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Benzylideneoxymorphone: A new lead for development of bifunctional mu/delta opioid receptor ligands

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ABSTRACT

Opioid analgesic tolerance remains a considerable drawback to chronic pain management. The finding that concomitant administration of delta opioid receptor (DOR) antagonists attenuates the development of tolerance to mu opioid receptor (MOR) agonists has led to interest in producing bifunctional MOR agonist/DOR antagonist ligands. Herein, we present 7-benzylideneoxymorphone (**6**, UMB 246) displaying MOR partial agonist/DOR antagonist activity, representing a new lead for designing bifunctional MOR/DOR ligands.

Chronic severe pain is a considerable burden facing public health, costing the United States an estimated $560–635 billion in health care costs and loss of productivity in 2011.[1] Opioid analgesics remain the standard treatment for severe pain, due to their ability to inhibit nociceptive processing and maintain the patient in a state of well-being.[2] Despite their benefits and widespread use, long-term use of opioids such as morphine and oxycodone is hindered by the rapid development of analgesic tolerance. Tolerance to intestinal motility does not develop as rapidly as analgesic tolerance; this "differential tolerance" results in severe constipation arising from the increased doses required to compensate for the diminished analgesic effect.[3] Analgesics that produce a blunted tolerance effect would be expected to lower healthcare costs and provide beneficial outcomes to patients.

Three opioid receptor (OR) types have been cloned and characterized, namely mu (MOR), delta (DOR), and kappa (KOR), and are members of the G protein-coupled receptor (GPCR) superfamily. All approved opioid analgesics are MOR agonists, and produce analgesic and euphoric effects. Bifunctional opioid receptor ligands possessing dual profiles of OR type agonism and antagonism may also offer certain pharmacologic advantages. For example, buprenorphine (Suboxone; Subutex) is a MOR partial agonist/KOR antagonist that is used as an alternative to methadone for treating opioid dependence.[4]

There is considerable evidence that concomitant DOR antagonism attenuates the development of tolerance to MOR agonists. Studies using DOR knockout mice[5] and DOR-specific antibodies,[6] as well as pharmacologic inhibition of DOR using naltrindole,[7] support this hypothesis. These proof-of-concept studies have led to the development of ligands that are bifunctional MOR agonists and DOR antagonists. Building upon the success of DIPP-NH_2[Ψ], the first bifunctional, tetrapeptide MOR agonist/DOR antagonist to produce antinociception with a low propensity to produce tolerance and dependence,[8] several small-molecule bifunctional MOR/DOR probes have been produced (Fig. 1) and demonstrate efficacy *in vitro* and *in vivo*.[9–12] To overcome limitations of solubility, oral bioavailability, and receptor-type potency, we have set out to identify new chemical leads for bifunctional MOR agonist/DOR antagonist ligands.

The DOR antagonist benzylidenenaltrexone (BNTX, Fig. 2) was first described as a DOR-selective antagonist.[13] This compound was rationalized using the "message-address concept," whereby the naltrexone "message" was selectively targeted to DOR by the

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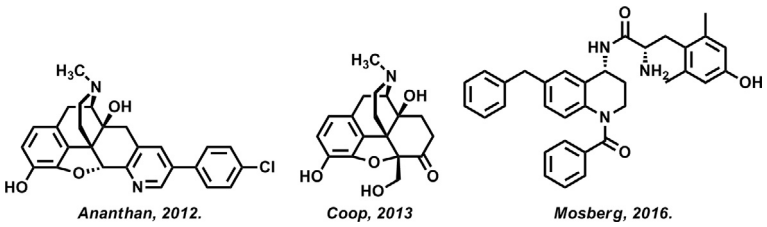


Fig. 1. Three examples of small-molecule, bifunctional MOR agonist/DOR antagonist lead molecules.[10–12]

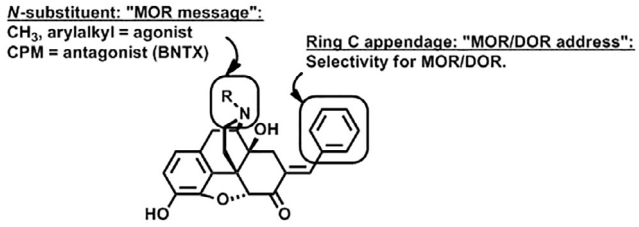
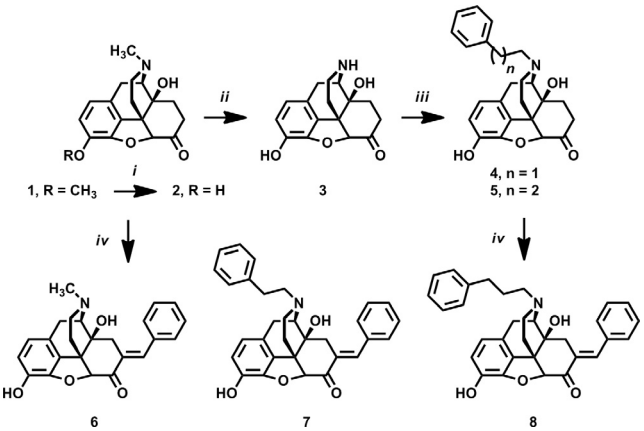


Fig. 2. Schematic describing the "message-address" rationale for designing bifunctional MOR agonist/DOR antagonist probes based on modification of oxymorphone. CPM = cyclopropylmethyl.

7-benzylidene "address." Subsequent reports[14,15] demonstrate BNTX binds with similar affinity to MOR and antagonizes the effects of MOR agonists with similar potency as DOR agonists.[16] Thus, we consider BNTX to be a MOR/DOR antagonist. The *N*-substituent frequently modulates efficacy in the 4,5-epoxymorphinan series of MOR analgesics. According to a ligand-based, quantitative conformationally-sampled pharmacophore describing DOR ligands,[17] *N*-alkyl substitutions do not alter predicted efficacy in this same series. Applying the message-address concept,[18,19] we hypothesize that substitution of the *N*-cyclopropylmethyl "message" of BNTX with *N*-alkyl groups satisfying the "message" (Fig. 2) would enhance MOR efficacy and maintain low efficacy at DOR, resulting in MOR agonist/DOR antagonist bifunctional ligands.

Synthesis of compounds **6–8** was achieved from oxycodone as shown in Scheme 1. Oxycodone (**1**) was converted to oxymorphone (**2**) using BBr_3 in the usual manner.[20] To synthesize *N*-substituted analogues **7** and **8**, **2** was *N*-demethylated to noroxymorphone (**3**) using α-chloroethyl chloroformate[21] and *N*-alkylated using



Scheme 1. Synthesis of **6–8**. Reagents and conditions: i) BBr_3, $CHCl_3$, 0 °C, 30 min; NH_4OH(aq), 0 °C, 30 min. ii) Ac_2O, reflux, 24 h; 1-chloroethyl chloroformate, K_2CO_3, $Cl(CH_2)_2Cl$, reflux, 24 h; NaOH, MeOH, reflux, 3 h. iii) R-Br, K_2CO_3, RT, 24 h. iv) PhCHO, NaOH, MeOH, 0 °C, 18 h

the appropriate alkyl bromide. Intermediates **2**, **4**, and **5** were converted to C7-benzylidene analogues **6–8** under basic Claisen conditions.[13] Purified final products were converted to water-soluble salts (HCl, oxalate) prior to pharmacologic evaluation.[22]

OR binding results are shown in Table 1. Compound **6** bound preferentially to MOR and DOR over KOR. Affinity for MOR and DOR generally decreased as a function of *N*-arylalkyl chain length, whereas KOR affinity was improved for **7** and **8** compared to **6**. This caused a net decrease in MOR/DOR preference over KOR, similar to BNTX.[14] The *N*-methyl analogue (**6**) exhibited the highest affinity for MOR and DOR of the series, approximately 3- and 20-fold greater than *N*-phenylethyl (**7**) and *N*-phenylpropyl (**8**) analogues for MOR, respectively. This was unexpected, as *N*-phenylethyl and *N*-phenylpropyl 4,5-epoxymorphinans are generally higher affinity MOR agonists than their parent *N*-methyl equivalents.[23,24]

Table 2 shows the results of a [^{35}S]GTPγS assay to determine the relative efficacy of compounds at MOR and DOR. Compounds **6** and **7** were found to be MOR partial agonists with **6** demonstrating approximately 3-fold higher potency than **7**. Efficacy data for **8** could not be determined due to low potency. Compounds **6** and **7** possess no significant agonist activity at DOR. Compound **6** was tested further to determine DOR antagonist potency. In the GTPγS functional assay, **6** caused a parallel rightward shift in the SNC80 concentration–response curve with Ke of 138 ± 24 nM (Table 2). Taken together, compound **6** (benzylideneoxymorphone, UMB 246) exhibited a profile of MOR/DOR preferential binding affinity, partial agonist effects at MOR, and antagonist activity at DOR. Compound **6** was therefore selected for further characterization *in vivo*.

Acute antinociception assays in the mouse were performed following subcutaneous (*s.c.*) administration of increasing doses of **6**.[24,25] As shown in Fig. 3, **6** produced an increase in E_{max} to approximately 40% MPE with T_{max} of 50 min in the hot plate nociception test. Peak antinociception effects for UMB2 46 were evident at 50 min post-administration for the 60 mg/kg treatment group. Repeated measures ANOVA revealed a significant difference in latency for hot plate nociception testing among treatment groups ($p < 0.01$) and among time points ($p < 0.001$). Bonferroni post hoc analysis revealed that the 50 mg/kg treatment group had significantly greater antinociception, compared to saline control, at 30 ($p < 0.05$) minutes post-administration (Fig. 3). Bonferroni post hoc analysis revealed that the 60 mg/kg treatment group had significantly greater antinociception, compared to saline control, at 30 ($p < 0.01$), 50 ($p < 0.001$), and 70 ($p < 0.05$) minutes post-administration.

Fig. 4 shows the results from the tail-flick nociceptive test. Peak antinociception effects for **6** were evident at 30 min post-administration for the 60 mg/kg treatment group. Repeated measures ANOVA revealed a significant difference in latency for tail-flick noticeptive testing among treatment groups ($p < 0.01$) and among time points ($p < 0.001$). Bonferroni post hoc analysis revealed that the 50 mg/kg treatment group had significantly greater antinociception, compared to saline control, at 70 ($p < 0.01$) minutes post-administration (Fig. 4). Bonferroni post analysis revealed