3.71 (s, 3 H, OCH_3), 4.36 (d, J = 3 Hz, 1 H, C-5 H), 5.35 (d, J = 8 Hz, 1 H, C-19 H), 5.64 (dd, J = 6 and 10 Hz, 1 H, C-18 H); UV λ_{max} (log ϵ) 240 nm (3.69), 288 (3.15); IR ($CHCl_3$) 2950, 1640, 1605 cm^{-1}; $[\alpha]^{23}_D$ −160° (c 0.40, $CHCl_3$). Anal. ($C_{26}H_{35}NO_3$) C, H, N.

(S)-15: yield 0.64 g (33%); mp 48–51 °C; ^1H NMR δ 0.87 (t, J = 7 Hz, 3 H, CH_2CH_3), 1.07 (s, 3 H, CCH_3), 2.32 (s, 3 H, NCH_3), 3.70 (s, 3 H, OCH_3), 4.35 (d, J = 3 Hz, 1 H, C-5 H); UV λ_{max} (log ϵ) 240 nm (3.72), 288 (3.17); $[\alpha]^{23}_D$ −119° (c 0.50, $CHCl_3$). Anal. ($C_{26}H_{35}NO_3$) C, H, N.

19(*R*)-Butyl-6-demethoxy-7α-orvinol [4,5α-Epoxy-3-hydroxy-α-*n*-butyl-α,17-dimethyl-6,14-ethenomorphinan-7α-(*R*)-methanol; (*R*)-16]. A solution of 0.42 g (1.0 mmol) of (*R*)-15 in 10 mL of dimethylformamide was stirred as 0.1 g (4.2 mmol) of degreased sodium hydride was added in small portions, followed by 0.25 g (3.3 mmol) of propanethiol. The mixture was heated at reflux for 1 h, then allowed to cool to room temperature, and poured into 1 M aqueous phosphoric acid. The aqueous solution was extracted with ether, adjusted to pH 11 with aqueous ammonium hydroxide, and extracted with chloroform. The combined organic extracts were washed with water and brine and dried over sodium sulfate. Evaporation gave a solid residue and chromatography on alumina with 1% methanol/chloroform gave (*R*)-16 as colorless crystals: yield 0.28 g (69%); mp 93–95 °C; ^1H NMR δ 0.96 (s, 3 H, CCH_3), 2.40 (s, 3 H, NCH_3), 4.50 (d, J = 3 Hz, 1 H, C-5 H), 5.30 (s, 1 H, OH), 5.45 (d, J = 8 Hz, 1 H, C-19 H), 5.74 (t, J = 8 Hz, 1 H, C-18 H); UV λ_{max} (log ϵ) 240 nm sh (3.36), 290 (2.84).

19(*S*)-Butyl-6-demethoxy-7α-orvinol [(*S*)-16]. Demethylation of (*S*)-15 by the above procedure gave phenol (*S*)-16 in 52% yield: mp 87–90 °C; ^1H NMR δ 1.07 (s, 3 H, CCH_3), 2.40 (s, 3 H, NCH_3), 4.50 (d, J = 3 Hz, 1 H, C-5 H), 5.30 (s, 1 H, OH).

Pharmacological Methods. Sprague–Dawley rats weighing 180–210 g restrained in cages in the dark were used in the tail-flick test.[17] Test compounds were prepared in 0.01 N aqueous hydrochloric acid and were administered in this solution at 1 mL/kg, subcutaneously. Hot water (54–55 °C) provided the stimulus and reaction times were measured 10 min after injection for a maximum of 15 s. Seven rats at seven different dose levels were used for each compound. Percent response is defined as the reaction time minus the control time (1.5 s) as a percent of the maximum response time minus the control time (15–1.5 s).

Acknowledgment. We are grateful to Dr. E. T. Wei and Donna Seid for assistance in the biological evaluations. This research was supported in part by the National Institute on Drug Abuse.

Opiate Receptor Interaction of Compounds Derived from or Structurally Related to Fentanyl

Marinus W. Lobbezoo,* Willem Soudijn,

Department of Pharmaceutical Chemistry, University of Amsterdam, Plantage Muidergracht 24, 1018 TV Amsterdam, The Netherlands

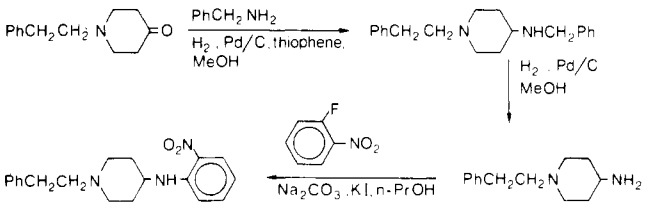
and Ineke van Wijngaarden

Janssen Pharmaceutica, B-2340 Beerse, Belgium. *Received August 18, 1980*

The opiate receptor affinity of compounds derived from or structurally related to fentanyl (1) was determined by in vitro receptor binding assays. The relatively high affinity of fentanyl (3 times morphine) was hardly influenced by the introduction of a 2-CH_3, 2-OCH_3, or a 2-Cl substituent into the anilino phenyl and was moderately reduced by 2-C_2H_5, 2-OC_2H_5, and 2,6-$(CH_3)_2$ substitution in this ring. Removal of the *N*-propionyl group of the 2-OCH_3 derivative, fixation of the anilino phenyl in fentanyl to the propionyl group or the piperidine ring, and replacement of the amide N by C all caused a sharp decline of receptor affinity. Examination of molecular models seemed to indicate that optimal opiate receptor interaction of fentanyl and its derivatives requires a virtually perpendicular position of the anilino phenyl with respect to the amide function.

Fentanyl (1) is a highly potent narcotic analgesic agent[1,2] which is widely used in anesthesiology. Structurally it belongs to the 4-anilinopiperidines. The analgesic potency of fentanyl (300 times morphine in the tail withdrawal test in rats[1]) could be further enhanced (up to 10 000 times morphine) by the introduction of an appropriate substituent (e.g., 3-CH_3, 4-$COOCH_3$, or 4-CH_2OCH_3) into the piperidine ring.[3-5] In vitro receptor binding studies have shown that these derivatives possess a very high opiate receptor affinity (ORA) with K_I values in the subnanomolar range.[6,7]

Scheme I



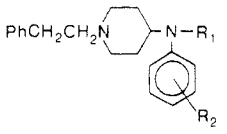
12

In a previous paper[8] we described the synthesis and in vitro opiate receptor affinity of a series of phenolic hydroxy derivatives of fentanyl and the corresponding methoxy derivatives. Since the ORA did not increase after the introduction of a phenolic OH, we concluded that it is very unlikely that, with respect to drug–receptor interaction,

(1) P. A. J. Janssen, C. J. E. Niemegeers, and J. G. H. Dony, *Arzneim.-Forsch. (Drug Res.)*, **13**, 502 (1963).
(2) P. A. J. Jansen and C. A. M. van der Eycken, in "Drugs Affecting the Central Nervous System", Vol. 2, A. Burger, Ed., Marcel Dekker, New York, 1968, p 25.
(3) W. F. M. van Bever, C. J. E. Niemegeers, and P. A. J. Janssen, *J. Med. Chem.*, **17**, 1047 (1974).
(4) W. F. M. van Bever, C. J. E. Niemegeers, K. H. L. Schellekens, and P. A. J. Janssen, *Arzneim.-Forsch. (Drug Res.)*, **26**, 1548 (1976).
(5) P. G. H. van Daele, M. F. L. de Bruyn, J. M. Boey, S. Sanczuk, J. T. M. Agten, and P. A. J. Janssen, *Arzneim.-Forsch. (Drug Res.)*, **26**, 1521 (1976).
(6) J. E. Leysen and P. M. Laduron, *Arch. Int. Pharmacodyn. Ther.*, **232**, 243 (1978).
(7) K. D. Stahl, W. van Bever, P. Janssen, and E. J. Simon, *Eur. J. Pharmacol.*, **46**, 199 (1977).
(8) M. W. Lobbezoo, W. Soudijn, and I. van Wijngaarden, *Eur. J. Med. Chem.*, **15**, 357 (1980).

0022-2623/81/1824-0777$01.25/0

Table I. Structure and Opiate Receptor Affinity of Fentanyl Derivatives Substituted in the Anilino Phenyl Ring



no.	R_1	R_2	IC_{50},[a] nM	n[b]	rel ORA[c]	p[d]
1	COC_2H_5	H	1.14	5	3.0	
2	COC_2H_5	2-OH	10.2	3	0.33	<0.05
3	COC_2H_5	2-OCH_3	0.84	3	4.0	NS
4	COC_2H_5	2-OC_2H_5	1.99	3	1.7	<0.05
5	COC_2H_5	2,4-$(OCH_3)_2$	4.48	3	0.75	<0.05
6	COC_2H_5	2-OCH_3, 4-OH	1.36	3	2.5	NS
7	COC_2H_5	2-CH_3	1.56	3	2.2	NS
8	COC_2H_5	2,6-$(CH_3)_2$	2.48	3	1.4	<0.05
9	COC_2H_5	2-C_2H_5	11.9	3	0.28	<0.05
10	COC_2H_5	2-Cl	1.64	3	2.1	NS
11	H	2-OCH_3	980	3	0.0034	<0.05
12	H	2-NO_2	2140	2	0.0016	<0.05

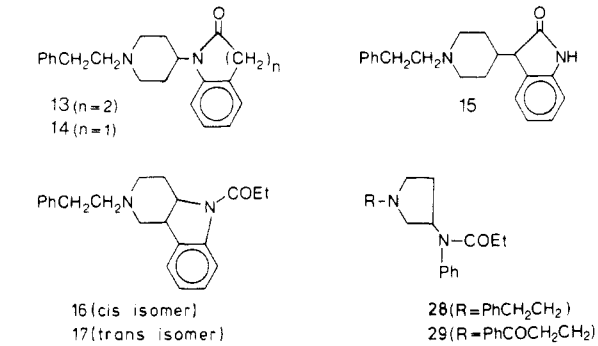
[a] Concentration causing 50% inhibition of [^{3}H]fentanyl receptor binding. [b] Number of determinations. [c] Opiate receptor affinity relative to morphine (IC_{50} = 3.37 nM; n = 4). [d] p value for the difference with fentanyl (see Experimental Section).

one of the phenyl rings in fentanyl would correspond with the phenyl ring in morphine or dihydromorphine.[9] Unexpectedly, it appeared that the introduction of 2-OCH_3 into the anilino phenyl ring tended to enhance ORA, whereas the corresponding 2-OH derivative possessed only 0.1 times the ORA of fentanyl. In order to elucidate the optimal structural requirements of substituents in this ortho position for the opiate receptor interaction, a series of derivatives covering a wide variety of chemical substituent features (e.g., steric bulk, electron donating, electron withdrawing) was synthesized and tested for ORA. Other structural variations in the anilino part, like ring closure between the ortho position and the piperidine ring or the propionyl chain and replacement of the nitrogen atom by a tetrahedral carbon atom, were also included in this study to obtain a better understanding of the structural features governing the opiate receptor interaction of fentanyl-like analgesics.

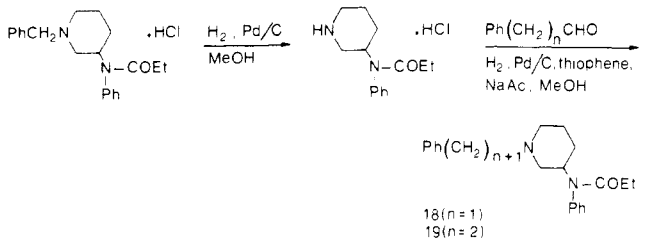
Chemistry. Generally, derivatives of fentanyl with substituents in the anilino phenyl nucleus can be prepared from the appropriately substituted aniline and 1-(2-phenylethyl)-4-piperidone, as has been described for the methoxy derivatives previously.[8] By this procedure all but two compounds (**2** and **12**) listed in Table I could be synthesized. The synthesis of **2** has already been described,[8] whereas **12** was prepared by the route shown in Scheme I. The N-depropionyl analogue **12** could not be prepared in the usual way because of the very low yield and unsatisfactory purification of the product. Attempts to convert **12** into the 2-nitro derivative of fentanyl by propionylation of the secondary amine group with propionic acid anhydride or propionyl chloride in benzene or toluene were unsuccessful, even after prolonged reaction times at reflux temperature, probably due to the low electron density at the secondary amine nitrogen atom.

Compound **6** was prepared from **5** by demethylation of the 4-OCH_3 group with BBr_3 at room temperature.[10] This

Chart I



Scheme II



simple method for demethylation of aromatic methyl ethers failed to demethylate the 2-OCH_3 group in **3**. After treatment with BBr_3, **3** was regained unaltered, which explains why the 2-methoxy-4-hydroxy derivative **6** was obtained as the reaction product of **5** instead of the 2,4-dihydroxy derivative.

The ring-closed analogues of fentanyl, **13** and **14** (Chart I), were prepared as described by Klein et al.[11] Compound **15** was synthesized by analogy of 1,3-dihydro-3-(1-methyl-4-piperidinyl)-2*H*-indol-2-one,[12] whereas **16** (INE 4884) and **17** (INE 4459) were synthesized[13] and generously donated by Endo Laboratories, Garden City, NY.

Compounds **18** and **19**, derived from fentanyl by displacement of the 4-substituent of the piperidine ring to the 3 position, were obtained by the reactions shown in Scheme II: catalytic debenzylation of *N*-phenyl-*N*-[1-(phenylmethyl)-3-piperidinyl]propanamide (prepared from 1-(phenylmethyl)-3-piperidone and aniline by analogy of the fentanyl derivatives[8]), followed by reductive alkylation with either phenylacetaldehyde or phenylpropionaldehyde.

The fentanyl analogue **20**, in which the amide nitrogen atom has been replaced by a carbon atom, and its methoxy derivatives **21–23** were synthesized according to Scheme III. After condensation of 1-(2-phenylethyl)-4-piperidone with phenylacetonitrile or methoxyphenylacetonitrile in methanol in the presence of sodium methanolate, the ensuing α,β-unsaturated nitrile was (without isolation and purification) reduced with $NaBH_4$ in 2-propanol. Alkylation of the nitrile group with the Grignard reagent ethylmagnesium bromide, followed by acidic hydrolysis, resulted in **20–23**. The phenolic hydroxy derivatives **24–26** were prepared by demethylation of the methoxy groups of **21–23** with BBr_3 in chloroform.[10] Without precautions, the benzofuran derivative **27** was obtained as the product of **21** in this reaction. The 2-OH derivative **24** could, however, be isolated when a slightly modified workup procedure was applied (see Experimental Section).

(9) J. Reden, M. F. Reich, K. C. Rice, A. E. Jacobson, A. Brossi, R. A. Streaty, and W. A. Klee, *J. Med. Chem.*, **22**, 256 (1979).
(10) K. C. Rice, *J. Med. Chem.*, **20**, 164 (1977).
(11) W. Klein, W. Back, and E. Mutschler, *Arch. Pharm. (Weinheim, Ger.)*, **307**, 360 (1974).
(12) G. H. Walker, R. T. Smith, and B. N. Weaver, *J. Med. Chem.*, **8**, 626 (1965).
(13) J. G. Berger, F. Davidson, and G. E. Langford, *J. Med. Chem.*, **20**, 600 (1977).

Scheme III

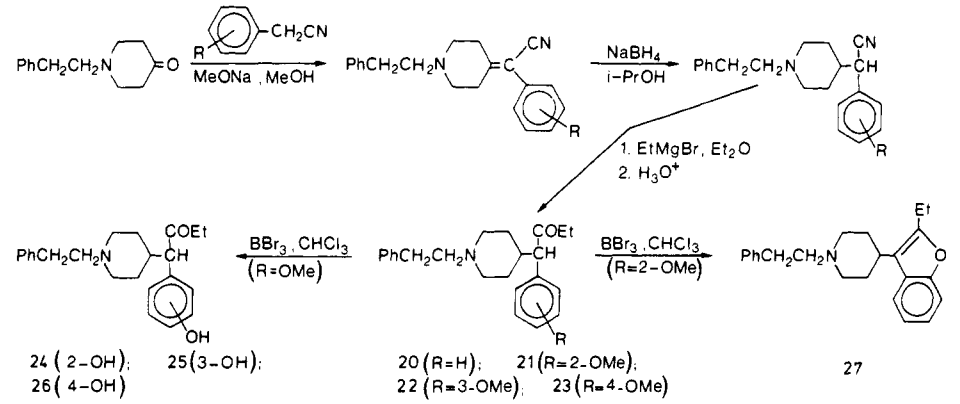


Table II. Opiate Receptor Affinity of Compounds Structurally Related to Fentanyl

no.[a]	IC_{50},[b] nM	rel ORA[c]	p
13	109	0.031	<0.05[d]
14	290	0.012	<0.05[d]
15	767	0.0044	<0.05[d]
16	721	0.0047	<0.05[d]
17	889	0.0038	<0.05[d]
18	345	0.0098	<0.05[d]
19	101	0.033	<0.05[d]
20	71	0.047	<0.05[d]
21	29	0.12	<0.05[e]
22	250	0.013	<0.05[e]
23	810	0.042	<0.05[e]
24	46	0.073	NS[e]
25	86	0.039	NS[e]
26	490	0.0069	<0.05[e]
27	61	0.055	NS[e]

[a] See Schemes II and III and Chart I for chemical structures. [b] See footnote *a* to Table I; for all compounds, $n = 3$. [c,d] See footnotes *c* and *d* to Table I. [e] *p* value for the difference with **20**.

Biological Evaluation. The ORA of all experimental compounds and some reference compounds was measured as their inhibiting potency (IC_{50}) in a binding assay with [^{3}H]fentanyl as the labeled opiate receptor ligand. IC_{50} values thus obtained are supposed to be proportional to the dissociation constants of the inhibitor–receptor complex,[14] so that the ORA of a test compound relative to morphine can be expressed as the following ratio: IC_{50} of morphine/IC_{50} of test compound. The results of these binding assays are summarized in Tables I and II.

Data on the analgesic activity of some compounds described here were available from literature references and personal communications[15] and are mentioned briefly for the purpose of comparison with the present data.

Results and Discussion

In the anilino ortho-substituted series (Table I) the relative ORA of **3** (2-OCH_3), **7** (2-CH_3), and **10** (2-Cl) is not significantly different from that of the parent compound fentanyl (**1**), indicating that the electronic nature of the ortho substituent has no relevance for the opiate receptor interaction. Lengthening of the substituent from methyl (**7**) to ethyl (**9**) and from methoxy (**3**) to ethoxy (**4**) causes a significant decrease of ORA. The 14-fold difference in ORA between **3** (2-OCH_3) and **9** (2-C_2H_5) indicates, however, that the observed substituent effects are not only related to their chain length or their total volume. The significant loss of ORA which occurs when the 2-methoxy group (**3**) is replaced by a 2-hydroxy group (**2**) would be explainable by assuming intramolecular hydrogen bond formation between the phenolic hydroxyl group and the amide carbonyl. This type of hydrogen bond formation could reduce the fraction of molecules which can adopt the conformation required for receptor binding. However, an IR analysis of **2** did not show the occurrence of hydrogen bonds of the intramolecular type but only of the intermolecular type.[16] Introduction of a second *o*-methyl group causes a slight decrease of ORA (cf. **7** and **8**). In the 2,4-disubstituted derivatives **5** and **6**, both possessing the favorable 2-methoxy group, the effect of the 4-substituent seems to predominate, since the IC_{50} of the 4-OCH_3 and 4-OH derivatives in this binding assay amounted to 11.5 and 1.22 nM, respectively.[8]

Removal of the *N*-propionyl group of **3**, resulting in **11**, is accompanied by a dramatic loss of ORA to less than 0.001 times the original value. The ORA of the 2-nitrodepropionyl analogue **12** is also in this range. Therefore, the *N*-propionyl group in fentanyl and its derivatives seems to be essential for their opiate receptor interaction.

Our present results with the above-mentioned derivatives seem to be in agreement with literature data[17] on the analgesic activity of a series of similar ortho-substituted derivatives (2-$COOCH_3$; 2-$COOC_2H_5$; 2-$COOC_3H_7$) of fentanyl. These compounds were approximately equipotent to morphine with decreasing activity at increasing chain length of the ester function, whereas the *N*-depropionyl analogues were inactive.

The ORA of a series of analogues with a modified basic structure is given in Table II. By attaching the anilino phenyl ring, via its ortho position, to the propionyl side chain (**13** and **14**) or to the 3 position of the piperidine ring (**16** and **17**), semirigid structures are obtained in which the anilino phenyl ring is nearly in the plane of the amide function. The ORA of the quinolinone derivative **13** and the indolinone derivative **14** is 100 and 250 times less than the ORA of fentanyl, respectively, whereas **15**–**17** show hardly any affinity (500 to 800 times less than fentanyl). In vivo, these compounds lack analgesic activity.[13,18] In the structure of "isofentanyl" (**18**) all functional groups

(14) L. T. Williams and R. J. Lefkowitz, "Receptor Binding Studies in Adrenergic Pharmacology", Raven Press, New York, 1978, Chapter 4.
(15) P. A. J. Janssen, personal communication.
(16) The relative intensity of the hydrogen bond absorption (3200–3300 cm^{-1}) in the IR spectrum of **2** in CCl_4 strongly diminished upon diluting the sample. In a KBr disk of **2** a very sharp carbonyl absorption was present at 1650 cm^{-1}; W. A. Seth Paul, personal communication.
(17) A. Burkartsmaier and E. Mutschler, *Arch. Pharm.* (*Weinheim, Ger.*), **311**, 843 (1978).
(18) W. Klein, W. Back, and E. Mutschler, *Arch. Pharm.* (*Weinheim, Ger.*), **308**, 910 (1975).

Table III. Fentanyl Analogues

no.	(re)crystn solvent[a]	yield, %	mp, °C	formula	anal.
1[b]				$C_{22}H_{28}N_2O \cdot C_6H_8O_7$[c]	
2	A	55	149-150.5	$C_{22}H_{28}N_2O_2$	H, N; C[d]
3	B	53	93.5-94	$C_{23}H_{30}N_2O_2$	C, H, N
4	C	57	181-182 dec	$C_{24}H_{32}N_2O_2 \cdot C_2H_2O_4$[e]	C, H, N
5	D	46	209-210	$C_{24}H_{32}N_2O_3 \cdot HCl$	H, N; C[f]
6	E	64	159-160	$C_{23}H_{30}N_2O_3 \cdot 0.5C_4H_8O$[g]	C, H, N
7	C	65	195-197	$C_{23}H_{30}N_2O \cdot HCl$	C, H, N
8	F	67	170.5-171.5	$C_{24}H_{32}N_2O \cdot HCl \cdot H_2O$	C, H, N
9	C	71	178-180	$C_{24}H_{32}N_2O \cdot HCl$	H, N; C[h]
10	D	44	193-195	$C_{22}H_{27}ClN_2O \cdot HCl$	C, H, N
11	E	33	206-206.5	$C_{20}H_{26}N_2O \cdot HCl \cdot 0.5H_2O$	H, N; C[i]
12	G/H[j]	34	79.5-81	$C_{19}H_{23}N_3O_2$	C, H, N
13	I	26	>250	$C_{22}H_{26}N_2O \cdot HCl$	C, H, N
14	J	21	145-146	$C_{21}H_{24}N_2O$	C, H, N
15	E	77	160-161	$C_{21}H_{24}N_2O$	H, N; C[k]
16[l]				$C_{22}H_{26}N_2O$	
17[l]				$C_{22}H_{26}N_2O \cdot HCl$	
18	C	50	214-215	$C_{22}H_{28}N_2O \cdot HCl$	C, H, N
19	C	46	162 dec	$C_{23}H_{30}N_2O \cdot C_2H_2O_4$[e]	C, H, N
20	H	72	141 dec	$C_{23}H_{29}NO \cdot C_2H_2O_4$[e]	H, N; C[m]
21	H	48	170 dec	$C_{24}H_{31}NO_2 \cdot C_2H_2O_4$[e] $\cdot 0.3H_2O$	C, H, N
22	H	37	191-193	$C_{24}H_{31}NO_2 \cdot HCl$	C, H, N
23	H	61	213-214	$C_{24}H_{31}NO_2 \cdot HCl$	C, H, N
24	F	10/15	156.5-158	$C_{23}H_{29}NO_2$	C, H, N
25	H	79	167.5-168.5	$C_{23}H_{29}NO_2$	H, N; C[n]
26	H	87	158-159.5	$C_{23}H_{29}NO_2$	C, H, N
27	H	65	>250	$C_{23}H_{27}NO \cdot HCl$	C, H, N

[a] A, cyclohexane; B, petroleum ether (60-80 °C); C, acetone; D, 4-methyl-2-pentanone; E, butanone; F, ethyl acetate; G, petroleum ether (40-60 °C); H, 2-propanol; I, methanol-absolute ether; J, methanol-water. [b] Fentanyl; donated by Janssen Pharmaceutica, Beerse, Belgium. [c] Citrate. [d] C: calcd, 74.96; found, 74.28. [e] Oxalate. [f] C: calcd, 66.58; found, 65.85. [g] 0.5 mol of butanone. [h] C: calcd, 71.89; found, 71.43. [i] C: calcd, 67.50; found, 68.23. [j] Crystallization from solvent G, recrystallization from solvent H. [k] C: calcd, 78.72; found, 78.10. N: calcd, 8.74; found, 8.03. [l] Donated by Endo Labs, Garden City, NY; synthesis described in ref 13. [m] C: calcd, 70.57; found, 69.95. [n] C: calcd, 78.60; found, 78.15.

of fentanyl are present, but the distance between the anilino phenyl and the piperidine nitrogen atom is reduced. This modification causes a significant 300-fold decrease of ORA, which is only partly overcome by increasing the distance between both phenyl rings to that in fentanyl (**19**). In the binding assay, **19** is about 3.5 times more active than the *N*-(phenylethyl) analogue **18** but still 90 times less active than fentanyl. Compound **18** has no analgesic activity, whereas **19** has some activity at 40 mg/kg sc (mouse hot plate).[15] In contrast, the structurally related pyrrolidinylanilides **28** and **29** possess moderate analgesic activity (0.9 and 3.4 times morphine, respectively).[19] Although data on the in vitro ORA of the ring-contracted fentanyl analogues **28** and **29** are not available, the difference in analgesic activity between these pyrrolidines and the piperidines **18** and **19** probably reflects a substantial difference in receptor affinity, which might be related to the larger dimensions of the piperidine ring or to conformational differences between both ring systems. Replacement of the amide nitrogen in fentanyl by a tetrahedral carbon atom (**20**) causes a complete loss of analgesic activity in the mouse hot-plate test[15] and a 60-fold decrease of ORA, which can be explained by the different orientation of the substituents at the tetrahedral carbon atom. The changes in ORA, caused by the introduction of a hydroxyl group into the phenyl ring at the butanone moiety of **20** (Ph_A), do not run parallel with the effects of hydroxylation in the anilino phenyl of fentanyl.[8] The ORA of **24** (2-OH) is not significantly different from that of the unsubstituted parent compound **20**, and the ORA decreases when the hydroxyl group is moved from the 2 position (**24**), via the 3 position (**25**), to the 4 position (**26**). However, in the fentanyl series the 2-hydroxy derivative (**2**) is far less active than the parent compound (Table I), and the ORA increases when the hydroxy group is moved from the 2 position, via the 3 position, to the 4 position.[8] Therefore, with respect to opiate receptor binding, Ph_A cannot be regarded as the equivalent of the anilino phenyl in fentanyl. Moreover, Ph_A is not equivalent to the morphine phenyl ring, which has to be hydroxylated for binding with high affinity, the methoxy analogue (codeine) and the deoxy analogue possessing substantially lower affinities.[8,9] The ORA of **27**, a benzofuran derivative obtained by ring closure and dehydration of **24**, is similar to that of the parent compounds **20** and **24**.

Some steric properties of fentanyl and its derivatives were compared by means of CPK and Dreiding models, in which a piperidine chair conformation and an equatorial orientation of the anilido moiety[20] were maintained. The "rotational freedom" of the anilino phenyl, which exists in the unsubstituted fentanyl molecule to a certain degree, was diminished after, for example, 2-methyl (**7**), 2-methoxy (**3**), or 2-chloro (**10**) substitution and was strongly inhibited after 2,6-dimethyl substitution (**8**), due to steric interactions between the ortho substituents and the axial hydrogens at the 3, 4, and 5 positions of the piperidine ring. In view of these observations, it seems plausible that conformations, in which the anilino phenyl is perpendicular to the plane of the amide function, become strongly preferred in the ortho-substituted derivatives. Yet, the ORA of **3**, **7**, **8**, and **10** is of the same order of magnitude as the ORA of fentanyl. Therefore, one might speculate that this

(19) G. C. Helsley, C. D. Lunsford, W. J. Welstead, Jr., R. F. Boswell, Jr., W. H. Funderburk, and D. J. Johnson, *J. Med. Chem.*, 12, 583 (1969).

(20) T. N. Riley and J. R. Bagley, *J. Med. Chem.*, **22**, 1167 (1979).

perpendicular conformation represents or approximates the optimal conformation for opiate receptor binding of fentanyl-like structures. This conclusion is consistent with results of X-ray crystallography and minimum energy calculations on fentanyl and some of its highly potent analogues.[21,22] Strikingly, semirigid analogues like 13–17, in which the perpendicular conformation of the anilido moiety is excluded, hardly bind to opiate receptors. It must be kept in mind, however, that the low ORA of these analogues can also be the result of a number of other deviations from the structure of the parent molecule.

Experimental Section

All target compounds were purified by (re)crystallization and/or preparative layer chromatography (PLC) on silica gel plates (Merck). Purity of compounds was checked by TLC (silica gel plates, Merck). Melting points were measured on a Tottoli apparatus (Buchi) and are uncorrected. Identity of compounds was checked routinely by spectroscopic methods (IR, UV, and NMR). IR spectra of KBr disks were recorded on a Beckman IR20A spectrometer, UV spectra of MeOH solutions were recorded on a Unicam SP 800 double-beam spectrophotometer, and NMR spectra of $CDCl_3$ or Me_2SO solutions with Me_4Si as the internal standard were recorded on a Varian EM 360 spectrometer. Spectroscopic properties of all compounds were compatible with the proposed structures. In a few cases, additional evidence was obtained by MS (Department of Organic Chemistry, University of Amsterdam). Characteristic shifts of UV absorption maxima of phenolic compounds were observed upon the addition of a few drops 1 N NaOH to the sample. Analyses of C, H, and N were carried out at the Analytical Department of Janssen Pharmaceutica, Beerse, Belgium. Generally, results were within ±0.4% of the theoretical values. Catalytic reductions in the presence of Pd/C or Pt catalyst were carried out at the Organic Chemistry Department of Janssen Pharmaceutica. All organic starting materials were obtained from Aldrich Chemical Co. (Beerse, Belgium).

***N*-(Alkylphenyl)-, *N*-(Alkoxyphenyl)- and *N*-(Chlorophenyl)-*N*-[1-(2-phenylethyl)-4-piperidinyl]propanamides (3–5 and 7–10).** To a solution of the substituted aniline (28 mmol) and *p*-toluenesulfonic acid (10 mg) in toluene (50 mL), heated at reflux under continuous separation of H_2O, was added dropwise a solution of 1-(2-phenylethyl)-4-piperidone (25 mmol) in toluene (15 mL) in ca. 15 min. The reaction mixture was heated at reflux for 5 h and, after the addition of 20 g of a molecular sieve (4Å), for 8 h. After cooling to room temperature, the mixture was filtered and the solvent was evaporated in vacuo. The residual oil was dissolved in MeOH (35 mL) and brought to reflux. Solid $NaBH_4$ (27.5 mmol) was added gradually, followed by heating at reflux for 2 h. The mixture was concentrated in vacuo to about 10 mL, diluted with H_2O (90 mL), and extracted with benzene (3 × 50 mL). The combined organic layers were washed with H_2O (2 × 50 mL), dried over $MgSO_4$, and concentrated in vacuo, leaving an oil from which the intermediate substituted *N*-phenyl-1-(2-phenylethyl)-4-piperidinamines (e.g., 11·HCl) could be isolated as their HCl salts in 30–50% yields. Propionylation of the free bases of the secondary amines was achieved by adding propionic acid anhydride (15 mmol) to a solution of the 4-piperidinamine (10 mmol) in anhydrous benzene (60 mL) at reflux, followed by heating at reflux for 16 h. The mixture was extracted with 20% NaOH (75 mL), washed with H_2O (3 × 30 mL), dried over $MgSO_4$, and concentrated in vacuo. The target compounds (3–5 and 7–10) were isolated from the residual oils usually as their HCl or $H_2C_2O_4$ (oxalic acid) salts.

***N*-(2-Methoxy-4-hydroxyphenyl)-*N*-[1-(2-phenylethyl)-4-piperidinyl]propanamide (6).** Demethylation of the 4-methoxy group of the free base of **5** was achieved by the method of Rice,[8] using BBr_3 in $CHCl_3$ at room temperature.

***N*-(2-Nitrophenyl)-1-(2-phenylethyl)-4-piperidinamine (12).** To a mixture of 1-(2-phenylethyl)-4-piperidone (15 g, 74 mmol) and benzylamine (10 g, 93 mmol) dissolved in MeOH (200 mL) were added 10% Pd/C catalyst (2 g) and 4% thiophene solution in MeOH (1 mL). This mixture was hydrogenated at room temperature and atmospheric pressure until 1 equiv of H_2 had been consumed. The poisoned catalyst was removed by filtration and replaced by fresh 10% Pd/C catalyst (2 g), followed by hydrogenation until again 1 equiv of H_2 had been taken up. The product of this reaction, 1-(2-phenylethyl)-4-piperidinamine, was isolated by filtration to remove the catalyst and evaporation of the solvent in vacuo (GLC: 98.7% pure) and dissolved (2.0 g, 6.8 mmol) in *n*-PrOH (50 mL) together with 1-fluoro-2-nitrobenzene (1.4 g, 9.9 mmol). Na_2CO_3 (1.2 g) and KI (10 mg) were added, and the mixture was heated at reflux until the starting material was no longer detectable by TLC. Inorganic salts were removed by filtration, and the filtrate was concentrated in vacuo. The remaining material was extracted with benzene (50 mL). The extract was washed with 0.1 N NaOH, dried over $MgSO_4$, and evaporated to dryness in vacuo. The residual oil crystallized from petroleum ether (40–60 °C). Recrystallization from *i*-PrOH yielded **12**.

***N*-Phenyl-*N*-[1-(phenylalkyl)-3-piperidinyl]propanamides (18 and 19).** A solution of *N*-phenyl-*N*-[1-(phenylmethyl)-3-piperidinyl]propanamide hydrochloride (1.0 g, 2.8 mmol) in MeOH (100 mL) was hydrogenated in the presence of 10% Pd/C catalyst (1 g) at room temperature. After 1 equiv of H_2 had been consumed, phenylacetaldehyde (**18**) or 3-phenylpropionaldehyde (**19**) (4.2 mmol), NaAc (0.5 g, 6.1 mmol), and 4% thiophene in MeOH (0.5 mL) were added. This mixture was hydrogenated until 1 equiv of H_2 had been consumed. The catalyst was removed by filtration, and the solvent by evaporation in vacuo. The residue was acidified with 5 N citric acid and extracted with ether (2 × 25 mL). The ether layers were discarded, and the aqueous layer was alkalized with 28% NH_3 and extracted with ether (2 × 25 mL). The combined alkaline ether extracts were dried over $MgSO_4$ and concentrated in vacuo. The residual oil was dissolved in acetone and acidified with either HCl/*i*-PrOH (**18**) or $H_2C_2O_4$ (oxalic acid)/*i*-PrOH (**19**) to yield the pure target compounds as the HCl and the $H_2C_2O_4$ salt, respectively.

1-Phenyl- and 1-(Methoxyphenyl)-1-[1-(2-phenylethyl)-4-piperidinyl]-2-butanones (20–23). A solution of *N*-(2-phenylethyl)-4-piperidone (4.1 g, 20 mmol) and phenylactonitrile or methoxyphenylacetonitrile (20 mmol) in absolute MeOH (3.0 mL) was added dropwise to 4.0 mL of a freshly prepared solution of Na (5.1 g) in absolute MeOH (120 mL). The mixture was heated at reflux for 6 h and, after cooling to room temperature, poured on a mixture of ice (10 g) and acetic acid anhydride (3.0 mL). The ensuing solid was isolated by filtration, washed with H_2O and *i*-PrOH, and dried at reduced pressure, yielding nearly pure α,β-unsaturated nitrile (yield 55–70%; IR sharp band between 2180 and 2230 cm^{-1}, α,β-unsaturated CN), 10 mmol of which was reduced in *i*-PrOH (25 mL) by gradually adding $NaBH_4$ (0.43 g, 11 mmol), followed by heating at reflux for 24 h. The reaction mixture was poured into a solution of NH_4Cl (1.4 g) in H_2O (10 mL) and stirred. After about 25 mL of H_2O was added to the solution, the compound precipitated and could be collected by filtration. The collected material consisted of nearly pure α,β-saturated nitrile (yield 75–95%; IR sharp band between 2240 and 2260 cm^{-1}, α,β-saturated CN) and was not purified further. The α,β-saturated nitrile (4 mmol) was dissolved in dry ether (50 mL) and added dropwise to the Grignard reagent EtMgBr [freshly prepared from Mg (0.48 g, 20 mmol) and EtBr (2.2 g, 20 mmol)] in dry ether (30 mL). After heating at reflux for 6 h, the mixture was poured on ice (20 g) and 36% HCl (3.3 mL) and stirred at room temperature for 90 min. After adjusting to pH 9 (3 N NaOH), the ether layer was separated and the aqueous layer was extracted with ether (2 × 50 mL). The collected ether layers were washed with H_2O and dried over $MgSO_4$. The solvent was evaporated in vacuo to leave an oil from which the target compounds were isolated as their HCl or $H_2C_2O_4$ salt by (re)crystallization from *i*-PrOH.

1-(Hydroxyphenyl)-1-[1-(2-phenylethyl)-4-piperidinyl]-2-butanones (24–26) and 4-(2-Ethyl-3-benzofuranyl)-1-(2-phenylethyl)piperidine (27). The phenolic compounds **25** and **26** were prepared in high yield (80–90%) by demethylation of the

(21) J. P. Tollenaere, H. Moereels, and L. A. Raymakers, "Atlas of the Three Dimensional Structure of Drugs"; Janssen Research Foundation Series, Vol. 1, Elsevier, Amsterdam, 1979.

(22) M. H. J. Koch, C. J. de Ranter, M. Rolies, and O. Dideberg, *Acta Crystallogr., Sect. B*, **32**, 2529 (1976).

free bases of **22** and **23** with BBr_3 in $CHCl_3$ according to Rice.[10] Application of this procedure to **21** normally yielded **27**. However, **24** could also be isolated after BBr_3 treatment of **21** by adding the theoretical amount of NH_3 to the ice-cooled reaction mixture and sufficient H_2O to dissolve the precipitating material. After the mixture was shaken for about 1 min, the $CHCl_3$ layer was separated and "dried" by filtration through several paper filters and evaporated under a stream of N_2. The target compound was isolated from the residue by PLC (10% MeOH in $CHCl_3$; R_f 0.35) and purified by crystallization from ethyl acetate.

Receptor Binding Assay. Binding experiments were performed, as previously described in more detail,[8] by incubating fixed amounts of a mitochondrial–synaptosomal fraction of rat brain homogenate in a medium of 50 mM Tris-HCl buffer of pH 7.4 at 25 °C, in the presence of 0.4 nM [^{3}H]fentanyl and either 40 nM levomoramide or 40 nM dextromoramide, to differentiate between opiate receptor binding and non-opiate-receptor-binding. Inhibitors were tested at three to five concentrations, and all incubations were carried out in duplicate. Incubations were terminated by rapid filtration through Whatman GF/B filters. Radioactivity on the filters was measured by liquid scintillation counting. Each experiment was repeated several times.

Statistical Evaluation of Receptor Binding Data. The combined variance of log IC_{50} determinations in our receptor binding assay was calculated from 137 independent observations on 47 test compounds by means of the SPSS program ONE WAY ANALYSIS OF VARIANCE (Version 70).[23] The combined variance was used in the Student's t test to evaluate the statistical significance of differences in ORA. In the case of two compounds both with $n = 3$, the difference is significant ($p < 0.05$) when the ratio of their mean IC_{50} values is larger than 1.563 or smaller than 0.640. Otherwise, it is denoted as nonsignificant (NS). In Tables I and II, most compounds are compared with fentanyl as the reference compound.

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(23) N. H. Nie, C. H. Hull, J. G. Jenkins, K. Steinbrenner, and D. H. Brent, Eds., "SPSS, Statistical Package for the Social Sciences", 2nd ed., McGraw-Hill, New York, 1975, Chapter 22.

Allylprodine Analogues as Receptor Probes. Evidence That Phenolic and Nonphenolic Ligands Interact with Different Subsites on Identical Opioid Receptors

Philip S. Portoghese,* Bipin D. Alreja, and Dennis L. Larson

Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota 55455.
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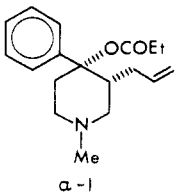
The *m*-hydroxy analogues of allylprodine and related structures have been synthesized and tested for narcotic agonist and antagonist activity on the electrically stimulated guinea pig ileum and by the hot-plate procedure in mice. It has been found that *m*-hydroxyallylprodine (α-**2**) is neither an agonist nor antagonist. Other phenolic congeners similarly have little or no activity. The fact that these results are in dramatic contrast with the structure–activity profile of morphine and closely related opiates has led to the proposal that the interaction of morphine and allylprodine (α-**1**) with the μ opioid receptor differs. This difference is postulated to arise from the recognition of the aromatic groups of morphine and α-**1** by different aromatic-binding subsites of the receptor. These subsites are suggested to be identical with those which recognize the aromatic rings of the Tyr^1 and Phe^4 of the enkephalins and endorphins. A receptor model consistent with these results is proposed.

The role of the phenolic OH in enhancing the agonist potency of opiates and closely related compounds is well recognized. It has been proposed that the phenolic OH effects this enhancement by functioning as a hydrogen-bonding proton donor in the ligand–receptor association process.[1] The fact that the phenolic series often possess structure–activity profiles which differ substantially from the nonphenolic series has been attributed to divergent ligand–receptor binding modes.[1-3]

In order to investigate this phenomenon further we have synthesized and biologically evaluated phenolic analogues of allylprodine (α-**1**)[4-6] and its congeners. Allylprodine was selected for modification because it is considerably more potent than morphine.[5,6] Hence, it was of interest to determine the effect of a meta OH on agonist activity by analogy with the phenolic OH in morphine. If the phenolic analogue interacts with opioid receptors in a fashion similar to that of morphine, then such a modification should enhance potency. On the other hand, a substantial diminution of activity would be a manifestation of divergent modes of interaction with opioid receptors.



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In this article we present evidence which suggests the latter possibility. A model consistent with the structure–activity relationship of the enkephalins[7] is proposed in order to account for the profoundly different structure–activity profiles between the allylprodine series and morphine-type compounds.

Chemistry. The first step leading to the piperidinol intermediates **11–13** in the synthesis of the target compounds (Table I) involved the condensation of *m*-anisyl-

(1) P. S. Portoghese, *J. Med. Chem.*, **8**, 609 (1965).
(2) P. S. Portoghese, *J. Pharm. Sci.*, **55**, 865 (1966).
(3) P. S. Portoghese, *Acc. Chem. Res.*, **11**, 21 (1978).
(4) A. Ziering, A. Motchane, and J. Lee, *J. Org. Chem.*, **22**, 1521 (1957).
(5) K. H. Bell and P. S. Portoghese, *J. Med. Chem.*, **16**, 203 (1973).
(6) K. H. Bell and P. S. Portoghese, *J. Med. Chem.*, **16**, 589 (1973).
(7) J. S. Morley, *Annu. Rev. Pharmacol. Toxicol.*, **20**, 81 (1980).

0022-2623/81/1824-0782$01.25/0