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Pyrrolo- and Pyridomorphinans: Non-selective opioid antagonists and delta opioid agonists/mu opioid partial agonists

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Abstract

Opioid ligands have found use in a number of therapeutic areas, including for the treatment of pain and opiate addiction (using agonists) and alcohol addiction (using antagonists such as naltrexone and nalmefene). The reaction of imines, derived from the opioid ligands oxymorphone and naltrexone, with Michael acceptors leads to pyridomorphinans with structures similar to known pyrrolo- and indolomorphinans. One of the synthesized compounds, **5e**, derived from oxymorphone had substantial agonist activity at delta opioid receptors but not at mu and/or kappa opioid receptors and in that sense profiled as a selective delta opioid receptor agonist. The pyridomorphinans derived from naltrexone and naloxone were all found to be non-selective potent antagonists and as such could have utility as treatments for alcohol abuse.

1. Introduction

The pyrrolomorphinan and indolomorphinan pharmacophores are a feature of a range of ligands selective for the δ -opioid receptor (DOPr) and the κ opioid receptor (KOPr). Among these are naltrindole (**1a**; NTI) and norbinaltorphimine (**2a**; norBNI) the prototype selective ligands for DOPr and KOPr respectively.^{1, 2} N-Benzylnaltrindole (BNTI; **1b**) is a potent DOPr antagonist with long duration of action *in vivo* when administered intracerebroventricularly (*i.c.v.*).³ When administered peripherally, the related compound N-BenzylnorBNI (BnorBNI; **2b**) displays early onset MOPr agonist activity in the tail withdrawal antinociception assay that is additive with the KOPr agonist activity of U69593. However when administered *i.c.v.* **2b** selectively inhibited the antinociceptive activity of U69593.⁴

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Pursuing our interest in N-benzylpyrrolomorphinan structures we investigated reaction of the benzylimines and methylamines of oxymorphone and naltrexone with Michael acceptors. The reactions of γ -nitrostyrenes with the imines generated *in-situ* produced 4'-aryl-1-benzylpyrrolomorphinan ligands (**3**).⁵ These ligands had modest selectivity in receptor binding assays for DOPr over KOPr but not over MOPr. In the [³⁵S]GTP γ S assays they showed predominantly opioid receptor antagonist activity with substantial DOPr selectivity particularly over KOPr. The lead compound (**3a**) *in vivo* had slow onset as an antagonist of the selective DOPr agonist SNC80 with potency comparable to that of NTI.⁵

In addition to the synthesis of the arylpyrrolomorphinans (**3**) we have reported the reactions of imines derived from oxymorphone and naltrexone with other Michael acceptors.^{6, 7} With maleic anhydride the products were the 5'-hydroxypyrrolomorphinans (**4**); reaction with ethyl acrylate, methyl methacrylate and α -methylene- γ -butyrolactone gave pyridomorphinans of general structure **5**.⁶ The pharmacological evaluation of these ligands was not reported. Since then a further group of 1'-benzylpyridomorphinans (**5k-5p**) unsubstituted at the 5'-position of the pyridine ring but with variation of the 17- and 1-(N)-substituents has been prepared. The evaluation of the previously reported ligands along with the new series is described herein and comparison made with the known related series **6** and **7**.⁸

2. Results

2.1 Synthesis

The synthetic procedure for accessing the new compounds is shown in Scheme 1. Briefly, the appropriate oxymorphone was treated with an amine in refluxing ethanol under anhydrous conditions in the presence of *p*-toluene sulfonic acid. The resulting imine was reacted with ethyl acrylate to give the desired morphinans in 20 – 40% yield after purification.

2.2 Biological Evaluation

The test ligands were evaluated in binding assays in membranes prepared from human recombinant opioid receptors transfected into chinese hamster ovary (CHO) cells in which the displaced radioligands were [³H]DAMGO (MOPr), [³H]CI-DPDPE (DOPr), [³H]U69593 (KOPr) and [³H]nociceptin (Table 1). Details of these assays have been described previously.⁹

The pyridomorphinans (**5**) generally had significantly higher affinity for opioid receptors than did the pyrrolomorphinans (**4**) when comparing ligands with the same 17(N)-substituent. In the 17-methyl series (**5a-f**) MOPr affinity was high (0.29 – 12.7 nM), similar to DOPr affinity (0.92 – 9.39 nM) and significantly higher than KOPr affinity (1.98 – 182 nM). Benzyl substitution on the pyridinonyl (1'-) nitrogen (**5b**, **5e**, **5f**) generally produced higher affinity for all receptor types than did equivalent methyl substitution (**5a**, **5c**, **5d**). Substitution in the 5'-position of the pyridinone ring with the hydroxyethyl group reduced MOPr and KOPr affinity with only minor effects on DOPr affinity, with the 5' β -substituted ligands (**5c**, **5e**) having somewhat higher affinity than equivalent 5' α -substituted ligands (**5d**,

5f). The affinity of the 17-cyclopropylmethyl series (**5g-5k**, **5n**) for all opioid receptors was low nanomolar to subnanomolar with no significant selectivity. The binding affinities for all the pyridinones (**5**) followed a general SAR pattern in which 5'-substitution in the pyridinonyl ring reduced affinity for all three opioid receptors with the least effect shown at DOPr.

The *in vitro* functional response of the previously synthesised ligands⁶ was determined in [³⁵S]GTPγS assays using membranes from cloned human receptors transfected into CHO cells⁹ where the reference ligands for efficacy comparison and/or determination of antagonism potency (Ke/nM) were DAMGO (MOPr), DPDPE (DOPr) and U69593 (KOPr). In order to expedite the evaluation process a primary screen for the newly synthesized ligands (**5k-5p**) was put in place with efficacy determination as before, but testing at a single high dose (10μM) to determine maximal efficacy for MOPr, KOPr and the nociceptin/orphanin FQ peptide receptor (NOPr) (Table 3). Efficacy at NOPr was screened for due to our interest in the possibility of developing MOPr/KOPr antagonists with NOPr partial agonist activity as potential relapse prevention agents for the treatment of drug addiction.^{10, 11} A limited selection of compounds from the screen (**5k**, **5n**) were then evaluated more fully (Tables 1 and 2).

The *in vitro* opioid receptor profiles of the pyrolomorphinans (**4**) were markedly influenced by the nature of the 17(N)-substituent. The 17-methyl derivative (**4a**) was a low potency partial agonist, but with a maximal response approaching that seen with the standard full agonists at MOPr (86% stimulation) and DOPr (74% stimulation) and a very low potency and very low efficacy KOPr partial agonist (23% stimulation). In contrast the 17-CPM analogue (**4b**) showed no evidence of agonism, but was an antagonist with nM affinity for all three opioid receptors.

All of the original pyridinones (**5a-5j**) were KOP receptor antagonists with marked difference of potency between the 17-methyl (**5a – 5f**) and 17-CPM (**5g – 5k**) groups (Table 2). Thus **5k** was over 160-fold more potent as an antagonist (Ke 0.13nM) than **5b** (Ke 21nM) and **5i** was 40-fold more potent (Ke 0.73nM) than **5e** (Ke 29nM). Whereas all the 17-CPM pyridinones (**5g – 5k**) were potent antagonists at all three opioid receptors, the 17-methyl series (**5a – 5f**) were partial agonists at MOPr of moderate efficacy and potency with the exception of the 5'β-hydroxyethyl derivative **5e** which had low MOPr efficacy (17% of DAMGO) and so acted as a MOPr antagonist (Ke 33nM), though of two orders of magnitude lower potency than the equivalent 17-CPM ligand (**5i**; Ke 0.35nM). The 17-methyl pyridinones (**5a – 5f**) were also DOPr partial agonists generally of higher efficacy (66 - 94% of DPDPE) and potency than as MOPr partial agonists (17 – 52% of DAMGO).

As there was no evidence that having a 5'-substituent was beneficial to activity at opioid receptors, the new group of 17-CPM pyridomorphinans were unsubstituted in the 5'-position in the pyridinone ring (**5k**, **5l**, **5n**, **5o**, **5p**) and the 17-allyl analogue **5m**. These new ligands showed no evidence of agonist efficacy for MOPr and KOPr at a 10μM concentration and even showed signs of inverse agonist activity at KOPr (Table 3). They also showed very low levels of efficacy for NOPr. Variation of the 1'-N substituent on the pyridinone ring, the

17(N)-substituent or substitution in the phenyl ring of the 1'-benzyl group had very little effect on the screened profile of the new ligands (Table 3).

3. Discussion

The known pyrrolomorphinan **6a** is a non-selective opioid antagonist¹² whereas the equivalent indolomorphinan NTI (**1a**) is a highly selective DOPr antagonist.² The difference is due to substantially lower DOPr potency and higher MOPr and KOPr antagonist potency of **6a**. DOPr antagonist potency and selectivity was restored in **6a** by pyrrole-ring substitution in the 4' and 5'-positions as in **6b**,¹³ with further enhancement of DOPr potency by introduction of a 1'(N)-benzyl substituent as in **3a**.⁵ The herein reported example (**4b**) is a non-selective opioid antagonist which is best compared with **6b** since a side chain CO₂Et is regarded as an isostere of a phenyl group¹⁴ so that the 4'-benzyl group of **6b** is comparable to the 4'-CH₂CO₂Et group of **4b**. Despite the structural differences between **6b** and **4b** in the 1'-(N) and 5'-positions (substituents R¹ and R³ in structure **6**) they have comparable DOPr antagonist potency (Ke **6b** 2.1nM, **4b** 6.8nM) but equally high MOPr (6.6 nM) and KOPr (4.3 nM) antagonist potencies of **4b** means DOPr antagonist selectivity in **4b** is lost whereas **6b** was 50-fold selective over both MOPr and KOPr.¹³ Since it was established that the 1'-(N)-benzyl group had a positive effect particularly on DOPr antagonist potency,⁵ it appears that the 5'-hydroxyl group has the opposite effect.

It is of interest to compare the pyridinones reported herein (**5**) with the pyridomorphinans (**7**) reported by the Ananthan group.⁸ Most of the attention in ligands of structure **7** was directed at ligands with 5'-aryl or heteroaryl substituents giving the lead compound SoRI9409 (**7c**),¹⁵ a MOPr agonist and DOPr antagonist showing antinociception with minimal tolerance in multiple dosing regimes. In contrast, substituents in the 5'-position in the pyridinones (**5**) are alkyl, particularly methyl and hydroxyethyl. Thus comparison will be made between ligands from the two series with no 5'-substituent. This means comparing **5b** with **7a** and **5k** with **7b**. **5b** has highest binding affinity of MOPr (K_i 0.29 nM) with around 3-fold lower affinity at DOPr and 6-fold lower at KOPr; **7a** also has highest affinity at MOPr but that is 27-fold lower than the MOPr affinity of **5b**. DOPr and KOPr affinity of **7a** is also very much lower than that of **5b**, 20-fold and 130-fold respectively.¹⁶ The effect of replacing the 17-methyl group of **5b** with the CPM group (**5k**) is relatively small since both ligands have high affinity for all three opioid receptors. Similar enhancement of binding affinity is seen in **7b** in comparison with **7a**; in both series improvement in KOPr affinity is most pronounced.

The *in vitro* profile in [³⁵S]GTPγS assays of the 17-(N)-methyl pyridinone **5b** is KOPr antagonist, MOPr partial agonist and DOPr full agonist giving a clear rank order of efficacy DOPr>MOPr>KOPr. The pyridomorphinan **7a** was reported as having no DOPr or KOPr agonist activity (though no antagonist activity could be detected) and extremely low potency MOPr partial agonist activity in a [³⁵S]GTPγS assay (EC₅₀ 3000nM).¹⁶ In isolated tissue assays the MOPr agonist activity of **7a** was reported as being confirmed (IC₅₀ 523 nM) in the guinea pig *ileum* (GPI: a tissue that is sensitive to agonists acting at MOPr and KOPr) but in mouse *vas deferens* (MVD: a tissue particularly sensitive to DOPr agonists, but also to MOPr and KOPr agonists) a DOPr partial agonist response was recorded (49% of

maximum).¹⁶ Thus the rank order of efficacies for opioid receptors of **7a** appears to be MOPr>DOPr>KOPr with the major difference from **5b** being the very low potency for all opioid receptor activity and particularly at DOPr where the efficacy difference was also very pronounced.

As shown in tables 1 and 2, the 17(N)-CPM pyridinone (**5k**) is an extremely potent non-selective opioid receptor antagonist. The equivalent pyridomorphinan (**7b**) in the GPI assay showed MOPr antagonist activity with $K_e = 164 \text{ nM}$ ⁸ representing 800-fold lower potency than that of **5k** in the [³⁵S]GTP γ S assay.

The effect of substitution in the 5'-position of the pyridinones (**5**) and pyridomorphinans (**7**) is interesting though the nature of the substituents in the two series is different. In the 17(N)-Me group of pyridinones (**5a-5f**) 5'-substitution of methyl and hydroxyethyl groups has a substantial negative influence on affinity for all three opioid receptors and in [³⁵S]GTP γ S assays results in much lower potencies without changing the rank order of efficacy DOPr>MOPr>KOPr. Aryl substitution in the 5'-position of **7a** and **7b** has a major effect on opioid receptor binding profile by substantially increasing DOPr affinity and reducing MOPr affinity resulting in DOPr selectivity.¹⁶ *In vitro* the 5'-aryl analogues of **7a** are not dissimilar to the parent.

4. Conclusions

The evaluation of the opioid receptor binding and *in vitro* activities of series of pyridinone and pyrrolomorphinans are herein reported. In both series the biggest effect on opioid receptor binding is the nature of the N(17)-substituent, with the 17-methyl ligands having substantially lower binding affinities and *in vitro* potencies than their 17-cyclopropylmethyl equivalents but very much higher efficacy for MOPr and DOPr. The pyrrolomorphinans had substantially lower binding affinities and *in vitro* potencies than the pyridinones. No ligand had binding selectivity for a single opioid receptor. The 17-CPM and 17-allyl pyridinones were all non-selective potent antagonists. The slight shift in profile from somewhat more active against MOPr (naltrexone) to somewhat more active against KOPr (e.g. **5g**) might mean these ligands are even better tolerated than naltrexone as treatment agents for alcohol abuse due to the reported anti-anxiety effects of KOPr antagonists.¹⁷ There was significant selectivity of agonist action in the 17-methyl analogues with one, **5e**, having only significant efficacy at DOPr and in that sense profiles *in vitro* as a selective DOPr agonist.

5. Experimental Section

5.1. General

Reagents and solvents were purchased from Sigma-Aldrich or Alfa Aesar and used as received. ¹H and ¹³C NMR spectra were obtained with a Bruker-400-MHz instrument (¹H at 400 MHz, ¹³C at 100 MHz); δ in ppm, J in Hz with TMS as an internal standard. ESIMS: microTOF (BRUKER). Microanalysis: Perkin-Elmer 240C analyzer. Column Chromatography was performed using pre-packed column in combi flash instrument. Ligands were tested as their hydrochloride salts, prepared by adding 5 equivalent of HCl (1N solution in diethyl ether) in a solution of compound in anhydrous methanol. Synthetic

methods are detailed for one example in each series. Full details for all experiments can be found in the supporting information. All reactions were carried out under an inert atmosphere of nitrogen unless otherwise indicated. The procedure and analysis for the new compounds, **5k** – **5p** is given below, **5a** – **5j** have been reported previously.⁶

5.2 Synthesis

5.2.1 General Procedure—Naltrexone (0.80g, 2.35 mmol) was dissolved in 2 ml of anhydrous ethanol under a nitrogen atmosphere. Catalytic p-toluene sulfonic acid (10 mg) and activated molecular sieves (50mg) were added. The reaction mixture was stirred for 10 min and the appropriate amine (RNH₂; 2.35 mmol) was added. The reaction mixture was refluxed for 5 hr before adding ethyl acrylate (0.26ml, 2.35 mmol) and again refluxing for 18 hr. After completion, the reaction mixture was cooled to room temperature, filtered and washed with dichloromethane (50 ml) and the organic layer was washed with water (2×20 ml) and brine (20 ml). The organic layer was dried over magnesium sulphate, concentrated under reduced pressure to obtain crude product which was purified by flash chromatography using methanol:dichloromethane:ammonium hydroxide (1:98.5:0.5). Yield (20-40 %)

5.2.2. 1'-Benzyl-17-cyclopropylmethyl-6,7-didehydro-4,5-epoxy-4',5'-dihydro-3,14-dihydroxy-pyrido[2,3: 6,7]-morphinan-6(1H)-one (5k)—White Solid; ¹H NMR (CDCl₃) δ 0.10–0.13 (2H, m), 0.51-0.54 (2H, m), 0.80-0.83 (1H, m), 1.54 (2H, d, *J*=13.00 Hz), 2.10-2.26 (4H, m), 2.34-2.46 (4H, m), 2.56-2.63 (3H, m), 3.01 (1H, d, *J*=18.48 Hz), 3.12 (1H, d, *J*=6.44Hz), 4.93 (1H, d, *J*=16.72Hz), 5.04 (1H, s), 5.33 (1H, d, *J*=16.72Hz), 6.52 (1H, d, *J*=8.16 Hz), 6.61 (1H, d, *J*=8.16 Hz), 7.19-7.25 (2H, m), 7.31-7.53 (3H, m); ¹³C NMR, (CDCl₃) δ 3.60, 3.96, 9.29, 22.82, 26.22, 30.85, 31.28, 36.92, 43.44, 44.37, 46.68, 59.31, 61.20, 70.09, 86.52, 116.80, 118.71, 118.97, 124.92, 125.82, 126.75, 128.55, 128.76, 131.02, 138.75, 138.94, 142.38, 170.41. HRMS, *m/z* for (C₃₀H₃₂N₂O₄Na) [MNa]⁺, calcd-507.2260, found-507.2242. Anal. (C₃₀H₃₂N₂O₄ ·HCl·2.5H₂O) C, H, N.

5.2.3. 1'-Phenylethyl-17-cyclopropylmethyl-6,7-didehydro-4,5-epoxy-4',5'-dihydro-3,14-dihydroxy-pyrido[2,3: 6,7]-morphinan-6(1H)-one (5l)—White Solid; ¹H NMR (CDCl₃) δ 0.13-0.13 (2H, m), 0.52-0.57 (2H, m), 0.83-0.86 (1H, m), 1.62 (1H, d, *J*=10.28 Hz), 1.82-1.85 (1H, m), 2.18-2.25 (5H, m), 2.32-2.49 (3H, m), 2.59-2.68 (2H, m), 2.77-2.91 (3H, m), 3.04 (1H, d, *J*=18.48 Hz), 3.16 (1H, d, *J*=6.44Hz), 3.67-3.71 (1H, m), 4.42-4.45 (1H, m), 5.03 (1H, s), 6.54 (1H, d, *J*=8.16 Hz), 6.66 (1H, d, *J*=8.16 Hz), 7.15-7.21 (2H, m), 7.26-7.30 (3H, m); ¹³C NMR, (CDCl₃) δ 3.61, 4.02, 9.32, 22.84, 25.86, 30.73, 31.60, 35.37, 36.73, 42.36, 43.55, 46.59, 59.34, 61.16, 69.94, 85.83, 117.11, 118.89, 119.27, 124.71, 126.27, 128.40, 128.61, 128.89, 131.07, 138.89, 139.35, 142.70, 170.16. HRMS, *m/z* for (C₃₁H₃₄N₂O₄Na) [MNa]⁺, calcd-521.2416, found-521.2420. Anal. (C₃₁H₃₄N₂O₄ ·HCl·2.75H₂O) C, H, N.

5.2.4. 1'-Phenylethyl-17-allyl-6,7-didehydro-4,5-epoxy-4',5'-dihydro-3,14-dihydroxy-pyrido[2,3: 6,7]-morphinan-6(1H)-one (5m)—White Solid; ¹H NMR (CDCl₃) δ 1.61-1.65 (2H, m), 1.83-1.86 (1H, m), 2.17-2.25 (5H, m), 2.36-2.40 (1H, m), 2.56-2.66 (2H, m), 2.84-2.91 (2H, m), 2.97 (1H, d, *J*=6.20 Hz), 3.08-3.14 (3H, m), 3.66-3.72 (1H, m), 4.41-4.46 (1H, m), 4.64 (1H, bd), 5.02 (1H, s), 5.16 (2H, t, *J*=9.12 Hz), 5.78-5.82

(1H, m), 6.56 (1H, d, $J=8.16$ Hz), 6.67 (1H, d, $J=8.16$ Hz), 7.21-7.30 (5H, m); ^{13}C NMR, (CDCl_3) δ 22.92, 25.90, 30.82, 31.43, 35.39, 36.69, 42.36, 43.24, 46.55, 57.77, 61.40, 70.02, 86.01, 116.93, 118.00, 118.97, 119.17, 124.91, 126.30, 128.33, 128.47, 128.84, 131.01, 135.11, 138.91, 139.07, 142.53, 170.06. HRMS, m/z for ($\text{C}_{30}\text{H}_{32}\text{N}_2\text{O}_4\text{Na}$) $[\text{MNa}]^+$, calcd-507.2260, found-507.2269. Anal. ($\text{C}_{30}\text{H}_{32}\text{N}_2\text{O}_4 \cdot \text{HCl} \cdot 1.9\text{H}_2\text{O}$) C, H, N.

5.2.5. 1'-Cyclohexylmethyl-17-cyclopropylmethyl-6,7-didehydro-4,5-epoxy-4', 5'-dihydro-3,14-dihydroxy-pyrido[2,3: 6,7]-morphinan-6(1H)-one (5n)—White Solid; ^1H NMR (CDCl_3) δ 0.11-0.15 (2H, m), 0.52-0.56 (2H, m), 0.83-0.85 (1H, m), 1.01-1.05 (2H, m), 1.17-1.25 (3H, m), 1.45-1.52 (1H, m), 1.65-1.78 (7H, m), 2.03-2.36 (5H, m), 2.38-2.43 (4H, m), 2.54-2.65 (2H, m), 3.04 (1H, d, $J=18.52$ Hz), 3.13 (1H, d, $J=6.36$ Hz), 3.20 (1H, dd, $J=5.84$ and 14.32 Hz), 4.17 (1H, dd, $J=8.76$ and 14.32 Hz), 5.08 (1H, s), 6.53 (1H, d, $J=8.16$ Hz), 6.66 (1H, d, $J=8.16$ Hz); ^{13}C NMR, (CDCl_3) δ 3.71, 3.89, 9.35, 22.90, 25.50, 25.74, 26.00, 26.39, 30.40, 30.83, 30.94, 31.64, 36.61, 37.80, 43.50, 46.21, 46.52, 59.40, 61.38, 69.67, 85.39, 117.10, 118.81, 119.51, 124.59, 128.91, 131.15, 139.43, 142.67, 170.78. HRMS, m/z for ($\text{C}_{30}\text{H}_{38}\text{N}_2\text{O}_4\text{Na}$) $[\text{MNa}]^+$, calcd-513.2729, found-513.2741. Anal. ($\text{C}_{30}\text{H}_{38}\text{N}_2\text{O}_4 \cdot \text{HCl} \cdot \text{H}_2\text{O} \cdot 0.7\text{CH}_2\text{Cl}_2$) C, H, N.

5.2.6. 1'-(*m*-Chlorobenzyl)-17-(cyclopropylmethyl)-6,7-didehydro-4,5-epoxy-4', 5'-dihydro-3,14-dihydroxy-pyrido[2,3: 6,7]-morphinan-6(1H)-one (5o)—White Solid; ^1H NMR (CDCl_3) δ 0.11-0.13 (2H, m), 0.51-0.54 (2H, m), 0.80-0.83 (1H, m), 1.54 (2H, d, $J=12.92$ Hz), 2.05-2.26 (4H, m), 2.34-2.41 (4H, m), 2.56-2.65 (3H, m), 3.02 (1H, d, $J=18.48$ Hz), 3.14 (1H, d, $J=6.44$ Hz), 4.81 (1H, d, $J=16.76$ Hz), 4.96 (1H, s), 5.38 (1H, d, $J=16.76$ Hz), 6.53 (1H, d, $J=8.16$ Hz), 6.64 (1H, d, $J=8.16$ Hz), 7.08 (1H, d, $J=7.12$ Hz), 7.21 (3H, m); ^{13}C NMR, (CDCl_3) δ 3.61, 3.97, 9.29, 22.82, 26.06, 30.69, 31.37, 36.82, 43.43, 43.88, 46.67, 59.32, 61.16, 70.03, 86.21, 116.94, 119.06, 119.08, 124.08, 124.86, 126.35, 127.07, 128.55, 129.83, 130.97, 134.52, 139.08, 140.74, 142.38, 170.52. HRMS, m/z for ($\text{C}_{30}\text{H}_{32}\text{ClN}_2\text{O}_4$) $[\text{MH}]^+$, calcd-519.2051, found-519.2052. Anal. ($\text{C}_{30}\text{H}_{31}\text{ClN}_2\text{O}_4 \cdot \text{HCl} \cdot \text{H}_2\text{O} \cdot 0.4\text{CH}_2\text{Cl}_2$) C, H, N.

5.2.7. 1'-(*m*-Fluorobenzyl)-17-(cyclopropylmethyl)-6,7-didehydro-4,5-epoxy-4', 5'-dihydro-3,14-dihydroxy-pyrido[2,3: 6,7]-morphinan-6(1H)-one (5p)—White Solid; ^1H NMR (CDCl_3) δ 0.11-0.13 (2H, m), 0.51-0.54 (2H, m), 0.81-0.83 (1H, m), 1.54 (2H, d, $J=12.92$ Hz), 2.05-2.21 (4H, m), 2.34-2.45 (4H, m), 2.56-2.65 (3H, m), 3.02 (1H, d, $J=18.48$ Hz), 3.13 (1H, d, $J=6.44$ Hz), 4.84 (1H, d, $J=16.72$ Hz), 4.98 (1H, s), 5.39 (1H, d, $J=16.72$ Hz), 6.54 (1H, d, $J=8.16$ Hz), 6.64 (1H, d, $J=8.16$ Hz), 6.90-6.95 (2H, m), 6.99 (1H, d, $J=7.60$ Hz), 7.26-7.30 (1H, m); ^{13}C NMR, (CDCl_3) δ 3.62, 3.95, 9.29, 22.83, 26.12, 30.73, 31.34, 36.86, 43.43, 43.93, 46.68, 59.32, 61.19, 70.03, 86.34, 112.96, 113.18, 113.66, 113.87, 116.86, 119.01, 121.48, 124.94, 128.55, 130.00, 130.98, 138.98, 141.37, 142.33, 170.44. HRMS, m/z for ($\text{C}_{30}\text{H}_{32}\text{FN}_2\text{O}_4$) $[\text{MH}]^+$, calcd-503.2346, found-503.2330. Anal. ($\text{C}_{30}\text{H}_{31}\text{FN}_2\text{O}_4 \cdot \text{HCl} \cdot \text{H}_2\text{O} \cdot 0.3\text{CH}_2\text{Cl}_2$) C, H, N.

5.3 Biological Assays

5.3.1 Cell culture—All receptors were in CHO cells transfected with human receptor cDNA. The cells were grown in Dulbecco's Modified Eagle's Medium with 10% fetal

bovine serum, in the presence of 0.4 mg ml⁻¹ G418 and 0.1% penicillin/streptomycin, in 100-mm plastic culture dishes. For binding assays, the cells were scraped off the plate at confluence.

5.3.2 Receptor binding—Binding to cell membranes was conducted in a 96-well format. Cells were removed from the plates by scraping with a rubber policeman, homogenized in 50 mM Tris pH 7.5, using a Polytron homogenizer, then centrifuged once and washed by an additional centrifugation at 27 000 g for 15 min. The pellet was resuspended in Tris, and the suspension incubated with [³H]DAMGO ([d-Ala², N-Me-Phe⁴, Gly⁵-ol]enkephalin) (51 Ci mmol⁻¹, 1.6 nM), [³H]Cl-DPDPE ([d-Pen², d-Pen⁵]enkephalin) (42 Ci mmol⁻¹, 1.4 nM), [³H]U69593 (41.7 Ci mmol⁻¹, 1.9 nM) or [³H]nociceptin (120 Ci mmol⁻¹, 0.2 nM) for binding to, MOP, DOP, KOP and NOP receptors, respectively. Nonspecific binding was determined with 1 μM of unlabelled DAMGO, DPDPE, ethylketocyclazocine and N/OFQ, respectively. Total volume of incubation was 1.0 ml and samples were incubated for 60 min at 25 °C. The amount of protein in the binding assay was 15 μg. The reaction was terminated by filtration using a Tomtec 96 harvester (Orange, CT, USA) through glass fibre filters. Bound radioactivity was counted on a Pharmacia Biotech beta-plate liquid scintillation counter (Piscataway, NJ, USA) and expressed in counts per minute. IC₅₀ values were determined using at least six concentrations of each compound and calculated using Graphpad/Prism (ISI, San Diego, CA, USA).

5.3.3 [³⁵S]GTPγS binding—[³⁵S]GTPγS binding was conducted basically as described by Traynor and Nahorski. Cells were scraped from tissue culture dishes into 20 mM HEPES, 1 mM EDTA, then centrifuged at 500 g for 10 min. Cells were resuspended in this buffer and homogenized using a Polytron Homogenizer. The homogenate was centrifuged at 27 000 g for 15 min, and the pellet resuspended in buffer A containing 20 mM HEPES, 10 mM MgCl₂, 100 mM NaCl, pH 7.4. The suspension was recentrifuged at 27 000 g and suspended once more in buffer A. For the binding assay, membranes (8–15 μg protein) were incubated with [³⁵S]GTPγS (50 pM), GDP (10 μM) and the appropriate compound, in a total volume of 1.0 ml, for 60 min at 25 °C. Samples were filtered over glass fibre filters and counted as described for the binding assays. Statistical analysis was conducted using the program Prism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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7. References

1. Portoghese PS, Lipkowski AW, Takemori AE. Life Sci. 1987; 40:1287. [PubMed: 2882399]
2. Portoghese PS, Sultana M, Takemori AE. J. Med. Chem. 1990; 33:1714. [PubMed: 2160538]
3. Korlipara VL, Takemori AE, Portoghese PS. J. Med. Chem. 1994; 37:1882. [PubMed: 8021928]

4. Chauvignac C, Miller CN, Srivastava SK, Lewis JW, Husbands SM, Traynon JR. *J. Med. Chem.* 2005; 48:1676. [PubMed: 15743210]
5. Srivastava SK, Husbands SM, Aceto MD, Miller CN, Traynor JR, Lewis JW. *J. Med. Chem.* 2002; 45:537. [PubMed: 11784158]
6. Shefali, Srivastava SK, Hall LD, Lewis JW, Husbands SM. *Helv. Chim. Acta.* 2002; 85:1790.
7. Srivastava SK, Shefali, Miller CN, Aceto MD, Traynor JR, Lewis JW, Husbands SM. *J. Med. Chem.* 2004; 47:6645. [PubMed: 15588100]
8. Ananthan S, Kezar HS, Carter RL, Saini SK, Rice KC, Wells JL, Davis P, Xu H, Dersch CM, Bilsky EJ, Porreca F, Rothman RB. *J. Med. Chem.* 1999; 42:3527. [PubMed: 10479286]
9. Spagnolo B, Calo G, Polgar WE, Jiang F, Olsen CM, Berzetei-Gurske I, Khroyan TV, Husbands SM, Lewis JW, Toll L, Zaveri NT. *Brit. J. Pharmacol.* 2008; 153:609. [PubMed: 18059322]
10. McCann DJ. *Clinical Pharmacology & Therapeutics.* 2008; 83:627. [PubMed: 18212797]
11. Husbands, SM. *Research and Development of Opioid-Related Ligands.* Vol. Vol. 1131. American Chemical Society; 2013. p. 127
12. Portoghese PS, Nagase H, Lipkowski AW, Larson DL, Takemori AE. *J. Med. Chem.* 1988; 31:836. [PubMed: 2832604]
13. FarouzGrant F, Portoghese PS. *J. Med. Chem.* 1997; 40:1977. [PubMed: 9207938]
14. Feldman PL, James MK, Brackeen MF, Bilotta JM, Schuster SV, Lahey AP, Lutz MW, Johnson MR, Leighton HJ. *J. Med. Chem.* 1991; 34:2202. [PubMed: 2066993]
15. Rothman, RB.; Ananthan, S.; Bilsky, EJ. *Opiate Receptors and Antagonists: From Bench to Clinic.* Humana Press; 2009. Novel Opioid Antagonists with Mixed/Dual Selectivity; p. 137-151.
16. Ananthan S, Khare NK, Saini SK, Seitz LE, Bartlett JL, Davis P, Dersch CM, Porreca F, Rothman RB, Bilsky EJ. *J. Med. Chem.* 2004; 47:1400. [PubMed: 14998329]
17. Van't Veer A, Carlezon W Jr. *Psychopharmacol.* 2013; 229:435.

Compounds of structure 5

	R	R ¹	R ²	R ³		R	R ¹	R ²	R ³
(a)	Me	Me	H	H	(i)	CPM	Bn	(CH ₂) ₂ OH	H
(b)	Me	Bn	H	H	(j)	CPM	Bn	H	(CH ₂) ₂ OH
(c)	Me	Me	(CH ₂) ₂ OH	H	(k)	CPM	Bn	H	H
(d)	Me	Me	H	(CH ₂) ₂ OH	(l)	CPM	PhEt	H	H
(e)	Me	Bn	(CH ₂) ₂ OH	H	(m)	Allyl	PhEt	H	H
(f)	Me	Bn	H	(CH ₂) ₂ OH	(n)	CPM	c-HexMe	H	H
(g)	CPM	Bn	Me	H	(o)	CPM	m-ClBn	H	H
(h)	CPM	Bn	H	Me	(p)	CPM	m-FBn	H	H

Scheme 1.i) R'NH₂, PTSA, mol. sieves, EtOH, reflux

Table 1Binding (K_i /nM) affinities to opioid receptors (and for selected compounds, to NOP receptors^a)

	R	R1	R2	R3	MOPr	Ki (nM) $\pm$ SEM^b KOPr	DOPr
5a	Me	Me	H	H	1.23 $\pm$ 0.02	20.4 $\pm$ 1.3	9.39 $\pm$ 2.1
5b	Me	Bn	H	H	0.29 $\pm$ 0.04	1.98 $\pm$ 0.19	0.92 $\pm$ 0.13
5c	Me	Me	(CH ₂) ₂ OH	H	2.37 $\pm$ 0.28	48.7 $\pm$ 1.3	3.59 $\pm$ 0.97
5d	Me	Me	H	(CH ₂) ₂ OH	12.8 $\pm$ 3.0	182 $\pm$ 63	9.25 $\pm$ 2.6
5e	Me	Bn	(CH ₂) ₂ OH	H	2.03 $\pm$ 0.47	4.68 $\pm$ 0.02	1.02 $\pm$ 0.06
5f	Me	Bn	H	(CH ₂) ₂ OH	3.00 $\pm$ 0.69	15.6 $\pm$ 2.02	2.85 $\pm$ 0.80
5g	CPM	Bn	Me	H	0.56 $\pm$ 0.03	0.95 $\pm$ 0.13	0.85 $\pm$ 0.05
5h	CPM	Bn	H	Me	0.42 $\pm$ 0.03	0.71 $\pm$ 0.01	1.58 $\pm$ 0.08
5i	CPM	Bn	(CH ₂) ₂ OH	H	0.43 $\pm$ 0.10	1.29 $\pm$ 0.18	0.70 $\pm$ 0.03
5j	CPM	Bn	H	(CH ₂) ₂ OH	1.54 $\pm$ 0.35	2.76 $\pm$ 0.91	2.38 $\pm$ 0.45
5k ^a	CPM	Bn	H	H	0.18 $\pm$ 0.05	0.15 $\pm$ 0.03	0.36 $\pm$ 0.01
5n ^a	CPM	cHexMe	H	H	0.11 $\pm$ 0.02	0.33 $\pm$ 0.05	0.28 $\pm$ 0.05
4a	Me	Bn	-	CH ₂ CO ₂ Et	13.2 $\pm$ 0.32	159 $\pm$ 22	82.1 $\pm$ 9.1
4b	CPM	Bn	-	CH ₂ CO ₂ Et	8.65 $\pm$ 0.03	4.79 $\pm$ 1.20	10.0 $\pm$ 1.3
Naltrexone	-	-	-	-	0.20 $\pm$ 0.0	0.40 $\pm$ 0.1	10.8 $\pm$ 3.0
Naltrindole	-	-	-	-	6.30 $\pm$ 2.3	10.1 $\pm$ 0.65	0.20 $\pm$ 0.05
norBNI	-	-	-	-	21.0 $\pm$ 5.0	0.20 $\pm$ 0.05	5.07 $\pm$ 0.9

^a **5k**, NOP Ki 1300 $\pm$ 800 nM; **5l**, NOP Ki 1500 $\pm$ 90 nM; **5n**, NOP Ki 880 $\pm$ 140 nM^b Affinities versus [³H]DAMGO (MOPr), [³H]U69593 (KOPr), [³H]Cl-DPDPE (DOPr) and [³H]nociceptin (NOPr)

Table 2

Functional activity; agonist stimulation of, and antagonist inhibition of, [³⁵S]GTPγS binding in cloned human opioid receptors

	R	R¹	R²	R³	EC₅₀ (nM) %stim^a or (Ke/nM) MOPr	EC₅₀ (nM) %stim^a or (Ke/nM) KOPr	EC₅₀ (nM) %stim^a or (Ke/nM) DOPr
5a	Me	Me	H	H	80.0±12 40	(392±66)	25.5±0.76 67
5b	Me	Bn	H	H	4.97±0.03 52	(21.3±2.8)	3.00±0.93 94
5c	Me	Me	(CH ₂) ₂ OH	H	163±31 36	(321±31)	11.8±0.77 66
5d	Me	Me	H	(CH ₂) ₂ OH	709±235 37	(2140±128)	30.6±1.4 80
5e^d	Me	Bn	(CH ₂) ₂ OH	H	(32.9±1.21)	(29.3±5.9)	2.37±0.45 73
5f	Me	Bn	H	(CH ₂) ₂ OH	94.9±24 36	(16.8±3.0)	10.6±3.6 68
5g	CPM	Bn	Me	H	(0.50±0.06)	(0.23±0.02)	(0.23±0.02)
5h	CPM	Bn	H	Me	(0.60±0.04)	(2.03±0.16)	(0.57±0.06)
5i	CPM	Bn	(CH ₂) ₂ OH	H	(0.35±0.02)	(0.73±0.17)	(0.47±0.01)
5j	CPM	Bn	H	(CH ₂) ₂ OH	(1.62±0.19)	(2.22±0.06)	(0.93±0.14)
5k	CPM	Bn	H	H	(0.20±0.04)	(0.13±0.08)	ND
5n	CPM	cHexMe	H	H	(0.27±0.07)	(0.48±0.11)	ND
4a	Me	Bn		CH ₂ CO ₂ Et	70.5±5.6 86	434±114 23	42.5±33 74
4b	CPM	Bn		CH ₂ CO ₂ Et	(6.61±0.71)	(4.30±0.34)	(6.79±0.81)
NTX^b	-	-	-	-	(0.59±0.04)	(1.86±0.16)	5.44±0.75
NTI^c	-	-	-	-	(4.26±0.33)	(4.95±0.32)	(0.11±0.005)
norBNI	-	-	-	-	(18.9±1.80)	(0.039±0.004)	(4.42±0.38)

^a % stimulation relative to the standards DAMGO, U69593, DPDPE or antagonist Ke/nM (quoted in brackets) versus the same standards. Ke is the antagonist equilibrium dissociation constant determined against a single concentration of agonist according to the equation $Ke = [\text{antagonist}]/\text{dose-ratio} - 1$;

^b NTX, naltrexone;

^c NTI, naltrindole;

^d **5e** had low efficacy partial agonism at MOPr, 17% of DAMGO.

Table 3Maximal stimulation of [³⁵S]GTP γ S binding of analogues **5k** – **5o** to MOP, KOP and NOP receptors

	<u>Maximum % stim^a</u>		
	MOPr	KOPr	NOPr
5k	-1 $\pm$ 4	-20 $\pm$ 9	12 $\pm$ 3
5l	1 $\pm$ 6	-10 $\pm$ 4	5 $\pm$ 7
5m	-2 $\pm$ 2	-15 $\pm$ 7	4 $\pm$ 5
5n	-1 $\pm$ 1	-5 $\pm$ 8	5 $\pm$ 3
5p	-1 $\pm$ 1	-9 $\pm$ 8	7 $\pm$ 4
5o	-1 $\pm$ 1	-11 $\pm$ 10	7 $\pm$ 4

^a At 10 μ M compared to the standard agonists

DAMGO (MOP), U69593 (KOP), nociceptin (NOP)