Synthesis and Pharmacological Evaluation of a Series of New 3-Methyl-1,4-disubstituted-piperidine Analgesics

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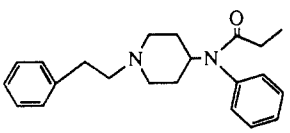
The synthesis and intravenous analgesic activity of a series of 3-methyl-4-(*N*-phenyl amido)piperidines, entries **34–79**, is described. The methoxyacetamide pharmacophore produced a series of compounds with optimal analgesic potency and short duration of action. *cis*-**42** was 13 036 times more potent than morphine and 29 times more potent than fentanyl; however, the corresponding diastereomer **43** was only 2778 and 6 times more potent, respectively. Compounds **40**, **43**, **47**, and **57** are extremely short acting; all had durations of action of about 2 min, which was about $^1/_5$ of that of fentanyl in the mouse hot-plate test at a dose equivalent to 2 times the ED_{50} analgesic dose. Among the many compounds that displayed exceptional analgesic activity, duration of action was one of the main factors for choosing a candidate for further pharmacological investigation. At present, *cis*-1-[2-(4-ethyl-4,5-dihydro-5-oxo-1*H*-tetrazol-1-yl)ethyl]-3-methyl-4-[*N*-(2-fluorophenyl)methoxyacetamido]piperidine hydrochloride (**40**) (Anaquest, A-3331.HCl, Brifentanil) is in clinical evaluation. Opiate analgesics that possess short duration of action are excellent candidates for short surgical procedures in an outpatient setting where a rapid recovery is required.

Introduction

In the search for the ideal analgesic devoid of the typical side effects common to all morphinomimetic compounds, investigators have modified the fentanyl structure (**1**) in various ways. Fentanyl is a well-known analgesic, 80–100 times more potent than morphine, with a fast onset and short duration of action. However, as with earlier opioids it produces profound respiratory depression, muscle rigidity, postoperative nausae, and physical dependence. As a result of this ongoing effort to develop the ideal analgesic, there are today an interesting number of novel compounds of the fentanyl family, all exhibiting an array of analgesic profiles. Carfentanil and sufentanil are the safest and most potent fentanyl congeners.[1,2] Afentanil is characterized by its short duration of action[3] while lofentanil is long-acting. Respiratory depression after an oral, subcutaneous or intravenous dose of lofentanil (**2**) of 0.7 mg/kg can last up to 48 h.[4]



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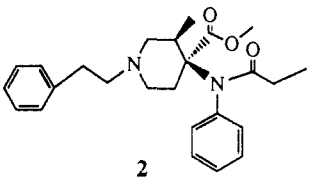

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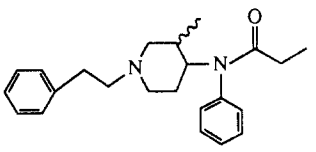

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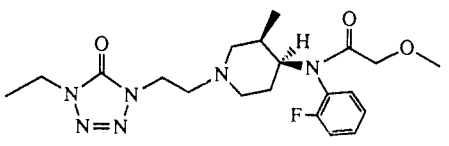

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As part of the continuing effort of our laboratories to develop therapeutically advantageous analgesics with rapid onset and short duration of action,[5] we initiated a research program based on the synthesis of a series of racemic cis and trans stereoisomers of 3-methyl-4-anilidopiperidine[6] (**3**). We wanted to evaluate the effect caused by changing the propionyl group to a methoxyacetyl, since this substitution is known to confer short duration of action in 4-anilidopiperidine analgesics.[7] It was foreseen that the highly lipophilic moieties would diffuse faster through the blood–brain barrier, while the more hydrophilic or ionized substituents would lead to analgesics with less lipid solubility (lower partition coefficients), little or no accumulation in fatty tissues, and rapid excretion. Opioid receptor binding affinities were determined for many of the compounds. These studies and secondary pharmacological results of selected compounds will be discussed herein.

Chemistry

The synthesis is outlined in Schemes I–III. All the compounds in Table I were synthesized with 1-benzyl-3-methylpiperidin-4-one (**5**) or with 1-phenethyl-3-methylpiperidin-4-one (**26**) as the starting material, which were prepared by the double Michael addition of *N*-benzyl- or

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(1) Van Bever, W. F. M.; Niemegeers, C. J. E.; Schellekens, K. H. L.; Janssen, P. A. J. *Arzneim.-Forsch. (Drug Res.)* **1976**, *26*, 1548.
(2) Niemegeers, C. J. E.; Schellekens, K. H. L.; Van Bever, W. F. M.; Janssen, P. A. J. *Arzneim.-Forsch. (Drug Res.)* **1976**, *26*, 1551.
(3) Niemergeers, C. J. E.; Janssen, P. A. J. *Drug Dev. Res.* **1981**, *1*, 83. Nauta, J.; DeLange, S.; Koopman, D.; Spierdijk, J.; Van Kleef, J.; Stanley, T. H. *Anesth. Analg.* **1982**, *61*, 267.
(4) Leysen, J. E.; Laduron, P. M. *Arch. Int. Pharmacodyn.* **1978**, *232*, 343.
(5) We have defined a compound to be short acting (S) if the effect is less than 6 min and intermediate (I) if between 6 and 15 min, and long duration (L) is anytime greater than 15 min, at a dose equivalent to 2 times the ED_{50} hot-plate analgesic dose.
(6) The term "cis" is applicable to those configurations in which the two functional groups in C-3 and C-4 lie on the same side of the plane of the ring and the term "trans" when the two groups lie on opposite sides of the plane. Janssen, P. A. J.; et al. U.S. Patent 3 907 813, 1975.
(7) Huang, B. S.; Terrell, R. C.; Deutsche, K. H.; Kudzma, L. V.; Lalinde, N. L. U.S. Patent 4 584 303, 1986.

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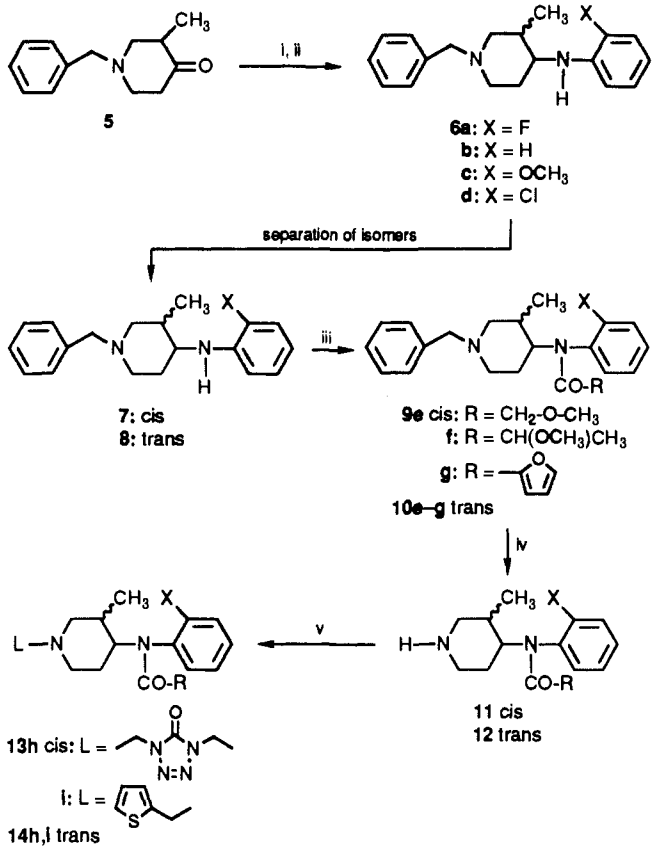
Scheme I[a]



[a] Reagents: (i) 2-X-aniline, $PhCH_3$, *p*-TsOH, Δ; (ii) $NaBH_3CN$, MeOH; (iii) RCOCl, CH_2Cl_2; (iv) 10% $Pd(OH)_2/C$, EtOH, H_2 (50 psi); (v) LBr, acetonitrile, NaI, Na_2CO_3.

N-phenethylamine, with methyl methacrylate and ethyl acrylate,[8] followed by cyclization and decarboxylation. By the use of *N*-benzyl-3-methyl-4-piperidin-4-one (**5**) as the starting material, it was possible to couple various piperidines (i.e., **11–23**) with different L substituents, such as 2-(chloroethyl)-2-thiophene and 1-(2-bromoethyl)-4-ethyl-1,4-dihydro-5*H*-tetrazol-5-one, as shown in Scheme I.

The synthesis of the cis isomers **36**, **37**, **45**, **46**, **51**, **53**, **61**, **62**, **70**, **76**, and **77** and of trans congeners **52**, **58**, **71**, and **73** is depicted in Scheme I. The condensation of the piperidone **5** with aniline or 1-substituted aniline, followed by reduction of the Schiff base with sodium borohydride, gave a 1-benzyl-3-methyl-4-(phenylamino)piperidine (**6**). Compounds **6** were produced in an approximate 7:3 (cis/trans) isomeric ratio. This was in agreement with previous literature reports.[9a] The cis isomer was always less polar than the trans isomer on silica, R_f (0.28) and (0.11), respectively (EtoAc/hexane, 1:4). Compounds **6** were separated by column chromatography into intermediates **7** and **8**, respectively. Acylation of **7** and **8**, with methoxyacetyl, furoyl, and methoxypropionyl chlorides, in methylene chloride, afforded intermediates **9** and **10**, respectively. Subsequent catalytic debenzylation produced the secondary amines **11** and **12**, which were N-alkylated in acetonitrile with appropriate L–X electrophiles.

In a different sequence of reactions (Scheme II, path A), intermediates **6** were first separated into isomers **15** and **16**, which in turn were treated with the corresponding acid chlorides, affording intermediates **17**, and **18**. The benzyl group of **17** and **18** was catalytically removed, giving rise to compounds **19** and **20**, which in turn were alkylated with the corresponding L–X groups (L = phenethyl, thienylethyl, and tetrazolinonylethyl) to give compounds **67** and **69**.

Compounds **21** were obtained by the acylation of the intermediates **6** (Scheme II). The products **21** were catalytically debenzylated as described above to give the nor compounds **22**. The intermediates **22** were utilized in two ways. Alkylation with appropriate L–X groups (path B) gave isomeric mixtures which were chromatographically separated to give compounds **25**. Specifically, compounds **40**,[9b] **42**, and **72** are of the cis form, and **41**, **43**, and **73** are their trans counterparts.

The final products, **56**, and **57**, were obtained by isomeric separation of the nor compound **22** (X = F) (Scheme II, path C) into intermediates **23** and **24**, followed by N-alkylation with 2-(chloroethyl)-2-thiophene and 1-(2-bromoethyl)-1,4-dihydro-5*H*-tetrazol-5-one, respectively.

All the compounds where L is phenethyl were prepared as indicated in Scheme III. The starting *N*-phenyethyl-3-methyl-4-piperidone (**26**) was condensed with aniline or 1-substituted aniline and reduced in the usual manner with sodium borohydride, producing the amines **27**. In one sequence of reactions, **27** was separated into compounds **28** and **29**. The final cis products **34**, **38**, **47**, **49**, **55**, **59**, **63**, **65**, **74**, and **78**, were produced through acylation of the amine **28** with the corresponding acid chlorides in dry methylene chloride. Compounds **35**, **39**, **48**, **60**, **64**, **66**, **75**, and **79** were the products of acylation of intermediate **29**. Compound **44** was obtained by a small variation in the reaction sequence. The intermediate **27** (X = OCH_3) was first acylated with the methoxyacetyl chloride, and then the resulting isomeric amides **30** were separated by column chromatography to afford **37**.

Pharmacology. Results and Discussion

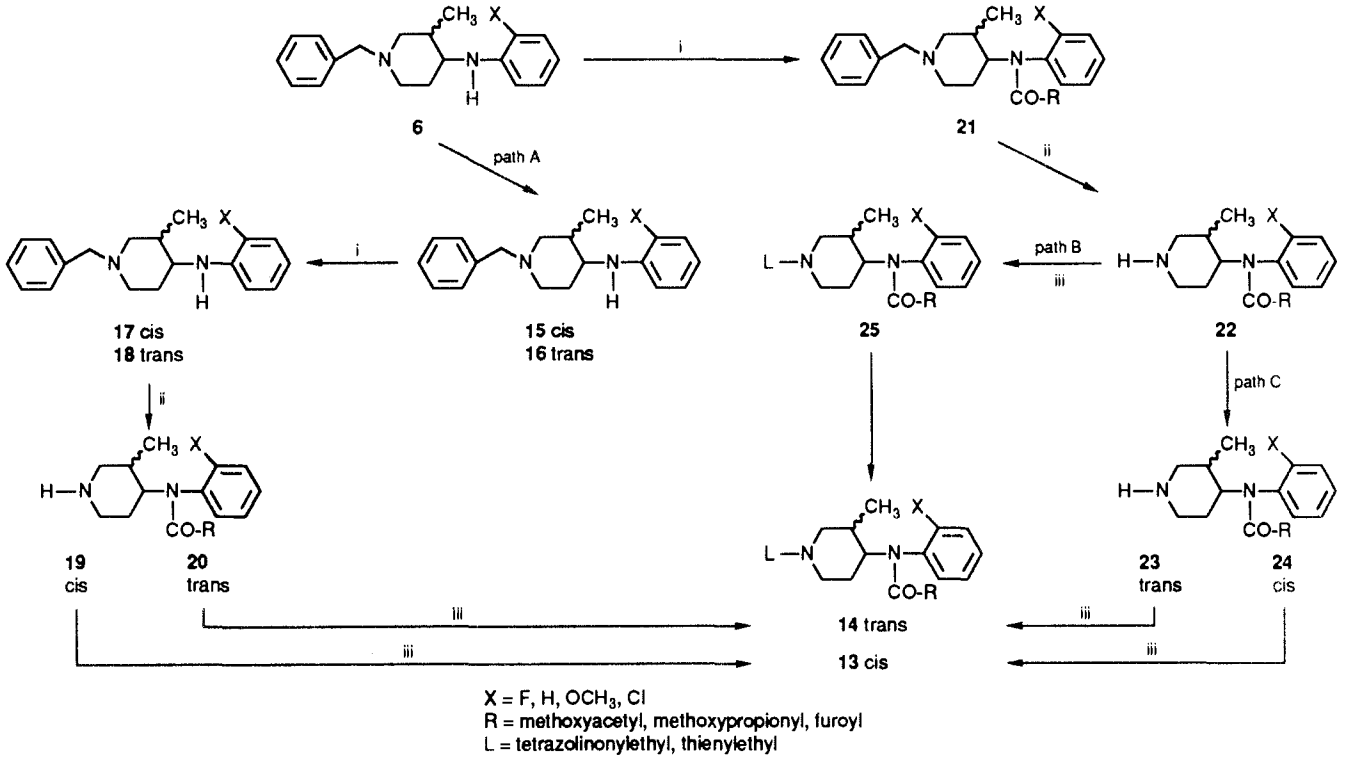
The analgesic properties of these compounds were evaluated by the mouse 55 °C hot-plate assay. An initial dose of 1 mg/kg was administered, and if 100% analgesia was observed, then lower dosing was continued until an ED_{50} was generated. If less than 100% analgesia was observed, then 5 mg/kg was administered. The duration of analgesia was also determined. Analgesics with relatively short duration of action, i.e., less than 6 min, at 2 × ED_{50} were again evaluated at doses equivalent to 8 times the ED_{50} analgesic dose, and those with durations of less than 9 min at 8 × ED_{50} were evaluated again at 16 times the ED_{50} analgesic dose. Many compounds were screened in vitro for their ability to displace [^{3}H]naloxone from its binding sites in rat brain membranes. Mouse ED_{50}'s and duration values are given in Table II.

The analgesic activity of compound **40**, along with that of fentanyl was evaluated in the mouse hot-plate (MHP), rat tail-flick (RTF), and rat hot-plate (RHP) tests (Table III). In addition, their anesthetic profile was evaluated

(8) (a) Beckett, A. H.; Casey, A. F.; Kick, G. *J. Med. Chem.* **1959**, *1*, 37. (b) Ziering, A.; Motchane, A.; Lee, J. *J. Med. Chem.* **1957**, *22*, 1521. (c) Ganellin, C. R.; Spickett, R. G. *J. Med. Chem.* **1965**, 8, 619. (d) Carabateas, P. M.; Grumback, L. *J. Med. Chem.* **1962**, *5*, 913.

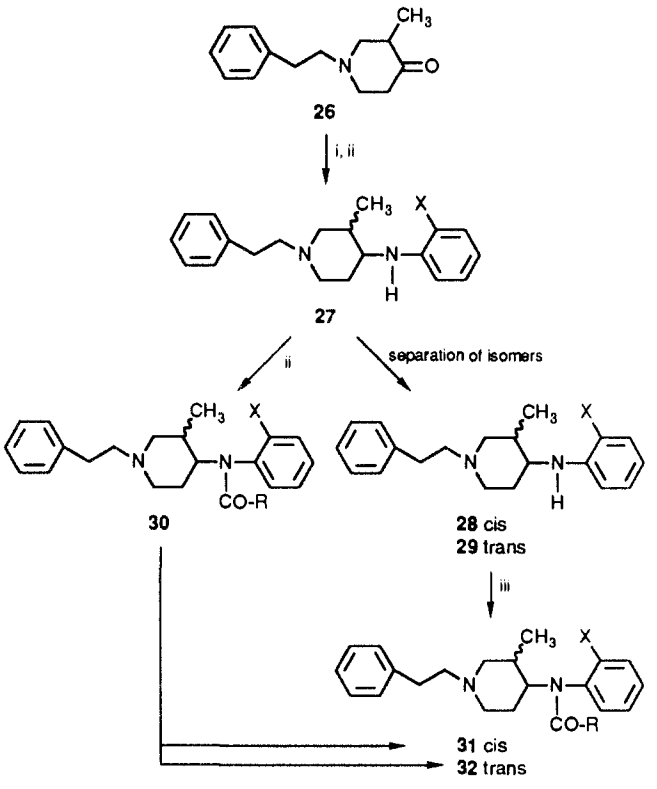
(9) (a) Van Bever, W. F. M.; Niemegeers, C. J. E.; Janssen, P. A. J. *J. Med. Chem.* **1974**, *17*, 1047. (b) Assuming a chair conformation for the piperidine ring, one would expect that the most predominant conformer would have an equatorial 4-N-($COCH_2OCH_3$) group with an equatorial 3-Me group for the trans compound and an axial 3-Me group for the cis compound.[9a] This was confirmed by the splitting pattern of the 4-proton on the piperidine ring. Compound **40** showed a multiplet, centered at δ 4.53, consisting of a doublet (J = 10.5 Hz) of triplets (J = 5.2 Hz). On the other hand, the trans compound **41** showed a multiplet centered, at δ 4.82, consisting of a triplet (J = 12.53 Hz) of doublets (J = 2.64 Hz).

Scheme II[a]



[a] Reagents: (i) RCOCl, CH_2Cl_2; (ii) 10% $Pd(OH)_2/C$, EtOH, H_2 (50 psi); (iii) LBr, 4-methyl-2-pentanone, NaI, Na_2CO_3.

Scheme III[a]



[a] Reagents: (i) 2-X-aniline, $PhCH_3$, *p*-TsOH, Δ; (ii) $NaBH_4$, MeOH; (iii) RCOCl, CH_2Cl_2.

in the rat loss of righting test (Table IV).

The methoxyacetamide pharmacophore produced a series of compounds with optimal analgesic potency and short duration of action. Compound **42** was 13 000 times more potent than morphine and 29 times more potent than fentanyl[10] in the mouse hot-plate test, while **43** was only 2778 and 6 times more potent, respectively (Table II). The furoyl analogues displayed potencies similar to that of fentanyl, and the methoxypropionyl moiety rendered compounds with significant diminution of analgesic activity (e.g., **55**, **57**, **58**, **59**, **60**, **62**, and **63**).

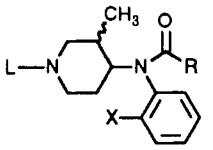
Within each series of compounds, the order of potency versus change of the ortho substituent on the anilido phenyl ring was observed to decrease in the order 2-F ≥ 2-H > 2-Cl > 2-OCH_3 (i.e., **42**, **37**, **47**, **46**). Although, the quantitative effect of the ortho fluorine on the analgesic potency was not as profound as reported by Huang[7] and Kudzma,[11] in most cases, it produced congeners with increased agonism, i.e., entries **35** and **39**. The duration of action was unaffected by those variations. In general, one could say that in an isomeric pair the greatest agonism was displayed by the cis isomer. Although in the instances where the ortho substituent was chlorine, the trans isomers possessed always higher analgesic potencies (e.g., **48** and **78**, **79**).

Closer examination of the series of compounds with an *o*-fluoro substituent in the anilido moiety reveals that the order of potency was directly dependent on the N-1 substituent, i.e., thienylethyl > phenylethyl > 4-ethyltetrazolinone (e.g., **42**, **38**, and **40**). This trend was not as rigorous for analogues with different ortho substituents, but in general, the introduction of a 2-phenylethyl or 2-thienylethyl group rendered compounds with appreciable agonist activity (e.g., **34** and **42**) vs the 4-ethyltetrazolinones analogues **40** and **41**.[12]

(10) Lalinde, N.; Spencer, K. H.; Wright, D. *Abstracts of Papers*, 193rd National Meeting of the American Chemical Society, Denver, CO, 1987; American Chemical Society: Washington, DC, 1987.

(11) Kudzma, L. V.; Severnak, S. A.; Benvenga, M.; Ezell, E. F.; Ossipov, M. H.; Knight, V. V.; Rudo, F. G.; Spencer, H. K.; Spaulding, T. H. *J. Med. Chem.* **1989**, *32*, 2534.

Table I. Chemical Properties of *N*-[1-(Substituted alkyl)-3-methyl-4-piperidinyl]-*N*-phenylalkanamides



entry	L	R	X	mp, °C	formula[a]
34 cis	phenylethyl	CH_2OCH_3	H	135–136	$C_{23}H_{30}N_2O_2 \cdot C_2H_2O_4$
35 trans	phenylethyl	CH_2OCH_3	H	190–191	$C_{23}H_{30}N_2O_2 \cdot C_2H_2O_4$
36 cis	tetrazolylethyl	CH_2OCH_3	H	145–146	$C_{20}H_{30}N_6O_3 \cdot C_2H_2O_4$
37 cis	thienylethyl	CH_2OCH_3	H	161–163	$C_{21}H_{28}N_2O_2 \cdot C_2H_2O_4$
38 cis	phenylethyl	CH_2OCH_3	F	168–169	$C_{23}H_{29}N_2O_4F \cdot C_2H_2O_4$
39 trans	phenylethy	CH_2OCH_3	F	180–181	$C_{23}H_{29}N_2O_2F \cdot C_2H_2O_4$
40 cis	tetrazolylethyl	CH_2OCH_3	F	151–153	$C_{20}H_{29}FN_6O_3 \cdot C_2H_2O_4$
41 trans	tetrazolylethyl	CH_2OCH_3	F	148–150	$C_{20}H_{29}N_6F \cdot C_2H_2O_4$
42 cis	thienylethyl	CH_2OCH_3	F	185–186	$C_{21}H_{27}FN_2O_2S \cdot C_2H_2O_4$
43 trans	thienylethyl	CH_2OCH_3	F	155–156	$C_{21}H_{27}FN_2O_2S \cdot C_2H_2O_4$
44 cis	phenylethyl	CH_2OCH_3	OCH_3	175–176	$C_{24}H_{32}N_2O_3 \cdot HCl$
45 cis	tetrazolylethyl	CH_2OCH_3	OCH_3	137–138	$C_{21}H_{32}N_6O_4 \cdot C_2H_2O_4$
46 cis	thienylethyl	CH_2OCH_3	OCH_3	194–195	$C_{22}H_{30}N_2O_3S \cdot HCl$
47 cis	phenylethyl	CH_2OCH_3	Cl	171–175	$C_{23}H_{29}N_2O_2 \cdot C_2H_2O_4$
48 trans	phenylethyl	CH_2OCH_3	Cl	171–172	$C_{23}H_{29}N_2O_2Cl \cdot C_2H_2O_4$
49 cis	phenylethyl	$CH(CH_3)OCH_3$	H	204–205	$C_{24}H_{32}N_2O_2 \cdot C_2H_2O_4$
50 trans	phenylethyl	$CH(CH_3)OCH_3$	H	167–169	$C_{24}H_{32}N_2O_2 \cdot C_2H_2O_4$
51 cis	tetrazolylethyl	$CH(CH_3)OCH_3$	H	147–148	$C_{21}H_{32}N_6O_3 \cdot C_2H_2O_4$
52 trans	tetrazolylethyl	$CH(CH_3)OCH_3$	H	164–165	$C_{21}H_{32}N_6O_3 \cdot C_2H_2O_4$
53 cis	thienylethyl	$CH(CH_3)OCH_3$	H	217–218	$C_{22}H_{30}N_2S \cdot C_2H_2O_4$
54 trans	thienylethyl	$CH(CH_3)OCH_3$	H	180–181	$C_{22}H_{30}N_2O_2S \cdot C_2H_2O_4$
55 cis	phenylethyl	$CH(CH_3)OCH_3$	F	179–180	$C_{24}H_{31}N_2O_2F \cdot C_2H_2O_4$
56 trans	tetrazolylethyl	$CH(CH_3)OCH_3$	F	206–209	$C_{21}H_{31}FN_6O_3 \cdot HCl$
57 cis	thienylethyl	$CH(CH_3)OCH_3$	F	204–205	$C_{22}H_{29}FN_2O_2S \cdot C_2H_2O_4$
58 trans	thienylethyl	$CH(CH_3)OCH_3$	F	203–204	$C_{22}H_{29}FN_2O_2SCC_2H_2O_4$
59 cis	phenylethyl	$CH(CH_3)OCH_3$	OCH_3	159–161	$C_{25}H_{35}N_2O_3 \cdot HCl$
60 trans	phenylethyl	$CH(CH_3)OCH_3$	OCH_3	223–224	$C_{25}H_{34}N_2O_4 \cdot C_2H_2O_4$
61 cis	tetrazolylethyl	$CH(CH_3)OCH_3$	OCH_3	220–222	$C_{22}H_{34}N_6O_4 \cdot C_2H_2O_4$
62 cis	thienylethyl	$CH(CH_3)OCH_3$	OCH_3	172–173	$C_{23}H_{32}N_2O_3S \cdot C_2H_2O_4$
63 cis	phenylethyl	$CH(CH_3)OCH_3$	Cl	212–213	$C_{24}H_{31}N_2O_2Cl \cdot C_2H_2O_4$
64 trans	phenylethyl	$CH(CH_3)OCH_3$	Cl	138–140	$C_{24}H_{31}N_2O_2Cl \cdot C_2H_2O_4$
65 cis	phenylethyl	furoyl	H	177–178	$C_{25}H_{28}N_2O_2 \cdot C_2H_2O_4$
66 trans	phenylethyl	furoyl	H	169–170	$C_{25}H_{28}N_2O_2 \cdot C_2H_2O_4$
67 cis	tetrazolylethyl	furoyl	H	124–125	$C_{22}H_{28}N_6O_3 \cdot C_2H_2O_4$
68 cis	thienylethyl	furoyl	H	201–204	$C_{23}H_{26}N_2O_2S \cdot C_2H_2O_4$
69 cis	phenylethyl	furoyl	F	208–212	$C_{25}H_2A_7N_2O_2F \cdot C_2H_2O$
70 cis	tetrazolylethyl	furoyl	F	154–155	$C_{22}H_{27}FN_6O_3 \cdot C_2H_2O_4$
71 trans	tetrazolylethyl	furoyl	F	174–175	$C_{22}H_{27}FN_6O_3 \cdot C_2H_2O_4$
72 cis	thienylethyl	furoyl	F	191–192	$C_{23}H_{25}FN_2SCC_2H_2O_4$
73 trans	thienylethyl	furoyl	F	168–169	$C_{23}H_{25}FN_2O_2S \cdot C_2H_2O_4$
74 cis	phenylethyl	furoyl	OCH_3	202–204	$C_{26}H_{30}N_2O_3 \cdot C_2H_2O_4$
75 trans	phenylethyl	furoyl	OCH_3	227–229	$C_{26}H_{30}N_2O_3 \cdot C_2H_2O_4$
76 cis	tetrazolylethyl	furoyl	OCH_3	150–151	$C_{23}H_{30}N_6O_4 \cdot C_2H_2O_4$
77 cis	thienylethyl	furoyl	OCH_3	195–196	$C_{24}H_{28}N_2O_3S \cdot C_2H_2O_4$
78 cis	phenylethyl	furoyl	Cl	201–202	$C_{25}H_{27}N_2O_2Cl \cdot C_2H_2O_4$
79 trans	phenylethyl	furoyl	Cl	187–189	$C_{25}H_{29}N_2O_2Cl \cdot C_2H_2O_4$

[a] Analytical results were within ±0.4% of the theoretical values. Recrystallized from methanol except where indicated. The compounds are either oxalates or hydrochloride salts.

The duration of action of **40** in the mouse hot-plate test, at the 2 × ED_{50} dose was 1.98 min, which was similar to that of compounds **42**, **57**, and **55**. However, superiority was clearly demonstrated at 8× and 16× MHP ED_{50} analgesic doses. At 8× MHP ED_{50}, **40** had a duration of action of only 9.00 min, and at the 16× MHP ED_{50} the duration was approximately and was comparable to that of fentanyl and sulfentanil at their 2× MHP ED_{50} analgesic doses (Table II). The onset of the antinociceptive activity of **40** in the mouse hot plate (MHP) occurred within 1 min after iv injection. From the series of compounds in Table II **40** was selected for further pharmacological investigation because it satisfied our criteria for short duration of action.

In the rat tail-flick experiments, at 2× ED_{50} **40** had a duration of action of 8.4 min, which was comparable to that of fentanyl (7.6 min), but at 8× RTF ED_{50} its duration of action was significantly shorter (20.8 min) and that of fentanyl (34 min). In the rat hot-plate test the duration of action of **40** at 2× RTF ED_{50} was 2.8 min and of fentanyl 11.62 min. At 8× RHP ED_{50} fentanyl had a duration of action of 32.35 min, which was 4.1 times longer than that of **40**, 7.9 min (Table III).

Due to its overall superior analgesic profile, the pharmacology of **40** was further scrutinized. The anesthetic activity of **40** and fentanyl was evaluated in the loss of righting test (LOR) in rats (Table IV). The ED_{50} for LOR in rats administered iv with **40** and fentanyl were calculated (percent responding vs dose) to be 0.152 and 0.0175 mg/kg, respectively. However at equi-efficacious doses for LOR (i.e., 100% responding), the duration of **40** was 1.7 min compared to 8.8 min for fentanyl. In addition, **40** appeared to produce less muscular rigidity and have a better behavioral syndrome, rated by the severity of pri-

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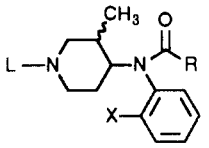
Table II. *N*-[1-(Substituted alkyl)-3-methyl-4-piperidinyl]-*N*-phenylalkanamides Preliminary Pharmacology



entry	L	R	X	ED_{50},[b] mg/kg	duration[b,c] 2*n*	8*n*	16*n*	K_i,[d] nM
34 cis	phenylethyl	CH_2OCH_3	H	0.0016 (0.001023–0.0024)	9.16	--	--	0.33
35 trans	phenylethyl	CH_2OCH_3	H	0.041 (0.029–0.06)	3.24	15.95	--	0.86
36 cis	tetrazolylethyl	CH_2OCH_3	H	inactive	--	--	--	ND[e]
37 cis	thienylethyl	CH_2OCH_3	H	0.0021 (0.00186–0.002508)	5.32	17.13	--	ND
38 cis	phenylethyl	CH_2OCH_3	F	0.0041 (0.002977–0.00551)	5.13	10.91	--	0.88
39 trans	phenylethyl	CH_2OCH_3	F	0.00069 (0.000535–0.00091)	25.40	--	--	0.19
40 cis	tetrazolylethyl	CH_2OCH_3	F	0.0980 (0.091–0.156)	2.00	9.00	12	2.00
41 trans	tetrazolylethyl	CH_2OCH_3	F	inactive	--	--	--	>100[f]
42 cis	thienylethyl	CH_2OCH_3	F	0.00056 (0.000453–0.000694)	16.00	--	--	ND
43 trans	thienylethyl	CH_2OCH_3	F	0.0027 (0.001085–0.00587)	2.33	9.30	--	ND
44 cis	phenylethyl	CH_2OCH_3	OCH_3	0.1125 (0.1016–0.1485)	8.60	--	--	ND
45 cis	tetrazolylethyl	CH_2OCH_3	OCH_3	inactive	--	--	--	ND
46 cis	thienylethyl	CH_2OCH_3	OCH_3	0.547	23.00	--	--	ND
47 cis	phenylethyl	CH_2OCH_3	Cl	0.078 (0.0724–0.12644)	1.41	9.70	--	1.10
48 trans	phenylethyl	CH_2OCH_3	Cl	0.00486 (0.00226–0.010463)	4.85	20.00	--	ND
49 cis	phenylethyl	$CH(CH_3)OCH_3$	H	0.035 (0.028406–0.044195)	7.70	--	--	4.20
50 trans	phenylethyl	$CH(CH_3)OCH_3$	H	0.575 (0.004–82.72)	6.10	--	--	32.2
51 cis	tetrazolylethyl	$CH(CH_3)OCH_3$	H	inactive	--	--	--	ND
52 trans	tetrazolylethyl	$CH(CH_3)OCH_3$	H	inactive	--	--	--	ND
53 cis	thienylethyl	$CH(CH_3)OCH_3$	H	0.0119 (0.006573–0.032742)	2.0	8.0	--	ND
54 trans	thienylethyl	$CH(CH_3)OCH_3$	H	0.0244 (0.0154–0.0388)	10.56	--	--	ND
55 cis	phenylethyl	$CH(CH_3)OCH_3$	F	0.410 (0.286–0.589)	11.54	--	--	8.10
56 trans	tetrazolylethyl	$CH(CH_3)OCH_3$	F	inactive	--	--	--	ND
57 cis	thienylethyl	$CH(CH_3)OCH_3$	F	0.0057 (0.00496–0.009692)	1.73	11.06	--	2.16
58 trans	thienylethyl	$CH(CH_3)OCH_3$	F	0.0244 (0.0154–0.0388)	10.56	--	--	ND
59 cis	phenylethyl	$CH(CH_3)OCH_3$	OCH_3	inactive	--	--	--	ND
63 trans	phenylethyl	$CH(CH_3)OCH_3$	OCH_3	0.651 (0.477–0.889)	10.20	--	--	7.62
61 cis	tetrazolylethyl	$CH(CH_3)OCH_3$	OCH_3	inactive	--	--	--	ND
62 cis	thienylethyl	$CH(CH_3)OCH_3$	OCH_3	2.5	--	--	--	11.2
63 cis	phenylethyl	$CH(CH_3)OCH_3$	OCH_3	0.669 (0.52–0.291)	6.88	--	--	ND
64 trans	phenylethyl	$CH(CH_3)OCH_3$	Cl	0.188 (0.122–0.291)	12.55	--	--	2.80
65 cis	phenylethyl	furoyl	H	0.005 (0.003–0.007027)	7.5	--	--	0.30
66 trans	phenylethyl	furoyl	H	0.082 (0.000896–0.6987)	7.8	--	--	0.40
67 cis	tetrazolylethyl	furoyl	H	0.638 (0.47–8.66)	6.46	--	--	ND
68 cis	thienylethyl	furoyl	H	0.0054 (0.0039–0.0073)	15.60	--	--	0.15
69 cis	phenylethyl	furoyl	F	0.041 (0.001–1.3)	11.55	--	--	ND
70 cis	tetrazolylethyl	furoyl	F	0.305 (0.298–0.451)	4.57	9.14	--	ND
71 trans	tetrazolylethyl	furoyl	F	inactive	--	--	--	ND
72 cis	thienylethyl	furoyl	F	0.004 (0.0008–0.018)	16.80	--	--	ND
73 trans	thienylethyl	furoyl	F	0.025 (0.01845–0.034407)	4.76	19.30	--	ND
74 cis	phenylethyl	furoyl	OCH_3	0.217 (0.156–0.303)	7.20	--	--	ND
75 trans	phenylethyl	furoyl	OCH_3	0.118 (0.11013–0.18712)	6.16	24.00	--	0.78
76 cis	tetrazolylethyl	furoyl	OCH_3	inactive	--	--	--	>100
77 cis	thienylethyl	furoyl	OCH_3	0.568 (0.434–0.743)	12.00	--	--	2.00
78 cis	phenylethyl	furoyl	Cl	1.96	--	--	--	ND
79 trans	phenylethyl	furoyl	Cl	0.247 (0.14948–0.40843)	26	--	--	0.13
morphine				7.3	>60.0	--	--	2.1
alfentanil				0.047 (0.034–0.065)	4.1	--	--	8.21
fentanyl				0.018 (0.014–0.023)	11.70	27.10	--	2.16
sufentanyl				0.0029	12.52	--	--	0.22

[a] ED_{50} for mouse hot plate with 95% confidence limits in parentheses; inactive refers to doses up to 5 mg/kg. [b] Duration in minutes at indicated multiples of the ED_{50} dose. [c] Analgesics with relatively short duration of action, less than 6 min at 2 times ED_{50} were evaluated at doses equivalent to 8 times the ED_{50} analgesic dose, and those with durations of less than 9 min, at 8 times ED_{50} were evaluated again at 16 times the ED_{50} analgesic dose. [d] K_i denotes the ability to displace [^{3}H]naloxone from the μ opioid receptor isolated from rat brain membranes. [e] ND = not determined. [f] A greater than sign (>) denotes no displacement of [^{3}H]naloxone at the concentration indicated.

mary overt effects (POE; e.g. ataxia, tremors, myotactic reflex, vascular tone, salivation), which was indicated by a lower behavioral or POE score (0.53 and 0.98 for **40** and fentanyl, respectively).

Many compounds were screened in vitro for their ability to displace [^{3}H]naloxone from its binding sites in rat brain membranes. Compound **40** exhibited a receptor-binding affinity (K_i, nmol) of 2.0, which was comparable to that of morphine (2.1) and fentanyl (2.16), though **40** was 70 times more potent than morphine and 6 times less potent than fentanyl. The variance in in vivo agonism with the in vitro data suggested that pharmacokinetic, rather than pharmacodynamic, factors are paramount.

The ability of iv naloxone to reverse the antinociceptive action of **40** was determined in the rat tail-flick test. Administration of the A_{99} dose, i.e., dose calculated to produce 99% of the maximal possible effect (99% MPE), of **40** (0.11 mg/kg, iv) followed by one injection of saline 1 min later, produced 81% maximum possible effect (MPE). The effect was significantly ($p < 0.05$) reduced to 25% and 3% MPE, following injections of 0.01 and 0.1 mg/kg of naloxone, respectively. These data suggest that

Table III. Potency and Duration of Action of **40**, Fentanyl, and Morphine in Various Tests

	40				fentanyl				morphine		
		durations[c]				durations[c]				durations[c]	
	ED_{50}[a,b]	2*n*	8*n*	16*n*	ED_{50}[a,b]	2*n*	8*n*	16*n*	ED_{50}[a,b]	2*n*	8*n*
mouse hot plate	0.098 (0.091–0.156)	1.98	9.0	12.6	0.018 (0.015–0.022)	7.9	27.1	41.07	4.68 (1.77 –12.39)	66.3	217.8
rat tail flick	0.059 (0.046–0.076)	8.4	20.8	--	0.0043 (0.003–0.0061)	7.6	34.0	--	1.11 (0.67–1.82)	18.5	102.2
rat hot plate	0.075 (0.049–0.114)	2.8	7.9	--	0.0086 (0.0065–0.010)	11.62	32.35	--	2.77 (2.32–3.30)	37.0	115.3

[a] Milligram/kilogram. [b] 95% confidence limits in parentheses. [c] Minutes, at the given multiple of the ED_{50}.

Table IV. Equiefficacious Dose and Duration of Action for the Lost of Righting in Rats following Intravenous **40** and Fentanyl

40			fentanyl		
dose[a,b]	duration[c]	POE score	dose[a,b]	duration[c]	POE score
0.152 (0.139–0.165)	1.7	0.53	0.0209 (0.0169–0.0249)	8.8	0.98

[a] Milligrams/kilogram. [b] LOR ED_{50} with 95% confidence limits in parentheses. [c] Minutes, at a given dose. 100% responding.

40 acts at the opioid receptor and that the effects of an overdose of **40** may be readily reversed by naloxone in the clinical setting.

In conclusion, our efforts in the search for a better analgesic agent have proven to be fruitful. Compound **40** (Anaquest, A-3331.HCl, Brifentanil, structure **4**) was chosen as a development compound because it met the criteria we had set forth at the beginning of the project. The short duration of analgesia and minimal opioid side effects gave this compound a unique position within the class of clinically useful opioid 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. ^{1}H NMR spectra were recorded on a Varian E 360 (60 MHz) spectrometer. The NMR data for the assignment of the cis and trans isomer was acquired on the JEOL GSX-270 and transformed by using an exponential broadening factor of 0.2 Hz and utilizing a trapezoidal window for resolution enhancement. IR spectra were recorded on a Perkin-Elmer 197 spectrophotometer. Conventional chromatography was 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. For pharmacological screening, oxalate salts were prepared by stirring a solution of the base, in 2-propanol, with excess oxalic acid at room temperature and recrystallizing the solids to analytical purity.

Starting Materials. 1-Phenethyl-3-methyl-4-piperidone (**5**) and 1-benzyl-3-methyl-4-piperidone (**26**) were prepared by standard procedures.[8] The acid chlorides were commercially available from Aldrich, Milwaukee, WI, and most did not require further purification.

3-Methyl-4-phenyl-4-anilinopiperidines. The procedure described is for the preparation of **40** (Scheme II, path B). Nevertheless it is representative of those depicted in Schemes I and II (path A) and Scheme III.

1-Benzyl-3-methyl-4-(2-fluoroanilino)piperidine (6). A mixture of **5** (25 g, 132 mmol), 2-fluoroaniline (23.25 g, 209 mmol), a few crystals of *p*-toluenesulfonic acid, and toluene (350 mL) was stirred under reflux overnight. This was the time required for collection of the theoretical quantity of water byproduct (2.2 mL) in a Dean–Stark trap. The reaction mixture was cooled and concentrated in vacuo to give a brownish oil which exhibited a strong C=N absorption band at 1665 cm^{-1} by IR analysis. The crude Schiff base was dissolved in methanol (250 mL) and then $NaBH_4$ (19 g) was added in small portions. The reaction mixture was stirred at room temperature overnight and concentrated in vacuo. Water (200 mL) was added, followed by extraction with toluene (450 mL). The organic layer was dried over $MgSO_4$. Concentration in vacuo left a brown oil; this was purified by vacuum distillation (0.1 mmHg, 140–170 °C), yielding 21.6 g (53%) of **6** as a pale yellow oil: ^{1}H NMR ($CDCL_3$) δ 1.05 (d, J = 6 Hz, 6 H), 1.67–2.75 (complex, 7 H), 3.58 (s, 2 H), 3.75–4.2 (br, 1 H), 6.58–7.29 (complex, 3 H), 7.39 (s, 5 H). Elemental analysis for $C_{19}H_{23}FN_2$. Calcd: C, 76.48; H, 7.77; N, 9.39. Found: C, 76.79; H, 7.83; N, 9.51.

1-Benzyl-3-methyl-4-[*N*-(2-fluorophenyl)methoxyacetamido]piperidine (21). A solution of **6** (10 g, 33 mmol), methoxyacetyl chloride (4.0 g, 37 mmol), and anhydrous THF (50 mL) was stirred at room temperature for 2 days. The acidic mixture was carefully alkalinized with 10% NaOH. The phases were separated, and the liberated free base was extracted with CH_2Cl_2 (100 mL). The organic extract was dried over $MgSO_4$. Concentration in vacuo yielded **21** as a pale yellow oil (12.33 g, 99%): ^{1}H NMR ($CDCl_3$) δ 1.29 (d, J = 6.5 Hz, 6 H), 2.45–3.21 (complex, 5 H), 3.42 (s, 5 H), 3.76 (2, 2 H), 4.3–4.75 (complex, 1 H), 7.19–7.4 (complex, 8 H). Elemental analysis for $C_{22}H_{27}FN_2O$. Calcd: C, 71.33; H, 7.35; N, 7.56. Found: C, 70.69; H, 7.27; N, 7.91.

3-Methyl-4-[*N*-(2-fluorophenyl)methoxyacetamido]piperidine (22). A mixture of **21** (12 g, 32 mmol) and palladium on carbon (0.5 g, 10%) in ethanol (50 mL) was shaken under a hydrogen atmosphere (50–60 psi) for 18 h. TLC (EtOAc, silica gel) indicated complete consumption of the starting material, and the slurry was filtered. The filtrate was concentrated in vacuo to afford a yellowish oil which was purified by column chromatography (methanol/NH_4OH 1:0.5 v:v) to yield 3.2 g (45%) of **22** (R_f 0.2): ^{1}H NMR ($CDCl_3$) δ 1.0 (d, J = 4.6 Hz, 6 H), 2.22–2.8 (complex, 5 H), 3.2 (s, 3 H), 3.62 (s, 2 H), 4.12–4.74 (complex, 1 H), 7.0–7.4 (complex, 4 H). Elemental analysis for $C_{15}H_{21}FN_2O$. Calcd: C, 64.27; H, 7.55; N, 9.99. Found: C, 63.94; H, 7.19; N, 9.71.

1-[2-(4-Ethyl-4,5-dihydro-1*H*-tetrazolyl)ethyl]-3-methyl-4-[*N*-(2-fluorophenyl)methoxyacetamido]piperidine (40). 1-(2-Chloroethyl)-4-ethyl-1,4-dihydro-5*H*-tetrazol-5-one[13] (1.68 g, 9.5 mmol) was added to a stirring solution of **22** (2.42 g, 8.6 mmol), powdered anhyrous potassium carbonate (11.9 g, 86 mmol), and a few crystals of potassium iodide in acetonitrile (60 mL). The reaction mixture was heated at reflux for 18 h, cooled, and filtered. The filtrate was concentrated in vacuo, affording the intermediate **25** as a yellowish oil. Column chromatography with EtOAc (100%) afforded 1.2 g of **13**, upper spot ($R_f \sim 0.3$), and 0.75 g of **14**, lower spot ($R_f \sim 0.24$). The combined total yield was 56%. Intermediate **13** was crystallized as the oxalate salt (mp 151–153 °C), affording 1.25 g of **40**. Note: The compounds where the N-1 substituent is phenethyl were prepared with 1-phenethyl-3-methyl-4-piperidone (**26**) as the starting material, which, in turn, was prepared according to ref 8.

***cis*-1-[2-(2-Thienyl)ethyl]-3-methyl-4-[*N*-(2-fluorophenyl)-2-methoxyacetamido]piperidinium oxalate (42):** ^{1}H NMR ($CDCl_3$) δ 1.28 (d, J = 6 Hz, 6 H), 1.78–3.2 (complex, 8 H), 3.45 (s, 3 H), 3.78 (s, 2 H), 4.3–4.85 (complex, 1 H), 6.8–7.6 (complex, 7 H).

***cis*-1-[2-(2-Thienyl)ethyl]-3-methyl-4-[*N*-(2-methoxyphenyl)-2-methoxypropionamido]piperidinium oxalate (62):** ^{1}H NMR ($CDCl_3$) δ 0.89–1.32 (complex, 6 H), 1.8–3.02 (complex,

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9 H), 3.25 (s, 3 H), 3.32–3.9 (complex, 4 H), 4.1–4.6 (complex, 1 H), 6.7–7.3 (complex, 7 H).

***cis*-1-(2-Phenylethyl)-3-methyl-4-(*N*-phenyl-*N*-2-furoylamino)piperidinium oxalate (65)**: ^{1}H NMR ($CDCl_3$) δ 1.3 (d, J = 6 Hz, 3 H), 1.7–3.2 (complex, 10 H), 4.1–4.7 (complex, 2 H), 5.45–6.65 (3 m, 3 H), 7.3–7.6 (complex, 10 H).

***cis*-1-[2-(2-Thienyl)ethyl]-3-methyl-4-(*N*-phenyl-*N*-2-furoylamino)piperidinium oxalate (68)**: ^{1}H NMR ($CDCl_3$) δ 1.2 (d, J = 6.5 Hz, 3 H), 1.6–3.2 (complex, 10 H), 3.8–4.9 (complex, 2 H), 5.5 (d, J = 4 Hz, 1 H), 6.15 (complex, 1 H), 6.7–7.7 (complex, 9 H).

Pharmacological Methods. In Vivo. Analgesic. A. 55 °C Mouse Hot Plate.[14] 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 maximum pharmacological effect (MPE) using the mouse hot-plate (MHP) assay, vehicle (saline) or drug solution (10 mL/kg) was injected into the lateral tail vein of groups of 10 mice and placed on the hot plate after 1 min. An initial dose of 1 mg/kg of compound was administered. If 100% MPE was observed, then lower dosing was continued until an ED_{50} was generated. If less than 100% MPE 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 10 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 30 s was reached. Antinociceptive effect was defined as a doubling of the latency time to paw-lick over control times.

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

The ED_{50} and 95% confidence limits were calculated by using a standard computer program of the method of Litchfield and Wilcoxin[15] fitted to a minicomputer.[16] Calculation of the ED_{50} (95% confidence limits) was corrected for base content of the salts.

Duration of Analgesia. Two times the ED_{50} was administered to 10 mice and the hot-plate latencies were determined at various times after injection in the lateral tail vein. The mean MPE was calculated for each time period and a time–effect curve was generated. A test compound was defined to be short acting if the duration of action to 50% MPE was less than 6 min, intermediate duration was 6.1–15 min, and long acting was a duration greater than 15.1 min.

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

C. Rat Tail Flick Median Effective Dose (ED_{50}) Determination.[17] Rat tail flick latencies were determined for 40 and drug standards as a measure of antinociceptive activity. Two pretreatment latencies were determined for each rat. They were then restrained gently and their tails were placed under a focused light that produced radiant heat. When the animals flicked their tail aside, a photocell was uncovered and the instrument's circuitry stopped a timer. Postinjection tail flick latencies were determined after iv injection. The quantal ED_{50}s were calculated by Litchfield and Wilcoxin's method.[15]

Naloxone Reversibility Test. Male Sprague–Dawley rats (150–250 g), housed with free access to food and water, were identified, weighed, and randomly assigned to a treatment group (N = 6). After the animals acclimated to the laboratory environment (1 h), pretreatment talil-flick latencies were determined for each animal. Drugs were dissolved in 0.9% saline or appropriate vehicle and administered in a volume of 1 mL/kg. All groups were administered the A_{99} dose of agonist followed 1 min later by either saline or naloxone. The animals posttreatment tail-flick latency was determined 1 min after naloxone (or saline) administration.

The percent maximal effect (% MPE) was determined for each animal by the equation

$$\% \text{ MPE} = \frac{\text{(posttreatment) - (control)}}{\text{(cut-off)- (control)}} \times 100$$

The mean % MPE was then determined for each treatment group. The T test for unpaired data was then used to determine whether the % MPE obtained after naloxone administration was significantly different from that obtained after saline administration. Treatment groups having a signficant difference (p = 0.05) were considered as naloxone reversible.

Inhibition of [^{3}H]Naloxone Binding. Compounds were studied for their ability to displace [^{3}H]naloxone from membrane binding sites. This assay is a good estimate of rank orders of potency for opiate agonists. It is based upon the method reported by Pasternak and his colleagues,[18] who used membrane fractions prepared from homogenates of rat brains. The membranes were incubated in Tris buffer in the presence of 1.0 nM [^{3}H]naloxone (50–60 Ci/mmol) and varying concentrations of either 40 or drug standards. After incubation at 25 °C for 30 min, the membranes with radioactivity bound to them were separated from free or unbound radioactivity by filtering the incubation mixtures over glass-fiber filters. After they were washed, the filters with adherent membranes and bound radioactivity were put into scintillation vials. Scintillation cocktail was added and radioactivity was estimated by liquid scintillation spectrometry. The results were plotted, and from the plots an IC_{50} for displacement was calculated. The K_i for inhibition was calculated by Cheng and Prusoff's method.[19]

Screen for Primary Overt Effects for Hypnotics/Anesthetics in the Rat. Six Sprague–Dawley rats (230–290 g) were used. Immediately following iv drug administration, the animal was removed from the restrainer and observed along with control (i.e., the next test animal) for 20 min. Righting is lost when the animal remains supine for at least 30 s. The ED_{50} for anesthesia was determined by linear regression. The confidence limits were determined as previously described.[20] Core temperature is obtained rectally prior to and during the 5 min postdrug period and the difference is recorded. Other primary overt effects are scored from 0 to plus or minus 3 (0 = normal, 1 = mild, 2 = moderate, 3 = marked) and recorded at 5-min intervals for 30 min.

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