Eur J Med Chem (1991) 26, 763–773

Structure–activity studies of morphine fragments. II. Synthesis, opiate receptor binding, analgetic activity and conformational studies of 2-R-2(hydroxybenzyl)piperidines

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(Received 13 August 1990; accepted 11 March 1991)

Summary — In this study, a series of 2-benzyl piperidines, that can be regarded as flexible fragments of fused ring opioids, have been synthesized and their pharmacological and conformational profiles determined. These combined studies reveal that, despite the weak activity of the only analog previously reported, modifications of it can lead to compounds with significant opioid receptor affinity and analgetic activity. Conformational studies of these compounds indicate that they can bind to these receptors in either an phenyl-axial and phenyl-equatorial conformer. Features such as the position of the phenolic OH group, the nature of the other 2-substituent and of the N-substituent appear to modulate receptor recognition and activation. However, the 2-benzyl piperidines do not appear to bind or act at the opioid receptors in the same conformation or orientation as their more rigid fused ring counterparts, the benzomorphans. In general, a change from *p*-OH to *m*-OH benzyl analogs reduces efficacy and its is possible that the *m*-OH analogs could be promising analgesics with low physical dependence liability.

Résumé — Études structure–activité de fragments de la morphine. II. Synthèse, liaison au récepteur opiacé, activité analgésique et études conformationnelles de 2-R-2-(hydroxybenzyl)pipéridines. *Dans cette étude, une série de 2-benzyl pipéridines qui peuvent être considérées comme des fragments flexibles d'opioïdes à anneaux fusionnés ont été synthétisées et leurs profils pharmacologiques et conformationnels ont été déterminés. Ces études combinées révèlent que malgré la faible activité du seul analogue signalé jusqu'ici, des modifications de celui-ci peuvent conduire à des composés ayant une affinité considérable pour les récepteurs opioïdes* μ *et* κ *de même qu'une activité analgésique. Des études conformationnelles de ces composés indiquent qu'ils peuvent se lier à ces récepteurs dans une conformation soit à phényle axial, soit à phényle équatorial. Des propriétés telles que la position du groupe phénolique OH, la nature de l'autre substituant en 2 et du N-substituant semblent moduler la capacité de reconnaissance et l'activation du récepteur. Cependant les 2-benzylpipéridines se semblent pas se lier ou agir sur les récepteurs opioïdes dans la même conformation ou orientation que les composés correspondants plus rigides avec des cycles accolés, les benzomorphanes. En général, un changement d'analogues benzylés* p-*OH à* m-*OH réduit l'efficacité et il est possible que les analogues* m-*OH puissent être des analgésiques prometteurs avec une faible tendance à engendrer la dépendance physique.*

opioid analgesic / 2-benzyl piperidine / receptor binding / energy-conformational profile

Introduction

While morphine-like fused ring analogs are the prototypical opioid analgesics, a variety of studies has established that more flexible analogs, which can be regarded as fragments of morphine, can also act as potent opioids [1]. In recent studies, we have systematically examined 4-phenyl piperidines[2,3] (D and E of fig 1), as well as 3-phenyl-piperidines [4, 5] which preserve the phenethyl amine moiety of morphine. In this study, we present theoretical and experimental results on a third morphine fragment, the 2-benzyl-piperidines, F, in figure 1. These conformationally flexible opioids are in principle structurally related to the more rigid fused ring benzomorphans (fig 1), but the anchoring 4-phenyl-piperidine bond has been cleaved. The question we wish to address then is whether releasing this constraint is compatible with high affinity and initiation of analgesic activity at any opioid receptor site. Another example of detaching ring A from conformational restraint is a study of *t*-4a-aryldecahydroisoquinolines [6].

Only one previous study of 2-benzyl-piperidines as possible analgesics has been reported, in which weak activity for one compound **1** (table I) was found [7]. This result implies that the enhanced flexibility of

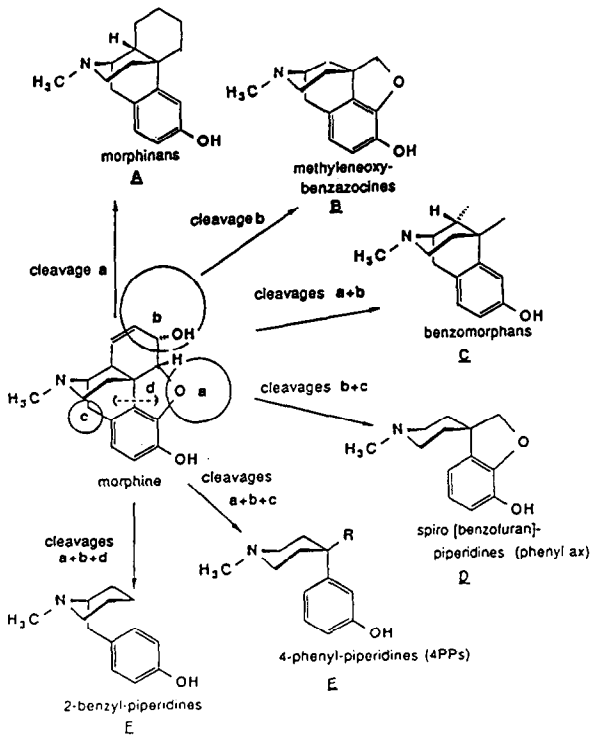


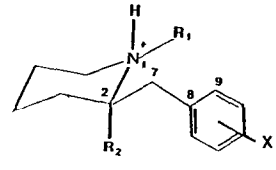
Fig 1. Schematic representation of the reduction of morphine into various substructures retaining opiate activity.

these compounds does not result in a conformer as favorable for recognition and activation as its more rigid fused ring counterpart. In the fused ring opiates, the 2-benzyl group is constrained in an axial position with the phenyl ring approximately perpendicular to the piperidine plane.

In this study, we address 2 related questions: the origin of the low activity of the known analog **1**; and whether it is possible to modulate its conformational profile to enhance recognition and activation at any opioid receptor. To this end, 3 types of substituent variation of **1** were considered.

In 1 type, a series of 2-benzyl piperidine derivatives with varying second substituents at the 2-position of increased bulk were were investigated including 2R = H, CH_3, *t*-butyl and a 2nd benzyl group (**1**, **2**, **8** and **4** in table I). This series was selected because of the possibility that variations in this position would modulate the relative energies of the benzyl-axial and benzyl-equatorial conformers. N-substituent variation from N-CH_3 to *N*-phenylethyl (±)**3** was also considered. Finally, we have considered whether modification of the *p*-OH benzyl analogs to an *m*-OH form would diminish efficacy and lead to *in vivo* anta-gonist activity, as had been observed for 4-phenyl and 3-phenyl piperidine compounds. To this end, the *m*-OH counterparts of (±)**1**, (±)**3**, and **4**, (±)**5**, (±)**6**, and **7** (table I) were prepared and examined.

Table I. Opioid receptor affinities of 2-benzyl-piperidine analogs studied.



Compound	R_1	R_2	*X*	*Binding (IC_{50} μM)*		
				[³H]DHM [b]	*[³H]DSLET* [b]	*[³H]U-69,593* [b]
(±)**1**	CH_3	H	*p*-OH	3.00 ± 0.8	7.10 ± 0.1	5.50 ± 0.7
(±)**2**	CH_3	CH_3	*p*-OH	3.65 ± 0.5	11.0 ± 0.1	5.50 ± 3.0
(±)**3**	Phenethyl	CH_3	*p*-OH	0.84 ± 0.4	0.74 ± 0.09	1.35 ± 0.5
(+)**3**	Phenethyl	CH_3	*p*-OH	0.33 ± 0.04	1.6 ± 0.6	1.20 ± 0.1
(−)**3**	Phenethyl	CH_3	*p*-OH	0.44 ± 0.2	0.95 ± 0.3	0.61 ± 0.3
4	CH_3	*p*-OH-benzyl	*p*-OH	0.51 ± 0.01	2.5 ± 1.0	6.85 ± 0.5
(±)**5**	CH_3	H	*m*-OH	1.20 ± 0.30	> 10	3.00 ± 0.1
(±)**6**	Phenethyl	CH_3	*m*-OH	0.21 ± 0.01	1.30 ± 0.1	0.29 ± 0.06
7	CH_3	*m*-OH-benzyl	*m*-OH	0.26 ± 0.01	0.80 ± 0.3	0.08 ± 0.02
8 [a]	CH_3	*t*-butyl	*p*-OH			
Morphine				0.003 ± 0.0002	0.20 ± 0.02	0.06 ± 0.002

[a]Not synthesized; computation only. [b]The concentration of radioligands used was [³H]DHM, 0.6 nM, [³H] DSLET, 1.5 nM and [³H] U69593, 1.2 nM.

Analogs **1–7** were synthesized and their receptor binding and *in vivo* activities determined. In addition, ±**3** was resolved in order to determine whether the well-known preference of 1 of the 2 enantiomers for binding at opiate receptor sites found in the morphine and benzomorphan families is also found in these less constrained 2-benzyl piperidine analogs. In addition to experimental characterization, the energy conformational profiles of these analogs were calculated in order to relate their structure to observed relative affinities and activities.

Methods

Chemistry

Synthesis

The 2-benzyl-piperidines **1–7** were prepared by 2 approaches. Method A (scheme 1) is the more general approach wherein an intermediate 2-cyano-2-benzyl analog (**9a** or **9b**) can be elaborated into various 2-substituted analogs through procedures which replace the 2-nitrile with H-, CH_3- or a second benzyl substituent: **1**, **2**, **4**, **5**, **7**.

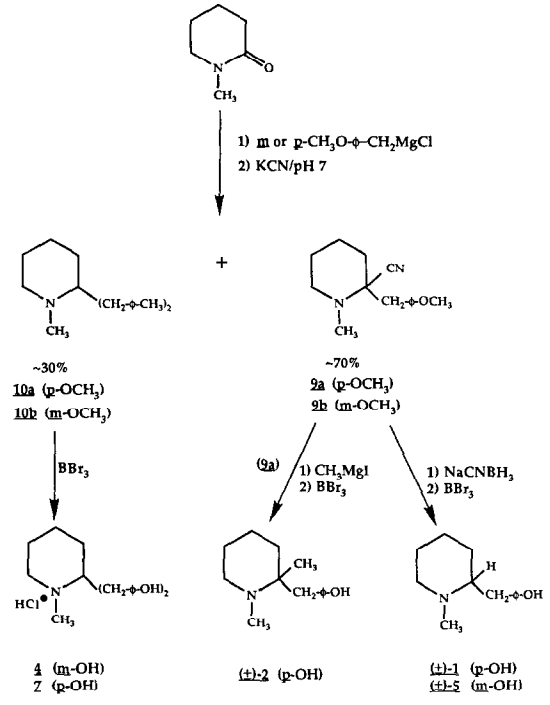
Method B (scheme 2) suffices for the situation where 2-CH_3-analogs are desired and only variation of the 2-benzyl-group is required: (±)**3**, (±)**6**.

Theoretical studies

Molecular mechanics calculations using MOLMEC [8], and employing MNDO [10] charges were carried out analogous to a previous study [2].

In all calculations, the piperidine ring was kept in a chair conformation and the nitrogen atom was protonated. Different conformations of the axial and equatorial 2-benzyl group due to rotations around the C_2-C_7 bond ($\tau 1$) and the C_7-C_8 bond (τ_2) (tables IV and V) have been systematically investigated for compounds **1**, **2**, **4** and **8**. The torsion angle τ_1 is defined by atoms $N_1C_2C_7C_8$ and values are given as the clockwise rotation of the $C_8C_7C_2$ plane into the $C_7C_2C_1$ plane. Simarly, τ_2 is defined by atoms $C_2C_7C_8C_9$ and values are given as the clockwise rotation of the $C_9C_8C_7$ plane into the $C_8C_7C_2$ plane.

Additional calculations were made for analog **3** in which the N-CH_3 group of compound **2** (2R = CH_3) was replaced by an *N*-phenylethyl moiety. In this study, the lowest energy equatorial conformer, **2a** (table IV) was the only conformer considered and all possible conformations of the *N*-phenylethyl substituent were calculated. In this manner, as shown in table VI, low energy forms were obtained for rotations around the N_1-C_{14} bound (τ_3), C_{14}-C_{15} bond (τ_4) and C_{15}-C_{16} bond (τ_5), where: $\tau_3 = C_2N_1C_{14}C_{15}$; $\tau_4 = N_1C_{14}C_{15}C_{16}$; $\tau_5 = C_{14}C_{15}C_{16}C_{17}$.

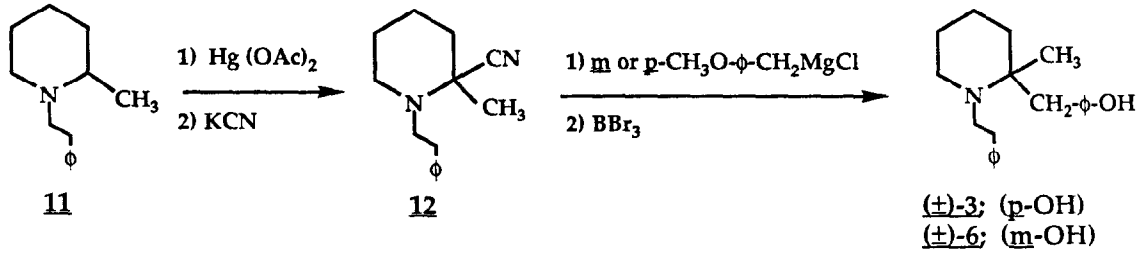


Scheme 1.

Pharmacology

Receptor binding

Receptor binding studies were conducted on guinea pig brain membranes isolated from whole guinea pig brains (Pel Freeze) using [^{3}H]DHM (dihydromorphine); [^{3}H]DSLET[Tyr-D-Ser-Gly-Phe-Leu-Thr] and [^{3}H]U-69, 593 [(5α, 7α, 8α)-(−)-*N*-methyl-N-[7-1-pyrrolidinyl)-1-oxaspiro(4,5)dec-8-4']benzeneacetamide.



Scheme 2

In vivo studies
Agonism and antagonism activity of the compounds under study was determined by the mouse tail-flick procedure. The effect of route of administration was investigated for some compounds by applying the intracerebroventricular (icv) administration procedure.

Results

Receptor binding

As shown in table I, analog **1** [(±)-2-(*p*-OH benzyl)-*N*-methyl-piperidine], reported to be inactive in early *in vivo* studies [5], binds very weakly to μ, δ and κ opiate receptors. Introduction of a methyl group at the 2-position of (±)**1** to give the analog (±)**2** made no significant difference in receptor selectivity or affinity. Only when the *N*-methyl group of (±)**2** was replaced by an *N*-phenethyl in (±)**3** was μ receptor affinity significantly enhanced. The 2 separated enantiomers of **3** had similar μ, δ and κ receptor affinities.

Substitution of a second 2-*p*-OH benzyl group into (±)**1** results in compound **4** which has 1 axial and 1 equatorial benzyl group. In this compound, affinity at μ is significantly increased compared to (±**1**) the 2-H, and (±**2**) the 2-CH_3 analog. Its affinity is comparable to that of the *N*-phenethyl analog (±)**3** and it is the most μ-selective compound made.

Compounds (±)**5**, (±)**6** and **7** are the *m*-OH isomers of (±)**1**, (±)**3** and **4** respectively. In each case, the change of the phenolic OH from a *para-* to a *meta-*position resulted in an increase in μ-affinity by a factor or 2, but an even greater increase in κ-affinity. This latter effect is particularly apparent for the 2 isomeric bis-benzyl compounds **4** and **7** in which a shift in the phenolic OH from a *p*-OH to a *m*-OH benzyl moiety leads to a 80-fold increase in κ affinity (from (6800 to 80 nM) and results in a change from a μ-selective to a κ-selective compound.

In vivo activity

The results of *in vivo* analgesic agonist and antagonist potency determinations by the mouse tail-flick procedure are summarized in table II.

The analogs with poorest affinities, (±)**1**, (±)**2** had no appreciable *in vivo* activity. Analog (±**3**) in which an *N*-phenylethyl is substituted for N-CH_3 of (±**2**) had a dramatically increased agonist activity with half the potency of morphine and is the most potent analog made. Interestingly, while further modifications increased affinity at the μ-receptor, all led to diminished activity, *ie*, compounds with lower apparent efficacy. In order to minimize differential effect of transport to the receptor site, the agonist activity of the analogs ±**3**, **4** ±**6** and **7** were also determined by an icv route of administration. Again, analog ±**3** was the most potent, 1/6 that of morphine.

If we define the apparent efficacy of each analog (i) relative to morphine as:

$$\text{Eff}_i = \frac{[(IC_{50}/ED_{50})\text{icv}]\ \text{compound i}}{[(IC_{50}/ED_{50})\text{icv}]\ \text{morphine}}$$

the resulting values are shown in table III. We see from this table that while analog ±**3** has a lower affinity and lower icv activity than morphine, it has a higher apparent efficacy. This could be due to a greater ability to activate the μ-receptor or to a

Table II. *In vivo* analgesic agonism and antagonism of 2-benzyl piperidines.

Compound	*Agonism* ED_{50} *(95% confidence limits) (μmol/kg)*		*Antagonism* [a] ED_{50} *(95% confidence limits) (μmol/kg, sc)*
	sc	*icv*	
(±)**1**	> 331[b] (42%)	–	NT
(±)**2**	> 313 (20%)	–	NT
(±)**3**	5.6 (3.0–10.1)	0.24 (0.07–0.85)	> 14[c,d] (0%)
4	> 230 (38%)	> 11.5[b,d] (16%)	> 230[c] (21%)
(±)**5**	NT	NT	NT
(±)**6**	24.7 (11.2–54.4)	0.86 (0.24–3.08)	> 29[c,d] (0%)
7	146 (60–355)	5.74 (3.25–12.03)	> 230[c] (0%)
Morphine	2.95 (1.84–4.72)	0.06	–
Nalorphine	828 (548–250)	–	2.01 (1.4–2.9)
Naloxone	–	–	0.2 (0.1–0.4)

[a]Antagonism of mouse tail-flick inhibition induced by 21.08 mol/kg (sc) of morphine administered immediately after test drug. [b]The highest dose evaluated and the percent agonism at that dose. [c]The highest dose evaluated and the percent antagonism at that dose. [d]Higher doses produced or convulsions. NT = not tested.

Table III. μ-Opioid receptor efficacies of ith analog relative to morphine derived from: $Eff_i = \frac{[IC_{50}/ED_{50}icv]i}{[IC_{50}/ED_{50}icv]morphine}$.

Analog	*Agonism* ED_{50} *(μ)mol/kg*		*Affinity (μ)*[c] IC_{50} *(nM)*	*'Efficacy' (relative to morphine)*	
	sc	*icv*		*sc*	*icv*
(±)**1**[a]	> 331	–	3000	≤ 0.1	–
(±)**2**[a]	> 313	–	3650	≤ 0.1	–
(±)**3**[a]	5.6	0.24	840	140	70
4[a]	> 230	> 23	440	≤ 2	≤ 2
Morphine	3	0.06	3	1	1
(±)**5**[b]	–	–	1200	–	–
(±)**6**[b]	25	0.86	210	≈ 0.1	0.4
(±)**7**[b]	146	5.74	230	≈ 2.0	0.6

[a]*p*-OH compounds; [b]*m*-OH compounds, [c]The concentration of [^{3}H]DHM was 0.6 nM.

residual advantage in transport to the receptor site. Comparing ±**3** with its *m*-OH analog, ±**6** (table III), we see that the latter has greatly reduced 'efficacy'; *ie*, higher μ-receptor affinity and weaker agonist activity, both sc and icv, than ±**3**. Since these compounds are isomers, this relative effect is unlikely to be due to transport or metabolism and its a reflection of important differences in receptor interactions leading to higher efficacy for the *p*-OH analog (±**3**) and lower efficacy for the *m*-OH analog (±**6**). This effect is similar to the pattern observed for 3-phenyl and 4-phenyl piperidines [1]. However, even analogs such as ±**6** and **7** with lower efficacy than morphine failed to demonstrate antagonism to morphine when they were injected subcutaneoulsy immediately prior to subcutaneous administration of morphine. To verify that (±)**3** was eliciting its analgesic activity through opiate receptors, we determined the ability of naloxone to inhibit its activity. Naloxone was found to fully antagonize the analgesic activity of (±)**3** with a median effective antagonist dose (AD_{50}) of 0.07 μmol/kg (0.025–0.198).

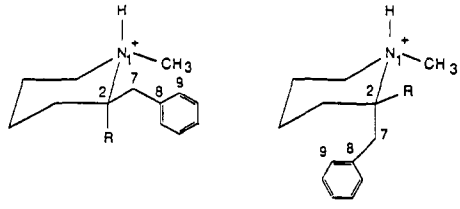
Theoretical studies

In the 2-benzyl piperidines studied, the phenyl group can be at each of 3 positions of the benzyl carbon atom C_7, resulting in 3 possible rotamers of this group. For each of these rotamers, systematic rotation of the benzyl group around the C_2-C_7 bond (τ_1) was performed and yielded 3 distinct local minima for the equatorial (a, b, c) and for the axial (a', b', c') position of the benzyl group. The resulting 6 conformation and relative energies obtained for them for analogs **1**, **2** and **8** are shown in table IV, each with optimized values for rotation about τ_1 and τ_2. Additional conformational minima were found with different values for rotation around τ_2, the C_7-C_8 bond of the benzyl group, but were much higher in energy than any of those reported. As shown in table IV, for analogs **1** and **2**, all equatorial conformers are comparable in energy and are lower than the benzyl axial ones. Structure **1c'** is the highest energy structure because of steric interaction between the phenyl and the piperidine rings. However, for both analogs **1** and **2** there is a very small energy difference between the lowest energy axial and equatorial conformers and the benzyl group can be axial or equatorial at room temperature. The benzyl group is also rather flexible in regard to rotation around the C_2-C_7 bound (τ_1).

Flexibility is dramatically reduced by the bulky 2-*t*-butyl group in analog **8** in which a single axial conformer **8c'** is the definitive lowest energy form, more than 4 kcal/mol lower than the next low-lying form. For the 3 rotamers, with the benzyl group in the axial position **8a'**–**8c'** steric repulsion between the *t*-butyl group and the 2-benzyl group is more important than the steric interaction with the piperidine ring. Thus, conformer **8c'** is favored, due to reduced steric repulsion between the benzyl and *t*-butyl group.

In the compound with 2 benzyl groups, 1 axial and 1 equatorial, nine rotamers are possible. As shown in table V, there are five 2,2-bis-benzyl conformations within 4 kcal/mol. Low energy conformations are found where steric repulsions both between the 2 benzyl groups and between each benzyl substituent and the piperidine ring is minimized. This effect is optimally achieved in conformer **4aa'**. The 3 lowest energy forms (**4aa'**, **4ab'** and **4ac'**) which differ only in the torsion angle of the axial benzyl group are shown in figures 2a–c. In one of these conformers (**4ac'**), the axial benzyl group is in the same position as one of the low energy forms of the 2-*t*-butyl compound **8c'**.

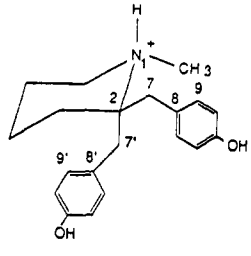
Table IV. Relative energies and conformations of 2-benzylpiperidines. τ_1 = N_1-C_2-C_7-C_8 in degrees; τ_2 = C_2-C_7-C_8-C_9 in degrees.



		-$CH_2\phi eq$				*-$CH_2\phi ax$* [a]		
		τ_1	*τ_2*	*ΔE (kcal/mol)*		*τ_1*	*τ_2*	*ΔE (kcal/mol)*
1 (R = H)	a	− 42	− 55	0.13	a'	+178	−105	1.77
	b	− 82	−115	0.34	b'	− 67	− 82	2.14
	c	+204	− 92	0.00	c'	+ 59	−135	4.21
2 (R = CH_3)	a	− 44	− 61	0.00	a'	+181	− 97	1.24
	b	+ 75	−105	1.77	b'	− 65	− 89	2.57
	c	−197	−102	1.18	c'	+ 62	−137	2.97
8 (R = *t*-bu)	a	− 45	− 60	4.70	a'	+186	− 72	12.26
	b	+ 77	− 92	23.20	b'	− 57	− 97	4.30
	c	+194	− 85	10.76	c'	+ 65	−138	0.00

[a]In benzomorphan ϕ is axial and $\tau_1 = 72°$, $\tau_2 = -20°$.

Table V. Relative energies and conformations of bis-(*p*-OH-benzyl)piperidine **4**. $\tau_1(°) = N_1C_2C_7C_8$; $\tau_2(°) = C_2C_7C_8C_9$; $\tau_1'(°) = N_1C_2C_7'C_8'$; $\tau_2'(°) = C_2C_7C_8'C_9'$.



Conformers	*-$CH_2\phi eq$*			*-$CH_2\phi ax$*	
	ΔE [a]	*τ_1*	*τ_2*	*τ_1'*	*τ_2'*
(a,a')	0.00	− 44	− 57	+180	−101
(a,c')	2.10	− 45	− 57	+ 60	−135
(a,b')	2.93	− 45	− 54	− 63	− 85
(c,c')	3.29	+185	− 95	+ 75	−114
(b,c')	3.62	+ 64	−103	+ 62	−129
(c,a')	4.60	+165	−145	+172	− 62
(b,a')	7.29	+ 49	−107	+172	− 64
(c,b')	9.13	+159	−147	+169	− 67
(b,b')	10.02	− 59	−100	− 55	− 93

[a]kcal/mol relative to conformer (a,a').

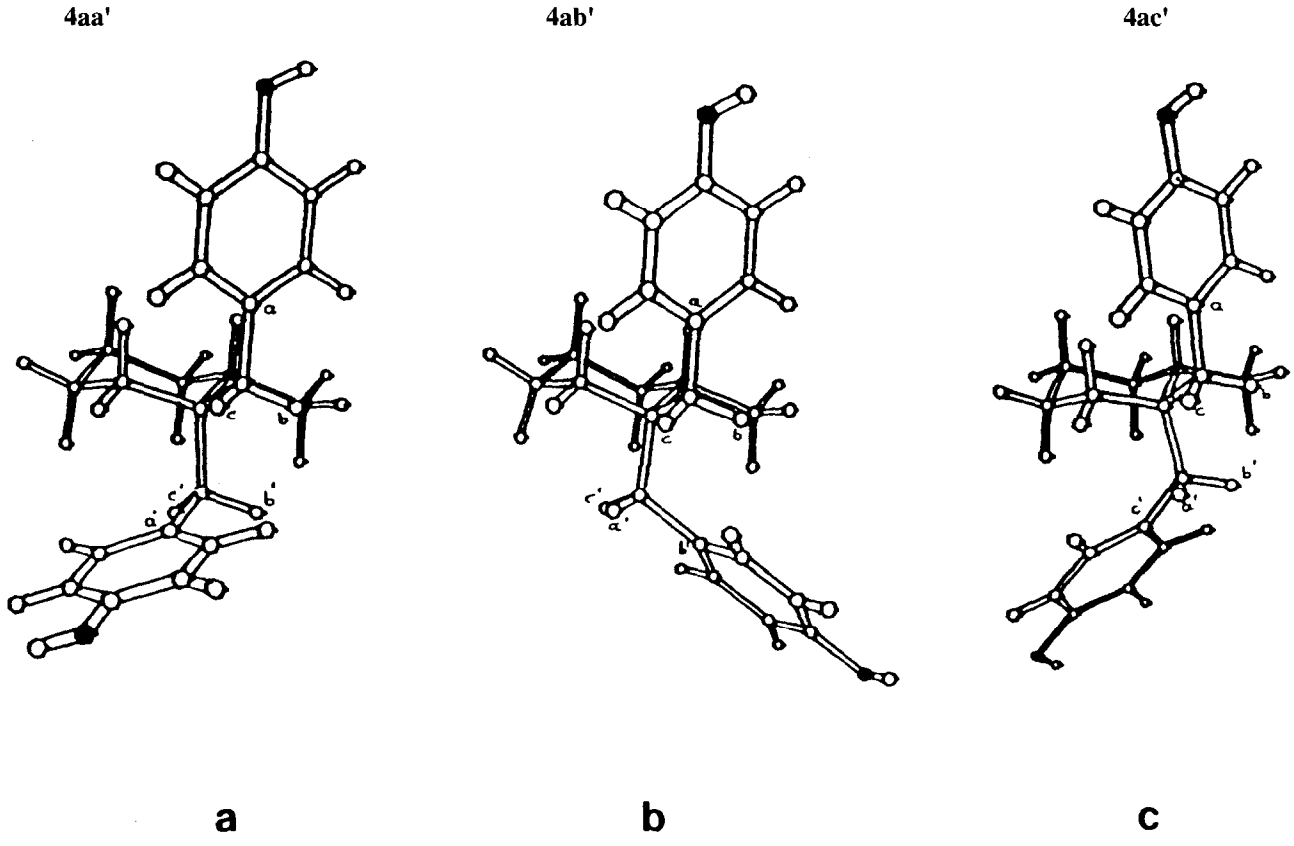


Fig 2. Three lowest energy forms of 2,2'-bis-(*p*-OH-benzyl)-piperidine (**4**). In each of these, the benzyl substituent that is equatorial has the same conformation while the one that is axial is in each of the 3 possible orientations.

We also investigated the effect of substitution of an *N*-phenethyl for an N-CH_3 group on the lowest energy conformer, **2a** of analog **2**. Using values of τ_1 and τ_2, found in **2a** for the 2-benzyl substituent, the *N*-phenethyl rotational profile of analog **3** was investigated with results shown in table VI. It is clear that the *N*-phenethyl substituent does not significantly interfere with the lowest energy conformer of the 2-benzyl piperidine moiety; τ_1 and τ_2 values are very similar to the *N*-methyl derivative **2a**. Within this most favorable 2-benzyl equatorial conformation, there are 3 favorable *N*-phenethyl conformers, each with a value of $\tau_3 \approx 180°$.

Discussion

In the only reported study [7], the 2-benzyl piperidine analog **1** was found to be a very weak opioid analgesic. No previous investigations were made of its binding affinity at any receptor subtype or of whether variations in it would improve its pharmacological profile. In this study, we have found that substituent variations in this 2-benzyl piperidine lead to analogs with significant affinity and varying selectivity at the μ and κ opioid receptors.

In addition, energy conformational studies of these analogs clearly show that in no low energy forms are they similar to their fused ring counterparts, the benzomorphans (fig 1). To achieve a benzomorphan-like conformation, the benzyl ring in these analogs must: (i), be in an axial position; (ii), adopt the a' or c' position of 3 rotamers, with respect to rotations around the C_2-C_7 bond; and (iii), have torsion angle values of $\tau_1 = 72°$ and $\tau_2 = 20°$. Conditions (i) and (ii) are fulfilled to some extent in all of the analogs studied, since all have a low energy axial conformation of the a' or c' type. However, condition (iii), *ie*, rotation of the axial phenyl group into the position found for benzomorphan, is not energetically accessible in any of them. In this conformer, these are strong steric repulsion between the hydrogen at the

meta-position of the phenyl ring, and the axial hydrogen at the 4-position of the pyridine ring. In fact, calculations with constrained optimization and rotation of the axial-phenyl group to this position in analog **1**, **2** and **8** yielded a conformational change of the piperidine ring, rather than a benzomorphan-like conformation. Thus, it is not possible for any of the compounds **1**–**8** to adopt a complete benzomorphan-like conformation. Similarly, for compound **4**, where, of the 2 benzyl groups, 1 is axial and 1 equatorial to the piperidine ring, constrained optimization showed a large energy requirement (> 30 kcal/mol), to adopt a benzomorphan-like conformation when condition (iii) was imposed on the axial benzyl group of **4**. These disparities with benzomorphan conformation could account for the rather low binding affinities of these compounds at the opiate receptor sites.

There are other qualitative differences in modulation of affinities and activities of analogs **1**–**7**, which further suggest that they bind at the receptors in different pharmacophores than benzomorphan. The observation that the *m*-OH analogs **5**, **6** and **7** had higher affinities than the *p*-OH analogs **1**, **3**, **4** would also seem to be more evidence against a benzomorphan-like interaction at the opiate receptor, since the analogous transposition of the 2'-OH to 1'- or 3'- position has been shown to dramatically reduce opiate affinity in the morphinans [9], a related fused-ring opioid. Finally, the nearly identical μ, δ and κ affinities of the enantiomers (+)**3** *vs* (–)**3** is another indication that these 2-benzyl-piperidines are not interacting in a benzomorphan-like mode, since benzomorphan optical antipodes have dramatic affinity differences.

The question remains in what pharmacophore are these analogs interacting at the opioid receptor. All analogs studied have low energy benzyl axial and equatorial forms either of which are available for binding to the receptor. Moreover, the 2,2-bis-benzyl compounds, **4** and **7**, with both axial and equatorial substituents, bind with higher affinity than their single benzyl counterpart (**2**). These results taken together clearly indicate that both axial and equatorial conformers can be accommodated at the receptor site. Moreover, very similar equatorial and axial conformers are accessible to all analogs. In all analogs, the preferred conformation for an equatorial 2-benzyl group is conformer a and for an axial 2-benzyl group is conformer a' or c'. For example, for the monosubstituted analogs, **1**, **2** and **3**, the 2-benzyl group in conformer a is the low energy equatorial position and conformer a' in an axial position (fig 2). In the 2-*t*-butyl analog **8** in which the 2-benzyl group is frozen in an axial position, it is in the c' conformation. Finally, in the 2,2-bis-benzyl analogs **4** and **7**, with both axial and equatorial groups, the 2 low energy conformers are aa' and ac'. Thus, the conformation of the 2-benzyl group in either an axial or equatorial position does not seem to be a primary determinant of receptor recognition or activation. This result would be further tested by synthesis and evaluation of analog **8**, which is frozen in a 2-benzyl axial conformation.

The results thus far point to 3 other modulators of receptor recognition and activation by the 2-benzyl piperidines. These are the different orientations and specific interactions associated with N-substituent variation, changes in the position of the phenol OH and addition of a second benzyl group. The *N*-phenethyl analog enhances affinity and greatly increases efficacy. This modulation by change in the N-substituent provides added evidence to that gathered over the years, that the interaction of the protonated amine group and its substituents with the receptor is central to the activation mechanism. The decrease in efficacy from the *p*-OH to *m*-OH analogs with the *N*-phenethyl group is an indication that symbiosis between the polar groups and amino group–receptor interaction modulates this activation. Further evidence for such symbiosis is that without the *N*-phenyl group to direct the compound to efficacious amine-group interaction with the receptor, changes from *p*-OH to *m*-OH (**4** to **7** and **1** to **5**) has little effect on activation.

In summary, these studies have shown that in contrast to the inactivity of the first analog reported, there is nothing in the 2-benzyl piperidine structure itself to prevent such compounds from having significant affinity at the μ and κ opioid receptors or *in vivo* analgesic activity.

We have, in fact, identified new analogs with moderate affinity and varying selectivity at these two receptors. Since simultaneous recognition of both μ or κ has been associated with lower physical dependance liability and reinforcement in monkey self-administration studies [8], it is possible that the *m*-OH analogs in particular could be clinically promising analgesics.

We have also determined that the 2-benzyl piperidines do not bind and act at the opioid receptors in the same conformation or orientation as their more rigid fused ring counterparts, the benzomorphans. Moreover, both axial and equatorial 2-benzyl conformers can be accommodated and there is no evidence thus far that either is preferred. Other variations among these analogs appear to be more significant modulators of recognition and activation.

The overall effect of substituents in the 2-benzyl piperidine series considered here has some general similarities with the other flexible series studied. In general, a change from *p*-OH to *m*-OH analogs in both this family and the 4-phenyl piperidine reduces efficacy. Also, when there are large substituents at several positions, for example, the *N*-phenethyl substituent and 2-substituent – the competitive directing effects of these groups appear to significantly modulate both affinity and efficacy.

Work is in progress to explicitly model opiate receptor binding sites using rigid opiates as initial templates. Preliminary results already show striking evidence for the effect of polar oxygen group–receptor site interaction on the amine–receptor interactions [16]. It is hoped that these studies together with continued experimental work will lead to an explicit model of each receptor type which is consistent with and can explain the more enigmatic results of families of flexible opioids.

Experimental protocols

Chemistry

All reactions were performed under an argon atmosphere, and solvents were removed on a rotary evaporator under vacuum. Melting points were taken in capillaries on a Mel-Temp apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra recorded on Varian 400 MHz and EM-360 instruments. 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 Radial-pak 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.

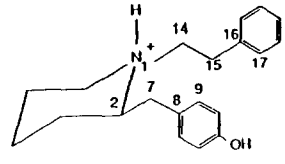
Method A–Scheme 1

N-Methyl-2-cyano-2-(p-methoxy-benzyl)-piperidine ***9a***
A solution of 4-CH_3O-φCH_2MgCl (≈ 0.03 mol) in 250 ml ether was prepared from 4-CH_3O-φCH_2Cl (18.5 g, 0.118 mol) and magnesium powder (6.0 g, 0.25 mol). To this solution was added *N*-methyl-2-piperidone (4.52 g, 0.04 mol, 33% excess) in 60 ml THF at room temperature and the mixture was stirred at reflux for 2 h, then quenched in 2 N HCl, extracted with hexane/ether 1:1 to remove non-basic by-products of Grignard formation. Then the aqueous layer was adjusted to pH 7 with $NaHCO_3$ and KCN (2.6 g, 0.04 mol) was added to trap the intermediate eneamine as the nitrile **9a**. The aqueous mixture was extracted with dichloromethane and the crude product as a mixture of **9a** and the bis-adduct **10a** was separated and purified by chromatograph (silica gel/ethyl acetate: triethylamine 99:1) **9a** was eluted first (R_f = 0.8) and isolated as a pale yellow oil. Yield **9a** 3.62 g (49%). ^{1}H-NMR ($CDCl_3$); δ 1.2–1.9 (m, 6H, $CH_2CH_2CH_2$), 2.45 (s, 3H, NCH_3), 2.7, 3.3 (2 doublets, 2H, CH_2Ar), 2.7–2.9 (m, 2H, CH_2N), 3.80 (s, 3H, OCH_3), 6.9 (d, 2H, arom), 7.3 (d, 2H, arom). M/S (DCI) 245 (M+1). Anal $C_{15}H_{20}N_2O$.

N-Methyl-2-cyano-2-(m-methoxy-benzyl)-piperidine ***9b***
As for **9a**, Grignard prepared from 3-CH_3O-φCH_2Cl (14.8 g, 0.094 mol) and magnesium powder (6.0 g, 0.25 mol) was treated with a THF solution of *N*-methyl-2-piperidone (4.52 g, 0.04 mol). After 2 h at reflux, reaction was quenched and the eneamine intermediate trapped by KCN addition to give the desired nitrile **9b** along with by-product **10b**. These were separated by chromatography and **9b** recovered as pale yellow oil. Yield 4.82 g (49%). ^{1}H-NMR ($CDCl_3$): δ 1.2–2.0 (m, 6H, $CH_2CH_2CH_2$), 2.47 (s, 2H, NCH_3), 2.5–3.0 (m, 2H, CH_2N), 2.7, 3.4 (2 doublets, 2H, CH_2Ar), 3.80 (s, 3H, OCH_3), 6.8–7.5 (m, 4H, arom). M/S (DCI) 245 (M+1). Anal $C_{15}H_{20}N_2O$.

N-Methyl-2,2-bis-(p-methoxy-benzyl)-piperidine ***10a***
From the chromatographic purification of **9a** a second major product was eluted (R_f = 0.5) which was the bis-benzyl adduct **10a**, a yellow oil. Yield 3.05 g (30%). ^{1}H-NMR ($CDCl_3$): δ 1.0–1.7 (m, 6H, $CH_2CH_2CH_2$), 2,3, 3.05 (2 d, 4H, CH_2Ar), 2.45 (s, 3H, NCH_3), 2.5–2.7 (m, 2H, CH_2N), 3.65 (s, 3H, OCH_3), 6.8 (d, 4H, arom), 7.15 (d, 4H, arom). M/S 339 (M+).

Table VI. Energy conformational profile of analog **3**.



Conformers	*ΔE* [a]	*(τ1, τ2)* [b]	*τ3(C2N1C14C15)*	*τ4(N1C14C15C16)*	*τ5(C14C15C16C17)*
1	0.0	(–47, –63)	181	297	95
2	1.8	(–42, –64)	175	191	94
3	2.4	(–40, –69)	184	70	67
4	6.3	(–41, –61)	–61	173	90
5	7.3	(–55, –72)	64	48	40
6	7.5	(–47, –65)	60	161	86
7	7.9	(–44, –62)	–57	286	123
8	8.6	(–43, –61)	–64	81	64

[a]kcal/mol, relative to lowest energy conformer found. [b]Initial τ_1 and τ_2 values for all conformers were taken from conformer 2a, the lowest energy conformer of **2**. ($\tau_1 = N_1C_2C_7C_8 = -44°$) ($\tau_2 = C_2C_7C_8C_9 = -61°$).

N-Methyl-2,2-bis-(p-OH-benzyl)piperidine·HCl **7**
Treatment of **11a** (0.61 g, 0.0018 mol) in dichloromethane with 1 m BBr_3 (6 ml, 0.006 mol) yielded, after standard workup, crude product as free base (0.45 g). This was precipitated from ethanol/ether (1:9) by the addition of HCl to afford **7** as a white powder, mp = 144–146°C. Yield 0.483 g (77%). ^{1}H-NMR ($CDCl_3/CD_3OD$ 1:1): δ 1.5–2.1 (m, 6H, $CH_2CH_2CH_2$), 2.62, 2.90, 3.25, 3.40 (4 doublets, 4H, CH_2Ar), 3.00 (s, 3H, NCH_3), 3.2, 3.4 (m, 2H, CH_2N), 6.8, 6.97 (q, 4H, arom), 6.8, 7.15 (q, 4H, arom). Note: benzyl groups are non-equivalent in **7** as HCl salt, even with sample at 65°C (sealed tube) no line broadening is noted in NMR. M/S EI 310 (M–1), DCI 312 (M+1). Anal $C_{22}H_{30}ClNO_2$ (C, H, N).

N-Methyl-2,2-bis-(m-methoxy-benzyl)-piperidine·HCl ***10b***
As described for the preparation of **10a** above, the *meta*-isomer **10b** which results as a by-product from Grignard addition to *N*-methyl-2-piperidine in the preparation of **9b**, is isolated by chromatography as a yellow gum. Yield 29%. ^{1}H-NMR ($CDCl_3$): δ 1.2–1.8 (m, 6H, $CH_2CH_2CH_2$), 2.5, 3.15 (2 doublets, 4H, CH_2Ar), 2.50 (s, 3H, NCH_3), 2.5–2.8 (m, 2H, CH_2N), 3.75 (s, 3H, OCH_3), 6.7–7.4 (m, 8H, arom). M/S 339 (M+).

N-Methyl-2,2-bis-(m-hydroxy-benzyl)-piperidine·HCl **4**
O-Demethylation of **10b** with BBr_3 gave expected bis-phenol **4** isolated from ethanol/ether 1:9 as the HCl salt, as a white powder, mp 215–217°C (dec). Yield 91%. ^{1}H-NMR ($CDCl_3/CD_3OD$ 1:1: δ 1.4–1.8 (m, 6H, $CH_2CH_2CH_2$), 2.56, 2.69, 2.98, 3.12 (4 doublets, 4H, CH_2Ar), 2.72 (s, 3H, NCH_3), 3.05–3.1 (m, 2H, CH_2N), 6.3–7.0 (m, 8H, arom). Note: As observed for **7**, **4** has non-equivalent benzyl groups at 70°C probe temperature (sealed tube). M/S EI 310 (M–1). DCI 312 (M+1). Anal $C_{22}H_{30}ClNO_2$ (C, H, N).

N-Methyl-2-(p-hydroxy-benzyl)piperidine·HCl (±)1
A solution of **9a** (0.50 g, 0.002 mol) in THF-*i*-propanol (1:1) was treated with excess $NaCNBH_3$ (1.0 g, 0.015 mol) and stirred at 50°C as acetic acid was added dropwise to maintain ≈ pH 5 in the reaction mixture. Reaction was complete in 10 min and after workup, the crude ether was recovered as a gum (0.35 g, –80% crude). This material was directly *O*-demethylated with BBr_3 as described for (±)**3** to afford the product (±)**1** (0.32 g, 66% from **9a**), mp = 220–222°C. ^{1}H-NMR ($CDCl_3$ on free base); δ 1.0–1.9 (m, 6H, $CH_2CH_2CH_2$), 2.0–2.6 (m, 3H, CHN, CH_2Ar), 2.47 (s, 3H, NCH_3), 3.0 (t, 2H, CH_2N), 6.9 (q, 4H, arom), 7.8 (s, 1H, OH). M/S (DCI) 206 (M+1). Anal $C_{13}H_{20}ClNO$ (C, H, N).

N-Methyl-2-(m-hydroxy-benzyl)piperidine·HCl (±)5
As for (±)**5** above, **9b** was reduced with $NaCNBH_3$ and the crude intermediate ether *O*-demethylated with BBr_3 to yield (±)**5** as a white powder when precipitated from ether as the HCl salt, mp = 205–207°C. Yield from **9b** 69/%. ^{1}H-NMR ($CDCl_3$ on free base): δ 1.0–2.0 (m, 6H, $CH_2CH_2CH_2$), 2.0–2.7 (m, 3H, CH_2Ar, N-CH), 2.45 (s, 3H, NCH_3), 3.1 (t, 2H, CH_2N), 6.6–7.3 (m, 4H, arom), 8.7 (s, 1H, OH). M/S (DCI) 206 (M+1). Anal $C_{13}H_{20}ClNO$ (C, H, N).

N-2-dimethyl-2-(p-hydroxy-benzyl)piperidine·HCl (±)2
A Grignard solution prepared from iodomethane (2.0 g, 0.014 mol) and magnesium (1.0 g, 0.041 mol) in 50 ml dry ether was added to a solution of **9a** (0.3 g, 0.0012 mol) in 25 ml THF. After 10 min, reaction was quenched and the crude intermediate ether recovered and *O*-demethylated in CH_2Cl_2 with **1** m BBr_3 solution (2.0 ml, 0.002 mol) at room temperature. After 45 min, reaction was worked up as usual and, following filtration through a silica gel pad (ethyl acetate), (±)**2** was precipitated as the HCl from ether as an off-white powder and recrystallized from methanol, mp = 251–253°C. Yield 0.124 g (40% from **9a**). ^{1}H-NMR ($CDCl_3$ on free base): δ 1.0 (s, 3H, CH_3), 1.2–1.9 (m, 6H, $CH_2CH_2CH_2$), 2.35 (s, 3H, NCH_3), 2.5–3.0 (m, 2H, CH_2N), 6.9 (br s, 1H, OH), 7.0 (q, 4H, arom). M/S (EI) 218 (M–1). Anal $C_{14}H_{22}ClNO$ (C, H, N).

Resolution of (±)3
A solution of L-di-*p*-toluoyltartrate (0.39 g, 0.00098 mol) in 10 ml CH_2Cl_2 was treated with free base (±)**3** (0.301 g, 0.00098 mol) in 5 ml CH_2Cl_2. On standing at room temperature, a crop of white crystals formed (0.45 g). These were recrystallized twice to constant rotation ($[\alpha]_D^{25°} = -71°C = 1$ in methanol) from 5 ml of hot chloroform. The final crop of (±)**3** as a tartrate salt was a white powder: 0.136 g (39% theory). This salt was neutralized with aqueous 1 N NH_4OH and the free base (±)**3** reprecipitated as the HCl salt (±)**3-HCl**, mp = 367–368°C, $[\alpha]_D^{25°} = +6.7°$ C = 1 in methanol).

The liquors from the first crystallization yielded (after neutralization with 1 N Na_2CO_3) 0.13 g (0.00042 mol) of enriched (–)**3**. This material was treated with D-di-*p*-toluoyltartrate (0.17 g, 0.00042 mol) in 10 ml CH_2Cl_2, resulting in formation of a crop of crystals, 0.178 g (60%). This salt was twice recrystallized from hot chloroform (3 ml) to constant rotation ($[\alpha]_D^{25°} = +70.6°C : 1$ in methanol). Final crop (–)**3** as a tartrate salt was a white powder: 0.081 g (27%).

Method B–Scheme 2

N-Phenethyl-2-cyano-2-methyl-piperidine ***12***
A solution of **11** (0.040 mol prepared by the alkylation of 2-methyl-piperidine with phenethyl bromide in the presence of $NaHCO_3$) in 300 ml of 5% acetic acid/H_2O was oxidized with 4.8 equivalents of mercuric acetate [$Hg(OAc)_2$] (0.192 mol) while stirred at 95°C for 90 min. The reaction mixture was then filtered to remove Hg(I) salts and 150 ml of 10% $NaHCO_3/H_2O$ added to adjust pH to 5. Then, potassium cyanide (19.5 g, 0.30 mol) was added which served to convert the intermediate tetrahydropyridium salts to the 2-cyano-derivative **12**. Sodium carbonate was then added to bring mixture to pH 8 and the nitrile product was removed by dichloromethane extraction which, after drying ($MgSO_4$) and solvent evaporation, **12** was recovered as a pale yellow oil: 7.3 g (80%). ^{1}H-NMR ($CDCl_3$): δ 1.30 (s, 3H, CH_3), 1.4–1.9 (m, 6H, $-CH_2CH_2CH_2-$), 2.4–3.2 (m, 6H, Ar CH_2CH_2, N-CH_2), 7.30 (s, 5H, arom). M/S (DCI) 229 (M+1).

N-Phenethyl-2-(p-OH-benzyl)-2-methyl-piperidine (±3)
The reaction of 4-methoxy-benzyl chloride (0.016 mol) with magnesium powder (0.125 mol) in dry ethyl ether produced 0.004–0.005 mol of the Grignard product 4-CH_3O-ϕ-CH_2MgCl, plus reduction and coupling by products. To this solution was added **12** (0.912 g, 0.004 mol) in 10 ml of THF. After 5 min, the reaction was quenched by addition of H_2O, and the organic layer separated, dried, and the crude product which was precipitated as the HCl salt from ether (1.25 g, 0.0035 mol–87% crude yield), was directly *O*-demethylated without further purification by exposure to 3 equivalents of 1 M boron tribromide (11.0 ml, 0.011 mol) at room temperature for 1 h in dichloromethane. Following the standard workup, crude (±)**3** was purified by chromatography (silica gel/ethylacetate–triethyl amine 99:1; 0 $R_f = 0.55$ to give 0.78 g of (±)**3** as an off-white solid (mp ≈ 68–70°C). (±)**3** was precipitated from CH_2Cl_2-Et_2O (1:3) with HCl/EtO_2 as the hydrochloride salt:

(±)**3 HCl**, mp = 128–130°C. NMR: (free base) ^1H ($CDCl_3$): δ 0.96 (s, 3H, CH_3), 1.1–1.9 (m, 6H, $-CH_2CH_2CH_2-$), 2.5–3.1 (m, 8H, ϕCH_2CH_2, CH_2N, CH_2Ar), 6.8 (d, 2H, arom), 7.05 (d, 2H, arom), 7.3 (br s, 5H, phenyl), 7.6 (s, 1H, OH). M/S 309 (M+). Anal $C_{21}H_{27}NO$ (C, H, N).

N-phenethyl-2-(m-OH-benzyl)-2-methyl-piperidine (±6)
As described above, the preparation of the *p*-OH isomer (±**3**), a Grignard reaction of *m*-CH_3O-ϕ-CH_2-MgCl with **12** (0.912 g, 0.004 mol) gave the intermediate ether which, again, was directly *O*-demethylated with BBr_3 in CH_2Cl_2 to afford the desired product (±)**6**, (0.89 g, 0.0029 mol) in 72% overall yield from **14** as a white solid (mp = 73–75°C). As HCl salt (±)**6-HCl**: mp 131–133°C. NMR: ^1H ($CDCl_3$): δ 0.97 (s, 3H, CH_3), 1.1–1.9 (m, 6H, $-CH_2CH_2CH_2-$), 2.5–3.2 (m, 8H, ϕCH_2CH_2, CH_2-N, CH_2Ar), 6.7–6.9 (m, 3H, arom), 7.1 (d, 1H, arom), 7.3 (br s, 5H, phenyl), 7.46 (br s, 1H, OH). M/S 309 (M+). Anal $C_{21}H_{27}NO$ (C, H, N).

Pharmacology

Receptor binding
Frozen guinea pig 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 tissue.

Binding incubations contained 12 mg tissue, the appropriate [^3H]ligand and unlabeled compound in a total volume of 2.0 ml. [^3H]DHM, [^3H]DSLET, and [^3H]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 μM of the unlabeled analog of the tritiated ligand. Incubations were maintained for 1 h at 25°C, at which time samples were filtered over glass fiber filters (Whatman GF/B). Filters were counted after sitting overnight in scintillation cocktail to extract the radioactivity.

In vivo studies
Agonism. Male Swiss–Webster mice weighing 21–28 mg were injected subcutaneously with a test compound (3 doses/compound, 10 animals/dose), standard or diluent 4–10% aqueous solution of ethanol) and the tail-flick test was administered at 10, 20, 30, 45 and 60 min after treatment. A 6.5-s cut-off time for tail-flick response was used. At the time of peak effect of each dose, the average increase in response time of each treated group was determined and the percent of the maximum possible increase in reaction time (percent agonism) was computed [12]. 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 [13].

The effect of route of administration on the observed analgesic activity of four of the 2-benzyl piperidine analogs (±)**3**, (±)**4**, (±)**6** and **7** were investigated by applying the intracerebroventricular (icv) administration procedure described by Haley and McCormick [14]. The compounds were dissolved in a 20% aqueous solution of dimethyl sulfoxide (DMSO) and injected icv at a standard volume of 4 μl/mouse. The animals were tested in the tail-flick apparatus 2, 5, 10, 20, 30, 45 and 60 min after treatment.
Antagonism. Antagonist activity of the compounds against 8 mg/kg (21.08 μmol/kg; ED_{50}) of morphine sulfate was determined by the tail-flick procedure. Mice were injected subcutaneously (sc) with a test substance, reference drug, or vehicle only and given immediately 8 mg/kg (sc) of morphine sulfate caudal to the site of the first injection. They were tested 10, 20, 30, 45 and 60 min after treatment. Percent agonism at the time of peak effect of each dose was computed. The percent antagonism was calculated for each dose level by applying the formula developed by Harris *et al* [15].

From a plot of percent antagonism *versus* the log dose, the median effective antagonist dose (AD_{50}) and 95% confidence limits were determined [9].

In order to confirm that the analgetic activity of this class of compounds is initiated by binding to the opioid receptors, the naloxone test of reversal of analgesic response was administered to the most potent analog, the *N*-phenethyl-2-methyl-2-(*p*-OH benzyl) piperidine [(±)**3**]. The effected doses of 0.275, 0.069, 0.034 and 0.014 μmol/kg of naloxone hydrochloride (4 mice/dose) were evaluated. The mice were injected subcutaneously (sc) with a dose of naloxone and immediately treated with 15.37 μmol/kg (sc) of *N*-phenethyl-2-methyl-2-(*p*-OH benzyl) piperidine. The tail-flick test was administered 5, 10, 20, 30, 45, 60 and 75 min after treatment.

Acknowledgment

Support for this work by National Institute of Drug Abuse Grant DA02622 is gratefully acknowledged.

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