0026-895X/92/010185-12$03.00/0

MOLECULAR PHARMACOLOGY, 41:185-196

Molecular Determinants of μ Receptor Recognition for the Fentanyl Class of Compounds

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Received July 3, 1991; Accepted October 17, 1991

SUMMARY

We report here a theoretical study of a series of fentanyl analogs with a wide range of affinities and selectivities at the μ receptor, designed to identify and characterize the molecular determinants of μ receptor recognition. In this work, a complete conformational search combining nested rotations and molecular dynamic simulations has been made, leading to identification of accessible conformers for all analogs and to the selection of a candidate bioactive form. In addition, electronic properties have been calculated and examined as possible modulators of recognition at the μ receptor. The results of these studies have led to a distinct pharmacophore for interaction at the μ receptor for this class of compounds, with the piperidine ring in a chair conformation and the *N*-phenethyl and 4-phenylpropanamide substituents both equatorial. Moreover, four key moieties necessary for optimum receptor recognition and a postulated role for each of them in this recognition have been identified. These are (i) a protonated amine nitrogen, assumed to be involved in an initial electrostatic interaction with a negatively charged site on the receptor; (ii) a polar function capable of hydrogen-bonding with an electrophilic site; (iii) an aromatic ring involved in lipophilic interaction with a similar moiety; and (iv) a second aromatic ring, most probably involved in electron transfer interaction with the receptor. These requirements, taken together, form the basis of our proposed mechanism for μ receptor recognition. Not only is the presence of these components required for recognition, but specific steric relationships between them have been determined, implying the appropriate arrangement for interaction with complementary receptor sites. These steric parameters are pseudobond angles and one torsion angle that determine the relative spatial arrangement of these four moieties. They are the angles θ_1 and θ_3, defining the relative position of the protonated nitrogen and the polar function with each of the two aromatic rings, and the torsion angle η_1, defining the orientation of the lone pair(s) on the polar proton-accepting function with respect to the lone pair on the piperidine nitrogen. This postulated mechanism of recognition provides a conceptual framework to understand why some compounds do and some do not recognize the μ receptor.

Of all the families of compounds that are known to bind at opiate receptors, the 4-anilido-piperidine class has proven to be one of the most intriguing and at the same time elusive. Ever since the synthesis of the parent compound of this series, fentanyl (1) (1), several structural variations of the basic 4-anilido-piperidine assembly have been probed, resulting in compounds showing activity over a wide range of structural modifications (see Ref. 2 for a review of most relevant structure-activity relationship data). The presence or absence of *in vivo* activity has been the only pharmacological characterization for many of these compounds, most of which were synthesized in the late 1970s. Little is known of their receptor binding profiles or their *in vitro* activity, and there are no known antagonists in this family of opiates.

Only fentanyl, sufentanil, and alfentanil, which are in clinical use, and the new analog ohmefentanyl (3) have been characterized as high affinity, selective μ receptor agonists (4–8). Without similar detailed *in vitro* studies, the same μ selectivity has been implicitly assumed for other members of the fentanyl class. However, recent reports on binding affinity at the μ and δ receptors for some of the most potent analogs (9–11) show that the assumption of μ receptor selectivity of the fentanyls needs to be reconsidered.

To more completely characterize some of these analogs and to provide a firmer basis for theoretical studies of the fentanyls, we have recently determined the binding affinities and *in vitro* activities of a number of analogs at μ, δ, and κ receptors.[1] Although all of the analogs studied had highest affinity for the μ receptor, they exhibited a wide range of receptor selectivity.

We report here a parallel theoretical study of a set of fentanyl analogs, among which are those found in our recent experimental studies to have high affinity and selectivity at the μ receptor.

This work was supported by a grant from the National Institute on Drug Abuse (DA02622).

[1] P. A. Maguire, N. Tsai, J. F. Kamal, C. Cometta-Morini, C. Upton, and G. H. Loew. Receptor affinities and agonist activities of a series of fentanyl analogs at the μ-, δ-, and κ-opioid receptors. *Submitted for publication.*

ABBREVIATIONS: CEA, chair-equatorial-axial; CEE, chair-equatorial-equatorial; HOMO, highest occupied molecular orbital; LUMO, lowest unoccupied molecular orbital; AM1, Austin Method 1.

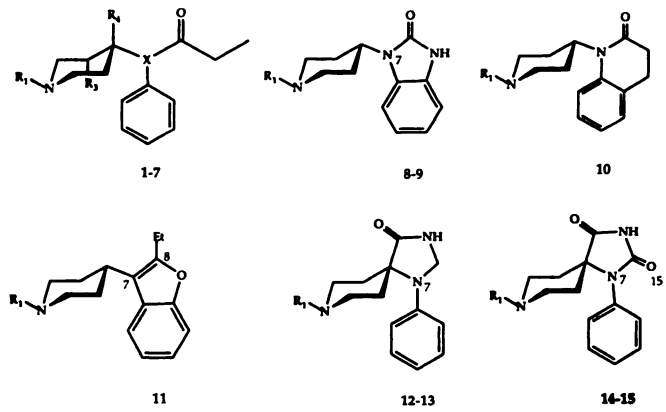
The work reported here is designed to further probe the molecular determinants of μ receptor recognition and activation.

A number of active conformations have been proposed in the past to explain the high affinity and *in vivo* potency of fentanyl itself and of some of its analogs (12–18). In these studies, all analogs characterized were implicitly assumed to be highly μ selective. The work presented here builds on previous studies in four ways. (i) It takes into account the increased knowledge of receptor affinity and activity of the analogs at each receptor gained from the experimental studies. (ii) It includes more diverse analogs. (iii) A more complete conformational search is made to determine the bioactive form that binds at the μ receptor. (iv) Electronic as well as steric factors are calculated for the first time and examined for their relevance as modulators of recognition at the μ receptor.

In a previous paper, as a first step in these studies, we have reported the results of an extensive conformational search of the parent compound fentanyl (19), which has led to the determination of the probable conformation of fentanyl in polar and nonpolar solvents and of three candidate conformers for its bioactive form.

Materials and Methods

The compounds selected for study are shown in Fig. 1, and their pharmacological profile, obtained from our own studies and other literature data, is summarized in Table 1. The set of analogs considered comprises flexible compounds, i.e., fentanyl (**1**), R30490 (**2**) (20), carfentanil (**3**) (21), lofentanil (**4**) (21), *N*-methylfentanyl (**5**) (22), *N*-methylcarfentanil (**6**) (21), and the CH-analog of fentanyl (**7**) (23), as well as conformationally constrained ones, i.e., the two 4-benzimidazolonopiperidine analogs **8** and **9** (24), the 3,4-dihydrocarbostyrile **10** (25), the benzofuryl derivative **11** (24), the two spirane analogs **12** and **13** (24), and the spirodiones **14** and **15**.



		R_1	R_3	R_4	X
1	fentanyl	CH_2CH_2Ph	H	H	N
2	R30490	CH_2CH_2Ph	H	CH_2OCH_3	N
3	carfentanil	CH_2CH_2Ph	H	$COOCH_3$	N
4	lofentanil	CH_2CH_2Ph	CH_3	$COOCH_3$	N
5	N-methyl fentanyl	CH_3	H	H	N
6	N-methyl carfentanil	CH_3	H	$COOCH_3$	N
7	CH-fentanyl	CH_2CH_2Ph	H	H	CH
8		CH_2CH_2Ph			
9		$CH(CH_3)Ph$			
10		CH_2CH_2Ph			
12		$CH(CH_3)Ph$			
13		CH_2CH_2Ph			
14		CH_3			
15		CH_2CH_2Ph			

Fig. 1. Fentanyl analogs selected for study.

All computations were performed on a Silicon Graphics IRIS 4D/220GTX workstation.

Conformational search. A detailed conformational search was performed for three of the flexible (R30490, carfentanil, and lofentanil) and two of the conformationally constrained (**8** and **12**) analogs. The results of these conformational searches could be used to deduce the energetically favored conformations for the remaining analogs. Subsequently, a series of electronic properties and environmental indices were computed for all analogs studied.

X-ray structures were used as starting structures for the computations for R30490 (26) and lofentanil (15). For the remaining compounds, initial structures were built by combining features of the crystal structures of fentanyl (27), R30490, and lofentanil.

All conformational studies were performed on the protonated form of the molecule. The pK_a values of the most potent derivatives (**1–4**) are all in the range of 7.8–8.4 (15). At these pK_a values, the compounds are predominantly in the protonated form (72–91%) at physiological pH (pH 7.4). On the basis of the extent of protonation, we assume that the protonated form dominates in solution and that it is the pharmacologically relevant species.

The conformational studies were performed using two techniques, molecular dynamics simulation and nested rotational searches. Molecular dynamics simulations were performed using the CHARMm force field (28), as available through the QUANTA molecular modeling system (Polygen Corp., Waltham, MA). The effect of a continuum polar solvent environment was taken into account by assuming a value of 78 Debye for the dielectric constant in the computations (dielectric constant of water at 25° (29).

As a first step, high temperature molecular dynamics simulations were performed for carfentanil, building on our previous detailed studies of the parent compound fentanyl (19). In that study, three candidate bioactive forms of fentanyl were identified. The lowest energy conformer, according to the AM1 computations, was one in which the *N*-phenethyl moiety was equatorial and the 4-phenylpropanamide substituent axial (CEA). In the other two, both the *N*-phenethyl and the 4-phenylpropanamide substituents were equatorial (CEE). In this study we explored the effect on the CEA and CEE conformations of adding the polar $COOCH_3$ substituent of carfentanil at the 4-position, in place of the H in fentanyl. The nature of the second 4-substituent has been shown to affect the phenyl equatorial-phenyl axial conformational ratio in the 4-substituted 4-phenylpiperidines (30). Using the CEE and the CEA conformations as initial structures, two 10-psec, high temperature (2000°K), molecular dynamics simulations of carfentanil were run, with structure collection every 10 fsec. These transient structures were fully optimized with CHARMm. The lowest energy CEE and CEA conformers were then reoptimized with AM1 (31). As a result of this procedure, the lowest energy CEE conformer was found to be 3.7 kcal/mol lower in energy than the CEA conformer. Because carfentanil has greatly enhanced μ affinity and activity, compared with fentanyl (Table 1), and the CEE is its lowest energy form, the inference can be made that the CEE structure, rather than the CEA one, is involved in recognition at the μ receptor. All further conformational studies were, therefore, limited to the CEE arrangement.

In the next step, using a CEE structure for all analogs, nested rotations were performed for carfentanil, lofentanil, R30490 (**2–4**), and the conformationally constrained analogs **8** and **12**, using the AM1 quantum mechanical method (31), specifically version 5.0 of the MOPAC molecular orbital package (32). The torsional angles that were varied for the five compounds, together with the values assigned, are given in Table 2. For each point in the grid, the particular torsion angle being scanned was given the initial value indicated but allowed to optimize along with all remaining geometrical variables.

All but analog **12** have the *N*-phenethyl substituent in common with fentanyl. For these analogs, assuming that the different substituent at position 4 of the ring will not affect the conformation of the *N*-

TABLE 1
Pharmacological data

	K_i			ED_{50}
	μ	δ	κ	
		nM		mg/kg
4 Lofentanil	0.023[a]	0.24[b]	0.60[c]	0.0006[d]
3 Carfentanil	0.024[a]	3.28[b]	43.1[c]	0.0004[d]
2 R30490	0.089[a]	23[b]	63.0[c]	0.0007[e]
1 Fentanyl	1.15[a]	178[b]	293[c]	0.011[d]
9	4.4[f]	ND[g]	ND	30× pethidine[h]
12	7.1[f]	ND	ND	60× pethidine[h]
11	39.6[f]	ND	ND	ND
7 CH-fentanyl	46.1[f]	ND	ND	Inactive[i]
6 *N*-Methylcarfentanil	42.1[a]	2.97 μM[b]	6.91 μM[c]	1.3[d]
10	70.7[f]	ND	ND	Inactive[k]
8	77.9[f]	ND	ND	Inactive[h]
13	975[a]	2.82 μM[b]	1.01 μM[c]	Inactive[h]
14 *N*-Methylspirodione	>100 μM[a]	>100 μM[b]	>100 mM[c]	ND
5 *N*-Methylfentanyl	17.6 μM[a]	11.2 μM[b]	26.6 μM[c]	Inactive[l]
15	>100 μM[a]	>100 μM[b]	>100 mM[c]	ND

[a] Inhibition of [^{3}H]DAGO ([D-Ala^2-Me-Phe^4-Gly-ol]enkephalin) binding in guinea pig whole brain.[1]
[b] Inhibition of [^{3}H]DPDPE ([D-Pen^2-D-Pen^5]-enkephalin) binding in guinea pig whole brain.[1]
[c] Inhibition of [^{3}H]U69593 binding in guinea pig whole brain.[1]
[d] Rat tail withdrawal (42).
[e] Rat tail withdrawal test (20).
[f] Inhibition of [^{3}H]fentanyl binding in rat brain (23).
[g] ND, not determined.
[h] Subcutaneously in mice (morphinomimetic potency) (24).
[i] Inhibition of [^{3}H]fentanyl binding in rat brain (41).
[l] Mouse hot plate (23).
[k] Mouse hot plate (25).
[l] Mouse hot plate (22).

substituent, we have used the results of our previous study of fentanyl (19) for the conformation of the phenethyl moiety. The conformational study performed here was thus restricted to the varying substituents in position 4. For analog **12**, with has a different *N*-substituent, i.e., α-methylbenzyl instead of phenylethyl, and an asymmetric carbon atom (C11), the conformational profile of the *N*-substituent was studied for only one enantiomer (*R*). A change in the chirality in the *N*-substituent does not affect the conformational profile of the substituent at position 4.

As a final step, molecular dynamics simulations were run at physiological temperature (312°K) for fentanyl, carfentanil, lofentanil, and R30490, to explore the conformational flexibility of their 1- and 4-piperidine ring substituents. The starting structures for the simulation were CHARMm-optimized X-ray structures for fentanyl, lofentanil, and R30490. For carfentanil, the starting structure was obtained from the X-ray structure of lofentanil. These simulations were run for 100 psec each, with structures collected every 50 fsec. These structures were optimized using the Fletcher and Powell algorithm (33), with full optimization of all geometrical variables.

Computation of electronic properties and environmental indices. As discussed, conformational studies were performed on the protonated species, because this form predominates in a polar environment and is most likely the initial species that binds to the receptor. However, because all opiates share the feature of a protonated amine, the assumption is made that this proton immediately transfers, without a barrier, to a nearby anionic site, resulting in neutralization. It is further assumed that subsequent specific interactions of each ligand with other receptor subsites occur that modulate its optimum orientation in the binding site. It is possible that the proposed steps in this sequential "zipper-type" recognition mechanism (34) are less distinct in the actual interaction in the binding pocket. To simulate a more simultaneous effect of proton transfer and other ligand-receptor interactions, we have recently used a simple explicit model for the binding pocket (35). The results of this study support the importance of the proton-transfer step in recognition. In an early study (36), it was also shown that the molecular electrostatic potential of the protonated forms of morphine, codeine, and other fused ring opioids in the presence of a model anionic site is very similar to the molecular electrostatic potential of their neutral forms. Therefore, the neutral form is the most realistic simulation of the ligand in the environment of an anionic receptor site. Electronic properties and environmental indices were computed for this form of the compound.

Among the electronic properties calculated as candidate modulators of ligand-receptor interaction was the heat of protonation at each of several competing sites for each analog. These quantities, defined as the difference between the heat of formation of the protonated and unprotonated forms of the compound, were computed using the AM1 method. They can be used as a measure of the tendency of different proton-accepting centers in the ligand to form hydrogen bonds with proton-donating atoms in the receptor binding site. Corresponding heats of deprotonation for analogs with proton-donating groups were also calculated.

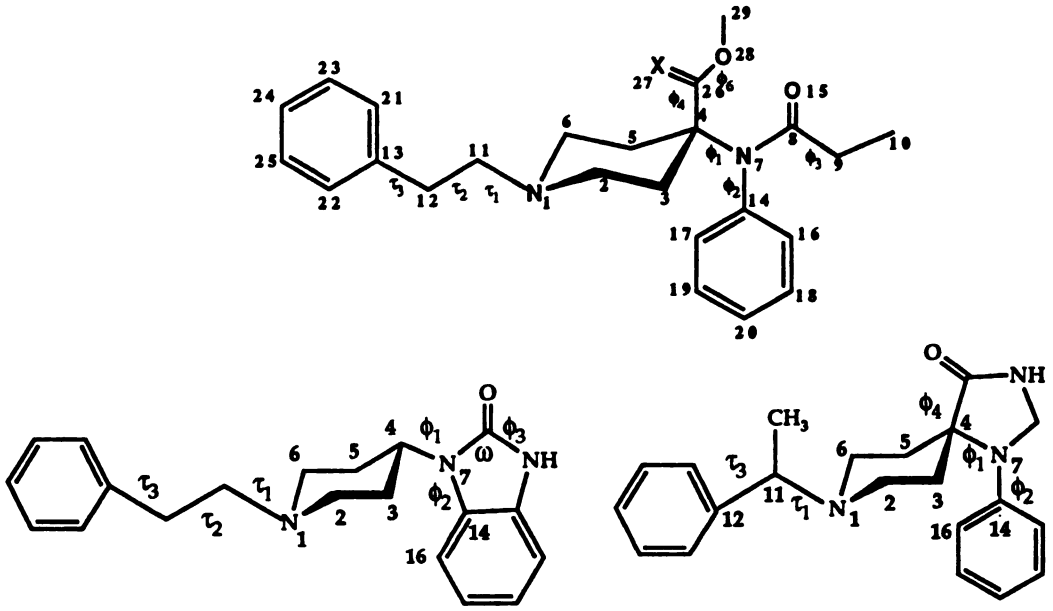
The nature and energy of the HOMO and of the LUMO were monitored as useful indicators of the site and relative ability to donate and accept electrons, respectively.

Dipole moments were also calculated as potential indicators of orientation in the binding site.

An additional property, the total polarizability volume, was determined for each analog, using an interpolation procedure of Fraga (37) together with the net atomic charges obtained from the AM1 computations. This procedure allows the determination of the individual atomic contributions to the polarizability volumes. In this way, the polarization of different regions in the molecule can be characterized separately. Such regional polarization values can be used as an indication of the likelihood of a given fragment of the ligand to be involved in stacking or other dispersion interactions with aromatic moieties in the receptor.

Molecular octanol-water partition coefficients were also computed, using a new method developed in our laboratory (38). In this procedure, each atom is assigned an atomic hydrophobicity index determined from its contribution to the total van der Waals area and its AM1 net atomic

TABLE 2
Torsion definitions and values in AM1 nested rotations search



8

12

Analog	X	Torsions varied	Values assigned
			degrees
Carfentanil	O	ϕ_1, 5-4-7-14	−60, 0, 120
		ϕ_2, 4-7-14-16	0, 90
		ϕ_4, 5-4-26-27	0, 180
R30490	H_2	ϕ_1, 5-4-7-14	−60, 0, 120
		ϕ_2, 4-7-14-16	0, 90
		ϕ_6, 5-4-26-28	0, 180
Lofentanil	O	ϕ_1, 5-4-7-14	−120, 0, 120
		ϕ_4, 5-4-26-27	0, 180
8		ϕ_1, 5-4-7-14	−60, 0, 120
12		ϕ_2, 4-7-14-16	180, 90
		τ_1, 12-11-1-6	−60, 180, 60

charge. The logarithm of the partition coefficient is then estimated as the sum of these individual contributions.

Results and Discussion

Conformational study. Table 3 summarizes the results of nested rotation searches of the CEE form for the six analogs studied. We see from this table that analogs with both rotatable and fused ring 4-substituents have several low energy rotamers of this type. The question remains which of these accessible conformations is the most favorable for μ receptor recognition.

All analogs listed in Table 3, except **12**, have *N*-phenethyl substituents. In a previous study of fentanyl, we have determined that there are two low energy forms of this equatorial *N*-substituent, an extended form ($\tau_2 \sim 180°$) and a bent form ($\tau_2 \sim 60°$) that is lower in energy by 2.1 kcal/mol (τ_2 is defined as C13-C12-C11-N1; see Table 2 for numbering). Despite this small energy difference, we have chosen the extended form as more relevant for recognition. This choice is based on several considerations. First, there are pharmacological data for conformationally constrained napthyl analogs (39) that are forced to have an extended arrangement of the phenethyl substituent and exhibit *in vivo* analgesic action. Second, computation of the solvent-accessible surfaces (40) for the two conformations in the unprotonated form of fentanyl shows that, when the phenethyl side chain is bent, access to the piperidine nitrogen proton is blocked by the Van der Waals envelope of the benzene ring. As discussed previously, the interaction of the protonated nitrogen is considered essential for recognition.

Analogs **9** and **12** are unique in that the *N*-phenethyl is replaced by an α-methyl branched *N*-benzyl substituent. For one of these analogs, **12**, as shown in Table 2, nested rotations were performed for τ_1, the major angle determining the conformation of the *N*-substituent. The results of the AM1 conformational study show that the branched *N*-substituent can also assume two low energy conformations, corresponding to a value of τ_1 of 64° and 157°. The two conformations have very similar energies, that with $\tau_1 = 64°$ being only 0.8 kcal/mol more stable than the conformation with $\tau_1 = 157°$, and either one is a possible candidate for the bioactive form.

For the important phenylpropamide moiety that defines this family of opioids, we see from Table 3 that there are four possible domains, labeled α, β, γ, and ω, defined by the major

TABLE 3
Summary of AM1 conformational studies

Analog	ϕ_1	ϕ_4	ϕ_6	$\Delta\Delta H_f$
		degrees		kcal/mol
1 Fentanyl				
α	−61			2.2
β	115			0.0
2 R30490				
ω	−127 (−127)[a]		170 (24)	0.0 (0.63)
β	117		−30	0.01
γ	16		−40	1.3
3 Carfentanil				
ω	−126 (−124)	41 (30)	−145	0.0 (0.7)
β	105	−8	167	0.08
γ	16	80	−96	0.12
4 Lofentanil				
ω	−135 (−133)	40 (178)	−143	0.0 (1.6)
β	83	−3	172	1.5
γ	14	90	−86	2.5
8				
α	−55			2.0
β	124			0.0
γ	−20			4.5
12				
ω	−114	63		0.0
γ	−8	58		0.1

[a] Values in parentheses correspond to a second minimum of the same type.

torsion angle ϕ_1. For all analogs with $R_4 \neq$ H (**2**, **3**, **4**, and **12**), the lowest energy arrangement for the phenylpropanamide moiety is the structure that we have called ω, corresponding to a value of the torsion ϕ_1 in the range −110° to −140°. However, in analog **8** and fentanyl, both of which have R_4 = H, the moiety can assume a different arrangement called α ($\phi_1 \sim -60°$). The ω conformer, with the larger value of ϕ_1, is favored in analogs with a second substitution at position 4, because of the repulsive interaction between the oxygen lone pairs of both substituents. The third possible structure, the β arrangement ($\phi_1 \sim 120°$), is allowed for the flexible analogs and for **8** but not for **12**, due to the ring fusion. The fourth arrangement of the phenylpropanamide function, called γ, is accessible to **2**, **3**, **4**, and **8**. It corresponds to a value of $\phi_1 = 0 \pm 15°$. Neither the γ nor the ω arrangement of the phenylpropanamide function corresponds to a minimum energy structure for fentanyl. However, AM1 computation of the conformational profile for rotation around ϕ_1 (data not shown) shows that both the γ and the ω conformation can be accessed from the α arrangement at negligible energy cost (ΔH_f = 1.1 kcal/mol for γ and ΔH_f = 0.5 kcal/mol for ω).

The torsional angle ϕ_2, which determines the position of the phenyl ring in the phenylpropanamide moiety, assumes a value of approximately ±90° in all conformers for all unconstrained analogs studied. Moreover, the barrier to rotation about this angle, computed for one of the analogs, **12**, is relatively high (~7 kcal/mol). Thus, the conformation of the phenyl ring is nearly perpendicular to the mean plane of the piperidine ring. In conformer **8**, it is constrained to a value of ~170°, i.e., nearly co-planar to the mean plane of the piperidine ring, through ring fusion.

The position of the second substituent at C4 is different for the four conformations of the phenylpropanamide moiety. For example, when the phenylpropanamide is in the ω conformation in lofentanil and R30490, two low energy orientations are allowed for the oxygen function of the other 4-substituent. These two orientations are defined by the torsion ϕ_4 for lofentanil and by the torsion ϕ_6 for R30490.

To help select a bioactive form that recognizes the μ receptor, it is helpful to know not only the energy-accessible conformational minima, as provided by the conformational search, but also their flexibility and the barriers separating them. To obtain this added insight, we performed low temperature (312°K) molecular dynamics simulation of the four flexible analogs, **1**–**4**.

The behavior of the *N*-phenethyl substituent in the molecular dynamics simulation is very similar in all four analogs. In the time span of the simulation (100 psec), this substituent assumes both the extended and the bent conformations and the benzene ring rotates freely.

More interesting is the conformational behavior of the substituents at position 4, which is different for the different analogs. This difference is shown by the variation of ϕ_1 in the simulation for fentanyl and by the scatter plots of ϕ_4/ϕ_6 versus ϕ_1 for the three remaining analogs (see Fig. 2). For the α form of fentanyl, ϕ_1 remains close to its initial value throughout the 100-psec simulation, varying in the narrow range $-65° \leq \phi_1 \leq -32°$. For the lowest energy ω forms of R30490 (Fig. 2A) and lofentanil (Fig. 2B), the value of ϕ_1 also varies very little from the initial value, as does the second C4 substituent angle, ϕ_4 and ϕ_6, respectively, in each. In contrast, in carfentanil (Fig. 2C), both ϕ_1 and ϕ_4 assume a wide range of values, with specific values of ϕ_4 found in combination with specific values of ϕ_1.

The higher conformational mobility of the two C4 substituents of carfentanil, compared with fentanyl or R30490, is surprising, because the presence of the bulky $COOCH_3$ substituent would be expected to hinder free rotation of the phenylpropanamide moiety more than an H (fentanyl) or a CH_2OCH_3 (R30490) substituent. The increased flexibility of these substituents in carfentanil could be due to a large contribution from the repulsive interaction between the carbonyl oxygens to the CHARMm total energy. The conformational rigidity of lofentanil, on the other hand, is not surprising, because the added methyl group in position 3 of the piperidine ring would be expected to impede free rotation of the phenylpropanamide moiety. The lower conformational flexibility of lofentanil with respect to carfentanil has been reported previously (15).

Conformation recognized by the μ receptor. Combination of the results of the conformational studies with the available pharmacological data allows us to identify a likely conformation in which the fentanyls are recognized by the μ receptor. Unlike in previous conformational studies (15), we can no longer assume that all congeners are highly μ selective, because both carfentanil and lofentanil bind with high affinity to the δ receptor and lofentanil binds with high affinity to the κ receptor.[1] Thus we have used the most μ-selective analog, R30490 (**2**), as the template for determining the electronic and conformational features modulating recognition at the μ receptor.

The lowest energy conformer of R30490 is the ω arrangement, but the β and γ arrangements are very close in energy. The ω conformation is also lowest energy for lofentanil (**4**), carfentanil (**3**), and analog **12**, again with the other arrangements being close in energy. The α conformer is not a minimum for these high affinity analogs, as it is for fentanyl and for analog **8**, with much lower μ affinity, and it can, therefore, be ruled out as the bioactive form. All conformers except the spirane

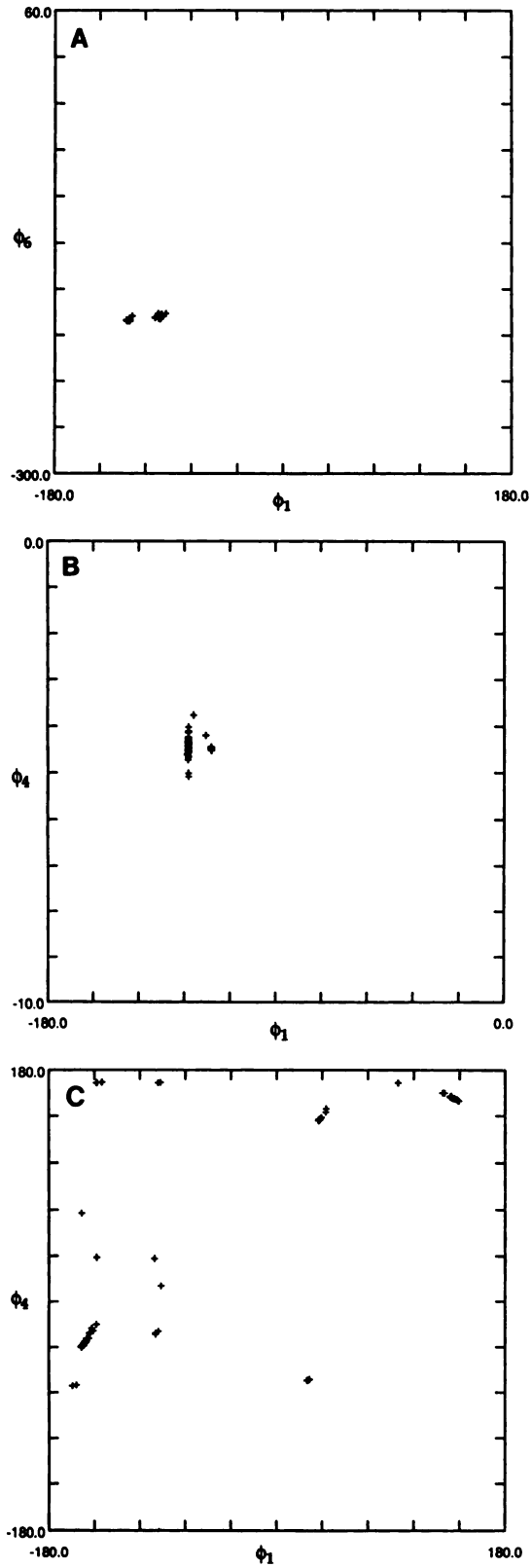


Fig. 2. Scatter plot of values assumed by torsions ϕ_4/ϕ_6 and ϕ_1 in the 100-psec 312°K molecular dynamics simulation. A, R30490, ϕ_6 versus ϕ_1; B, lofentanil, ϕ_4 versus ϕ_1; C, carfentanil, ϕ_4 versus ϕ_1.

12, due to ring fusion, can assume the β conformational arrangement. However, analog **12** is known from the literature to bind at the μ receptor with affinity comparable to that of fentanyl (41). On this basis, the β arrangement can also be ruled out for interaction at the μ receptor. Only the ω and γ conformations of the phenylpropanamide moiety are, therefore, candidates for recognition at the μ receptor.

For the three analogs with highest affinity at μ, R30490, carfentanil, and lofentanil, the ω conformation has lowest energy. In addition, for fentanyl and other analogs that energetically favor an α conformation of the phenylpropanamide moiety, the energy difference between the α and the ω conformation was determined to be $\leq$0.5 kcal/mol in all cases. Finally, the results of the CHARMm molecular dynamic studies of the conformational flexibility of analogs **1**–**4** indicate that, with the exception of carfentanil, at 312°K a molecule initially in the ω conformation will remain in that conformation because of a high energy barrier to conversion to other forms. For lofentanil, for example, starting from the ω conformation, conversion to the γ one would involve a rotation of 240° for ϕ_1, in order for the phenyl ring to avoid rotating past the 3-methyl substituent. Such a movement would require overcoming of a high energy barrier. Lofentanil is the analog with highest affinity at the μ receptor. Taken together, these results strongly suggest that it is the ω conformation of the phenylpropanamide moiety that is recognized by the μ receptor.

In summary, on the basis of our conformational studies and of the available pharmacological data, we propose that the fentanyls assume the bioactive conformation shown at the top of Table 2 (for R30490) and in Fig. 3 for recognition at the μ receptor. The piperidine ring is in the chair conformation and the two bulky substituents, *N*-phenethyl and 4-phenylpropanamide, are both in an equatorial arrangement. The phenethyl substituent is in the extended conformation ($\tau_2 \sim 180°$). The phenyl ring in this substituent is, however, free to rotate, and nothing can be said about its specific orientation at the receptor. The 4-phenylpropanamide substituent is in the ω conformation ($\phi_1 \sim 240°$). In this conformation the benzene ring points towards the (+)- and the carbonyl function towards the (−)-side of the piperidine ring. The specificity of orientation of the two functionalities on the amide nitrogen is reflected in the different μ-binding affinities of the two enantiomers of the 3-CH_3 derivatives of fentanyl and carfentanil (42). In the bioactive form, the benzene ring of the phenylpropanamide is nearly perpendicular to the plane bisecting the piperidine ring ($\phi_2 \sim \pm 90°$).

Electronic properties and environmental indices. Tables 4 through 9 show the results of the computation of a number of structural and electronic properties, as well as environmental indices, for all analogs in Fig. 1. These properties were evaluated for each analog in a low energy conformation most closely resembling the proposed active conformation.

Table 4 indicates the most favorable proton-accepting and -donating sites. The lower the value of $\Delta\Delta H_f$, the more favorable the site. As can be seen, the piperidine nitrogen is the best proton-accepting site ($\Delta\Delta H_{f_1}$) for almost all analogs. This is consistent with the results of NMR studies of the salts of fentanyl and *N*-methyl fentanyl (22), as well as with the experimentally known pK_a values (15). These results confirm the widespread assumption that this is the site of protonation at physiological pH. The only exception is *N*-methylcarfentanil

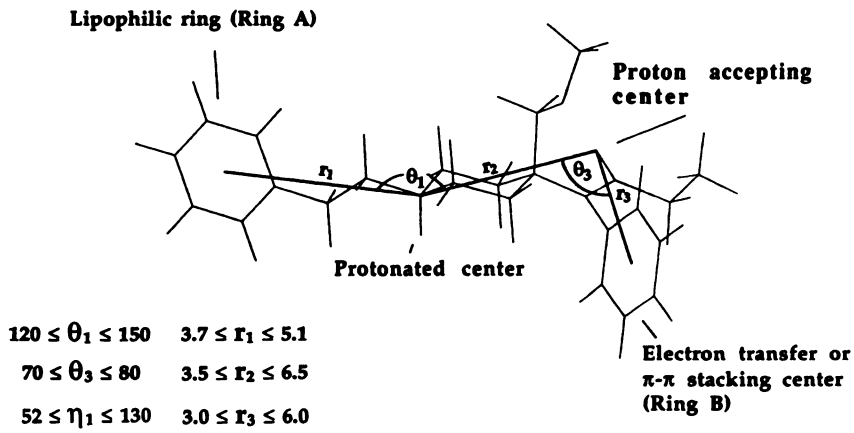


Fig. 3. Proposed model for the μ pharmacophore for the fentanyl class of compounds, shown for R30490. For the definition of angles θ_1 and θ_3 and distances R_1, R_2', and R_3, see Table 9.

TABLE 4
Proton affinities
See Table 1 for numbering.

	Proton affinity			
	$\Delta\Delta H_{f_1}$	$\Delta\Delta H_{f_2}$	$\Delta\Delta H_{f_3}$	$\Delta\Delta H_{f_4}$
	kcal/mol			
4 Lofentanil	139.4 (N_{pip})	144.7 (O_{R_4})	147.8 (O_{NCO})	
4 Carfentanil	140.2 (N_{pip})	141.7 (O_{R_4})	150.4 (O_{NCO})	
2 R30490	137.5 (N_{pip})	148.3 (O_{NCO})	163.7 (O_{ether})	
1 Fentanyl	141.7 (N_{pip})	149.2 (O_{NCO})		
9	142.3 (N_{pip})	154.2 (O_{NCO})		−26.7 (N_{NH})
12	134.4 (N_{pip})	152.7 (N7)	158.3 ($O_{C=O}$)	−17.1 (N_{NH})
11	140.5 (N_{pip})	161.6 (C8)	164.8 (C7)	
7 CH-fentanyl	139.9 (N_{pip})	168.7 (O)		
6 *N*-Methylcarfentanil	141.6 (O_{R_4})	144.2 (N_{pip})	148.6 (O_{NCO})	
10	143.7 (N_{pip})	152.8 (O)		
8	145.6 (N_{pip})	154.5 (O)	165.2 (N7)	−27.2 (N_{NH})
13	138.3 (N_{pip})	153.2 (N7)	158.8 (O_{NCO})	
14	149.7 (N_{pip})	159.2 (O_{NCO})	167.3 (N7)	−32.1 (N_{NH})
5 *N*-Methylfentanyl	146.2 (N_{pip})	149.6 (O_{NCO})		
15	146.0 (N_{pip})	159.6 (O_{NCO})	167.5 (N7)	−32.1 (N_{NH})

(6), for which this role is assumed by the carbonyl oxygen of the R_4 substituent. Comparison of the proton affinities of carfentanil and *N*-methylcarfentanil shows that their proton affinities at the carbonyl oxygen are comparable, whereas the proton affinity at the piperidine nitrogen decreases with substitution of the *N*-phenethyl by the *N*-methyl substituent.

Some differences can be observed among the analogs for the second best proton-accepting site ($\Delta\Delta H_{f_2}$), i.e., the site that is most likely to be involved in hydrogen bond formation with the receptor after the initial "neutralization" of the first protonated site. For the two analogs with a $COOCH_3$ substituent in position 4, the second best proton-accepting site is the carbonyl oxygen on this moiety. For analog 11 the second best proton-accepting site is one of the two vinyl carbon atoms (C8 in Fig. 1). The other vinyl carbon (C7) is also a good proton-accepting site. Both carbon atoms are better proton-accepting sites than the ether oxygen. For analogs 12 and 13 the second best proton-accepting site is the anilino nitrogen (N7 in Fig. 1), whose lone pair is not involved in an amide bond, as in the classical fentanyl structure. For the two spirodiones, 14 and 15, the second best proton-accepting site is the oxygen of the additional carbonyl function (C15 in Fig. 1). The higher proton affinity of this carbonyl oxygen, compared with the carbonyl oxygen on position 4 of the piperidine ring, is probably due to the additional stabilization, by the lone pair of the piperidyl nitrogen (N7 in Fig. 1), of the partial positive charge being transferred in the hydrogen bond-forming process.

In addition to proton-accepting sites, analogs 12–15 have a strong proton-donating center, the NH group, as shown by the value of heats of deprotonation ($\Delta\Delta H_{f_4}$ in Table 4). This quantity is defined as the difference between the heat of formation of the unprotonated drug and the heat of formation of the negatively charged species after deprotonation at NH.

Computation of the total polarizability volumes and of the separate contribution of the rings (Table 5) shows that, for all analogs with two benzene rings, that on the N1 substituent (ring A) is consistently more polarizable than the benzene ring substituent on position 4 (ring B). Ring A is also the most lipophilic of the two aromatic rings, from the computation of the regional contribution to the partition coefficients (Table 6).

As a measure of electron-accepting and -donating capabilities, we computed the energy of the LUMO and HOMO for all analogs (Table 7). For all analogs, HOMO corresponds to the piperidine nitrogen lone pair, as indicated by the computed frontier orbital charges (data not shown), making it the most favorable electron-donating site. The charge density in LUMO is centered on the carbon atoms of ring B, making it the most favorable electron-accepting center.

All analogs have a relatively small dipole moment (Table 8),

TABLE 5
Polarizability volumes
Volumes were computed according to the method of Fraga (37).

	Polarizability volume			
	Total	Ring A	Ring B	Ring C
	Å³			
4 Lofentanil	66.57	13.85	13.60	
3 Carfentanil	63.72	13.84	13.55	
2 R30490	64.28	13.78	13.61	
1 Fentanyl	57.69	13.85	13.61	
9	52.49	13.83	12.94	8.94
12	55.33	13.78	13.64	9.94
11	57.70	13.85	13.20	8.23
7 CH-fentanyl	59.07	13.83	13.76	
6 *N*-Methylcarfentanil	47.85		13.61	
10	56.58	13.84	13.06	13.07
8	52.51	13.80	12.98	9.01
13				
14				
5 *N*-Methylfentanyl	42.00		13.60	
15				

TABLE 6
Partition coefficients

Analog	log*P*				
	Experimental[a]	Computed[b]			
		Total	Ring A	Ring B	Ring C
4 Lofentanil	4.22	3.43	2.02	1.81	
3 Carfentanil	3.85	3.32	2.04	1.87	
2 R30490	4.18	3.74	1.93	1.89	
1 Fentanyl	4.05	4.37	2.04	1.88	
9		3.06	1.94	1.87	−1.19
12		2.89	1.92	1.90	−1.52
11		5.24	2.04	1.89	0.97
7 CH-fentanyl		4.94	2.02	1.85	
6 *N*-Methylcarfentanil		1.36		1.87	
10		3.96	2.03	1.77	−0.14
8		3.12	2.03	1.89	−1.17
13		2.99			
14					
5 *N*-Methylfentanyl		2.47		1.89	
15		2.54			

[a] Octanol-water partition coefficient, corrected for ionization (15).
[b] Conformation-dependent hydrophobicity index (38).

TABLE 7
E_{HOMO}/E_{LUMO} in the unprotonated form

	E_{HOMO}[a]	E_{LUMO}[b]
	eV	eV
4 Lofentanil	−9.060	−0.0122
3 Carfentanil	−9.0376	−0.0331
2 R30490	−8.8948	0.1374
1 Fentanyl	−9.1528	0.0856
9	−8.5735	0.2044
12	−8.9802	0.1608
11	−8.6696	−0.0142
7 CH-fentanyl	−9.0909	0.3256
6 *N*-Methylcarfentanil	−9.0904	−0.0259
10	−8.6865	0.1883
8	−8.6047	0.1803
13	−8.9726	0.1540
14		
5 *N*-Methylfentanyl	−9.1432	0.0976
15	−9.2684	0.0166

[a] For all analogs, HOMO corresponds to the piperidine lone pair.
[b] For all analogs, the charge density on LUMO is centered on the carbon atoms of ring B.

TABLE 8
Dipole moment

	Dipole moment		χ[b]
	Experimental[a]	AM1 computed	
	debye		
4 Lofentanil	3.23	4.25	111°
3 Carfentanil	3.45	4.29	112
2 R30490	2.80	2.30	86
1 Fentanyl	3.04	2.92	114
9		2.05	150
12		2.50	109
11		1.40	25
7 CH-fentanyl		2.25	90
6 *N*-Methylcarfentanil		4.33	111
10		1.59	156
8		2.07	152
13			106
14		2.48	106
5 *N*-Methylfentanyl		2.87	114
15		2.36	120

[a] Determined in benzene at 20° (15).
[b] χ, angle between dipole moment direction and nitrogen lone pair direction.

with the computed values being in reasonably good agreement with the available experimental values. The possibility that the dipole direction plays a modulatory role in recognition was investigated. If, after anchoring at the anionic receptor site, the molecule orients itself to obtain maximum alignment between the dipole moment and the direction of the electric field inside the receptor binding pocket, then the angle between the dipole moment direction and the direction of the piperidine nitrogen lone pair (χ in Table 8) should be a modulator of recognition. However, the value of the angle shows no relationship to receptor affinity.

Modulators of recognition at the μ receptor. A protonated amine and an aromatic ring are features common to all opioid drugs. In the case of the fentanyls, the results of our computations of proton affinities, as well as the pharmacological data, suggest four putative recognition points for interaction with the receptor. In addition to the protonated nitrogen, common to all opiates, there is a polar proton-accepting group, which could be involved in hydrogen bonding with a proton-donating (electrophilic site) site on the receptor. The polar function is a carbonyl oxygen (in the highest affinity analogs) or a nitrogen (in analogs **12** and **13**). There are also two aromatic rings, one on the *N*-substituent (ring A) and one in the phenylpropanamide moiety (ring B).

The possible role of the two aryl moieties of the fentanyls in the drug-receptor interaction can be inferred from a combination of the theoretical and experimental data. Comparison of the binding affinity at the three receptors for two pairs of analogs, fentanyl/*N*-methylfentanyl and carfentanil/*N*-methylcarfentanil (Table 1), shows that removal of the phenethyl substituent causes a similar loss of binding affinities at all three receptors. On the basis of these data, it can be inferred that the benzene ring of the *N*-phenethyl substituent (ring A) is important for recognition at each opiate receptor subtype. From the computation of the regional contributions to polarizability and hydrophobicity, we see that this ring is consistently more polarizable and more lipophilic than the 4-substituent one. In addition, there is no appreciable participation of ring A in either HOMO or LUMO, indicating that it is not a likely electron-donating or -accepting center. These results, taken

TABLE 9
Structural parameters

Analog	R_1 (R_1')[a]	R_2 (R_2')[b]	R_3[c]	θ_1[d]	θ_2[e]	θ_3'[f]	ξ[g]	η_1[h]
		Å						
4 Lofentanil	5.1 (5.9)	7.5 (5.9)	3.5	120°	149°	74°	148°	52°
3 Carfentanil	5.1 (5.9)	7.4 (5.9)	3.4	118	151	73	150	54
								−164
2 R30490	5.1 (5.8)	9.9 (4.9)	5.3	146	162	70	180	79
								145
1 Fentanyl	5.1 (5.7)	9.9 (4.9)	5.2	150	153	73	151	102
								149
9	3.7 (6.2)	9.1 (4.6)	5.7	119	147	81	216	93
							145	110
12	3.7 (5.9)	7.6 (2.8)	4.3	141	146	106	243	62
11	5.1 (6.2)	10.4 (3.4)	5.3	167	157	92	140	66
7 CH-fentanyl	5.1 (5.5)	10.1 (5.1)	5.3	154	153	67	201	130
								134
6 *N*-Methylcarfentanil	(5.8)	(6.0)	3.4			76		56
								−163
10	5.1 (6.0)	10.0 (4.9)	5.3	152	152	75	156	105
								118
8	5.1 (6.2)	10.3 (4.6)	5.7	155	157	79	160	81
								142
13	5.1 (5.8)	9.5 (2.8)	4.4	171	152	107	95	61
14	(5.8)	(2.8)	4.4			106		−14
								−179
5 *N*-methylfentanyl	(6.0)	(4.9)	5.5			72		89
								145
15	5.1 (5.8)	11.5 (3.9)	6.5	159	164	61	181	−1
								178

[a] R_1, distance between best proton-accepting site (PA_1) and center of most lipophilic ring (A). In parentheses, R_1', the distance between PA_1 and ring B.
[b] R_2, distance between second best proton-accepting site (PA_2) and center of most lipophilic ring (A). In parentheses, R_2', the distance between PA_2 and ring B.
[c] R_3, distance between two best proton-accepting sites.
[d] θ_1, angle between second accepting site, first accepting site, and center of aromatic ring A.
[e] θ_2, angle between center of ring A, piperidine nitrogen, and center of ring B.
[f] θ_3, angle between PA_1, PA_2, and center of ring B.
[g] ξ, torsion angle between ring A, PA_1, PA_2, and ring B (A-PA_1-PA_2-B).
[h] η_1, torsion angle defining spatial relationship between direction of amine nitrogen proton and direction(s) of protonation for second proton-accepting site PA_2, i.e., η_1 = H_p-N-PA_2-H.

together, suggest that ring A might be involved in hydrophobic rather than electron transfer interactions with an appropriate site on the receptor.

The benzene ring of the phenylpropanamide moiety (ring B) is almost perpendicular to the plane bisecting the piperidine ring in the chosen bioactive form at the μ receptor. From Table 1, we see that analogs **8–10**, in which benzene ring B cannot assume this orientation because of fusion with the propanamide moiety, have significantly reduced affinity. On this basis it can be inferred that ring B is another modulator of recognition. The fact that the LUMO is primarily localized on ring B makes it a potential electron-accepting center, suggesting its possible involvement in electron transfer interaction with an aromatic receptor site. Moreover, of these three analogs, **8** and **10** are inactive in the mouse hot plate test (24, 25), whereas **9** has some morphinomimetic potency (24). From these data, it appears that the position and orientation of the benzene ring in the phenylpropanamide moiety are very important not only for recognition but also for activation. This inference could be further tested by more definitive *in vivo* or *in vitro* measures of activation for these analogs. The role of ring B as an electron acceptor can also be further tested by synthesis of analogs with electron-donating or electron-accepting substituents on the ring, to determine their effect on recognition.

To determine more precise requirements for the relationship among the four proposed recognition points and to further substantiate their involvement in recognition at the μ receptor, we have computed a number of structural parameters for the analogs studied (Table 9). These parameters are a combination of steric and electronic criteria, because they define the spatial relationship between these four motifs, including the first and second best proton-accepting sites, the most lipophilic benzene ring (ring A), and the benzene ring (ring B) with highest concentration of charge in the LUMO.

The structural parameters shown in Table 9 were determined for all analogs in their conformation most closely resembling the proposed bioactive form. Analogs **9** and **12** have the unusual *N*-substituent α-methylbenzyl, with two almost equal energy conformers ($\tau_1 \sim 60°$ and 160°). Thus, to resolve this ambiguity, selection of the bioactive form was preceded by a detailed study on how variation of the angle τ_1 would affect the chosen structural parameters. Variation of τ_1 did not affect the values of the five distances R_1, R_1', R_2, R_2', and R_3 or the angle θ_3. However, as shown in Table 10, angle θ_1 for analog **9** and angle θ_2 for analog **12** varied. As can be seen from Table 10, for neither of the two analogs **9** and **12** are the two conformational minima (τ_1 = 60° and 150°) separated by a high energy barrier. Therefore, selection of the conformation for the *N*-α-methylbenzyl substituent in the bioactive form has been guided by comparison of the values assumed by θ_1 and θ_2 for different values of τ_1. From Tables 9 and 10 it is evident that both analog **9** and analog **12** can better fit the proposed bioactive conformation for values of τ_1 close to 60°, and this is the value used.

TABLE 10

Variation of structural parameters θ_1, θ_2, and θ_3 with torsion angle τ_1 in analogs 9 and 12

Analog	$\Delta\Delta H_f$	τ_1	θ_1	θ_2	θ_3
	kcal/mol				
9	0.0	60°[a]	119°	147°	81°
	0.1	90	142	136	79
	2.0	120	151	133	80
	0.6	150[a]	169	125	81
	1.4	180	165	123	80
12	0.0	60[a]	141	146	106
	0.7	90	144	133	106
	1.7	120	149	126	105
	0.4	150[a]	146	118	106
	0.9	180	141	116	106

[a] Conformational minima.

As can be seen from Table 9, no single distance can be used as a modulator of recognition. A distance of 5.1 Å between the protonated nitrogen and the center of ring A (R_1) is found for both high and low affinity analogs. Moreover, analogs **9** and **12**, for which this distance is only 3.7 Å, still have appreciable affinity for the μ receptor (Table 1). According to these results, R_1 cannot be considered a modulator of recognition, as proposed by Gero (43). Similarly, the distance between the protonated nitrogen and ring B (R_1') does not modulate recognition, remaining fairly constant (5.8–6.2 Å) over a wide range of affinities. The fact that no single distance modulates recognition indicates that more than two points are involved in recognition.

Similar to distances, no single angle seems to modulate recognition. As can be seen from Table 9, the range of values for angle θ_1 is the same among high affinity analogs as between high and low affinity analogs. The value of θ_2 remains fairly constant through a wide range of affinities. The value of angle θ_3 is different from the otherwise common range $70° \leq \theta_3 \leq 80°$ only for the spirane analogs **12–15**. Of these, however, **12** has high affinity, whereas both **13** and **14** have very low affinity and the receptor binding affinity of **15** is not known.

The fact that no single distance and no single angle modulate recognition indicates that recognition must occur through more than three points. Further support for this idea is the fact that the two angles defining the relative position of the protonated nitrogen and the polar function with respect to ring A (θ_1) and ring B (θ_3) and the torsion angle η_1 correlating the possible direction(s) of protonation on the polar proton-accepting function PA_2 and the direction of the piperidine nitrogen lone pair appear to be coupled determinants of recognition. From Table 9 we see that, for high affinity, the following conditions must be met: $118° \leq \theta_1 \leq 150°$, $70° \leq \theta_3 \leq 80°$, and $52 \leq \eta_1 \leq 130$. These results imply that the second hydrogen-bonding interaction is an important directional anchoring point, which then leads to favorable interaction with the two aromatic rings. Deviation from the allowed range for one or the other of the two angles results in some loss of affinity. The two angles coupled in recognition, θ_1 and θ_3, define a torsion angle ξ. From Table 9 we see that, for analogs recognizing the receptor, the value of ξ is in the range of 140–240°. Deviation from the allowed range for η_1 also results in loss of affinity, even if the values for θ_1 and θ_3 lie in the allowed range (see, for example, analog **15** in Table 9).

From the combined results, we are thus able to propose a model for the μ pharmacophore with specific steric and electronic properties, as shown in Fig. 3. In this proposed model, the distances between the four recognition points are allowed to vary as much as 2–3 Å, suggesting that the receptor binding pocket has the flexibility to expand and contract to accommodate one-dimensional variations in the ligand. However, the overall spatial requirements for recognition, as defined by the two angles θ_1 and θ_3 and by the torsion angle η_1, are quite rigid.

The proposed model for the μ pharmacophore is consistent with the observed pharmacological profile of all analogs listed in Table 1 and provides a mechanistically based self-consistent set of criteria that allow us to understand why some compounds do and some do not recognize the receptor. In particular, it can explain the lack of affinity of analogs **5**, **13**, **14**, and **15**. Analog **13** has the wrong spatial arrangement. Analogs **14** and **5** lack ring A. For analog **15**, the values assumed by θ_1 and θ_3 in the proposed bioactive form are very close to the allowed ranges (Table 9). However, neither one of the values assumed by η_1 lies in the allowed range, which explains the complete lack of recognition at the μ receptor for this analog.

The model also offers a possible explanation as to why *N*-methylcarfentanil binds with good affinity at the μ receptor, despite lacking ring A, whereas *N*-methylfentanyl does not. The computed proton affinities for the two analogs show that the carbonyl oxygen on the R_4 substituent of *N*-methylcarfentanil has much higher tendency to accept protons than the amide oxygen in *N*-methylfentanyl (Table 4). Despite the diminished interaction due to the absence of ring A, *N*-methylcarfentanil retains some affinity because of the presence of this favorable R_4 substituent, which is absent in the fentanyl analog.

In the spiranes **12** and **13**, substitution of the branched α-methyl *N*-substituent of **12** with a phenethyl substituent to give **13** is detrimental to recognition. The different modulation by the *N*-substituent can be attributed to two factors. (i) These compounds have an unusual proton-accepting site, an anilido nitrogen, instead of a carbonyl oxygen. (ii) Both analogs have a strong proton-donating group (NH) on the five-membered ring. Best interaction of ring A with a corresponding aromatic site on the receptor results in displacement of the anilido nitrogen from the position of best interaction with the proton-donating site on the receptor in the *N*-phenethyl but not in the α-methyl analog. Moreover, in the *N*-phenethyl compound the proton-donating group (NH) takes the place of the proton-accepting group (Fig. 4). These combined effects result in a dramatic loss of affinity.

Tollenaere *et al.* studied analog **7** (CH-fentanyl) previously and attributed its complete lack of morphinomimetic activity (15) to the high energy required for it to mimic their proposed

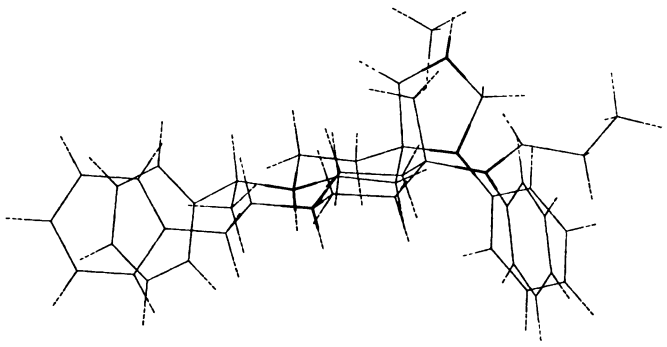


Fig. 4. Proposed overlap of compound **12** and analog R30490 in the bioactive conformation.

receptor-preferred conformation (15). However, our analysis of the conformational profile of both *R*- and *S*-enantiomers leads to a different conclusion. A low energy conformation of the *S*-enantiomer that was capable of good overlap with our proposed bioactive form was identified (Fig. 5), being only 0.6 kcal/mol higher in energy than the lowest energy arrangement. No such low energy conformation could be found for the *R*-enantiomer. These results suggest that the *S*-enantiomer should recognize the μ receptor. Consistent with these results, receptor binding studies have been reported that indicate that, despite its total inactivity in *in vivo* studies, CH-fentanyl recognizes the receptor with moderately good affinity (Table 1). These results, taken together, suggest that the lack of *in vivo* activity of CH-fentanyl is due to lack of activation of the receptor and not lack of recognition, as previously suggested (15).

Our calculations then, together with the pharmacological data available, strongly imply that three analogs studied, the *S*-enantiomer of analog **7**, analog **8**, and analog **10**, might be antagonists. Further pharmacological characterization of these fentanyl derivatives would help in clarifying this important issue. Until now, no antagonist had been found in the fentanyl class of compounds that is capable of matching fentanyl in potency. New fentanyl analogs have recently been reported to have antagonistic action against morphine *in vivo* (44) and *in vitro* (45). This development is promising, but the pharmacological data available are not yet sufficient to characterize them as fentanyl antagonists.

Conclusions

A combination of our conformational study of selected flexible and conformationally constrained analogs of fentanyl with analysis of the available pharmacological data has allowed us to propose a pharmacophore for interaction at the μ receptor for this class of compounds (Fig. 3). In this conformer the piperidine ring is in the chair conformation and the *N*-phenethyl and 4-phenylpropanamide substituents are both equatorial. The *N*-phenethyl substituent is extended, with a flexible phenyl ring, and the 4-phenylpropanamide is in the ω arrangement ($\phi_1 \sim 240°$).

Four anchoring points in a particular spatial relationship to each other are necessary for optimum receptor recognition, i.e., the protonated amine assumed to be involved in an initial electrostatic interaction with a negatively charged site on the receptor, a polar function capable of hydrogen bonding with an electrophilic site, an aromatic ring involved in lipophilic interaction with a similar moiety, and a second aromatic ring most probably involved in electron transfer interaction with the receptor. Two pseudo bond angles (θ_1 and θ_3) and one torsion angle (η_1) have been found to modulate recognition at the μ receptor. The angles define the relative position of the protonated nitrogen and the polar function with each of the two aromatic rings, respectively, and the torsion angle defines the relative orientation of the lone pair(s) of electrons on the polar function and the lone pair on the piperidine nitrogen.

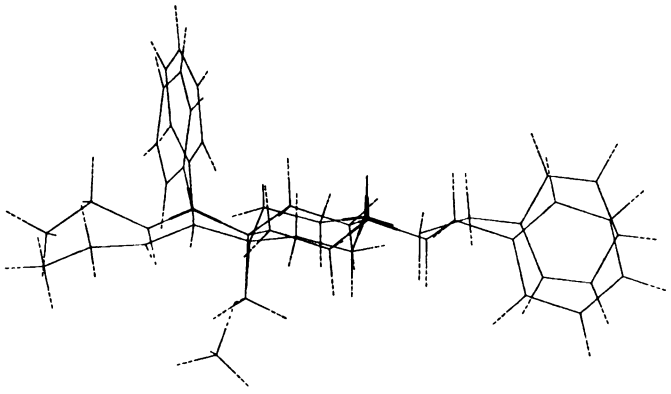


Fig. 5. Proposed overlap of the *S*-enantiomer of CH-fentanyl and analog R30490 in the bioactive conformation.

Three known analogs are postulated to be antagonists. Until this is confirmed and other analogs are made and assessed, the absence of a confirmed antagonist in this class of compounds precludes the determination of the steric and electronic modulators of activation.

The anomalously high affinity binding behavior of the *N*-methylcarfentanil analog can be explained on the basis of an increased contribution of the hydrogen-bonding interaction between the polar function and an electrophilic site on the receptor to the total enthalpy of binding. This fact reaffirms the necessity of including thermodynamic considerations in the study of the drug receptor interaction.

Acknowledgments

We are thankful to Dr. H. O. Villar for critically reading the manuscript. Analogs **14** and **15** were kindly synthetized by Dr. J. F. Kamal in the laboratories of Prof. A. F. Casy at the University of Bath (England).

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