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Selectively Promiscuous Opioid Ligands: Discovery of High Affinity/Low Efficacy Opioid Ligands with Substantial Nociceptin Opioid Peptide Receptor Affinity

Vinod Kumar,[†,⊥] Irna E. Ridzwan,[†] Konstantinos Grivas,[§] John W. Lewis,[†] Mary J. Clark,[‡] Claire Meurice,[‡] Corina Jimenez-Gomez,[‡] Irina Pogozheva,[∥] Henry Mosberg,[∥] John R. Traynor,[‡] and Stephen M. Husbands*[,†]

[†]Department of Pharmacy and Pharmacology, University of Bath, Bath, BA2 7AY, U.K.

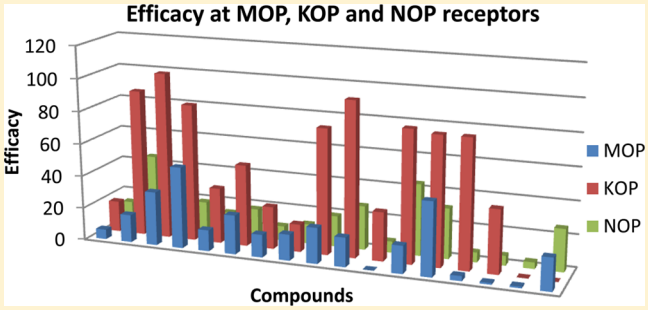
[‡]Department of Pharmacology, University of Michigan, Ann Arbor, Michigan 48109, United States

[§]School of Chemistry, University of Bristol, Bristol, BS8 1TS, U.K.

[∥]College of Pharmacy, University of Michigan, Ann Arbor, Michigan 48109, United States

S *Supporting Information*

ABSTRACT: Emerging clinical and preclinical evidence suggests that a compound displaying high affinity for μ, κ, and δ opioid (MOP, KOP, and DOP) receptors and antagonist activity at each, coupled with moderate affinity and efficacy at nociceptin opioid peptide (NOP) receptors will have utility as a relapse prevention agent for multiple types of drug abuse. Members of the orvinol family of opioid ligands have the desired affinity profile but have typically displayed substantial efficacy at MOP and or KOP receptors. In this study it is shown that a phenyl ring analogue (**1d**) of buprenorphine displays the desired profile in vitro with high, nonselective affinity for the MOP, KOP, and DOP receptors coupled with moderate affinity for NOP receptors. In vivo, **1d** lacked any opioid agonist activity and was an antagonist of both the MOP receptor agonist morphine and the KOP receptor agonist ethylketocyclazocine, confirming the desired opioid receptor profile in vivo.



INTRODUCTION

The orvinols are a group of ring-C bridged epoxymorphinan compounds that were originally synthesized by Bentley and co-workers[1−3] and developed by Reckitt and Colman.[4] The most studied members of the series include the very potent opiate antagonist diprenorphine (**1a**)[1] and buprenorphine (**1b**) (Chart 1), the clinical analgesic and treatment agent for opiate abuse and addiction.[5−8]

Buprenorphine displays a unique and complex pharmacology derived from the manner in which it binds to opioid receptors.[9−11] At the μ opioid (MOP) receptor it is a partial agonist with high affinity and slow onset and offset, thus having "irreversible" characteristics, manifested in its long duration of action and the mildness of abstinence effects when the drug is withdrawn following chronic administration. At the other opioid receptors, κ (KOP) and δ (DOP) buprenorphine has negligible efficacy and is a potent antagonist of KOP and DOP receptor agonists. In addition to its binding to these classical opioid receptors, buprenorphine also binds as a partial agonist of moderate affinity to the nociceptin opioid peptide (NOP) receptor that, though having a high degree of amino acid sequence homology with the classical opioid receptors, nevertheless has negligible affinity for most opioid ligands. Buprenorphine's KOP and DOP receptor antagonism and NOP receptor partial agonism appear to contribute to its demonstrated potential as a treatment for cocaine and ethanol abuse and dependence in addition to its approved use in opiate abuse and dependence that is derived from its MOP receptor partial agonism.[8]

Use of buprenorphine to treat cocaine and alcohol abuse would not be allowed in patients without concurrent opiate abuse problems, since buprenorphine treatment supports a significant level of MOP receptor dependence.[7] Thus, one of our medicinal chemistry objectives has been to discover, among structural analogues of buprenorphine, ligands having MOP and KOP receptor antagonist and NOP receptor agonist or partial agonist activity. Such a compound should be more widely useful than buprenorphine in the treatment of cocaine and alcohol abuse and dependence.

Structure−activity relationship studies in orvinols of structures **1** and **2** (Chart 1) based solely on in vivo antinociception data demonstrated that in structure **1** only when R^1 and R^2 were H or methyl was MOPr antagonist

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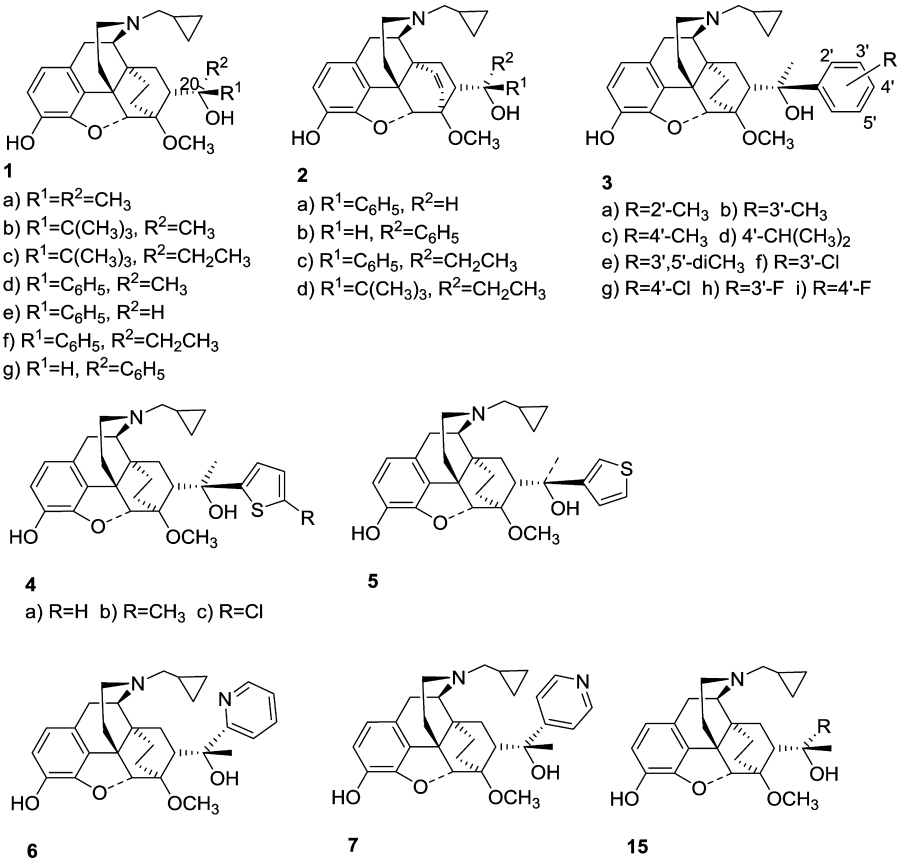
Chart 1



activity shown without accompanying antinociceptive activity.[1,12] These studies did not rule out the possibility that these MOPr antagonists may have had low efficacy KOPr and DOPr activity below the level required to produce an antinociceptive response in the mouse antiwrithing test. Nevertheless it was clear that when R^1 and R^2 in structure **1** were alkyl groups larger than methyl, substantial MOPr activity was normally found. Further insight into orvinol SAR has recently been provided with the first publication of affinity and efficacy data for a sizable series of predominantly branched-chain orvinols.[13] Substantial efficacy for MOPr and KOPr was found to be the norm. KOPr agonist efficacy, which would translate in humans as debilitating dysphoric side effects, is dominant. Twenty-five of the 39 compounds tested for KOPr efficacy showed full KOPr agonism (>85% of the response to the full KOPr agonist, U69593), and the others showed a partial KOPr agonist response (30−75% of U69593). The notable exception was buprenorphine, which gave zero KOPr agonist response. Three out of the 40 compounds tested had zero MOPr efficacy, and three had 80% or greater (compared to the full MOPr agonist DAMGO = 100%). The majority, including buprenorphine, demonstrated efficacy between these limits. A CoMFA (comparative molecular field analysis) model developed using this series of ligands suggested that a bulky group immediately adjacent to C20 was key to obtaining low efficacy at KOP receptors when R^1 in **1** was larger than methyl.

This led to an investigation of orvinol structures having a large lipophilic moiety directly attached to C20, during which we synthesized a set of phenyl orvinols (**1d−f**, **2a−c**). One of these ligands (**1d**) was proven to lack any significant MOPr or KOPr efficacy in vitro or in vivo. This led us to synthesize and evaluate a number of analogues. The results of these studies are reported below along with C20-ethyl homologues, as the effect of increasing lipophilicity/bulk through manipulation of the C20-methyl group has also not been explored previously.

SYNTHESIS

All tertiary alcohols were accessed by Grignard addition to the known methyl ketone (**8**), itself prepared by the recently reported methods of Greedy et al. (Scheme 1).[13] No addition could be achieved with pyridyl Grignard reagents, and so pyridyl lithium addition to the ketone was attempted. This was successful but interestingly gave the opposite diastereomer to that expected (Scheme 1), i.e., opposite to that obtained from standard Grignard addition (confirmed by X-ray crystallography; see Supporting Information). It appears that the reactive aryllithium addition follows the Felkin−Ahn model[14] such that the carbonyl oxygen would be oriented between the large (C6) and medium (C8) neighboring groups, orthogonal to the large (C6) group. Attack of the nucleophile then occurs *anti* to C6 (Figure 1a). Addition of the less reactive Grignard reagents appears to require initial formation of a six-membered chelate ring (Figure 1b), providing an ordered and activated complex, with nucleophilic addition then occurring from the less hindered (C7-H) face to afford the other epimer. Access to the secondary phenyl alcohols (**1e**, **1g**, **2a**, **2b**) was via the known aldehydes (**11a**,**b**)[13] (Scheme 2). Addition of phenylmagnesium bromide to **11a** and **11b** gave **12a** and **12b**, respectively, the opposite diastereoisomer to that obtained on Grignard addition to the methyl ketone (as shown in Scheme 1). Presumably, addition to the more reactive aldehyde does not require formation of the active complex, and so addition

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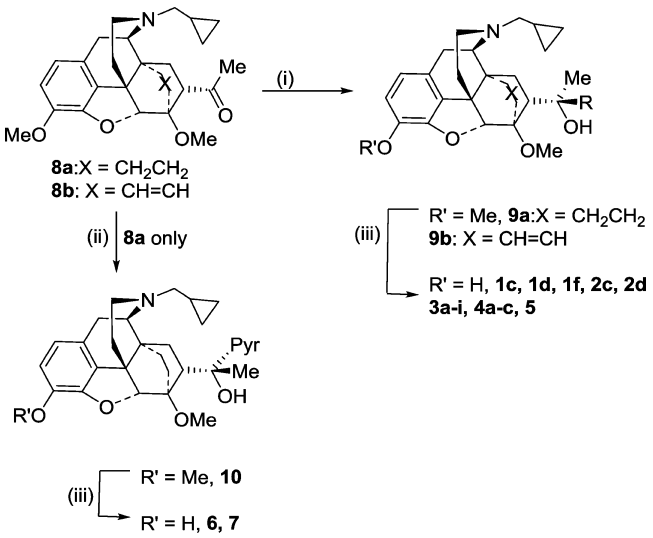
Scheme 1[a]



[a]Reagents and conditions: (i) RMgBr, THF, rt; (ii) 2-pyridyllithium or 4-pyridyllithium, Et_2O, THF, −78 °C → rt; (iii) PrSNa, HMPA, 110 °C or L-selectride, THF, reflux.

follows the Felkin−Ahn model. Subsequent oxidation to **13a** and **13b** followed by then reduction with lithium aluminum hydride provided the opposite diastereoisomers (**14a** and **14b**). Finally, 3-O-demethylation gave the desired phenolic products **1e** and **2a**.

RESULTS

In Vitro. Opioid receptor binding affinities of the first series of phenyl orvinol analogues (**1d−g**, **2a−d**) were determined by displacement of [^{3}H]DAMGO, [^{3}H]-DPDPE, [^{3}H]U69,593, and [^{3}H]N/OFQ from human opioid receptors transfected into Chinese hamster ovary (CHO) cells. Details of these assays have been described previously.[15]

As expected, in these assays the new compounds all had high affinity for all MOP, DOP, and KOP receptors with no evidence of any selectivity for an individual receptor type (Table 1). Changing R^2 from methyl to ethyl (**1d** to **1f**) had no effect on binding affinity, whereas secondary alcohol C20 groups were associated with a slightly lower affinity than tertiary alcohol groups (e.g., **1e** versus **1d**, **1f**), particularly at KOP and DOP receptors. The diasereoisomers **2a** and **2b** both displayed nonselective binding, though absolute affinities could not be compared because **2b** was evaluated in a separate assay. Nevertheless all binding affinities were in the nanomolar range.

In the [^{35}S]GTPγS assay for functional opioid activity at MOP, DOP, and KOP receptors using methods reported previously,[15] only **1d** was a potent MOP and KOP receptor antagonist (Table 1); it failed to show MOP receptor agonist activity and had very low level KOP receptor efficacy (Table 2). The other phenyl orvinols were MOP receptor partial agonists of efficacy ranging from the low (**1f**, **2a**) to moderately high (**2c**). They were generally high efficacy KOP receptor partial to full agonists. They showed a similar pattern of agonist efficacy at DOP receptors, at which **1d** was also a partial agonist of modest efficacy. It was striking that **1f** and **2c** which differ structurally only in the 6,14-bridge differed markedly in efficacy for MOP, DOP, and KOP receptor types with the etheno-bridged ligand **2c** having markedly higher efficacy.

The effect of replacing the R^2 methyl group in buprenorphine (**1b**) by ethyl was also investigated. The homologue (**1c**) had comparable affinities at MOP, DOP, and KOP receptors. Affinity for NOP receptors was also measured and compared with that of buprenorphine; again affinities were comparable (Table 1). The etheno analogue (**2d**) of **1c** was also evaluated. Binding affinity for MOP, DOP, and KOP receptors was similar to that of **1c**, but NOP receptor affinity was lower. In [^{35}S]GTPγS assays **1c** had somewhat higher MOP receptor efficacy than buprenorphine while the etheno analogue (**2d**) was an almost full MOP receptor agonist though with very modest potency. **1c** had high KOP receptor

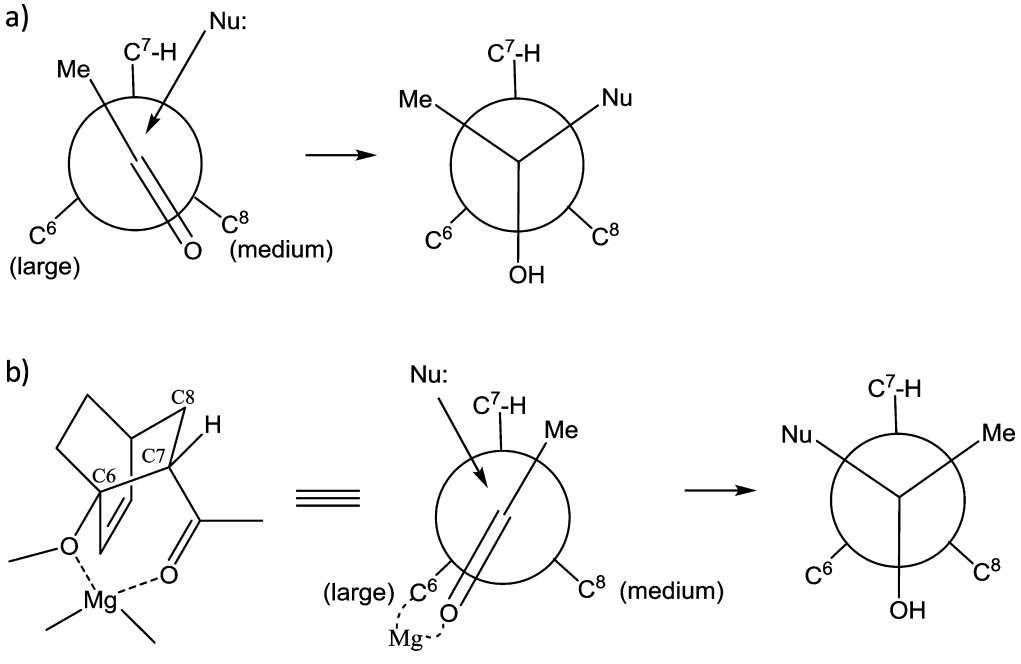


Figure 1. Nucleophilic addition (a) without chelation and (b) with chelation control.

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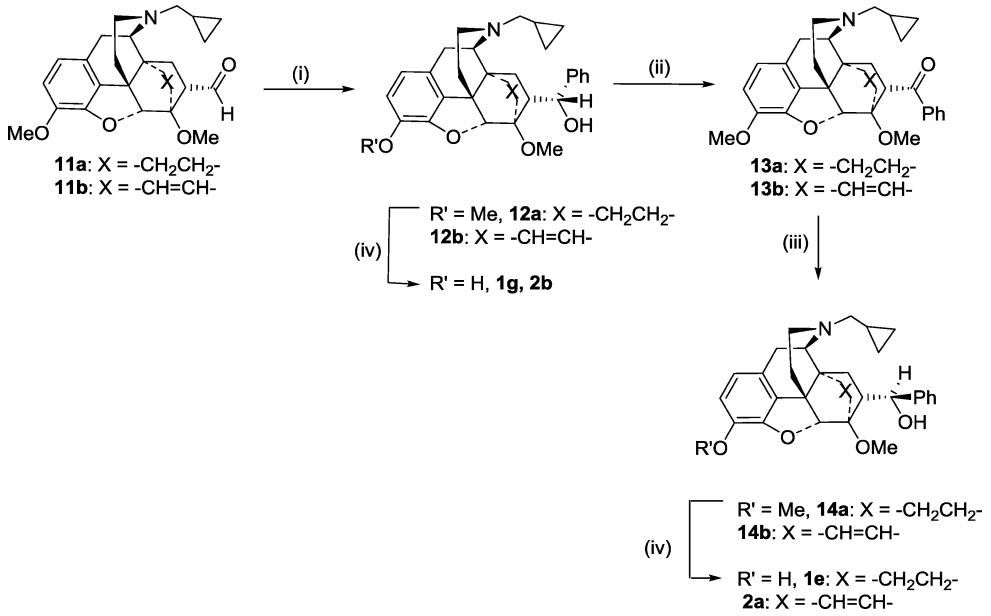
Scheme 2[a]



[a]Reagents and conditions: (i) PhMgBr, THF, rt; (ii) $(COCl)_2$, DMSO, DMSO, CH_2Cl_2, −78 °C; (iii) $LiAlH_4$, ether; (iv) PrSNa, HMPA, 110 °C or L-selectride, THF, reflux.

Table 1. Binding Affinities (K_i, nM) and stimulation of [^{35}S]GTPγS Binding of Series 1 and 2 to Opioid Receptors

	K_i, nM [a,e]				EC_{50}, nM; % stim[b] or [K_e, nM][c,e]		
	MOP	KOP	DOP	NOP	MOP	KOP	DOP
1b	1.5 ± 0.80	2.5 ± 1.2	6.1 ± 0.40	77 ± 16	10.2 ± 2.2; 29 ± 1.1	NS	NS
1c	4.0 ± 0.57	0.56 ± 0.01	0.86 ± 0.02	105 ± 4.0	50.2 ± 6.6; 40 ± 3.7	183 ± 9.4; 73 ± 17	>10000
1d	0.71 ± 0.17	0.49 ± 0.08	1.9 ± 0.33	NT	[0.47 ± 0.03]	[0.27 ± 0.03]	2.66 ± 0.34; 34 ± 8.0
1e	1.3 ± 0.39	4.4 ± 1.6	2.6 ± 0.22	NT	10.4 ± 2.7; 32 ± 5.9	1.09 ± 0.0; 67 ± 1.4	4.54 ± 0.58; 90 ± 5.1
1f	1.0 ± 0.15	0.36 ± 0.04	0.80 ± 0.05	396 ± 41	18.4 ± 5.7; 18 ± 1.0	249 ± 120; 22 ± 4.4	8.90 ± 1.8; 30 ± 7.5
1g	0.82 ± 0.30	0.88 ± 0.03	1.3 ± 0.36	NT	1.55 ± 0.28; 37 ± 1.1	0.36 ± 0.03; 79 ± 5.0	0.64 ± 0.17; 113 ± 3.8
2a	4.0 ± 0.63	3.8 ± 0.74	3.2 ± 0.48	NT	2.75 ± 1.05; 18 ± 0.9	2.10 ± 0.49; 70 ± 4.6	1.76 ± 0.46; 55 ± 0.82
2b[d]	0.80 ± 0.50	1.5 ± 0.95	0.40 ± 0.0	NT	NT	NT	NT
2c	3.2 ± 0.38	0.95 ± 0.26	1.2 ± 0.14	197 ± 0.21	31.8 ± 18.5; 56 ± 13	56.6 ± 11; 128 ± 2.4	10.2 ± 2.4; 123 ± 22
2d	4.2 ± 0.60	0.75 ± 0.11	1.2 ± 0.16	187 ± 27	238 ± 19; 84 ± 3.5	122 ± 63; 57 ± 5.6	314 ± 11; 115 ± 5.2

[a]Displacement of [^{3}H]DAMGO, [^{3}H]-DPDPE, [^{3}H]U69,593, and [^{3}H]N/OFQ from human opioid receptors transfected into Chinese hamster ovary (CHO) cells. [b]% maximal stimulation with respect to the standard agonists DAMGO (MOP), U69,593 (KOP), and DPDPE (DOP). [c]Values in brackets are antagonist K_e values versus the standard agonists DAMGO (MOP), U69,593 (KOP), and DPDPE (DOP). Values are the average ± SEM from three separate experiments. [d]Binding to Hartley guinea pig brain membranes, K_i (nM) versus [^{3}H]DAMGO, [^{3}H]DPDPE, [^{3}H]U69,593. [e]NS: no stimulation. NT: not tested.

efficacy, whereas buprenorphine is a KOP receptor antagonist; **1c** like buprenorphine has no efficacy for DOP receptors. In that respect there was a remarkable difference between **1c** and the etheno analogue (**2d**) which was a full DOP receptor agonist though of low potency.

We followed up on these findings by focusing on close analogues of **1d** to identify which had the desired profile of MOP and KOP receptor antagonism. To expedite this process, a primary assay was established whereby compounds were evaluated in the [^{35}S]GTPγS assay for MOP, KOP, and NOP receptor efficacy at a very high concentration (10 μM) to determine peak efficacy at each receptor (Table 2). Nine phenyl substituted analogues of **1d** and six analogues with the phenyl group of **1d** replaced by heteroaryl rings were evaluated. A limited number of compounds were also evaluated for affinity at MOP, DOP, and KOP receptors by measuring displacement of [^{3}H]diprenorphine binding from C6-rat glioma cells expressing recombinant rat MOP and DOP receptors and CHO cells expressing recombinant human KOP receptors, essentially to confirm the expected high affinity of the series at these receptors. NOP receptor binding affinity was measured by displacement of [^{3}H]N/OFQ from membranes of HEK cells expressing recombinant NOP receptor. Details of these assays have been described previously.[16]

With respect to MOP receptor efficacy, the rank order for the methyl substituted derivatives of **1d** was 4′ (**3c**) > 3′ (**3b**) > 2′ (**3a**) > H (**1d**) (Table 2). All three methyl substituted derivatives were full KOP receptor agonists, whereas NOP receptor efficacy was in the order 2′ > 3′ = 4′ = H. The 4′-isopropyl derivative (**3d**) had low MOP and NOP receptor efficacy and lower KOP receptor efficacy than the methyl derivative. The 3′- and 4′-chloro derivatives showed all-round low efficacy though slightly higher than for the parent. The 3′- and 4′-fluoro derivatives had low efficacy MOP and NOP

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Table 2. Binding Affinities (K_i, nM) and Maximal Stimulation of [^{35}S]GTPγS Binding of 1d and Analogues to Opioid Receptors

	% stim[a]			K_i, nM[b]			
	MOP	KOP	NOP	MOP	KOP	DOP	NOP
1d	6.0 ± 1	19 ± 4	14 ± 4	0.17 ± 0.05	0.044 ± 0.015		43.2 ± 13.4
3a	17 ± 4	90 ± 3	45 ± 4	0.19 ± 0.08	0.16 ± 0.09		
3b	33 ± 5	102 ± 1	22 ± 4				
3c	50 ± 2	84 ± 7	19 ± 5				
3d	13 ± 3	34 ± 3	14 ± 6				
3e	24 ± 7	50 ± 6	18 ± 2	0.28 ± 0.16	0.10 ± 0.04		
3f	14 ± 4	26 ± 2	9 ± 5				
3g	16 ± 4	17 ± 3	12 ± 4				
3h	22 ± 2	77 ± 1	19 ± 10				
3i	18 ± 4	95 ± 4	27 ± 2				
4a	0 ± 1	30 ± 6	7 ± 5	0.6 ± 0.14	2.8 ± 0.78	1.0 ± 0.22	75 ± 4.2
4b	17 ± 3	81 ± 1	44 ± 6				
4c	45 ± 3	79 ± 6	31 ± 4				
5	3 ± 2	79 ± 2	6 ± 4				
6	1 ± 3	39 ± 12	6 ± 3				
7	1 ± 3	−17 ± 7	4 ± 3	0.16 ± 0.04	0.39 ± 0.09	0.99 ± 0.43	4630 ± 380
1b	20 ± 6	0 ± 6	26 ± 2	0.13 ± 0.02	0.089 ± 0.023	0.48 ± 0.26	212 ± 7

[a]Percent maximal stimulation (% stim) at a single high dose (10 μM) with respect to the standard agonists DAMGO (MOP) and U69,593 (KOP) and nociceptin (NOP). Values are an average ± SEM from three separate experiments. [b]K_i (nM) versus [^{3}H]diprenorphine (for MOP and KOP receptors) and [^{3}H]N/OFQ (for NOP receptors). Values are an average ± SEM from three separate experiments.

receptor agonist activity but high efficacy KOP receptor agonist activity (Table 2).

Screening of the heteroaryl analogues (**4**, **5**, **6**, **7**) of **1d** was undertaken using the same protocols. The 2-thienyl ligand (**4a**) had zero MOP receptor efficacy in the [^{35}S]GTPγS assay, whereas its 5-methyl (**4b**) and 5-chloro (**4c**) substituted derivatives were MOP receptor partial agonists with efficacy respectively similar to and significantly higher than that of buprenorphine (Table 2). **4a** had modest KOP receptor efficacy, whereas **4b** and **4c** were almost full agonists. **4a** had low NOP receptor efficacy but had binding affinity for this receptor (K_i = 75 nM) equal to or better than that of buprenorphine (**1b**) (K_i = 212 nM). The 3-thienyl analogue (**5**) had MOP and NOP receptor efficacy similar to that of **4a** but higher KOP receptor efficacy.

The isomeric 2′- and 4′-pyridyl ligands (**6**, **7**) both had low efficacy for MOP receptor and NOP receptor, but whereas **7** also had no efficacy for KOP receptor, **6** showed distinct KOP receptor activity. In binding assays, **7** showed all-round high affinity for opioid receptors and affinity for NOP receptors equivalent to **1d**.

In Vivo. Compound **1d** was evaluated in vivo to confirm the lack of MOP and KOP receptor agonism. In the hot-plate test **1d** showed no antinociceptive activity and instead was an antagonist of both the MOP receptor agonist morphine and the KOP receptor agonist ethylketocyclazocine (EKC). At 10 mg/kg, **1d** caused a parallel shift to the right in the dose−response curve for morphine (Figure 2A), and for EKC there was a complete flattening of the dose−response curve (Figure 2B). The effect of **1d** antagonism at both receptors was gone by 24 h.

The hot-plate test uses heat as the nociceptive stimulus and so requires high agonist efficacy in a compound to provide antinociception.[17] Therefore, we checked for agonism in **1d** using the lower agonist efficacy requiring acetic acid stretch test. In this test **1d** also showed no agonist activity (Figure 3A) up to 32 mg/kg. In contrast buprenorphine was potent and fully efficacious in this assay (Figure 3B), affording an ED_{50} value

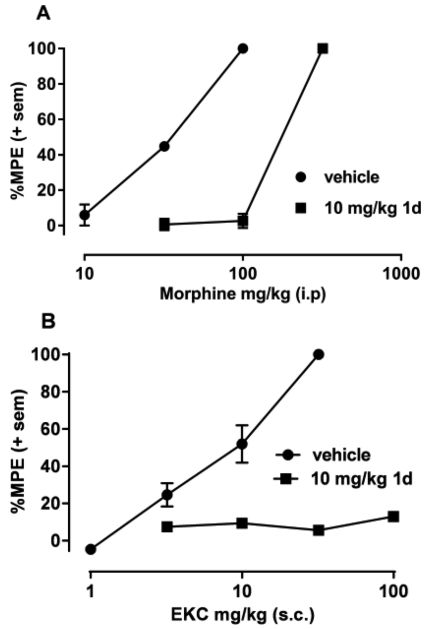

Figure 2. Antinociceptive effect using the hot-plate assay in mice of (A) morphine and (B) EKC in the absence and presence of 10 mg/kg **1d**. **1d** was given as a 30 min pretreatment. Morphine and EKC were administered by a cumulative dosing procedure by intraperitoneal (ip) and subcutaneous (sc) injections, respectively, as described.[28] **1d** was given ip. Vehicle is a 1:1:9 solution of ethanol, emulphor (oil), and sterile water.[29] Data represent the mean ± SEM from five to six mice.

(determined by nonlinear regression analysis) of 0.16 mg/kg, similar to the value (0.07 mg/kg) previously reported.[18] These findings confirm that **1d** has no, or extremely low, efficacy at MOP or KOP receptors in vivo.

DISCUSSION

In this study of analogues of buprenorphine the aim was to identify orvinols with zero or very low efficacy for MOP and

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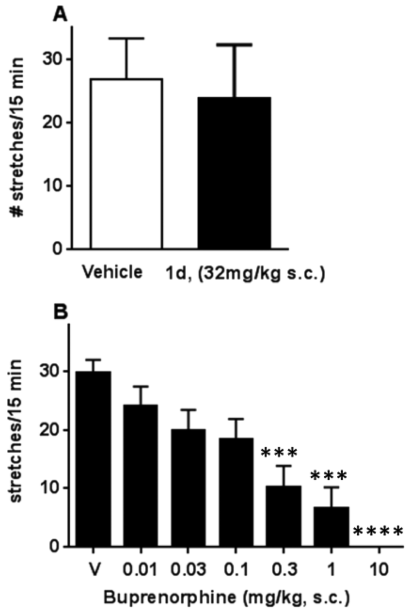

Figure 3. (A) Lack of antinociceptive effect of **1d** at 32 mg/kg in the acetic acid stretch assay in mice. (B) Buprenorphine is a full agonist in this assay. The assay was performed as described.[28] Separate groups of mice were used for each dose. Data represent the mean ± SEM from six mice. Vehicle is as in Figure 2. (***) $p < 0.001$; (****) $p < 0.0001$.

KOP receptors together with buprenorphine-like affinity and efficacy for NOP receptors. The criterion of low MOP and KOP receptor efficacy was achieved in several orvinols, but the NOP receptor criterion proved to be very difficult to achieve. The only ligands with NOP receptor efficacy equal or greater than that of buprenorphine (**1b**) had very much higher KOP receptor efficacy which would be associated with dysphoric side effects in clinical use. From the little data reported to date, finding either significant MOP or KOP receptor efficacy in orvinols that also have affinity and efficacy at NOP receptors is the norm.[19,20] The most interesting candidate is **1d** which satisfies the MOP and KOP receptor efficacy criteria and also has higher binding affinity for NOP receptors than buprenorphine. However, its NOP receptor efficacy is lower than that of buprenorphine. The lack of any activity in the acetic acid induced abdominal stretch assay, which would be expected to indicate even low level MOP or KOP receptor agonist activity and the 52 °C hot-plate antinociceptive assay, confirms, in vivo, the very low efficacy of **1d** at MOP and KOP receptors and the promise of **1d** as a lead for further investigation.

A surprising finding was the low efficacy of the pyridyl ligands **6** and **7**, in particular the latter, at both KOP and MOP receptors. It has previously been shown that the 2° alcohols having opposite relative stereochemistry to buprenorphine, i.e., **15** (Chart 1), are agonists at both receptors, typically full agonists at the KOP receptor.[13] This view was strengthened with the finding that **1g**, the phenyl ring analogue of **6** and **7**, had high efficacy at KOP receptors as predicted.

SAR at the KOP receptor was striking within this new series. Efficacy ranged from <20% to 100% with clear differences between type of substituent and less consistent differences due to substitution pattern. Of the monosubstituted phenyl analogues the larger substituents such as *i*-Pr (**3d**) and chloro (**3f**, **3g**) gave the lowest efficacy analogues while methyl (**3a** **3c**) and fluoro (**3h**, **3i**) gave full efficacy agonists. Molecular modeling of the small-molecule ligands in complex with KOP receptor in the inactive state (PBD code 4DJH) and in the activated conformation was performed as described.[21] The molecular models suggest that the ligands such as **1d** interact with the receptor in such a way as to orientate the phenyl ring into a pocket defined by residues from transmembrane helices II (Q115, L135), III (C210 and L135), VII (Y312), and extracellular loops 1 (W124) and 2 (V118, L212) (Figure 4a)

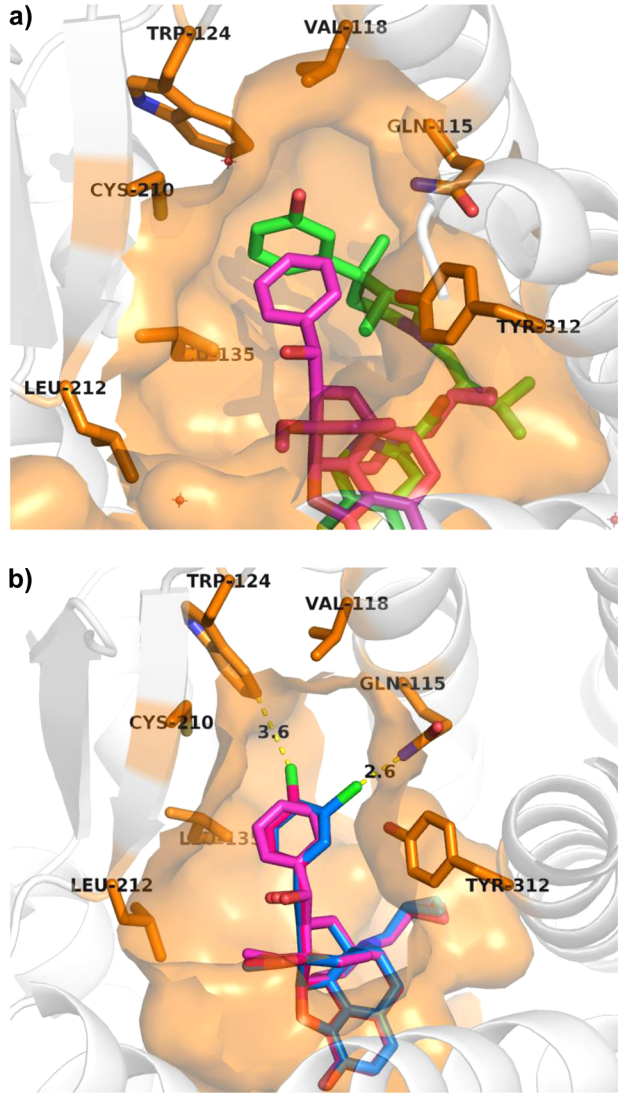

Figure 4. (a) Predicted binding mode for **1d** and analogues (magenta) in the KOPr in comparison to the crystal structure ligand JDTic (green). (b) Chloro-substituted analogues of **1d** and potential interactions with the KOPr.

This is the region occupied by the phenolic ring of JDTic in the reported crystal structure.[22] In this antagonist conformation of the receptor, the presence of substituents on the phenyl ring appears to be well tolerated, for example, potentially having interactions with W124 (for 4′-substituents) or Q115 (for 3′-substituents) or L212 and L135 (for 2′-substituents) (Figure 4b). The KOPr binding pocket in the antagonist conformation, defined by the binding of JDTic, is large but narrow and deep and somewhat covered by part of extracellular loop 3.[18] It has been proposed that the active (agonist bound) conformation of

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the receptor provides an even more restricted binding pocket.[21,23] It is therefore possible that the larger substituents, Cl and *i*-Pr in this study, cannot readily fit into the agonist conformation and are therefore predominantly antagonist in character, whereas the smaller substituents (CH_3 and F) are easily accommodated in the agonist conformation leading to strong binding to the agonist conformation. However, the low efficacy of the unsubstituted parent (**1d**) suggests that a small substituent is required for good binding to the agonist conformation.

The C20-ethyl analogues **1c**, **1f**, **2c**, and **2d** were all higher efficacy KOP receptor agonists than their C20-methyl homologues. Clearly the ethyl group does not provide the extra bulk around C20 that has been reported to minimize KOP receptor efficacy[13] but is more likely accessing the site below C8 previously identified as a region associated with KOP receptor activation.[24−26]

In vivo evaluation of the lead compound, **1d**, confirmed a lack of agonist activity in the hot-plate test and the acetic acid stretch assay which is responsive to low efficacy MOP and KOP receptor agonists. For example, buprenorphine was a fully effective agonist in this assay. The antagonist action of **1d** was observed as soon as 30 min after administration but dissipated by 24 h, confirming that **1d** is accessing the CNS, in line with its lower predicted log $D_{7.4}$ than buprenorphine (4.39 versus 4.81) and its predicted level of brain penetration (ACD/I-lab) sufficient for CNS activity.

The aim of generating a ligand with a buprenorphine-like profile but having substantially lower efficacy at MOP receptor has been achieved in part in the current study. **1d** is an antagonist at MOP and KOP receptors (though it does have some low efficacy at KOP receptors) and has good affinity, equivalent or better than buprenorphine, for NOP receptors. Efficacy at NOP receptors is, however, lower than displayed by buprenorphine so that the desired profile is not fully realized.

EXPERIMENTAL SECTION

Reagents and solvents were purchased from Sigma-Aldrich or Alfa Aesar and used as received. Buprenorphine (**1b**) was supplied by the National Institute on Drug Abuse, Bethesda, MD. ^{1}H and ^{13}C NMR spectra were obtained with a Bruker 400 MHz instrument (^{1}H at 400 MHz, ^{13}C at 100 MHz); δ in ppm, J in Hz with TMS as an internal standard. Instrumentation was as follows: ESIMS, microTOF (Bruker); EIMS, Fisons autosampler; microanalysis, PerkinElmer 240C analyzer. Column chromatography was performed using RediSep prepacked columns with a Teledyne Isco CombiFlash instrument. Ligands were tested as their hydrochloride salts, prepared by adding 5 equiv of HCl (1 N solution in diethyl ether) to a solution of compound in anhydrous methanol. All reactions were carried out under an inert atmosphere of nitrogen unless otherwise indicated. All compounds were >95% pure as determined by microanalysis. A representative synthesis for each series is reported here.

General Procedure A: 3-O-Demethylation with Propanethiolate and HCl Salt Formation. A solution of the appropriate thevinol (0.25 mmol) in anhydrous HMPA (1 mL) under an inert atmosphere was treated with sodium hydride (21 mg, 0.875 mmol) followed by 1-propanethiol (79 μL, 0.875 mmol). After the addition was complete, the reaction mixture was heated to 120 °C and stirred for 3 h. When the mixture was cooled to room temperature, NH_4Cl (sat., aq) was added and the mixture extracted with diethyl ether. The organic extracts were washed with water (3×) and brine. The organic phase was dried ($MgSO_4$), filtered, and evaporated to dryness. The residue was purified by column chromatography over silica gel. The HCl salts were prepared by the addition of 2 M HCl in diethyl ether (1.2 equiv) to a solution of the orvinol in diethyl ether. The white precipitate that formed was collected by filtration, washed with ether, and dried under high vacuum.

(1′*S*,5*α*,6*R*,7*R*,14*α*)-1′-(4,5-Epoxy-7,8-dihydro-3-hydroxy-6-methoxy-17-cyclopropylmethyl-6,14-ethanomorphinan-7-yl)-1′-phenylethan-1′-ol (1d). N-CPM dihydronorthevinone **8a** (220 mg, 0.52 mmol) in anhydrous toluene (5.2 mL) was treated with phenylmagnesium bromide (1.5 mL, 1.04 mmol) at room temperature for 22 h. Purification using column chromatography (30% EtOAc−petroleum ether−0.5% NH_3) gave thevinol **9a** (R = Ph), (110 mg, 42%). R_f (30% EtOAc−petroleum ether−0.5% NH_3) 0.7. δ_H ($CDCl_3$) 7.50 (2H, d), 7.33 (2H, t), 7.18−7.26 (1H, m), 6.69 (1H, d), 6.52 (1H, d), 5.50 (1H, s), 4.42 (1H, s), 3.87 (3H, s), 3.61 (3H, s), 2.91 (1H, d), 2.86 (1H, d), 2.39−2.44 (1H, m), 2.11−2.55 (5H, m), 1.87−1.99 (1H, m), 1.79−1.86 (2H, m), 1.79 (3H, s), 1.54−1.58 (1H, m), 0.77−1.07 (3H, m), 0.55−0.73 (1H, m), 0.33−0.39 (2H, m), −0.10 to −0.03 (2H, m). δ_C ($CDCl_3$) 147.46, 146.94, 141.66, 132.76, 128.98, 127.92, 126.79, 126.17, 119.18, 113.97, 97.14, 80.87, 59.54, 57.97, 56.90, 53.00, 48.57, 46.95, 43.52, 36.03, 35.70, 32.65, 30.06, 23.58, 22.72, 17.97, 9.35, 4.18, 3.32. m/z for $C_{32}H_{40}NO_4$, $[MH]^+$ calcd 502.2957. Found 502.2958. **9a** (R = Ph) (103 mg, 0.21 mmol) was treated as in procedure A to yield **1d** after silica gel chromatography (30% EtOAc−petroleum ether−0.5% NH_3) (40.0 mg, 39%). R_f (30% EtOAc−petroleum ether−0.5% NH_3) 0.2. δ_H ($CDCl_3$) 7.50 (2H, d), 7.32 (2H, t), 7.18−7.26 (1H, m). 6.62 (1H, d), 6.45 (1H, d), 5.58 (1H, s), 4.60 (1H, s), 4.42 (1H, s), 3.56 (3H, s), 2.89 (1H, d), 2.84 (1H, d), 2.40−2.42 (1H, m), 2.10−2.19 (5H, m), 1.90−2.08 (1H, m), 1.72−1.84 (3H, m), 1.80 (3H, s), 1.54−1.58 (1H, m), 1.02−1.10 (1H, m), 0.89−0.94 (1H, dd), 0.69−0.76 (1H, m), 0.56−0.65 (1H, m), 0.30−0.40 (2H, m), −0.1 to 0 (2H, m); δ_C ($CDCl_3$) 147.27, 132.44, 127.93, 126.83, 126.14, 119.56, 116.51, 97.39, 80.92, 59.52, 58.01, 52.91, 48.48, 47.24, 43.53, 36.10, 35.60, 32.60, 29.95, 23.59, 22.80, 17.97, 9.32, 4.15, 3.31. m/z found $[MH]^+$ 488.2778. $C_{31}H_{38}NO_4$ requires 488.2801. Anal. ($C_{31}H_{38}ClNO_4$) C, H, N.

(1′*S*,5*α*,6*R*,7*R*,14*α*)-1′-(4,5-Epoxy-7,8-dihydro-3-hydroxy-6-methoxy-17-cyclopropylmethyl-6,14-ethenomorphinan-7-yl)-1′-phenylmethanol (2a). The alcohol **14b** (500 mg, 1.03 mmol) was treated as in procedure A to yield **2a**, which was purified by gravity elution chromatography with MeOH−CH_2Cl_2 (1:20) (370 mg, 76%). R_f (MeOH−CH_2Cl_2, 1:10) 0.48. NMR δ_H ($CDCl_3$) 0.38−0.40 (2H, m), 0.40−0.53 (2H, m), 0.64−0.66 (1H, m), 3.01 (1H, d), 3.35 (1H, d), 3.80 (3H, s), 4.35 (1H, d), 4.65 (1H, d), 5.43 (1H, s), 5.56 (1H, d), 6.00 (1H, d), 6.43 (1H, d), 6.55 (1H, d), 7.26−7.32 (5H, m). δ_C ($CDCl_3$) 3.52, 3.98, 9.18, 23.03, 30.38, 33.00, 42.59, 43.83, 43.92, 47.76, 54.86, 57.04, 59.85, 77.70, 84.53, 97.79, 116.26, 119.83, 124.37, 125.77, 127.70, 128.09, 128.23, 134.32, 137.54, 137.78, 141.71, 146.33. m/z found M^+ for $C_{30}H_{33}NO_4$, 471.2404; calculated 471.2410. Mp (HCl salt) 227−231 °C (dec, EtOH). Anal. ($C_{30}H_{34}ClNO_4\cdot H_2O$) C, H, N.

(1′*R*,5*α*,6*R*,7*R*, 14*α*)-1′-(4,5-Epoxy-7,8-dihydro-3-hydroxy-6-methoxy-17-cyclopropylmethyl-6,14-ethenomorphinan-7-yl)-1′-phenylmethanol (2b). The alcohol **12b** (550 mg, 1.13 mmol) was treated as in procedure A to yield **2b** which was purified by gravity elution chromatography with MeOH−CH_2Cl_2 (1:20) (470 mg, 88%). R_f (MeOH−CH_2Cl_2, 1:10) 0.48. NMR δ_H ($CDCl_3$) 0.01−0.08 (2H, m), 0.45−0.49 (2H, m), 0.74−0.76 (1H, m), 1.37 (1H, dd), 3.05 (1H, d), 3.53 (1H, d), 3.69 (3H, s), 4.62 (1H, d), 5.20 (1H, s), 5.49 (1H, d), 5.81 (1H, d), 6.44 (1H, d), 6.58 (1H, d), 7.31−7.33 (5H, m). δ_C ($CDCl_3$) 3.34, 4.20, 9.30, 22.97, 24.99, 33.43, 43.04, 43.41, 44.01, 48.39, 52.24, 56.91, 59.85, 70.18, 80.85, 94.43, 116.41, 119.88, 125.72, 126.44, 126.84, 127.91, 128.18, 134.19, 136.89, 137.41, 143.32, 146.74. m/z found M^+ for $C_{30}H_{33}NO_4$, 471.2408; calculated 471.2410). Mp (HCl salt) 198−200 °C (dec, EtOH). Anal. ($C_{30}H_{34}ClNO_4\cdot 1.5H_2O$) C, H, N, Cl.

(1′*R*,5*α*,6*R*,7*R*,14*α*)-1′-(4,5-Epoxy-7,8-dihydro-3-hydroxy-6-methoxy-17-cyclopropylmethyl-6,14-ethanomorphinan-7-yl)-1′-(2-pyridyl)ethan-1′-ol (6). 2-Bromopyridine (1.13 mmol) in dry Et_2O was cooled to −78 °C under a nitrogen atmosphere. *n*-Butyllithium (1.13 mmol) was added dropwise and the mixture stirred for 10 min before adding N-CPM dihydronorthevinone (**8a**, 1 mmol) in dry THF. The reaction mixture was allowed to warm to room temperature and stirred for 20 h. After completion, the reaction

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mixture was quenched with saturated NH_4Cl solution (aqueous) and extracted with EtOAc. Organic layer was washed with brine, dried (Na_2SO_4), and evaporated to yield crude product (**10**) that was purified by flash chromatography using MeOH/CH_2Cl_2 (0.5:99.5) (35%). White solid. ^{1}H NMR ($CDCl_3$) δ 0.08−0.11 (2H, m), 0.45−0.52 (2H, m), 0.81−0.86 (2H, m), 1.18−1.22 (2H, m), 1.57−1.73 (4H, m), 2.21−2.41 (8H, m), 2.61−2.70 (2H, m), 2.91 (1H, d, J = 18.24 Hz), 3.02 (1H, d), 3.36 (3H, s), 3.82 (3H, s), 4.36 (1H, s), 5.87 (1H, s), 6.47 (1H, d), 6.62 (1H, d), 7.10−7.12 (1H, m), 7.59−7.63 (2H, m), 8.47 (1H, d). This was 3-O-demethylated using general procedure A to give **6** as a white solid. ^{1}H NMR ($CDCl_3$) δ 0.08−0.11 (2H, m), 0.40−0.51 (2H, m), 0.80−0.88 (2H, m), 1.20−1.27 (2H, m), 1.62 (1H, d), 1.67 (3H, s), 2.02−2.41 (8H, m), 2.61 (1H, dd), 2.78 (1H, dt), 2.91 (1H, d), 3.02 (1H, d), 3.35 (3H, s), 4.39 (1H, s), 4.55 (1H, bd), 5.83 (1H, s), 6.43 (1H, d), 6.61 (1H, d), 7.10−7.14 (1H, m), 7.62−7.64 (2H, m), 8.48 (1H, d). ^{13}C NMR, 400 MHz ($CDCl_3$) δ 3.21, 4.33, 9.35, 16.42, 22.45, 28.39, 28.86, 29.92, 35.24, 35.65, 43.81, 46.78, 49.36, 52.28, 57.95, 59.89, 80.1, 97.70, 116.01, 119.37, 120.54, 121.55, 128.54, 132.45, 135.78. 136.93, 145.30, 147.32, 166.46. HRMS, m/z for ($C_{30}H_{36}N_2O_4$), $[MH]^+$: calcd 489.2753, found 489.2821. Anal. ($C_{30}H_{36}Cl_2N_2O_4\cdot 3H_2O$) C, H, N.

***N*-Cyclopropylmethylnorisonepenthol (12b).** Aldehyde **11b**[13] (5.0 g, 1.61 mmol) was treated with PhMgBr (THF solution) in toluene (20 mL) for 24 h at room temperature. The reaction was quenched with a saturated NH4Cl solution and extracted with Et_2O to yield **12b** (2.97 g, 50%) which was purified by silica gel chromatography. ^{1}H NMR δ_H ($CDCl_3$) 0.01−0.08 (2H, m), 0.45−0.53 (2H, m), 0.74−0.76 (1H, m), 1.36 (1H, dd), 3.07 (1H, d), 3.53 (1H, d), 3.72 (3H, s), 3.83 (3H, s), 4.62 (1H, d), 5.23 (1H, s), 5.52 (1H, d), 5.88 (1H, d), 6.49 (1H, d), 6.61 (1H, d), 7.31−7.34 (5H, m). δ_C ($CDCl_3$) 3.39, 4.20, 9.34, 15.27, 23.03, 25.18, 33.64, 43.05, 43.95, 44.04, 48.20, 52.78, 56.65, 57.04, 59.93, 70.39, 80.73, 94.99, 113.47, 119.28, 125.81, 126.78, 128.16, 128.43, 134.46, 136.85, 141.87, 143.47, 148.30. Found M^+ for $C_{31}H_{35}NO_4$, 485.2566; calculated 485.2566

***N*-Cyclopropylmethylnornepenthol (14b).** Ketone **13b**[27] (800 mg, 1.61 mmol) in THF (10 mL) was added dropwise to a solution of $LiAlH_4$ (2.5 equiv) in THF (10 mL) at room temperature. The solution was allowed to stir for 16 h before quenching with a solution of Rochelle salt (10 mL). Extraction with Et_2O yielded **14b** (700 mg, 90%) which was purified by recrystallization, R_f (EtOAc−hexane, 1:1, 0.5% NH_3) 0.48. NMR δ_H ($CDCl_3$) 0.02−0.07 (2H, m), 0.37−0.45 (2H, m), 0.64−0.69 (1H, m), 3.03 (1H, d), 3.34 (1H, d), 3.83 (3H, s), 3.84 (3H, s), 4.34 (1H, d), 4.63 (1H, d), 5.37 (1H, s), 5.56 (1H, d), 6.04 (1H, d), 6.48 (1H, d), 6.62 (1H, d), 7.26−7.33 (5H, m). δ_C ($CDCl_3$) 3.50, 3.91, 9.24, 22.95, 30.43, 33.17, 42.55, 43.77, 44.13, 47.46, 54.97, 56.82, 57.05, 59.87, 77.70, 84.56, 97.62, 113.83, 119.33, 124.62, 127.58, 128.07, 128.17, 128.35, 134.65, 137.72, 141.88, 141.96, 147.82. m/z found M^+ for $C_{31}H_{35}NO_4$, 485.2588; calculated 485.2566.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Full experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 44-(0)1225-383103. E-mail: s.m.husbands@bath.ac.uk.

Present Address

⊥V.K.: Centre for Chemical and Pharmaceutical Sciences, Central University of Punjab, Bathinda, India.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

MOP, μ opioid; DOP, δ opioid; KOP, κ opioid; NOP, nociceptin opioid peptide; EKC, ethylketocyclazocine; N/OFQ, nociceptin/orphanin FQ

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