(50 mL × 5). This extract was dried over sodium sulfate and concentrated under pressure at room temperature. The crude product was purified on a silica gel plate (20 × 20 cm, 2 mm thick) with methanol-chloroform (3:7) as solvent. The red band gave the product in 50–58% yield after extraction with acetone and concentration. Table I gives the physical properties and NMR signals for the products. In all cases, the 7-methoxy group protons at δ 4.0 were absent. Compound **15** showed the following UV and visible absorption: (CH_3OH) λ_{max} 245 nm (ϵ 18000), 305 (ϵ 11600), 250 (ϵ 7200) sh, 518 (ϵ 1000).

Determination of the pK_a for 6 and 7. A solution of 2.4 mg (0.0076 mmol) of **6** or **7** in a solvent composed of 1 mL of CH_3OH and 90 mL of 0.1 M KCl was titrated with standardized 0.01 N HCl, added in 0.1-mL aliquots, and the pH was measured with a Chemcadet digital meter. After 1.3 mL had been added, the solution was titrated back with standardized 0.01 N NaOH. The pK_a, determined from inflection points, was 4.8 in the forward direction and 5.0 in the reverse direction for **6** and 5.25 and 5.20 for **7**.

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Registry No. 1, 4055-39-4; 2, 18209-14-8; 6, 5047-66-5; 7, 15973-07-6; 9, 103422-20-4; 10, 103422-21-5; 11, 103422-22-6; 12, 103422-23-7; 13, 103456-57-1; 14, 103422-24-8; 15, 103422-25-9; 16, 32633-56-0; aziridine, 151-56-4; 2-methylaziridine, 75-55-8; propargylamine, 2450-71-7; 2-(aminomethyl)furan, 617-89-0.

Irreversible Blockage of Opioid Receptor Types by Ester Homologues of β-Funaltrexamine

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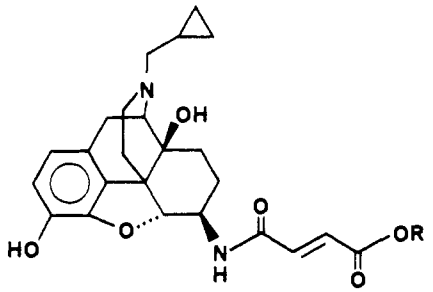
A series of ester homologues **2–5** of the μ receptor nonequilibrium antagonist β-funaltrexamine (**1**, β-FNA) was synthesized. These ligands were of interest in our investigation of the relationship between the structure of the ester function and the ability to irreversibly block μ opioid receptors. While all of the ligands were potent reversible agonists in the guinea pig ileum (GPI) and mouse vas deferens (MVD) preparations, most appeared to behave as irreversible antagonists of morphine. The benzyl **5** and phenethyl **6** esters possessed irreversible μ antagonist potency that was of similar magnitude to that of β-FNA in the GPI. In the MVD, all esters appeared to irreversibly block the agonist effect of morphine, but none of the compounds irreversibly antagonized [D-Ala^2,D-Leu^5]enkephalin to a significant degree. [^{3}H]Dihydromorphine displacement studies revealed no relationship between the affinity of the esters **1–6** and the irreversible blockage of μ receptors in the GPI or MVD. Possible reasons for the observed structure–activity relationship are discussed.

The nonequilibrium opioid antagonist β-funaltrexamine[1] (**1**, β-FNA) is employed widely as a pharmacologic probe.[2]



1, R = CH_3
2, R = CH_2CH_3
3, R = $(CH_2)_2CH_3$
4, R = $(CH_2)_3CH_3$
5, R = $CH_2C_6H_5$
6, R = $CH_2CH_2C_6H_5$

Its high selectivity for μ receptors both in vivo and in vitro has made β-FNA a useful ligand for investigating the involvement of μ receptors in opioid activity.

The high selectivity of β-FNA has been attributed to two recognition steps.[3] The first is manifested by the affinity of the ligand for the receptor, and the second involves the proper alignment of its electrophilic center with a proximal nucleophile on the receptor. This second recognition step appears to be particularly important when a selective electrophile is present, and in this regard it has been observed that the orientation and reactivity of a Michael acceptor group determines the effectiveness of irreversible blockage of μ receptors.

In an effort to reduce the number of variables in the structure–activity relationship of this series, we have examined β-FNA analogues **2–6** that differ only in the ester function. This series was of interest because it was anticipated that such modification should alter the steric and lipophilic parameters of the analogues without introducing significant changes in the intrinsic reactivity of the Michael acceptor moiety.

Chemistry. The β-FNA analogues **2–6** were synthesized by coupling 6β-naltrexamine[4] with the appropriate acid chlorides in aqueous THF containing K_2CO_3. Contaminants arising from acylation of the phenolic group were removed by a triethylamine-catalyzed ester interchange with the same alcohol as that contained in the fumaramate ester group. The acid chlorides for the above coupling were synthesized by first reacting maleic anhydride with an equimolar amount of the appropriate alcohol in a 10% molar excess of triethylamine. The resulting alkyl triethylammonium maleates were converted to the free carboxylates by extraction from aqueous HCl and heated with thionyl chloride to yield the acid chlorides.

The trans double bond in the acid chlorides was verified by NMR after they were reacted with 6β-naltrexamine to

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Table I. Activities of β-FNA Analogues on the GPI and Opioid Receptor Binding

no.	agonist potency ratio[a,b] ± SE	irreversible antagonist potency:[b,c] IC_{50} ratio ± SE (nM)	morphine	nalorphine	binding:[b,d] IC_{50}, nM
1 (β-FNA)	5.0 (5)	20	6.0 ± 0.6[e,f]	1.2 ± 0.2 (5)[f]	1.03 (2)
		100	12.0 ± 2.2[e,f]		
2	16.08 ± 1.38	20	1.75 ± 0.30[e]		0.64 ± 0.14
			2.22 ± 0.82[g]		
		200	3.94 ± 1.40[e]	1.67 (2)[e]	
			2.06 ± 0.26[g]		
3	147 ± 59.0	20	2.46 ± 0.58[e]		1.56 ± 0.64
			1.69 ± 0.46[g]		
		200	11.75 ± 2.11[e]	1.52 (2)[e]	
			7.69 ± 1.31[g]		
4	66.09 ± 8.21	20	5.05 ± 0.70[e]		1.65 (2)
			4.42 ± 2.24[g]		
		200	*h, e, g*	*h, e*	
5	1.41 ± 0.74	20	6.33 ± 0.60[e]		0.3 (1)
			5.85 ± 0.86[g]		
		200	6.69 ± 1.80[e]	0.81 (2)[e]	
			4.41 ± 2.14[g]		
6	10.40 ± 2.18	20	4.84 ± 1.39[e]		0.015 ± 0.01
			2.88 ± 0.92[g]		
		200	24.40 ± 7.08[e]	1.22 (2)[e]	
			19.05 ± 2.74[g]		

[a] The IC_{50} of morphine divided by that of the test compound on the same ileum preparation (morphine IC_{50} = (2.92 ± 0.71) × 10^{-7} M, n = 17). [b] Values represent means of three separate determinations unless otherwise noted by number in parentheses. [c] Morphine or nalorphine IC_{50} in the treated preparation divided by control IC_{50} values of the respective ligands in the same prepartion (morphine IC_{50} = (2.92 ± 0.71) × 10^{-7} M, n = 17; nalorphine IC_{50} = (5.13 ± 0.93) × 10^{-8} M, n = 20). [d] [^{3}H]Dihydromorphine binding to rat brain membranes. [e] Thorough washing (20 times) of preparation after incubation with test ligand for 30 min. [f] Taken from Takemori, A. E.; Larson, D. L.; Portoghese, P. S. *Eur. J. Pharmacol.* **1981**, *70*, 445. [g] Thorough washing (20 times) of preparation after incubation with test ligand for 30 min and displacement with naltrexone (200 nM, 5 min). [h] Inhibition of contraction did not recover sufficiently from the agonism of 4 to allow for testing of antagonism.

give **2–6**. The coupling constants of the vinyl protons were 15.4 Hz, which is consistent with this geometry.

Pharmacologic Results

Compounds **2–6** were first assessed for agonist and antagonist activity in the guinea pig ileal longitudinal muscle preparation (GPI) (Table I). The GPI assay was performed by the method of Rang.[5] The agonist potencies varied considerably, with the least potent ester **5** being equipotent with morphine and the most potent ester **3** having a potency approximately 150 times greater than that of morphine. In general, the longer aliphatic ester substituents conferred more potent agonist activity. The agonist activities were generally reversible, with the exception of the activity of **4**, where, after high concentrations, the agonist effect persisted even after extensive washing and attempts at displacement with naltrexone.

The compounds were assessed for irreversible antagonism by incubating with the GPI for 30 min, followed by extensive washing. The IC_{50} values for morphine and nalorphine were then determined and compared to control IC_{50} values determined in the same tissue and expressed as an IC_{50} ratio (treated/control). After incubation at 200 nM, the phenethyl ester **6** was found to be the most potent of the series as an irreversible antagonist of morphine, while the ethyl ester **2** was found to be the weakest. The antagonist activity of the butyl ester **4** could not be assessed at this concentration due to its persistent agonist activity. After treatment with **2**, **3**, **5**, and **6**, it was found that exposing the treated tissues to naltrexone reversed about 30% of the antagonist effect of these esters that could not previously be washed out. Like β-FNA, none of the target compounds blocked the effects of the κ agonist nalorphine.

The compounds **2–6** were assessed for agonist and antagonist activity on the mouse vas deferens preparation[6] (MVD) (Table II). All of the agents acted as reversible agonists on the MVD. Compounds **2–4** were slightly more potent agonists than β-FNA, while **6** was equipotent. Ester **5** exhibited partial agonist activity. As in the GPI, the longer aliphatic ester substituents conferred more potent agonism.

Table II. Activities of β-FNA Analogues on the MVD

no.	agonist potency:[a,b] IC_{50} ± SE, nM	irreversible antagonist potency:[c,d] IC_{50} ratio ± SE: morphine	DADLE
1 (β-FNA)	82 ± 35	10.6 ± 0.4	1.1 ± 0.2
2	13.0 ± 6.2	2.53 (2)	1.24 ± 0.33
3	21 ± 0.8	IC_{20}, 200 nM[e]	1.52 ± 0.24
4	7.0 ± 0.5	10.0 ± 5.9	1.75 ± 0.70
5	*f*	IC_{20}, 400 nM[g]	1.39 ± 0.49
6	70.1 ± 3.4	64.68 ± 16.8	3.73 ± 1.18
		24.38 ± 12.08[h]	

[a] Control morphine IC_{50} = (1.42 ± 0.32) × 10^{-6} M, n = 30. Control DADLE IC_{50} = (3.57 ± 0.47) × 10^{-10} M, n = 30. [b] Values represent means of three determinations unless otherwise noted by a number in parentheses. [c] See footnote *a* of Table I for definition of IC_{50} ratio. Tissues were treated with 200 nM test ligand. Where the standard agonist produced less than a 65% response following treatment, an estimate of an IC_{20} value was made from the plotted dose–response curve. [d] Value obtained following thorough washing (20 times) of preparation after incubation with test ligand for 30 min. [e] After treatment with **3**, morphine displaced partial agonism (maximum of 56.4 ± 3.9% at 3 × 10^{-5} M). [f] Compound displayed partial agonism, inhibiting contraction a maximum of 57 ± 8% at 1 × 10^{-7} M. [g] After treatment with **5**, morphine displayed partial agonism (maximum of 42 ± 4.8% at 1 × 10^{-5} M). [h] Value obtained following treatment described in footnote *c*, with subsequent displacement by naltrexone (200 nM, 5 min).

The μ receptor agonist morphine and the δ receptor agonist [D-Ala^2,D-Leu^5]enkephalin (DADLE) were em-

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ployed as standard agonists for assessing the irreversible antagonist activities of 2–6 on the MVD. Compound 5 was found to be the most potent irreversible morphine antagonist on the MVD. This contrasts with the results on the GPI, where 6 was the most potent irreversible morphine antagonist. Compounds 3 and 6 were somewhat more effective irreversible morphine antagonists than β-FNA, while 4 was equipotent with β-FNA. Surprisingly, the ester 2 most closely resembling β-FNA appeared to be the weakest irreversible morphine antagonist. As with the GPI, naltrexone could reverse some of the antagonist activity of 2–6 on the MVD that was not reversible by washing. With the possible exception of weak irreversible DADLE antagonism exhibited by 6, none of the compounds significantly antagonized DADLE irreversibly.

Reversible receptor binding at μ opioid receptors was evaluated from the displacement of [^{3}H]dihydromorphine. All of the esters except 6 were found to have affinity comparable to that of β-FNA. The phenethyl ester 6 possessed substantial affinity, differing from that of β-FNA by a factor of 69.

Discussion

Affinity labels may possess high receptor selectivity if their covalent binding entails a double recognition process. Enhanced selectivity can be obtained by incorporating electrophiles that preferentially react with particular types of receptor nucleophiles. For example, Michael acceptors react preferentially with sulfhydryl groups[7] that are strongly implicated as being constituents of opioid receptors.[8]

We chose to modify β-FNA by varying the ester substituents of its Michael acceptor group to explore the possibility that such modified ligands might selectively bind to subtypes of μ receptors presently undifferentiable with β-FNA. The additional selectivity could arise either from binding of the ester substituents to neighboring ancillary lipophilic areas or through steric constraints imposed by the receptor. In this regard, the existence of ancillary lipophilic areas has been suggested by studies of the 6,14-*endo*-ethenotetrahydrooripavines.[9]

The results of testing in both the GPI and MVD indicate that the longer aliphatic substituents confer greater agonist potency. These substituents could be enhancing receptor affinity through additional hydrophobic interaction. Since the agonist activity of β-FNA is mediated principally by κ receptors,[10] it is likely that the aliphatic esters 2–4 also are κ selective receptor agonists.

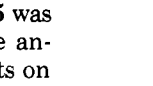
On both the GPI and MVD, the aralkyl esters 5 and 6 displayed substantially greater irreversible morphine antagonism than did their aliphatic counterparts 2–4. It is unlikely that differing intrinsic reactivities are responsible for this variation, since the Michael acceptor groups of all of the compounds probably have similar electrophilic properties. One possible explanation for this structure–activity profile is the existence of ancillary lipophilic binding subsites associated with the μ receptor system in these tissues. Such subsites might alter the reversible binding mode of the electrophilic moieties and thereby hinder covalent bonding of 2–4 by making the second recognition step less favorable. The observation that 2–4 reversibly bind to μ receptors with comparable affinity relative to β-FNA suggests that their decreased effectiveness in the irreversible blockage of morphine in the GPI is related to a deficient second recognition step and not to decreased affinity (first recognition step). On the other hand, binding of the aralkyl ester substituents of 5 and 6 to suitable subsites could serve to properly orient their Michael acceptor groups for reaction with a proximal nucleophile. However, this enhancement in the irreversible blockage of morphine may also be related to their greater affinity for μ receptors.

Another possibility is that β-FNA and the aralkyl esters (5, 6) bind preferentially to an allosteric, antagonist regulatory site that is coupled to an agonist site in the μ receptor system. Evidence for such a regulatory site has been reported.[11] This regulatory site may differ from the agonist site in that it has a sulfhydryl group readily available for reaction with an opioid affinity labeling group. It is apparent that in this case, just as in the previous case, two sequential recognition steps are required for irreversible blockage of the μ receptor system. The fact that the inhibition of [^{3}H]dihydromorphine binding by 1–6 does not parallel irreversible blockage of the agonist effect of morphine is consistent with this possibility.

Interestingly, in the GPI the phenethyl ester 6 was the most potent antagonist, while in the MVD the benzyl ester 5 was the most potent. This suggests a difference between the μ receptors of the two tissues and is consistent with the results of studies conducted with other affinity labeling opiates.[3,12,13]

As with β-FNA, none of the target compounds irreversibly antagonized to a significant degree the κ agonist nalorphine in the GPI nor the δ agonist DADLE in the MVD. Though studies with the nitrogen mustard ligand β-chlornaltrexamine indicate that nucleophiles exist on or near these receptors,[14,15] apparently the Michael acceptor

Table III. (*E*)-4-Chloro-4-oxy-2-butenoic Acid Esters



R	bp, °C	yield, %
C_2H_5	83–84 (10 mmHg)[a]	42[b]
$(CH_2)_2CH_3$	79–80 (6 mmHg)[c]	40[b]
$(CH_2)_3CH_3$	92–94 (3.5 mmHg)[d]	10
$CH_2C_6H_5$	128–130 (0.02 mmHg)	16
$(CH_2)_2C_6H_5$	122–124 (0.01 mmHg)[e]	40

[a] Bp 74–75 °C (10 mmHg) (Eisner, V.; Elvidge, J. A.; Linstead, R. P. *J. Chem. Soc.* **1951**, 1501). [b] By modified preparation procedure: In a one-pot reaction, maleic anhydride and an equimolar amount of the appropriate alcohol were brought to reflux temperature for several hours, after which an equivalent of thionyl chloride was added, followed by further refluxing. The product was purified from the resulting mixture by two successive fractional distillations. [c] Bp 96–98 (14 mmHg) (Pianka, M.) U.S. Patent 3 714 230, 1973). [d] Bp 105–107 (15 mmHg) (Carlsohn, H.; Hipler, U.-C.; Beuer, A.; Hartmann, M.; Schultz, H. *Z. Chem.* **1981**, *21*, 283). [e] Bp 178 (15 mmHg) (see footnote *d*).

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Table IV. (*E*)-4-[[(5α,6β)-17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan-6-yl]amino]-4-oxo-2-butenoic Acid Esters

no.	mp, °C	$[\alpha]_D$, deg (*c* 1.0, MeOH)	yield, %	formula[a]
2	123–125	−182[b]	21	$C_{26}H_{32}O_6N_2$
3	123–125	−171[c]	30	$C_{27}H_{34}O_6N_2$
4	117–119	−148[c]	42	$C_{28}H_{36}O_6N_2$
5	107–109	−148[b]	11	$C_{31}H_{34}O_6N_2$
6	90–92	−161[d]	45	$C_{32}H_{36}O_6N_2$

[a] All compounds werre within ±0.4% of theory for C, H, and N analyses. [b] 22 °C. [c] 21 °C. [d] 23 °C.

groups of **2–6** are not suitable for reaction with these nucleophiles.

Experimental Section

(*E*)-4-Chloro-4-oxo-2-butenoic Acid Esters. To a stirred slurry of maleic anhydride (10.0 g, 101.9 mmol) in the appropriate alcohol (101.9 mmol) at 0 °C was added triethylamine (15.6 mL, 112.1 mmol). After 1 h of stirring at 25 °C, the reaction mixture was dissolved in water (70 mL) and washed with CH_2Cl_2 (10 mL × 3). The aqueous solution was acidified with HCl and extracted with CH_2Cl_2 (30 mL × 3). The acidic organic layers were pooled, dried (Na_2SO_4), filtered, and evaporated to leave the free maleic acid monoester as an oil. To a solution of the maleic acid monoester in toluene (80 mL) was added thionyl chloride (13.4 g, 8.2 mL, 112.1 mmol) slowly with stirring at 25 °C. The mixture was stirred for 2 h at 100 °C. Toluene and unreacted thionyl chloride were removed in vacuo. The residue was subjected to two successive fractional vacuum distillations to yield the pure acid chloride as a clear liquid. Yields and physical constants for the acid chlorides **8–12** appear in Table III.

(*E*)-4-[[(5α,6β)-17-(Cyclopropylmethyl)-4,5-epoxy-3,14-dihydroxymorphinan-6-yl]amino]-4-oxo-2-butenoic Acid Esters (2–6). To a stirred solution of the dihydrochloride salt of 6β-naltrexamine (300 mg, 0.7 mmol), K_2CO_3 (0.45 g), water (7.5 mL), and THF (10 mL) was added a solution of the appropriate acid chloride (0.72 mmol) in THF (5 mL), dropwise over a 15-min period. To the reaction mixture was added water (15 mL), and the resulting solution was extracted with CH_2Cl_2 (30 mL × 3). The organic layers were pooled, dried (Na_2SO_4), filtered, and evaporated in vacuo to leave a residue. This was dissolved in the same alcohol as that comprising the ester substituent (20 mL) and triethylamine (1 mL). The solution was stirred at 25 °C for 24 h. The solvent then was removed in vacuo and the residue subjected to an acid–base extraction. The basic organic layers were pooled, and the solvent was removed in vacuo. The residue was purified by dry column chromatography ($EtOAc/NH_4OH$, 100:1) to yield the analytically pure ester. Yields and physical constants for the esters **2–6** appear in Table IV.

Binding Studies. Rats were sacrificed by decapitation, and the forebrain was rapidly removed and homogenized (Brinkman Polytron, setting 6; 30 s) in 20 vol (w/v) of ice-cold Tris·HCl buffer (pH 7.4; 0.05 M). The suspension was washed twice by centrifugation (Beckman J2-21M; 4 °C; 10 min) with intermittent resuspension in fresh buffer. Following a preincubation (10 min; 37 °C), the pellet was washed a final time as described above and resuspended (10 mg/mL) in fresh buffer for use in the assay. For assay, 1-mL portions of the tissue suspension were added in triplicate to tubes containing various concentrations of the drugs of interest, 1 nM [^{3}H]dihydromorphine (New England and Nuclear, Boston, MA; sp act. = 71 Ci/mmol), and 1 mL of assay buffer. Incubations were continued for 40 min at room temperature, and the reaction was terminated with use of vacuum filtration and a modified Brandell Cell Harvester (Brandell Instruments, Gaithersburg, MD). Filters (Whatman GF/B) were washed with 2 × 5 mL of ice-cold buffer. Radioactivity was determined by using conventional liquid scintillation counting. Naloxone (10^{-6} M) was used to determined nonspecific binding. Where shown, values are the mean ± SEM of three separate experiments using at least seven concentrations of drug.

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