New 4-(heteroanilido)piperidines, structurally related to the pure opioidagonist fentanyl, with agonist and/or antagonist properties

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The final pellets were frozen at -20 °C and thawed the next day for [^{3}H]QNB binding experiments as described below. Other experiments in which receptor alkylation by **2A** and **4A** was studied at 0 °C were performed similarly except that the homogenates were kept on ice.

Muscarinic Receptor Binding Assays. All binding assays were run in triplicate. Nonspecific binding was estimated by carrying out incubations in the presence of 10 μM atropine. The pellets from the homogenates treated with **2**, **3**, and **4** were thawed and resuspended to a concentration of 10 mg of original wet tissue weight/mL of phosphate buffer. The binding of the specific muscarinic antagonist (-)-[^{3}H]QNB (34.7 Ci/mmol, New England Nuclear) was measured by the rapid filtration method of Yamamura and Snyder[24] with minor modifications. Homogenates (0.1 mL) were incubated with (-)-[^{3}H]QNB (0.4 nM) in a final volume of 2 mL of 50 mM phosphate buffer (pH 7.4). Incubations lasted 1 h at 37 °C. Tissue-bound (-)-[^{3}H]QNB was trapped by vacuum filtration of the incubation mixture over Whatman glass fiber filters (GF/B).

The binding of the specific muscarinic antagonist (-)-[^{3}H]NMS (87 Ci/mmol, New England Nuclear) was measured similarly. Measurements of the competitive inhibition of (-)-[^{3}H]NMS binding by **2A** and **4A** were carried out at 0 °C. Incubations lasted 1 h. Binding parameters were determined by unweighted nonlinear regression analysis. A two-site equation was fitted to the **2A** and **4A**/(-)-[^{3}H]NMS competition data to provide estimates of the apparent dissociation constants at the high (K_H') and low (K_L') affinity binding sites.[20] The apparent dissociation constants were corrected for receptor occupancy by (-)-[^{3}H]NMS according to the relationship[25] $K = K'/(1 + y/K_{NMS})$ were K is the true dissociation constant (K_H or K_L), y is the concentration of (-)-[^{3}H]NMS (0.3 nM), and K_{NMS} is the dissociation constant of (-)-[^{3}H]NMS (0.055 nM). The latter was determined independently by nonlinear regression analysis of seven-point (-)-[^{3}H]NMS binding isotherms.

Analysis of Receptor Inactivation Data. Alkylation of muscarinic receptors by **2**, **3**, and **4** was analyzed in terms of the model used earlier to describe the interaction of **1** with the receptor.[14] It was assumed that the reversible interaction of the aziridinium ion with the receptor was sufficiently fast to allow it to be essentially in equilibrium with the receptor and that the rate of the covalent reaction, described by the rate constant k_3, was proportional to the amount of reversibly bound ligand. The resulting equation, integrated over the time interval beginning at $t = 0$, is

$$Y = \left(\frac{A_0^{-k_0 t} + K_A}{A_0 + K_A} \right)^{k_3/k_0} \quad (1)$$

where Y is the proportion of receptors remaining unalkylated, t is time, A_0 is the concentration of the aziridinium ion at time zero, K_A is the apparent dissociation constant of the reversible drug-receptor complex, and k_0 is the rate constant for the exponential decay of the aziridinium ion. Values for k_0 were obtained from Table I ($k_0 \sim k_1$ for **1** and **3** and $k_0 = k_2$ for **2** and **4**). A_0 was calculated from the initial concentration of the parent haloethylamine and the peak level of the aziridinium ion (Table I). This equation (eq 1) was fitted to data from experiments in which residual (-)-[^{3}H]QNB binding was measured after 30 min of exposure to various concentrations of precyclized **2**, **3**, and **4** by unweighted nonlinear regression.

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New 4-(Heteroanilido)piperidines, Structurally Related to the Pure Opioid Agonist Fentanyl, with Agonist and/or Antagonist Properties

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A research program based on certain heterocyclic modifications (**12**-**50**) of the fentanyl (**1**) molecule has generated a novel class of opioids. In the mouse hot-plate test, these compounds were weaker analgesics than **1**. Two types of antagonists were observed in morphine-treated rabbits: those (e.g., **28**) that reversed both respiratory depression and analgesia and those (e.g. **32**) that selectively reversed respiratory depression. Evaluation of in vitro binding affinities to rat brain opioid receptors was inconclusive for a common locus of action for the agonist as well as the antagonist component. Further pharmacological evaluation of **32**, *N*-(2-pyrazinyl)-*N*-(1-phenethyl-4-piperidinyl)-2-furamide, in the rat showed it to be a potent analgesic (ED_{50} = 0.07 mg/kg, tail-flick test) with little cardiovascular and respiratory depression when compared to fentanyl.

Intravenous infusion of opioids with a skeletal muscle relaxant and an inhalation anesthetic is a widely accepted practice in surgical procedures. Among many anesthesiologists, fentanyl (**1**), the prototype of the 4-anilidopiperidine class of analgesics, has become the first-choice opioid for this regimen.[1] In recent years a number of new 4-anilidopiperidines exhibiting an array of analgesic profiles have been introduced,[2] and among these, the development of alfentanil (**2**) warrants special consideration.[2,3] While less potent than **1**, **2** has a more rapid onset and shorter duration of action. In addition, the respiratory depressant effect of **2** is less severe than that of **1**.[4,5] With the growing volume of outpatient surgical procedures, such

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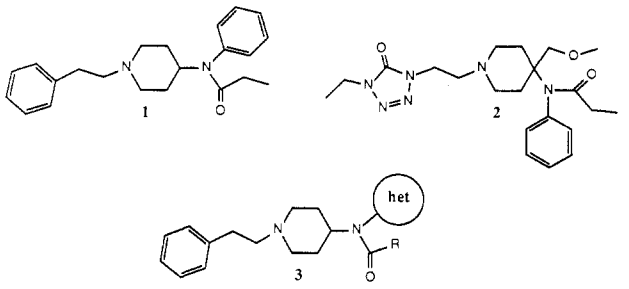
analgesics are much in demand.

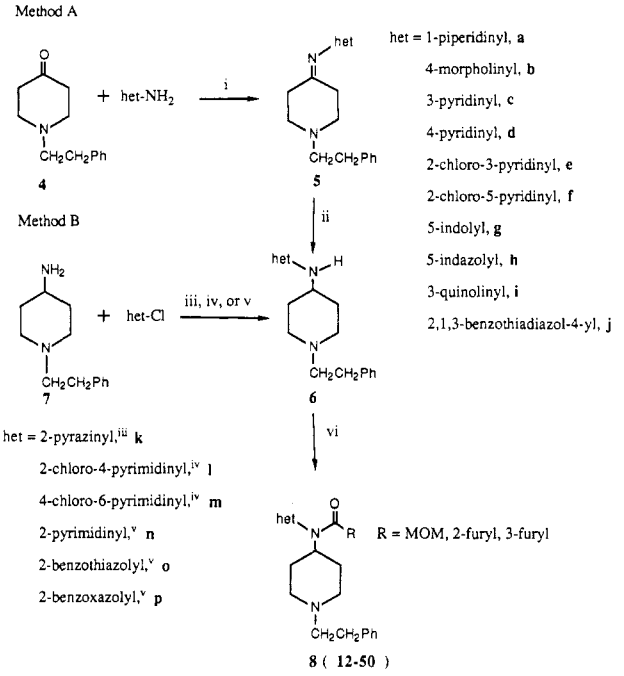


We initiated a research program to find new analgesics (**3**) based on a more structurally restricted approach than in the development of **2** by concentrating on two modifications of the propionanilido group of **1**. The first is to replace the benzene ring with various heterocycles, an approach that has been successful in the retention of analgesic activity though limitedly employed.[6,7] The second is to replace the propionyl group with methoxyacetyl, a substitution known to confer short duration of action in 4-anilidopiperidine analgesics[8] and to utilize the 2- and 3-furoyl groups as rigid alkoxyalkyl chain moieties. It was decided to screen the 4-(heteroanilido)piperidines for morphine antagonism also. Opioid receptor binding affinities were determined for many of the compounds. These studies and secondary pharmacological studies of selected compounds will be discussed herein.

Chemistry. The intermediate 4-(heteroanilino)-piperidines (**6**) were prepared by two major methods as depicted in Scheme I. *p*-Toluenesulfonic acid catalyzed condensation of 1-phenethyl-4-piperidone (**4**) with heteroanilines of sufficient nucleophilicity provided Schiff bases **5**, which were reduced to **6a–j** with sodium borohydride. Alternatively, where the point of attachment involved an electropositive carbon, intermediates **6k–p** were prepared by coupling of 1-phenethyl-4-aminopiperidine (**7**) with the appropriate heteroaryl chloride under conditions dictated by the particular ring system. A variation of this route was offered wherein the C–Cl bond was activated by an adjacent bipolar *N*-oxide bond with deoxygenation affected later with phosphorus trichloride (Scheme II). Acylation of **6** and **11** with the appropriate acid chloride in the presence of triethylamine afforded the target compounds **12–50** (series **8**). Pertinent spectral and physical data for these compounds are presented in Table I.

Pharmacology. Results and Discussion. The compound series **8** were evaluated for analgesia in mice by the 55 °C hot-plate assay. An initial dose of 1 mg/kg was administered, and if 100% analgesia was observed, then lower dosing was continued until an ED_{50} was generated. If less than 100% analgesia was observed, then 5 mg/kg was administered. Many compounds were screened in vitro for their ability to displace [^{3}H]naloxone from μ opioid receptor sites in rat brain membranes. The heterocyclic substitutions for the phenyl ring of the propionanilido group of **1** structurally illustrated in Table I provided a systematic pattern of variation of this ring system: simple saturated and unsaturated six-membered heterocycles, peripherally substituted congeners of the latter, and graduation to the benzofused (or extended phenyl) ring systems. Inspection of the percentages of analgesia recorded in the hot-plate test after administration of 1 mg/kg of target compounds showed that in the mouse the combinations of these heterocycles with the R groups selected, about the amido nitrogen, resulted in a significant diminution of analgesic activity (<50%) relative to **1** (Table II). The sole exception among the heterocyclic families was the 2-pyridino member. It was the most pharmacologically accommodating and provided the most potent of each of the types of amides (**16–18**). The overall results were unlikely to be related to pharmacokinetic factors. The time-dependent effects on biological response of peripheral tissue and/or plasma protein binding, metabolism, and excretion were not operative here since all

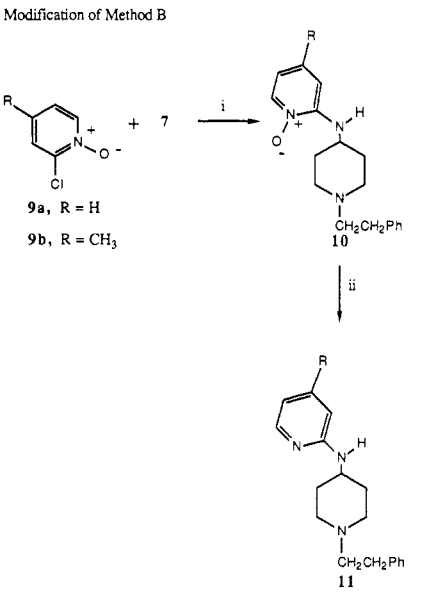
Scheme I



i, TsOH, $C_6H_5CH_3$; ii, $NaBH_4$, MeOH; iii, Cu pwd, 170-180°; iv, EtOH, Et_3N;

v, $(CH_3)_2CHCH_2CH_2OH$, Na_2CO_3; vi, RCOCl, Et_3N, $CHCl_3$ (or $C_6H_5CH_3$).

Scheme II



i, $(CH_3)_2CHCH_2CH_2OH$, KI; ii, PCl_3, $CHCl_3$

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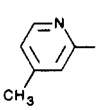
Table I. 4-(Heteroanilido)piperidines: Spectral and Physical Characteristics

het	R[a]	no.	^{1}H NMR signals for aromatics[b]	yield, %[c]	formula[d]	mp, °C (recrystn)[e]
	MOM	12	7.36, s, 5 H	44	$C_{21}H_{33}N_3O_2 \cdot C_2H_2O_4$	212–212.5 (A)
	2-fur	13	6.45–7.50, m, 8 H	45	$C_{23}H_{31}N_3O_2 \cdot C_2H_2O_4$	229–230 (A)
	MOM	14	7.36, s, 5 H	19	$C_{20}H_{31}N_3O_3 \cdot C_2H_2O_4$	180–181.5 (B)
	2-fur	15	6.72–7.92, m, 8 H	24	$C_{22}H_{29}N_3O_3 \cdot C_2H_2O_4$	203.5–205.5 (B)
	MOM	16	7.12–8.12, m, 8 H; 8.60, br s, 1 H	43	$C_{21}H_{27}N_3O_2 \cdot C_2H_2O_4$	197–199 (C)
	2-fur	17	6.08–6.30, m, 2 H; 7.05–7.95, m, 9 H; 8.62, br s, 1 H	94	$C_{23}H_{25}N_3O_2 \cdot C_2H_2O_4$	209–210.5 (A)
	3-fur	18	6.00, s, 1 H; 6.88–7.85, m, 10 H; 8.60, br s, 1 H	78	$C_{23}H_{25}N_3O_2 \cdot C_2H_2O_4$	210.5–202 (A)
	2-fur	19	6.20, s, 1 H; 7.20–7.60, m, 10 H; 8.65, d, 2 H	72	$C_{23}H_{25}N_3O_2 \cdot C_2H_2O_4$	195–195.5 (B)
	2-fur	20	6.20–6.50, m, 2 H; 7.15–7.50, m, 8 H; 8.70, d, 2 H	74	$C_{23}H_{25}N_3O_2 \cdot C_2H_2O_4$	181–183 (B)
	3-fur	21	6.12, s, 1 H; 7.00–7.30, m, 10 H; 8.70, d, 2 H	43	$C_{23}H_{25}N_3O_2 \cdot C_2H_2O_4$	192–195 (B)
	MOM	22	7.20, s, 5 H; 7.50–8.00, m, 2 H; 8.46, d, 1 H	100	$C_{21}H_{26}N_3ClO_2 \cdot C_2H_2O_4$	149–151 (C)
	2-fur	23	6.25–6.45, m, 2 H; 7.20, s, 5 H; 7.50–8.70, m, 4 H	50	$C_{23}H_{24}N_3ClO_2 \cdot C_2H_2O_4$	177–179 (B)
	3-fur	24	6.20, s, 1 H; 6.80–7.80, m, 9 H; 8.48, br s, 1 H	71	$C_{23}H_{24}N_3ClO_2 \cdot C_2H_2O_4$[f]	118–123 (B)
	MOM	25	7.20, s, 5 H; 8.50–8.80, m, 2 H; 8.40, br s, 1 H	83	$C_{21}H_{26}N_3ClO_2 \cdot C_2H_2O_4$	180–182 (C)
	2-fur	26	6.40, s, 1 H; 7.20–8.00, m, 9 H; 8.40, br s, 1 H	72	$C_{23}H_{24}N_3ClO_2 \cdot C_2H_2O_4$	213–214 (C)
	3-fur	27	6.12, s, 1 H; 6.88–7.50, m, 9 H; 8.20, br s, 1 H	27	$C_{23}H_{24}N_3ClO_2 \cdot C_2H_2O_4$[g]	191.5–192 (C)
	2-fur	28	6.08, d, 1 H; 6.25, br s, 1 H; 7.00–7.35, m, 8 H; 8.50, d, 1 H (2.38, s, $ArCH_3$)	75	$C_{24}H_{27}N_3O_2 \cdot C_2H_2O_4$	198–199.5 (B)
	MOM	29	7.20, s, 5 H; 7.40, s, 1 H; 8.78, d, 2 H	89	$C_{20}H_{26}N_4O_2 \cdot C_2H_2O_4$	188–190 (B)
	2-fur	30	6.32, s, 1 H; 6.85, br s, 1 H; 7.10–7.40, 8 H; 8.70, d, 1 H	82	$C_{22}H_{24}N_4O_2 \cdot C_2H_2O_4$	211–212 (D)
	3-fur	31	5.96, s, 1 H; 7.10–7.35, m, 8 H; 8.70, d, 2 H	50	$C_{22}H_{24}N_4O_2 \cdot C_2H_2O_4$	211–214 (A)
	2-fur	32	6.20, br s, 1 H; 6.48, d, 1 H; 7.05, s, 1 H; 7.22, s, 5 H; 8.40, s, 1 H; 8.50, s, 2 H	45	$C_{22}H_{24}N_4O_2 \cdot C_2H_2O_4$	206–207 (D)
	3-fur	33	6.00, s, 1 H; 7.00–7.30, m, 7 H; 7.40, d, 2 H; 7.50, s, 1 H	55	$C_{22}H_{24}N_4O_2 \cdot C_2H_2O_4$	204.5–207.5 (E)
	2-fur	34	6.40, br s, 1 H; 6.70–7.00, m, 3 H; 7.20, s, 5 H; 8.42, d, 1 H	40	$C_{22}H_{23}N_4ClO_2 \cdot C_2H_2O_4$	173–174.5 (D)
	3-fur	35	6.18, s, 1 H; 6.78, d, 1 H; 7.20–7.55, m, 7 H; 8.45, d, 1 H	55	$C_{22}H_{23}N_4ClO_2 \cdot C_2H_2O_4$	177–178 (D)
	MOM	36	7.20, s, 5 H; 7.40, s, 1 H; 8.98, s, 1 H	62	$C_{20}H_{25}N_4ClO_2 \cdot C_2H_2O_4$	163.5–164 (A)
	2-fur	37	6.38, s, 1 H, 6.90–7.32, m, 8 H; 8.40, s, 1 H	62	$C_{22}H_{23}N_4ClO_2 \cdot C_2H_2O_4$	193–195 (F)
	3-fur	38	6.10, s, 1 H; 7.00–7.48, m, 8 H; 8.90, s, 1 H	55	$C_{22}H_{23}N_4ClO_2 \cdot C_2H_2O_4$[h]	185–186.5 (F)
	MOM	39	7.20–8.85, m, 9 H	69	$C_{23}H_{27}N_3O_3 \cdot C_2H_2O_4$	205.5–206.5 (B)
	2-fur	40	6.30, s, 1 H; 6.85, d, 1 H; 7.10–7.76, m, 10 H	85	$C_{25}H_{25}N_3O_3 \cdot C_2H_2O_4$	210–211.5 (B)
	3-fur	41	6.18, s, 1 H; 7.25, s, 5 H; 7.35–7.85, m, 6 H	78	$C_{25}H_{25}N_3O_3 \cdot C_2H_2O_4$	219–219.5 (G)
	MOM	42	7.20, s, 5 H; 7.38–8.12, m, 4 H	61	$C_{23}H_{27}N_3O_2S \cdot C_2H_2O_4$	220–222 (A)
	2-fur	43	6.20, s, 1 H; 6.85, d, 1 H; 7.10–8.18, m, 10 H	68	$C_{25}H_{25}N_3O_2S \cdot C_2H_2O_4$	226.5–227 (G)
	3-fur	44	7.30, s, 1 H; 7.18–8.15, m, 11 H	29	$C_{25}H_{25}N_3O_2S \cdot C_2H_2O_4$	232–232.5 (H)
	MOM	45	6.50–7.45, m, 10 H	64	$C_{24}H_{29}N_3O_2 \cdot C_2H_2O_4$	130–134 (C)
	MOM	46	6.80–7.50, m, 8 H; 8.00, s, 1 H	57	$C_{23}H_{28}N_4O_2 \cdot C_2H_2O_4$[i]	150–152 (C)
	MOM	47	7.20, s, 5 H; 7.40–8.18, m, 3 H	73	$C_{22}H_{26}N_4O_2S \cdot C_2H_2O_4$	176–178 (C)
	2-fur	48	6.20, br s, 1 H; 7.20, s, 5 H; 7.60–8.20, m, 5 H	60	$C_{24}H_{24}N_4O_2S \cdot C_2H_2O_4$	142–145 (B)
	3-fur	49	6.05, s, 1 H; 6.85–8.20, m, 10 H	69	$C_{24}H_{24}N_4O_2S \cdot C_2H_2O_4$	191–192 (E)
	2-fur	50	6.12, s, 1 H; 7.12–8.32, m, 12 H; 8.72, br s, 1 H	85	$C_{27}H_{27}N_3O_2 \cdot C_2H_2O_4$	171.5–175 (B)

[a] MOM = methoxymethyl, 2-fur = 2-furyl, 3-fur = 3-furyl. [b] Spectra were recorded in $CDCl_3$ with $(CH_3)_4Si$ as reference. [c] Yields were not optimized. [d] All compounds were analyzed for C, H, and N and results were within ±0.4% of theoretical values unless otherwise indicated. [e] Recrystn = recrystallization solvent, i.e., A = 2-PrOH–MeOH, B = 2-PrOH–2-Pr_2O–MeOH, C = 2-PrOH–2-Pr_2O, D = 2-PrOH–MeOH–H_2O, E = 2-PrOH–2-Pr_2O–MeOH–H_2O, F = 2-PrOH–2-Pr_2O–EtOH–H_2O, G = 2-PrOH, H = 2-PrOH–EtOH–H_2O. [f] H: calcd, 5.24; found, 5.72. [g] C: calcd, 60.06; found, 60.48. [h] C: calcd, 57,54; found, 57.96. [i] H: calcd, 6.27; found, 6.75.

compounds were administered intravenously and hot-plate measurements recorded 1 min later. A lack of bioavailability due to inadequate absorption across the blood–brain barrier is unlikely as well since these compounds are extensively π electron rich and should be highly lipophilic. Inconclusive as well was the lack of correlation between in vitro K_i values and degree of analgesia for those compounds screened for μ opioid receptor binding. Compounds **17** and **49** exhibited receptor affinities approximating that of **1**. However, **17** was only one-fifth as potent as an analgesic by comparison of ED_{50} values and **49** was not analgesic at 1 mg/kg. Generally, these compounds did not offer an advantageous side effect profile over **1**, with rigidity and respiratory depression being most noticeable, especially at 5 mg/kg.

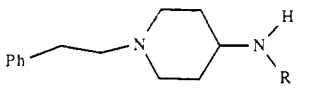
The compound series **8** was also screened for antagonism of the effects of morphine in rabbits. Antagonism of morphine-induced analgesia by a compound was reflected in its ability to decrease the voltage required for the characteristic lick/chew response to noxious electrical stimulus to the maxillary central incisor tooth pulp in this species. Antagonism of morphine-induced respiratory

Table II. 4-(Heteroanilido)piperidines: Preliminary Pharmacology

no.	% analgesia[a] 5 mg/kg	% analgesia[a] 1 mg/kg	K_i,[b] nM	% reversal[c] rpm[e]	% reversal[c] analg[f]	reversal type[d]
12	100	0	>100[g]	29–66[h]	no[i]	1
13	80	0	7.7	60–100	no	1
14	0	0	>100	28–22	no	0
15	80	0	>100	40–75	no	1
16	0.455 (0.314–0.659) mg/kg[j]		12.7	no	no	0
17	0.081 (0.059–0.109) mg/kg[j]		1.9	43–86	no	1
18	100	73	ND[k]	39–92	no	1
19	100	33	8.42	41–103	no	1
20	100	28	ND	23–91	100–0[l]	2
21	80	0	>100	43–100	100–44	2
22	58	8	ND	no	no	0
23	50	4	ND	29–88	100–0	2
24	40	0	21.3	43–103	no	1
25	3	0	ND	47–82	no	1
26	72	14	ND	38–118	no	1
27	40	0	>100	28–97	no	1
28	0	0	4.05	31–115	100–45	2
29	100	30	15.6	56–91	91–88	1
30	39	31	13.9	60–100	100–52	2
31	100	0	35.4	27–50	no	1
32	100	0	7.5	17–114	no	1
33	51	11	ND	34–91	no	1
34	0	0	22.2	42–83	no	1
35	100	0	>100	29–71	no	1
36	20	23	214	56–94	no	1
37	0	0	18.5	32–100	100–54	2
38	80	0	>100	28–103	no	1
39	100	33	ND	34–26	no	0
40	40	0	ND	43–103	no	1
41	49	0	ND	36–92	no	1
42	100	14	ND	31–43	no	0
43	ND	ND	ND	25–112	no	1
44	0	0	ND	42–72	no	1
45	30	0	ND	27–20	no	0
46	10	19	ND	no	no	0
47	100	19	ND	15–50	no	0
48	59	20	ND	53–127	100–22	2
49	100	0	2.64	14–100	no	1
50	100	60	ND	43–95	no	1
51a	0	0	>100	29–103	no	1
51b	11	0	>100	18–129	no	1
52	0	0	>100	no	no	0
1	0.018 (0.014–0.023)[j]		2.16	no	no	0
53			1.07	30–100	50–0	2

[a] Mouse hot plate. [b] K_i denotes the ability to displace [^{3}H]naloxone from the μ opioid receptor isolated from rat brain membranes. [c] Initial morphine reversal screen in rabbits. [d] 0 = no reversal, 1 = reversal of morphine respiratory depression only, 2 = reversal of both morphine respiratory depression and analgesia. [e] Respirations per minute. [f] Tooth-pulp assay. [g] A greater than sign (>) denotes no displacement of [^{3}H]naloxone at the concentration indicated. [h] First value is % RPM of control after morphine and second value is % RPM of control after test compound. [i] No = absolutely no % change. [j] ED_{50} with 95% confidence limits. [k] ND = not determined. [l] First value is % analgesia after morphine and second value is % analgesia after test compound.

depression was manifested in the ability of a compound to increase respirations per minute by observation of the inspirations of the rib cage muscles. There was observed an inherent ability of **8** to alter the effects of morphine during these qualitative assays (Table II). The majority of the compounds (80%) exhibited morphine antagonism, and of these, most (77%) selectively antagonized the respiratory depression. Bearing a methoxymethyl chain attached to the amide carbonyl group was not predictive of opioid antagonism since two-thirds of these compounds were not antagonists. Propionyl (R = CH_2CH_3) congeners of such compounds have been reported elsewhere.[6,7] Those containing the 2-pyridinyl, 4-pyridinyl, and 2-pyrimidinyl rings were found to be inactive as antagonists as well (data not shown). Thus the presence of an acyclic chain within the amido substructure of 4-(heteroanilido)piperidines is likely to produce pure agonists as with fentanyl. In contrast, a 2- or 3-furyl attachment conferred antagonsim, and within the furan family of compounds, there existed the preponderance for selectively reversing respiratory depression. Even in the absence of a heterocycle attached to the amido nitrogen (**51**), antagonism remained, whereas subsequent removal of the furan (**52**) abolished activity



51a, R = 2-furoyl

51b, R = 3-furoyl

52, R = H

(Table II). Evidence suggests that there are two distinct μ opioid receptor subtypes.[9] The μ-1 receptor is implicated in mediating supraspinal analgesia and the μ-2 receptor in respiratory depression. As discussed in the mouse findings, the results obtained from the opioid receptor binding studies made it clear that any generality as to μ receptor involvement was not warranted since μ binding affinities as reflected in K_i values ranged from strong (1.9

(9) Pasternak, G. W.; Childers, S. R.; Snyder, S. H. *J. Pharmacol. Exp. Ther.* **1980**, *214*, 455.

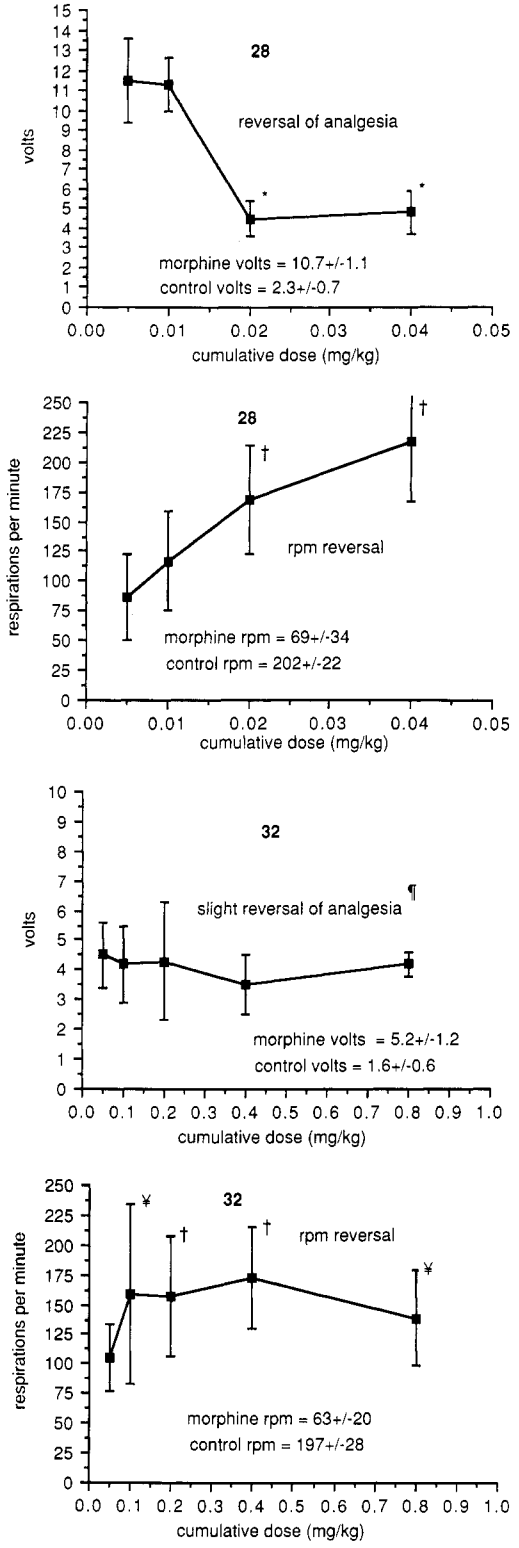


Figure 1. Comparative effects of **28** and **32** on morphine-induced analgesia and respiratory depression in rabbits. The compounds were cumulatively administered (iv) 10 min after that of morphine sulfate (4 mg/kg). The lick/chew volts and respirations per minute were recorded 1 min after each compound dose. Data points are mean values (±SEM), $N = 4$. (*) Significantly different from morphine ($p \leq 0.001$). (†) Significantly different from morphine ($p \leq 0.05$). (¶) All data points significantly different from control ($p \leq 0.05$) and not significantly different from morphine. (¥) Significantly different from morphine ($p \leq 0.10$).

nM) to very weak (>100 nM). Receptor selectivity for these compounds has not been established and is a subject for future investigation.

Table III. Antinociceptive Effects of **28** and **32** in Different Species Assays

	doses, mg/kg	
	28	**32**
rabbit tooth pulp ($N = 4$)	30% MPE[a] cumulative dose 7.7	57% MPE cumulative dose 0.8
rat tail flick ($N = 6$)	ED_{50} = 0.177 (0.101–0.308)[b]	ED_{50} = 0.07 (0.032–0.15)
rat hot plate ($N = 6$)	not active up to 10.0	ED_{50} = 0.585 (0.236–1.45)
mouse hot plate ($N = 5$)	not active up to 5.0[c]	100% MPE at 5.0[c]

[a] Maximum pharmacological effect. [b] ED_{50} values with 95% confidence limits. [c] From Table II.

Many of the compounds precipitated convulsions, tremors, and rigid limb extensions in the initial morphine reversal screen studies. Two antagonists (**28** and **32**), which displayed high safety margins characterized by a normal gait, good facial and auricular color, and alertness, were selected for further study. In the quantal rabbit tooth pulp assay both exhibited analgesia (**28**, 30% of maximum pharmacological effect (MPE) with a cumulative dose of 7.7 mg/kg; **32**, 57% of MPE with a cumulative dose of 0.8 mg/kg.). However, each displayed different antagonistic profiles as shown in Figure 1. As an antagonist, **28** resembled naloxone in reversing both morphine-induced analgesia and respiratory depression. Compound **32**, however, reduced morphine analgesia slightly while completely reversing respiratory depression. Investigations of the effects of **28** and **32** in the rat were then undertaken. In tail-flick experiments, ED_{50} values of 0.177 and 0.07 mg/kg were determined for **28** and **32**, respectively. In hot-plate experiments, an ED_{50} of 0.585 mg/kg was determined for **32**, while **28** was inactive up to 10 mg/kg. Thus the results from antinociception tests in three species indicated that **32** was consistent in eliciting a superior analgesic profile (Table III). The quantitative differences between **28** and **32** in their antagonism of the analgesic effects of morphine in the rat supported the findings of the rabbit studies. In hot-plate experiments, a 10-fold shift to the right of the morphine analgesia versus dose–response curve was observed on pretreatment with **28** at a dose of 2.0 mg/kg. However, pretreatment with **32** at doses of 0.5, 1.0, and 4.0 mg/kg did not significantly affect the morphine curve (curves not shown). The analgesic antagonist property of **28** clearly demonstrated in the rabbit and rat may explain the weaker to inefficacious analgesia of **28** relative to **32**.

Due to its overall superior analgesic profile, the pharmacology of **32** was further scrutinized. The cardiorespiratory effects of **32** ($N = 3$ per dose), fentanyl (**1**, $N = 2$–4 per dose), and alfentanil (**2**, $N = 3$–5 per dose) in the conscious freely moving rat were measured. The effects on respiration, as measured by changes in arterial blood CO_2 levels versus multiples of rat tail-flick (RTF) ED_{50} values are graphically illustrated in Figure 2. At 28 × RTF ED_{50}, **32** caused less than a 50% increase in blood CO_2 levels while **1** and **2** began to adversely affect respiration early on. After administration of doses of up to respective 10 × RTF ED_{50} values, significant elevations in blood CO_2 levels were detected for **1** and **2** relative to **32**. Thus the profound respiratory depression in the rat characteristic of μ agonists[10] is not a property of **32**. Within the same dose interval as depicted in Figure 2, **32** decreased mean arterial blood pressure to 40 (±5%) from control. This

(10) Van Den Hoogen, R. H. W. M.; Colpaert, F. C. *J. Pharmacol. Exp. Ther.* **1986**, *237*, 25.

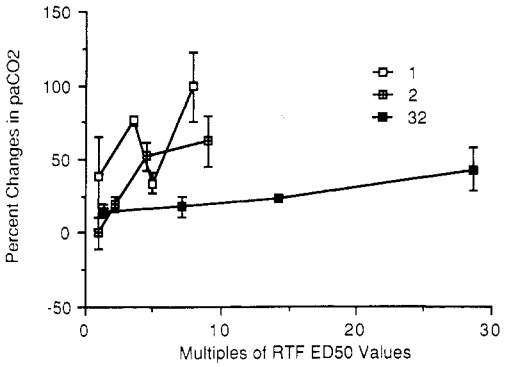
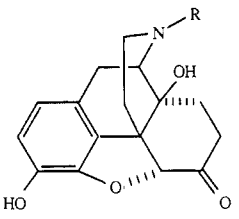


Figure 2. Effects on respiration in conscious freely moving rats after iv administration of fentanyl (**1**), alfentanil (**2**), and **32**. $paCO_2$ = pressure in millimeters of mercury of CO_2 gas in arterial blood. Respiratory effects were measured as percent changes in $paCO_2$ after control blood samples were taken and each animal served as its own control. Data points are mean values (±SEM), N = 2–5.

magnitude of decrease in blood pressure is characteristic of opioids,[11-13] as evident by the effect of **1**, which reduced mean arterial blood pressure to 49 (±8%). Heart rate decreases to 36 (±16%) from control by **32** were also observed. However, this is not characteristic of opioids since these have been shown to cause larger decreases in heart rate in animals.[11-13] In this study, **1** and **2** reduced heart rate to 54 (±12%) and 67 (±0%), respectively. Overall, the pharmacological findings thus far gathered for this structurally unique fentanyl analogue reveal a new central analgesic with an extraordinarily high degree of therapeutic safety.

Summary. Efforts to create opioid reversal agents from fentanyl-type agonists by classical antagonism-conferring substitutions at the piperidino nitrogen as those present in naloxone (**53**) and naltrexone (**54**) have been unsuccessful.[14,15] Structural modifications at the other sector of the fentanyl molecule have now generated the first class of opioid antagonists incorporating a 4-anilidopiperidine-type pharmacophore. Bearing in mind the predominance of a furan ring among these molecules, it is tempting to speculate as to its importance as a cognitive anchor for a complementary subsite within a distinct receptor.



53, R = allyl

54, R = cyclopropylmethyl

While naloxone is used primarily in the treatment of postanesthetic respiratory depression due to opioids, it is disadvantageous.[16] The anesthesiologist must titrate its intravenous dosage to avoid reversal of analgesia and the problems of sudden arousal of pain. Adverse effects reported are hypertension, pulmonary edema, and cardiac arrhythmias and may be the result of arousal-induced catecholamine release.[17] In addition, it exhibits a relatively short duration of action which can be the cause for recurrence of the respiratory depression characteristic of opioids such as fentanyl and morphine, which are commonly employed during surgical procedures. Central analgesics devoid of significant cardiovascular and respiratory depressant effects offer a less complex drug regimen for the maintenance of pain relief during the entire perioperative spectrum of acute care. The discovery of the structurally novel class of morphine antagonists and the pharmacological profile of **32** reported herein provides a lead toward such analgesics.

Experimental Section

General Information. Melting points were recorded on a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were obtained from the Analytical Services Division, BOC Technical Center, Murray Hill, NJ, and from Galbraith Laboratories, Knoxville, TN. ^1H NMR spectra were recorded on a Varian EM 360 (60 MHz) spectrometer. IR spectra were recorded on a Perkin-Elmer 197 spectrophometer. Conventional and flash column chromatography were performed with fine silica (EM Science, 230–400 mesh). Reaction progress and purity of products were checked by analytical TLC using Analtech (GHLF) silica-coated glass plates. Spots were visualized with UV_{254} light or iodine. Tetrahydrofuran (THF) was freshly distilled from $LiAlH_4$ and triethylamine from KOH.

Starting Materials. Most starting materials were commercially available and did not require further purification. 1-Phenethyl-4-piperidone (**4**, Aldrich) was recrystallized from hexane. The solid oxime (mp 132–134 °C) of **4** was prepared by the standard procedure[18] and used directly after filtering and drying (80 °C, 0.10 mmHg). 1-Phenethyl-4-aminopiperidine (**7**, bp 142 °C, 0.10 mmHg; bp[7] 141–142 °C, 0.6 mmHg) was prepared by $LiAlH_4$ (25% molar equiv excess) reduction under nitrogen of the oxime in refluxing THF (15 mL/g of oxime), followed by aqueous NaOH quenching[19] necessary for liberation of the free-base product. This highly basic diamine is air-sensitive. It can be efficiently weighed out if this is done quickly and returned to freezer storage. However, prolonged exposure to the atmosphere will result in copious formation of carbonic acid salts. *N*-Oxide **9b** was synthesized by the following procedure. 2-Chloro-4-methylpyridine[20] (16 g, 0.125 mol) was stirred with 3-chloroperoxybenzoic acid (80%, 27 g, 0.125 mol) in refluxing chloroform (250 mL) for 4 h. The mixture was cooled and partially concentrated to precipitate 3-chlorobenzoic acid byproduct. The suspension was filtered and the filtrate washed with 6 N NaOH, water, and brine and dried over Na_2SO_4. Purification by flash chromatography (400 g of fine silica; $CHCl_3$–MeOH–NH_3, 100:1:0.1) yielded 6.2 g (35%) of **9b** as a red oil. 3-Furoyl chloride was prepared from 3-furoic acid (Aldrich). The acid was suspended in a mixture of 0.10% DMF in CH_2Cl_2 (1 g of acid/12.5 mL of medium). This was chilled to 0 °C and oxalyl chloride (1.1 molar equiv) was added dropwise with stirring under nitrogen. After the mixture was stirred at room temperature for 44 h, gaseous HCl and CO evolution ceased. Concentration in vacuo left a brown oil, which on distillation (bp 155–158 °C, 1 atm) yielded a colorless oil that required constant vigil to prevent solidification and clogging of the condenser. **CAUTION**! This acid chloride is a severe lachrymator.

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(12) Eisele, J. H.; Reitan, J. A.; Torten, M.; Miller, C. H. *Br. J. Anaesth.* **1975**, *47*, 937.
(13) Liu, W. S.; Bidwai, A. V.; Stanley, T. H.; Isern-Amaral, J. *Anesth. Analg. (N.Y.)* **1976**, *55*, 168.
(14) Riley, T. N.; Bagley, J. R. *J. Med. Chem.* **1979**, *22*, 1167.
(15) Riley, T. N.; Finney, Z. N. *J. Med. Chem.* **1980**, *23*, 895.
(16) Smith, G.; Pinnock, C. *Brit. J. Anaesth.* **1985**, *57*, 547.
(17) Wood, K. M. *Trends Intravenous Anaesthesia*; Aldrete, Stanley, Eds.; Symposia Specialists Inc.: Chicago, 1980; p 501.
(18) Riley, T. N.; Bagley, J. R. *J. Heterocycl. Chem.* **1982**, *19*, 485.
(19) Micovic, V. M.; Mihailovic, M. L. J. *J. Org. Chem.* **1953**, *18*, 1190.
(20) 2-Chloro-4-methylpyridine was prepared by diazotization of 2-amino-4-methylpyridine (Aldrich) by a procedure similar to that for 3-bromo-2-chloro-4-methylpyridine except that product was extracted from the aqueous ammoniated medium with CH_2Cl_2 and subsequently purified by vacuum distillation (bp 100–110 °C, 30 mmHg). Link, W. J.; Setliff, F. L.; Borne, R. F. *J. Heterocycl. Chem.* **1967**, *4*, 641.

4-(Heteroanilino)piperidines. The procedures described are representative of those depicted in Schemes I and II.

Method A. Via $NaBH_4$ Reduction of Schiff Bases 5. Example. 1-Phenethyl-4-[*N*-(2,1,3-benzothiadiazol-4-yl)amino]piperidine (6j). A mixture of 1-phenethyl-4-piperidone (17.4 g, 85.6 mmol), 4-amino-2,1,3-benzothiadiazole (15 g, 99.2 mmol), a few crystals of *p*-toluenesulfonic acid, and toluene (170 ml) was stirred under reflux for 4 days. This was the time required for collection of the theoretical quantity of water byproduct (1.54 mL) in a Dean–Stark trap. The reaction mixture was cooled and concentrated in vacuo to give a reddish-brown oil which exhibited a strong C=N absorption band at 1665 cm^{-1} by IR analysis. The crude Schiff base was dissolved in MeOH (150 mL) and then $NaBH_4$ (3.7 g, 98 mmol) was added in portions. The reaction mixture was stirred under reflux for 2 h, cooled, and concentrated in vacuo. Water (100 mL) was added, followed by extraction with toluene (200 mL). The organic extract was washed with water (2 × 100 mL) and dried over Na_2SO_4. Concentration in vacuo left 33.5 g of a dark brown oil. This was purified by flash chromatography (800 g of fine silica; hexane–ethyl acetate–triethylamine, 150:100:1), leaving a red oil which crystallized on standing at room temperature. TLC analysis (100:100:1) showed this to contain a trace of 4-amino-2,1,3-benzothiadiazole. A homogeneous product was obtained after recrystallization from hexane, yielding 8.8 g (30%) of golden needles (mp 85–88 °C).

Method B. Via Aromatic Nucleophilic Substitution. Coupling of Amine 7 with Heteroaromatic Chlorides. Example 1. 1-Phenethyl-4-[*N*-(2-pyrazinyl)amino]piperidine[21] (6k). A mixture of 7 (13 g, 64 mmol), chloropyrazine (3.6 g, 32 mmol), and copper powder (2.0 g, 32 mmol) was stirred under nitrogen at 170–180 °C for 6 h. On cooling, a green solid formed. The solid was broken into chunks with a spatula and gradually churned into thick soup in 10% HCl (100 mL). This was filtered of insolubles and the filtrate extracted with ether (52 mL). Alkalinization with 12 N NaOH liberated the free base, which was extracted with methylene chloride (2 × 50 mL). The organic extract was washed with water (50 mL) and brine (50 mL) and dried over Na_2SO_4. The green solid left after evaporation of solvent was eluted through 600 g of silica. This required successive passages of $CHCl_3$–MeOH–NH_3 (4000 mL of 100:1:0.1, 1800 mL of 90:1:0.1, 1600 mL of 80.1:0.1, 4000 mL of 70:1:0.1) to yield 4.0 g (44%) of **6k** as a beige solid.

Example 2. 1-Phenethyl-4-[*N*-(4-chloropyrimidin-6-yl)amino]piperidine (6m). A mixture of 7 (1.0 g, 4.9 mmol), 4,6-dichloropyrimidine (0.8 g, 5.4 mmol), triethylamine (3 mL), and ethanol (15 mL) was stirred under reflux. After 20 h TLC analysis ($CHCl_3$–MeOH–NH_3, 95:5:0.5) indicated an absence of 7 with emergence of a major spot at R_f 0.41. The mixture was concentrated in vacuo and the residue partitioned between 10% HCl (40 mL) and ether (2 × 40 mL). The aqueous phase was alkalinized with 12 N NaOH and extracted with CH_2Cl_2 (2 × 50 mL). The organic extract was washed with water (50 mL) and brine (50 mL) and dried over Na_2SO_4. The crude concentrate was purified by flash chromatography (60 g fine silica; $CHCl_3$–MeOH–NH_3, 30:1:0.1) to yield 1.2 g (77%) of **6m** as a cream-colored solid.

Example 3. 1-Phenethyl-4-[*N*-(2-benzothiazolyl)amino]piperidine (6o). A mixture of 7 (1.0 g, 4.9 mmol), 2-chlorobenzothiazole (1.0 g, 5.5 mmol), Na_2CO_3 (1.5 g, 14 mmol), and 3-methyl-1-butanol (15 mL) was refluxed overnight. TLC inspection ($CHCl_3$–MeOH–NH_3; 95:5:0.5) showed an absence of 7 and emergence of a major spot at R_f 0.35. The suspension was filtered and the filtrate concentrated in vacuo. The concentrate was partitioned between 10% HCl (30 mL) and ether (2 × 30 mL). The aqueous phase was alkalinized with 12 N NaOH and extracted with $CHCl_3$ (2 × 50 mL). The organic extract was dried over Na_2SO_4. The crude concentrate was purified by flash chromatography (50 g of fine silica; $CHCl_3$–MeOH–NH_3, 40:1:0.1) to yield 1.2 g (71%) of **6o** as a pale yellow solid.

Example 4. 1-Phenethyl-4-[*N*-(4-methylpyridin-2-yl)amino]piperidine (11b). A mixture of 7 (9.2 g, 45 mmol), **9b** (6.2 g, 43 mmol), Na_2CO_3 (23 g), KI (200 mg), and 3-methyl-1-butanol (150 mL) was stirred under reflux for 48 h and then cooled and filtered. The filtrate was concentrated in vacuo and the residue partitioned between 10% HCl (100 mL) and ether (100 mL). The aqueous phase was alkalinized with 12 N NaOH and extracted with CH_2Cl_2 (2 × 100 mL). The organic extract was washed with water (100 mL) and brine (50 mL) and dried over Na_2SO_4. Concentration in vacuo left a purple oil which was eluted through fine silica (650 g) with $CHCl_3$–MeOH–NH_3 (20:1:0.2) to yield **10b** as a viscous red oil (3.5 g, 11.2 mmol, 26%). This was dissolved in $CHCl_3$ (80 mL), followed by chilling in an ice–brine bath. Phosphorus trichloride (11.3 mL) was then added in a slow dropwise fashion while the internal temperature was maintained at 0 °C. After this, the reaction mixture was stirred under reflux for 2 h, cooled, and poured into a 100-mL beaker of ice. The acidic mixture was cautiously alkalinized with 20% NaOH. The liberated free base was extracted with CH_2Cl_2 (100 mL), and the organic extract was washed with water (100 mL) and brine (100 mL) and dried over Na_2SO_4. Initial purification by flash chromatography (120 g of fine silica; $CHCl_3$–MeOH–NH_3, 25:1:0.1) yielded 2.4 g of a tan solid, which required further chromatography (as previously), finally yielding **11b** as a pale yellow solid (2.0 g, 61%).

4-(Heteroanilido)piperidines. General Procedure. To a stirring mixture of 1 g of the 4-(heteroanilino)piperidine, 1.0 mL of triethylamine and 8 mL of $CHCl_3$ was added, via a disposable pipet, a solution of the acid chloride (1.2 molar equiv) in 2 mL of $CHCl_3$. The mixing was usually mildly exothermic, and after stirring at room temperature for 30 min, if TLC analysis ($CHCl_3$–MeOH–NH_3, 95:5:0.5) indicated incomplete reaction, the mixture was stirred under reflux until completion was achieved. Compounds **6d**, **6m**, **6o**, **6p** for methoxyacetyl chloride and **6e** and **6j** for 3-furoyl chloride required refluxing toluene. On completion, the mixture was concentrated in vacuo and the residue partitioned between 10% HCl (50 mL) and ether (50 mL). The aqueous phase was alkalinized with 12 N NaOH and extracted with CH_2Cl_2 (2 × 50 mL), and the organic extract was washed with water (50 mL) and brine (50 mL) and dried over Na_2SO_4. Purification of the amides was accomplished by flash chromatography over fine silica with $CHCl_3$–MeOH–NH_3. Hydrogen oxalate salts were prepared from 1 molar equiv of oxalic acid in 2-PrOH or 2-PrOH–2-Pr_2O followed by recrystallization from the appropriate medium (Table I).

Pharmacological Methods. In Vivo. Analgesic. A. 55 °C Mouse Hot Plate.[22] The hot-plate assay utilized nonfasted male mice (Swiss-Webster) weighing between 18 and 22 g. The surface of the hot-plate apparatus was maintained at 55 ± 0.5 °C. To determine the percentage of analgesia using the MHP assay, vehicle (saline) or drug solution (10 mL/kg) was injected into the lateral tail vein of groups of five mice and placed on the hot plate after 1 min. An initial dose of 1 mg/kg of compound was administered. If 100% analgesia was observed, then lower dosing was continued until an ED_{50} was generated. If less than 100% analgesia was observed, then 5 mg/kg was administered. In addition to analgesia, side effects were noted. These were chiefly categorized as rigidity, sedation, respiratory depression, tremors, convulsions, and cyanosis. For each experiment, control latency times were determined in five mice and treatment latency times determined in additional groups after each dose of compound. The response latency was the time between the initial contact on the hot surface and the first paw-lick response. Animals were removed from the hot plate immediately after a response or until the cut-off time of 30 s was reached. Antinociceptive effect was

(21) A recommended procedure is composed of the following modifications: (a) phenyl ether as solvent; (b) use of a constant-temperature apparatus (175 °C) for a 24-h reaction time; (c) 10% molar equiv excess of chloropyrazine; (d) dilute cool reaction mixture with hexane and filter insolubles containing unreacted Cu, Cu salts, and **6k**·HCl; (e) suspend these in CH_2Cl_2 and filter Cu, then extract residual Cu salts with concentrated NH_4OH, which simultaneously provides the basic **6k** in the CH_2Cl_2. Purification of **6k** consisted of addition of decolorizing charcoal to the organic extract, filtration through Celite, crystallization of the concentrate with MeOH–H_2O (1:1) and recrystallization from toluene. Schwartz, M.; Dulina, R. G.; Arvizzigno, J. A.; Sondheimer, S. J. Unpublished results.

(22) Wynn, R. L.; Ford, R. D.; McCourt, P. J.; Ramkumar, V.; Bergman, S. A.; Rudo, R. G. *Drug Dev. Res.* **1986**, *9*, 233.

defined as a doubling of the latency time to paw lick over control times. The ED_{50} and 95% confidence limits were calculated by

$$\% \text{ analgesia} = \frac{\text{test time} - \text{control time}}{30 \text{ s} - \text{control time}} \times 100$$

using a standard computer program of the method of Litchfield and Wilcoxon[23] fitted to a minicomputer.[24] Calculation of the ED_{50} (95% confidence limits) was corrected for base content of the salts.

B. 55 °C Rat Hot Plate. This assay was performed similarly to the above with six male Sprague–Dawley rats weighing between 300 and 400 g.

C. Rat Tail Flick. A modification of the D'Armour–Smith tail-flick method was employed in the evaluation of analgesic activity.[25] Male Sprague–Dawley rats weighing between 150 and 200 g were given a thermal stimulus challenge 1 min postadministration in the laterall tail vein of the test compounds. Groups of at least six animals were used in the determination of ED_{50} values, which were calculated in a manner similar to that described above.

D. Rabbit Tooth Pulp.[26] New Zealand rabbits (1.5–3 kg) were utilized. Under fentanyl analgesia, the pulps of the maxillary central incisors were exposed. Three days later, an electric stimulator and one set of fine wire platinum electrodes were used to stimulate the pulp. Threshold voltage to elicit the lick/chew (L/C) response and respirations per minute (RPM) were recorded. RPM were determined by the observation of the number of inspirations of the rib cage muscles.

1. Initial Screen for Reversal Activity. Morphine sulfate (5 mg/kg) was administered at the lateral marginal ear vein. Ten minutes later threshold L/C volts and RPM were measured and recorded. The analgesic dose in mice or 1 mg/kg of the test compound was given iv. One minute after this dose, and each subsequent test drug dose, L/C volts and RPM were measured. If no reversal of the analgesic or respiratory depressant effects of morphine were noted after the first dose, subsequent doses, double the previous dose, were administered at 2-min intervals until 16 mg/kg was given. If an effect was noted after the first dose, $^1/_{10}$ of that dose was given to a second rabbit and doubled every 2 min until an effect was observed. A decrease in morphine threshold L/C volts and an increase in morphine RPM values were an indication of analgesia reversal and respiratory depression reversal, respectively. If morphine reversal was observed, dosing was continued at 2-min intervals until maximum respiratory depression reversal occurred or the maximum cumulative dose was given.

2. Quantal Assay for Reversal of Morphine. Four rabbits were prepared as above for each compound to be tested (i.e., **28** and **32**). Four milligrams/kilogram of morphine was preadministered this time, and the starting dose of the test compound was $^1/_{10}$ the maximal reversal dose as previously determined in the initial rabbit screen. Measurements of threshold L/C volts and RPM were determined as described above. Dosing was continued in an attempt to reverse threshold L/C volts and RPM to control values or until a maximum cumulative dose of 10 mg/kg was administered. The cumulative doses to achieve maximum respiratory reversal and analgesic reversal expressed as percentages of control RPM and threshold L/C volts were determined by inspection of the dose–response curves.

3. Analgesic Assay. Four rabbits were prepared as above for **28** and **32**. Threshold volts and RPM were measured and recorded prior to drug injection. An initial dose of test compound the same as that determined for the initial screen for reversal activity was injected. If there was elevated L/C threshold volts above control with this dose in the first rabbit, then one-half that dose was given and so on in additional rabbits or until a starting dose was selected that had no effect on L/C threshold volts. One minute after administration of each 2-min iv incremental dose, threshold volts were observed and recorded. Each incremental dose, double the prior dose, was administered until either a peak effect was observed or a cumultive dose of 10 mg/kg was injected. Antinociception was calculted as the percentage maximum possible effect (% MPE) given by the expression:

$$\% \text{ MPE} = \frac{\text{test volts} - \text{control volts}}{10 \text{ V} - \text{control volts}} \times 100$$

where 10 V was the maximum threshold voltage utilized.

Cardiorespiratory Measurements in the Conscious Freely Moving Rat. In the method described, direct recordings of arterial blood pressure, heart rate, ECG, and respiratory blood gases ($paCO_2$, paO_2, and pH) were used. Male Sprague–Dawley rats about 300–400 g were allowed free access to food and water and housed five per cage in a vivarium with a light dark cycle of 12 h. Prior to testing (24 h) the animals were chronically implanted with heparinized catheters in the left internal jugular vein and right common carotid artery under 2% isoflurane anesthesia. The catheters were exteriorized through the nap of the neck and secured with surgical silk. Two braided strands of aluminum wire were threaded subcutaneously on either side of the chest in the shoulder area for the ECG recording. On the day of the experiment, the catheters were flushed with heparinized saline, and the arterial catheter was connected to a P 50 pressure transducer. The pressure signal was displayed as systolic, diastolic, and mean blood pressure on a physiological recorder. Heart rate was recorded from the pulse pressure signal and channeled through a tachograph for recording. Two standard limb leads were connected to the subcutaneous electrodes for lead II ECG recording through a 7P4G Grass preamplifier. Control blood samples were taken prior to the test drug administration for measurement of arterial blood gases, i.e., each animal served as its own control.

In Vitro. Opioid Receptor Binding. The method was based upon that described by Pasternak[27] and used crude membrane fractions prepared from freshly harvested rat brains. Incubation mixtures contained [^{3}H]naloxone and increasing concentrations (1, 10, 100 nM) of competing ligand. Nonspecific binding was determined from incubations containing [^{3}H]naloxone and radioinert levorphanol. The effect on naloxone binding of each concentration was determined in triplicate. The K_i for inhibition of [^{3}H]naloxone binding was calculated according to the method of Cheng and Prussof.[28]

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Registry No. **1**, 437-38-7; **4**, 39742-60-4; **5a**, 118142-33-9; **5b**, 118142-39-5; **5c**, 118142-40-8; **5d**, 118142-41-9; **5e**, 118142-42-0; **5f**, 118142-43-1; **5g**, 118142-44-2; **5h**, 118142-45-3; **5i**, 118142-46-4; **5j**, 117523-89-4; **6a**, 118142-34-0; **6b**, 118142-47-5; **6c**, 69492-61-1; **6d**, 69492-63-3; **6e**, 118142-48-6; **6f**, 118142-35-1; **6g**, 118142-49-7; **6h**, 118142-50-0; **6i**, 118142-51-1; **6j**, 117523-90-7; **6k**, 117523-88-3; **6l**, 118142-52-2; **6m**, 118142-53-3; **6n**, 79278-72-1; **6o**, 104588-49-0; **6p**, 104588-08-1; **7**, 51448-56-7; **7** (oxime), 79278-71-0; **9a**, 2402-95-1; **9b**, 52313-61-8; **10a**, 118142-36-2; **10b**, 117523-86-1; **11a**, 55692-29-0; **11b**, 117523-87-2; **12**, 117523-54-3; **12**·oxalate, 118142-54-4; **13**, 117523-49-6; **13**·oxalate, 118142-55-5; **14**, 117523-55-4; **14**·oxalate, 118170-18-6; **15**, 117523-56-5; **15**·oxalate, 118170-19-7; **16**, 117550-18-2; **16**·oxalate, 118142-56-6; **17**, 117523-57-6; **17**·oxalate, 118142-57-7; **18**, 117523-58-7; **18**·oxalate, 118142-58-8; **19**,

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Studies on Prodrugs. 11. Synthesis and Antimicrobial Activity of *N*-[(4-Methyl-5-methylene-2-oxo-1,3-dioxolan-4-yl)oxy]norfloxacin

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The chemical oxidation of *N*-[(5-methyl-2-oxo-1,3-dioxol-4-yl)methyl]norfloxacin (**2**) was carried out to afford *N*-[(4-methyl-5-methylene-2-oxo-1,3-dioxolan-4-yl)oxy]norfloxacin (**4**). In vitro, **4** exhibited lower activity than that of norfloxacin (NFLX, **1**) for both Gram-positive and Gram-negative bacteria. However, in vivo the activity of **4** was higher than that of NFLX. Bioavailability studies in mice showed that **4** liberated a higher concentration of NFLX in plasma than NFLX itself when administered orally. From these data, **4** obtained by the chemical oxidation of **2** functioned as a prodrug of NFLX as well as did **2**. The mechanism of the formation of **4** is interpreted in terms of [2,3]-sigmatropic rearrangement.

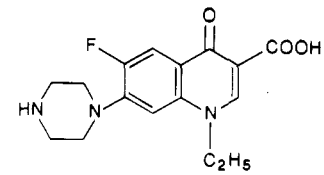
Norfloxacin (NFLX, **1**) is a new quinolone which has been shown to be a clinically effective antibacterial agent.[1] On the basis of pharmacokinetic studies in animals,[2] we have applied the prodrug technique to NFLX.[3-5] Recently, we have reported on the N-masked NFLX prodrug **2**[3] which is transformed into **1** in vivo by the cleavage of the C–N bond.

Various biological N-dealkylations are oxidatively catalyzed by cytochrome P-450.[6] For the purpose of understanding the metabolic mechanism of conversion of **2** to **1** in vivo, we first decided to examine chemical models for the oxidation of **2**.

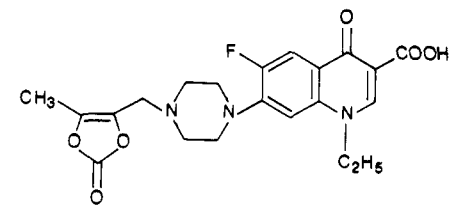
We observed that the nonbiological oxidation of N-masked NFLX (**2**) with *m*-chloroperbenzoic acid (MCPBA) at low temperatures (<5 °C) afforded allylic *N*-oxide **3**. This allylic *N*-oxide (**3**) rearranged at approximately 50 °C to give the corresponding *O*-allylhydroxylamine **4**.

This paper describes the synthesis and the mechanism of the formation of **4** and its antibacterial activity both in vitro and in vivo.

Chart I



1



2

Chemistry

N-[(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl]norfloxacin (**2**) was synthesized according to our previous report.[3]

The oxidation of **2** with MCPBA in dry chloroform at 50 °C under an argon atmosphere afforded **4** (Scheme I). This new compound was characterized by NMR analyses (^{1}H, ^{13}C), IR spectroscopy, and mass spectrometry. In particular, the methyl protons of the 2-oxo-1,3-dioxolane moiety in **4** were observed at higher field (δ 1.76) than the methyl protons of the 2-oxo-1,3-dioxole in **2** (δ 2.15). In addition, two doublets due to the exocyclic methylene of **4** were recorded at 4.95 and 5.10 ppm (J = 4.0 Hz). The IR spectra of **2** and **4** showed characteristic five-membered ring carbonyl bands at 1815 and 1845 cm^{-1}, respectively. The ^{13}C NMR spectrum of **4** was completely consistent with the proposed structure, the most pertinent points of

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