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SYNTHESIS AND BIOLOGICAL EVALUATION OF 14-ALKOXYMORPHINANS. 12.[1] A PHENETHYL ANALOGUE OF THE μ-SELECTIVE OPIOID RECEPTOR ANTAGONIST CYPRODIME

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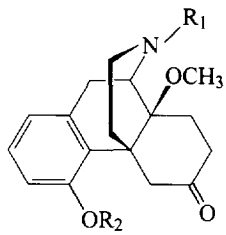
Abstract: The *N*-phenethyl analogue (compound **4**) of the μ-selective opioid antagonist cyprodime (**1**) has been prepared and evaluated in radioligand binding, bioassays (guinea-pig ileum myenteric plexus and mouse vas deferens preparations) and the rat tail flick test. It was found to possess remarkable affinity and high preference for μ opioid receptors and high antinociceptive potency which is comparable to its *N*-methyl analogue **3**.

The nonpeptide, competitive μ opioid receptor antagonist cyprodime (**1**)[2] has become a valuable tool in opioid research.[3-5] Recently a new and more efficient synthesis of cyprodime has been reported.[6] Cyprodime has been tritium labelled and has become available for radioligand binding assays especially since it is commercially available.[7]

An extensive study on cyprodime-related compounds revealed that several changes to the cyprodime molecule (e. g. an additional methoxy group at C-3, different substituents at C-4, a 14-ethoxy group, a *N*-allyl group) yielded compounds with either less μ selectivity or partial agonist activity.[3] Increasing the chain length at C-4 (compound **2**) resulted in higher affinity for μ receptors (ca. 2-fold) but very little change in either selectivity or intrinsic activity. A *N*-methyl group instead of the *N*-cyclopropylmethyl group afforded a compound (**3**) with very high antinociceptive potency.[8]

In the series of morphinans having a 4,5-epoxy bridge it was found that a *N*-phenethyl group could increase the antinociceptive potency ca. 10-fold in comparison to their *N*-methyl counterparts.[9] In an effort to investigate the role of a *N*-phenethyl group in morphinans lacking the 4,5-epoxy bridge we synthesized 4,14-dimethoxy-17-(2-phenylethyl)-morphinan-6-one (**4**) and performed *in vitro* and *in vivo* studies.

The synthesis of compound **4** was accomplished starting from 4,14-dimethoxy-17-methylmorphinan-6-one (**3**) which is available from oxymorphone in 6 steps.[8,10] In modification of the original procedure,[2] compound **3** was *N*-demethylated by an improved procedure using 1-chloroethyl chloroformate[11] instead of 2,2,2-trichloroethyl chloroformate. Thus, treatment of **3** with 1-chloroethyl chloroformate gave carbamate **5** as an oil which was not further purified and characterized. Refluxing this oil in MeOH afforded the *N*-normorphinan **6** as the hydrochloride salt.[12] **6**.HCl was alkylated with 2-phenylethyl bromide in DMF at elevated temperature to yield compound **4** which was isolated as the hydrobromide salt.[13]



1: R_1 = cyclopropylmethyl, $R_2 = CH_3$
2: R_1 = cyclopropylmethyl, R_2 = n-C_4H_9
3: $R_1 = R_2 = CH_3$
4: $R_1 = CH_2CH_2Ph$, $R_2 = CH_3$
5: $R_1 = CO_2CHClCH_3$, $R_2 = CH_3$
6: R_1 = H, $R_2 = CH_3$

The *in vitro* studies performed were radioligand binding and bioassays (guinea-pig ileum myenteric plexus preparation (GPI) and mouse vas deferens preparation (MVD)). The inhibitory effects of **4**.HBr and cyprodime were assessed in homogenates of guinea-pig brain in Tris.HCl buffer (50 mM, pH 7.4)[14] employing [^{3}H]DAMGO (μ agonist), [^{3}H]DPDPE (δ agonist) and [^{3}H]U69593 (κ agonist) as radioligands (Table 1). Introduction of a *N*-phenethyl group to the cyprodime molecule greatly enhanced μ affinity and afforded a compound with 50-fold preference for μ over δ receptors and 700-fold preference for μ over κ receptors. A similar μ affinity was observed with the *N*-methyl analogue **3** but without showing preference for μ over κ receptors.

Table 1. Opioid Receptor Binding of Compounds **1** (Cyprodime), **3** and **4**.

	K_i (nM) ± SEM		
Compound	[^{3}H]DAMGO (μ)	[^{3}H]DPDPE (δ)	[^{3}H]U69593 (κ)
4.HBr	0.9 ± 0.25	48.6 ± 10.1	690 ± 66.5
Cyprodime (**1**.HBr)	23.7 ± 6.3	105 ± 9.7	61.1 ± 12.2
3	0.7[a]	46.2[a,b]	1.5[a,c]

[a] Rat brain homogenates were used. [b] [^{3}H]Deltorphin was used. [c] [^{3}H]EKC was used: μ and δ sites were blocked.[15]

The bioassays (GPI and MVD) were performed as described previously.[14,16] Compound **4**.HBr was a potent agonist in both GPI and MVD. In both tissues the ability of naloxone to antagonize the responses to **4**.HBr was similar to values determined against the μ agonist DAMGO, but different to the K_e for naloxone determined against the κ agonist U69593 and the δ agonist DPDPE, confirming that the compound was acting as a μ agonist in both tissues (Table 2). The mouse vas deferens has high δ receptor reserve but considerably lower μ receptor population. The μ activity of **4** in this tissue therefore confirms the high selectivity of the compound for μ over δ opioid receptors.

Table 2. Agonist Potencies and Antagonism by Naloxone of **4**.HBr and Selected Agonists (DAMGO, μ; U69593, κ; DPDPE, δ) in GPI and MVD.

	GPI		MVD	
Compound	EC_{50} (nM) ± SEM	Naloxone K_e (nM) ± SEM	EC_{50} (nM) ± SEM	Naloxone K_e (nM) ± SEM
4.HBr	17.4 ± 3.0	0.54 ± 0.1	11.8 ± 3.14	1.67 ± 0.57
DAMGO	11.8 ± 1.2	1.6 ± 0.1	24.7 ± 3.0	1.9 ± 0.6
U69593	2.2 ± 0.4	9.0 ± 2.1	30.0 ± 7.4	14.9 ± 3.0
DPDPE	no agonism		2.8 ± 0.6	20.4 ± 2.6

Compound **4**.HBr was tested *in vivo* in the rat tail flick test[17] and exhibited remarkable antinociceptive potency which is comparable to its *N*-methyl analogue **3**. When compared to morphine, **4**.HBr shows approximately 50 times the potency of this reference drug (Table 3).

Table 3. Antinociceptive Effects of **4**.HBr, **3** and Morphine in the Rat Tail Flick Test.

Compound	Rat Tail Flick Test ED_{50}, mg/kg, s.c. (95% confidence limits)
4.HBr	0.037 (0.019-0.070)
3	0.021 (0.014-0.028)
Morphine.HCl	1.8 (1.28 - 2.52)

Replacement of the *N*-cyclopropylmethyl moiety of cyprodime with phenethyl to afford **4**.HBr or with methyl to afford **3** markedly improved μ opioid receptor affinity in spite of the lack of a 3-OH function, without altering δ affinity thus resulting in improved μ/δ selectivity. Unlike **3** however substitution with phenethyl led to a loss of affinity for κ receptors such that **4**.HBr had a very high preference for μ over κ receptors, resulting in a compound with much improved overall μ selectivity. In marked contrast to cyprodime, **4**.HBr was a potent

agonist in both biossays (GPI and MVD) and was found to exhibit a high preference for μ opioid receptors in these tissues. **4**.HBr possessed also high agonist potency *in vivo* in the rat tail flick test comparable to its *N*-methyl analogue **3** as might be expected from the similar affinities of the compounds for the μ opioid receptor.

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12. **6**.HCl: mp 155-157 °C; free-basing gave **6** which was identical by mixed mp, IR and ^{1}H NMR with authentic material.[2]
13. **4**.HBr: mp 243-248 °C; IR (KBr): 3480 ($^+$NH) and 1715 (CO) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d$_6$): δ 8.95 (broad s, $^+$NH), 7.38-7.20 (m, 6 arom. H), 6.88 (d, J = 8.2 Hz, 1 arom. H), 6.84 (d, J = 8.2 Hz, 1 arom. H), 3.77 (s, CH_3O-C(4)), 3.42 (s, CH_3O-C(14)); CI-MS: 406 (M^++1); satisfactory elemental analysis (CHN) was obtained.
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