Six Charles River rats were used for each determination.

Reverse Passive Arthus Reaction. This assay was performed according to the procedure of Chang and Otterness.[24] The animals used were injected intravenously through the tail vein with 1.0 mL of 0.9% saline containing 2.5 mg of Evans Blue and 5.0 mg of ovalbumin, followed immediately by intracutaneous injection of 0.03 mL of rabbit antiovalbumin antiserum diluted with sufficient 0.9% saline to contain 3.65 mg of antibody/mL. The compound to be tested (11, **22**, or **53**) (30 mg/kg of body weight) was administered orally in a 1:1 *N*-methyl-D-glucamine and water solution 1 h before the injection of the ovalbumin. The mean index of the reaction was calculated 3-h postinjection as the product of the diameter of the reaction site (indicated by the accumulation of Evans Blue) and the intensity score of the reaction (a subjective score of 1–4). Five Charles River rats were used for each determination.

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Registry No. 1, 100-47-0; **2**, 874-90-8; **3**, 2024-83-1; **4**, 1885-35-4; **5**, 18039-42-4; **6**, 6926-51-8; **7**, 10493-27-3; **8**, 91759-55-6; **9**, 51517-88-5; **10**, 91759-56-7; 11, 91759-57-8; **12**, 21054-65-9; **13**, 76765-82-7; 14, 91759-58-9; **15**, 91759-59-0; **16**, 21743-68-0; **17**, 91759-60-3; **18**, 91759-61-4; **19**, 91759-62-5; **20**, 91759-63-6; **21**, 91759-64-7; **22**, 91759-65-8; **23**, 65-85-0; **24**, 100-09-4; **25**, 93-07-2; **26**, 118-41-2; **27**, 93-89-0; **28**, 94-30-4; **29**, 3943-77-9; **30**, 6178-44-5; **31**, 613-94-5; **32**, 3290-99-1; **33**, 41764-74-3; **34**, 3291-03-0; **35**, 13153-01-0; **36**, 91759-66-9; **37**, 91759-67-0; **38**, 70452-38-9; **39**, 6596-82-3; **40**, 91759-68-1; 41, 91759-69-2; **42**, 70452-47-0; **43**, 91759-70-5; 44, 91759-71-6; **45**, 91759-72-7; **46**, 70452-56-1; **47**, 54559-45-4; 48, 18204-66-5; **49**, 91759-73-8; **50**, 18204-69-8; **51**, 91759-74-9; **52**, 91759-75-0; **53**, 91759-76-1; NaN_3, 26628-22-8; ethyl bromoacetate, 105-36-2; phenyl isothiocyanate, 103-72-0; superoxide, 11062-77-4.

Supplementary Material Available: Tables containing complete spectral and physical data for all compounds synthesized (19 pages). Ordering information is given on any current masthead page.

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Probes for Narcotic Receptor Mediated Phenomena. 7.[1] Synthesis and Pharmacological Properties of Irreversible Ligands Specific for μ or δ Opiate Receptors

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Syntheses of affinity reagents for opiate receptors based on the fentanyl, *endo*-ethenotetrahydrooripavine, and etonitazene carbon–nitrogen skeletons are described. The isothiocyanate, bromoacetamido, and methylfumaramido alkylating functions were employed in these compounds, some of which had previously been shown to be μ specific (**12**, BIT) and δ specific (**8**, FIT and **19**, FAO) in vitro. Antinociceptive activity of the title compounds was determined in the mouse hot-plate test, which revealed that certain compounds in each class showed morphine-like activity. The binding EC_{50} values against [^{3}H]Dalamid for opiate receptors in NG108-15 (δ receptors) and rat brain membranes (μ + δ receptors) are also reported. With this type of experiment, it was possible to independently measure the apparent affinity of the etonitazene congeners **12**–**14** for the μ and δ receptors.

The concept of multiplicity of opiate receptors, originally proposed by Martin[2] to account for differing pharmacological effects of several opiates in the spinal dog, has received much support from binding and other pharmacological studies.[3-8] The three major types of opioid receptors in the CNS are commonly referred to as μ, δ, κ.[9] Some opioids display considerable selectivity in their interaction with these receptor types. The endogenous opioid peptides enkephalin and dynorphin are relatively selective ligands for δ and κ receptors, respectively.[9,10] Important questions raised by these observations are whether the receptors are structurally related, and what are the physiological roles of these receptors. Answers to these questions can come from experiments performed with opioids which bind irreversibly to one or another of the receptor types to the exclusion of the others. Many attempts at the preparation of affinity reagents for opioid receptors have been made;[11-22] some of those based on the 3,14-dihydroxy-4,5-epoxymorphinan system are useful probes for the μ receptor.[16,17]

With the goal of obtaining opioids specific for opiate receptor subpopulations, we have prepared three classes

† Deceased, June 7, 1983.

‡ National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases.

§ National Institute of Mental Health.

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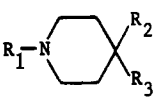
of alkylating opioids closely related to fentanyl, etonitazene, and etorphine which are known to be extremely potent in vivo and have high affinity for opioid receptors in vitro. Several of these derivatives have been found to exhibit the desired selectively for irreversible binding to μ or δ receptor types, and the preparation of one of the δ-specific ligands with high specific activity ^3H incorporation has previously permitted our identification of a M_r 58000 subunit of the δ receptor.[23] The δ and μ receptor selective agents described here are, we believe, uniquely useful agents for studying questions related to the structure and function of opioid receptor subtypes, as demonstrated in our preliminary communications.[23,24]

Chemistry. Our synthetic strategy for preparation of each of the three classes of alkylating opioid involved incorporation of a strategically located amino substituent and subsequent conversion to an alkylating function. The alkylating functions employed were methylfumaramido, previously utilized by Portoghese and his collaborators for synthesis of the μ-specific β-FNA,[16] and the bromoacetyl and isothiocyanate functions. The latter two functions were selected since we have previously found that the isothiocyanate function served admirably as the acylating group in the first specific irreversible inhibitor of the benzodiazepine receptor,[25] and in a subsequent study with the corresponding bromoacetyl derivative,[26] we were able to detect heterogeneity of benzodiazepine receptors by virtue of different reactivities of the two alkylating drugs. In the latter study,[26] the bromoacetyl and isothiocyanate functions were found to be particularly suitable candidates for the alkylating moieties since neither reacts to any significant extent with hydroxyl groups as shown by their high stability in 70% ethanol at 25 °C. This property thus confers stability in the aqueous media used for receptor binding studies provided no reactive primary or secondary amine-based buffers are used.

In addition, these two functions have distinctly different reactivities toward amino and sulfhydryl functions in this medium.[26] The isothiocyanate function reacted completely with a moderate excess of diethylamine or 1-propanethiol at 25 °C within 5 min. Under the same conditions, the bromoacetyl function required ~17 h for complete reaction with diethylamine and little, if any, reaction with 1-propanethiol occurred during the same period. Similar reactivity (or lack of) for hydroxyl, amino, and sulfhydryl groups present in the receptor matrix may also be obtained for the bromoacetyl and isothiocyanate functions in vitro or in vivo although changes in the order of reactivity cannot be definitely ruled out since enzymatic-like rate enhancements probably occur.

Synthesis of the alkylating fentanyl derivatives was straightforward using a modification of the previously reported route[27] to fentanyl and congeners. Reaction of commercially available 4-piperidone hydrochloride with (4-nitrophenyl)acetyl chloride afforded amide **2** in which the protecting (4-nitrophenyl)acetyl function served as precursor for the (4-nitrophenyl)ethyl group (see below). Treatment of **2** with aniline under dehydrating conditions gave Schiff base **3** which was reduced directly with sodium borohydride to **4**. Borane reduction of the amide function in **4** then gave diamine **5**. Acylation of **5** with propionic anhydride afforded the known[28] nitrofentanyl **6**. Catalytic reduction of **6** over Pd/C provided the key aminofentanyl **7**,[28] which was converted to the corresponding isothiocyanate (**8**, fentanyl isothiocyanate, FIT) or bromoacetamido derivative **9** by reaction with a slight excess of thiophosgene or bromoacetic anhydride, respectively, in a two-phase chloroform–aqueous sodium bicarbonate system. For preparation of isothiocyanate **8**, and in general, the thiophosgene should be redistilled in order to remove the dimer[29] which accumulates on standing and complicates purification of the isothiocyanate.



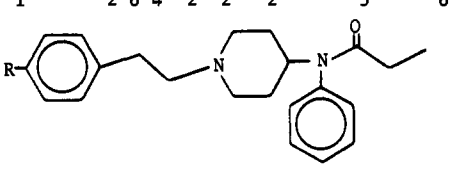
1: R_1 = H, R_2, R_3 = O

2: R_1 = 4-$NO_2C_6H_4CH_2CO$, R_2, R_3 = O

3: R_1 = 4-$NO_2C_6H_4CH_2CO$, R_2, R_3 = NC_6H_5

4: R_1 = 4-$NO_2C_6H_4CH_2CO$, R_2 = H, R_3 = NHC_6H_5

5: R_1 = 4-$NO_2C_6H_4CH_2CH_2$, R_2 = H, R_3 = NHC_6H_5



6: R = NO_2

7: R = NH_2

8: R = NCS

9: R = $NHCOCH_2Br$

In the benzimidazole series, etonitazene methylfumaroyl was hydrogenated as the methanesulfonate salt over Pd/C to afford the amino derivative **11**[30] isolated as the same salt. This salt was converted to the isothiocyanate and bromoacetamido derivatives **12** (benzimidazole isothiocyanate, BIT) and **13**, respectively, essentially as described for **8** and **9**. Reaction of aminobenzimidazole **11** with methylfumaroyl chloride[31] in the chloroform–aqueous so-

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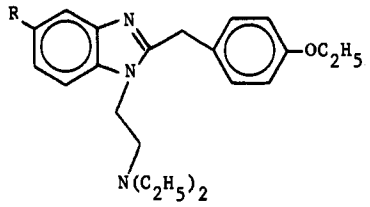
dium bicarbonate system afforded the corresponding methylfumaramido **14**.



10: R = NO_2

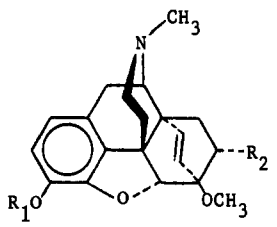
11: R = NH_2

12: R = NCS

13: R = $NHCOCH_2Br$

14: R = $NHCOCH{=}^{t}CHCO_2CH_3$

Synthesis of the 7-amino-6,14-*endo*-ethenomorphinan **15** was accomplished in three steps from thebaine as previously described.[32] Selective *O*-demethylation of the phenolic ether function of **15** proceeded smoothly with boron tribromide as in the conversion of codeine to morphine[33] to afford **16**, which was remethylated to **15** with diazomethane in order to insure that no carbon–nitrogen skeleton rearrangement had occurred during *O*-demethylation. The conversion of **16** to the isothiocyanate, bromoacetamido, and methylfumaramido derivatives **17**, **18**, and **19** (fumaramidooripavine, FAO), respectively, was then easily accomplished as described above in the fentanyl and benzimidazole series.



15: $R_1 = CH_3$, $R_2 = NH_2$

16: $R_1 = H$, $R_2 = NH_2$

17: $R_1 = H$, $R_2 = NCS$

18: $R_1 = H$, $R_2 = NHCOCH_2Br$

19: $R_1 = H$, $R_2 = NHCOCH{=}^{t}CHCO_2CH_3$

Biological Results. We have previously presented evidence that the alkylating compounds prepared in this study react irreversibly with opioid receptors[23,24] and that BIT shows specificity for μ receptors and FIT and FAO are specific for δ receptors.[24] Direct evidence for covalent binding of [^{3}H]FIT to receptors from neuroblastoma × glioma hybrid NG 108-15 cells was obtained by showing that the complex formed between [^{3}H]FIT and a protein migrating upon electrophoresis in $NaDodSO_4$ gels with a mobility corresponding to a molecular weight of 58 000 is stable to denaturation by $NaDodSO_4$ and to boiling in SDS, urea, dithiothreitol mixtures.[23] Formation of this complex could be blocked by active but not inactive enantiomers of opiates and is, therefore, receptor linked. Secondly, we have shown that incubation of membranes isolated from neuroblastoma × glioma hybrid NG108-15 cells with the alkylating ligands followed by thorough washing results in the loss of receptor binding sites without change in the affinity of any remaining opioid binding sites.[24] A similar decrease in μ or δ receptor number also occurs in membranes from rat brain after removal of the free alkylating ligand by extensive washing.[24] Thus, even through no free alkylating ligand remains after washing, receptor numbers are diminished. These results strongly suggest that covalent bonds have indeed been formed between the receptors and the ligands.

A summary of some of the biological activities of the compounds prepared in this study is presented in Table I. Interestingly, compounds in each of the series have morphine-like antinociceptive potencies even though some, like BIT, are μ selective whereas others like FIT and FAO are δ selective in vitro.[24] Of course, we cannot say, from these experiments, whether the compounds reach the brain as such or as metabolites of unknown selectively. In binding studies against [^{3}H]Dalamid which binds equally well to μ and δ receptors, the fentanyl and oripavine derivatives bind to membranes of NG108-15 cells with good affinity, whereas the etonitazene derivatives do not. The observations are consistent with our previous finding[24] that FIT and FAO and their congeners are δ selective and BIT is μ selective because NG108-15 membranes have only δ receptors.[34]

With brain membranes it is possible to measure etonitazene congener binding to μ and δ receptors independently against [^{3}H]Dalamid since affinities for these differ by 2 orders of magnitude (or more in the case of the parent compound).[24] Thus, BIT and its relatives bind much more avidly to μ than to δ receptors, as expected. It is not possible with this kind of experiment to separate μ and δ binding with the other compounds, but our earlier observations showed that FIT and FAO are strongly δ selective.[24] The binding EC_{50} values measured here with brain membranes for FIT and FAO and their congeners included variable contributions of binding to μ and δ sites.

Experimental Section

Melting points were determined on a Fisher-Johns apparatus and are corrected. NMR spectra were recorded with a Varian 220-MHz spectrometer. Electron-ionization mass spectra (EIMS) were obtained with a Hitachi Perkin-Elmer RMU-6E spectrometer (70 eV). Chemical-ionization mass spectra (CIMS) were obtained with a Finnigan 1015D spectrometer with a Model 6000 data collection system and high-resolution mass spectra were obtained with a V.G. Micromass 7070F spectrometer. Column chromatography was performed with 230–400- mesh EM silica gel. Mass spectra and elemental analysis were obtained from the Section on Analytical Services and Instrumentation, NIADDK. All compounds gave NMR and IR spectra consistent with their structure.

1-[(4-Nitrophenyl)acetyl]-4-piperidone (2). Powdered piperidin-4-one hydrate hydrochloride (1; 19.18 g, 124 mmol) was added to a mechanically stirred biphasic mixture of (4-nitrophenyl)acetyl chloride (24.78 g, 124 mmol) in CH_2Cl_2 (300 mL) and $NaHCO_3$ (31.88 g, 374 mmol) in H_2O (300 mL). After 1 h the CH_2Cl_2 layer was collected, combined with a CH_2Cl_2 (100 mL) extract of the aqueous phase, washed with 2 N HCl (100 mL), dried ($MgSO_4$), and evaporated to an oil which crystallized. The crystals were washed with ether (200 mL) to yield pure **2** as a yellow crystalline solid (29.0 g, 89%): mp 102.9–104.3 °C; EIMS,

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Table I. Properties of Irreversible Opiate Ligands[a]

compd	antinociceptive act.:[b] ED_{50}, μmol/kg, sc	binding EC_{50},[d] nM		
		NG108-15	rat brain μ	rat brain δ
8 (FIT)	5.0 (3.6-7.4)[e]	8	300	
9·HCl	>20	200	500	
12 (BIT)	2.7 (2.1-3.9)[c]	280	10[e]	800[e]
13	>102[c]	200	40	1500
14	13.6 (9.6-17.6)[c]	240	10	800
17·HCl	3.6 (2.6-5.0)	20	50	
18	4.7 (3.4-6.5)[c]	50	70	
19 (FAO)	3.5 (2.2-5.8)[c]	50	50	
(−)-morphine sulfate	2.9 (2.5-3.3)			

[a] See biological methods in the Experimental Section. [b] 95% confidence limits shown in parentheses, as obtained through computerized probit analysis. [c] Dissolved by addition of 1.0 equiv of aqueous HCl and further diluted. [d] The EC_{50} values are based on two or more determinations and are considered reliable within a factor of 2. [e] Because etonitazene and its congeners bond to μ sites much more tightly than to δ sites, displacement of the nonselective ligand [^{3}H]-D-Ala^2-Met^5-enkephalinamide by these compounds is biphasic and can easily be separated into two classes.[24] This is not the case with the other ligands tested, which are primarily directed at δ sites as shown by our earlier studies.[24]

m/e 262 (M^+). Anal. ($C_{13}H_{14}N_2O_4$) C, H, N.

1-[(4-Nitrophenyl)acetyl]-4-(phenylamino)piperidine (4). A mixture of **2** (29.0 g, 111 mmol), aniline (11.1 mL, 122 mmol), toluene (450 mL), and a few crystals of *p*-TsOH was refluxed with a Dean–Stark trap for 5 h. The mixture was cooled, decanted from a residual tar, and evaporated to give crude **3** as a red oil. The oil was taken up in MeOH (200 mL) and stirred for 45 min with $NaBH_4$ (7.07 g, 186 mmol). The mixture was then diluted with H_2O (300 mL), extracted with CH_2Cl_2 (2 × 300 mL), dried ($MgSO_4$), and evaporated to a red oil. Trituration with ether (200 mL) gave product **3** as a yellow solid, which was collected, dissolved in MeOH (500 mL), concentrated to 200 mL, and diluted with ether (300 mL). Cooling (−70 °C) yielded pure **4** as yellow crystals: 12.6 g (35% yield); mp 159–160 °C; CIMS (NH_3), *m/e* 340 ($M + 1)^+$. Anal. ($C_{19}H_{21}N_3O_3$) C, H, N.

1-[2-(4-Nitrophenyl)ethyl]-4-(phenylamino)piperidine (5). A solution of BH_3 (100 mmol) in THF (100 mL) was added slowly to a stirred solution of **4** (11.15 g, 34.3 mmol) in THF (100 mL). The solution was stirred at reflux for 2 h, then diluted with MeOH (20 mL), and stirred an additional 30 min. Evaporation of solvent gave an orange solid, which was heated at reflux for 1 h with a mixture of MeOH (100 mL) and 2 N HCl (100 mL). The solution was made alkaline by addition of concentrated aqueous NaOH, extracted with CH_2Cl_2 (2 × 100 mL), washed with brine (100 mL), dried ($MgSO_4$), and evaporated to an oil. Crystallization (−70 °C; ether–ligroin, 1:1) gave **5** as yellow crystals (4.4 g). Purification of the filtrate by silica gel flash chromatography (3% MeOH in CH_2Cl_2) gave additional **5** (1.8 g; 6.2 g total yield, 56%): mp 90–92 °C; CIMS (NH_3), *m/e* 326 ($M + 1)^+$. Anal. ($C_{19}H_{23}N_3O_2$) C, H, N.

***N*-[1-[2-(4-Nitrophenyl)ethyl]-4-piperidinyl]-*N*-phenylpropanamide (6).** A solution of **5** (5.09 g, 15.7 mmol) and propionic anhydride (4.2 g, 32 mmol) in toluene (30 mL) was refluxed for 3 h, diluted with 30% aqueous NH_4OH (2 mL), stirred at 20 °C for 5 min, then partitioned between saturated aqueous $NaHCO_3$ (100 mL) and CH_2Cl_2 (2 × 100 mL), dried ($MgSO_4$), and evaporated to an orange oil. Crystallization (ether–ligroin) gave **6** as light yellow crystals (4.73 g, 79%), mp 114–116 °C. A sample was recrystallized for analysis: mp 117–119 °C (lit.[28] mp 114–119 °C); EIMS, *m/e* 381 (M^+). Anal. ($C_{22}H_{27}N_3O_3$) C, H, N.

***N*-[1-[2-(4-Aminophenyl)ethyl]-4-piperidinyl]-*N*-phenylpropanamide Hydrochloride (7·HCl).** A solution of **6** (4.5 g, 10.6 mmol) in EtOH (200 mL) was hydrogenated at 40 psi of H_2 over 5% Pd/C (400 mg) for 1.5 h. Filtration through Celite and evaporation of solvent gave a white solid, which was crystallized from ether (−70 °C) to yield **7** (3.30 g) as white crystals, mp 150–153 °C (lit.[28] mp 150–151 °C). Conversion to the hydrochloride salt and crystallization from ether–MeOH yielded **7**·HCl (3.4 g, 93%): mp 203–212 °C; high-resolution MS ($C_{22}H_{29}N_3O$) calcd 351.2310, found 351.2303.

***N*-[1-[2-(4-Isothiocyanatophenyl)ethyl]-4-piperidinyl]-*N*-phenylpropanamide (FIT, 8).** A biphasic mixture of **7**·HCl (500 mg, 1.18 mmol) and $NaHCO_3$ (693 mg, 8.25 mmol) in H_2O (10 mL) and $CHCl_3$ (30 mL) was stirred with ice-bath cooling while redistilled thiophosgene (108 μL, 1.41 mmol) was added. After 15 min the $CHCl_3$ layer was removed, combined with a $CHCl_3$ extract (15 mL) of the aqueous layer, dried ($MgSO_4$), and evaporated to a yellow oil. The oil was dissolved in ether (3 mL), diluted with ligroin (20 mL), and filtered to remove an insoluble residue (18 mg). The filtrate was cooled, yielding **8** as a pure crystalline solid (234 mg, 50%): mp 88–91 °C; EIMS, *m/e* 393 (M^+). Anal. ($C_{23}H_{27}N_3SO$) C, H, N.

***N*-[1-[2-[4-(Bromoacetamido)phenyl]ethyl]-4-piperidinyl]-*N*-phenylpropanamide Hydrochloride (9·HCl).** A biphasic mixture of **7**·HCl (424 mg, 1.0 mmol) and $NaHCO_3$ (588 mg, 7.0 mmol) in $CHCl_3$ (30 mL) and H_2O (10 mL) was stirred with ice-bath cooling while bromoacetic anhydride (345 mg, 1.33 mmol) in $CHCl_3$ (1 mL) was added. After 20 min the reaction was worked up as above to yield a foam which solidified upon trituration with ether (−70 °C). The solid was dissolved in MeOH (5 mL) and acidified with ethereal HCl (10 mL), yielding crude **9**·HCl as a solid. Recrystallization from MeOH–ether gave **9**·HCl as pure white crystals (135 mg, 26%): mp 221–224 °C; CIMS (CH_4) *m/e* 472, 474 ($M + 1)^+$. Anal. ($C_{24}H_{30}BrN_3O_2$·HCl) C, H, N.

5-Amino-1-[2-(diethylamino)ethyl]-2-(4-ethoxybenzyl)-benzimidazole (11). A solution of 1-[2-(diethylamino)ethyl]-2-(4-ethoxybenzyl)-5-nitrobenzimidazole methanesulfonate (**10**·CH_3SO_3H, etonitazene methanesulfonate)[30] (5.00 g, 10.2 mmol) in H_2O (50 mL) was diluted with EtOH (80 mL) and hydrogenated for 2 h at 45 psi of H_2 over 10% Pd/C (500 mg). Filtration through Celite and evaporation of solvent gave a syrup which was crystallized from 2-propanol (30 mL)–ether (130 mL) to yield **11**·CH_3SO_3H as white crystals (4.0 g, 83%): mp 203–206 °C dec; high-resolution MS ($C_{22}H_{30}N_4O$) calcd 366.2419, found 366.2419. This compound was previously reported as the base·H_2O.[30]

1-[2-(Diethylamino)ethyl]-2-(4-ethoxybenzyl)-5-isothiocyanatobenzimidazole (BIT, 12). To a biphasic mixture of **11**·CH_3SO_3H (2.0 g, 4.33 mmol) and $NaHCO_3$ (2.96 g, 35.4 mmol) in H_2O (20 mL) and $CHCl_3$ (50 mL) was added redistilled thiophosgene (512 μL, 6.7 mmol). After 20 min the $CHCl_3$ layer was collected, combined with a $CHCl_3$ extract (50 mL), dried ($MgSO_4$), and evaporated. Crystallization of the residue from ether (−70 °C) gave **12** as analytically pure crystals (530 mg, 30%): mp 67–68 °C; EIMS, *m/e* 408 (M^+). Anal. ($C_{23}H_{28}N_4OS$) C, H, N.

5-(Bromoacetamido)-1-[2-(diethylamino)ethyl]-2-(4-ethoxybenzyl)benzimidazole (13). To a biphasic mixture of **11**·CH_3SO_3H (924 mg, 2.0 mmol) and $NaHCO_3$ (1.17 g, 13.9 mmol) in H_2O (20 mL) and (50 mL) was added with ice-bath cooling a solution of bromoacetic anhydride (728 mg, 2.8 mmol) in $CHCl_3$ (2 mL). After 45 min the $CHCl_3$ layer was removed, combined with a $CHCl_3$ extract of the aqueous phase (25 mL), dried ($MgSO_4$), and evaporated, yielding a foam. Trituration with ether gave **13** as a white solid (935 g, 80%): mp 96 °C, resolidifying, then decomposing above 220 °C. Anal. ($C_{24}H_{31}BrN_4O_2$) C, H, N. This compound forms dimeric and trimeric quaternary salts after standing in solution or prolonged storage as crystalline material.

1-[2-(Diethylamino)ethyl]-2-(4-ethoxybenzyl)-5-(methylfumaramido)benzimidazole (14). A solution of 11·CH_3SO_3H (1.38 g, 3 mmol) and $NaHCO_3$ (1.76 g, 21 mmol) in H_2O (20 mL) was stirred on an ice bath with $CHCl_3$ (30 mL) and methylfumaroyl chloride[31] (660 mg, 4.5 mmol) in $CHCl_3$ (5 mL) was added over 10 min. After 20 min the $CHCl_3$ layer was removed, combined with a $CHCl_3$ (40 mL) extract of the aqueous layer, washed with saturated aqueous $NaHCO_3$ (40 mL) and brine (30 mL), decolorized with charcoal, dried ($MgSO_4$), and evaporated to a brown foam. The foam was mixed with ether, decanted from an insoluble residue, and allowed to stand at 20 °C, yielding 14 as white crystals (340 mg, 25%): mp 143–146 °C; high-resolution MS ($C_{27}H_{34}N_4O_4$) calcd 478.2580, found 478.2550. Anal. ($C_{27}H_{34}N_4O_4$) C, H, N.

7α-Amino-6,14-*endo*-ethenotetrahydrooripavine (16). A solution of 7α-amino-6,14-*endo*-ethenotetrahydrothebaine (15[32]) (4.95 g, 14 mmol) was added during 2 min to a well-stirred solution of BBr_3 (21.25 g, 84.8 mmol) in $CHCl_3$ (80 mL) maintained at 23–24 °C. After 15 min the reaction mixture was poured into a well-stirred mixture of 30% aqueous NH_4OH (50 mL) and ice (200 g). The $CHCl_3$ layer was removed, reduced in volume to 20 mL, and shaken with the aqueous phase. The resulting crystalline material was filtered and washed with H_2O. The crude product was dissolved in a minimum volume of MeOH, filtered, and evaporated to an oil which was crystallized from cold acetone to yield 16 as colorless prisms (3.24 g, 68%): mp 139–140 °C; CIMS (NH_3), m/e 341 $(M + 1)^+$. The dioxalate was crystallized from a MeOH–acetone mixture for analysis: mp 246–248 °C dec. Anal. ($C_{20}H_{24}N_2O_3 \cdot 2(CO_2H)_2$) C, H, N. The hydrochloride salt was crystallized from EtOH as colorless prisms; mp >300 °C dec.

7α-Isothiocyanato-6,14-*endo*-ethenotetrahydrooripavine Hydrochloride (17·HCl). A solution of 16·HCl (413 mg, 1 mmol) in H_2O (15 mL), $CHCl_3$ (30 mL), and $NaHCO_3$ (504 mg, 6 mmol) was stirred for 5 min and then treated with redistilled thiophosgene (126 mg, 1.1 mmol). After 15 min the $CHCl_3$ layer was removed, washed successively with dilute aqueous NH_4OH (2 × 25 mL) and H_2O (2 × 25 mL), dried (Na_2SO_4), treated with methanolic HCl, and evaporated. Crystallization from MeOH–acetone gave 17·HCl as colorless prisms (265 mg, 63%); mp >300 °C dec; CIMS (NH_3), m/e 383 $(M + 1)^+$. Anal. ($C_{21}H_{22}N_2O_3 \cdot HCl \cdot 2H_2O$) C, H, N.

7α-(Bromoacetamido)-6,14-*endo*-ethenotetrahydrooripavine (18). In a manner similar to above, 16·HCl (413 mg, 1 mmol) was treated with bromoacetic anhydride (286 mg, 1.1 mmol) in a mixture of H_2O (15 mL), $CHCl_3$ (30 mL), and $NaHCO_3$ (504 mg, 6 mmol). The crude product was purified by flash chromatography and crystallized from ether to afford 18 as colorless prisms (280 mg, 61%): mp 297–301 °C dec; CIMS (NH_3), m/e 461, 463 $(M + 1)^+$. Anal. ($C_{22}H_{25}N_2O_4Br \cdot {}^1/_2H_2O$) C, H, N.

7α-(Methylfumaramido)-6,14-*endo*-ethenotetrahydrooripavine (FAO, 19). In a manner similar to above, reaction of 16·HCl (413 mg, 1 mmol), $NaHCO_3$ (504 mg, 6 mmol), and methylfumaroyl chloride[31] (148 mg, 1 mmol) in H_2O (15 mL) with $CHCl_3$ (30 mL) afforded a crude product (494 mg), which was purified by silica gel flash chromatography to afford pure material. Crystallization from CH_2Cl_2–ligroin gave 19 as colorless flakes (375 mg, 82%); mp 165–166 °C; CIMS (NH_3), m/e 453 $(M + 1)^+$. Anal. ($C_{25}H_{28}N_2O_6 \cdot {}^1/_2H_2O$) C, H, N.

Biological Methods. Antinociceptive activities of the compounds were measured after subcutaneous injection in the mouse hot-plate assay as previously described.[34-36]

Opiate binding was measured to membranes of neuroblastoma × glioma hybrid cell NG108-15 membranes[37] which contain only δ receptors[38] and to rat brain membranes[39] which contain both μ and δ receptors. In all experiments the radiolabeled ligand used was [^{3}H]-D-Ala^2-Met^5-enkephalinamide (Dalamid), a ligand with equal affinities for μ and δ sites.[6] Membranes were incubated with 2 nM [^{3}H]Dalamid in 10 mM Tris·Cl, pH 8, at 37 °C in the presence of competing ligand at concentrations between 10^{-10} and 10^{-5} M. At the end of the incubation, the samples were rapidly cooled to 4 °C in ice and membrane-bound ligand was separated from free with the aid of prepacked, 9-mL columns of Sephadex G-25 as described previously. The membranes with bound ligand emerge in the first 4.5 mL eluted from these columns and are collected directly into scintillation vials and assayed for radioactivity after addition of 12 mL of Aquasol. Etonitazene and its derivatives displace Dalamid from brain membranes in a biphasic manner, and the curves are easily dissected into μ and δ components as shown earlier[24] and as indicated here. Other evidence for type specificity of these compounds was presented earlier.[24]

Registry No. 1, 41979-39-9; 2, 91742-66-4; 3, 91742-67-5; 4, 91742-68-6; 5, 91742-69-7; 6, 1640-11-5; 7, 1169-73-9; 7·HCl, 91742-70-0; 8, 85951-63-9; 9, 91742-72-2; 9·HCl, 91742-71-1; 10·CH_3SO_3H, 58-75-3; 11, 91742-73-3; 12, 85951-65-1; 13, 91742-80-2; 14, 91742-81-3; 15, 24501-03-9; 16, 87453-63-2; 16·2$(CO_2H)_2$, 91742-74-4; 16·HCl, 91742-75-5; 17, 91742-77-7; 17·HCl, 91742-76-6; 18, 91742-78-8; 19, 91742-79-9; (4-nitrophenyl)acetyl chloride, 50434-36-1; aniline, 62-53-3; propionic anhydride, 123-62-6; thiophosgene, 463-71-8; bromoacetic anhydride, 13094-51-4; methylfumaroyl chloride, 17081-97-9.

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