2O	EtOH/Et_2O	C, H, N, Cl
3k	2,3-$(CH_3)_2$Ph	30	180–182	$C_{28}H_{32}N_2O_4$·$C_2H_2O_4$·H_2O	EtOH/Et_2O	C, H, N
3l		18	> 150 (dec)[c]	$C_{30}H_{34}N_2O_4$·HCl·H_2O	EtOH/Et_2O	C, H, N, Cl
3m	2-CF_3Ph	40	> 150 (dec)[c]	$C_{27}H_{27}N_2O_4F_3$·HCl·0.5H_2O	EtOH/Et_2O	C, H, N, Cl
3n	4-CF_3Ph	58	220–211	$C_{27}H_{27}N_2O_4F_3$·HCl	EtOH/Et_2O	C, H, N, Cl
3o	4-CNPh	62	205–207	$C_{27}H_{27}N_3O_4$·HCl·0.5H_2O	EtOH/Et_2O	C, H, N, Cl
3p	4-Phthalimido-CH_2Ph	47	178–180	$C_{35}H_{33}N_3O_6$·HCl·1.5H_2O	THF/EtOAc	C, H, N, Cl
3q	4-H_2NCH_2Ph	18	> 150 (dec)[c]	$C_{27}H_{31}N_3O_4$·2HCl·H_2O	EtOH/Et_2O	C, H, N, Cl
3r	4-$H_2NC(=NH)$Ph	71	278–280	$C_{27}H_{30}N_4O_4$·2HCl·H_2O	EtOH/Et_2O	C, H, N, Cl
3s	Adamantyl	50	305–307	$C_{30}H_{38}N_2O_4$·HCl	EtOH/Et_2O	C, H, N, Cl

[a]All yields are for analytically pure product. [b]All analyses were within 0.4% of calculated values. [c]This compound was obtained as an amorphous, non-crystalline solid of indefinite mp. Cited dec values are approximate.



Scheme 2. *Methods A* and *B* (R groups are as defined in table I).

Ala[2]-MePhe[4]-Gly-ol[5]-enkephalin (DAMGO) [21] and [^{3}H]-D-Ala[2]-D-Leu[5]-enkephalin (DADLE binding) [22], respectively, to rat forebrain homogenates. Binding to κ opioid receptors was evaluated by inhibition of [^{3}H]U-69593 [23] binding to guinea-pig cerebellar homogenates. Results of the opioid receptor binding studies are presented in table II as affinity constants (K_is) calculated as described in the *Experimental protocols* [24]. Selectivity ratios δ/κ and μ/κ, calculated from the K_i values at the indicated subtypes, provide a measure of κ *versus* δ and μ receptor selectivities. Opioid antagonist activity was determined by the ability of a compound to antagonize morphine-induced analgesia in a tail-flick test [25] in rats. The ED_{50}s were calculated as described in the *Experimental protocols* and the results are tabulated in table III. Agonist potencies, as measured by the compound's ability to block a tail-flick response following ip administration to rats and to antagonize acetic-acid-induced abdominal constriction following sc administration to mice, were measured as ED_{50} values determined as described in the *Experimental protocols*. The results are shown in table IV.

Table II. Opioid binding affinity and selectivity.

Compound	*K_i (nM)*			*Selectivity ratios*	
	κ	μ	δ	κ/δ	κ/μ
3a	0.69 ± 0.09	0.43 ± 0.02	2.1 ± 0.3	3	0.6
3b	0.71 ± 0.07	0.46 ± 0.13	1.2 ± 0.1	2	0.6
3c	2.7 ± 0.4	1.3 ± 0.2	1.8 ± 1.2	0.7	0.5
3d	0.06 ± 0.02	0.89 ± 0.11	1.4 ± 0.2	23	15
3e[b]	0.04 ± 0.02	0.88 ± 0.09	0.60 ± 0.04	15	22
3f	0.04 ±0.01	0.4 ± 0.08	0.89 ± 0.22	22	10
3g	2.9 ± 0.5	0.58 ± 0.12	0.12 ± 0.04	0.04	0.2
3h	3.8 ± 0.5	1.3 ± 0.15	0.40 ± 0.11	0.1	0.3
3i[a]	1.6 ± 0.3	0.33 ± 0.04	0.68 ± 0.07	0.4	0.2
3j	1.6 ± 0.4	1.7 ± 0.4	4.8 ± 1.1	3	1
3k	2.2 ± 0.3	1.7 ± 0.6	1.4 ± 0.3	0.6	1
3l	1.5 ± 0.3	2.5 ± 0.2	5.7 ± 1.7	4	2
3m	12 ± 0.5	4.1 ± 0.4	1.6 ± 0.3	0.1	0.3
3n	2.4 ± 0.6	7.0 ± 0.7	24 ± 3	10	3
3o	5.2 ± 0.6	3.3 ± 0.5	7.7 ± 0.3	1.5	0.6
3p	8.2 ± 0.8	6.4 ± 1.1	2.2 ± 0.4	0.3	0.8
3q	0.19 ± 0.05	0.29 ± 0.05	1.6 ± 0.6	8	1.5
3r	12 ± 0.04	0.87 ± 0.15	2.2 ± 0.5	0.2	0.1
3s	2.9 ± 0.4	6.5 ± 0.7	3.2 ± 0.3	1	2
Naltrexone[a]	0.51 ± 0.12	0.60 ± 0.07	1.40 ± 0.71	3	1

The values represent the mean ± SEM of at least 3 separate experiments performed in triplicate. See *Experimental protocols, Pharmacology* for a description of methods. [a]Data taken from reference [14]. [b]Data taken from reference [15].

Table III. Antagonism of morphine-induced analgesia in the tail-flick assay by oxime derivatives of naltrexone.

Compound	ED_{50} *(mg/kg)*	
	ip	*po*
3a	0.30	> 10
3c	0.19	> 5
3d	0.55	> 30
3e	> 5	> 100
3f	> 10	> 20
3g	0.11	2.8
3i[a]	0.07	0.82
3j	0.02	2.53
3k	0.12	> 5
3l	0.02	1.11
3m	1.71	> 30
3n	2.17	ND
3o	1.79	14.1
3p	2.19	> 100
3q	0.06	14.9
3r	2.89	> 45
3s	0.98	> 10
3w	1.31	> 5
Naltrexone[a]	0.04	0.27

Data represent the mean of at least 4 rats per dose; ND = not determined; [a]data taken from reference [14].

Consistent with earlier observations [10] that oximination of **1** has little influence on opioid-like activity, naltrexone oxime **3a** and its methyl ether **3b** had receptor-binding properties similar to those of naltrexone. Etherification of **3a** with larger alkyl and aryl groups (**3c**, **3g–l**) generally decreased affinity for κ opioid receptors. This decrease was more pronounced for bulky alkyl, *eg*, **3c**, **3g**, **3h** and **3s**, and phenyl, *eg*, **3i–l**, oximino ethers. Substitution of the phenyl group with electron-withdrawing moieties, *eg*, **3m–p**, **r**, further decreased, whereas substitution with an electron-releasing group, *ie* **3q**, enhanced the affinity of naltrexone oxime ethers for κ opioid receptors. In contrast to the alkyl and aryl derivatives, the affinity of aralkyl ethers **3d–f** for these receptors was increased by about one order of magnitude. With the exception of **3i** and **3q**, the affinity of the oximino ethers for the μ opioid receptor subpopulation was less than that of **3a**. At δ receptors, the affinity was markedly increased by cycloalkyl substitution, *eg*, **3g** and **3h**, whereas it was markedly decreased by electronegative substitution, *eg*, **3n** and **3o**, of the phenyl groups of **3i** [14]. These results suggest a hydrophobic bonding region with steric and electronic requirements that differ for the opioid receptor subpopulations in a location complementary to the 6 position of the 14-hydroxydihydromorphinones. More favorable κ receptor accommodation of aralkyl substituents in this vicinity results in the κ preferring oximino ethers **3d–f**. Decreased accommodation of cycloalkyl groups by the κ subtype, coupled with the relatively favored bonding of these substituents by the μ and δ subtypes, provides oximino ethers, *eg*, **3g** and **3h**, with increased selectivity for μ and δ *versus* κ receptors.

As indicated by the data in table III, following ip administration, the oximino ethers antagonized morphine-induced analgesia in a tail-flick test in rats; however, with the exception of **3j** and **3l**, they were less effective than naltrexone. The aralkyl derivatives **3e** and **3f** in fact has relatively little effect on morphine-induced analgesia, suggesting that this substitution actually conferred opioid intrinsic activity. All of the test compounds were significantly less potent than naltrexone following oral administration in this paradigm.

Consistent with their potency at both μ and κ opioid receptors, as indicated by the data in table IV, the aralkyl oximino ethers **3d–f** were 10–25 times more potent than morphine in blocking the abdominal constriction response to acetic acid in mice. Com-

Table IV. Agonist properties of oxime derivatives of naltrexone.

Compound	*Acetic acid-induced abdominal constriction in mice*	*Tail-flick response in rats*
	ED_{50} *(mg/kg) sc*	ED_{50} *(mg/kg) ip*
3a	> 100	ND
3c	3.69	> 30
3d	0.03	> 30
3e*	0.05	4.02
3f*	0.02	2.24
3g	4.14	> 50
3h	19.9	ND
3i	15.9	ND
3k	9.32	ND
3l	> 30	ND
3p	> 100	ND
3q	5.28	> 30
3r	9.50	ND
3s	9.84	ND
Morphine*	0.50	2.60

*Data taken from reference [15].

pounds **3e** and **3f** [15] had potencies approximating that of morphine in blocking a tail-flick response in rats. The analgesia elicited by **3e** has been partially attributed to its μ opioid activity, while the effects of **3f** appear to be due primarily to κ opioid agonism [15]. The high κ and μ opioid receptor affinities of **3e** and **3f** [15], coupled with their *in vivo* analgesic activity, suggests that these compounds may be useful prototypes for the design of new drugs, which alleviate pain while having diminished side-effect liability.

Experimental protocols

Melting points were determined in open glass capillaries using a Thomas Hoover Unimelt apparatus; they are uncorrected. Infrared (IR) spectra were recorded on a Beckman FT 1300 spectrophotometer. Proton magnetic resonance (^{1}H-NMR) spectra were recorded on a Varian A-60A spectrometer with Me_4Si as the internal standard. Chemical shifts are reported as parts per million (δ) downfield relative to the standard. Chromatographic separations were performed on a silica-gel column (Kieselgel 60, finer than 230 mesh, Merck). Analytical thin-layer chromatography (TLC) was carried out on precoated glass plates (silica gel, 60 F-254). TLC spots were visualized with UV light or iodine vapor. Elemental analyses were carried out by Atlantic Microlabs, Inc, Atlanta, GA, and were within 0.4% of theoretical values unless indicated otherwise. All spectral data for reported compounds were evaluated as consistent with the assigned structures; TLCs were single spots. All radioligands were purchased from New England Nuclear (Boston, MA).

Chemistry

General Methods

Naltrexone Oxime Ethers 3. A solution of equimolar amounts of naltrexone and *O*-substituted hydroxylamine (base or acid addition salt) in ethanol was adjusted to pH 4 by dropwise addition of concentrated hydrochloric acid. The reaction mixture was stirred at ambient temperature for 15 h, concentrated *in vacuo*, and the residue was suspended in a small volume of water. The suspension was adjusted to pH 7 by addition of a 5% aqueous solution of sodium bicarbonate. Solid products were filtered. Others were extracted with ethyl acetate, and the extracts were dried ($MgSO_4$) and concentrated to afford crude product. Products were purified by column chromatography (silica gel, chloroform/methanol/concentrated aqueous ammonia 90:10:1 or ethyl acetate/methanol/concentrated aqueous ammonia 90:10:1). As described in table I, **3a** [10] and **3b** [10] were isolated as bases. The remaining naltrexone oxime ethers (**3c–s**) were converted to salts and recrystallized from the solvents indicated in table I. The ^{1}H-NMR spectrum (AC 400 MHz, Bruker) of **3b** (**2**, R = CH_3) gave the following characteristic peaks; δ = 6.72 (d, J = 8.0 Hz, 1H); 6.57 (d, J = 8.0 Hz, 1H); 4.98 (s, 1H); 3.9 (s, 3H); 6.08 (m, 2H); 2.8–2.2 (m, 8H); 1.61–1.56 (m, 2H); 1.39–1.30 (m, 1H); 0.88–0.80 (m, 1H); 0.56–0.52 (m, 2H) and 0.15–0.11 (m, 2H). The ^{13}C-NMR spectrum (AC 400 MHz) recorded the following peaks: 156.745, 143.551, 138.609, 130.349, 124.783, 119.393, 117.411, 87.266, 70.054, 62.069, 61.831, 59.285, 48.328, 43.804, 31.532, 28.247, 22.780, 18.825, 9.424, 4.079 and 3.724.

*Method A. O-Alkylhydroxylamines (**2e–h**)*

N-(3-Phenylpropoxy)phthalimide. This was prepared by the following procedure: 5 N KOH (20 ml) was added dropwise to a stirred mixture of 16.3 g (0.1 mol) *N*-hydroxyphthalimide and 19.9 g (0.1 mol) of 1-bromo-3-phenylpropane in 300 ml DMF at 65°C over a period of 1 h. The reaction mixture was cooled

Table V. ^{1}H-NMR data for oxyamines **2** R-O-NH_2.

Compound	*Solvent*	*Signals (δ)*
2e	$CDCl_3$	7.5–7.2 (m, 5H); 5.5 (s, 2H); 3.9 (t, 2H, J = 6.9 Hz); 2.9 (t, 2H, J = 6.9 Hz).
2f	$CDCl_3$	7.31–7.16 (m, 5H); 5.35 (s, 2H); 3.66 (t, 2H, J = 6.5 Hz); 2.68 (t, 2H, J = 7.8 Hz); 1.95–1.85 (m, 2H)
2g	$CDCl_3$	5.25 (s, 2H); 4.2–4.13 (m, 1H); 1.75–1.47 (m, 8H)
2h	$CDCl_3$	5.26 (s, 2H); 3.52–3.47 (m, 1H); 1.97–1.22 (m, 10H)
2j	$CDCl_3$	7.2–6.8 (m, 3H); 5.7 (s, 2H); 2.1 (s, 3H); 2.15 (s, 3H)
2k	$CDCl_3$	7.35–6.65 (m, 3H); 5.75 (s, 2H); 2.3 (s, 3H); 2.1 (s, 3H)
2m	$CDCl_3$	7.6–8.8 (m, 4H); 5.95 (s, 2H)
2n	$CDCl_3$	7.4 (d, 2H, J = 9 Hz); 7.20 (d, 2H, J = 9 Hz); 5.8 (s, 2H)
2o	$CDCl_3$	7.58 (d, 2H, J = 9 Hz); 7.23 (d, 2H, J = 9 Hz); 5.98 (s, 2H)
2p	$CDCl_3$	7.84–7.81 (m, 2H); 7.71–7.68 (m, 2H); 7.36 (d, 2H, J = 8.7 Hz); 7.07 (d, 2H, J = 8.7 Hz); 5.82 (s, 2H); 4.78 (s, 2H)
2q	$CDCl_3$	7.22 (d, 2H, J = 8.7 Hz); 7.1 (d, 2H, J = 8.7 Hz); 5.86 (s, 2H); 3.80 (s, 2H); 1.60 (s, broad, 2H)
2r	d^{6}DMSO	8.8–7.7 (s, broad, 4H); 7.9 (d, 2H, J = 9 Hz); 7.6 (d, 2H, J = 9 Hz); 7.5 (s, broad, 3H)
2s	$CDCl_3$	4.8 (s, 2H); 2.1 (s, 3H); 1.8 (s, 6H); 1.7 (s, 6H)

R is given in table I for the corresponding compounds **3**.

to room temperature and poured into 1 ml water. The aqueous mixture was extracted with methylene chloride, the combined extracts were washed with water, and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue was recrystallized from ethanol to afford 16.5 g (58.7%) of colorless crystals, mp 68–69°C.

N-(Cyclopentyloxy)phthalimide. Diethyl azodicarboxylate (57.5 g, 330 mmol) was added dropwise to a mixture of 25.8 g (300 mmol) cyclopentanol, 78.7 g (300 mmol) triphenylphosphine, and 48.9 g (300 mmol) of *N*-hydroxyphthalimide in 800 ml THF under argon over a period of 1 h. The mixture was stirred for 20 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel with hexane/ether (9:1) as eluent. The product was recrystallized from ethanol to afford 56.8 g (82%) of colorless crystals, mp 81–82°C.

N-(Phenylethoxy)phthalimide. This was prepared from 2-phenethyl bromide and *N*-hydroxyphthalimide by an identical procedure, to afford colorless crystals (from ethanol), mp 91–92°C.

Hydrazinolysis of alkyloxyphthalimides. This is illustrated by the following example. A mixture of 56 g (242 mmol) *N*-(cyclopentyloxy)phthalimide and 14.5 g hydrazine hydrate in 300 ml ethanol was refluxed for 1 h. The reaction mixture was diluted with 500 ml 5% sodium carbonate and the resulting precipitate was filtered. The filtrate was extracted with ether and this extract was washed with water and dried ($MgSO_4$). The ether was removed under reduced pressure and the residue was distilled at atmospheric pressure using a Vigreux column to afford 20.1 g (82%) of a colorless liquid, bp 97–97°C.

*Method B. O-Aryloxyamines (**2j–p**)*
This general method is illustrated by the synthesis of *O*-(4-cyanophenyl)oxyamine **2o**. Potassium *tert*-butoxide (1.1 g, 10 mmol) was added to a stirred solution of 1.2 g (10 mmol) 4-cyanophenol in 50 ml methanol at 25°C. After removal of the solvent under reduced pressure, the residue was dissolved in 10 ml DMF. The solution was cooled to 0°C and a solution of 2.15 g (10 mmol) *O*-mesitylenesulfonyloxylamine [18] in 10 ml DMF was added dropwise with stirring. After the mixture was stirred for 30 min at 0°C, it was poured into 300 ml water. The mixture was extracted with methylene chloride. The extracts were dried ($MgSO_4$) and concentrated. The residual semi-solid was chromatographed on a silica-gel column using methylene chloride to elute. Concentration of the eluate *in vacuo* gave a solid. Recrystallization from methylene chloride afforded 0.82 g (60.7%) of colorless crystals, mp 108–109°C (table V).

4-(Triisopropylsilyloxy)benzonitrile. After a mixture of 12.1 g (101.7 mmol) cyanophenol, 17.3 g (254 mmol) imidazole, and 23.5 g (122.1 mmol) triisopropylsilyl chloride in 100 ml DMF had been stirred for 24 h at 25°C, it was poured into 1 l water. The mixture was extracted with methylene chloride. After the extracts were washed with 2 N HCl and water, they were dried ($MgSO_4$) and concentrated. The residue was distilled to give 29.0 g (92%) of a colorless liquid, bp (0.25 Torr) 165–167°C.

4-(Triisopropylsilyloxy)benzylamine. A mixture of 26.2 g (95 mmol) of 4-(triisopropylsilyloxy)benzonitrile and 2.6 g 10% palladium-on-carbon catalyst was hydrogenated by shaking for 4 h on a Parr apparatus under an initial pressure of 50 psi hydrogen. The mixture was filtered and the filtrate was concentrated. The residual liquid was distilled to give 16.2 g (61%) of a colorless liquid, bp (0.2 Torr) 123–125°C.

N-[4-(Triisopropylsilyloxy)benzyl]phthalimide. A stirred mixture of 16.2 g (58 mmol) 4-(triisopropylsilyloxy)benzylamine and 8.6 g (58 mmol) phthalic anhydride was refluxed azeotropically for 16 h. The toluene solvent was distilled under reduced pressure and the residue was chromatographed on a silica-gel column using methylene chloride to elute. Concentration of the eluate afforded 21.3 g (89%) of crystalline solid, mp 99–101°C.

4-[(N-Phthalimido)methyl]phenol. *N*-[4-(Triisopropylsilyloxy)benzyl]phthalimide (17.0 g, 41.4 mmol) was added, in portions, to 50 ml of a stirred 1 M solution of tetrabutylammonium fluoride in 200 ml THF at 25°C. After being stirred for 3 h at 25°C, the mixture was poured into 1 l water. The precipitated solid was filtered and recrystallized from methanol to afford 7.9 g (75%) of white crystals, mp 208–210°C.

*O-[(N-Phthalimido)methylphenyloxyamine **2p**.* This was prepared from 4-[(*N*-phthalimido)methyl]phenol and *O*-mesitylenesulfonyloxyamine in 53% yield by general *Method B*, mp 126–127°C.

*O-[4-(Aminomethyl)phenyl]oxyamine **2q**.* This was prepared by hydrazinolysis of **2p** as described in general *Method A*. It was an amorphous solid.

*O-(4-Amidinophenyl)oxyamine **2r**.* A slow stream of hydrogen chloride gas was bubbled through a solution of 1.4 g (10.5 mmol) of *O*-(4-cyanophenyl)oxyamine **20** in 50 ml ethanol for 30 min. After allowing the mixture to stand at 25°C for 16 h, ether was added to precipitate imino ether hydrochloride. The precipitate was filtered and dissolved in 50 ml ethanol. A saturated solution of ammonia in 50 ml ethanol was added to the imino ether solution. After being stirred for 18 h, the mixture was filtered and the filtrate was concentrated to about 15 ml. The colorless crystals (1.8 g, 95%), mp > 200°C (dec) were collected.

N-(1-Adamantyloxy)phthalimide. A mixture of 4.1 g (19.2 mmol) of 1-adamantyl bromide, 3.1 g (19.2 mmol) of *N*-hydroxyphthalimide, 3.7 g (19.2 mmol) of silver tetrafluoroborate, and 200 ml ether was stirred under a nitrogen atmosphere for 15 h. The precipitated solid (AgBr) was filtered and the filtrate was concentrated. Residual solid was chromatographed on silica gel using methylene chloride as eluent to give 2.0 g (63%) of white crystals, mp 189°C, after recrystallization from ethanol.

*O-(1-Adamantyl)oxyamine hydrochloride **2s**.* This was prepared by hydrazinolysis of *N*-(1-adamantyloxy)phthalimide as described in general *Method A*. Crude product was chromatographed in silica gel using methanol/methylene chloride 3:97 as eluent. The residue obtained by concentration of the eluate was dissolved in ether and treated with ethereal hydrogen chloride. Recrystallization of the precipitated solid from ethanol gave 90% of colorless crystals, mp 205–206°C.

Pharmacology

Receptor binding studies
κ opioid receptor binding. After guinea pigs were killed by decapitation, cerebella were removed and homogenized in cold HEPES-KOH buffer (50 mM, pH 7.4 at room temperature) at a

concentration of 20 mg/ml with a Brinkmann Polytron (setting 7, 30 s). After the homogenate was centrifuged at 48 000 *g* for 10 min at 4°C, the supernatant was decanted and the residual pellet was resuspended by means of the Polytron. The mixture was centrifuged again and the pellet was resuspended at a concentration of 20 mg/ml. Each assay tube contained 100 µl of [^{3}H]U-69,593 [23] at a final concentration of 1 nM, 100 µl test compound, 1 ml tissue homogenate at a final concentration of 10 mg/ml, and sufficient buffer for a final assay volume of 2 ml. Nonspecific binding was determined in the presence of 5 µM bremazocine. All incubations, conducted in triplicate, were in tubes at 30°C for 2 h and reactions were terminated by rapid filtration over Whatman GF/B glass filters that were presoaked in a 0.05% polyethyleneimine solution for at least 2 h. The filters were washed 3 times with 4 ml Tris-HCl buffer (50 mM, pH 7.4 at room temperature). The amount of bound radioactivity was determined by liquid scintillation spectroscopy. IC_{50} values were calculated from competition curves by means of the EBDA program [26]. Apparent affinity constants (K_i) were determined according to the method of Cheng and Prusoff [24].

µ and δ opioid receptor binding. Rats were killed by decapitation. Forebrain (whole brain minus cerebellum and brainstem) was dissected and homogenized in 30 volumes (w/v) of 50 mM Tris-HCl (pH 7.4) at 25°C using a Brinkmann Polytron. The homogenate was centrifuged at 48 000 *g* for 10 min at 4°C. The supernatant was discarded, and the pellet was resuspended in 30 volumes of 50 mM Tris-HCl at 37°C for 10 min. All incubations, performed in triplicate, were carried out in tubes containing 1 ml of the tissue homogenate and 100 µl of either [^{3}H]DAMGO [20] or [^{3}H]DADLE [22] for a final concentration of 1 nM and 100 µl of the test compound in a 2 ml final incubation volume. Nonspecific binding was determined in the presence of 10 µM naloxone. After incubation for 90 min at room temperature, the reaction was terminated by rapid vacuum filtration over Whatman GF/B glass fiber filters. The filters were washed 3 times with 5 ml ice-cold 50 mM Tris-HCl buffer (pH 7.4 at room temperature). The amount of bound radioactivity was determined by liquid scintillation spectrometry. IC_{50} values were calculated from competition curves by means of the EBDA program [26]. Apparent affinity constants (K_i) were determined according to the method of Cheng and Prusoff [24].

In vivo studies

Antagonism of morphine-induced analgesia. [14] Tail-flick latencies were determined in rats as described previously [25], and were quantified using a Columbus Instrument Analgesia Testing Device (Columbus, OH) with the lamp intensity set to produce a flick latency of 4.0–5.0 s in naive rats. A flick latency of 15 s was considered a maximal response, and the trial was terminated. After baseline flick latencies were measured, the animals were treated with the test compound administered either po to rats that were food-deprived overnight or ip. Immediately following dosing with the test compound, the rats were given 4 mg/kg morphine, sc. Animals were tested at 40 min following morphine administration. Data for tail-flick latencies were expressed as a percentage of the maximum possible effect (% MPE), calculated as:

$$\%\ \text{MPE} = 100 \times \frac{(\text{post drug latency} - \text{baseline latency})}{(15 - \text{baseline latency})}$$

ED_{50} values (table III) for the antagonist were calculated using the % MPE values from the log-dose–response curves by least squares regression analysis and represent the mean of at least 5 rats per group.

Acetic-acid-induced abdominal constriction. [14] Mice were divided into treatment groups and administered various doses of test compounds in a volume of 1 ml/kg sc 30 min prior to testing. At 5 min prior to testing, a 0.6% acetic acid solution was administered ip in a volume of 10 ml/kg. Mice were then placed into observation chambers, and the number of abdominal constriction responses was recorded for 5 or 10 min. A abdominal constriction response consisted of full hind-limb extension and retraction. The number of abdominal constriction responses was calculated for vehicle-control mice. The percentage inhibition (%I) of abdominal constriction was calculated for each drug-treated mouse using the following formula:

$$\%\text{I} = 100 \times \frac{\text{mean control abdominal constriction responses} - \text{individual test responses}}{\text{mean control writhing responses}}$$

ED_{50} values given in table IV were calculated from the mean %I values for groups of at least 8 rats using least squares analysis.

Antagonism of tail-flick response. [14] Male rats (Sprague-Dawley, Charles River) were randomly assigned to treatment groups and tail-flick latencies [25] were quantified using a Columbus Instrument Analgesia Testing Device (Columbus, OH). A flick latency of 15 s was considered a maximal response and the trial was terminated. Tail-flick latencies were determined prior to, as well as 40 min after, administration of the test compound to groups of at least 5 rats. Individual post-drug latencies were determined and the % MPE and ED_{50}s presented in table IV were calculated as described in the antagonism of morphine-induced analgesia procedure.

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