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Communications to the Editor

Structural Determinants of Efficacy for κ Opioid Receptors in the Orvinol Series: 7,7-Spiro Analogues of Buprenorphine

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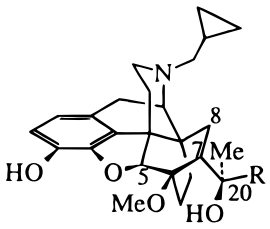
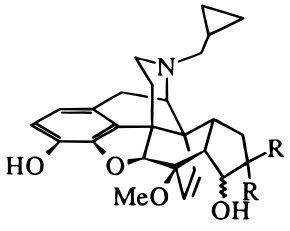
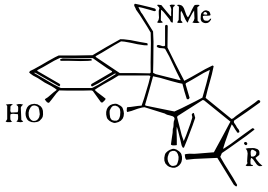
The clinically important, potent analgesic buprenorphine (**1a**) has generated considerable interest in recent years as a potential pharmacotherapy for opiate abuse and dependence. Buprenorphine has a unique pharmacological profile, μ partial agonist, κ, δ antagonist, that sets it apart from other orvinols of general structure (**1**)



1a: R = ^{t}Bu, buprenorphine
1b: R = ^{n}Pr



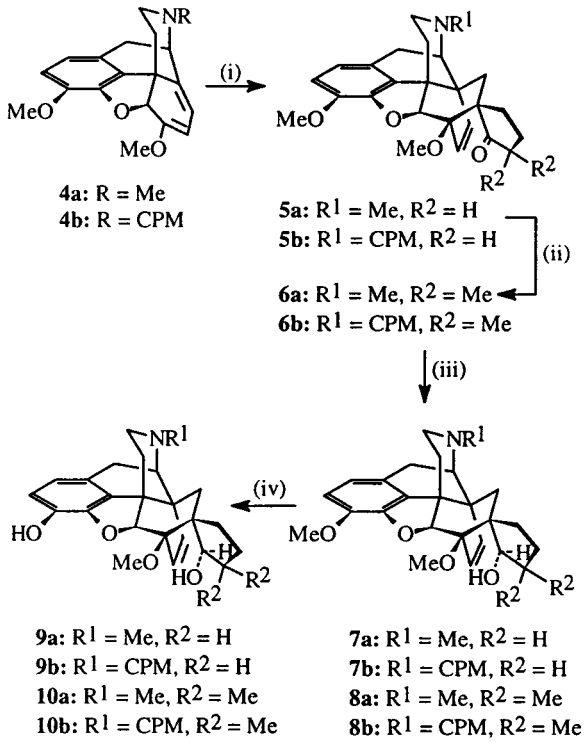
2: R = H or Me



3: R = H or Me

that also display agonist activity at the κ opioid receptor.[1] Buprenorphine's profile is believed to be derived in part from the location of the *tert*-butyl group attached to C20, although it is still not certain what conformation is adopted on binding to the receptor. In order to better define the role of this moiety we have prepared ring constrained analogues of buprenorphine in which the *tert*-butyl group is incorporated into a rigid ring system.[2,3] In the series of 7,8-fused analogues (**2**)[3] the ring is held in the region below C7,C8—an area proposed by Rapoport to be critical for the expression of agonist activity in the orvinol and related series.[4] Surprisingly, in vitro pharmacological analysis of this series showed only small differences between the unsubstituted (**2**, R = H) and disubstituted (**2**, R = Me) compounds at any of the three receptors, each displaying κ agonist activity in the guinea pig ileum (GPI) and δ agonist activity in the mouse vas deferens (MVD).[3] Additionally our own

Scheme 1. Synthesis of 7,7-Spiro Analogues **9a**−**10b**[a]



[a] Reagents: (i) 2-methylenecyclopentanone, toluene; (ii) NaH, MeI, THF; (iii) LAH, THF; (iv) PrSNa, HMPA.

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Table 1. Binding Affinity to Cloned Human Opioid Receptors Transfected into Chinese Hamster Ovary Cells

			K_i (nM)[a]		
	R^1	R^2	μ	κ	δ
9a	Me	H	0.26 ± 0.16	0.87 ± 0.13	5.52 ± 0.25
10a	Me	Me	0.50 ± 0.11	1.28 ± 0.01	8.47 ± 0.51
9b	CPM	H	0.32 ± 0.05	1.04 ± 0.01	0.69 ± 0.15
10b	CPM	Me	0.59 ± 0.03	3.12 ± 0.20	5.42 ± 0.54
1a			1.5 ± 0.8	0.8 ± 0.05	4.5 ± 0.4

[a] K_i (nM) versus [^{3}H]DAMGO (μ), [^{3}H]U69593 (κ), and [^{3}H]-DPDPE (δ). Mean of two experiments, each carried out in triplicate.

studies with furanomorphides (**3**) have indicated that the presence or absence of methyl groups attached to C20 has no affect on their activity in vitro.[2] Together, the above results lead us to conclude that the area below C7,C8 can accommodate alkyl groups but does not play a critical role in determining the pharmacological profile of the molecules. Thus buprenorphine's unique profile and in particular its lack of κ agonist effects is unlikely to be associated with interactions at this site. In order to more clearly resolve this matter, analogues were required in which the *tert*-butyl group was constrained in the same conformation as proposed for the active conformation of buprenorphine. 7,7-Spiro analogues **9a**, **9b** and **10a**, **10b** meet the requirement and represent the closest structurally constrained analogues of buprenorphine yet reported.

Chemistry. The synthesis of the intermediate ketones **5** and **6** has been reported previously and utilizes the Diels−Alder reaction of thebaine (**4a**) or NCPM northebaine (**4b**) with in situ generated 2-methylenecyclopentanone.[5] This yields **5** with the C20 carbon in the α position (below the plane of the ring) along with its β analogue (not shown). Compound **6** is then formed by dimethylation of **5** with NaH/MeI.[6] Reduction of both ketones with lithium aluminum hydride (LAH) in THF resulted in selective reduction from the less hindered *Si* face to yield the *R* isomers **7** and **8**.[7] Subsequent 3-O-demethylation of the secondary alcohols yielded the constrained orvinol analogues **9a**, **9b** and **10a**, **10b** (Scheme 1).

Results. The new analogues were evaluated in vitro in both binding and functional assays. K_i values for binding to μ, κ, and δ opioid receptors were determined in cloned human opioid receptors transfected into chinese hamster ovary (CHO) cells. The standard tritiated ligands used for these assays were [^{3}H]DAMGO (μ), [^{3}H]-U69,593 (κ), and [^{3}H]DPDPE-Cl (δ).[8] Each of the spiro analogues displayed nanomolar or subnanomolar affinities for all three receptor types, resulting in little selectivity, which is usual for orvinols and related series (Table 1).[2,9,11] In general, the unsubstituted analogues (**9a** and **9b**) had marginally higher affinity for each receptor than the dimethyl analogues (**10a** and **10b**).

In vitro functional activity was determined by measuring stimulation of [^{35}S]GTPγS binding in membranes from cells transfected with human opioid receptors. A standard prototypical agonist (DAMGO, DPDPE, and U69,593 for μ, δ, and κ receptors) is tested in every experiment so that percent maximal stimulation, with respect to the standard agonist, can be determined.[8] Both EC_{50} (potency) and percent stimulation (efficacy) are reported for each compound at each of the three receptors (Table 2). Each of the compounds showed agonist effects at all three receptors, but with varying degrees of potency and efficacy. Of the NMe compounds, **9a** was a full agonist at μ, κ, and δ receptors, while **10a** displayed full efficacy at μ and δ but only very low efficacy at κ. Converting NMe to NCPM substantially reduced the efficacy of **9a** and **10a** at μ receptors, while at κ receptors the change of NMe to NCPM resulted in a dramatic increase in potency of 260-fold for **9a** to **9b** and 16-fold for **10a** to **10b**. For δ receptors the predominant effect was again to increase potency by 20-fold (**9a** to **9b**) and 17-fold (**10a** to **10b**). Although similar trends have been noted before for other opioid series,[9] this is the clearest demonstration yet of the effect of converting NMe to NCPM.

The most intriguing result comes from comparison of the unsubstituted (**9a** and **9b**) ligands with their disubstituted analogues (**10a** and **10b**). While there is little effect on μ efficacy and potency, there is a substantial reduction in κ efficacy in both the NMe and NCPM compounds as a result of the methylations. A similar effect on δ agonist efficacy is seen in the NCPM analogues (**9b** to **10b**).

Discussion. Loew and Berkowitz[10] have suggested that the steric bulk of the *tert*-butyl moiety effectively locks buprenorphine into conformation (**1a**), with restriction of rotation about C7,C20. Thus, buprenorphine, unlike less substituted analogues, would not be able to access the region below C8. It was tempting to use this argument to explain the differences in efficacy for κ opioid receptors between buprenorphine and its close analogues (e.g., **1b**). The latter can access the region below C8 which could be designated as a κ agonist site. This was partly supported by the profiles of the constrained analogues **2**. However, the results for the present series do not support this hypothesis since the unmethylated NCPM derivative (**9b**) has a profile (κ/δ agonist; low efficacy at μ) very similar to **1b** and **2** but without the possibility of any significant interaction with a lipophilic site below C8. The most significant SAR in the present series is the effect of methyl substitution in the spiro ring in attenuating κ efficacy. Thus, it

Table 2. GTPγS Binding in Cloned Human Opioid Receptors Transfected into Chinese Hamster Ovary Cells

			μ		κ		δ	
	R^1	R^2	EC_{50} (nM)	% stim[a]	EC_{50} (nM)	% stim[a]	EC_{50} (nM)	% stim[a]
9a	Me	H	3.7 ± 0.50	87.0 ± 14.0	10.5 ± 3.0	88.0 ± 1.0	6.1 ± 1.8	111.0 ± 6.0
10a	Me	Me	0.8 ± 0.03	99.5 ± 18.5	1.6 ± 0.4	21.0 ± 1.0	18.6 ± 5.2	100 ± 1.5
9b	CPM	H	2.8 ± 1.1	32.0 ± 19.0	0.04 ± 0.0	89.0 ± 3.0	0.3 ± 0.1	104.5 ± 12.5
10b	CPM	Me	4.2 ± 1.4	21.0 ± 1.0	0.1 ± 0.04	40.0 ± 6.0	1.1 ± 0.2	45.5 ±2.5
1a			2.3 ± 1.7	66 ± 36		<20%		<20%

[a] Percent maximal stimulation with respect to the standard agonists DAMGO (μ), U69593 (κ), and DPDPE (δ). Mean of two experiments, each carried out in triplicate.

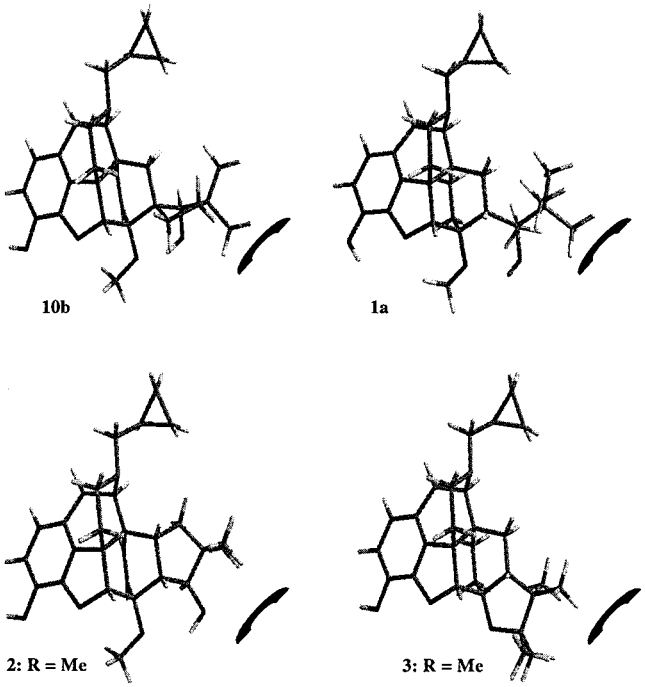

Figure 1. Energy minimized conformations of **1a**, **10b**, **2**, and **3** indicating negative interactions between **10b** and **1a** with the receptor, but not between **2** and **3** and the receptor.

appears that the dimethyl analogues may share with buprenorphine the structural feature that is detrimental to κ efficacy. From molecular modeling studies (Figure 1) it is apparent that there is excellent overlap between the *tert*-butyl group of **1a** and the substituted ring of **10b**, but only a partial overlap between **1a** and the other ring constrained analogues **2** and **3**.[12] In particular, a single methyl group extending away from C7 (Figure 1) is shared between **1a** and **10b**, but crucially not with the other analogues. It can be concluded that occupation of this region of space results in a negative interaction and the loss in κ-efficacy. Thus, it is this region and not that below C8 that is critical in defining buprenorphine's unique pharmacological profile, and **10b** provides a viable model for the active conformation of buprenorphine.

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Supporting Information Available: Spectra and detailed experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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