of **37b** and **38b** with a linear MeOH gradient (0–25%, v/v; 0.6 L) in $CHCl_3$ gave the individual nucleosides **37b** and **38b**.

38a: R_f 0.29 (C); UV (20% EtOH in H_2O) 241 nm shoulder (ϵ 7020), λ_{max} 280 nm (ϵ 7400); UV (0.1 N NaOH) 237 nm shoulder (ϵ 8600), λ_{max} 274 nm (ϵ 6200); UV (0.1 N HCl) λ_{max} 258 nm (ϵ 9200), λ_{max} 269 nm (ϵ 8600).

37b: UV (20% EtOH in H_2O) λ_{max} 254 nm (ϵ 15 200), 268 nm shoulder (ϵ 11 500); UV (0.1 N NaOH) broad max 258–270 nm (ϵ 13 700); UV (0.1 N HCl) λ_{max} 258 nm (ϵ 15 000), 274 nm shoulder (ϵ 10 960).

38b: UV (20% EtOH in H_2O) λ_{max} 287 nm (ϵ 7400), 238 nm shoulder (ϵ 6150); UV (0.1 N NaOH) λ_{max} 283 nm (ϵ 6900) 236 nm shoulder (ϵ 9100); UV (0.1 N HCl) λ_{max} 259 nm (ϵ 8600) and 267 nm (ϵ 8070).

Deprotection of 0.1 g of **32a** afforded 40 mg of **37a**: R_f 0.2 (C); UV (20% EtOH in H_2O) λ_{max} 254 nm (ϵ 14 500), 269 nm shoulder (ϵ 12 000); UV (0.1 N NaOH), broad max 260–270 nm (ϵ 9600); UV (0.1 N HCl) λ_{max} 259 nm (ϵ 12 400), 274 nm shoulder (ϵ 8900).

Inhibition of L1210, Raji, Molt/4F, and MT-4 Cell Proliferation. All assays were performed in flat-bottomed microtests III Plates (96 wells) as previously described.[48] Briefly, the cells were suspended in growth medium and added to the microplate wells at a density of 5×10^4 L1210 or Molt/4F cells/well (200 μL), 6.25×10^4 MT-4 cells/well or 7.5×10^4 Raji cells/well in the presence of varying concentrations of the test compounds. The cells were then allowed to proliferate for 48 h (L1210 cells), 72 h (Molt/4F and Raji cells), or 120 h (MT-4 cells) at 37 °C in a humidified CO_2-controlled atmosphere. At the end of the incubation period, the cells were counted in a Coulter counter (L1210, Raji, Molt/4F) or a blood cell counting chamber by trypan blue dye exclusion (MT-4). The IC_{50} was defined as the concentration of compound that reduced the number of viable cells by 50%.

Antiviral Assays. The antiviral assays, other than HIV-1, were based on an inhibition of virus-induced cytopathogenicity in either HeLa cell, Vero cell, or primary rabbit kidney cell cultures, following previously established procedures.[49] Briefly, confluent cell cultures in microtiter trays were inoculated with 100 $CCID_{50}$ of virus, 1 $CCID_{50}$ being the virus dose required to infect 50% of the cell cultures. After a 1-h virus adsorption period, residual virus was removed, and the cell cultures were incubated in the presence of varying concentrations (400, 200, 100, ... μg/mL) of the test compounds. Viral cytopathogenicity was recorded as soon as it reached completion in the control virus-infected cell cultures.

Inhibition of HIV-1-Induced Cytopathogenicity in MT-4 Cells. Human 5×10^5 MT-4 cells were infected with 100 $CCID_{50}$ HIV-1 (strain $HLTV\text{-}III_B$)/mL and seeded in 200 μL wells of a microtiter plate, containing appropriate dilutions of the test compounds.[50] After 5 days of incubation at 37 °C, the number of viable cells was determined in a blood cell counting chamber by trypan blue dye exclusion.

Acknowledgment. This work was supported by grants from the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek (Project no. 3.0040.83 and 3.0040.87), the Belgian Geconcerteerde Onderzoeksacties (Conventie no. 85/90-79), and the AIDS Basic Research Programme of the European Communities). We would like to thank Lizette van Berckelaer and Ann Absillis for excellent technical assistance. I.A.M. is deeply grateful to the Alexander von Humboldt-Stiftung (Bonn-Bad-Godesberg, Germany) for partial financial support.

Registry No. **5a**, 14980-09-7; **5b**, 28867-47-2; **6a**, 126716-23-2; **6b**, 126716-24-3; **7a**, 124939-87-3; **7b**, 124939-88-4; **8b**, 133776-28-0; **9a**, 126737-61-9; **9b**, 126716-26-5; **10a**, 126716-27-6; **10b**, 112695-36-0; **11a**, 133814-60-5; **11b**, 122654-34-6; **12**, 133776-08-6; **13**, 133776-09-7; **14**, 133776-10-0; **15a**, 133776-11-1; **15b**, 133776-29-1; **16**, 112668-58-3; **17**, 133776-12-2; **18**, 133776-13-3; **19**, 112668-60-7; **20**, 133776-14-4; **21**, 133776-15-5; **22**, 133776-16-6; **23**, 57994-13-5; **24**, 133776-17-7; **25**, 123402-20-0; **26**, 75059-22-2; **27**, 123402-21-1; **28**, 133794-39-5; **29a**, 133776-18-8; **29b**, 133776-30-4; **30a**, 133776-19-9; **30b**, 133776-31-5; **31a**, 133776-20-2; **31b**, 133776-32-6; **32a**, 133776-21-3; **32b**, 133776-33-7; **33a**, 133776-22-4; **33b**, 133776-34-8; **34a**, 133776-23-5; **34b**, 132776-27-3; **35a**, 133776-24-6; **35b**, 133776-35-9; **36a**, 133776-25-7; **36b**, 123334-78-1; **37a**, 133776-26-8; **37b**, 133776-36-0; **38a**, 133776-27-9; **38b**, 133776-37-1; bis(trimethylsilyl)uracil, 10457-14-4; bis(trimethylsilyl)cytosine, 18037-10-0; bis(trimethylsilyl)-N^6-benzoyladenine, 18055-46-4; bis(trimethylsilyl)-N^2-acetylguanine, 133776-38-2; bis(trimethylsilyl)-N^2-lauroylguanine, 133776-39-3; bis(trimethylsilyl)-N^4-benzoylcytosine, 133776-40-6.

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Design, Synthesis, and Pharmacological Evaluation of Ultrashort- to Long-Acting Opioid Analgetics

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In an effort to discover a potent ultrashort-acting μ opioid analgetic that is capable of metabolizing to an inactive species independent of hepatic function, several classes of 4-anilidopiperidine analgetics were synthesized and evaluated. One series of compounds displayed potent μ opioid agonist activity with a high degree of analgesic efficacy and an ultrashort to long duration of action. These analgetics, 4-(methoxycarbonyl)-4-[(1-oxopropyl)phenylamino]-1-piperidinepropanoic acid alkyl esters, were evaluated in vitro in the guinea pig ileum for μ opioid activity, in vivo in the rat tail withdrawal assay for analgesic efficacy and duration of action, and in vitro in human whole blood for their ability to be metabolized in blood. Compounds in this series were all shown to be potent μ agonists in vitro, but depending upon the alkyl ester substitution the potency and duration of action in vivo varied substantially. The discrepancies between the in vitro and in vivo activities and variations in duration of action are probably due to different rates of ester hydrolysis by blood esterase(s). The SAR with respect to analgesic activity and duration of action as a function of the various esters synthesized is discussed. It was also demonstrated that the duration of action for the ultrashort-acting analgetic, **8**, does not change upon prolonged infusion or administration of multiple bolus injections.

Fentanyl, a potent short-acting analgetic, is used clinically during surgical procedures as an adjunct to gaseous anesthesia.[1] As a result of fentanyl's clinical success and the desire to more clearly define the structural require-

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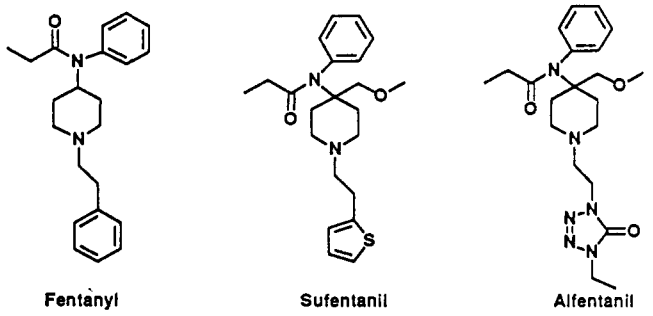
ments necessary for its μ opioid agonist efficacy, extensive efforts have been devoted to developing the SAR of the 4-anilidopiperidine class of analgetics.[2-4] As a result of these efforts two congeners of fentanyl, alfentanil[4,5] and sufentanil,[6] were discovered and have found clinical utility as anesthesia adjuncts. In comparison with fentanyl, alfentanil has a shorter duration of action and sufentanil has 5–10-fold greater potency.[6,7]

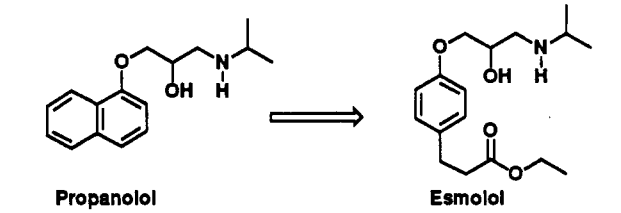


Fentanyl Sufentanil Alfentanil

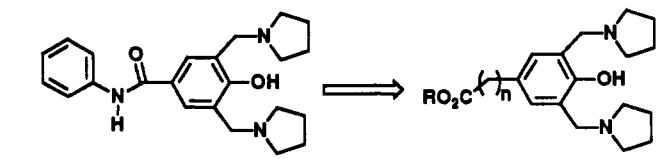
With the emphasis in anesthetic and surgical practice focusing on shorter and outpatient surgical procedures, the need for an ultrashort acting opioid analgetic has surfaced.[8] Although alfentanil is considered an ultrashort acting agent, its terminal half-life in humans, approximately 70–90 min, is longer than desired.[7] Our research was based upon the premise that the ideal analgetic would have a biological half life ranging from 10–30 min. Rapid elimination or biotransformation of such an agent to inactive or less active products would minimize accumulation and subsequent redistribution with prolonged or repeated administration. Furthermore, respiratory depression and muscle rigidity, two well-documented μ opioid effects with agonists of high intrinsic efficacy, would be of short duration. Thus, these side effects, if present, would not be considered a major drawback due to the presence and routine use of respiratory support and muscle relaxants in the surgical setting.

In order to discover an analgetic with the desired profile, we modified the 4-anilidopiperidine structure of analgetics such that the metabolism could be achieved through a rapid enzymatic reaction in the blood. By employing this strategy the analgetic could be rapidly inactivated independent of hepatic function.[9] Furthermore, rapid degradation of the analgetic to inactive or less active metabolites in the blood would allow more predictable correlation of dose with duration of pharmacologic effect.[10]

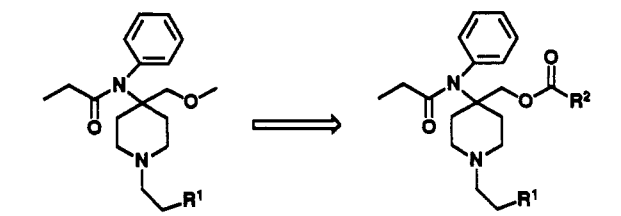
This strategy is elegantly illustrated by the short-acting β-blocker esmolol.[11] It has been demonstrated that blood esterases hydrolyze the ester function of esmolol to produce the resultant carboxylic acid which is devoid of activity.



Propanolol Esmolol



ACC-9358



R^1= 2-thienyl, phenyl (1)
R^2= Me, Et, OMe, OEt

More recently, it was reported that the antiarrythmic ACC-9358 could be transformed into ultrashort-acting agents through a similar approach by substituting the formanilide portion of ACC-9358 with alkyl esters.[12] In-

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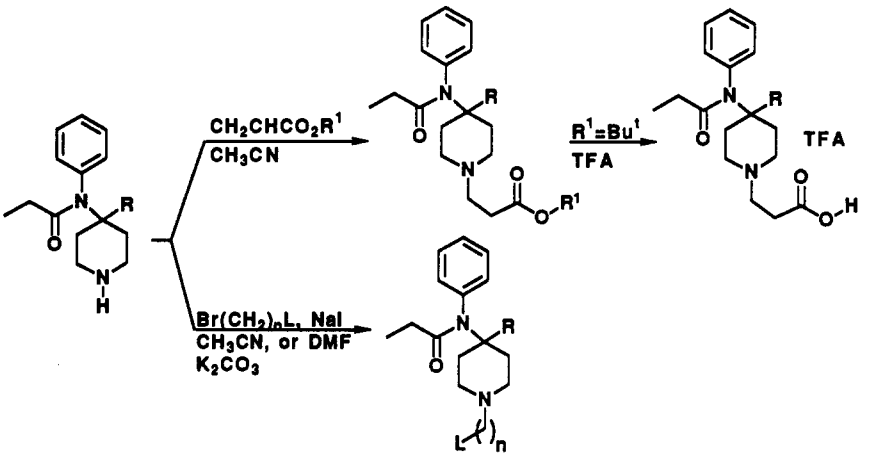
Scheme I



R= H, CH_2OMe, CO_2Me
n= 1,3,4
R^1= Alkyl
L= see Table 1

deed this strategy has also been employed with the 4-anilidopiperidine class of analgetics, however, with only marginal success.[4f] Replacement of the 4-methoxymethyl substituent of sufentanil and 1 with 4-carboalkoxy esters and carbonates produced potent analgetics, but the durations of action were longer than desired.

In order to discover 4-anilidopiperidine analgetics which retain the high degree of potency of the prototypes and have the targeted pharmacokinetic profile, judicious incorporation of an ester function was required. Review of the many SAR studies conducted on this class of analgetics revealed that relatively little has been published on the SAR of the pendant piperidine nitrogen substituent (NR).[3c,d,4h,k] The two most common NRs used are the phenethyl and 2-thienylethyl. Both of these groups are very lipophilic and this undoubtedly reflects the complementary lipophilic binding site they occupy in the μ receptor. In order to achieve the set goals, our strategy involved replacing the aryl groups with a lipophilic group that would still retain the binding characteristics of the aryl groups, but upon enzymatic degradation would yield a polar group with less affinity for the μ receptor, and greater chances for rapid elimination. The chemcial approach examined to achieve these goals is discussed in the results and discussion section.

Chemistry

Methyl 4-bromobutanoate and methyl 5-bromopentanoate were made according to literature protocols.[13] The acrylates used that were not commercially available were synthesized by adding triethyl amine to acryloyl chloride and the corresponding alcohol in dichloromethane at 0 °C. The 4-substituted and 4,4-disubstituted piperidines used in the alkylation and Michael addition reactions were prepared by using known procedures.[3b,6,14] Alkylation of these piperidines was achieved in yields generally ranging from 60–90% by treating the secondary amines with alkyl bromides in acetonitrile or dimethyl formamide in the presence of potassium carbonate and sodium iodide. For those compounds where two methylene units separated the piperidine nitrogen and the ester moiety, the Michael reaction with the acrylates was employed to give the products in 80–100% yields. The carboxylic acid salts, **6** and **12**, were synthesized by treating the corresponding *tert*-butyl esters with neat trifluoroacetic acid. See Scheme I. Table I lists the final products synthesized along with some of the physical and biological data collected.

Pharmacology and Metabolism

Compounds were tested for opioid activity in vitro in the isolated guinea pig ileum[15] (GPI) and for analgesic efficacy and duration of action in vivo by using a modified rat tail withdrawal (RTW) reflex model.[16] For some of the preferred compounds, the half-lives were determined in vitro in human whole blood. The biological results are shown in Table I. See the Experimental Section for the details of how the assays were conducted.

Results and Discussion

The SAR of the compounds was determined by the in vitro potency for inhibiting contractions of the electrically stimulated GPI and the in vivo potency and duration of action in the RTW assay. Ideally, we wished to discover compounds as potent as fentanyl and its congeners both in vitro and in vivo, but with a duration of action in the rat of less than 15 min. For those compounds with the desired profile in the primary assays, the in vitro half-lives in human whole blood were determined in order to assess the ability of the compounds to be metabolized by esterases in the blood.

After several unsuccessful approaches at replacing the NR substituent on the fentanyl nucleus with a hydrolytically labile phenyl bioisostere, we found that appending alkyl esters onto the piperidine nitrogen, compounds **2–5**, yielded analgetics with weak in vitro and in vivo potency, but with the desired duration of action in vivo. It has been shown in the literature that replacement of the fentanyl nucleus, R = H, with the carfentanil piperidine, R = CO_2CH_3, increases the analgesic potency of this class of compounds.[6a] Indeed, upon substituting the alkyl ester groups for the phenethyl moiety of carfentanil, compounds **7**, **8**, **10**, **11**, and **13–26**, potent analgetics with most having an ultrashort duration of action in the RTW and the hu-

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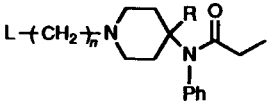
Table I. Physical and Biological Properties of Substituted 4-Anilidopiperidines



cmpd	*n*	L	R	mp, °C	formula	analysis[a,b]	in vitro EC_{50}, M[c]	in vivo ED_{50}, mg/kg[d]	duration in vivo, min[d]	human blood metabolism: $t^1/_2$, min
2	1	CO_2CH_3	H	130–133	$C_{17}H_{24}N_2O_3 \cdot C_4H_4O_4$	C,H,N	$1.27 \pm 0.07 \times 10^{-5}$	>60	–	–
3	2	CO_2CH_3	H	117–119	$C_{18}H_{26}N_2O_3 \cdot C_4H_4O_4$	C,H,N	$1.66 \pm 0.59 \times 10^{-6}$	3.2	10–15	–
4	3	CO_2CH_3	H	102–104	$C_{19}H_{28}N_2O_3 \cdot C_4H_4O_4$	C,H,N	$3.60 \pm 0.30 \times 10^{-6}$	3.4	10	–
5	4	CO_2CH_3	H	105–106	$C_{20}H_{30}N_2O_3 \cdot C_4H_4O_4$	C,H,N	$5.44 \pm 1.51 \times 10^{-6}$	49.4	45	–
6	2	CO_2H	H	187–189	$C_{17}H_{24}N_2O_3 \cdot C_2HF_3O_2$	C,H,N	1.87×10^{-5}[f]	>30	–	–
7	1	CO_2CH_3	CO_2CH_3	130–135	$C_{19}H_{26}N_2O_5 \cdot C_2H_2O_4$	*a*	$5.39 \pm 1.02 \times 10^{-6}$	39.1	60	–
8	2	CO_2CH_3	CO_2CH_3	168–170	$C_{20}H_{28}N_2O_5 \cdot C_2H_2O_4$	C,H,N	$3.55 \pm 0.23 \times 10^{-9}$	0.0044	15	37
9	2	CO_2CH_3	CH_2OCH_3	180–182	$C_{20}H_{30}N_2O_4 \cdot C_2H_2O_4$	C,H,N	$1.03 \pm 1.00 \times 10^{-5}$	–	–	–
10	3	CO_2CH_3	CO_2CH_3	153–155	$C_{21}H_{30}N_2O_5 \cdot C_2H_2O_4$	C,H,N	$1.50 \pm 0.98 \times 10^{-8}$	0.0016	20	–
11	4	CO_2CH_3	CO_2CH_3	162–164	$C_{22}H_{32}N_2O_5 \cdot C_2H_2O_4$	C,H,N	$1.02 \pm 0.65 \times 10^{-8}$	0.8	30	–
12	2	CO_2H	CO_2CH_3	189–190	$C_{19}H_{26}N_2O_5 \cdot C_2HF_3O_2$	C,H,N	$1.95 \pm 0.08 \times 10^{-6}$	1.6	35	–
13	2	$CO_2CH_2CH_3$	CO_2CH_3	166–167	$C_{21}H_{30}N_2O_5 \cdot C_2H_2O_4$	C,H,N	$4.88 \pm 1.17 \times 10^{-8}$	0.0017	10	37
14	2	$CO_2(CH_2)_2CH_3$	CO_2CH_3	169–170	$C_{22}H_{32}N_2O_5 \cdot C_2H_2O_4$	C,H,N	$1.79 \pm 0.09 \times 10^{-9}$	0.14	20	20
15	2	$CO_2CH(CH_3)_2$	CO_2CH_3	176–177	$C_{22}H_{32}N_2O_5 \cdot C_2H_2O_4$	C,H,N	$1.08 \pm 0.39 \times 10^{-9}$	<0.03	10	63
16	2	$CO_2(CH_2)_3CH_3$	CO_2CH_3	153–154	$C_{23}H_{34}N_2O_5 \cdot C_2H_2O_4$	C,H,N	$1.78 \pm 0.96 \times 10^{-8}$	0.17	5	10
17	2	$CO_2CH(CH_3)CH_2CH_3$	CO_2CH_3	160–161	$C_{23}H_{34}N_2O_5 \cdot C_2H_2O_4$	C,H,N	$8.65 \pm 1.86 \times 10^{-10}$	0.046	5	77
18	2	$CO_2CH_2CH(CH_3)_2$	CO_2CH_3	177–178	$C_{23}H_{34}N_2O_5 \cdot C_2H_2O_4$	C,H,N	$4.35 \pm 1.84 \times 10^{-9}$	0.015	5	8.3
19	2	$CO_2C(CH_3)_3$	CO_2CH_3	157–158	$C_{23}H_{34}N_2O_5 \cdot C_2H_2O_4$	*a*	$1.04 \pm 0.16 \times 10^{-9}$	21 ng/kg	85	–
20	2	$CO_2(CH_2)_4CH_3$	CO_2CH_3	141–142	$C_{24}H_{36}N_2O_5 \cdot C_2H_2O_4$	C,H,N	$1.08 \pm 1.00 \times 10^{-8}$	0.17	15	32
21	2	$CO_2CH_2CH(CH_3)CH_2CH_3$	CO_2CH_3	160–161	$C_{24}H_{36}N_2O_5 \cdot C_2H_2O_4$	C,H,N	$4.50 \pm 0.90 \times 10^{-7}$	0.18	5	12
22	2	$CO_2(CH_2)_2CH(CH_3)_2$	CO_2CH_3	148–149	$C_{24}H_{36}N_2O_5 \cdot C_2H_2O_4$	C,H,N	$9.70 \pm 8.99 \times 10^{-8}$	0.16	15	26
23	2	$CO_2CH_2C(CH_3)_3$	CO_2CH_3	157–158	$C_{24}H_{36}N_2O_5 \cdot C_2H_2O_4$	C,H,N	$1.50 \pm 0.27 \times 10^{-8}$	0.22	10	67
24	2	$CO_2(CH_2)_5CH_3$	CO_2CH_3	141–142	$C_{25}H_{38}N_2O_5 \cdot C_2H_2O_4$	C,H,N	$3.30 \pm 0.85 \times 10^{-8}$	0.4	25	122
25	2	$CO_2(CH_2)_6CH_3$	CO_2CH_3	129–130	$C_{26}H_{40}N_2O_5 \cdot C_2H_2O_4$	C,H,N	$4.20 \pm 0.54 \times 10^{-8}$	1.7	10	154
26	2	$CO_2(CH_2)_7CH_3$	CO_2CH_3	141–142	$C_{27}H_{42}N_2O_5 \cdot C_2H_2O_4 \cdot {}^1/_2H_2O$	C,H,N	$6.70 \pm 5.00 \times 10^{-8}$	>3.0	–	–
fentanyl	2	Ph	H	*e*	*e*	*e*	$1.76 \pm 0.36 \times 10^{-9}$	0.0046	60	*g*
sufentanil	2	2-thienyl	CH_2OCH_3	131–133	$C_{22}H_{30}N_2O_2S \cdot C_4H_4O_4$	C,H,N	$7.43 \pm 1.53 \times 10^{-9}$	0.0013	80	*g*
alfentanil	2	4-(1-ethyl)-Δ^2-tetrazolin-5-one	CH_2OCH_3	*e*	*e*	*e*	$2.01 \pm 0.12 \times 10^{-9}$	0.0045	55	*g*
carfentanil	2	Ph	CO_2CH_3	182–184	$C_{24}H_{30}N_2O_3 \cdot C_2H_2O_4$	C,H,N	$1.30 \pm 0.15 \times 10^{-9}$	520 ng/kg	45	–

[a] A satisfactory C, H, N analysis was not obtained. [b] All values were within ±0.4 of theoretical. [c] EC_{50} values for inhibition of electrically evoked contraction in guinea pig ileum were estimated by fitting mean concentration–response data from four or more tissues to the logistic function: response = $(M[A]^n)/([A]^n + k^n)$ where: M = maximal response produced in the tissue by the test compound; [A] = concentration of test compound; n = apparent kinetic order of the response at low concentrations of test compound; and k = concentration of test compound required to produce a half-maximal response (EC_{50}).[20-22] Goodness of fit can be shown by the standard error of estimate of these parameters. Variability in the mean data points used for curve fitting were generally 1–5% of maximal response or less than 10% of response. [d] ED_{50} values for analgesic effects of the test compound in the rat tail withdrawal assay were determined by first calculating the change in tail withdrawal latency produced by a dose of test compound (mean values from three or more rats). These values were then expressed as percentages of the maximum effect (15 s (cutoff value) – baseline latency). The percentage changes in the tail withdrawal latency were then plotted and the ED_{50} was determined by graphical interpolation. Standard errors of the mean for the individual data points were generally less than 2.0 s, i.e., less than 13% of maximal effect. Variability tended to be greatest during the recovery phase of response and not at maximal or baseline responses used to calculate the dose–response curves. Curve fitting was not used due to the generally small number of data points available for fitting. [e] Purchased from commercial suppliers. [f] Maximum inhibition of contraction was only 17.4%. [g] The standards, fentanyl, sufentanil, and alfentanil, were not incubated in human whole blood, but were tested in rat blood and compared with 8. In this study more than 90% of 8 disappeared after incubation for 30 s, whereas 75%, 65%, and 85% of fentanyl, sufentanil, and alfentanil, respectively, were still present after 1 h. [h] Dashed lines mean that the data was not obtained.

man whole blood assays were obtained. Varying the length of the methylene tether, compounds **7**, **8**, **10**, and **11** revealed that two methylene units between the piperidine nitrogen and the methyl ester were optimal for added potency and decreased duration of action. Use of the sufentanil nucleus, R = CH_2OCH_3, with the methyl propanoate NR, **9**, gave a weak opioid agonist. The carboxylic acid **12**, resulting from hydrolysis of the esters **8** and **13–26**, was approximately 1000 times less potent in the GPI assay and 350 times less potent in the RTW assay than **8**. Therefore, the hydrolysis of the alkyl propanoate esters to **12** is a mechanism by which opioid activity is rapidly lost. Indeed, it was demonstrated that **8** is nearly quantitatively converted to **12** in vitro in human whole blood with a half life of approximately 35 min.

The effects of the alkyl ester portion on potency and duration of action was revealed in compounds **8** and **13–26**. All of the esters were potent μ opioid agonists as demonstrated by the EC_{50} values in the GPI. However, variation in the size and substitution pattern of the ester produced interesting effects on both in vivo potency and in vivo and in vitro (human whole blood assay) half-lives. The most potent compounds in the GPI and RTW assays were those which have a methine or quaternary carbon alpha to the ester oxygen. The increased in vivo activity was partially due to the decreased rate of ester hydrolysis. Since the hydrolysis of most of the compounds in this series was rapid in the rat, the in vivo activity reflects a combination of the potencies of both the ester and the resultant hydrolysis product, carboxylic acid **12**.[17] Thus, since carboxylic acid **12** shows weak activity in vivo, the esters which hydrolyze extremely rapidly typically had decreased in vivo potency relative to esters which hydrolyze slowly.[18] This effect is notably manifested by comparing the RTW assay potency (ED_{50}) of the extremely slowly metabolized *tert*-butyl ester **19** with any of the other ester's ED_{50} values even though they all have comparable potencies in the GPI assay.

For the straight chain esters the potencies in the RTW assay decreased as chain length increased from two to eight carbons (ethyl to octyl). This trend cannot be solely rationalized by an increase in ester hydrolysis rate with increasing chain length. In human whole blood, the rate of hydrolysis increased in the series methyl to butyl, but increasing the ester chain length beyond five carbons markedly decreases the hydrolysis rate. The measurement of the half-lives of this series of compounds in human blood revealed the substrate specificity of the blood enzyme(s) for hydrolyzing the various esters. Also, these results demonstrated that within one series of compounds potent analgetics with durations of action ranging from ultrashort to long are available depending upon the choice of the alkyl ester.

It is known that with the more lipophilic analgetics, fentanyl and sufentanil, the rate of elimination of the drugs

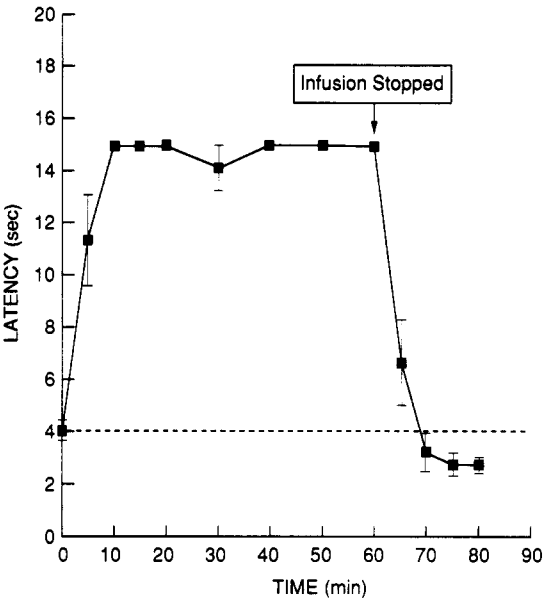


Figure 1. Effects of infusion of 8·HCl on rat tail withdrawal latency. 8·HCl was infused into the femoral vein of three rats for 60 min at a dose of 2 μg/kg per min. During and after the infusion, analgesic effects were measured with a tail withdrawal assay. Analgesia dissipated rapidly after termination of the infusion with a time course similar to that seen after a single bolus injection. These studies showed no evidence for accumulation of 8·HCl during a 60-min infusion.

is limited by redistribution.[7] For an ideal ultrashort acting agent elimination should not be dependent upon redistribution since this would cause prolongation of pharmacologic effects upon infusion or repeated administration. In order to assess whether the metabolism of these ultrashort acting agents was limited by redistribution, two experiments were conducted with 8·HCl. Rats were infused with 2 μg/kg per min 8·HCl for 60 min. This treatment yielded a maximal analgesic effect over the infusion period. Upon ceasing the infusion, the rate of loss of pharmacologic effect was no different than with a single bolus injection. Furthermore, administration of multiple bolus injections of 8·HCl in rapid succession to rats with doses that give maximal latency in the RTW assay did not effect the rate in which tail withdrawal latency returned to control values. These results indicated that 8·HCl shows no evidence for accumulation. See Figures 1 and 2.

Additional pharmacological experiments with **8** demonstrated that the major μ opioid side effects, respiratory depression, bradycardia, and muscle rigidity, were also of short duration. In a study performed in conscious dogs, the respiratory effects of 8·HCl and sufentanil were monitored by measuring levels of oxyhemoglobin saturation using pulse oximetry.[19] Spontaneous respiration was suppressed for 37.7 ± 14.8 min (n = 3) in dogs which received sufentanil (7.3 nmol/kg) and were given respiratory support. Oxyhemoglobin saturation fell to a similar level in these dogs (64.7 ± 3.8%) and dogs which received a 40 times greater dose of 8·HCl (53.5 ± 5.5%, 290 nmol/kg, n = 4). After receiving 8·HCl, oxyhemoglobin saturation returned to control levels in less than 15 min without respiratory assistance. Although direct comparison

(17) For this series of compounds the half-life in rat blood for the conversion of the esters to the carboxylic acid **12** was much shorter than in human blood. The half-life of **8** in vitro in rat blood was approximately 30 s compared to 37 min in human blood.

(18) The in vivo degradation rate constants of several esters have been estimated and shown to be in agreement with the experimental results by using a combined pharmacokinetic and pharmacodynamic model. The qualitative argument given in the text is commensurate with the mathematical modeling results. (a) Lutz, M. W.; Morgan, P. H.; James, M. K.; Bilotta, J. M. *FASEB J.* **1990**, *4*, A563. (b) Lutz, M.; Morgan, P.; Bilotta, J. M.; Schuster, S. V.; Lahey, A. P.; James, M. K.; Feldman, P. L. Unpublished results.

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(22) James, M. K.; Morgan, P. H.; Leighton, H. J. *J. Pharmacol. Exp. Ther.* **1989**, *249*, 61.

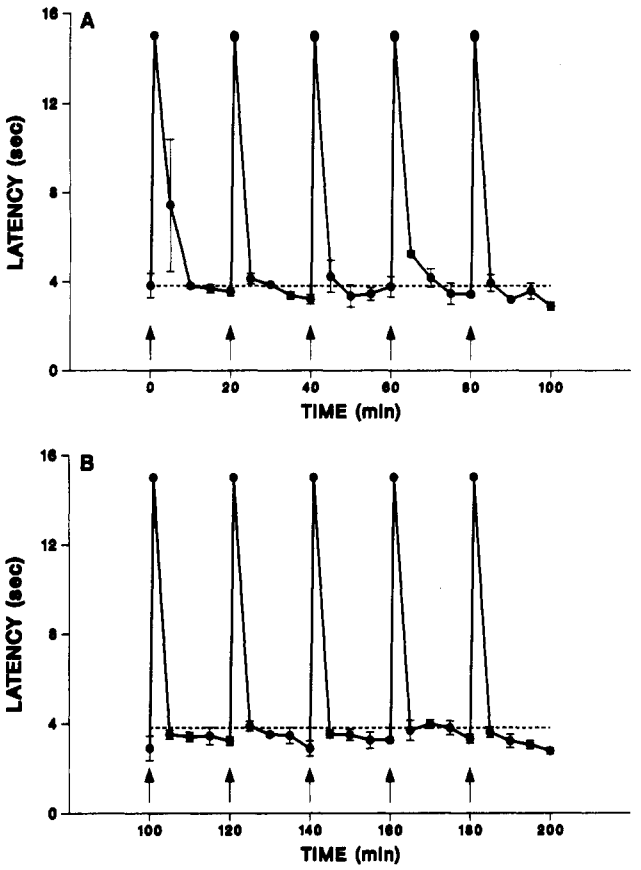

Figure 2. Effects of multiple bolus doses of 8·HCl on rat tail withdrawal latency. A total of 10 intravenous bolus doses of 8·HCl (10 μg/kg) were given in rapid succession in order to assess the propensity of 8·HCl to accumulate on repeated dosing (n = 3). Accumulation would be indicated in this study by broadening of the response peaks (longer periods of elevated tail withdrawal latency). No evidence for accumulation of 8·HCl was seen in this study.

is difficult, these data suggest that 8·HCl is less potent at producing respiratory depression and the effects are much shorter in duration than sufentanil.

In summary, several of the 4-anilido-4-(methoxycarbonyl)piperidines substituted with alkylpropanoate chains on the piperidine nitrogen are potent ultrashort-acting analgetics which possess a nonhepatic means of inactivation via ester hydrolysis. The carboxylic acid resulting from ester hydrolysis is a weak opioid agonist relative to the parent esters. Durations of action, as measured in the RTW assay, range from extremely short to long (5 to 85 min) depending upon the substitution of the alkyl portion of the ester. For the ultrashort-acting analogue 8, no evidence for accumulation of the drug was detected after prolonged infusion or administration of multiple bolus injections in rats. As a result of these unique pharmacokinetic properties coupled with a high degree of potency these analgesics have potential as clinically useful additions to the narcotic analgesic family.

Experimental Section

Representative examples of experimental procedures used to synthesize all of the compounds listed in Table I are given. All of the final synthesized products shown in Table I were characterized by obtaining melting points (uncorrected), ^{1}H and ^{13}C NMR spectra (obtained on the free base samples), and C, H, and N elemental analyses unless noted otherwise. ^{1}H and ^{13}C NMR were determined using a Varian spectrometer (300-MHZ superconducting, FT instrument), with $CDCl_3$ as the solvent, unless noted otherwise, and are expressed in ppm downfield from internal tetramethylsilane. Elemental analyses were performed by Atlantic Microlab Inc., Norcross, GA. Reagents were used as received from commercial suppliers. Concentration of the reaction mixtures was performed by using a rotary evaporator at water aspirator pressure. Flash silica gel chromatography was conducted with 32–63 μm grade Universal Scientific grade silica gel.

4-[(1-Oxopropyl)phenylamino]-1-piperidinebutanoic Acid Methyl Ester (4). A mixture of 4-[(1-oxopropyl)phenylamino]piperidine (250 mg, 1.08 mmol), methyl 4-bromobutanoate[13] (224 mg, 1.23 mmol, 115 mol%), NaI (81 mg, 0.54 mmol, 50 mol%) and K_2CO_3 (298 mg, 2.15 mmol, 200 mol%) in acetonitrile (CH_3CN) (1.1 mL) was stirred at 23 °C for 5 h. The mixture was diluted with H_2O and ethyl acetate (EtOAc). The phases were separated and the aqueous extracted with EtOAc (2×). The combined organics were washed with brine, dried (Na_2SO_4), and concentrated. The residue was chromatographed on silica gel (EtOAc) to give the free base of 4 (223 mg, 62%) as an oil: ^{1}H NMR δ 7.35–7.31 (m, 3 H), 7.04–7.01 (m, 2 H), 4.65–4.55 (m, 1 H), 3.53 (s, 3 H), 2.85–2.82 (bd, 4 H, J = 7.31 Hz), 2.23 (q, 4 H, J = 7.31), 2.00 (bt, 2 H, J = 2.1), 1.88–1.83 (q, 2 H, J = 7.32), 1.73–1.70 (m, 4 H), 1.68–1.66 (m, 2 H), 0.98–0.93 (t, 3 H, J = 7.32); ^{13}C NMR δ 173.7, 173.3, 139.1, 130.4, 129.1, 128.1, 57.5, 53.0, 52.4, 51.2, 32.0, 30.6, 28.4, 22.4, 9.5. The oil was dissolved in EtOAc and an equimolar amount of maleic acid in ether was added to give the maleate salt as a white solid.

4-(Methoxycarbonyl)-4-[(1-oxopropyl)phenylamino]-1-piperidinepropanoic Acid Methyl Ester (8). To a solution of 4-(methoxycarbonyl)-4-[(1-oxopropyl)phenylamino]-piperidine[6,14] (200 mg, 0.68 mmol) in CH_3CN (1.1 mL) was added methyl acrylate (124 μL, 1.36 mmol, 200 mol%) at 23 °C. The solution was stirred at 50 °C for 2 h, cooled to room temperature, and concentrated. The residue was chromatographed on silica gel (EtOAc) to give 8 free base (253 mg, 97%) as an oil: ^{1}H NMR δ 7.38–7.33 (m, 3 H), 7.26–7.23 (m, 2 H), 3.62 (s, 3 H), 3.57 (s, 3 H), 2.61–2.53 (m, 4 H), 2.39–2.34 (m, 4 H), 2.32–2.19 (bd, 2 H, J = 14 Hz), 1.84–1.79 (q, 2 H, J = 7.5), 1.59–1.49 (m, 2 H), 0.92–0.87 (t, 3 H, J = 7.5); ^{13}C NMR δ 174.3, 174.2, 173.1, 139.9, 131.0, 129.5, 128.9, 63.2, 53.6, 52.2, 51.7, 49.9, 33.9, 32.5, 29.3, 9.44. An equimolar amount of oxalic acid in ether is added to a solution of the free base of 8 dissolved in EtOAc. The oxalate salt 8 precipitates as an analytically pure white solid.

1-Piperidine-4-[(1-oxopropyl)phenylamino]propanoic Acid (6). A solution of 4-[(1-oxopropyl)phenylamino]piperidine[3b] (500 mg, 2.15 mmol) and *tert*-butyl acrylate (0.37 mL, 2.58 mmol, 120 mol%) in CH_3CN (2.5 mL) was stirred at 23 °C for 24 h. The solution was concentrated and the residue chromatographed on silica gel (EtOAc) to give the Michael adduct (605 mg, 78%) as an oil. To the ester (309 mg, 0.85 mmol) was added trifluoroacetic acid (4 mL) at 23 °C. The solution was stirred at 23 °C for 1 h and then concentrated. The residue was triturated with ether to give 6 (316 mg, 88%) as a analytically pure white solid: mp 187–189 °C; ^{1}H NMR δ 7.34–7.29 (m, 3 H), 6.98–6.95 (m, 2 H), 4.80–4.75 (m, 1 H), 3.44–3.43 (bd, 2 H, J = 2.2 Hz), 3.17–3.13 (t, 2 H, J = 7.1), 2.70–2.78 (bt, 2 H, J = 2.5), 2.71–2.66 (t, 2 H, J = 7.1), 1.91–1.87 (m, 4 H), 1.86–1.85 (bq, 2 H, J = 7.5), 0.94–0.92 (t, 3 H, J = 7.5); ^{13}C NMR δ 171.5, 137.3, 129.4, 129.3, 128.6, 51.8, 51.6, 49.1, 28.7, 28.0, 27.2, 9.1.

2-Methylbutyl Acrylate. To a stirred solution of 2-methyl butanol (2.0 g, 22.6 mmol) and acryloyl chloride (2.05 g, 22.6 mmol) in dichloromethane (5 mL) at 0 °C was added triethylamine (3.2 mL, 22.7 mmol) dropwise. The solution was stirred 30 min at 23 °C and then concentrated. The residue was extracted with hexanes (2 × 10 mL), the combined organics were concentrated, and the residue was purified by passing it through a short plug of silica gel (hexanes) giving 3.0 g (93%) of 2-methylbutyl acrylate as an oil.

Guinea Pig Ileum Assay (in Vitro). Compounds were tested for opioid activity in the isolated guinea pig ileum.[15] The terminal ileum was removed from male Hartley guinea pigs after sacrifice. The isolated ileum was washed and placed in Krebs–Henseleit buffer oxygenated with 95% O_2 and 5% CO_2 mixture and maintained at 37 °C. The washed ileum was cut into segments (2.0–2.5 cm) and mounted on platinum ring electrodes. The ileal segments were then placed in 10-mL temperature-controlled tissue baths containing oxygenated Krebs–Henseleit buffer. The tissues were connected to force–displacement transducers and stretched

to a resting tension of 1.0 g. The composition of Krebs–Henseleit buffer was as follows (millimolar): NaCl, 118.1; KCl, 4.15; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.23; $NaHCO_3$, 25.5; and glucose, 11.1.

The ileal segments were stimulated at 0.1 Hz, 0.5 ms duration at supramaximal voltage to induce contractions. Opioid activity in the test compounds was manifested as inhibition of electrically evoked contractions. A noncumulative concentration–effect curve for each test compound was performed to assess the ability of the compound to inhibit contraction in the guinea pig ileum. After the concentration–effect curve was completed, naloxone was added to the tissue baths to determine if the compound-induced inhibition of contraction was reversed. Antagonism of the inhibition by naloxone confirms that the inhibitory effects of the compounds are mediated through opioid receptors. Assay results are expressed as EC_{50} values (a measure of potency), defined as the concentration of the compound producing 50% of the maximal response, and are expressed in molar units.

Rat Tail Withdrawal Assay. The analgesic efficacy of test compounds were evaluated in a rat tail withdrawal assay.[16] Male Sprague-Dawley rats were anesthetized and implanted with femoral vein cannulae and allowed to recover overnight. After recovery, the test compounds were administered intravenously through the cannula and effects on tail withdrawal latency were measured. Tail withdrawal latency was measured as the time to tail movement after exposure of the tail to a radiant heat source. The heat source was calibrated to produce a temperature of 62 °C after 15 s. Tail withdrawal latency in the absence of drugs was 4–7 s. Test compounds demonstrating analgesic activity prolong tail withdrawal latency beyond that seen in the absence of drugs. A maximal latency cutoff of 15 s was imposed to prevent tissue damage. The assay was verified with known opioids as standards. Results of these studies are expressed as ED_{50} values, calculated as the dose producing a tail withdrawal latency equal to half the difference between the maximum latency (15 s) and the baseline latency (4–7 s). ED_{50} values are expressed as milligrams of compound/kilogram of body weight. Duration of action is defined as the time (in minutes) necessary for the tail withdrawal response to return to baseline values after being elevated in response to drug administration. Duration of action is measured at the lowest dose producing a 15-s (maximum) tail withdrawal latency.

Human Whole Blood Metabolism Assay for 8 and 13–26. The assay using human blood was performed as follows. Fresh, heparinized human blood (20 mL) was placed in a temperature-controlled water bath at 37 °C for 10 min. At that time, 400 μL of a 2 mg/mL solution in deionized water of the test compound was added to the blood to give a blood concentration of 40 μg/mL of the test compound. Solutions of the test compounds were prepared just before the assay was started. Two 500-μL aliquots of blood were withdrawn at various timepoints for measurement of the concentrations of the test compound and the propanoic acid (**12**) formed by ester hydrolysis. A trial run was performed with each test compound and thereafter timepoints were set for sampling over 2–3 half-lives.

Table II

A. group 1		B. group 2	
compd	t_R, min	compd	t_R, min
propanoic acid **12**	5.1	*n*-pentyl ester **20**	14.3
methyl ester **8**	7.5	*n*-hexyl ester **24**	17.3
internal standard (**10**)	8.6	*n*-heptyl ester **25**	20.5
ethyl ester **13**	9.0	isopentyl ester **22**	14.1
isopropyl ester **15**	11.1	neopentyl ester **23**	13.8
propyl ester **14**	11.8	2-methylbutyl ester **21**	14.6
sec-butyl ester **17**	13.9	isobutyl ester **18**	12.2
isobutyl ester **18**	14.4	propanoic acid **12**	5.7
butyl ester **16**	14.9	internal standard (**10**)	7.9
		methyl ester **8**	7.2

CH_3CN (700 μL) was added to the samples removed from the incubation mixture along with 50 μL of a solution of the internal standard 4-(methoxycarbonyl)-4-[(1-oxopropyl)phenylamino]-1-piperidinebutanoic acid methyl ester (**10**), (0.12 mg/mL in CH_3CN). Samples were mixed and immediately centrifuged at 30000*g* for 10 min. The supernatant was removed and cooled to 8 °C. A 20-μL sample of the supernatant was injected into the high-performance liquid chromatograph (HPLC) for analysis.

The esters were analyzed by HPLC in two distinct groups. In group 1, a Keystone 5 μm Spherisorb CN (250 × 4.6 mm) column, a flow rate of 2 mL/min and a mobile phase of CH_3CN and 0.1 M phosphate buffer (pH 2.0) in a gradient elution (10% to 11% CH_3CN from 0 to 5 min, to 16% by 10 min and maintained to 12 min), provided baseline resolution for the propanoic acid **12** and internal standard **10** for all but the ethyl ester **13**. The peaks eluted as listed below in group 1 with a total run time of 16 min. By reducing the flow rate to 1 mL/min, baseline separation was obtained between the ethyl ester and the internal standard. Under these conditions, the compounds of interest eluted as follows: **12** at 10.1 min, **10** at 15.1 min, and **13** at 15.8 min. The analysis was monitored by UV at 220 nm.

For group 2 esters, the mobile phase was changed to 25% CH_3CN from 0 to 5 min increasing to 60% by 7 min and held to 18 min. Total run time was 25 min and peaks eluted as shown in group 2 of Table II.

Data from the HPLC assay was analyzed by a simple first-order kinetic model for disappearance of the test compounds and appearance of the propanoic acid **12**. First-order rate constants were calculated for each test compound from these data along with the apparent half-lives in minutes ($t_{1/2}$).

Acknowledgment. We thank Ms. Suzanne Collins for preparing the manuscript.