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MORPHINAN CYCLIC IMINES AND PYRROLIDINES CONTAINING A CONSTRAINED PHENYL GROUP: HIGH AFFINITY OPIOID AGONISTS.

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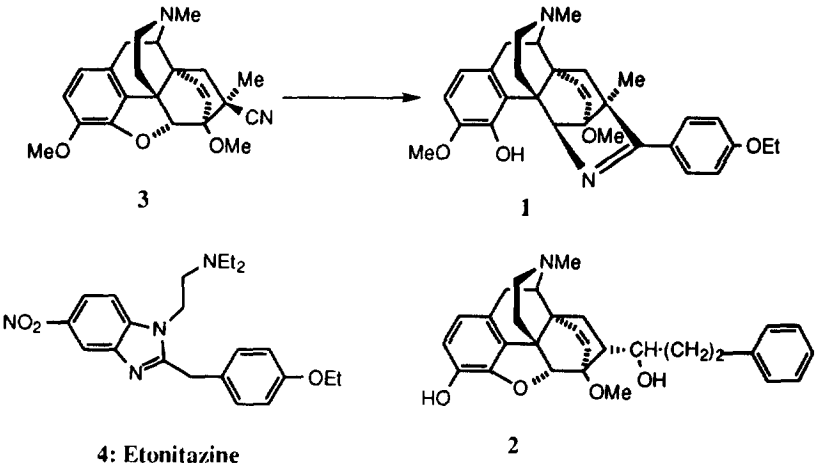
Abstract: A series of morphinan derivatives which are cyclic imines and pyrrolidines containing a constrained phenyl group has been synthesised and tested for their opioid receptor binding affinity. In opioid binding assays the ligands displayed very high affinity particularly for μ receptors and some showed substantial μ selectivity.

In a continuing search for pharmacological treatments for opiate abuse[1] we are interested in selective agonists for the μ opioid receptor with slow receptor kinetics to provide substitution therapy of long duration. One approach to achieving μ selectivity was expected to be conformationally constraining a phenyl group to interact with the lipophilic site on the opioid receptor which we identified as crucial to the opioid actions of the orvinols.[5] Portoghese *et al* [2,3] have demontrated the validity of this approach in the design of δ-selective ligands including those with δ_1 and δ_2 subtype selectivity. We realised that the phenyl ring of the cyclic imine (**5**)[4] occupied a region in space that can also be occupied by the phenyl group of the extremely potent opioid agonist **2**.[5] The cyclic imine is known to be relatively potent as an analgesic in the rat tail pressure test (25 x morphine), but until now no work has been carried out to determine its relative affinity or efficacy at the three opioid receptor types (μ, δ and κ).[4] We hoped that the constrained phenyl group in **5** and some close analogues would impart some selectivity, particularly for the μ receptor. We here report that such selectivity has been achieved in **5**, the *p*-ethoxy derivative (**1**), and the quaternary iminium salt (**13**).

The cyclic imine **5** and a range of close analogues were synthesised as shown in schemes 1 and 2. The *p*-ethoxy analogue of **5** was synthesised due to the high potency of etonitazine (**4**). Structure-activity relationships in the etonitazine series showed that the *p*-ethoxy group afforded the highest potency.[6] Since the aromatic ring of etonitazine can occupy a similar region in space to the phenyl ring of the cyclic imine it seemed possible that a similar modification of the cyclic imine might yield a more potent ligand. Thus the nitrile was treated under the standard conditions (for the formation of **5**) to give **1** in 60% yield (scheme 1). Initially it was hoped to 3-O-demethylate **5** (prepared as previously described[4]) as morphinan 3-phenols traditionally display higher affinity (but lower efficacy) for μ opioid receptors than the corresponding methyl ethers.[7] However, treatment with propane thiolate failed to yield any product, the likely reason being the instability of the catechol formed.

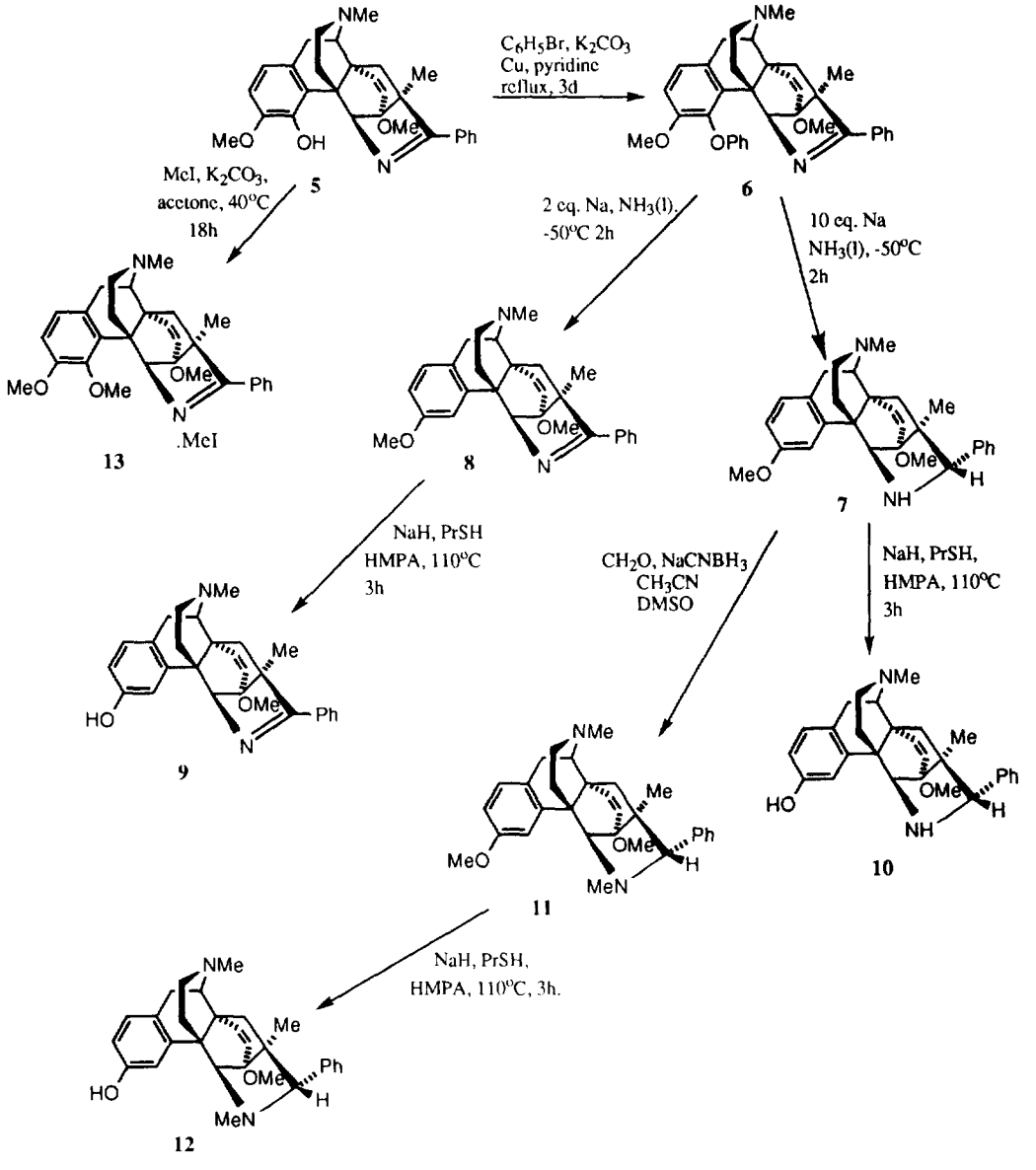


Scheme 1: The synthesis of **1** and the structures of etonitazine (4) and the phenethyl-orvinol (2).

The presence of an OH group at C-4, unlike at C-3 appears to be detrimental to potency. In view of this and the instability of the catechol system in the 3,4-dihydroxy derivatives it was decided to prepare 4-deoxy analogues. Various methods are available for the removal of phenolic OH groups. Formation of the phenyltetrazolyl ether followed by catalytic hydrogenation (10% Pd/C)[8] gave the product of reduction at both the 6,14-etheno bridge and the imine function but leaving the tetrazole ether intact. The ease of reduction of the etheno bridge is in sharp contrast to the difficulty in reducing the etheno bridge in the related α-series of orvinols.[9] To avoid the need for catalytic hydrogenation the phenyl ether (**6**) was formed by a standard method and then reduced using sodium in liquid ammonia (scheme 2).[10] With a large excess of sodium the pyrrolidine (**7**) was the product. The reaction was stereospecific with reduction occuring from the upper face (NOE studies[11]). By reducing the amount of sodium the 4-deoxy cyclic imine (**8**) could be obtained as the major component of a mixture with **7**. Using less sodium simply resulted in the recovery of some starting material along with **7** and **8**. The mixture was not separable at this point but 3-O-demethylation yielded an easily separable mixture containing **9**, (60%, over 2 steps). Demethylation of the pyrollidine (**7**) gave the phenol (**10**) (51% over 2 steps). To provide some insight into whether the pyrrolidine was acting as a proton donor in hydrogen bonding at the receptor the amino group was methylated using formaldehyde and sodium borohydride.[12] Again demethylation was performed to yield **12** (66% from **7**).

As well as 4-deoxy compounds being, in general, more potent than 4-phenols, the corresponding 4-methoxy compounds also tend to show increased potency.[13] Therefore **5** was methylated using methyl iodide to give **13**. As can be seen (scheme 2), the methylation could not be carried out without quaternisation occurring. It appears from ^{1}Hnmr that the quaternisation took place on the imine nitrogen rather than the pyrrolidine nitrogen. Interestingly when **5** was treated with the same reagents at room temperature, only quaternisation occurred, confirming both the ease of quaternisation and the also the relative difficulty in methylating this C-4 phenol.

Binding assays in Hartley guinea pig membranes were conducted using standard procedures.[14] Compounds were investigated for their ability to displace [^{3}H]DAMGO (μ), [^{3}H]Cl-DPDPE (δ), [^{3}H]U69,593 (κ1) and bremazocine (in the presence of 100nM DAMGO, DPDPE and U69,593 to block μ, δ and κ receptors respectively) for κ2; the data are shown in the table. All six ligands tested displayed subnanomolar affinity for the μ receptor and somewhat lower affinity for the other opioid receptors. The parent of the series (**5**) was μ selective having ten fold selectivity for μ/δ, nearly seventy fold for μ/κ1 and nearly 1300 fold for μ/κ2.



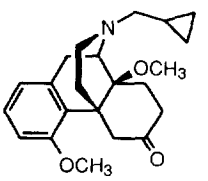
Scheme 2: The synthesis of **9**, **10**, **12** and **13**.

Similar μ selectivity was shown by the ethoxy derivative (1). The quarternary iminium salt (**13**) was also μ selective but in this case the μ/κ_1 ratio (~eight) was lower than μ/δ (~sixty). The retention of opioid receptor affinity in the quaternary iminium salt is in contrast to the quaternisation of the piperidine basic nitrogen in morphine and other opioids which results in substantial loss of affinity.[15] In the three tested derivatives in which the C_4-phenolic group had been removed there was no μ selectivity though affinity for μ receptors was highest in each case. These 3-hydroxymorphinan derivatives showed subnanomolar affinity for μ,δ and κ_1 receptors. Affinity for κ_2 receptors was approximately an order of magnitude lower than for κ_1 which was also true for the three derivatives having C_4-O functions.

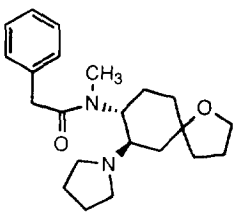
	IC_{50} (nM)			
ligand	[^{3}H]DAMGO (μ)	[^{3}H]Cl-DPDPE (δ)	[^{3}H]U69593 (κ_1)	bremazocine* (κ_2).
5	0.25	2.44	16.84	319.5
9	0.33	0.69	0.52	6.2
10	0.15	0.32	0.26	2.6
12	0.36	0.71	0.52	9.2
1	0.89	11.62	96.89	330.8
13	0.56	34.00	4.67	379.5

Table 1: Binding affinity to Hartley guinea pig brain membranes. * carried out in the presence of 100nM each of DAMGO, DPDPE and U69,593 to block μ, δ and κ_1 receptors.

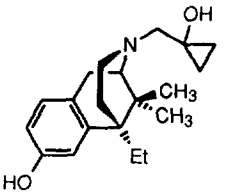
The effect of hydrogenation of the imine (**9**) to the pyrrolidine (**10**) was to increase affinity for all the receptors about two fold but N-methylation of **10** reversed this effect. The effect of the presence of a C_4-O function in conferring μ selectivity has not previously been highlighted though cyprodime (**14**) a selective μ antagonist has a C_4-methoxy group and no C_3-O function.[16] It appears that the conformationally constrained phenyl group in these ligands does not confer μ selectivity since it is present in the non-selective 3-hydroxymorphinans **9**, **10** and **12**. In these derivatives the presence of the phenolic group in the position it occupies in the natural opiates confers increased affinity at all the opioid receptors but with less effect at μ than at δ and κ. The retention of opioid receptor affinity in the quarternary iminium salt may be the effect of enhanced affinity on methylation of the C_4-hydroxy function offsetting the expected loss of affinity caused by quaternisation.



14: Cyprodime



15: U69593



16: Bremazocine

The substantially higher affinity of these morphinan derivatives for κ_1 than for κ_2 subtypes is interesting. This suggests that the lipophilic binding site for the prototypic κ_1-agonist U69593 (**15**) is more accessible to them than the unique binding site related to the hydroxycyclopropylmethyl group of the κ_2 agonist bremazocine (**16**).

In conclusion it appears that fixing the position of the phenyl group in this series of morphinan derivatives did not confer selectivity in opioid binding assays. However, the presence of a C_4-oxygen function did confer μ selectivity. It remains to be determined by functional assays and *in vivo* evaluation whether this selectivity is of utility for the design of a treatment for opioid abuse.

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14. **Receptor binding:** Binding studies were conducted on Hartley guinea pig membranes. Guinea pigs were decapitated and the brains quickly removed and weighed, then homogenized in 50mM Tris HCl, pH 7.5, using a Polytron homogenizer. The homogenate was centrifuged at 40,000g for 15min, rehomogenized and centrifuged once more. The final pellet was resuspended in Tris HCl, pH 7.5, at a final concentration of 6.67mg original wet weight of tissue per milliliter. This crude membrane preparation was used for determination of binding to each receptor site. Assays were conducted using [^{3}H]DAMGO (μ), [^{3}H]Cl-DPDPE (δ), [^{3}H]U69,593 (κ_1) and [^{3}H]bremazocine in the presence of DAMGO, Cl-DPDPE and U69,593 for κ_2. 1.8ml of homogenate from the crude membrane preparation

with 0.1ml of the test compound for 1h at 25°C. Brain membranes were incubated with the test compounds at concentrations ranging from 10^{-5} to 10^{-10} nM. After the incubation, samples were filtered through glass fibres on a 48-well Brandel cell harvester. Filters were left overnight in plastic scintillation vials containing 5ml of scintillation fluid, to elute the radioactivity out of the membrane, before radioactivity levels were determined. Nonspecific binding was determined by using 1.0μM of the unlabelled counterpart of each radioligand. Full characterisation of compounds included two full inhibition curves and analysis of the data for IC_{50} values and Hill coefficients by using the program ALLFIT.

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