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Structure-Activity Studies of Morphine Fragments. I. 4-Alkyl-4-(*m*-hydroxy-phenyl)-piperidines

GILDA H. LOEW, JOHN A. LAWSON, EDWARD T. UYENO, LAWRENCE TOLL, GERNOT FRENKING, WILLMA POLGAR, L. Y. Y. MA, NORMAN CAMERMAN, and ARTHUR CAMERMAN

SRI International, Life Sciences Division, Menlo Park, California 94025, Department of Biochemistry, University of Toronto, Toronto, Canada, and University of Washington, Medical School, Seattle, Washington

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SUMMARY

The 4-(*m*-OH-phenyl)piperidines are a flexible fragment of the morphine/benzomorphan fused-ring opioids. Analogs in this family were synthesized with varying 4-alkyl substituents increasing in bulk from H through methyl, *n*-propyl, to *t*-butyl, each with the three *N*-substituents methyl, allyl, and phenethyl. These twelve compounds were evaluated for analgetic agonism in mice using two different models for antinociceptive activity, acetic acid writhing and tail-flick, the latter by both subcutaneous and intracerebroventricular routes of administration. Antagonism to morphine analgesia was also measured by the mouse tail-flick procedure. Binding affinities of these new analogs to different opioid receptor subtypes were determined. Energy conformational calculations on these compounds were also carried out using the empirical energy program called MOLMEC, in order to better understand how the 4-*R* substituents modulate receptor binding affinities and efficacies. The results obtained show that, in general, the compounds studied are μ-selective and vary in agonist potency from weak to morphine-like. Significant differences in rank order of analgetic potencies and their relationship to receptor affinities were obtained from the results of subcutaneous and intracerebroventricular administration. Results of energy-conformational calculations for twelve *N*-methyl compounds indicate that those with 4-alkyl substituents favor a common, non-morphine-like phenyl axial conformation. The 4-*t*-butyl compounds are, in fact, the first simple mono-alkyl-substituted 4-phenylpiperidines predicted to definitely exist in a phenyl axial conformation, as confirmed by X-ray analysis. On the basis of this common phenyl axial conformation, the observed variation in μ receptor affinities and efficacies of the 4-methyl, 4-*n*-propyl, and 4-*t*-butyl compounds could be understood and the behavior of 4-ethyl and 4-isopropyl analogs predicted. Two equatorial conformers (rotamers) were found to be the preferred forms of the analogs with 4-*R* being H or an ester group, or with a 3-methyl group added *trans* (β) to the 4-*R* group. Taking into account the rotational flexibility of these analogs, these two conformers could be used to understand differences in high and low efficacy compounds observed among analogs with preferred phenyl equatorial conformations. None of the analogs exhibit a fused-ring-like *N*-substituent modulation of efficacy. This result can, perhaps, be understood by their inability in any proposed conformer to totally mimic key receptor interactions of both the phenol-OH and *N*-substituent portions of the fused compounds.

The modulation of the molecular structure of morphine with the aim of finding a nonaddictive opiate has been a major topic of structure-activity research in drug design (1). A vast number of candidate compounds have been synthesized and tested for analgesic activity. One strategy that has been used is the synthesis of compounds that can be regarded as fragments of the fused-ring morphine structure. This approach has a long history. In the 1940s, a group at Hoffmann-LaRoche (2, 3) recognized that such structures as 4PPs and 2-benzyl piperidines are sub-structures of morphine and a few members of these families were then synthesized. Fig. 1 shows how fragmentation of morphine leads to these two families (E and F) and a number of others. This paper is the first in a series considering three such families, 4PPs (E), 2-benzyl piperidines (F), and spiro-piperidines (D) in which we have combined experimental and theoretical methods to further elucidate their pharmacological behavior and the molecular features that can modulate them.

Since first recognized as promising, a large number of 4PPs with varying 4-substituents, including 4-esters such as meperidines or bemidones (4), 4-reverse esters such as prodines (5), 4-keto (ketobemidones) (6), and 4-alkyls (7), have been synthesized. One of the underlying objectives in the search for a clinically useful analgesic was to design an analog with analgetic activity that could, nevertheless, antagonize morphine-induced analgesia in an appropriate test. The basis of this reasoning was the example that such so-called mixed agonist/antagonists, in the fused-ring opioids, had been found to have little or no

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ABBREVIATIONS: 4PP, 4-phenyl piperidine; SC, subcutaneous; ICV, intracerebroventricular; THF, tetrahydrofuran; DADL, D-Ala^2-D-Leu^5 enkephalin; EKC, ethylketocyclazocine; DHM, dihydromorphine; CPPD, Committee on Problems of Drug Dependence; RMS, root mean square.

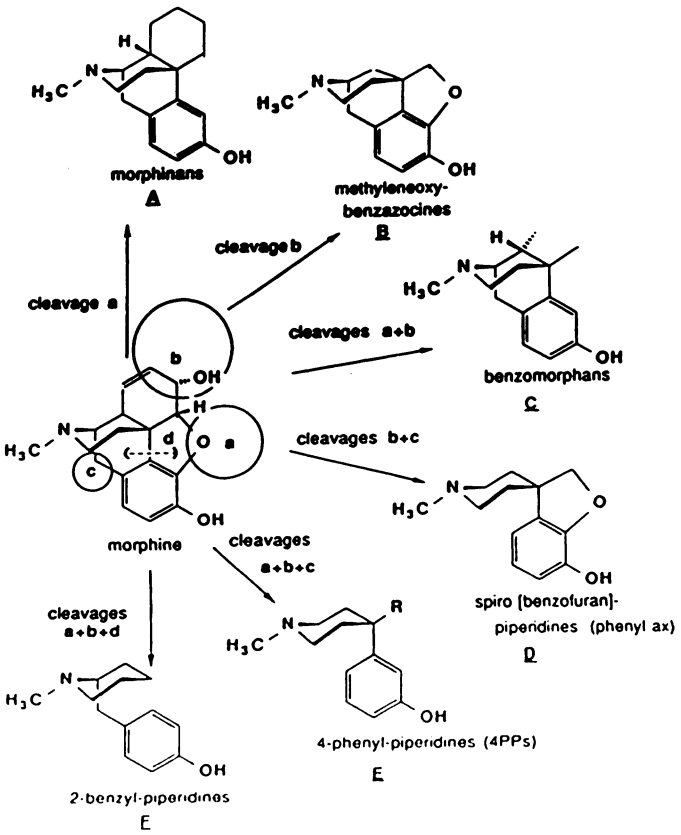
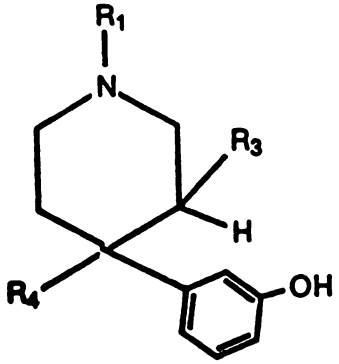


Fig. 1. Schematic representation of the reduction of morphine into various substructures retaining opiate activity.

addiction liability. Initially, the 4PP analogs synthesized were found to be pure agonists. These include those compounds with an *N*-substituent, which promotes antagonism in morphine-like rigid opioids (e.g., *N*-allyl and *N*-cyclopropyl methyl). However, as new analogs were prepared with a *m*-OH substituent on the phenyl ring, agonist and antagonist activity was observed in some compounds, primarily the 4-carbomethoxy analogs (8). Zimmerman and colleagues (9) subsequently synthesized a new family of 4PPs with a 3-methyl substituent that were pure potent antagonists. These results led to the suggestion that one possible role of the 3-methyl substituent in reducing agonist efficacy is to favor the phenyl equatorial conformation, a suggestion that is contrary to the earlier notion (8) that such conformations would not lead to antagonism.

In order to further distinguish the role of the 3-methyl group from that of other key substituents of 4PPs, particularly the 4-*R* and *N*-*R* groups, and to better understand how the relative stabilities of phenyl axial versus phenyl equatorial conformers modulate relative receptor affinities and efficacies, a series of 4-(*m*-OH-phenyl)-derivatives was selected for study, in which the 4-*R* group alone could affect the conformational preference of the analogs. A series of analogs with 4-*R* of H, methyl, *n*-propyl, and *t*-butyl were prepared. For each 4-*R* group, three *N*-substituent variations were also investigated. The resulting twelve compounds (**1–4, a, b,** and **c**, Table 1) were tested for antinociceptive agonist activity in mice by both abdominal writhing and tail-flick procedures. The tail-flick test was administered by both SC and ICV routes in order to explore the dependence of rank order potencies on pain stimuli and route of administration. Antagonism to morphine-induced tail-flick

TABLE 1
4-Alkyl-4-(m-OH-phenyl)piperidines investigated



Compound	R_4	R_1	R_3
1a	H	CH_3	H
1b	H	Allyl	H
1c	H	Phenethyl	H
2a	CH_3	CH_3	H
2b	CH_3	Allyl	H
2c	CH_3	Phenethyl	H
3a	*n*-C_3H_7	CH_3	H
3b	*n*-C_3H_7	Allyl	H
3c	*n*-C_3H_7	Phenethyl	H
4a	*t*-C_4H_9	CH_3	H
4b	*t*-C_4H_9	Allyl	H
4c	*t*-C_4H_9	Phenethyl	H
10α	CH_3	CH_3	CH_3 (*cis*)
10β	CH_3	CH_3	CH_3 (*trans*)
11α	*n*-C_3H_7	CH_3	CH_3 (*cis*)
11β	*n*-C_3H_7	CH_3	CH_3 (*trans*)
12	$O_2CC_2H_5$	CH_3	H
13α	$O_2CC_2H_5$	CH_3	α-CH_3 (*cis*)
13β	$O_2CC_2H_5$	CH_3	β-CH_3 (*trans*)
14	$CO_2C_2H_5$	CH_3	H

analgesia was also evaluated. Opiate receptor binding experiments were performed to determine affinities of these analogs at multiple opiate receptors. In addition, energy conformational profiles were determined for the four *N*-methyl analogs synthesized (**1a, 2a, 3a,** and **4a**), and for additional 4PPs, to help identify the molecular properties and modes of receptor binding that modulate relative receptor affinities and their ability to initiate antinociceptive activity.

The underlying property that we implicitly wish to address in these structure-activity studies is efficacy. As defined by Stephenson (10) and elaborated in a classic paper by Furchgott and Burszty (11), efficacy relates the affinity of a drug for a receptor to its ability to activate it. In principle, relative efficacy can be measured directly by combining studies of affinity with measures of agonist activity in the same system. For drugs such as opioids with multiple receptors, additional procedures are needed to determine efficacy at each receptor. Such additional studies are becoming increasingly important for opioids because there is some evidence that varying relative efficacy at μ and κ receptors can also modulate addiction liability (12). In practice, these very difficult studies have not yet been systematically reported for any family of opioids. Most studies of 4PPs do not include systematic determination of affinities at any opioid receptor and commonly include only *in vivo* agonist and antagonist potency determinations for morphine or meperidine analgesia.

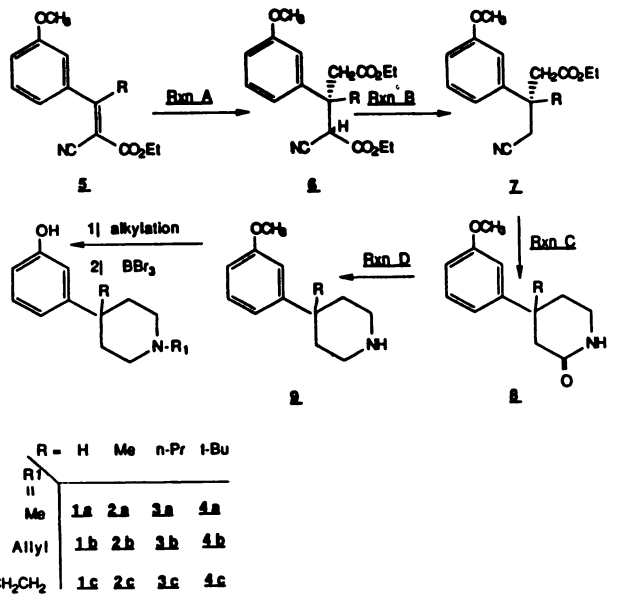
In this study we have used the ratio of receptor affinity to *in*

vivo antinociceptive agonist potencies as a qualitative measure of efficacy. This definition suffers from the weakness that potencies are measured in different systems and under different conditions than receptor affinities and are dependent upon such features as routes of administration, pain stimulus, and species. To somewhat compensate for these weaknesses, we have examined the dependence of the *in vivo* results on route of administration and pain stimuli and have chosen to use the ICV route of administration and a mouse tail-flick model for antinociceptive activity, in estimating relative efficacies, and to relate these to insights gained from the conformational studies.

Materials and Methods

Chemical Synthesis

All reactions were performed under an argon atmosphere, and solvents were removed on a rotary evaporator under vacuum. Melting points were taken on a Mel-Temp apparatus and are uncorrected. NMR spectra were recorded on a Varian EM-360 instrument. Chemical shift values are reported in parts per million (δ) relative to trimethylsilane. Mass spectra were determined on an LKB 9000 spectrometer equipped with a gas chromatograph and a PDP12 computer. Analytical high pressure liquid chromatography was carried out on a Waters Radialpak Column, and preparative liquid chromatography was performed on a Waters Prep LC/500 system. Elemental analyses were performed by Galbraith Laboratories, Inc. (Knoxville, TN) and are within 0.4% of theoretical values. Mass spectral analyses of all products **8a–d** and **1–4, a, b,** and **c** (Scheme 1) showed parent ion peaks at the expected mass, with consistent fragmentation for assumed structure. Physical data on the final products are shown in Table 2.



R = H Me n-Pr t-Bu

R1				
Me	1a	2a	3a	4a
Allyl	1b	2b	3b	4b
cCH2CH2	1c	2c	3c	4c

Scheme 1.

In Scheme 1 is shown the synthetic route used to prepare the desired piperidines **1–4, a, b,** and **c**. Because the various analogs were produced by similar steps, only the synthesis of the 4-*t*-butyl analog will be described in detail. As we were preparing this publication, a report (13) appeared that described reaction A, using a slight variation of our method, also leading to compound **6b**.

The required starting olefins **5a–d** resulted from the condensation of the corresponding *m*-CH_3O-ϕ-CO-R with ethyl cyanoacetate in a procedure similar to that reported (13) for the synthesis of **5b**.

Reaction A: 1-cyano-2-*t*-butyl-2-(3′-methoxy-1,3-phenyl) propanecarboxylic acid diethylester (6d). To a mixture of 11.0 g (109 mmol) diisopropylamine, 11.6 ml (110 mmol) 9.5 N butyllithium, and 10.0 g (113

TABLE 2

Physical properties of 4PP analogs *1–4, a, b,* and c

Compound	NMR (Characteristic Peaks)	Yield[a]	m.p. (Acid)
	ppm (δ)	%	°C
1a	9.5 (s, 1*H*, O*H*), 2.9–3.2 (m, 4H, CH_2, NCH_2), 2.24 (s, 3H, CH_3)	92	159.5–160.5[b]
1b	10.3 (s, 1H, OH), 4.9–6.2 (m, 3H, olefinic), 2.8–3.3 (m, 4H, CH_2-CH = CH, CH^{α}-N-CH^{α})	90	201–202 (dec) (·HBr) 223.5–24.5[b]
1c	3.0–3.3 (m, 2H, CH^{α}-N-CH^{α}), 2.6–2.9 (m, 4H, CH_2 CH_2 N)	83	219–220[b]
2a	8.9 (s, 1H, OH), 2.6–3.2 (m, 4H, CH_2N CH_2), 2.5 (s, 3H, CH_3), 1.20 (s, 3H, CH_3)	61	194–96 (dec) (·HCl)
2b	9.05 (s, 1H, OH), 4.9–6.2 (m, 3H, olefinic), 3.06 (d, 2H, CH_2-CH_2)	47	192–4 (dec) (·HCl)
2c	2.5–3.0 (m, 4H, N-CH_2CH_2), 1.20 (s, 3H, CH_3)	89	258–60 (dec) (·HBr)
3a	8.8 (s, H, OH), 2.5–3.0 (m, 4H, CH_2N CH_2), 2.25 (s, 3H, CH_3)	67	90–95 (·HCl)
3b	9.1 (s, 1H, OH), 4.9–6.2 (m, 3H, olefinic), 3.06 (d, 2H, N-CH_2-CH = CH_2)	60	120–24 (·HCl)
3c	8.95 (s, 1H, OH), 2.4–3.1 (m, 4H, N-CH_2 CH_2-ϕ)	79	108–110 (·HCl)
4a	2.5–2.9 (m, 2H, CH^{α}-N-CH^{α}), 2.15 (s, 3H, CH_3)	62	262–4 (dec) (·HBr)
4b	4.9–6.2 (m, 3H, olefin), 2.93 (m, 2H, CH^{α}-N-CH^{α}), 8.83 (s, 9H, $[CH_3]_3C$)	51	195–97 (dec) (·HBr)
4c	2.4–3.0 (m, 4H, d CH_2CH_2N), 0.83 (s, 9H $[CH_3]_3C$)	76	282–84 (dec) (·HBr)

[a] Yield for *N*-alkylation and *O*-demethylation steps from precursors **8a–d**.
[b] Free base.

mmol) of ethyl acetate in 600 ml of THF, stirred 30 min at approximately −70° (dry ice bath), was added 12.2 g (42 mmol) of the olefin **5d** in 50 ml of THF. After 2 hr, the bath was removed, and the reaction mixture was allowed warm to −45°, then maintained at this temperature by partial immersion in a bath for 45 min. (Note: reactions with olefins **5a–5c** are faster. The bath is removed after olefin addition, and reaction is complete when the temperature reaches approximately −45°.) Then the reaction was quenched by the addition of excess 3 N hydrochloric acid solution. The organic layer was separated, dried over $MgSO_4$, and concentrated by spin evaporation. The resulting material was then partially distilled under vacuum to remove volatile impurities, including unreacted **5d** (~ 1mm of Hg, pot temperature up to ~170°). The pot residue, crude adduct product, is used without further purification. Yield: **6d**, 13.9 g (NMR suggests 50% purity, ~44% yield); **6a**, 68%; **6b**, 54%; and **6c**, 73%.

Reaction B: 3-*t*-butyl-3-(3′-methoxyphenyl)-4-cyano-butanoic acid ethyl ester (7d). To a stirred solution of 9.60 g (~12.8 mmol, based on assumed 50% purity) of semipurified adduct **6d** in 70 ml of dimethyl sulfoxide was added 14.0 g (101 mmol) of lithium iodide (anhydrous powder), then the slurry was heated to gentle reflux (~170°) and CO_2 evolution became vigorous. After 30 min, the mixture was allowed to cool, then partitioned in H_2O washes and filtered through a pad of silica gel (~200 ml), and the eluate was evaporated to give ~6 g of orange gel. This was distilled and the product was collected (3 mm of Hg; head temperature, 130–150°) as 2.05 g of yellow oil. Yield: **7d**, 51%; **7a**, 50%; **7b**, 64%; and **7c**, 56%. All four nitrile products **7a–d** failed to crystallize and were characterized by NMR and gas chromatography-MS. Distinguishing NMR features for **7a–d** are as follows. **7a** ($CDCl_3$): δ 3.6 (pentuplet, 1H, ArC*H*), 2.75, 2.68 (overlapping doublets, 4H, CH_2CN, CH_2 CO_2Et); **7b:** δ 2.96 (s, 2H, CH_2CO_2Et), 2.75 (s, 2H, CH_2CN), 1.60 (s, 3H, CH_3); **7c:** δ 3.13 (q, 2H, CH_2CO_2Et, gem coupling J = 17 Hz), 2.87 (broad s, 2H, CH_2CN), 1.7–2.0 (m, 4H, CH_2CH_2), 0.9 (m, 3H, CH_3-CH_2CH_2); **7d:** δ 3.40 (q, 2H, CH_2CO_2Et, J = 17 Hz), 2.23 (q, 2H, CH_2CN), and 0.93 (s, 9H, $[CH_3]_3$C).

Reaction C: 4-*t*-butyl-4-(3′-methoxyphenyl)-2-piperidone (8d). A mixture of 0.47 g (1.55 mmol) of **7d** and 0.5 g of platinum oxide (lesser amounts result in a very slow hydrogenation) in 100 ml of acetic acid was shaken on a Parr shaker under 60 pounds H_2 pressure for 24 hr. (Reductions on less hindered analogs **7a–c** are complete in 6–14 hr.) After filtration of platinum, the solution was spin evaporated to a gum that was largely unlactamized amine-acetate salt by NMR. However, continued distillation at higher heat (pot temperature, ~150–170°) resulted in the evolution of ethanol/acetic acid as lactam formed *in situ*. When bubbling ceased, the residue was dissolved in ethyl ether with immediate formation of a white crystalline powder; **8d**, 0.232 g (m.p., 173–74°). Yields: **8d**, 57%; **8a**, 42%, (m.p., 163–163.5°); **8b**, 52%, (m.p., 80–81°); and **8c**, 83% (oil). NMR: **8a:** δ 7.7 (broad, 1H, N*H*), 3.43 (m, 2H, CH_2N), 3.03 (m, 1H, Ar-C*H*); **8b:** δ 7.7 (broad, 1H, N*H*), 3.1 (m, 2H, CH_2N), 2.7, 2.4 (doublets, 2H, CH_2 —C = 0, gem coupling J = 17 Hz), 1.30 (s, 3H, CH_3); **8c:** δ 7.9 (broad, 1H, N*H*), 3.1 (m, 2H, CH_2N), 2.9, 2.5 (doublets, 2H, CH_2 —C = 0, gem coupling J = 17 Hz); **8d:** δ 7.4 (broad, 1H, N*H*), 3.2 (m, 2H, CH_2N), and 0.9 (s, 9H, $(CH_3)_3$C).

Reaction D: 4-*t*-butyl-4-(3′-methoxyphenyl)piperidine (9d). A solution of 0.507 g (1.94 mmol) of **8d** in 50 ml of THF was reduced with diborane in THF as described by Brown and Heim (14) to give 0.336 g of **9d** as the HCl salt from ethyl ether. Yield: **9d·HCl**, 61%, m.p., 206–7°; **9a**, 60%, m.p., 48–50°; **9b·HCl**, 85%, m.p., 173–4° (dec.); and **9c HCl**, 71%. NMR: **9a:** δ 3.16 (d of t, 1H, C*H*N), 2.80 (d of d, 1H, C*H*N), 2.18 (s, 1H, NH); **9b:** 2.6–3.0 (m, 4H, CH_2NCH_2), 2.5 (broad s, 1H, NH), 1.18 (s, 3H, CH_3); **9c:** 2.6-3.3 (m, 4H, C*H*NCH_2), 2.35 (broad s, 1H, NH); **9d:** 3.3 (s, 1H, NH), and 0.80 (s, 9H, $[CH_3]_3$C).

The methods of *N*-alkylation of **9a–d** using formalin/$NaBH_3CN$, allyl bromide, and phenethyl bromide and subsequent *O*-demethylations by BBr_3 are fully described in our recent paper (15) on 3-phenylpiperidines. The final products **1–4, a, b,** and **c** were all fully characterized as free bases and acid salts (see Table 2).

Receptor Binding

Opiate receptor binding assays and data analysis were performed as described previously (15). Briefly, rat (Sprague-Dawley) whole brain homogenates were prepared, washed by centrifugation twice, preincubated at 37° for 1 hr and resuspended in Tris, pH 7.7, at 6.7 mg of tissue per ml. Receptor-binding incubations contained 1.8 ml of tissue suspension, 0.1 ml of labeled ligand and 0.1 ml of unlabeled drugs. The tubes were incubated in triplicate at 25° for 1 hr before filtration over glass fiber filters and scintillation counting.

In order to determine affinities at μ and δ sites, self- and cross-competition experiments were conducted using two different concentrations of four tritiated ligands. These labeled ligands were naloxone, DADL, EKC, and DHM. In addition to the resulting four-by-four "matrix" of competitive inhibition behavior, inhibition of binding of all four labeled ligands with each of the twelve 4PP analogs was performed at two labeled ligand concentrations. In general, the two concentrations of labeled ligand used were approximately 0.5 and 1.5 nM. Thirteen concentrations of unlabeled ligand were used in each inhibition experiment.

Data obtained were analyzed for affinity at μ and δ by a modified version of the program LIGAND (16), which predicts a set of self-consistent receptor binding affinities and capacities, assuming different receptor site models, by using a weighted, nonlinear, least-squares regression analysis procedure. Despite the presence of experiments using [^{3}H]EKC, due to the very low level of κ receptors in rat brain an excellent binding model for the determination of affinities at μ and δ receptors, but not κ-receptors, was obtained.

Because of the lack of ability to obtain κ affinity in rat brain, κ affinities were determined for these analogs by inhibition of [^{3}H]U69,593 binding in guinea pig brain membranes. Binding was conducted as described for rat brain membranes. We have determined that, under these conditions, binding affinities found in rat and guinea pig brain are very similar for all opioid receptor types. The use of guinea pig brain membranes thus facilitates the determination of κ affinities.

Materials

[^{3}H]Naloxone, [^{3}H]DADL, [^{3}H]EKC, and [^{3}H]U69,593 were from New England Nuclear (Boston, MA). [^{3}H]DHM was from Amersham (Arlington Heights, IL). Naloxone was from Endo Laboratories (Garden City, NY), DADL from Peninsula Laboratories (Belmont, CA), EKC from Sterling Winthrop (Rensselaer, NY), and DHM from the National Institute on Drug Abuse.

In Vivo Pharmacology

Because some of the compounds were not readily soluble in saline solution, all were dissolved in a 4% aqueous solution of ethanol. At least three dose levels (10 mice per level) of each compound, injected at a standard volume of 10 ml/kg, were bioassayed. Meperidine hydrochloride, morphine sulfate, and *N*-allylnormorphine hydrochloride were tested as reference drugs.

The following two tests of analgesic agonism have been administered: 1) the mouse acetic acid writhing (chemical stimulus) test and 2) the mouse tail-flick (heat stimulus) test. The acetic acid writhing test described by Koster *et al.* (17) was slightly modified and applied. Basically, the mice were dosed intraperitoneally with 60 mg/kg 0.6% aqueous solution of acetic acid. Immediately after the onset of writhing, i.e., stretching, contraction of the abdomen, or twisting of a hind leg inward, they were injected SC with a dose of a test compound. Thirty minutes later the number of writhes shown by each animal was counted during the 10-min test session. The average number of writhes in the control group (C) and the mean number of writhes in each of the treated groups (T_i) were computed. Then per cent agonism at each dose level was calculated as:

$$\% \text{ Agonism} = \frac{C - T_i}{C} \times 100\%$$

Per cent agonism was plotted against log dose. The ED_{50} values and 95% confidence limits were calculated according to the method of Litchfield and Wilcoxon (18).

The mouse tail-flick test (19–21) has been adapted for use in our laboratory (15). After determining an effective analgesic dose range for each compound, time-course and dose-response experiments (three doses/compound; 10 animals/dose) were conducted. The animals were injected SC with a test chemical, standard, or diluent and the tail-flick test was administered at 10, 20, 30, 45, and 60 min after treatment. A 6.5-sec cut-off time for tail-flick latency response was observed. The analgesic response was expressed as the percentage of the maximum possible increase in reaction time (21). Six and one half seconds minus the reaction time of the control group was considered to be the maximum possible increase in response time. At the time of peak effect of each dose, the average increase in response time of each group was determined and the percent of the maximum possible increase in reaction time (per cent agonism) was computed. The percentages were plotted versus the log-dose on probit paper, and the median effective dose (ED_{50}) and the 95% confidence limits were calculated by the method of Litchfield and Wilcoxon (18).

Antagonist activity of the test samples against 8 mg/kg (21.08 μmol/kg; ED_{80}) of morphine sulfate was determined by the tail-flick procedure developed by Harris *et al.* (22). A mouse was injected SC at the nape and immediately dosed with 8 mg/kg (SC) of morphine sulfate caudal to the nape. It was tested at 10, 20, 30, 45, and 60 min after treatment in order to record the time of peak antagonist effect. Per cent antagonism (23) at the peak time was computed for each dose. From the plot of per cent antagonism versus the log dose, the median effective antagonist dose (AD_{50}) and the 95% confidence limits were calculated (18) for the three analogs (**1c**, **2b**, and **2c**) for which significant antagonism was found. For these analogs peak time of antagonist activity was 30, 20, and 20 min after injection, respectively.

ICV Administration

The analgesic potency of 4-(*m*-hydroxyphenyl)piperidine analogs with *N*-methyl and *N*-phenethyl substituent variations was evaluated further by applying the ICV injection method developed by Haley and McCormick (24). At least three doses (10 mice/dose) of each chemical were bioassayed. The samples were dissolved in 20% aqueous solution of DMSO and injected at a standard volume of 4 μl/mouse. The tail-flick test was administered at 2, 5, 10, 20, 30, 45, and 60 min after treatment. Per cent agonism at the peak time was computed at each dose level of each agent and plotted versus the log dose to derive the ED_{50} values. The time of peak activity by ICV administration was 20 min, except for **1a** and **3c** (10 min) and **4a** (30 min).

The antagonist activity of two analogs, **1c** and **4a**, against 8 mg/kg (21.08 μmol/kg; ED_{80}) of morphine sulfate was evaluated further by applying the modification of tail-flick procedure developed by Harris *et al.* (22) and Li *et al.* (25). At least three doses (10 mice/dose) of each chemical were bioassayed. The samples were dissolved in a 20% aqueous solution of DMSO. A mouse was injected SC with 8 mg/kg of morphine sulfate caudal at the nape. The putative antagonist, the standard, or the vehicle only was injected ICV 28 min later (at the time of peak agonism of morphine) at a standard volume of 4 μl/mouse. The tail-flick test was administered at 2, 5, 10, 20, 30, 45, and 60 min after ICV treatment. Per cent antagonism at the peak time was computed at each dose level of each agent. Neither **1c** nor **4a** had significant antagonist activities at the maximum dose tested and AD_{50} values could not be determined.

Theoretical Studies

All calculations were performed using the empirical energy program called MOLMEC, described in detail elsewhere (26). In this program, developed jointly by Dr. DuChamp and collaborators at Upjohn and Dr. Tetsuro Oie and collaborators at the University of Kansas, the energy is expressed as the sum of seven terms:

$$E = E^{b} + E^{ang} + L^{op} + E^{tor} + E^{nb} + E^{hb} + E^{el} \quad (1)$$

The terms in Eq. 1 have the following meaning. The first four terms describe the intramolecular motions due to bond stretching (E^{b}), angle bending (E^{ang}), out of plane bending (E^{op}), and torsional rotation (E^{tor}). The last three terms in Eq. 1 describe nonbonding interactions between atoms not bonded to each other (E^{nb}), hydrogen-bonding (E^{hb}), and Coulombic interaction (E^{el}). The analytical functions for these expressions can be found in the detailed description of the method (26).

In the studies reported here, the initial geometry used was taken from an X-ray crystal structure of prodine. Total geometry optimizations were first performed without the electrostatic term. Then, using these optimized geometries, partial atomic charges were obtained from the semi-empirical quantum mechanical procedure MNDO (27). These charges were then used in the electrostatic term of the seven-term energy formula in MOLMEC and the geometries were reoptimized. All the results reported are for the final MOLMEC optimizations, which include the electrostatic term. This iterative procedure insures self-consistency in use of charges in this program. In general, it was found that the addition of the electrostatic term had little effect on the geometries and relative energies obtained.

The calculations reported here are for the 12 *N*-methyl derivatives listed in Table 1. Two types of studies were performed. In the first study, the starting point was a set of initial geometries for phenyl axial, phenyl equatorial, piperidine chair, and piperidine boat conformers, with varying values of torsion angle $\tau = C_8C_7C_4X$, where X is the first atom of the 4-substituent and varies for different analogs (X = H, C, O). These geometries were completely optimized with respect to all variables, i.e., bond lengths, bond angles, and dihedral angles. In this way, a set of energy-optimized conformers of each type were obtained. For 4-*R* substituents such as ethyl, *n*-propyl, and isopropyl, which are lacking rotational symmetry, several conformational minima were found, corresponding to rotamers of the substituent. In the second type of study, rotational energy profiles were obtained for compounds in both phenyl axial and phenyl equatorial forms, by performing constrained optimizations at fixed values of the τ dihedral angle.

Results

The affinities of the 4PPs at the μ, δ, and κ sites are shown in Table 3. Affinities at μ and δ were obtained from a three-site fit of binding data determined in rat brain membranes using LIGAND. In this analysis, the third site is a high capacity site with low affinity for all ligands. As clearly indicated, all analogs have high to moderate affinity at μ with values at δ being 1 to 2 orders of magnitude lower. Also shown in this table are affinities of the 4PPs at the κ site as determined by inhibition of binding of the highly κ-selective ligand [^{3}H]U69,593 in guinea pig brain. These studies were conducted in guinea pig brain because κ receptor levels are higher in guinea pig than in rat and values obtained for [^{3}H]U69,593 binding were more reliable in guinea pig brain. This data was analyzed using a one-site model, which was superior to a two-site binding model. For all 4-alkyl PPs, affinities at κ and δ are much less than those at μ, making them μ-selective analogs. In the 4H series, particularly **1b** and **1c**, affinities at κ are slightly higher than at μ receptors.

In general, for a given *N*-*R* derivative, increase in size of the 4-*R* group enhanced μ affinity, an effect seen most clearly for the four *N*-methyl compounds **4a** > **3a** > **2a** ≫ **1a**. In fused-ring and some 4PP opioids, an *N*-phenethyl substituent is known to increase μ affinity over that of its *N*-methyl analog (9, 28). Interestingly, for the compounds studied here, as the 4-*R* group increases in size, the effect on μ affinities of this *N*-*R* variation reverses. The presence of an *N*-phenethyl substituent greatly increases μ affinity relative to the *N*-methyl group, when 4-*R* is H or CH_3. An *N*-phenethyl substituent has little

TABLE 3

Receptor affinities and maximum binding capacities at μ and δ receptors

Values were determined by using the curve-fitting program LIGAND for multiple binding experiments as described in Materials and Methods (three-site fit). Approximate total specific binding and the per cent specific binding for 0.5 nM of each labeled ligand are as follows: [^{3}H]naloxone, specific binding 4500 cpm, 88% specific; [^{3}H]DADL, 1800 cpm specific binding, 85% specific; [^{3}H]EKC, specific binding 1100 cpm, 90% specific; [^{3}H]DHM, specific binding 2400 cpm, 70% specific.

	K_D[a]		
	μ	δ	κ[a]
	nM		
Naloxone	1.1 ± 0.03	33 ± 2.4	
DADL	7.1 ± 0.17	1.6 ± 0.34	
EKC	4.2 ± 0.13	6.7 ± 1.89	
DHM	1.5 ± 0.03	230 ± 12.7	
U69,593			3.3 ± 0.62
4PP[b]			
1a	1640 ± 155	18,000 ± 3600	7,100 ± 2,200
1b	910 ± 99.1	5,000 ± 761	630 ± 170
1c	135 ± 5.3	2,130 ± 265	59 ± 15
2a	35.7 ± 6.07	1,450 ± 190	630 ± 140
2b	170 ± 46.0	3,700 ± 565	910 ± 180
2c	4.5 ± 0.63	360 ± 58.1	91 ± 23
3a	3.3 ± 0.37	45.5 ± 6.87	670 ± 150
3b	12.2 ± 1.59	220 ± 30.7	260 ± 66
3c	4.5 ± 0.56	27.8 ± 4.14	34 ± 8.6
4a	1.2 ± 0.07	83.3 ± 11.1	190 ± 49
4b	9.1 ± 0.67	400 ± 59.6	190 ± 44
4c	10.6 ± 3.55	170 ± 27.0	100 ± 27
B_{max} (pmol/g)	14.7 ± 0.25	4.2 ± 0.92	5.0 ± 0.63

[a] Affinities at κ determined by competitive inhibition of the κ-selective compound [^{3}H]U69,593 in guinea pig brain and analyzed by a one-site model using LIGAND.
[b] For computer analysis, each of the 4PP was used to inhibit the four labeled ligands, naloxone, DADL, EKC, and DHM.

effect on affinity when 4-*R* is *n*-propyl and, surprisingly, decreases affinity when 4-R is *t*-butyl. Thus, optimimum binding at the μ receptor appears to entail a competition for binding sites between the 4-*R* and *N*-*R* substituent.

In contrast to its variable effect on μ receptor affinity, an *N*-phenethyl substituent enhances κ affinity in each 4-*R* series, indicating less competition between the 4-*R* and *N*-*R* substituents for binding at this site. As we have seen in our binding studies of fused ring opiates, *N*-substituent variations have a differential effect on μ and κ affinities.

The results of *in vivo* studies of analgesic agonist and antagonist potencies are shown in Table 4. These results are also presented in Table 5 by rank order of potency obtained using each test for antinociceptive agonist activity. As seen from these tables, all compounds exhibited more activity in the mouse abdominal constriction (writhing) test than in the mouse tail-flick model for antinociception. This result is consistent with recent studies of both μ-selective and κ-selective opiates (12), which showed that most compounds had greater activity in the writhing test. Moreover, as shown in Table 5, there is generally good agreement between the rank order of potency found in writhing and tail-flick models when the compounds are administered SC.

The results in Tables 4 and 5 show a striking effect of different routes of administration on rank order of antinociceptive agonist activity for the 4-H-series (**1a,b,c**). Although none of the 4-H compounds had measurable agonist activity when administered SC, both **1c** and, particularly, **1a** have considerable agonist activity ICV, the latter being 1/10 as potent as morphine. These compounds are the most susceptible to enzymatic metabolism because of the labile benzylic 4-H group. It is possible that such transformations are responsible for the inactivity when the compounds are administered SC and that these compounds remain intact when injected ICV. Although it appears that the 4-H compounds are degraded when injected SC, we have determined that there is no degradation of the compounds during the binding incubation *in vitro*. Thus, the binding K_D values obtained appear to be those for the intact compounds. The *N*-allyl compound **1b** appears to be an intrinsically inactive analog, having no appreciable potency in both models for antinociceptive activity and by both routes of administration. The *N*-allyl analogs, in general, seem to be weak agonists or inactive.

The effect of different routes of administration on the remaining 4-*R* analogs is not as dramatic. The 4-*t*-butyl compounds remain among the least active, whereas the 4-*n*-propyl compounds remain the most active, with the 4-methyl compounds of intermediate potency. There are some significant changes in rank order among these compounds. The most striking is the particular enhancement of agonist activity by ICV administration of the **2c** analog, which was found by us as well as by Dr. Jacobsen[1] at the National Institutes of Health to be nearly devoid of agonist activity after SC administration.

Not only does the route of administration affect relative agonist potencies, but it also affects the apparent antagonism of morphine-induced analgesia by the tail-flick procedure. By the procedure used when the two drugs are administered simultaneously SC, three compounds, **1c, 2b,** and **2c**, were found to have antagonist activity, all very weak compared with nalorphine. When **1c** was studied by the ICV route of administration, no significant antagonism to morphine-induced tail-flick analgesia was obtained. The only other compound for which measurement of antagonism by an ICV route of administration was attempted was **4a**. This compound was chosen because it has the highest receptor affinity and lowest agonist activity ICV. In spite of this apparent low efficacy, no significant antagonist activity was detected at the highest concentration of this compound that could be used.

In addition to the rank order of agonist activities, Table 5 lists the compounds in order of decreasing affinity at μ receptor sites. Because only one compound has highest affinity at the κ receptor and the remainder are relatively μ-selective, we make the assumption that the *in vivo* analgesic activities measured are initiated by binding to μ receptors. The only possible exception would be **1c**, which binds with somewhat higher affinity to κ than to μ. Its activity could be due to binding at both sites. This possibility does not change the inferences made from our results. Comparing the activities and affinities given in Table 5, several trends and anomalies may be observed. Except for the low potency of the 4-*t*-butyl compounds, SC agonist activity, determined by both writhing and tail-flick tests, varies consistently with μ affinity. For example, the four inactive analogs have the lowest affinity. This relationship changes when ICV agonist potencies are compared with μ receptor affinities. There are two dramatic disparities between ICV agonism and μ affinities. The highest affinity analog, **4a**, has the lowest measured ICV agonist activity and the lowest affinity analog, **1a**, has a relatively high ICV agonist potency. It is generally assumed that agonist activities, for closely related analogs, obtained by an ICV route of administration are better

[1] Personal communication.

TABLE 4

Analgesic narcotic agonist and antagonist potencies of 4-(m-OH-hydroxy-phenyl)piperidines evaluated in mice

Compound	Agonism, ED_{50} (95% Confidence limit)[a]			Antagonism,[b] AD_{50} (95% Confidence limit)[a]	
	Writhing, SC	Tail-flick, SC	Tail flick, ICV	Tail flick, SC	Tail-flick, ICV
			μmol/kg		
4-Hydrogen					
1a *N*-Methyl	48 (30–75)	>419	0.5 (0.2–1.5)	>419	
1b *N*-Allyl	>302	>336	>33 (43%)	>336	
1c *N*-Phenethyl	8 (5–14)	>285	5.9 (2.9–12.2)	142 (92–219)	>25 (16%)[c]
4-Methyl					
2a *N*-Methyl	9 (5–17)	25 (14–45)	0.2 (0.1–0.5)	>279	
2b *N*-Allyl	96 (58–160)	>299		149 (60–373)	
2c *N*-Phenethyl	1.0 (0.5–4)	70 (29–168)[d]	0.7 (0.2–2.4)	44 (19–101)[e]	
4-*n*-Propyl					
3a *N*-Methyl	0.9 (0.5–1.6)	2.8 (1.7–4.6)	0.9 (0.4–2.1)	>255	
3b *N*-Allyl	4.6 (2.6–8.4)	6.1 (3.1–12.3)		>271	
3c *N*-Phenethyl	1.0 (0.6–1.6)	2.1 (1.3–3.2)[f]	0.2 (0.1–0.5)	>198[g]	
4-*t*-Butyl					
4a *N*-Methyl	6 (3–11)	27 (15–48)	12.5 (7.8–20)	>244	>18.3 (8%)[h]
4b *N*-Allyl	7 (5–12)	23 (13–42)		>226	
4c *N*-Phenethyl	11 (6–22)	48 (33–69)	4.5 (1.61–12.6)	>191	
Morphine	1.0 (0.5–2.1)	3.0 (1.8–4.7)	0.06 (0.03–0.15)		
Meperidine	23 (15–34)	25 (14–43)		None	
Nalorphine	2.0 (1.1–3.5)	828 (550–1250)	19.3 (8–47)	2 (1.4–2.9)	

[a] Recorded for peak time of agonist or antagonist potency.
[b] Antagonism of mouse tail-flick inhibition induced by 21.08 μmol/kg (SC) of morphine.
[c] Maximum saturated concentration in 20% aqueous solution of DMSO.
[d] Value reported by CPPD (as transmitted by Dr. Arthur Jacobson, National Institutes of Health): inactive ≤80 μmol/kg.
[e] CPPD value: 17 (5–61) μmol/kg.
[f] CPPD value: 6 (4–10) μmol/kg.
[g] CPPD value: >80 μmol/kg.
[h] At this dose 6/10 animals convulsed and died.

TABLE 5

Rank order of agonist activities and μ receptor affinities

Antinociceptive Agonism						μ Receptor affinities			
Writhing, SC		Tail-flick, SC		Tail-flick, ICV		Compound	K_D	Compound	Efficacy[a]
Compound	ED_{50}	Compound	ED_{50}	Compound	ED_{50}				
	μmol/kg		μmol/kg		μmol/kg		nM		
3a	0.9	3c	2.1	3c	0.22	4a	1.2	1a	3000
3c	1.0	3a	2.8	2a	0.24	3a	3.3	1b	<22
2c	1.0	3b	6.1	1a	0.54	2c	4.5	1c	23
3b	4.6	4b	23	2c	0.76	3c	4.5	2a	162
4a	6	2a	25	3a	0.89	4b	9.1	2c	6
4b	7	4a	27	4c	4.5	4c	10.6	3a	4
1c	8	4c	48	1c	5.9	3b	12.2	3c	20
2a	9	2c	70	4a	12.5	2a	35.7	4a	0.1
4c	11	2b	IA[b]	1b	>33	1c	135	4c	2
1a	48	1c	IA			2b	170		
2b	96	1b	IA			1b	910		
1b	>302	1a	IA			1a	1640		
Morphine	1		3.0		0.06				333[c]
Meperidine	23		25						
Nalorphine	2		828		19.3				

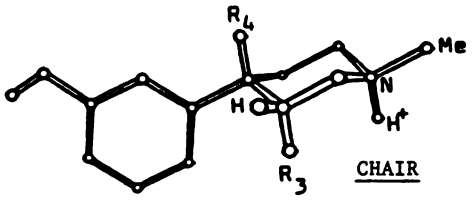
[a] Efficacies defined as: $\frac{K_D\ (\mu)}{\text{Tail-flick } ED_{50} \text{ (ICV)}}$
[b] IA, inactive.
[c] Affinity of morphine (20 nM) as determined in guinea pig brain membranes (Toll *et al.*, manuscript submitted).

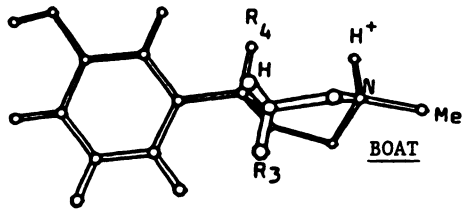
measures of their relative intrinsic agonist activity, because differences among analogs due to metabolic transformations and transport, although not completely eliminated, are minimized. Thus, the ratio of μ affinities to ED_{50} ICV agonist potencies (Table 5) can serve as an approximate measure of relative efficacy.

The results of energy conformation studies of 12 *N*-methyl, 4-(*m*-OH-phenyl)piperidines with varying 4-*R* groups are summarized in Table 6. Shown in this table, for eight of the analogs, are the relative energies of the lowest energy chair and twist boat conformations of the piperidine ring with the 4-phenyl group in both an axial and equatorial position. Also given for all of the compounds is the relative energy of the most stable phenyl axial and phenyl equatorial conformations. Finally, the inter-ring torsion angle $\tau = C_8C_7C_4X_4$, for all rotamers that are equi-energy with the lowest energy rotamer are indicated in this table for both the phenyl equatorial and phenyl axial piperidine chair conformers.

TABLE 6

Calculated relative energies for geometry-optimized conformers of protonated 4R-4-(m-OH-phenyl) piperidines





Compound	R_3	R_4	ϕ_{eq} (chair)		ϕ_{eq} (boat), ΔE	ϕ_{ax} (chair)		ϕ_{ax} (boat), ΔE
			ΔE	τ^a		ΔE	τ^a	
			kcal/mol					
1a	H	H	0.0	0°	7.1	3.7	−57° (−123°)	9.2
2a	H	Methyl	0.9	76° (104°)	6.8	0.0	−81° (−99°)	6.7
	H	Ethyl	1.1	71° (109°)	7.6	0.0	−79° (−101°)	6.8
3a	H	*n*-Propyl	0.8	73° (107°)	9.2	0.0	−78° (−102°)	8.4
	H	*i*-Propyl	1.8	89° (91°)	9.1	0.0	−89° (−91°)	6.9
4a	H	*t*-Butyl	5.1	88° (92°)	11.9	0.0	−89° (−91°)	7.0
10α	α-CH_3	Methyl	0.0	0°	NC[b]	1.8	−70°	NC
10β	β-CH_3	Methyl	0.0	68°	NC	2.3	−123°	NC
11α	α-CH_3	*n*-Propyl	0.7	19°	NC	0.0	−85°	NC
11β	β-CH_3	*n*-Propyl	0.0	71°	NC	2.2	−119°	NC
12	H	OCOethyl	0.0	52° (128°)	6.6	2.2	−62° (−118°)	9.0
13α	α-CH_3	OCOethyl	0.0	138°	NC	1.3	−66°	NC
13β	β-CH_3	OCOethyl	0.0	59°	NC	3.9	−59°	NC
14	H	COOethyl	0.0	60° (120°)	7.6	1.5	−70° (−110°)	7.8
Metazocine							−145°	
Morphine							−172°	

[a] $\tau = X_4C_4 - C_7C_8$ (Table 1) with clockwise rotation of the plane defined by $C_8C_7C_4$ into the plane defined by $C_7C_4X_4$. Values of τ in parentheses are additional rotamers with $\Delta E = 0$. For every value of τ listed, there is an equi-energy conformer with $\tau \pm 180$ because of the symmetry of the phenyl ring.

[b] NC, not calculated.

The results given in Table 6 clearly show that for each of the eight compounds studied, the chair conformation is lower in energy than the twist boat form, regardless of type and position (axial or equatorial) of the 4-*R* substituent. The energy differences, after total geometry optimizations, are 6–8 kcal/mol, making it very unlikely that there is a significant population of the twist boat conformation at room temperature.

The results indicate how variations in the 4-*R* group alone can influence the relative stabilities of the phenyl axial versus phenyl equatorial conformations. For the five 4-alkyl compounds studied, the phenyl axial conformation is preferred, the bulky 4-*t*-butyl compound being most definitively in a phenyl axial form. This is the first mono-alkyl-substituted 4-(*m*-OH-phenyl)piperidine predicted to be definitively in a phenyl axial conformation. Previously, the only examples of substituted piperidine compounds that have been reported to have a strong phenyl axial preference were 3,4,6-trimethyl compounds (29). Fig. 2 shows that the structure of **4a**, as determined by X-ray crystallography, is essentially identical to the calculated structure. The phenyl ring was found to be axial and the relative orientation of the phenyl ring to the piperidine ring is identical, with a τ value of 91° from X-ray analysis corresponding to one of the lowest energy conformers from the MOLMEC results (Table 6). The other torsion angles, as well as bond angles and bond lengths, were also found to be very similar. Coordinates from both studies are available upon request from the authors.

For the five alkyl compounds, not only is a phenyl axial conformer preferred but the inter-ring torsion angle is very similar for all of them. Thus, Fig. 2 represents the lowest energy conformers for all of these analogs. All have two equi-energy minima ($\Delta E = 0$) with one τ value in the range of 80–90° and the other 90–100°. In addition, because of the symmetry of the phenyl ring, there is an equi-energy conformer with $\tau = \pm 180°$ for these minima.

As a measure of the flexibility of analogs **2a**, **3a**, and **4a** to inter-ring rotation, rotations about the inter-ring axis ($\tau = C_8C_7C_4X_4$) were performed in 20° intervals with total geometry optimization of all the other parameters. The results are shown in Fig. 3. As indicated there, for each of these three analogs, the phenyl group can be rotated by ±15° from the low energy minimum with an energy input of less than 1 kcal/mol energy. Thus, these three compounds can be regarded as having a common energy-accessible phenyl axial conformer, with $\tau = 90° \pm 15°$, available for binding at the μ receptor site. This conformer is, however, different from that in the fused-ring opiates, for example metazocine or morphine, in which the phenyl ring is constrained to be in an axial conformation. In metazocine, the same torsion angle is 32° and in morphine it is 18°. These values for the two fused-ring analogs were obtained by total geometry optimization using the same method (MOLMEC) as for the 4PPs, starting with standard bond lengths and bond angles. As seen in Fig. 3, to rotate **2a** and **3a** to a metazocine- or morphine-like conformation requires 4–6 kcal/mol, whereas to rotate the bulkier **4a** analog to these values requires 14–15 kcal/mol.

As shown in Table 6, in contrast to the 4-alkyl analogs, an equatorial conformer of the phenyl group is preferred for analogs with 4-*R* of H or the ester groups $CO_2C_2H_5$ and $OCOC_2H_5$.

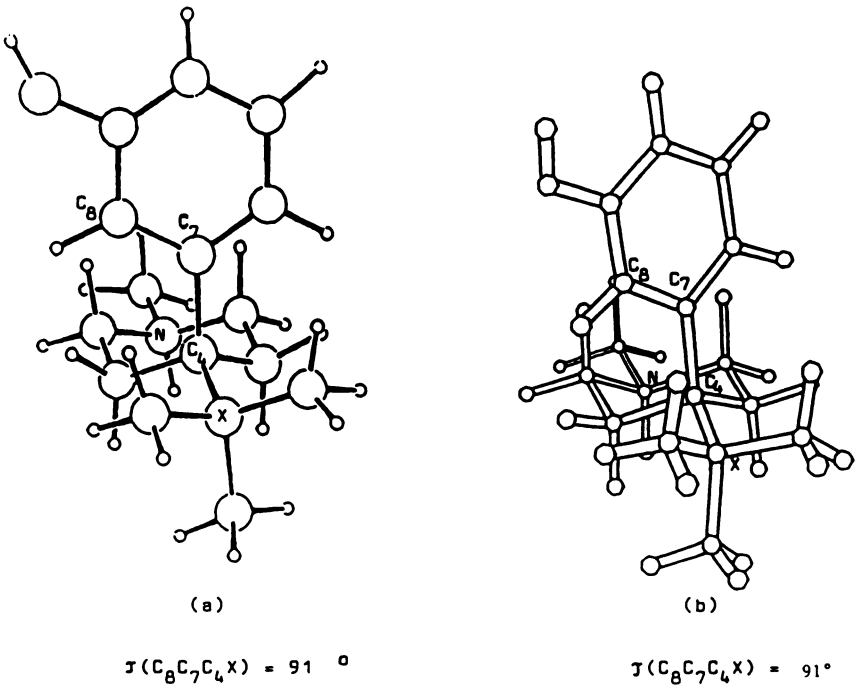


Fig. 2. Structure of analog **4a** as determined by (a) X-ray crystallography and (b) computation using the program MOLMEC. This is common low energy conformer for all 4-alkyl analogs studied.

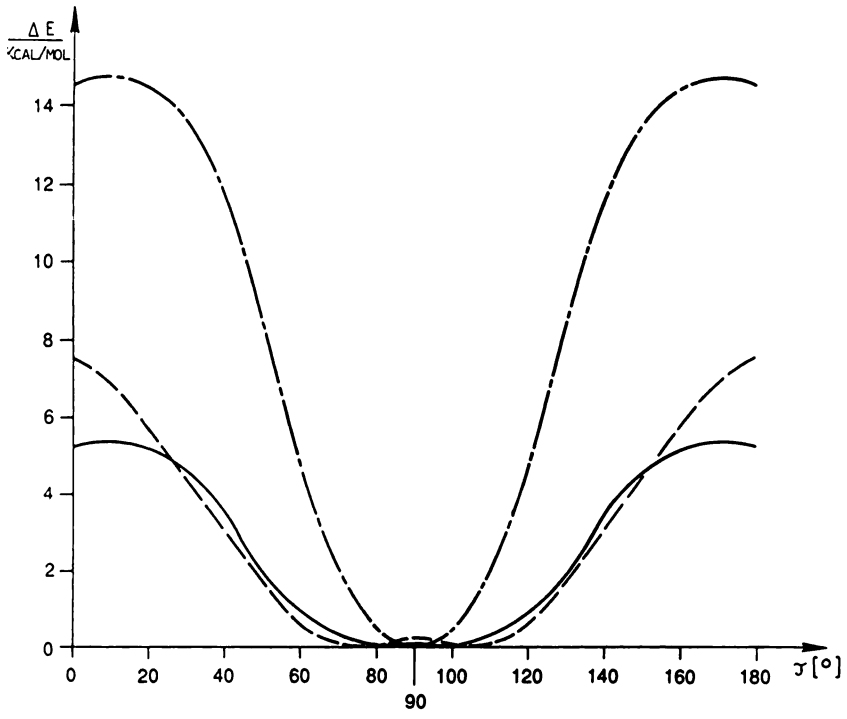


Fig 3. Rotational energy profiles for 4-alkyl analogs (**2a**, **3a**, and **4a**). ——**2a**, – – –, **3a**; – – – –,**4a**.

This conformer is also the preferred one when a 3-methyl group is added in either a **cis** (α) or *trans* (β) position relative to the 4-*R* group, for 4-*R* of methyl or $OCOC_2H_5$. Only when 4-*R* is *n*-propyl and the 3-methyl is *cis* is a phenyl axial conformer preferred.

As indicated in Table 6, for the seven analogs with a preferred phenyl equatorial conformer, two types of low energy rotamers were found, one with $\tau \sim 0°$ and one with $\tau \sim 60°$. For all such analogs, the energy required to rotate each of these compounds from one to the other rotamer (τ, 0° to 60°, or 60° to 0°) was determined, as shown in Table 7. These results indicate that the three des-3-CH_3 analogs can assume either low energy conformer at an energy cost of only 1.7–3.0 kcal/mol. Addition of a 3-CH_3 group and, particularly, one *trans* (β) to the 4-*R* group, favors the $\tau \sim 60°$ rotamer by more than 4 kcal/mol and makes the analog more rigid. The two types of low energy conformers are illustrated in Fig. 4, a and b with analog **1a** in Fig. 4 representing the $\tau = 0°$ type, and analog **10**β representing the $\tau \sim 60°$ type of low energy conformer. Although there are no crystal structure determinations for any of these compounds, the X-ray structure of an analog similar to **10**β but with a 3-ethyl rather than 3-methyl group is known[2] and has a nearly

[2] Dr. D. Zimmerman, Lilly Research Laboratories, Personal Communication.

TABLE 7

Relative energies calculated by MOLMEC for a partial rotation of the phenyl group in the equatorial conformations of compounds with phenyl equatorial conformers lowest energy form

Analog	° ΔE			
	τ = minimum	τ = 0°	τ = 30°	τ = 60°
	kcal/mol			
1a	0.0 (0°)	0.0	0.5	2.0
12	0.0 (52°)	3.1	0.9	0.1
14	0.0 (60°)	1.7	0.6	0.0
10β	0.0 (68°)	4.6	1.7	0.2
11β	0.0 (71°)	6.0	2.0	0.2
13β	0.0 (59°)	6.5	2.0	0.0
10α	0.0 (0°)	0.0	2.0	4.2
13α	0.0 (−42°)	4.0	0.4[a]	0.5[a]

[a] Calculated at −30° and −60°, respectively. Value of −42° is equivalent to 138°, −30° to 150°, and −60° to 120°.

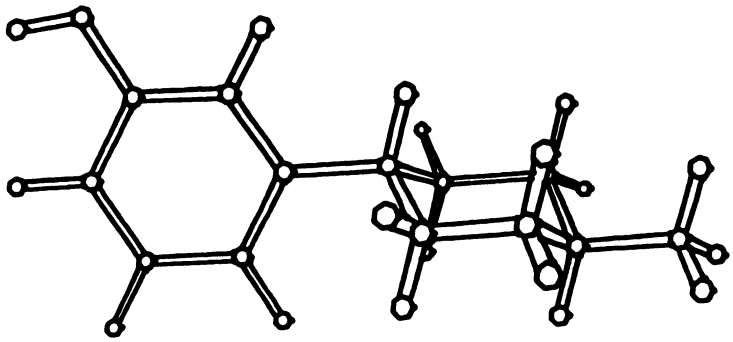
identical structure to that calculated for **10β**, with a phenyl equatorial conformation and a torsion angle of 64°.

Discussion

One of the goals of this investigation was to further explore the relationship between phenyl axial and phenyl equatorial conformations of 4PPs and their relative receptor affinities, selectivities, and efficacies. The combined theoretical and experimental results allow the identification of a single common low energy, nonmorphine-like, phenyl axial conformer (90° ± 15°) for the 4-alkyl analogs leading to relatively high affinity μ receptor binding. By contrast, binding to the μ receptor of analogs with a preferred phenyl equatorial conformation leads to lower μ receptor affinity, regardless of the torsion angle. This effect is exemplified by the micromolar binding affinity of the 4-*H* compound, **1a**, which has a preferred angle of 0°. Although little or no systematic binding studies have been made for those 4PP compounds predicted here to have a definitively preferred phenyl equatorial conformation with τ = 60–70°, such as prodines, IC_{50} results reported[2] for **10β** of 400 nM versus [^{3}H]naloxone also suggest low μ affinities. Thus, our energy conformational studies have identified two qualitatively different conformers of the 4-(*m*-OH-phenyl)piperidines leading to selective binding to the μ receptor, one phenyl axial with high and one phenyl equatorial with low affinity. In either mode, however, factors other than conformation, e.g., *N*- and 4-substituents with varying polarity and steric requirements, can produce localized interactions with specific receptor sites, and, thereby, modulate relative receptor affinities.

In the phenyl axial pharmacophore of 4-alkyl-4-(*m*-OH-phenyl)piperidines, competition for local optimization of 4-*R*

a)



b)

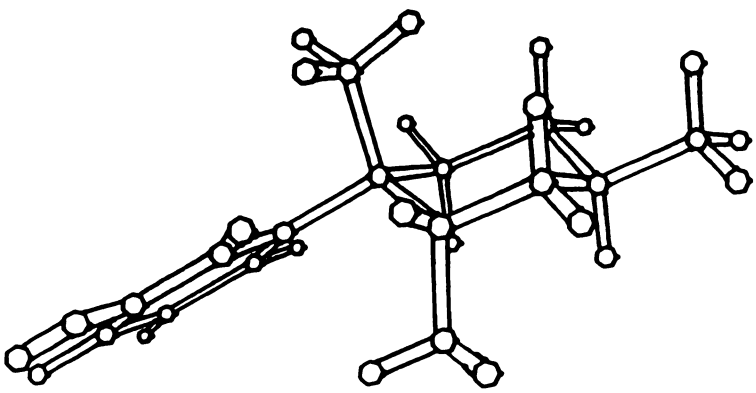


Fig. 4. Two types of low energy rotamers found for analogs with preferred phenyl equatorial conformations. a, Analog **1a** (τ = 0°) b, Analog **10β** (τ = 60°).

and *N*-*R* binding appears to occur. The bulkier the 4-*R* group, the higher the receptor affinities, suggesting a hydrophobic receptor pocket in which the *t*-butyl group can have a most favorable interaction. Apparently, the tight requirement for the *t*-butyl group at the receptor locks in the ligand and does not allow the flexibility necessary for the *N*-phenethyl group in **4c** to reach a propitious binding site. The looser fit of the 4-methyl group in **2c** appears to allow more flexibility, resulting in accommodation of the *N*-phenethyl group. As a result, **4c** has decreased affinity compared with **4a**, whereas **2c** has greatly increased affinity compared with **2a**. The 4-*n*-propyl analogs **3a** and **3c** are intermediate in flexibility of fit, with approximately equal affinities as a result.

Although all the 4-alkyl-4-(*m*-OH-phenyl)piperidine analogs studied have a common low energy phenyl axial form leading to relatively high receptor affinities, the relative ability of these compounds to initiate the changes in the μ receptor leading to antinociceptive activity, i.e., their efficacy, varies considerably (Table 5). The question then arises as to what features of their binding modulate this efficacy relative to morphine.

For the purpose of discussing activities of these compounds, we have defined relative efficacy as the ratio of K_D at μ receptors to ED_{50} by an ICV route of administration. The 4-*t*-butyl-N-CH_3 analog **4a** has the highest affinity at the μ receptor and is also the compound with the lowest efficacy, with a K_D (μ)/ED_{50} (ICV) ratio of 0.10 compared with 333 for morphine. The decreased efficacy could be caused by formation of a more rigid drug-receptor complex due to the steric requirements of the bulky *t*-butyl group.

A plausible mode of binding of the 4-*t*-butyl analog to the μ receptor relative to the fused-ring opiate metazocine is shown in Fig. 5. As shown, despite the difference in piperidine ring-phenyl ring torsion angle, the two key moieties, the *N*-*H* and the O-*H* groups of both analogs, can be superimposed with an RMS overlap for these four atoms of 0.156 Å. This value is similar to that of 0.202 Å obtained for morphine overlap. Such an overlap would allow these moieties to interact with similar proton-donating and proton-accepting receptor binding sites. However, in this mode of binding, the *N*-*R* group, as well as the remaining portions of each molecule, would not bind at exactly the same place in the receptor. Inasmuch as the 4-*t*-butyl analog retains high affinity, the *t*-butyl group obviously finds an appropriate hydrophobic pocket in the receptor. However, this match could also prevent the receptor from responding to the initial drug receptor interaction in a manner leading to activation. The high affinity and decreased efficacy of the 4-*t*-butyl compounds could then be due to a combination of the requirement for binding of the 4-*R* group and the displacement of the *N*-*R* group, both of which might interfere with the activation step.

We propose that the mode of binding relative to fused-ring opioids, shown in Fig. 5, represents the universal phenyl axial 4PP pharmacophore and that this is the mode of binding of all the 4-alkyl analogs for which this conformer is the lowest energy. Thus, we are suggesting that analogs **2a** and **3a**, as well as the 4-ethyl and 4-isopropyl compounds not yet synthesized, bind in this mode, even though the energy required to reach a phenyl equatorial conformation (<1 kcal/mol) is much less than for the *t*-butyl compound.

Using this most conservative hypothesis, which does not invoke any higher energy forms in binding to the receptor, the various activities induced by both 4-*R* and *N*-*R* substituent

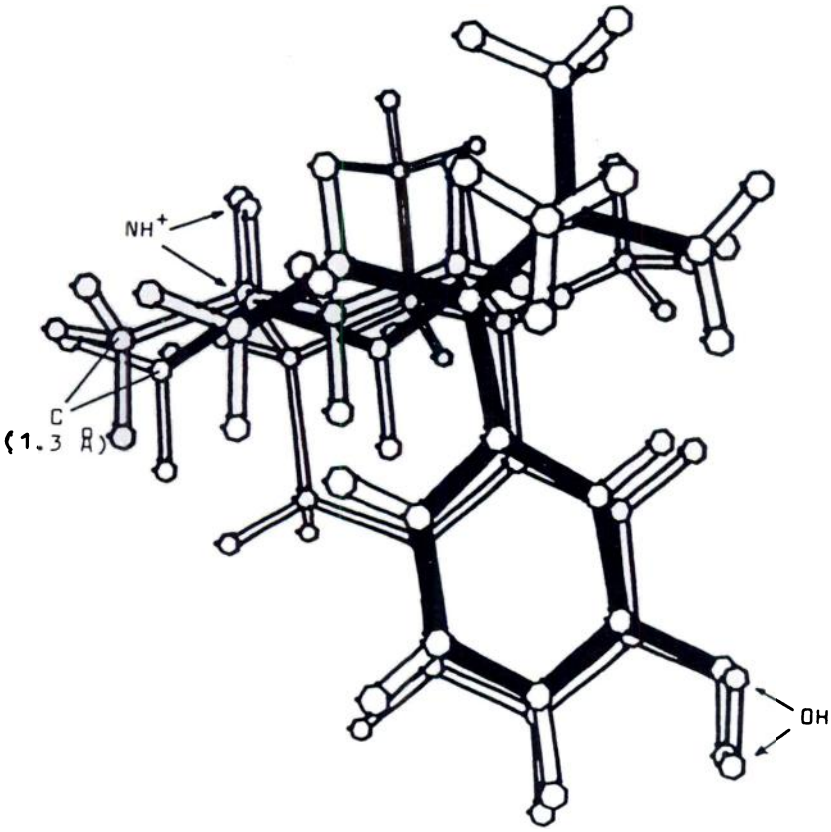


Fig. 5. Overlap of low energy phenyl axial conformer of **4a** with metazocine to optimize protonated NH^+ and phenolic OH. 4-atom overlap RMS = 0.156.

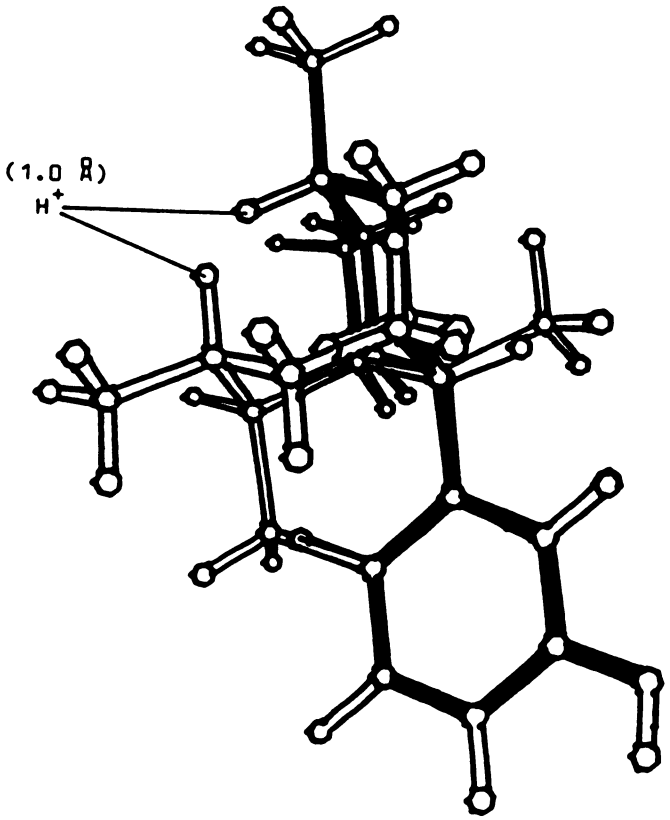


Fig. 6. Low energy phenyl equatorial conformer of **1a** with $\tau = 30°$, with phenol ring overlaped with that of metazocine.

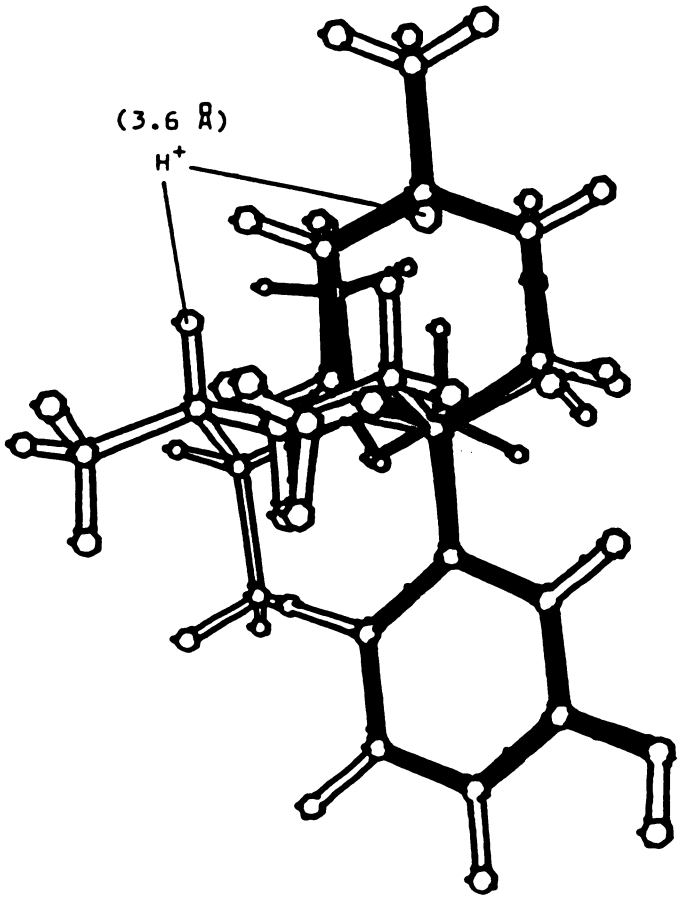


Fig. 7. Low energy conformer of **10**β with $\tau = 68°$, with phenol ring overlaped with that of metazocine.

variations can be rationalized. It is proposed that, although all the analogs bind in essentially the same manner shown in Fig. 5, the variations in 4-*R* and *N*-*R* substituents and the competition between them in finding an optimum binding site do cause small changes in orientation that can affect both affinity and efficacy. For the *N*-methyl analogs, although the μ affinity increased with the bulkiness of the 4-*R* group (**2a** < **3a** **4a**), the relative efficacy decreases from 162 to 4 to 0.11. This reverse trend tends to support the idea that the *t*-butyl group finds an optimum binding site, but at the expense of the flexibility needed for receptor activation. When the strict steric requirement and favorable hydrophobic contribution to affinity are diminished as the 4-*R* group is decreased in size, flexibility of movement of either the drug or receptor is restored and the ability of the receptor to respond to the effect of the drug complex is increased. If this hypothesis is correct, then the μ receptor affinity and efficacy of the *N*-methyl derivatives of the unknown compound with 4-*R* being ethyl should be between that of **2a** and **3a**, whereas that of the *N*-methyl derivative of the unknown compound with 4-*R* being isopropyl is predicted to be between that of **3a** and **4a**.

For each 4-*R* group, the effects of the *N*-*R* variation on affinities and efficacies can be rationalized by a 4-*R*, *N*-*R*′ competition. With the small 4-methyl group, the larger *N*-phenethyl group, **2c** has the flexibility to find an optimum binding site that, however, appears to interfere with the ability to activate the receptor. This adjusted fit produces the increase in affinity of **2c** over **2a** as well as the decrease in efficacy from 162 to 6. For the bulky *t*-butyl group of **4c**, its requirements dominate and the *N*-phenethyl group does not find an optimum binding site, resulting in diminished affinity but enhanced efficacy compared with **4a**. For the intermediate sized *n*-propyl group, affinity is barely affected in going from the *N*-methyl to *N*-phenethyl compound, and **3a** and **3c** have comparable efficacies. If these competing 4-*R*/*N*-*R* effects on the orientation of essentially the same pharmacophore are correct, then *N*-substituent effects on affinity and efficacy of the 4-ethyl compounds are predicted to be intermediate between those of the 4-methyl and 4-*n*-propyl compounds, whereas the *N*-phenethyl analog of the 4-isopropyl compound is predicted to have about 5- to 10-fold enhanced efficacy over the *N*-methyl compound and 2- to 3-fold diminished μ affinity.

Another interesting example of a compound with a preferred phenyl axial pharmacophore is **11**α, the (*cis*)-3-methyl-4-*n*-propyl-*N*-methyl compound. This is the only 3-methyl compound we have calculated to have a preferred phenyl axial conformation. Both the racemic mixture and the separated isomers of **11**α have been studied in detail by Zimmerman and colleagues at Eli Lilly (30). They report very similar μ receptor affinities for the two enantiomers, 7–8 nM, similar to that for our des-3-methyl analog **3a** (3.3 nM). Interestingly, one enantiomer [(+)·**11**α] has morphine-like agonist activity similar to **3a**, suggesting a common conformer. However, the other isomer, [(−)·**11**α], has zero efficacy, i.e., it is a pure antagonist in the rat tail heat procedure reported. This dramatic modulation of efficacy by the position of the 3-*cis* methyl group could be caused by the specific local requirement for binding of the methyl group, apparently in a tight hydrophobic pocket matched in the unique phenyl axial conformation of this com-

pound. If **11**α does indeed bind in a manner similar to **3a**, then the unreported *N*-phenethyl analog is predicted to have very similar μ affinity and agonist analgetic activity to the *N*-methyl compound.

Turning to the compounds with a phenyl equatorial lowest energy conformer, the simplest analog **1a** requires about 4 kcal/mol to be transformed to a phenyl axial conformer. For the 4-ester compounds, **12**, **13**α, **13**β, and **14**, the phenyl equatorial conformer is preferred by 1.3 to 3.9 kcal/mol. This result, as well as previous studies (31, 32) indicate an inverse correlation between reported agonist potencies (33) and the energy required to attain phenyl axial conformation. Thus, it can be assumed that these analogs bind and act at the μ receptor in their lowest energy phenyl equatorial conformation. Although a phenyl equatorial rotamer for **1a** with $\tau = 0°$ is the lowest energy form, as shown in Table 7, rotation of 30° can be achieved with negligible energy cost (≤0.5 kcal/mol). Shown in Fig. 6, when **1a** with a torsion angle of 30° is superimposed with overlapping phenol groups on metazocine, the amine protons can occupy similar positions 1 Å apart. The distance decreases to 0.67 Å in comparison with morphine, which has a torsion angle of 18°. However, the *N*-*R* substituents are in very different places.

We propose that the phenyl equatorial pharmacophore shown in Fig. 6 with variable torsion angles between 0° and 30° is plausible for those 4PPs with a rotamer minimum near 0° and nearly free rotation, as in the 4-*H* compounds. This pharmacophore could be responsible for low affinity and moderate to high efficacy. Because the OH and NH overlap is close, both the fused-ring and 4PP opioids can have similar protonated amine anionic receptor interactions. However, the different position and directionality of the *N*-*R* substituent could result in a different *N*-substituent modulation of affinities and efficacies, compared with fused-ring opioids. Affinity increases for the *N*-phenethyl analog **1c** whereas efficacy is greatly diminished from 3000 for **1a** to 23 for **1c**, further evidence for different position of the *N*-*R* group in this analog relative to fused-ring compounds.

For 4-*R* of methyl, *N*-propyl, or $OCOC_2H_5$, when a 3-CH_3 group is added *trans* to the 4-*R* substituent, the lowest energy rotamer has a torsion angle of ~60° with at least 2 kcal/mol required to transform it to the rotamer at $\tau = 30°$ and 4–6 kcal/mol to that with a value of 0°. Thus, both the greater energy required for rotation in these compounds and the anchoring effect of the added 3-CH_3 groups, or 4-ester group limit the free inter-ring rotation possible for the 4-H compounds **1a**–**1c**. Unlike the 4-*H* compounds, all of these compounds have been shown to have zero efficacy, i.e., to be pure antagonists. As shown in Fig. 7, when the phenol OH group of this conformer of analog **10**β is superimposed on that of metazocine, not only the *N*-*R* groups but the amine protons thought to be essential for significant receptor activation are in very different positions, 3.6 Å apart. Adjustment of the overlap to bring the crucial NH moieties closer results in loss of OH overlap. This mismatch could be the reason for the lack of efficacy of these compounds. This phenyl equatorial compound **10**β with a τ ~ 60° is proposed, as it has been in the past (31, 32), to model the low efficacy (antagonist) pharmacophore for the 4-(*m*-OH-phenyl)piperidines. Although *N*-substituents can increase the affinity of these compounds, i.e., **10**β is found to have 400 nM affinity whereas its *N*-phenethyl analog has 2 nM affinity,[2] the efficacy is not enhanced, an observation that can be explained by the proposed pharmacophore, because the mismatch of the amine protons or phenyl OH is still present.

Conclusion

For the 4-alkyl-4-(*m*-OH-phenyl)piperidines with *N*-*R* variations, our combined experimental and theoretical results indicate that selective high affinity μ receptor binding can occur in a universal phenyl axial pharmacophore, which is different from that of fused-ring opioids. Bulky groups in the 4-*R* position, such as the *t*-butyl group, lead to a definitive preference for this pharmacophore and appear to enhance affinity but diminish efficacy. In the context of this universal pharmacophore, invoked as well for the 4-alkyl compounds, which show less-decisive preference for it, the effect of both 4-*R* and *N*-*R* variations on affinity and efficacy can be consistently understood in terms of a competition for optimum receptor interactions of these two varying groups. Based on this hypothesis, the relative affinities and efficacies of the analogs with 4-*R* of ethyl and isopropyl have been predicted.

For 4-*R* of H, $CO_2C_2H_5$, and $OCOC_2H_5$, as well as for all compounds with a 3-methyl group *trans* to the 4-*R* group, phenyl equatorial conformers are preferred. These could be subclassified into two types of pharmacophores, depending on the lowest energy rotamer found. One, with a minimum at $\tau = 0°$, with relatively free rotation of 30°, leads to a pharmacophore in which the phenol and amine protons can interact with a receptor as do the respective protons in fused-ring opiates. This pharmacophore can result in high efficacy compounds even with low affinity, such as **1a**. By contrast, the second phenyl equatorial pharmacophore with a minimum at τ ~ 60° and with more restricted rotation, does not allow simultaneous overlap of both phenyl rings and amine protons. This lack leads to analogs with little or no efficacy. No pharmacophore found for 4PPs possesses both the *N*-*R* substituent and the phenol ring overlap as in fused-ring compounds. This result explains why no *N*-substituent variation in 4PPs effects affinity and efficacy in a manner analogous to those found in fused-ring opiates.

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Send reprint requests to: Gilda H. Loew, SRI International, Life Sciences Division, Menlo Park, CA 94025.