3-methylpyrrolo[2,1-*a*]isoquinoline-1,2-dicarboxylate[5] (23.4 g, 0.078 mol) in dry dichloromethane was added dropwise over a period of 1 h to a stirred suspension of lithium aluminum hydride (6.84 g, 0.18 mol) in anhydrous ether (210 mL) at room temperature. The mixture was stirred 1 h beyond completion of the addition; workup followed the procedure described under the reduction of **4**. The mixture was concentrated in vacuo to give a solid that was crystallized from tetrahydrofuran–petroleum ether (2:1) to yield **12** as white crystals (16.4 g, 87%): mp 140–142 °C; IR 3600–3000, 2950, 2900, 1600, 1530, 1480, 1340, 930 cm^{-1}; NMR δ 2.26 (s, 3 H), 2.69–2.93 (s, 2 H, br overlapping t), 2.96 (t, J = 6 Hz, 2 H), 4.93 (t, J = 6 Hz, 2 H), 4.6 (s, 2 H), 4.83 (s, 2 H), 6.96–7.49 (m, 3 H), 7.56–7.86 (m, 1 H). Anal. ($C_{15}H_{17}NO_2$) C, H, N.

1,2-Bis(hydroxymethyl)-5,6-dihydro-3-methylpyrrolo-[2,1-*a*]isoquinoline Bis(methylcarbamate) (13a). A solution of **12** (4.76 g, 0.0196 mol) and triethylamine (1.0 mL) was heated (50 °C) and treated portionwise with methyl isocyanate (2.8 g, 0.049 mol) over a period of 4 h. The mixture was stirred 36 h and concentrated in vacuo to give a brown gum, which yielded white crystals overnight from a minimum volume of cold tetrahydrofuran (2.18 g, 31.2%): mp 162–163 °C dec; IR 3400, 3050 (sh), 2990, 1720, 1640, 1530, 1260, 940 (br), 780–680 cm^{-1}; NMR δ 2.26 (s, 3 H), 2.8 (d, J = 6 Hz, 6 H, overlapping t), 2.53–3.2 (t, 2 H), 2.93 (t, J = 6 Hz, 2 H), 4.43 (s, 2 H), 4.56–5.20 (s, 2 H, overlapping s), 5.23 (s, 2 H), 7.00–7.40 (m, 3 H), 7.46–7.73 (m, 1 H). Anal. ($C_{19}H_{23}N_3O_4$) C, H, N.

1,2-Bis(hydroxymethyl)-5,6-dihydro-3-methylpyrrolo-[2,1-*a*]isoquinoline Bis(cyclohexylcarbamate) (13c). A stirred solution of **12** (3.0 g, 0.012 mol) and triethylamine (0.5 mL) in dry tetrahydrofuran (50 mL) heated under reflux was treated with cyclohexyl isocyanate (3.87 g, 0.031 mol) over a period of 45 min. The mixture was stirred 7 h and concentrated in vacuo to give a solid that was recrystallized from tetrahydrofuran–petroleum ether (4:1) to give **13c** as white crystals (2.78 g, 47%): mp 205–208 °C dec; IR 3350, 2950, 2900, 1690, 1530, 1320, 1290, 1250, 1230, 1040 cm^{-1}; NMR δ 0.73–2.20 (m, 20 H), 2.33 (s, 3 H), 3.03 (t, J = 6 Hz, 2 H), 4.60 (s, 1 H, br overlapping s), 4.73 (s, 1 H, br overlapping s), 5.20 (s, 2 H), 5.33 (s, 2 H), 7.09–7.49 (m, 3 H), 7.49–7.75 (m, 1 H). Anal. ($C_{29}H_{39}H_3O_4$) C, H, N.

1,2-Bis(hydroxymethyl)-5,6-dihydro-3-methylpyrrolo-[2,1-*a*]isoquinoline Bis(ethylcarbamate) (13b). A solution of **12** (4.4 g, 0.018 mol) and triethylamine (0.5 mL) in dry tetrahydrofuran (50 mL) was treated portionwise with ethyl isocyanate (3.2 g, 0.045 mol) over a period of 6 h. The mixture was heated under reflux for 26 h and concentrated to give a dark gum. This was crystallized from a minimum volume of ethyl acetate to yield white crystals (2.84 g, 41%): mp 175–175 °C dec; IR 3300, 3060 (w), 3000–2900, 1690, 1540, 1260, 1000 cm^{-1}; NMR δ 1.20 (t, J = 6 Hz, 6 H), 2.29 (s, 3 H), 2.80–3.56 (m, 6 H), 4.93 (t, J = 6 Hz, 2 H), 4.53–5.00 (s, 2 H, br), 5.20 (s, 2 H), 5.33 (s, 2 H), 7.03–7.46 (m, 3 H), 7.49–7.76 (m, 1 H). Anal. ($C_{21}H_{27}N_3O_4$) C, H, N.

Acknowledgment. This investigation was supported by Public Health Service Grants CA 22935 and CA 09166 awarded by the National Cancer Institute, DHHS.

Registry No. **3**, 1700-37-4; **4**, 24550-32-1; **5**, 588-05-6; **6**, 91523-50-1; **7**, 91523-51-2; **8**, 91523-52-3; **9**, 91523-53-4; **10**, 91523-54-5; **11a**, 91523-55-6; **11b**, 91523-56-7; **12**, 91523-57-8; **13a**, 91523-58-9; **13b**, 91523-60-3; **13c**, 91523-59-0; $PhCH_2O$-*m*-C_6H_4-$(CH_2)_2NH_2$, 51061-22-4; $MeOC(O)C{\equiv}CC(O)OMe$, 762-42-5; CH_3NO_2, 75-52-5; *i*-PrNCO, 1795-48-8; MeNCO, 624-83-9; EtNCO, 109-90-0; dimethyl 5,6-dihydro-3-methylpyrrolo[2,1-*a*]isoquinoline-1,2-dicarboxylate, 53927-34-7; glyoxylic acid, 298-12-4; cyclohexyl isocyanate, 109-90-0.

Design and Synthesis of Naltrexone-Derived Affinity Labels with Nonequilibrium Opioid Agonist and Antagonist Activities. Evidence for the Existence of Different μ Receptor Subtypes in Different Tissues

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A series of β-funaltrexamine (**2**, β-FNA) analogues (**3–14**) were synthesized that contain a variety of electrophilic groups attached at the 6β-position of the opiate. The opioid agonist and antagonist activities of these ligands were evaluated in the guinea pig ileum (GPI) and mouse vas deferens (MVD) in vitro assays. Several of the compounds behaved like β-FNA in that they exhibited reversible agonist activity at κ opioid receptors and irreversible antagonist activity at μ opioid receptors. The rank order of irreversible antagonism for a series of related Michael acceptors did not parallel their intrinsic chemical reactivity, confirming that the degree of covalent binding is in part dependent on the spatial disposition of the electrophilic center relative to the receptor nucleophile (secondary recognition). The maleimidoacetamide **8** behaved very differently from β-FNA in that it exhibited considerably greater irreversible μ antagonism in MVD relative to the μ blockage in the GPI. This suggests that different proportions of μ receptor subtypes exist in the two tissues. Several of the agents tested, including some nonreactive control compounds, displayed an unusual type of persistent κ agonist activity in the GPI. This activity, which was reversed by addition of naloxone, reappeared upon washing. Receptor models have been presented to explain this effect. A few of the reactive ligands displayed a true nonreversible κ agonist activity, suggesting a covalent association with the receptor. Of note in this regard was the propiolamide **6**, which appeared to be an irreversible mixed agonist–antagonist at κ and μ receptors.

We have recently reported on the opioid receptor activity of several epimeric pairs of moderately reactive affinity labels derived from naltrexone (**1**) that differed in stereochemistry of attachment of the electrophilic moiety at C-6.[1] Although both α and β epimers were shown to be recognized by different opioid receptor types, covalent bonding capacity resided mainly in the β series and showed a high preference for labeling of the μ receptor system. This behavior was typified by β-funaltrexamine (**2**, β-FNA), which has been shown to be a reversible κ agonist and a specific irreversible μ antagonist in vivo[2] as well as

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(1) Sayre, L. M.; Larson, D. L.; Fries, D. S.; Takemori, A. E.; Portoghese, P. S. *J. Med. Chem.* **1983**, *26*, 1229.

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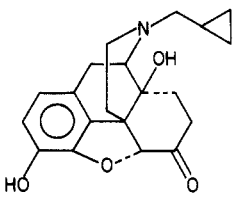

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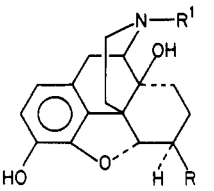
in vitro.[3] We also obtained evidence for differences between the μ receptor systems present in the guinea pig ileum (GPI) and mouse vas deferens (MVD) tissue preparations.[4] These results supported the notion that two consecutive recognition processes must be fulfilled to obtain covalent labeling: (1) a high-affinity (reversible) recognition of the agent for the receptor site and (2) the alignment of the electrophilic center in the ligand with a compatible, proximal receptor nucleophile. The second recognition step is a function of the intrinsic reactivity of the electrophilic moiety as well as the frequency of productive encounters with the receptor nucleophile. These conclusions were in conformity with the principles put forth by Baker in connection with active-site-directed irreversible inhibition of enzymes.[5]

In an effort to develop new ligands that are highly selective for different receptor types and subtypes, we have conducted a structure–activity study with analogues of β-FNA involving a more extensive variation of the electrophilic functionality attached to the 6β-nitrogen atom than in our previous work. The variables that have been examined included (1) intrinsic reactivity, (2) chemical constitution of the electrophile, and (3) the "distance" between the electrophilic center and the opiate nucleus. The present study presents additional evidence for the concept of secondary recognition discussed in our previous reports and demonstrates the feasibility of designing ligands to distinguish between different subtypes of μ receptors. In addition, a thorough pharmacologic evaluation of the target and control compounds has revealed the operation of several types of agonistic phenomena at κ receptors, including both reversible and irreversible activities.

Design Rationale

The target compounds that were synthesized fall into two major groups. Ligands in the first group (3–8) have in common a Michael acceptor moiety. The second group (9–12) is comprised of compounds that contain their electrophilic centers in moieties of diverse constitution. In addition, nonreactive ligands (13, 14) bearing some structural similarity to selected compounds in both groups were prepared for comparison purposes.

Michael Acceptors. The purpose of selecting the Michael acceptors 3–8 as target compounds was to investigate the role of reactivity and geometry of the electrophilic group in the irreversible blockage of opioid receptors. As with other Michael acceptors, these electrophiles were presumed to have selectivity for the sulfhydryl group. Also, since β-FNA (2) is a specific alkylator of the μ opioid receptor system, it was of interest to determine whether replacement of the cyclopropylmethyl by an allyl



$R^1 = CH_2CH(CH_2)_2$ unless otherwise indicated

R

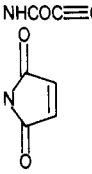
2 NHCOC(H)=CCOOMe

3 NHCOC(H)=CCOOMe ($R^1 = CH_2CH=CH_2$)

4 NHCOC(H)=CCOMe

5 $NHCOCCl=CH_2$

6 $NHCOC\equiv CH$



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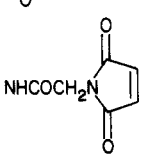
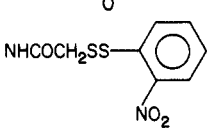


8 $NHCOCH_2N$



9 $NHCOCH_2SS$ (NO_2)

10 $NHCOCH_2HgCl$

11 $NHCOCOC_6H_5$

12 $NHCOCH_2Br$

13 $NHCOC_6H_5$

14 $NHCOCH_2CH_2COOMe$

15 $NHCOCH_2I$

16 NH_2

17 NH_2 ($R^1 = CH_2CH=CH_2$)

18 NHCOC(H)=C(H)COOMe

group (3) would alter the specificity and potency of the ligand.

It can be noted that the Michael acceptors 4–6 are closely related to β-FNA (2) but should differ somewhat with respect to their reactivity. Other electrophilic groups (7, 8) in this series differ even more markedly from β-FNA, both in their greater reactivity[6] and the disposition of their reactive centers.

Our working hypothesis that very closely related recognition sites (e.g., μ opioid receptor subtypes) possess a high degree of homology at their recognition locus, but considerably less similarity at some distance from the site, provided the basis for the design of 8. As illustrated (Figure 1), such a difference can in principle lead to the alkylation of one receptor subtype (A_1) in the presence of a second (A_2) if the electrophilic group of the ligand can

(2) Ward, S. J.; Portoghese, P. S.; Takemori, A. E. *J. Pharmacol. Exp. Ther.* **1982**, *220*, 494.
(3) Takemori, A. E.; Larson, D. L.; Portoghese, P. S. *Eur. J. Pharmacol.* **1981**, *70*, 445. Ward, S. J.; Portoghese, P. S.; Takemori, A. E. *Eur. J. Pharmacol.* **1982**, *80*, 377.
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(5) Baker, B. R. "Design of Active-Site-Directed Irreversible Enzyme Inhibitors"; Wiley: New York, 1967.
(6) Anderson, B. M.; Tanchoco, M. L.; Pozzo, A. D. *Biochim. Biophys. Acta* **1982**, *703*, 204.

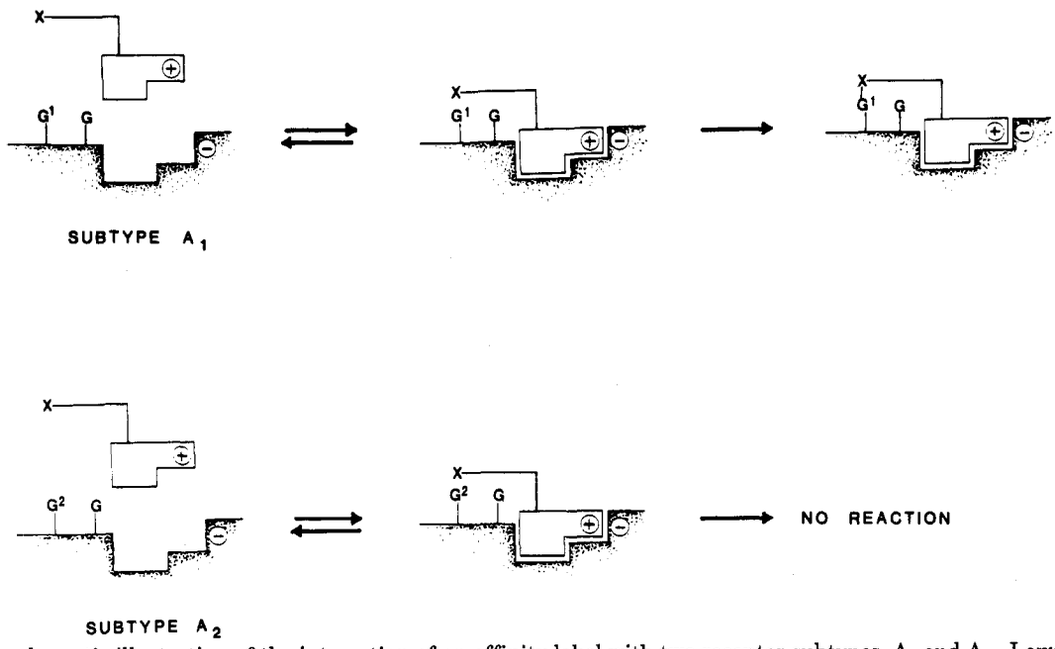
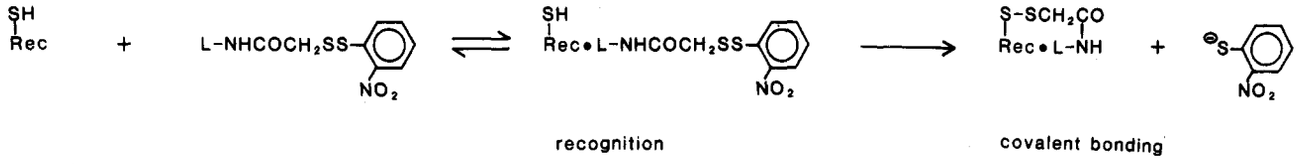
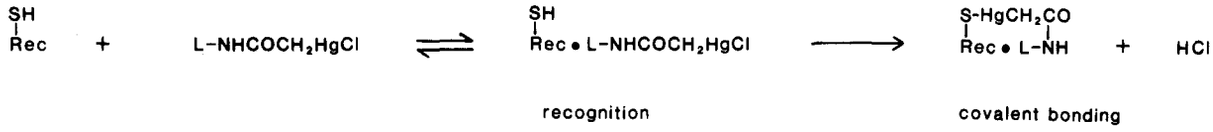


Figure 1. A schematic illustration of the interaction of an affinity label with two receptor subtypes, A_1 and A_2. Lower homology is symbolized by the presence of different nucleophiles (G^1 and G^2) as the distance from the recognition locus is increased.

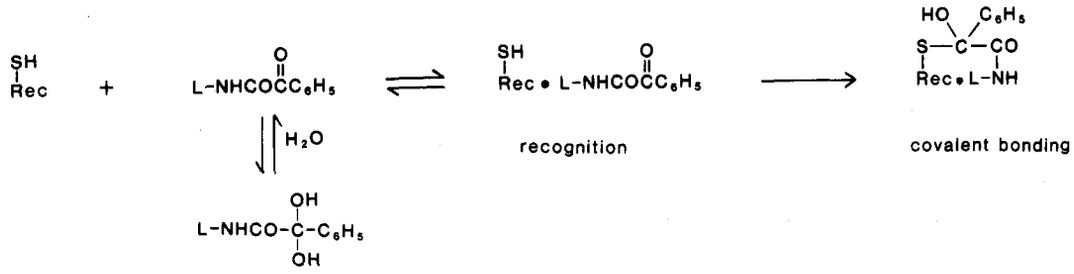
Scheme I



Scheme II



Scheme III



reach regions of low homology with different distributions of nucleophiles. For this reason, an acetamide group was employed as a spacer to place the maleimide moiety in **8** at a greater distance from the pharmacophore than in **7** or β-FNA (**2**).

Miscellaneous Electrophiles. Because of the possible involvement of a sulfhydryl group in the covalent attachment of the Michael acceptor moiety of β-FNA to μ opioid receptors, we investigated other electrophiles (**9–12**) that have known selectivity for this nucleophile. On the basis of the knowledge that sulfhydryl groups undergo exchange with disulfides, we decided to prepare **9** in hope that its reaction with a receptor sulfhydryl would promote the departure of *o*-nitrothiophenolate with the concomitant formation of a disulfide bond linking the ligand to the receptor (Scheme I).

Another approach involved the use of an organomercury electrophile **10**, which has high selectivity for sulfhydryl over other nucleophiles (Scheme II).

The benzoylformamide **11** was synthesized as an example of a ketone that is activated toward nucleophilic addition by the presence of an adjacent electron-withdrawing group. A 1,2-dicarbonyl compound of this type is substantially hydrated in water at equilibrium, and in the presence of sulfhydryl groups, the addition product is formed stoichiometrically (Scheme III).[7]

The bromoacetamide **12** was synthesized because of our finding that the iodoacetamide **15** irreversibly and selectively blocks μ opioid receptors in the MVD but not in the GPI.[4] We therefore were interested in determining if the

(7) Kanchuger, M. S.; Byers, L. D. *J. Am. Chem. Soc.* **1979**, *101*, 3005. Burkey, T. J.; Fahey, R. C. "Abstracts of Papers", 179th National Meeting of the American Chemical Society, Houston, March 1980; American Chemical Society: Washington, DC, 1980; ORGN 79.

less reactive bromoacetamide group might exhibit greater selectivity.

Chemistry

Synthesis of Target Compounds. Compounds 3–15 were prepared from β-naltrexamine (16), which was obtained stereospecifically from naltrexone (1).[8] The *N*-allyl analogue 3 of β-FNA (2) was prepared from β-naloxamine (17)[9] by the same procedure used to synthesize 2. The *trans*-3-acetylacrylamide 4 was prepared by two alternative routes from the corresponding commercially available acid. Treatment of *trans*-3-acetylacrylic acid with oxalyl chloride[10] afforded the desired acid chloride, which was employed in the acylation of 16 to afford 4. Alternatively, 4 could be prepared by treating 16 with the product obtained from coupling *trans*-3-acetylacrylic acid to 1-hydroxybenzotriazole (HOBt) with dicyclohexylcarbodiimide (DCC).[11] When isolated as its HCl salt, 4 was found to be unstable in aqueous or methanol solution, forming an insoluble white colloidal substance, which from elemental analysis appeared to contain additional oxygen. Suspecting an autoxidative decomposition, we prepared an alternative salt of 4 with the oxidation inhibitor gallic acid. This latter salt was found to afford stable solutions.

For the preparation of the 2-chloroacrylamide 5, we first attempted to utilize either HOBt or *N*-hydroxysuccinimide (HOSu) esters of 2-chloroacrylic acid. However, when the active esters were treated with amine 16, the major reaction pathway appeared to involve conjugate addition of the amine function of 16 to the carbon–carbon double bond. The acrylamide 5 ultimately was obtained by treatment of the sodium salt of 2-chloroacrylic acid with 1 equiv of oxalyl chloride in THF and in situ reaction of the resulting acid chloride mixture with the amine 16.

The propiolamide 6 was prepared by coupling 16 directly to propiolic acid with DCC in the presence of a slight excess of HOBt. Although HOBt was required for the desired coupling to proceed, it was found necessary to remove it rapidly in order to prevent it from adding to the unsaturated center in the product 6.[12]

The synthesis of the maleimide 7 posed a special challenge. The conventional approach involved reaction of the amine 16 with maleic anhydride followed by cyclization of the resulting maleamic acid. However, several mild cyclization procedures were found to afford the kinetically favored isomaleimides.[13,14] More stringent dehydrating conditions are known to give the thermodynamically favored maleimides[15] but because these procedures involve acid anhydrides or acid halides, they could not be employed in the present case. It has been reported that DCC/HOBt will convert *N*-arylmaleamic acids to *N*-arylmaleimides,[16] but this procedure was found to be ineffective for *N-alkyl*maleamic acids. There is literature precedent for the formation of maleimides from maleamate esters in the presence of alumina.[14] We found that ester 18 was slowly but cleanly converted to 7 in acetonitrile containing a trace of cesium carbonate.

The maleimidoacetamide 8 was synthesized from 16 and the known HOSu ester[17] of maleoylglycine.[18] Similarly, the bromoacetamide 12 was obtained from 16 via the reported HOSu ester of bromoacetic acid.[19]

The (*o*-nitrophenyl)dithioacetamide 9 was prepared from 16 and the corresponding HOSu active ester, which was synthesized as follows: Treatment of mercaptoacetic acid with a 2.6-fold excess[20] of *o*-nitrobenzenesulfenyl chloride in ether gave mainly the desired (*o*-nitrophenyl)dithioacetic acid but which cocrystallized with *o*-nitrophenyl disulfide, which formed as byproduct (the latter comprised 20 molar percent by NMR). On the basis of this composition, the mixture was reacted with the appropriate amounts of DCC and HOSu, and after removal of DCU, the desired active ester was easily separated from the more soluble *o*-nitrophenyl disulfide by crystallization.

The (chloromercuri)acetamide 10 was prepared from 16 and the corresponding HOBt active ester. The starting (chloromercuri)acetic acid was synthesized from ketene and mercuric chloride according to the method of Tilander[21] and isolated as its methanesulfonate salt.

The benzoylformamide 11 was prepared from the corresponding acid chloride which was prepared via the sodium salt–oxalyl chloride method. The usual thionyl chloride route could not be employed, since it was accompanied by partial decarbonylation.

The benzamide 13 was prepared in a straightforward fashion from benzoyl chloride, with base workup to hydrolyze the phenol ester byproduct. The methyl succinamate 14 was prepared by catalytic hydrogenation of 2.

Kinetic Study of Michael Acceptors. Model kinetic studies were performed with most of the Michael acceptors in order to assess their intrinsic reactivities with a sulfhydryl group. Accordingly, the pseudo-first-order rates of reaction of Michael acceptors with a 13-fold excess of cysteine as model nucleophile were determined. The half-lives and relative rates are listed in Table I. These measurements in several cases were conducted at lower pH values and extrapolated to pH 7.4 because of the rapidity of the reaction. These data indicate that the intrinsic reactivities of the *trans*- and *cis*-3-carbomethoxyacrylamides 2 and 18 and 2-chloroacrylamide 5 are comparable. A considerably greater reactivity was displayed by the *trans*-3-acetylacrylamide 4 and the maleimides 7 and 8.

Table I. Rates of Reaction of Michael Acceptors with Cysteine

compd	half-life,[a] s	rel rate[b]	compd	half-life,[a] s	rel rate[b]
2 (β-FNA)	18	1	7	0.04[c]	450
4	0.08[c]	225	8	0.04[c]	450
5	42	0.4	18	36	0.5

[a] Reaction at pH 7.4 and 25 °C in the presence of a 13-fold excess of cysteine. [b] Relative to β-FNA (2). [c] Determined at pH 2.4 and 3.0 and extrapolated to pH 7.4.

Pharmacology

GPI Agonist Activities. The agonist potencies of the target compounds in the guinea pig ileal longitudinal

(8) Sayre, L. M.; Portoghese, P. S. *J. Org. Chem.* **1980**, *45*, 3366.
(9) Jiang, J. B.; Hanson, R. N.; Portoghese, P. S.; Takemori, A. E. *J. Med. Chem.* **1977**, *20*, 1100.
(10) Use of thionyl chloride produced a rearranged material, identified as the lactone of 4-chloro-4-hydroxy-*cis*-2-pentenoic acid, on the basis of NMR.
(11) The active acylating intermediate appeared to be a mixture of *O*- and *N*-acyl reactive species, as is often the case with HOBt (Konig, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788).
(12) The adduct was characterized by IR, NMR, and mass spectroscopy. The separation of HOBt could not be achieved by liquid–liquid extraction and was ultimately accomplished by immediate chromatography of the crude reaction mixture.
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(14) Paul, R.; Kende, A. S. *J. Am. Chem. Soc.* **1964**, *86*, 4162.
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(20) Harris, J. F. *Chem. Abstr.* **1961**, *55*, 7747. Harris, J. F.; Carmack, M. *Chem. Abstr.* **1959**, *53*, 3148.
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Table II. Activities of 6β-Substituted 4,5α-Epoxy-3,14-dihydroxymorphinans on the GPI

	agonism		irreversible antagonism		
compd	potency[a] (*n*)	persistent effect[b] (*n*)	concn, nM	morphine IC_{50} ratio[c] ± SE (*n*)	ethylketazocine[d] IC_{50} ratio[c] ± SE (*n*)
2 (β-FNA)[e]	5.0 (5)	none (100 nM)	20	6.0 ± 0.6 (6)	1.0 ± 0.1 (5)
			1000	18.8 ± 4.3 (3)	1.3 ± 0.1 (3)
3	5.8 (5)	40% (200 nM) (8)	20	1.6, 2.2	1.0, 0.9
			200	6.4 ± 1.3[f] (4)	1.9 ± 0.4[f] (4)
4	4.5 (2)	none (20 nM)	20	12.5 ± 3.3[g] (3)	0.85, 0.90
5	1.1 (4)	none (200 nM)	20	13.4 ± 0.8[h] (4)	0.93 ± 0.08 (4)
			200		0.88 (1)
6	56 (4)	53% at 20 nM (5)	20	2.6 ± 0.1 (8)	0.84 ± 0.12 (4)
			200	*i*	*i*
7	2 (4)	none (200 nM)	20	1.3, 0.98	0.94, 0.59
			200	1.3 ± 0.2 (3)	1.4 ± 0.3 (3)
8	0.6 (5)	21% at 20 nM (2)	20	1.5 (1)	0.71 (1)
		59% at 200 nM (3)	200	3.3 ± 1.1 (6)	0.92 ± 0.11 (4)
9	1.1 (3)	none (200 nM)	20	1.3, 0.75	0.5, 1.4
			200	2.5 ± 0.15 (3)	1.3 ± 0.5 (3)
10	50% max	26% at 200 nM (7)	10[j]	0.75 ± 0.09 (3)	0.74 (1)
	IC_{30} = 300 nM (5)		20	1.2, 1.1	1.0 (1)
			200	4.2 ± 1.2 (4)	1.3 ± 0.2 (4)
11	24.2 (4)	22% at 20 nM (9)	20	1.48 ± 0.1 (4)	0.93 ± 0.2 (4)
12	58% max at	none (200 nM)	20[j]	3.3 ± 1.1 (4)	0.73 ± 0.18 (3)
	500 nM (3)		200	1.7 ± 0.1 (5)	0.78 ± 0.06 (5)
13	201 (4)	39% at 20 nM (9)	20	1.4 ± 0.5 (3)	0.91 ± 0.2 (4)
14	5.3 (3)	10% at 200 nM (3)	20	0.92 ± 0.4 (3)	1.0 ± 0.1 (3)

[a] Relative to morphine = 1 (IC_{50} = 2.92 ± 0.71 × 10^{-7}, *n* = 17) on the same ileum preparation; inhibition of contraction is greater than 80%. Values in parentheses are the number of determinations. [b] Percent inhibition of twitch that is not reversed by ≥20 washings following 30-min incubations at the concentrations indicated. Values in parentheses are the number of determinations. [c] Agonist IC_{50} (after 30-min incubation of the GPI with the test compound followed by washing 20 times) divided by control agonist IC_{50} in same preparation. Values in parentheses are the number of determinations. [d] Ethylketazocine IC_{50} = 9.73 ± 0.82 × 10^{-10} (*n* = 21). [e] Data from ref 1. [f] Reliable data at higher concentrations could not be obtained due to persistent effect in which the twitch was less than one-half the control value after 60-min incubation and washing (20 times). [g] This value was reduced to 6.6 ± 3.3 (5) on incubation with 100–1000 nM naloxone for 10–20 min following incubation with the test compound. [h] Reduced to 8.4 on naloxone incubation (see footnote *g*). [i] Value could not be determined due to a complete inhibition of twitch that was not reversed by washing or naloxone displacement. [j] Fifty-minute incubation.

muscle preparation (GPI) were determined relative to morphine as control (Table II). Compounds **6**, **11**, and **13** were notable in being considerably more potent than morphine (24-201X). When the GPI preparation was depleted of functional μ receptors by prior treatment with the nonequilibrium μ receptor blocker, β-FNA (**2**), the potencies of **6**, **11**, and **13** were not substantially changed.[22] This supports previous findings suggesting that the agonist activity of naltrexamine derivatives is mediated by κ receptors in GPI.[1]

A number of compounds (**3**, **6**, **8**, **10**, **11**, **13**) exhibited persistent agonism after washing. The propiolamide **6** displayed the most potent persistent effect (~50% permanent inhibition at 20 nM). Further studies demonstrated that the persistent agonistic effect was of two types. In one type (characteristic of compounds **3**, **11**, and **13**), treating the tissue with naloxone or naltrexone restored the full twitch response, but upon washing the tissue the inhibition of twitch returned. Similar observations have been reported previously with other opioids.[23] In the other type (characteristic of compounds **6**, **8**, and **10**), washing or treatment with naloxone and naltrexone was ineffective in restoring the normal twitch. For the case of the most potent agonist **6**, it was found that the irreversible agonism was still evident after β-FNA pretreatment.

GPI Antagonist Activities. Irreversible antagonist activity at μ and κ receptors was evaluated by determining the ileal response to μ (morphine) and κ [ethylketazocine (EKC)] agonists after a 30-min incubation with the test compound (followed by thorough washing). The IC_{50} values were compared to the control responses obtained prior to incubation and are presented in Table II as IC_{50} ratios (treated/control). More than one concentration of the test compound was examined in order to determine whether or not irreversible blockage was concentration related.

None of the *N*-cyclopropylmethyl compounds displayed irreversible antagonism at κ receptors, but several did so at μ receptors. The Michael acceptors **4** and **5** exhibited the greatest activity, about twice that of the previously reported for β-FNA (**2**). Significant irreversible activity also was displayed by propiolamide **6**. The Michael acceptor **8** and the mercurial **10** exhibited weaker activity which was evident only at 200 nM. Weak antagonism at 200 nM was observed for **9**.

The irreversible antagonist activity of the *N*-allyl compound **3** differed from that of β-FNA (**2**) in being one-tenth as effective a μ antagonist. Also in contrast to β-FNA, **3** exhibited persistent agonism in the GPI (vide supra).

In general, it was not possible to determine whether the test compounds produced *reversible* antagonism, on account of their potent agonist activities. However, in the case of the feeble agonists, reversible IC_{50} ratios (obtained after incubation *without* washing) could be measured. Accordingly, the bromoacetamide **12** displayed potent μ-selective antagonism (morphine IC_{50} ratio = 14.7 at 20 nM, 0.81 for EKC), which was reversed upon washing.

MVD Agonist Activities. The target compounds afforded a pattern of agonist activities on the mouse vas deferens preparation (MVD) (Table III) that was similar to that displayed in the GPI except that they possessed diminished potency and many exhibited partial agonism. Several compounds were considerably more potent than

(22) The agonist potencies (relative to morphine) for **6**, **11**, and **13** after preincubation with 200 nM **2** for 60 min were 21 (5), 21.8 (4), and 341 (4), where the value in parentheses equals the number of determinations.

(23) Cowie, A. L.; Kosterlitz, H. W.; Watt, A. J. *Nature (London)* **1968**, *220*, 1040.

Table III. Activities of 6β-Substituted 4,5α-Epoxy-3,14-dihydroxymorphinans on the MVD

			irreversible antagonism		
compd	agonist potency[a] (n)	persistent effect[b]	concn, nM	morphine IC_{50} ratio[c] ± SE	DADLE IC_{50} ratio[c] ± SE
2 (β-FNA)[d]	IC_{50} = 82 ± 35 nM (4)	none	100	3.9 ± 0.8 (3)	1.8 ± 0.4 (3)
			200	10.6 ± 0.4 (3)	1.1 ± 0.2 (3)
3	34% (3)	none	30[e]		1.7 ± 0.4 (3)
			200	4[f] (3)	1.1 ± 0.3 (5)
4	31% (3)	none	200	30 (34% max)[g] (4)	5.0 ± 0.9 (3)
5	44% (4)	none	200	6.0 ± 2.7 (3)	1.6 ± 0.4 (3)
6	IC_{50} = 23 ± 7 nM (3)		3[e]		1.5 ± 0.5 (3)
		50%	20	5 (56% max)[g] (3)	1.06, 1.13
			200		1.3, 1.5
7	52% (3)	35%	200	1.1 ± 0.4 (3)	0.81 ± 0.08 (3)
8	IC_{50} = 240 ± 35 nM (4)	none	200[h]	30 (55% max)[g] (4)	1.8 ± 0.4 (4)
9	70% (4)	none	200	1.8[f] (4)	0.98 ± 0.05 (4)
10	IC_{50} = 54 ± 18 nM (3)	20%	3[e]		1.1 ± 0.3 (4)
			20		2.6 ± 0.9 (3)
			200	0.57 ± 0.14 (4)	0.90, 0.85
11	51% (4)		10[e]		0.56 ± 0.1 (4)
		44%	20		0.51, 0.39
			200	6.5 ± 0.1 (3)	0.87, 1.6
12	31% (3)	20%	200	1.4 ± 0.6 (3)	1.5 ± 0.5 (3)
13	IC_{50} = 29 ± 18 (5)	50%	1[e]		0.78 ± 0.2 (3)
			20		2.3 ± 0.6 (3)
			200	1.9 ± 0.6 (3)	1.0 (1)

[a] In those cases where a concentration–response study was performed, IC_{50} values are reported unless the maximum inhibition of contraction was less than 70%. In such cases agonist potency was assessed by measuring the inhibition of contraction at a single concentration (200 nM). Values in parentheses are the number of determinations. [b] Percent inhibition of contraction that is not reversed significantly by ≥20 washings following a 30-min incubation at 200 nM. [c] See footnote c, Table II, for definition of IC_{50} ratio. Morphine IC_{50} = 1.42 ± 0.32 × 10^{-6} M (n = 30); [D-Ala², D-Leu⁵]enkephalin (DADLE) IC_{50} = 3.57 ± 0.47 × 10^{-10} (n = 30). [d] Data in part from ref 1. [e] Fifty min incubation. [f] The insensitivity of the tissues to morphine prevented the obtainment of a parallel concentration–response curve shift at 50% inhibition of contraction, and a curve shift was approximated in a parallel region at 30–40% inhibition of contraction. [g] The concentration–response curve experienced a rightward shift accompanied by significant flattening. The values given represent an approximate IC_{25} ratio and the maximum percent inhibition of contraction observed for morphine after treatment. [h] The IC_{50} ratio for EKC was 0.71 ± 0.22 (3).

morphine in this tissue (e.g., 62 times for **6**, 26 times for **10**, 49 times for **13**), but none rivaled the potency of [D-Ala²,D-Leu⁵]enkephalin (DADLE, 64× that of **6**). Several of the compounds that displayed persistent agonist effects in the GPI also did so in the MVD, in particular **6**, for which the inhibition of twitch was three-quarters permanent at its IC_{50} concentration.

MVD Antagonist Activities. On the MVD preparation, compounds **3–6**, **8**, and **11** behaved as irreversible antagonists of morphine agonism but, with the exception of **4**, had relatively little ability to block the effect of DADLE. It is noteworthy that the maleimidoacetamide **8**, which displayed only weak irreversible morphine antagonism in the GPI, exhibited a large nonparallel shift of the morphine dose–response curve in the MVD but only a slight shift of the response to DADLE and had no effect on the response to EKC. In addition, although both **4** and **5** were twice as potent as β-FNA (**2**) in blocking morphine agonism in the GPI, in the MVD their potency order differed (**4** > **2** > **5**).

Discussion

Studies on the GPI Preparation. We recently have reported on the importance of C-6 chirality in conferring irreversible μ receptor antagonism to naltrexone-derived affinity labels that contain moderately reactive electrophiles. That dihydro-β-FNA (**14**) was not able to irreversibly antagonize the agonist effect of morphine on the GPI in the present study provides additional evidence for the covalent attachment of β-FNA to the μ receptor system and complements the results of our reported binding experiments.[24]

In an effort to obtain additional information concerning the spatial confines and nature of the receptor nucleophile(s) involved in covalent bonding, we have investigated additional opiates **3–12** that possess a variety of selective electrophilic moieties attached to the 6β nitrogen atom. These electrophiles presumably have selectivity for a sulfhydryl group that has been implicated[25] as a receptor nucleophile. The fact that the relative reactivities (Table I) of the opiates containing Michael acceptor groups (**7**, **8** > **4** >> **2** > **5**, **18**) did not parallel their irreversible blockage of μ opioid receptors in the GPI (**4**, **5** > **2** >> **8** > **7**, **18**) (Table II and ref 1) indicates that intrinsic reactivity is not the only factor that contributes to covalent bonding to the recognition site. The most notable discrepancy in the above rank orders are the highly reactive maleimides **7** and **8**. One possible explanation for their inability to irreversibly block μ receptors in the GPI may be related to an inefficient secondary recognition step. Thus, in the case of **8**, the acetamide spacer may position the maleimide moiety distal to the receptor nucleophile. The inactivity of **7** also probably arises on account of the electrophilic center being constrained to an unfavorable orientation for reaction with the receptor nucleophile. This may be due to a conformational preference that restricts the maleimide moiety perpendicular to ring C of the opiate. The inactivity of the cis isomer **18**[1] also can be rationalized on this basis, in view of the evidence for a maleamate ester being restricted to a cyclic hydrogen-bonded structure.[26]

Inasmuch as the propiolamide **6** possesses a geometry similar to that of the acrylamide **5** and is presumed to be

(24) Ward, S. J.; Fries, D. S.; Larson, D. L.; Portoghese, P. S.; Takemori, A. E. *Pharmacologist* **1980**, *22*, 799.

(25) Smith, J. R.; Simon, E. J. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *77*, 281.

(26) Portoghese, P. S.; Telang, V. G.; Takemori, A. E.; Hayashi, G. *J. Med. Chem.* **1971**, *14*, 144.

a highly reactive Michael acceptor, it was surprising that it displayed weak irreversible antagonism of morphine (at 20 nM). However, in view of the highly potent agonism of **6** (56 times morphine) and the fact that it displayed considerable irreversible agonism at 20 nM, it is possible that this masked most of the antagonist effect. It was not possible to measure antagonist activity at a higher concentration of **6** since the twitch was inhibited completely and could not be reversed by washing or by naloxone treatment. This irreversible agonism may have arisen through a covalent process. It was noted that neither the reversible nor irreversible agonist potencies of **6** were significantly changed when the GPI preparation was depleted of functional μ receptors by incubation with β-FNA. This implicates the involvement of κ receptors in the agonist effects.

The *N*-allyl analogue **3** of β-FNA differed from its *N*-cyclopropylmethyl counterpart **2** in displaying a 10-fold lower irreversible antagonism of morphine as well as exhibiting persistent agonist activity. The reason for these differences is not immediately apparent but may indicate how this change in N-substituent can alter the mode of binding and/or receptor subtype preference.

In an attempt to elucidate the chemical identity of the μ receptor nucleophile(s) involved in covalent binding, we prepared and tested the sulfhydryl-specific disulfide **9** and mercurial **10** compounds. The electrophilic centers in these ligands (alkyl sulfur atom in **9** and mercury atom in **10**) are located at approximately the same distance from the opiate nucleus as are the electrophilic carbons in the potent, irreversible μ antagonists **2**, **4**, and **5**. Both ligands (**9**, **10**) displayed some persistent morphine-antagonist effects in the GPI but only at 200 nM. Due to the low potencies at relatively high concentration, we were unable to arrive at a definitive conclusion regarding the involvement of a sulfhydryl group in this effect.

No evidence for covalent attachment of the benzoylformamide **11** or bromoacetamide **12** to opioid receptors in the GPI was observed. The weak agonist activity of **12** (at 20 nM) allowed us to check for *reversible* antagonist activity. The finding of a potent μ-selective antagonism indicates that the lack of irreversible activity is *not* due to poor primary recognition (i.e., binding).

It is noteworthy that **11** is a highly potent agonist (24 times morphine) and that its benzamide analogue **13**, which was synthesized for comparison purposes, was over 200 times more potent than morphine. This agonist effect appeared to be mediated through κ receptors, since the potency was undiminished when the preparation was depleted of functional μ receptors by treatment with β-FNA. We ascribe the extremely high potency of **13** to the presence of a lipophilic region on the κ receptor that accommodates the benzamide phenyl ring.

Studies on the MVD. Since the MVD contains a minor fraction of μ and κ opioid receptor types together with a preponderance of δ receptors,[27] it is instructive to compare any irreversible antagonism of the target ligands to their action on the GPI, which is believed to contain mainly μ and κ receptors.

In this regard, the action of maleimide **8** was particularly significant, as it produced a large irreversible (~30-fold) shift of the morphine concentration–response curve in the MVD but a relatively weak antagonist effect in the GPI.[4] This was in contrast to the irreversible μ antagonist activity of β-FNA, whose effectiveness was greater on the GPI than on the MVD. Also, the shift of the morphine concentration–response curve in MVD was accompanied by flattening in the case of **8** but not in the case of β-FNA. The involvement of non-μ opioid receptor types in the irreversible effect of **8** seems unlikely in view of the absence of significant irreversible blockage of EKC (κ agonist) and DADLE (δ agonist).

The divergent profiles of irreversible μ blockage by **8** and β-FNA suggest that the GPI and MVD contain different proportions of μ receptor subtypes (μ_G and μ_M), both recognizing morphine. Our results can then be rationalized if one assumes that β-FNA alkylates preferentially the μ_G subtype and the maleimide **8** only the μ_M subtype. The μ_G subtype predominates in the GPI but is only a minor component in the MVD, explaining the lesser effect of β-FNA in the latter tissue. Since the μ_M subtype predominates in the MVD, alkylation by **8** would be expected to produce the large shift and flattening of the morphine concentration–response curve observed. A similar activity to that of **8** has been reported for the iodoacetamide **15**.[1]

The large shift and flattening of the morphine concentration–response curve in the MVD by the acetylacrylamide **4** may in part be due to blockage of δ receptors because of its 5-fold shift of the DADLE IC_{50} (Table III). In addition, **4** might be acting at both μ receptor subtypes (μ_M and μ_G).

It is noteworthy that the benzoylformamide **11** but not the benzamide **13** exhibited significant persistent morphine antagonism at 200 nM in the MVD preparation. A comparison with the GPI was not possible since the strong agonist activities of **11** and **13** in that tissue precluded the evaluation of irreversible antagonism at a concentration higher than 20 nM. Nonetheless, the data in MVD are consistent with the ketone group of **11** acting as an electrophilic acceptor for a nucleophile on the μ receptor.

Of interest was the observation that the bromoacetamide **12** (200 nM) displayed no significant irreversible antagonism of either morphine or DADLE in the MVD. In contrast, the iodoacetamide **15** (200 nM) was reported to produce a 20-fold shift of the morphine concentration–response curve.[1] It is possible that the activity difference between these compounds is related to the greater reactivity of **15** relative to **12**.

Persistent Agonist Activities. Many of the target compounds exhibited potent opioid agonist activity in the GPI and MVD preparations. In a number of such cases difficulty was encountered in washing out the agonism completely. Normally, extensive washing or treatment with naloxone (or naltrexone) resulted in a complete reversal of the agonist effect. However, in several cases such treatment reversed the agonist response only while naloxone was in the tissue bath, but upon washing, the inhibition of the twitch returned to nearly the same level as before the antagonist was added. The fact that the ligands that produced this effect do not all contain electrophilic moieties suggests a noncovalent mechanism. This phenomenon was described previously by Cowie et al.,[23] who suggested that naloxone exerts its antagonist action at a site different from that of the agonists they employed.

Since the agonist activity of our target compounds and those reported[23] are apparently mediated through interaction with κ receptors, we offer two possible explanations for this phenomenon. The first explanation, illustrated schematically in Figure 2, is a two-site model that contains a κ receptor and a neighboring regulatory recognition site for the antagonist. Binding of the antagonist to this neighboring site R allosterically inhibits biochemical conversion without reducing the affinity of the agonist ligand

(27) Robson, L. E.; Paterson, S. J.; Kosterlitz, H. W. *Handb. Psychopharmacol.* **1983**, *17*, 13.

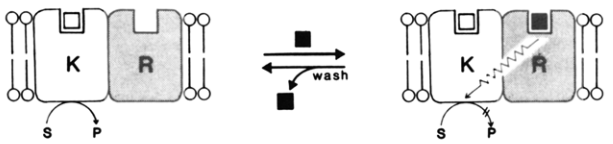


Figure 2. A schematic illustration of a two-site model for the κ receptor (K) and its associated regulatory (R) subunit. Occupation of the regulatory site by an antagonist ligand (■) noncompetitively inhibits the agonist (□) response that stimulates a biochemical reaction (S → P) that leads to pharmacological effect. The agonist is bound more tightly than the antagonist; consequently washing reverses the antagonism with the reappearance of the agonist effect.

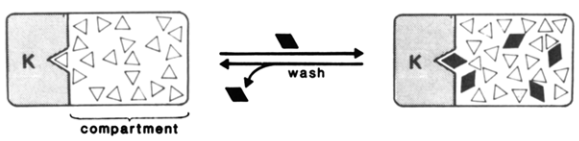


Figure 3. A single site model to explain persistent κ agonist activity. Agonist (△) localized in a membrane compartment associates with an adjacent κ receptor (K) to produce an agonist effect. Addition of naloxone (♦) displaces the agonist from the receptor, thereby reversing the agonist effect. When the tissue is washed, naloxone is removed selectively and the receptor becomes reoccupied by residual agonist.

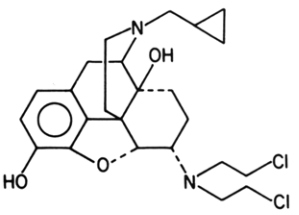
for the κ receptor K. Upon washing, the antagonist is selectively removed, thereby restoring the agonism of the bound ligand.

An alternate, though not mutually exclusive explanation, involves the localization of the compound in a membrane domain that allows high accessibility to the κ receptor (Figure 3). The introduction of an antagonist such as naloxone into this domain displaces the receptor-bound κ agonist, thereby antagonizing its effect. However, washing is more effective in removing the antagonist, permitting reassociation of the residual κ agonist with the receptor. Such a differential washout could result from a greater lipophilicity of the agonist relative to that of naloxone. This scheme essentially implies that ligand–receptor interaction is comprised of two consecutive steps, as has been described previously by Perry et al.[28]

Of the compounds that produced persistent agonism, some could not be temporarily reversed by exposure to naloxone. The compounds observed to produce this irreversible agonist effect were those that contained electrophilic groups, suggesting a covalent association with opioid receptors. Presumably this irreversible agonism is mediated by κ receptors, and supporting evidence was obtained from experiments with the propiolamide **6**. We determined that there was no difference in the reversible agonist potency or the development of irreversible agonism in the normal GPI preparation and in one that was devoid of functional μ receptors (β-FNA treated). Since **6** also produces irreversible antagonism of morphine, this compound may be an irreversible mixed agonist–antagonist at κ and μ receptors, respectively. In this respect its behavior is similar to that previously reported for the nitrogen mustard α-CNA (**19**).[29]

Conclusions

The lack of correlation between irreversible μ antagonism and reactivity of Michael acceptor ligands suggests that proper alignment of an electrophilic center with a proximal nucleophile on the receptor (secondary recognition) is required for covalent binding. The sulfhydryl group appears to be a good candidate for this nucleophile, in view of the known selectivity of many of the electrophiles that were attached to the ligands in the present study.



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On the basis of the concept that there is less homology between different μ receptor subtypes as the distance from the recognition locus is increased, a ligand (**8**) whose electrophile is attached to a spacer group was synthesized and found to block μ receptors in the MVD selectively with minimal blockage of the μ subtype in the GPI. This profile differs from that of β-FNA (**2**), which is more selective for GPI μ receptors. If the μ receptor system is viewed as being comprised of descrete agonist and antagonist (regulatory) sites that are allosterically coupled,[30] then the difference in selectivity between maleimide **8** and β-FNA may reflect covalent attachment at different regulatory sites rather than at the associated μ receptor subunit (Figure 4).

Several of the reactive and unreactive ligands produced persistent κ-mediated agonism which was antagonized while naloxone was present in the tissue bath but which returned to the original agonist response on thorough washing. This is consistent either with (a) a noncompetitive mechanism for κ antagonism or (b) a great difference between the ease of removal of naloxone and the κ agonists from a receptor compartment. Irrespective of which mechanism may be operative, this phenomenon has not been observed for other opioid receptor systems, suggesting an organization of κ receptors distinct from that of the other opioid receptors. For a few of the reactive ligands, the persistent κ-mediated agonism was not affected by naloxone, suggesting an irreversible covalent association.

Experimental Section

General Procedures. 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, Phoeniz, AZ, and were within ±0.4% of the theoretical values. IR spectra were obtained from KBr pellets with a Perkin-Elmer 281 instrument. NMR spectra were recorded at ambient temperature on Varian A-60D and T-60 instruments with tetramethylsilane and DSS as internal standard in nonaqueous media and D_2O, respectively. Optical rotations were determined with a Perkin-Elmer 141 polarimeter. Mass spectra were obtained on a AEI MS-30 instrument. All TLC data were determined with Eastman "Chromogram" 13181 plastic-backed sheets (silica gel), and the eluant EMA refers to EtOAc–MeOH–NH_4OH. Unless otherwise stated, all reagents and solvents used were reagent grade, without subsequent purification. Other abbreviations employed are DCC, dicyclohexylcarbodiimide; DCU, *N,N'*-dicyclohexylurea; HOSu, *N*-hydroxysuccinimide; HOBt, 1-hydroxybenzotriazole.

17-Allyl-4,5α-epoxy-3,14-dihydroxy-6β-[*trans*-3-(methoxycarbonyl)acrylamido]morphinan Hydrochloride (3·HCl). A slurry of **17**·2HCl[9] (0.401 g, 1 mmol) and triethylamine (0.354 g, 3.5 mmol) in 20 mL of CH_3CN–THF (1:2) was stirred over 3-Å sieves for 45 min and cooled to −10 °C. With continued stirring,

(28) Perry, D. C.; Mullis, K. B.; Oie, S.; Sadée, W. *Brain Res.* **1980**, *199*, 49.

(29) Sayre, L. M.; Takemori, A. E.; Portoghese, P. S. *J. Med. Chem.* **1983**, *26*, 503.

(30) Portoghese, P. S.; Takemori, A. E. *J. Med. Chem.* **1983**, *26*, 1341.

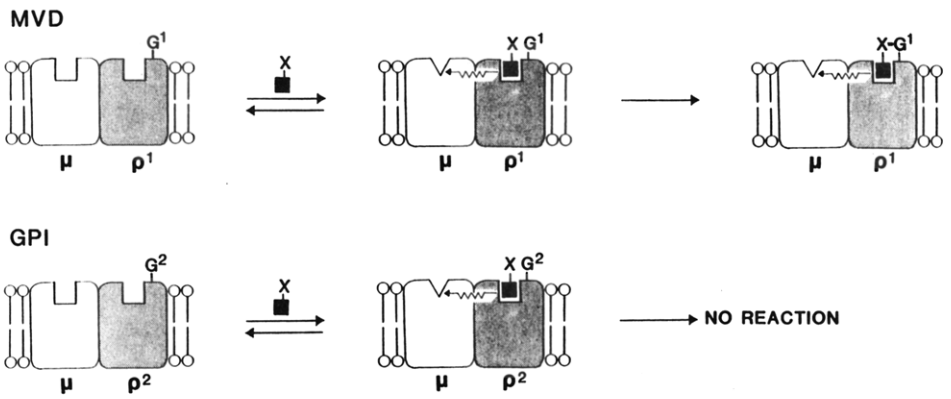

Figure 4. A schematic illustration of the selective, covalent affinity labeling of different μ receptor subtypes in the MVD and GPI. Note that the electrophilic moiety X alkylates a nucleophile G^1 associated with the regulatory subunit ρ rather than the receptor subunit (μ). The differential irreversible blockage is depicted as being related to the different properties (G^1 vs. G^2) of the regulatory subunits (ρ^1 and ρ^2) that are part of the μ receptor system.

a solution of *trans*-3-(methoxycarbonyl)acryloyl chloride (0.178 g, 1.2 mmol) in THF (3 mL) was added dropwise. After an additional 1 h of stirring at 25 °C, MeOH (1 mL) and Et_3N (0.2 mL) were added, and stirring was continued 48 h. The resulting mixture was coarsely filtered, concentrated, and partitioned between $CHCl_3$ and dilute aqueous NH_4OH. The $CHCl_3$ layer was concentrated and diluted with MeOH, HCl was added to pH 1.5, and the solvent was evaporated. The residue was crystallized from MeOH–CH_3CN, yielding 333 mg of **3** (70%) in the first crop: decomposes without melting at 280 °C; EIMS (30 eV) m/e 440 (M^+, 19%); IR cm^{-1} 1720, 1675, 1649; R_f 0.50 (EMA 95:5:2); $[\alpha]^{22}_D$ −160° (*c* 1.2, MeOH). Anal. ($C_{24}H_{29}N_2O_6Cl·0.25H_2O$) C, H, N.

***trans*-3-Acetylacryloyl Chloride.** The anhydrous sodium salt of *trans*-3-acetylacrylic acid (from Na_2CO_3 and the acid, 2.28 g, 20 mmol) was suspended in dry THF (50 mL). A solution of oxalyl chloride (3.81 g, 30 mmol) in THF (10 mL) was added dropwise with stirring, with protection from atmospheric moisture. The resulting solution was stirred 16 h and filtered, yielding 1.15 g of NaCl (98%). The filtrate was taken to dryness, diluted with dry toluene (20 mL), and again taken to dryness. The toluene "chase" was repeated two more times, yielding 2.31 g of residue (87%): NMR δ 2.42 (s, 3 H), 7.25–6.55 (2 d, 2 H, J = 15.5 Hz). The residue was diluted with dry THF (17 mL) to give a 1.0 M stock solution.

6β-(*trans*-3-Acetylacrylamido)-17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan Gallate (4·$C_7H_6O_5$). The free base **16**[8] (0.616 g, 1.8 mmol) and Et_3N (0.232 g, 2.3 mmol) was dissolved with stirring in distilled THF (25 mL) over 3-Å sieves at −20 °C. To this was added dropwise over 20 min a 1 M THF solution (2.25 mL) of *trans*-3-acetylacryloyl chloride (2.25 mmol) in THF (8 mL). After the addition was complete, the resulting mixture was stored at −10 °C overnight, filtered, treated with a trace of hydroquinone, and concentrated. The residue was chromatographed on silica gel (CH_2Cl_2–acetone–Et_3N, 65:35:1), and the fractions containing the desired product (R_f 0.35, CH_2Cl_2–acetone–Et_3N, 50:50:1) were pooled. Toluene (30 mL) was added and all low boiling solvent (and Et_3N) was removed at reduced pressure. To the resulting concentrate was added a solution of gallic acid–water (0.35 g) in acetone (30 mL). The mixture was concentrated until solid appeared, which, after cooling, was filtered and discarded (gallic acid). Further concentration of the filtrate afforded 0.30 g in two crops (27%) of the desired 1:1 salt. An additional 40% was obtained from the mother liquor. Recrystallization from acetone–isopropyl ether yielded 4·$C_7H_6O_5$·0.33H_2O: decomposes without melting at 150–200 °C; $[\alpha]^{25}_D$ −128° (*c* 1.0, MeOH); EIMS (for CH_3SO_3H salt), m/e 438 (M^+, 66%); IR cm^{-1} (for CH_3SO_3H salt) 1660 (br, sh at 1700). Anal. ($C_{32}H_{36}N_2O_{10}·0.33H_2O$) C, H, N.

6β-(2-Chloroacrylamido)-17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan Hydrochloride (5·HCl). The anhydrous sodium salt of 2-chloroacrylic acid (Polysciences) was prepared from 1.6 g of the acid (15 mmol) and Na_2CO_3 (0.795 g, 7.5 mmol). To a stirred slurry of this salt in distilled THF (30 mL) was added a solution of oxalyl chloride (2.79 g, 22 mmol) in THF (8 mL), dropwise until evolution of gas ceased and then the remainder in one portion. The resulting mixture was stirred at 25 °C for 24 h, refrigerated for 3 h, and filtered. The NaCl was washed with 30 mL of THF–toluene (2:1), and the filtrate was concentrated to 4 mL and then rediluted with THF to 12 mL. This solution was found to be about 0.62 M in CH_2=C(Cl)COCl, corresponding to a 50% retention of the desired acid chloride. To a solution of **16** free base (0.342 g, 1.0 mmol) and Et_3N (0.23 g, 2.3 mmol) in THF (20 mL) containing 3-Å sieves at −20 °C was added dropwise 2.5 mL of the above solution of CH_2=C(Cl)COCl (1.5 mmol) over 15 min. The mixture was then stirred at 25 °C for 20 h and concentrated, and the residue was partitioned between $CHCl_3$ and aqueous $NaHCO_3$. The organic extracts were evaporated, the residue was taken up in MeOH, concentrated HCl was added to pH 1.5, the solvent was removed, and the residue was dried (anhydrous EtOH). Slow crystallization from CH_3CN–EtOH yielded 0.36 g of **5**·HCl in two crops (76%). Recrystallization from CH_3CN–MeOH afforded analytically pure material, which analyzed as the hydrate–hemimethanolate: decomposes without melting above 210 °C; mass spectrum (70 eV), m/e 430 (M^+, 87%); $[\alpha]^{22}_D$ −132° (*c* 1.2, CH_3OH); IR cm^{-1} 1657; R_f 0.63 (EMA 80:20:2). Chromatography and reprecipitation yielded methanol-free material. Anal. ($C_{23}H_{28}N_2O_4Cl_2·1.5H_2O$) C, H, N.

17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-6β-propiolamidomorphinan (6). A slurry of **16** free base (0.68 g, 2 mmol), propiolic acid (0.147 g, 2.1 mmol), and HOBt (0.34 g, 2.2 mmol) in CH_3CN (30 mL) and DMF (12 mL) was stirred over 3-Å sieves for 1 h. A solution of DCC (0.454 g, 2.2 mmol) in CH_3CN (10 mL) was added, and the resulting mixture was stirred at room temperature for 2 days with protection from moisture and light. The mixture was diluted (MeOH) and filtered, and the filtrate was concentrated. The remaining DMF was removed under high vacuum. The residue was partitioned between aqueous $NaHCO_3$ and $CHCl_3$, and the material obtained upon evaporation of the combined organic extracts was chromatographed on silica gel (CH_2Cl_2–acetone–hexanes, 6:1:3). Fractions containing mainly the material of R_f 0.5 (EMA 95:5:2) were pooled and evaporated. Recrystallization from toluene–petroleum ether yielded 0.145 g in two crops (18%): decomposes without melting at 237 °C; EIMS (30 eV), m/e 394 (M^+, 92%); IR cm^{-1} 2093, 1665, 1624; $[\alpha]^{25}_D$ −187° (*c* 0.8, CH_3CN). Anal. ($C_{23}H_{26}N_2O_4·0.5H_2O$) C, H, N.

17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-6β-maleimidomorphinan (7). A slurry of 6β-(*cis*-3-carboxyacrylamido)-17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan[1] (1.5 g, 3.4 mmol) and Cs_2CO_3 (0.554 g, 1.7 mmol) in MeOH (10 mL) and DMF (50 mL) was warmed to 35 °C and concentrated at reduced pressure for 1 h. Toluene and DMF (10 mL each) were added, and the resulting solution was again concentrated [35 °C (30 mmHg), 1 h]. After cooling, a solution of MeI (0.523 g, 3.68 mmol) in DMF (10 mL) was added, and the mixture was kept in a closed vessel for 24 h at 25 °C (forming the ester intermediate **18**). This was followed by addition of 4-Å molecular sieves, Et_3N (1.5 mL), and Cs_2CO_3 (30 mg), and the resulting mixture was stirred at 25 °C for 3 weeks. The sieves were removed by coarse filtration, the filtrate was evaporated to dryness at high vacuum, and the residue was chromatographed

on silica gel with use of CH_2Cl_2–acetone–hexanes (1:1:1). Fractions containing the fastest moving material (R_f 0.61, EMA 95:5:2) were combined, toluene (10 mL) was added, and the solution was concentrated to the first hint of cloudiness. After cooling and filtration of the resulting crystals, two additional crops were obtained by enriching the mother liquor in toluene. Total yield, 1.1 g (72%): decomposes without melting at 239 °C; EIMS (20 eV), m/e 422 (M^+, 85%); IR cm^{-1} 1770, 1703 (br), 1660; IR (HCl salt) cm^{-1} 1710 (br), 1632 (br); NMR ($CDCl_3$–5% Me_2SO-d_6) δ 6.89 (s, 2 H, vinyls), 6.75 (AB q, 2 H, Ar H), 5.14 (d, 1 H, J = 8 Hz, C-5 H); $[\alpha]^{25}_D$ –67.0° (c 1.0, CH_3CN). Anal. ($C_{24}H_{26}N_2O_5 \cdot H_2O$) C, H, N.

17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-6β-(maleimidoacetamido)morphinan Hydrochloride (8·HCl). To a solution of maleimidoacetic acid[18] (2.5 g, 16.1 mmol) and HOSu (1.88 g, 16.3 mmol) in 75 mL of THF–acetone (4:1) at 0 °C was added DCC (3.43 g, 16.6 mmol) in THF (15 mL), and the mixture was kept at 4 °C for 48 h. The resulting slurry was filtered, and the precipitate was washed and dried, giving 6.2 g. Evaporation of the mother liquor and crystallization of the residue from i-PrOH–hexane afforded a second crop of 0.87 g. The first crop was stirred in $CHCl_3$–acetone for 24 h, and filtration yielded 3.62 g of DCU. The residue obtained from this mother liquor was combined with the second crop and recrystallized from i-PrOH–EtOH, yielding 2.33 g (57%) of the HOSu ester of maleimidoacetic acid:[17] IR cm^{-1} 1830, 1760–1700 (br); NMR δ 7.01 (s, 2 H, vinyls), 4.67 (s, 2 H, glycyl CH_2), 2.87 (s, 4 H, CH_2CH_2). A mixture of this ester (0.48 g, 1.9 mmol) and **16** free base (0.582 g, 1.7 mmol) was dissolved in THF (40 mL), and following overnight refrigeration, the solvent was removed and the residue was loaded onto a silica gel column. The column was eluted with $CHCl_3$–acetone (3:1), initially containing a trace of Et_3N, until a fast-moving yellow band was removed, and then with a mixed solvent gradient starting at 1:1 and ending with straight acetone. The fractions containing the desired compound (R_f 0.64, EMA 80:20:2) were pooled, toluene (30 mL) was added, and the $CHCl_3$–acetone (and any Et_3N) was removed. THF (70 mL) was added, and a solution of HCl in THF was added until no further precipitation occurred. The solid (0.62 g, 71%) was collected and recrystallized from MeOH–i-PrOH: foams at 230 °C; $[\alpha]^{25}_D$ –130° (c 1.2, CH_3OH); EIMS (20 eV), m/e 479 (M^+, 51%); IR cm^{-1} 1720 (s, br), 1780 (w); NMR δ 6.95 (s, 2 H, vinyls), 6.81 (s, 2 H, Ar H), 4.31 (s, 2 H, glycyl CH_2). Anal. ($C_{26}H_{30}N_2O_6Cl \cdot 1.5H_2O$) C, H, N.

17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-6β-[(2-nitrophenyl)dithioacetamido]morphinan Hydrochloride (9·HCl). (o-Nitrophenyl)dithioacetic acid[20] (2.32 g, containing 20% of o-nitrophenyl disulfide contaminant, 7.2 mmol), HOSu (0.84 g, 7.3 mmol), and DCC (1.5 g, 7.3 mmol) were dissolved in THF (60 mL), the solution was allowed to stand 24 h at 25 °C, and the DCU (1.7 g, 100%) was removed by filtration. Evaporation of the filtrate and crystallization from THF–$CHCl_3$ (1:1) yielded 2.14 g (87%) of N-[(o-nitrophenyl)dithioacetoxy]succinimide: mp 164 °C; NMR (acetone-d_6) δ 7.63–8.4 (m, 4 H, Ar H), 4.05 (s, 2 H, $SCH_2C(=O)$), 2.86 (s, 4 H, CH_2CH_2).

To a solution of **16** free base (0.32 g, 0.92 mmol) in THF–EtOH (25 mL, 2:1) cooled to 0 °C was added 0.40 g of the above active ester (1.05 mmol) in small portions as a solid over 30 min with stirring. After the latter had all dissolved, the solution was stored at 5 °C for 12 h and then evaporated. The residue was partitioned between $CHCl_3$ and aqueous $NaHCO_3$, and the organic layer was concentrated. Redilution with MeOH, addition of HCl to pH 1.5, addition of a little EtOH–toluene, evaporation, and recrystallization from MeOH afforded 0.52 g (90%) of **9** in two crops: decomposes without melting at 230 °C; $[\alpha]^{24}_D$ –89.3° (c 1.0, 92% aqueous MeOH); EIMS (20 eV), m/e 416 ($MH^+ - C_6H_4NO_2S$, 4.4%);[12] IR cm^{-1} 1655 (br), 1590; R_f 0.64 (EMA 80:20:2). Anal. ($C_{28}H_{32}N_3O_6S_2Cl \cdot 2CH_3OH$) C, H, N.

1-[(Chloromercuri)acetoxy]benzotriazole. (Chloromercuri)acetic acid[21] (6.0 g 20.3 mmol), HOBt (3.11 g, 20.3 mmol), and DCC (4.46 g, 21.6 mmol) were stirred at 25 °C in THF–EtOAc (200 mL, 3:1) for 60 h. The solution was cooled, the DCU was filtered (4.7 g, 100%), and the filtrate was diluted with petroleum ether until cloudy. Upon cooling to –5 °C, a first crop of 2.7 g (32%) was obtained: mp 178–180 °C; IR (KBr) cm^{-1} 1716, 1622, 1592, 1492, 1417 (br); NMR (Me_2SO-d_6) δ 7.95–7.05 (m, 4, Ar H), 2.99 [s, $J_{^{199}Hg-C-^1H}$ = 320.5 Hz, 2 (with satellites), CH_2Hg].

6β-[(Chloromercuri)acetamido]-17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan Methanesulfonate (10·CH_3SO_3H). 1-[(Chloromercuri)acetoxy]benzotriazole (0.41 g, 1 mmol) and **16** free base (0.34 g, 1 mmol) were stirred together in THF (35 mL) at room temperature. After stirring for 12 h, Et_2O (5 mL) was added, and the solution was cooled and filtered. The filtrate was brought to pH 1.8 with a solution of CH_3SO_3H in MeOH. This solution was concentrated by rotary evaporation, rediluted with EtOH–toluene (9:1), and concentrated until cloudy. Warming to clarity and cooling yielded 0.58 g (81%) of **10**. The product was recrystallized from 2-propanol: mp 278 °C dec; R_f 0.14 (EMA 80:20:2); $[\alpha]^{25}_D$ – 129° (c 1.0, CH_3OH); IR (KBr) cm^{-1} 1625, 1509, 1410, 1200 (broad); EIMS, m/e 385 (7), 384 (19), 383 (8, M^+ – HgCl), 382 (14%); NMR (Me_2SO-d_6, D_2O exchanged) δ 6.73 (br s, 2, Ar H), 2.56 (s, 3, CH_3SO_3), 2.45 (br s, 1.7, CH_2Hg, $J_{^{199}Hg-C-^1H}$ = 293 Hz). Anal. ($C_{23}H_{32}N_2O_7SHgCl$) C, H, N.

6β-(Benzoylformamido)-17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan Hydrochloride (11·HCl). Benzoylformic acid (2.4 g, 16 mmol) was converted to its sodium salt with Na_2CO_3 (0.85 g, 8 mmol) and was made anhydrous by azeotropic removal of water. To a suspension of the salt in dry THF (50 mL) was added a solution of oxalyl chloride (3.05 g, 24 mmol) in THF (50 mL) was added a solution of oxalyl chloride (3.05 g, 24 mmol) in THF (10 mL) with efficient stirring and protection from atmospheric moisture. After stirring overnight, the solvent was removed and the residue was taken up in dry toluene (20 mL) and reevaporated. The toluene "chase" was repeated twice more, and the residue was diluted to 15 mL with THF. This solution was assumed to be about 0.9 M in acid chloride.

To a solution of **16** free base (0.62 g, 1.8 mmol) and Et_3N (0.28 g, 2.8 mmol) in dry THF (15 mL) was added dropwise 2.7 mL of the 0.9 M acid chloride solution (diluted to 8 mL with dry THF) at 0 °C under moisture-exclusion conditions. Stirring was continued overnight, allowing the mixture to come to room temperature. After filtration to remove NaCl and Et_3NHCl and removal of solvent, the residue was chromatographed on silica gel ($CHCl_3$–EtOH–Et_3N, 90:10:1). The first material to elute was the desired product (R_f 0.69, EMA 95:5:2). Conversion to the HCl salt and recrystallization from i-PrOH–MeOH yielded 0.48 g (52%) in two crops: decomposes without melting at 205 °C; $[\alpha]^{25}_D$ – 167° (c 1.1, CH_3OH); EIMS (70 eV), M/e 474 (M^+, 85%); IR cm^{-1} 1690–1620. Anal. ($C_{28}H_{31}N_2O_5Cl \cdot H_2O$) C, H, N.

6β-(Bromoacetamido)-17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan Hydrobromide (12·HBr). A solution of **16** free base (0.616 g, 1.8 mmol) in THF–i-PrOH (25 mL, 1:1) and a solution of N-(bromoacetoxy)succinimide[19] [0.59 g (containing 10% DCU), 2.3 mmol] in THF (25 mL) were each cooled to 0 °C and then mixed together. The resulting solution was stored at 5 °C for 20 h, filtered, concentrated, and partitioned between $CHCl_3$ and aqueous $NaHCO_3$. The organic layer was concentrated and rediluted with MeOH, and HBr was added to pH 1.8. The solution was taken to dryness, and the residue was recrystallized from MeOH–CH_3CN–toluene (7:2:1), yielding 0.542 g (55%) of **12**·HBr in two crops: decomposes without melting at 275 °C; EIMS (20 eV), m/e 462/464 (M^+, 64%/65%); IR cm^{-1} 1660 (br); R_f 0.66 (EMA 80:20:2); $[\alpha]^{22}_D$ –115° (c 1.05, MeOH–H_2O, 5:1). Anal. ($C_{22}H_{28}N_2O_4Br_2 \cdot 1.5H_2O$) C, H, N.

6β-Benzamido-17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan (13). A solution of **16**·2HOAc (0.301 g, 0.65 mmol) in 50% aqueous THF (10 mL) was adjusted to pH 11 was 2 N NaOH. A solution of benzoyl chloride (0.21 g, 1.5 mmol) in THF (5 mL) was added dropwise, while the pH was maintained at 11.0 ± 0.5 by addition of base. The mixture was then kept at pH 11.5 for 4 days, at which point selective solvolysis of the N,O-diacyl byproduct was complete. The mixture was adjusted to pH 10, concentrated, and extracted with $CHCl_3$. After removal of solvent, the product was crystallized from aqueous MeOH and then recrystallized from toluene–ether, affording 0.226 g (76%): mp 160–163 °C; 1641 cm^{-1} IR; $[\alpha]^{25}_D$ –168° (c 1.0, $CHCl_3$); EIMS, m/e 446 (M^+, 48), 105 ($PhC{=}O^+$, 100). Anal. ($C_{27}H_{30}N_2O_4 \cdot 0.5H_2O$) C, H, N.

17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-6β-[(3-methoxycarbonyl)propionamido]morphinan Hydrochloride (14·HCl). A solution of 170 mg of **2**·HCl[1] in MeOH (60

mL) containing 30 mg of 10% Pd/C was hydrogenated in a Parr apparatus at 45 psi for 5 h. The solution was filtered and evaporated, and the residue was recrystallized from CH_3CN containing a trace of MeOH. Yield of two crops 122 mg (71%): foams 250 °C; EIMS (20 eV), *m/e* 456 (M^+, 20%); IR cm^{-1} 1719, 1705, 1664, 1649; R_f 0.33 (EMA 95:5:2); $[\alpha]^{25}_D$ −132° (*c* 0.9, MeOH). Anal. ($C_{25}H_{33}N_2O_6Cl \cdot H_2O$) C, H, N.

Model Kinetic Studies. To a rapidly stirred solution (2.1 μM) of the naltrexamine derivative (Table I) in 0.025 M NaH_2PO_4 buffer (0.24 mL) of different pH values (7.4, 6.1, 5.5, 5.0, 3.0, or 2.4) was quickly added 10 μL of 0.65 M cysteine in same phosphate buffer. Rapid stirring was continued at 25 °C for a specific time interval (0, 10, 15, 30, or 60 s), and then the reaction mixture was quenched by acidification to pH 1. The amount of compound remaining was determined by using reversed-phase HPLC (25 × 0.46 cm Ultrasphere ODS, 5 μ; isocratic MeOH–KH_2PO_4 buffer, pH 4.4 (65:35) at 1.3 mL/min; UV, 254 nm) using an internal standard. Buffer solutions were purged with nitrogen before use, and each compound was assessed separately for hydrolytic decomposition, which was found not to be significant under the experimental conditions employed. Four time intervals were examined in duplicate for each compound, and a half-life estimate was calculated from the slope of a linear regression of the log amount remaining vs. time.

Pharmacology. The GPI and MVD assays were prepared and performed according to the methods of Rang[31] and Henderson,[32] as described previously.[29]

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Registry No. **2**, 72782-05-9; **2**·HCl, 72786-10-8; **3**·HCl, 91409-37-9; **4**, 91409-38-0; **5**, 91409-40-4; **5**·HCl, 91409-39-1; **6**, 91409-41-5; **7**, 91409-42-6; **8**, 91409-44-8; **8**·HCl, 91409-43-7; **9**·HCl, 91409-45-9; **10**·CH_3SO_3H, 91423-93-7; **11**, 91409-47-1; **11**·HCl, 91409-46-0; **12**, 91409-49-3; **12**·HBr, 91409-48-2; **13**, 91409-50-6; **14**, 91409-52-8; **14**·HCl, 91409-51-7; **16**, 67025-97-2; **18**, 83514-37-8; HOSu, 6066-82-6; HOBt, 2592-95-2; CH_2=C(Cl)COCl, 21369-76-6; *trans*-3-(methoxycarbonyl)acryloyl chloride, 17081-97-9; *trans*-3-acetylacrylic acid, 14300-75-5; sodium *trans*-3-acetylacrylic acid, 91409-53-9; sodium 2-chloroacrylic acid, 32997-86-7; propiolic acid, 471-25-0; 6β-(*cis*-3-carboxyacrylamido)-17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan, 91409-54-0; maleimidoacetic acid HOSu ester, 55750-61-3; *N*-[(*o*-nitrophenyl)dithioacetoxy]succinimide, 91409-55-1; 1-[(chloromercuri)acetoxy]benzotriazole, 91409-56-2; benzoylformic acid, 611-73-4; benzoylformic acid chloride, 25726-04-9; *N*-(bromoacetoxy)succinimide, 42014-51-7; benzoyl chloride, 98-88-4.

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Latent Inhibitors of Aldehyde Dehydrogenase as Alcohol Deterrent Agents

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A series of compounds structurally related to pargyline (*N*-methyl-*N*-propargylbenzylamine, **4**) were synthesized with the propargyl group replaced by a cyclopropyl, allyl, or 2,2,2-trichloroethyl group and, additionally in several cases, with the methyl group replaced by H. The rationale for their preparation was based on the expectation that, like pargyline, which gives rise to propiolaldehyde, oxidative metabolism of the above compounds by the hepatic cytochrome P-450 enzymes would lead to the generation in vivo of the aldehyde dehydrogenase (AlDH) inhibitors, cyclopropanone, acrolein, or chloral. These compounds were evaluated for inhibition of liver AlDH in vivo by measuring the elevation of ethanol-derived blood acetaldehyde in rats and in vitro by the rate of oxidation of acetaldehyde by intact and osmotically disrupted liver mitochondria. Administration of *N*-methyl-*N*-cyclopropylbenzylamine (**5**) and its nor-methyl analogue (**8**) to rats raised blood acetaldehyde levels significantly over controls at 2 h. This effect was more pronounced at 9 h, with blood acetaldehyde levels reaching 19 to 27 times control values and approaching the values induced by pargyline. Other compounds elicited significant elevations in ethanol-derived blood acetaldehyde only at 9 h. We suggest that latent inhibitors of AlDH such as **5** or **8** might be useful as alcohol deterrent agents.

The biochemical mechanism underlying the multitude of reported alcohol-drug interactions giving rise to the disulfiram–ethanol reaction (DER) is generally believed to be due to inhibition of one or more of the hepatic aldehyde dehydrogenase (AlDH) isozymes.[1] Our interest in drugs that produce a clinical DER is based on the expectation that understanding of these mechanisms at the molecular level should greatly aid in the design of alcohol deterrent agents for the treatment of alcoholism. Indeed, such deterrent drugs as disulfiram and citrated calcium carbimide were discovered accidentally when they were found to be incompatible with alcohol ingestion.[2]

Of the several known potent inhibitors of AlDH only disulfiram and chloral hydrate appear to be direct acting, while the others require prior metabolism to produce the active inhibitors. Thus, the mechanism of AlDH inhibition by disulfiram is by direct interaction with the SH group at the active site of the enzyme to form initially a covalently linked mixed disulfide, followed by intraenzyme displacement of diethyl dithiocarbamate by a second proximate SH group.[3] Cyanamide, the active component in citrated calcium carbiimide, requires activation by liver

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