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Structure–activity studies of morphine fragments. III. Synthesis, opiate receptor binding, analgetic activity and conformational studies of spiro-[tetralin-1,4'-piperidines]

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Summary — A series of 5 spiro compounds, a new class of conformationally restricted analogs of 4-alkyl-4-(*m*-OH-phenyl) piperidines, have been synthesized and their affinities for μ, δ and κ opioid receptor sites and *in vivo* analgetic activities determined. All compounds show rather low affinities for the 3 receptors, with some modulation by the N-substituent and by the position of the phenolic group. To help understand the origin of this poor affinity compared to the unrestricted 4-alkyl-4-phenyl piperidines, energy conformation calculations were performed which indicated that all the analogs favor a phenyl equatorial over a phenyl axial conformer. Significant differences in the lowest energy conformation were found between these spiro analogs and both morphine and 4-*n*-propyl-4-(*m*-OH-phenyl) piperidines, **18** and **19** which are conformationally unrestricted, closely related analogs with high μ-affinity. These differences could account for their lower affinities. To continue the search for more active members of the family, structure variations which favor a phenyl-axial conformation have been identified and proposed for further study.

Résumé — Études structure–activité de fragments de la morphine. III. Synthèse, liaison au récepteur opiacé, activité analgésique et études conformationnelles de spiro-[tétraline-1,4'-pipéridines]. *Une série de cinq spiro-[tétraline-1,4'-pipéridines], une nouvelle classe d'analogues à conformation restreinte des 4-alkyl-4-(*m*-OH-phényl) pipéridines ont été synthétisées, et leurs affinités aux sites récepteurs opioïdes μ, δ et κ ainsi que leurs activités analgésiques ont été déterminées. Tous les composés montrent des affinités plutôt faibles pour les trois récepteurs, avec quelque modulation par le substituant de l'azote et par la position du groupe phénolique. Pour aider à comprendre l'origine de cette faible affinité, des calculs de l'énergie de conformation ont été effectués indiquant que les cinq analogues favorisent un phényle équatorial plutôt qu'un phényle axial. Des différences considérables de conformation d'énergie la plus faible ont été trouvées entre ces analogues et la morphine ainsi que les 4-*n*-propyl-4-(*m*-OH-phényl) pipéridines, proches analogues à conformation non restreinte avec forte affinité μ, qui pourraient expliquer leurs plus faibles affinités. Pour poursuivre la recherche de membres plus actifs de cette famille, des composés qui favorisent une conformation à phényle axial ont été identifiés et proposés pour des études supplémentaires.*

spiro piperidine / opioid receptor binding / conformational profile

Introduction

The modulation of the molecular structure of morphine with the aim of finding a non-addictive opiate has been a major topic of structure–activity research in drug design [1, 2]. A vast number of candidate compounds have been synthesized and tested for analgetic activity. One strategy in the design of analgesics is the partial or total cleavage of various fragments from the multi-ring morphine molecule. This approach has had a long history. In the 1940's, a Hoffman/LaRoche group recognized that such structures as 4-phenyl piperidines (4PP) and 2-benzyl-piperidines are substructures of morphine and members of these families were then synthesized [3, 4]. In a companion paper, we described our recent investigation of the 2-benzyl-piperidine substructure [5]. Figure 1 shows how fragmentation of morphine leads to these 2 families (E and F), and a number of others.

In a recent study [6], we calculated conformational energy profiles for a series of 4PPs and performed receptor binding and *in vivo* analgesic activity studies. The results of these and other previous studies [7, 8], led to the conclusion that such compounds can bind to the μ-receptor in either a phenyl-axial or phenyl-equa-

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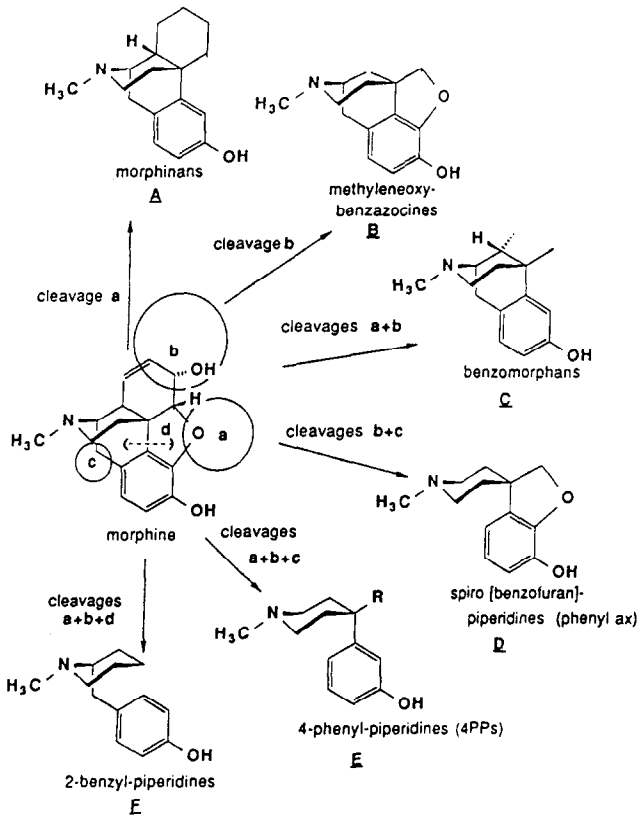
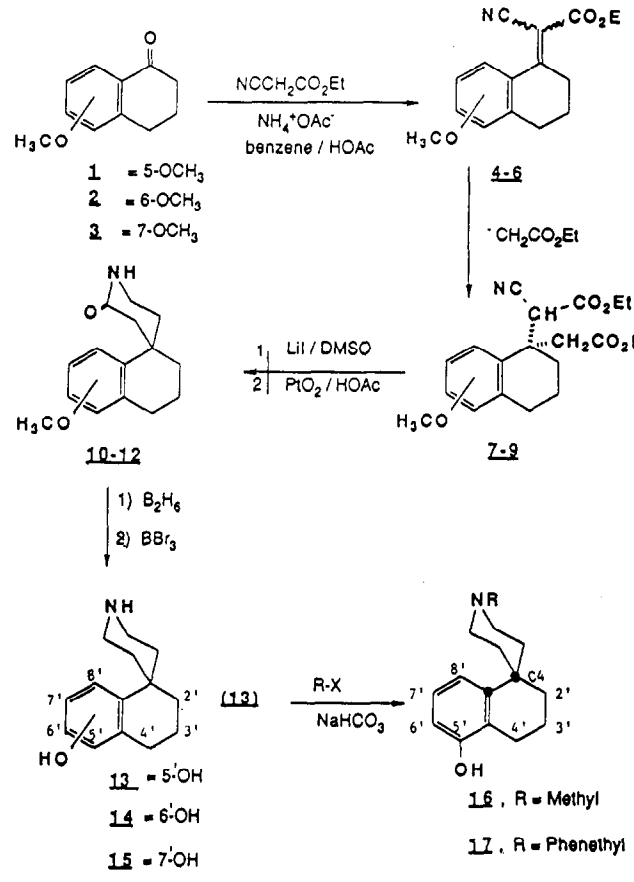


Fig 1. Schematic representation of the reduction of morphine into various substructures retaining opiate activity.



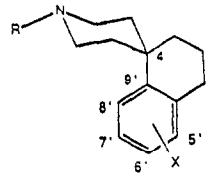
Scheme 1.

torial conformer, depending on the nature of the second 4-R substituent. Where R is an alkyl group, phenyl-axial conformers are favored, the more so with bulky R-substituents such as *t*-butyl. When R represents an ester (COOEt) group, such as in substituted meperidines or a retro ester (OCOEt) as in prodines, phenyl-equatorial conformers are favored. Moreover, for the 4PP analogs for which a phenyl-equatorial conformer is favored, the inter-ring torsion angle appears to be a modulator of efficacy. Compounds for which this angle is approximately ± 30° have high efficacy and those for which this angle is ± 60° have lower efficacy. A possible origin of this modulation is that analogs with a torsion angle τ ($C_8C_7C_4N$, table II) of 30° between the phenyl and piperidine ring can bind to the μ-receptor in an orientation similar to fused ring opiates, while phenyl equatorial analogs with τ = ± 60° cannot. Similar ideas have been suggested in the work of Froimowitz [9, 10] and Portoghese [11, 12].

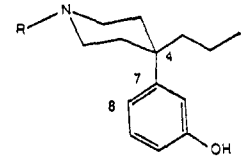
In the study reported here, we continue to probe the effect of changes in the relative orientation of the phenyl and piperidine rings on receptor recognition and *in vivo* activity. The approach taken was to synthesize conformationally restricted analogs of 4-phenyl-piperidines by bridging the phenyl group to the piperidine ring, thus restricting its torsion angles to values near 0°C. The morphine fragment D in figure 1, resulting from cleavages b and c, is such a bridged structure. Its close relationship to the 4PPs is evident, wherein the 4-R group has been tied to the *ortho*-position of the phenyl group. A similar approach has been undertaken by Rice *et al* [13] in their synthesis of conformationally restricted 5-phenyl-morphans and in a number of other reported studies [14–16] of related 4-spiro-substituted piperidines.

In this work, we have synthesized the spiro[tetralin-1, 4'-piperidines] analogs (**13–17**, table I) which resemble fragment D, but have a propylene bridge between the phenyl ring and the piperidine ring, rather than the oxymethylene found in substructure D. Thus, as shown in table I, the compounds made are fused analogs of 4-*n*-propyl-4-phenyl piperidine. Since ro-

Table I. Receptor binding and *in vivo* activity of a series of spiro-[tetralin-1,4'piperidines].



13–17 (spiro)



18, 19 (4PP)

			Guinea pig brain IC_{50} *(nM)*[c]			*Mouse tail-flick* ED_{50} AD_{50} *μmol/kg (sc)*	
	R	*X*	*[³H]DHM*[d]	*[³H]DSLET*[d]	*[³H]U-69*[d]	*Agonism*	*Antagonism*
Spiro analogs							
13	H	5'-OH	710 ± 150	25 000 ± 7000	4500 ± 200	ND	ND
14	H	6'-OH	1600 ± 400	21 000 ± 1400	9500 ± 3000	ND	ND
15	H	7'-OH	4800 ± 1900	26 000 ± 8000	9500 ± 700	ND	ND
16	CH_3	5'-OH	2100 ± 600	31 500 ± 9000	3850 ± 500	ND	ND
17	Phenethyl	5'-OH	360 ± 220	6 500 ± 700	1100 ± 90	> 50[a]	>223[a]
4PP analogs[b]							
18	CH_3		37.5 ± 18	43 ± 9	1400 ± 800	2.8	> 80
19	Phenethyl		27 ± 8	25 ± 7	150 ± 90	2.1	> 71
Metazocine			12 ± 6	53 ± 20	26 ± 21		
Morphine			4.7 ± 0.3	150 ± 28	140 ± 60		

[a]When administered icv in 20% DMSO saturated solution, 50 μmol/kg produces 43% agonism and no measurable antagonism. [b]These are 4PPs from a previous study, included for comparison since they have a free 4-*n*-propyl substituent which has been cyclized in the corresponding spiro analogs (**16**, **17**). [c]Values shown are the average: SD for at least 2 experiments conducted in triplicate. [d]DHM-dihydromorphine; DSLET = Tyr-DSer-Gly-Phe-Leu-Thr; U-69-593 = (5a, 7a, 8β)-(–)-*N*-methyl-*N*-(7-(1-pyrrolidinyl)-1-oxaspirol(4.5)dec-8-yl) benzeneacetamide.

tation is restricted, there are 2 possible *meta*-positions (5', 7') on the phenyl ring and both compounds (**13**, **15**) were synthesized along with the *para* analog **14**. Previous spiro-piperidine analogs have been reported [8, 13–15] only with the equivalent of a 7'-hydroxy group, thus not addressing the relative importance of the two positions.

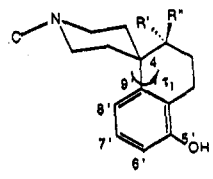
Receptor binding studies of these analogs to μ-, δ-, and κ-opioid receptors were carried out and *in vivo* analgesic agonist and antagonist activities were determined for the highest affinity analog. Energy conformational studies were carried out for analog **16** to help interpret the pharmacological results. Additional calculations were also made with the goal of identifying other spiro analogs for future synthesis which would have more promising pharmacological profiles. In particular, we investigated the conformational effects of introducing 1 and 2 methyls to the linking bridge leading to structures **20**, **21** (table II), as well as the effect caused by the shortening of the bridging propylene chain to ethylene, with and without methyl substitution, leading to structures **22** and **23** (table II).

Results

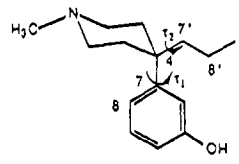
Chemistry

The synthetic procedures for the spiro compounds (scheme 1) were based on those used in our synthesis

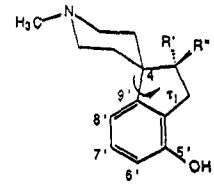
Table II. Calculated conformational energies of a series of spiro-[tetralin-1,4'-piperidines].



16, 20, 21



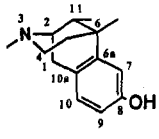
19, 19



22, 23

Analog	*Substituents* R	R''	*Conformation* Φ	τ_1	*ΔE kcal/mol*
16eq	H	H	eq	7°[a]	0.0
16ax	H	H	ax	17°[a]	2.1
18eq	H	H	eq	73°[b] (τ_2 = 122°)[c]	0.0
18ax	H	H	ax	− 78°[b] (τ_2 = 124°)[c]	− 0.6
20eq	H	CH_3	eq	7°[a]	0.0
20ax	H	CH_3	ax	18°[a]	0.8
21eq	CH_3	CH_3	eq	5°[a]	0.0
21ax	CH_3	CH_3	ax	22°[a]	− 2.1
22eq	H	H	eq	10°[a]	0.0
22ax	H	H	ax	15°[a]	1.7
23eq	CH_3	CH_3	eq	14°[a]	0.0
23ax	CH_3	CH_3	ax	20°[a]	− 0.5
Metazocine			ax	35°[d]	

a) τ_1 = $C_8C_9C_4N$
b) τ_1 = $C_8C_7C_4N$
c) τ_2 = $C_8C_7C_4N$
d) τ_1 = $C_{10a}C_{6a}C_6N$



of 4-alkyl-4-phenyl-piperidines [6, 17]. The olefins (**4–6**) resulting from the condensation of the various methoxy-1-tetralones and ethyl cyanoacetate smoothly underwent Michael addition by the lithium salt of ethyl acetate anion, leading to the diesters **7–9** as a mixture of diastereomers, resulting from the 2 chiral centers generated. Since subsequent reactions will eliminate these chiral centers, separations were unnecessary. The ester group activated by the α-nitrile was labile, and decarboxylation in the presence of anhydrous lithium iodide in DMSO was facile above 140°C. The resulting crude nitrile-ester was directly reduced to the corresponding amino-ester (H_2/PtO_2/HOAc), with lactamization occurring as the solvent was evaporated. The resulting racemic lactams **10–12** were crystalline and readily characterized. Diborane reduction and *O*-demethylation under standard conditions afforded the parent spiro-compounds **13–15** [18]. The HO-isomer most analogous to morphine is **13**. Since, as expected, **13** had the highest affinity of the three analogs, it was N-alkylated to give the *N*-methyl (**16**) and *N*-phenethyl (**17**) derivatives for comparison with the corresponding ring-open compounds **18** and **19** (table I).

Pharmacology

The affinities of the 5 new spiro analogs **13–17**, and, for comparison, the related 4-phenyl-piperidines (4PPs) **18** and **19** as well as metazocine and dihydromorphine (DHM) are given in table I. The values shown represent IC_{50} values in competititon with μ-selective (DHM), δ-selective (DSLET; [Tyr-DSer-Gly-Phe-Leu-Thr], and κ-selective (U69, 593) ligands

in guinea pig brain. As indicated in this table, the spiro-analogs **13–17** bind with 100–1000-fold lower affinity at μ-receptors than both the fused ring opioids or the related flexible 4PP opiate analogs **18** and **19**. They show no appreciable affinity at δ- or κ-receptors. In common with morphine and other fused-ring opiate families, but not with the 4PPs, **18** and **19**, the *N*-phenethyl analog **17** has enhanced affinity compared to its *N*-methyl analog **16** and was the highest affinity spiro analog obtained. The N-H analog **13** also had higher affinity than **16**. However, the highest affinity analog **17** had no significant *in vivo* analgesic agonist or antagonist activity by either subcutaneous or intracerebroventricular (icv) administration.

Energy conformational studies

Shown in table II are the calculated energy differences between phenyl–axial and phenyl–equatorial conformers for compound **16** and its unconstrained 4PP counterpart, **18**. Also given are the torsion angles for the lowest energy of each form.

In contrast to its unconstrained 4PP counterpart **18**, in the constrained analog **16** (fig 2b) the phenyl axial conformer is higher in energy by 2.1 kcal/mol than the phenyl equatorial conformer (fig 2a). We ascribe this destabilization of the axial conformer of **16** to a repulsive interaction between the *ortho*- (8)-aromatic hydrogen and the 2 axial piperidine protons (at C-2', C-6'; fig 2b), which is enforced by the spiro bridge. As can be seen in figure 3b, this interaction is avoided in the ring-open 4PP analog **18ax** by rotation of the phenyl group, making it lower energy than the **18eq** rotamer (fig 3a).

Both conformers of **16** have values of τ_1 close to zero and are thus qualitatively different from conformer **18eq**. Although the phenyl ring **18** is free to rotate, all other minima found with different τ_1 values for both **18eq** and **18ax**, had energies > 6 kcal/mol higher than the lowest energy forms given. Thus, a single rotamer with definitive value of $\tau_1 = \pm 73°$ was found.

These significant differences in conformational profiles suggest that the poor binding affinity of the spiro analogs (**13–17**) could be due either to the higher energy (2.1 kcal/mol) required to obtain an axial conformation compared to the ring-open analog **18** or, if the equatorial form binds to the receptor, to the different values of τ_1 of the spiro-analogs in its lowest energy phenyl equatorial form.

In order to further explore additional modifications that could alter the relative stabilities of the phenyl–axial and phenyl–equatorial conformation, we also calculated phenyl equatorial and phenyl axial energies and torsion angles for spiro analogs with methyl substitution on the bridge (**20**, **21**) and a shorter bridge length (**22**, **23**) as candidates for future synthesis.

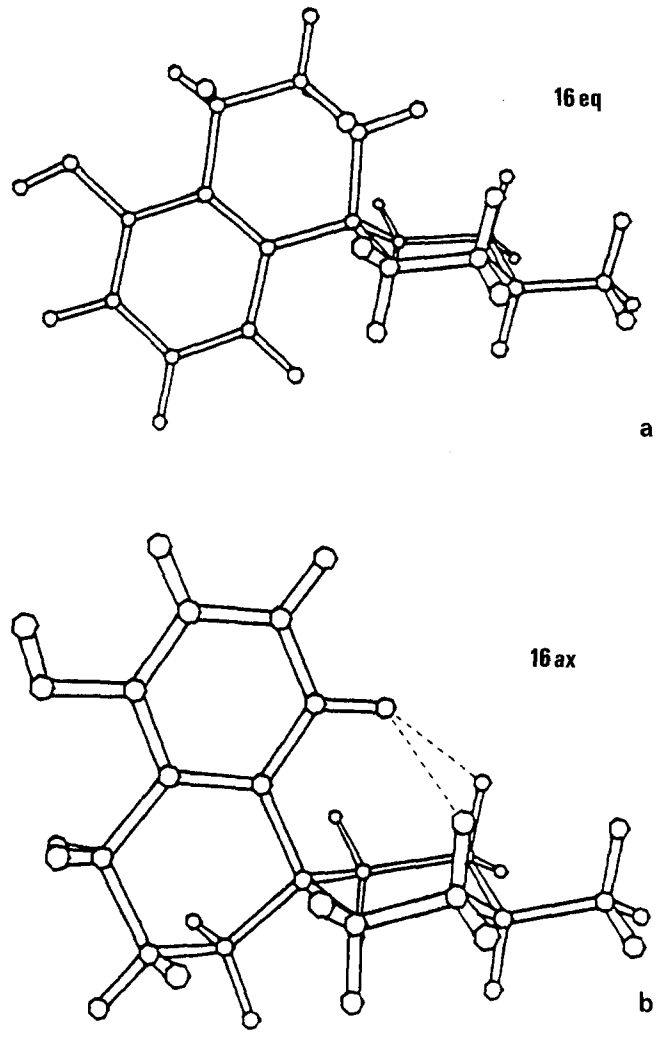


Fig 2. Optimized structures of (a) **16eq** and (b) **16ax**.

Table II gives the calculated energies of axial/equatorial conformers for the compounds **20–23**. Like the unsubstituted 6-membered ring, spiro structure **16**, the 5-membered analog **22** is also predicted to favor a phenyl equatorial conformation (by 1.7 kcal/mol). With one methyl group in the 2'-position of the propylene bridge, analog **20** is still calculated to favor a phenyl equatorial form, but by only 0.8 kcal/mol. Addition of 2 methyl groups at the 2'-position of the propylene bridge in analog **21**, reverses this and the phenyl axial form is favored by 2.1 kcal/mol.

Figure 4a, b shows why addition of 2 methyl groups at the 2' position of the propylene bridge in analog **21** has this effect. As shown in this figure, in the phenyl equatorial form, **21eq** (fig 4a) there is

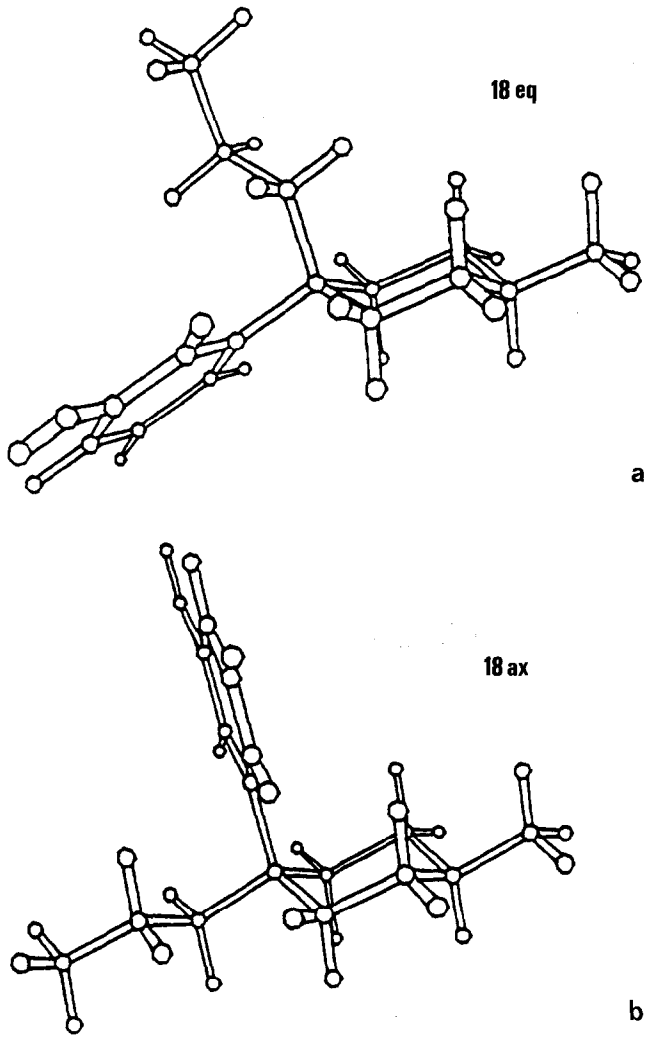


Fig 3. Optimized structures of (a) **18eq** and (b) **18ax**.

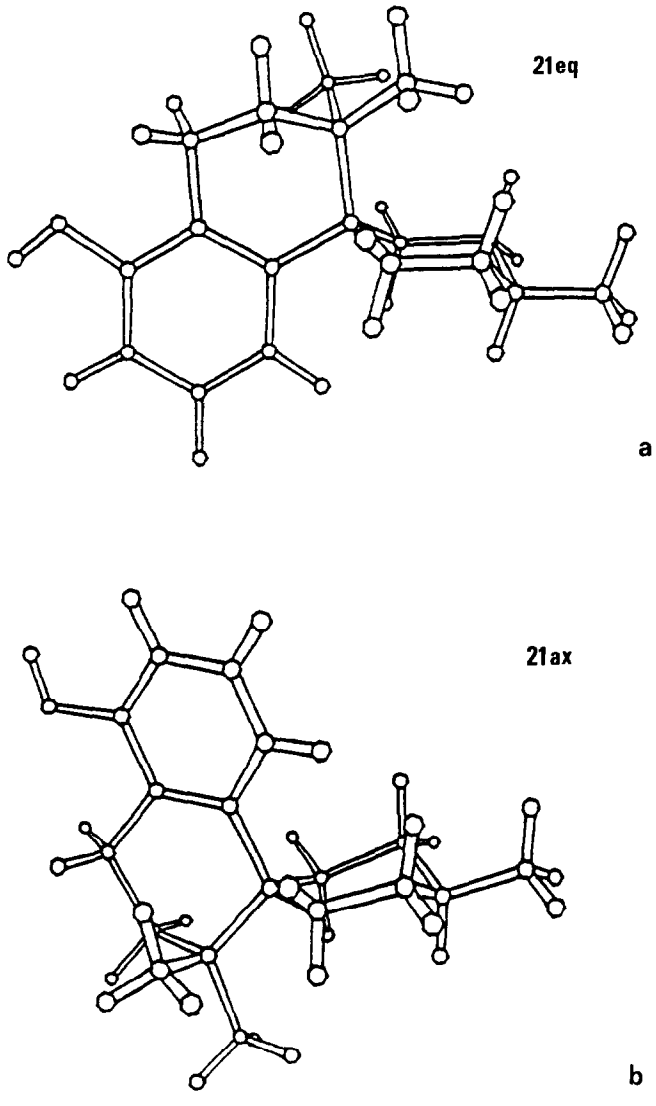


Fig 4. Optimized structures of (a) **21eq** and (b) **21ax**.

steric repulsion between the added methyl groups and the nearby axial hydrogen atoms of the piperidine ring. Consequently, the axial form, **21ax** (fig 4b), is lower in energy by 1.7 kcal/mol than the equatorial form.

Discussion

The results obtained for the binding affinities of the 5 spiro compounds synthesized (**13–17**, table I) show that all have greatly diminished affinities compared to both ring-open derivatives (**18, 19**) and the fused-ring opioids, morphine and metazocine. This poor affinity is in contrast to the high affinity of the 'ring-opened' analogs **18** and **19**. In a previous study [6] of a series of unconstrained 4PP including analogs **18** with varying 4-alkyl groups, the phenyl–axial conformer was found to be the preferred form increasingly favored as the size of this group increased. Since neither affinity nor efficacy were modulated by the energy required to attain the phenyl-equatorial form, the preferred phenyl–axial conformer was chosen as the bioactive form.

Unlike the 4-alkyl-4PP analogs **18** and **19** and fused-ring opioids, the spiro compounds **13–17** have an energetically favored phenyl equatorial conformer with the inter-ring torsion angle in these constrained

analogs forced to be near 0°. While there are many well-known examples of phenyl equatorial 4PP with high affinity at opioid receptor [7, 9, 10, 15–21], in all of these, the calculated torsion angle τ_1 of the preferred conformer is ≥ 30°. The torsion angle between the phenyl group and the piperidine in the phenyl equatorial analogs has been proposed as a crucial modulating property since it determines the position of the basic amine nitrogen with respect to the *m*-OH-phenyl, 2 groups thought to make important receptor contacts. Thus, there are 2 possible reasons for the lower affinity of the spiro compounds: they either bind poorly to the receptor in an unfavorable low energy equatorial conformers or in a more favorable but higher energy axial conformer. Both possibilities appear plausible.

As shown in figure 5, when the equatorial *m*-OH phenyl groups of the constrained spiro-analog **16eq** and the unconstrained 4PP analog **18eq** are overlapped, a dramatic effect of their torsion angle differences on the relative position of the protonated amine is apparent. The nitrogens do not overlap, nor are the N-protons directed at a common receptor site. Thus, if these compounds bind in their lowest energy form, it is possible that the lower affinity of **16** is due to this mismatch.

A similar effect of the torsion angle τ_1 of the phenyl ring on affinities has been reported in another phenyl equatorial piperidine family, the bridged 5-phenylmorphans [13]. One analog with a calculated τ_1 value of ≈ 4° (similar to that of **13–17**) showed a very poor affinity (IC_{50}, 1700 nM), while another analog with a phenyl torsion angle of $\tau \approx 82°$, similar to the value found for **18**, was reported to have a much higher affinity (IC_{50}, 96 nM) [9]. The same result has been found by the analysis of calculated and experimental values of methyl-substituted 4PPS [16]. These findings suggest that phenyl equatorial compounds with values τ_1 = 0–25° result in poor receptor affinities.

Comparison of the phenyl equatorial conformer of the spiro compound **16eq** with fused-ring opioids also reveals a mismatch. Shown in figure 6 is **16eq** and morphine in an orientation in which the phenol groups and the amine protons are overlapped. As a measure of similarity, the optimum root mean square (RMS) deviation from perfect overlap of designated pairs of equivalent atoms in each compound was obtained. While the RMS value is small (0.25) , both the piperidine ring and the N-substituent of **16eq** are significantly displaced from those in morphine. The poor affinity of the 5'-OH spiro-compounds compared to morphine could be due to the combined effect of these 2 differences. The relative displacement of the N-substituents is also consistent with the difference in how they modulate affinity in the 2 families. In the spiro-compounds, affinity of **13** (N-H) is enhanced over **16** (N-CH_3), an effect opposite to that observed in morphine-like fused ring opiates. Also, the enhanced affinity of the *N*-phenethyl compound (**17**) over the N-CH_3 (**16**) is similar, but less than that observed in fused-ring opioids. Thus, if the spiro-

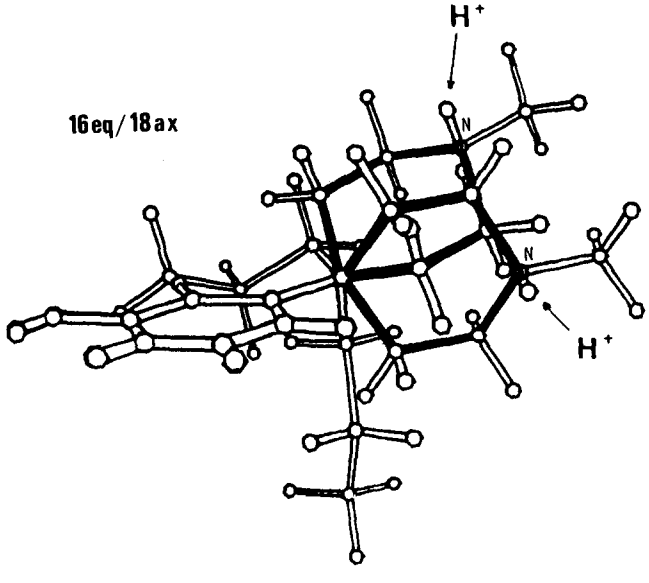


Fig 5. Overlapping of the phenyl rings of structures **16eq** and **18eq**.

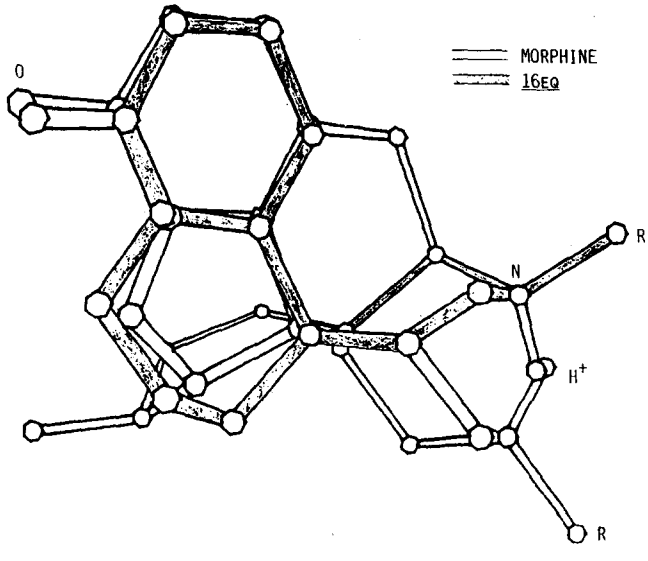


Fig 6. Overlapping the phenyl rings and N-protons of morphine and structure **16eq**. With the exception of N-protons, the hydrogens are omitted from the figure. RMS = 0.25 for 7 atom overlap (6 phenol ring carbons and proton on nitrogen).

compounds bind to the μ-receptor in their lowest energy phenyl equatorial form, their disparate pharmacological behavior, relative to both the 4PPs and fused-ring opioids, can be understood.

It is also possible, however, that these compounds bind to the receptor in the phenyl–axial conformer, the bioactive conformer of their unconstrained 4PP counterpart, **18**. However, the higher energy axial conformer **16ax** does not overlap well with the preferred 4-phenyl piperidine axial conformer **18ax**. There is, again, a disparity due to torsion angle differences (see figs 2b, 4b and table II). However, the overlap of **16ax** with metazocine is much better than with **18ax** as the torsion angles are much closer: τ_1 (**16ax**) = 17°, τ_1 (metazocine) = 35°. This suggests that the higher energy (+ 2.1 kcal) phenyl axial conformer could also be a candidate for binding to the μ-receptor in a fused ring pharmacophore. This possibility is further supported by the observation that the receptor affinity of the spiro-analogs diminishes in the order **13** > **14** > **15**, *ie*, 5'-OH > 6'-OH > 7'-OH, in a parallel fashion to the order found for the equivalent morphinan analogs (3-OH > 2-OH > 1-OH) [22]. The relatively poor affinities of the 5'-OH spiro-analogs could be explained as a result of the required energy (2.1 kcal) to reach the phenyl axial conformer.

Further resolution of whether the phenyl–axial or phenyl–equatorial form of these analogs binds to the receptor would be aided by the synthesis and evaluation of a variation of **16** which has a lower energy phenyl axial conformer, compared to the phenyl-equatorial. Our calculations suggest that one such analog is **21**, the bis-methylated analog of **16**, which favors the phenyl-axial conformer by 2.1 kcal/mol (table II). When **21ax** is overlapped with morphine (fig 7), there is not only a high degree of overlapping of the phenol ring (RMS = 0.185) but also the piperidine rings and the N-CH_3 groups are found close to each other.

Until these further experiments are carried out, there is no compelling evidence to support a role for the higher energy phenyl axial conformer in binding of these spiro compounds to the receptor. Thus, while this is a plausible alternative, the more conservative explanation at present for the poor μ-affinity of these spiro-compounds is the poor fit at the receptor for their lowest energy phenyl-equatorial conformers compared to both fused-ring and more flexible 4-phenyl piperidines.

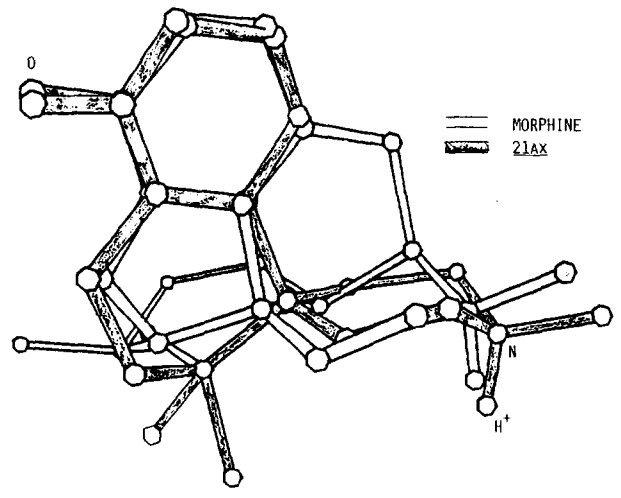


Fig 7. Overlapping the phenyl rings and N-protons of morphine and structure **21ax**. With the exception of the N-protons, the hydrogens are omitted from the figure.

Experimental protocols

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. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian EM-360 instrument. Chemical shift values are reported in parts per million (δ) relative to Me_4Si. Mass spectra (MS) were determined on an LKB 9000 spectrometer equipped with a gas chromatograph and a PDP12 computer. Analytical high pressure liquid chromatography (HPLC) 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.

*1-(1'-Cyano-1'-carboethoxy)methylene-5-methoxy-1,2,3,4-tetrahydronaphthalene (**4**)*

A mixture of 5-methoxy 1-tetralone (25.0 g, 0.142 mol), ammonium acetate (20 g, 0.26 mol), and ethyl cyanoacetate (20 g, 0.177 mol) in benzene (150 ml) was heated with stirring to reflux into a Dean–Stark trap. After 24 h aqueous distillate volume reached 17 ml. After an aqueous wash, the organic layer was separated, dried and distilled to remove unreacted 2 sm and other volatiles. The pot residues, the crude desired product as a mixture of isomers, was a yellow gum. It was carried on without further purification. Yield 19.1 g, 49%. ^{1}H-NMR ($CDCl_3$): δ 1.0–1.5 (q–overlapping triplets, 3H, CH_3CH_2), 3.85 (s, 3H, OCH_3), 4.0–4.5 (pentuplet-overlapping quartets, 2H, OCH_2CH_3), 6.7–7.3 (m, 2H, C6, C7 aromatic), 7.35 (d, 1/2H, C8 aromatic isomer A), 7.7 (d, 1/2H, C8 isomer B). Ratio isomer A: isomer B ≈ 3:4. M/S 271 (M^+).

*1-(1'-Cyano-1'-carboethoxy)methylene-6-methoxy-1,2,3,4-tetrahydronaphthalene (**5**)*

Under conditions identical to those used for the synthesis of **4** above, 6-methoxy-1-tetralone (2.4 g, 0.137 mol) was condensed with ethyl cyanoacetate to give the desired olefin **5** as a yellow gum. Yield 18.7 g, 50%. ^{1}H-NMR ($CDCl_3$): δ 1.1–1.5

(q–2 overlapping triplets, 3H, CH_2CH_3), 3.85 (s, 3H, OCH_3), 4.1–4.5 (m–2 quartets, 2H, OCH_2CH_3), 6.6–7.2 (m, 2H, C5, C7 aromatic), 7.4 (d, 1/3H, C8 aromatic isomer A), 8.2 (d, ≈ 2/3H, C8 aromatic isomer B). Ratio isomer A: isomer B ≈ 1:2. M/S 271 (M^+).

*1-(1'-Cyano-1'-carboethoxy)methylene-7-methoxy-1,2,3,4-tetrahydronaphthalene (**6**)*
As for **4** and **5** above, 7-methoxy-1-tetralone (25.2 g, 0.143 mol) was condensed with ethyl cyanoacetate to give **6** as a yellow gum. Yield 25.1 g, 64%. ^{1}H-NMR ($CDCl_3$): δ 1.1–1.5 (q–overlapping triplets, 3H, CH_2CH_3), 3.8 (s, 1/3 3H, OCH_3–isomer A), 3.9 (s, 2/3 3H, OCH_3–isomer B), 4.1–4.6 (m, 2H, OCH_2CH_3), 6.7–7.1 (m, 2H, C5, C6 aromatics), 7.15 (d, 2/3 1H, C8 aromatic isomer B), 7.75 (d, 1/3 1H, C8 aromatic, isomer A). Ratio isomer A:B ≈ 1:2. M/S 271 (M^+).

*5-Methoxy-1,2,3,4-tetrahydronaphthalene-1-acetic acid-1-(2'-cyano) acetic acid diethyl ester (**7**)*
To a THF solution of the lithium salt of ethyl acetate (prepared by the mixture of diisopropylamine (8.0 g, 0.079 mol), 10 *N*-butyl lithium (10 ml, 0.10 mol) at – 70°C followed by ethyl acetate (11.0 g, 0.125 mol) was added to a THF solution of **4** (16.2 g, 0.060 mol). TLC indicated that the addition to the olefin is complete in < 5 min. After quenching with 3 N HCl (aqueous) the organic layer was separated, dried, evaporated to give 20.0 g of a mixture of product isomers and sm isomers. This crude mixture was partially purified by preparative HPLC (dichloromethane (CH_2Cl_2)/ethyl acetate 75:1) on silica gel. Collected were 5.82 g of **7** and 5.2 g of recovered sm **4**. Yield **7**, 40% (based on recovered **4**). ^{1}H-NMR ($CDCl_3$): δ 1.15 (t, 6H, CH_2CH_3), 1.6–2.9 (m, 6H, $-CH_2CH_2CH_2-$), 3.05 (m, 2H, CH_2CO_2), 3.8 (s, 3H, OCH_3), 3.9–4.3 (m, 4H, CH_2CH_3), 4.6 (s, H, $NCCHCO_2$), 6.65–7.2 (m, 3H, arom). M/S 359 (M^+).

*6-Methoxy-1,2,3,4-tetrahydronaphthalene-1-acetic acid-1-(2'-cyano) acetic acid diethyl ester (**8**)*
Using the identical conditions as for **7** above, 0.14 mol of ethyl acetate anion was treated at – 70°C with **5** (18.7 g, 0.069 mol). After workup, the crude product (23.4 g) was purified by chromatography. 4.1 g of sm **5** and purified **8** (14.2 g) were recovered. Yield **8**, 73% (based on recovered **5**). ^{1}H-NMR ($CDCl_3$): δ 1.0–1.3 (t, 6H, CH_2CH_3), 1.7–2.8 (m, 6H, $-CH_2CH_2CH_2-$), 2.8–3.0 (m, 2H, CH_2CO_2), 3.66 (s, 3H, CH_3O), 3.9–4.3 (m, 4H, CH_2CH_3), 4.45 (s, H, $NCCHCO_2$), 6.5–7.5 (m, 3H, arom). M/S 359 (M^+).

*7-Methoxy-1,2,3,4-tetrahydronaphthalene-1-acetic acid-1-(2'-cyano) acetic acid diethyl ester (**9**)*
As for **7** above, a THF solution of **6** (25.0 g, 0.092 mol) was added to 0.15 mol ethyl acetate anion. After workup, 27.5 g crude oil was recovered. Following preparative HPLC, 8.0 g of **6** and 14.2 g of product **9** (isomers) were recovered. Yield **9** (based on recovered **6**) 63%. ^{1}H-NMR ($CDCl_3$): δ 1.0–1.3 (m, 6H, CH_2CH_3), 1.7–2.4 (m, 6H, $-CH_2CH_2CH_2-$), 3.0 (m, 2H, CH_2CO_2), 3.7 (s, 3H, OCH_3), 3.9–4.35 (m, 4H, CH_2CH_3), 4.55 (s, 1H, $NCCHCO_2$), 6.6–7.2 (m, 3H, arom). M/S 359 (M^+).

*Spiro-[5-methoxy-tetralin-1,4'-piperidin-3'-one] (**10**)*
A mixture of **7** (6.2 g, 0.017 mol) and anhydrous lithium iodide (LiI, 4.0 g, 0.03 mol) in dry dimethylsulfoxide (DMSO) was heated to 170°C for 30 min, until gas evolution ceased. The reaction mixture was left to cool, quenched in H_2O and product extracted into hexane/ethyl acetate 3:1, dried, filtered through a silica gel pad, and solvent evaporated to yield 4.9 g crude nitrile–ester (100%). Without further purification, this oil (4.7 g, 0.016 mol) was shaken in 75 ml acetic acid with platinum oxide (PtO_2 1.0 g, 0.0044 mol) in a Parr shaker under 21.3 kg of hydrogen for 24 h. Then the mixture was filtered and spin-evaporated to drive off volatiles. As pot temperature rose, lactamization occurred and further volatiles were observed to distil (ethanol, HOAc). The pot residue (3.7 g) was dissolved in ethanol to give pure **10** as white crystals, mp = 186–188°C. Yield 2.45 g (59%). ^{1}H-NMR ($CDCl_3$): δ 1.3–2.4 (m, 6H, CH_2), 2.5–2.9 (m, 4H, $-CH_2CO$, CH_2-Ar), 3.2–3.6 (m, 2H, CH_2N), 3.85 (s, 3H, CH_3O), 6.8 (d, 1H, C-6-*H*), 7.0–7.4 (m, 2H, arom), 7.15 (s, 1H, NH). M/S 245 (M^+). Anal $C_{15}H_{19}NO_2$ (C, H, N).

*Spiro-[6-methoxy-tetralin-1,4'-piperidin-2'-one] (**11**)*
Under similar conditions which afforded **10**, **8** (14.1 g, 0.039 mol) and LiI (7.0 g, 0.052 mol) were decarboxylated in DMSO, and the recovered crude nitrile (9.36 g, 83%) was reduced in HOAc with PtO_2 (2.0 g, 0.0088 mol). The resulting lactam **11** was crystallized from ethanol in several crops. Analytical sample of **11**, mp = 187–188°C. Yield: 3.68 g (38%). ^{1}H-NMR ($CDCl_3$): δ 1.3–2.4 (m, 6H, CH_2), 2.5 (m, 2H, CH_2CO), 2.6–2.9 (m, 2H, CH_2Ar), 3.2–3.6 (m, 2H, CH_3N), 3.75 (s, 3H, CH_3O), 6.66 (br s, H, C-5-H), 6.75 (d, 1H, C-7-H), 7.2 (d, 1H, C-8-H), 7.3 (s, 1H, N*H*). M/S 245 (M^+). Anal $C_{15}H_{19}NO_2$ (C, H, N).

*Spiro-[7-methoxy-tetralin-1,4'-piperidin-2'-one] (**12**)*
As for **11** above, **9** (14.1 g, 0.039 mol) was decarboxylated and reduced to give **12**. Yield: 4.7 g (49%). Analytical sample crystallized from ethanol/cyclohexane, mp = 154–155°C. ^{1}H-NMR ($CDCl_3$): δ 1.14–2.5 (m, 6H, CH_2), 2.5–3.0 (m, 4H, CH_2CO, CH_2Ar), 3.3–3.7 (m, 2H, CH_2N), 3.80 (3H, CH_3O), 6.8 (d, 1H, C-6-H), 6.9 (br s, 1H, C-8-H), 7.15 (d, 1H, C-5-H), 8.0 (s, 1H, NH). M/S 245 (M^+). Anal $C_{15}H_{19}NO_2$ (C, H, N).

*Spiro-[5-hydroxy-tetralin-1,4'-piperidine. HCl] (**13-HCl**)*
To a solution of **10** (2.39 g, 0.0098 mol) in THF (20 ml) was added 1 M diborane solution in THF (20 ml, 0.010 mol) and solution heated at reflux for 1 h, then worked up in the standard manner [4] to give the crude amine (1.86 g as HCl salt, 70%). 1.2 g (0.0045 mol) of this amine salt was immediately *O*-demethylated in dichloromethane (30 ml) at room temperature by the addition of 1 M BBr_3 (13 ml, 0.013 mol). Reaction was complete in 1 h. After routine workup, **13-HCl** was precipitated from ether as the HCl salt, mp = 310–312°C dec. Yield **13**, 0.84 g (73% from ether, 51% from **10**). ^{1}H-NMR ($CDCl_3$/DMSO–d_6): δ 1.5–3.5 (m, 14H, CH_2), 6.55 (d, 1H, C-6-H), 6.8–7.4 (m, 2H, arom). M/S 217 (M^+-HCl). Anal $C_{14}H_{20}ClNO$ (C, H, N).

*Spiro-[6-hydroxy-tetralin-1,4'-piperidine. HCl] (**14-HCl**)*
As above, **11** was reduced with diborane and *O*-demethylated with BBr_3. The salt **14-HCl** was isolated in overall yield from **11** of 53%, mp = 317–319°C dec. ^{1}H-NMR ($CDCl_3$/DMSO–d_6) similar to **13-HCl**, except in aromatic region: δ 6.55 (br s, 1H, C-5-H), 6.7 (d, 1H, C-7-H), 7.4 (d, 1H, C-8-H). M/S 217 (M^+-HCl). Anal $C_{14}H_{20}ClNO$ (C, H, N).

*Spiro-[7-hydroxy-tetralin-1,4'-piperidine. HCl] (**15-HCl**)*
As above, **12** was converted to 15 in overall yield of 50%, mp = 297–299°C dec. ^{1}H-NMR ($CDCl_3$/DMSO–d_6) similar to **13-HCl** except: δ 6.6–6.9 (m, 2H, C-6, C-8-H), 7.2 (d, 1H, C-5-H). M/S 217 (M^+-HCl). Anal $C_{14}H_{20}ClNO$ (C, H, N).

*Spiro-[5-hydroxy-tetralin-1'-methyl-1,4'-piperidine] (**16**)*
13 (0.314 g, 0.00124 mol) was *N*-methylated under Borch conditions [11] with formalin and $NaCNBH_3$. The free base crystallized from ethyl acetate as prisms, mp = 225–226°C. Yield 0.24 g (84%). ^{1}H-NMR ($CDCl_3$): δ 1.3–3.2 (m, 14H, CH_2), 2.5 (s, 3H, NCH_3), 6.7 (t, 1H, C-7-H), 7.1 (d, 2H, C-6, C-8-H), 7.9 (br s, 1H, OH). M/S 231 (M^+). Anal $C_{15}H_{21}NO$ (C, H, N).

*Spiro-[5-hydroxy-tetralin-1'-phenethyl-1,4'-piperidine] (**17**)*
A mixture of **13** (0.0308 g, 0.0012 mol) and $NaHCO_3$ (3.0 g, excess) in 2-butanone (10 ml) was treated with phenethyl bromide (0.33 g, 0.0018 mol) at reflux for 6 h. After purification on silica gel column (ethyl acetate/hexane 1:1), pure **17** was recovered as plates from ethanol/cyclohexane 1:1, mp = 183–184°C. Yield 0.231 g (60%). ^{1}H-NMR ($CDCl_3$): δ 1.3–3.3 (m, 18H, CH_2), 6.7 (t, 1H, C-7-H), 7.1 (d, 2H, C-6, C-8-H), 7.35 (s, 5H, arom), 7.8 (br s, 1H, OH). M/S 321 (M^+). Anal $C_{22}H_{27}NO$ (C, H, N). Synthesis of compounds **10** and **11** is described in [4].

Receptor binding

Receptor binding studies were conducted on brain membranes isolated from frozen guinea pig brains (Pel Freeze). Frozen brains were thawed, homogenized in a Polytron homogenizer (Brinkman), and centrifuged, in Tris buffer pH 7.7 at 40 000 *g*. The pellets were homogenized and centrifuged once more. The final pellets were homogenized and suspended in 150 ml Tris per g of tissue.

Binding incubations contained 12 mg tissue, the appropriate [^{3}H]ligand, and unlabeled compound in a total volume of 2.0 ml. [^{3}H]DHM, [^{3}H]DSLET, and [^{3}H]U-69, 593, at concentrations of ≈ 0.6, 1.5 and 1.2 nM were used to label μ, δ and κ receptors respectively. At these concentrations, these ligands bind virtually totally to single receptor sites. Non-specific binding was determined by using 1.0 nM of the unlabeled analog of the tritiated ligand. Incubations were for 1 h at 25°C, at which time samples were filtered over glass fiber filters. Filters were counted after sitting overnight in scintillation cocktail to extract the radioactivity.

In vivo evaluation study

For the subcutaneous (sc) evaluation of agonist activity, the compound was dissolved in 4% aqueous solution of ethanol and the analgesic evaluation used was the mouse tail-flick test described by D'Amour and Smith [23], Howes *et al* [24], and Li *et al* [25].

The protocol for determining the antagonist activity against 8 mg/kg of morphine sulfate (80% agonist effect of morphine in mice) was a modification of the tail-flick test reported by Harris *et al* [26, 27].

The analgesic activity of **17** was also investigated by applying the icv administration procedure described by Haley and McCormick [28]. All procedures used are described in more detail in the companion paper (IIIa).

Theoretical methods

All theoretical data reported in this study are based on molecular mechanics calculations using the program MOLMEC [29]. The geometries have been subject to a complete optimization of all variables. The partial charges for the Coulomb term were taken from MNDO [30] calculations using geometries which were optimized by MOLMEC without the charge term.

Calculations have been performed for the spiro compounds **16**, **20–23**, as shown in table II. For comparison, the results previously obtained for the related 4PP compound **18** with a free 4-*n*-propyl group are also shown in table II. In all these studies, the piperidine ring was kept in a chair conformation and the nitrogen atom was protonated, since it was assumed that this is the bioactive form of the opioids, *ie*, the form in which it binds to the receptor. This assumption is justified considering that the known pKa values of related 4-phenyl piperidines lie between 9.7–8.72 and also that interaction of the protonated amine with an anionic receptor site [1], is generally thought to be important. Initial geometries were constructed from standard bond lengths and angles for each moiety. For all 6 compounds, optimized geometries and energies have been computed for conformations with the phenyl group in an equatorial (**16eq**, **18eq–23eq**) and axial position (**16ax**, **18ax–23ax**).

For the 4PP structure, **18**, the rotational profiles of the *n*-propyl (τ_2) and *m*-OH-phenyl groups (τ_1) have been calculated. The most favorable axial rotamers for the phenyl axial and phenyl equatorial conformers are given in table II.

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