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Opioids and efflux transporters. Part 3: P-glycoprotein substrate activity of 3-hydroxyl addition to meperidine analogs

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Abstract—Numerous studies have shown that many clinically employed opioid analgesics are substrates for P-glycoprotein (P-gp), suggesting that up-regulation of P-gp may contribute to the development of central tolerance to opioids. The studies herein focus on the development of SAR for P-gp substrate activity in the meperidine series of opioids. Addition of a 3-OH to meperidine and the ketone analog of meperidine yielding bemidone and ketobemidone, respectively, significantly increased P-gp substrate affinity. The results of this study have implications in the development of novel analgesics to be utilized as tools to study the contribution of P-gp on the development of central tolerance to opioids.

Introduction. There is a growing body of evidence that suggests efflux transporters, specifically P-glycoprotein (P-gp), may play a role in the development of opioid related central tolerance and constipation.[1–6] Recent studies have shown that opioids are substrates for P-gp, although to differing extents,[7] and P-gp is up-regulated at the blood-brain barrier (BBB) of morphine[3] (**1**) and oxycodone[2] (**2**) (Fig. 1) tolerant rats. Upon chronic administration, the up-regulated P-gp would be expected to result in lower brain concentrations of opioid, thereby exacerbating tolerance to the central analgesic effects. P-gp knockout animals[8] are available and offer a useful model to study the effects of P-gp on opioids; however, an alternative approach in wild-type animals is the development of mu opioid receptor agonists which are not P-gp substrates. These compounds would allow a full investigation of the contribution of up-regulated P-gp to opioid tolerance, as full cross-tolerance between morphine and the opioid lacking P-gp substrate activity would not be anticipated to occur. Additionally, opioids lacking P-gp substrate activity may potentially be developed into analgesics with lower degrees of tolerance.

Meperidine (**3**), a moderately potent mu opioid analgesic, [9,10] has been reported to possess low P-gp substrate activity.[7] Therefore, our investigations are focused on delineating the structure-activity relationship (SAR) for the addition of a *m*-OH, while increasing mu opioid potency based on known SAR for this series.[10]

Results and discussion. The compounds synthesized are readily known in the literature as mu opioid analgesics;[10] however, the syntheses described here are novel approaches. Meperidine (**3**) was prepared from nitrile **4** (obtained from Sigma–Aldrich, Inc.), via alkylation with MeI in DMF in the presence of K_2CO_3, followed by aqueous NH_4Cl hydrolysis of the *N*-methyl nitrile **5** to the ethyl ester through treatment with H_2SO_4 and EtOH. Treatment of **5** with EtMgBr, via a Grignard reaction,[11] produced the ketone meperidine analog **6** (Scheme 1).

Bemidone **9** was prepared from the condensation of mechloroethamine hydrochloride and 3-methoxyphenyl-

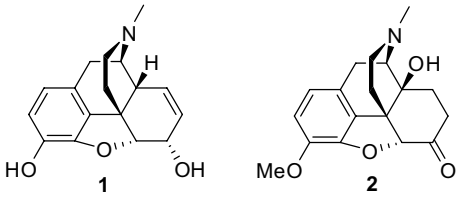

Figure 1. Morphine (**1**) and oxycodone (**2**).

Keywords: Meperidine; P-glycoprotein; Opioids; Tolerance.
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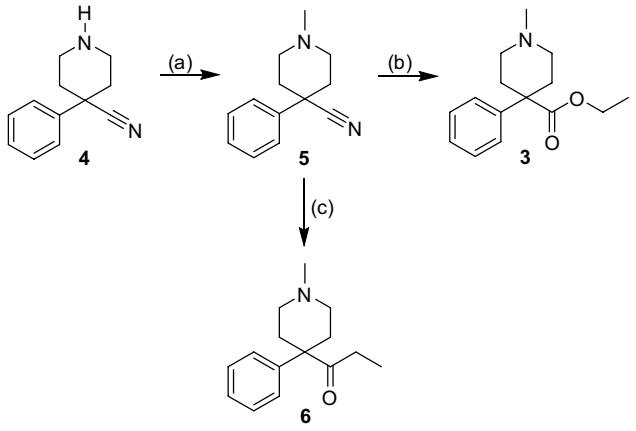


Scheme 1. Reagents and conditions: (a) MeI, K_2CO_3, DMF; (b) H_2SO_4, EtOH, reflux; (c) EtMgBr, NH_4Cl hydrolysis.

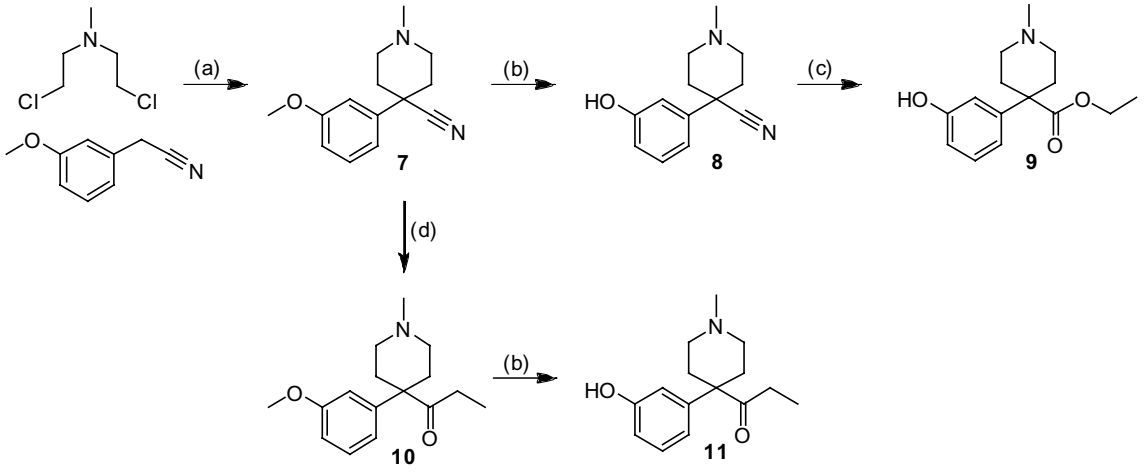
acetonitrile (both reagents obtained from Sigma–Aldrich, Inc.) with NaH and NaOH to yield **7**. *O*-dealkylation of **7** was performed with BBr_3 and NH_4OH,[12] converting the methoxy group to a phenol **8**, followed by nitrile hydrolysis to give the *m*-OH ethyl ester as previously described.[4] Treatment of **7** with an EtMgBr Grignard reagent,[11] followed by aqueous NH_4Cl hydrolysis produced **10**, which then underwent treatment with BBr_3 to produce ketobemidone (**11**) (Scheme 2).

All compounds were converted to their respective salts (see Table 1). Drug stimulated P-gp ATPase activity was estimated using the Pgp-Glo assay system[13] (Promega, Madison, WI) and the results are shown in Table 1. Briefly, this method relies on the ATP dependence of the light-generating reaction of firefly luciferase where ATP consumption is detected as a decrease in luminescence, the greater the decrease in signal the higher the P-gp activity. Sodium orthovanadate was used as a P-gp ATPase inhibitor, whereas verapamil was used as a positive control. All test compounds were analyzed at 200 μM and fold stimulation values were calculated using Eq. 1. Fold stimulation values greater than 2.0 indicate a P-gp substrate.[14]

$$\text{Fold stimulation by a test compound} = \frac{\text{Test compound stimulated P-gp activity}}{\text{Basal P-gp activity}} \quad (1)$$

The addition of a *m*-OH into the phenyl ring significantly increases the P-gp fold stimulation of meperidine analogs. Meperidine itself has a P-gp fold stimulation value of 1.78 and increases to 2.64 with the *m*-OH addition (bemidone, **9**). Whereas the ketone analog **6**, with a P-gp fold stimulation value of 1.37, increases to 4.89 with the *m*-OH addition (ketobemidone, **11**). Thus, the addition of a *m*-OH increases the P-gp substrate activity of these meperidine analogs, which are members of the 4-phenylpiperidine class of opioids.

The hydroxylated meperidine analogs were initially pursued to investigate the relationship between P-gp and increased opioid potency. Interestingly, these results are consistent with previous studies in our laboratory which



Scheme 2. Reagents: (a) NaH, NaOH; (b) BBr_3, NH_4OH; (c) H_2SO_4, EtOH; (d) EtMgBr, NH_4Cl hydrolysis.

Table 1. Fold stimulation values of test compounds prepared, salt form, yield, and melting point

Compound	Name	Salt	Yield (%)	Mp (°C)	Fold stimulation ± SEM
	Non-treated (control)				1.00
3	Meperidine	Oxalate	7	190–192	1.78 ± 0.39*
6	Ketone analog	Citrate	56	170–171	1.37 ± 0.19*
9	Bemidone	Oxalate	36	200–202	2.64 ± 0.82*
11	Ketobemidone	Oxalate	51	233–235	4.89 ± 1.94*

All compounds assayed at 200 μM. Data are represented as fold stimulation ± SEM ($n = 3$). *Significant difference ($p < 0.05$) from control (non-treated) as determined from *t*-test. All compounds gave satisfactory CHN (±0.4%) and spectral analysis (see Supporting Information).

showed that removal of the 3- and 6-OH from morphine resulted in decreased P-gp substrate activity,[5] as morphine is a P-gp substrate.[7] These studies attest that the *m*-OH substituent increases P-gp substrate activity across the phenylpiperidine and morphinan classes of opioids. Furthermore, the development of opioids lacking P-gp substrate activity should not possess a *m*-OH substituent. The interaction between opioids and P-gp is currently under investigation and these results aid in further SAR development. The ultimate goal is to develop a potent opioid with low P-gp substrate activity for use as a tool to study the contribution of P-gp up-regulation to the development of opioid tolerance and cross-tolerance between opioids with P-gp substrate activity and those without.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.04.046.

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