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# Opioids and efflux transporters. Part 1: P-Glycoprotein substrate activity of N-substituted analogs of meperidine

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**Abstract**—P-Glycoprotein (P-gp) is an efflux transporter which is up-regulated at the blood–brain barrier in both morphine- and oxycodone-tolerant rats. Numerous studies have shown that many clinically employed opioid analgesics are substrates for 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 compounds, and show that a meperidine analog of greater potency, *N*-phenylbutyl-*N*-normeperidine, has low activity as a P-gp substrate and has the potential to be utilized as a tool to study the contribution of P-gp to the development of central tolerance to opioids.


The development of improved agents for the treatment of chronic pain remains an important goal in public health.[1] The vast majority of currently employed agents for the treatment of severe chronic pain are opioid analgesics, which act as agonists at mu opioid receptors in the CNS.[2] Unfortunately, clinically employed opioid analgesics suffer from the development of tolerance, necessitating escalating doses to maintain the patient in a pain-free state,[3] thereby leading to escalated side-effects such as constipation.[4,5] Numerous mechanisms at the receptor and cellular level have been indicated in the development of tolerance to mu opioid receptor agonists,[6] but recent reports have suggested that efflux transporters at the blood–brain barrier (BBB) may also contribute toward the development of central tolerance.[7,8] P-glycoprotein (P-gp) is an efflux transporter which is located in numerous tissues,[9] and its function at the BBB is to actively remove xenobiotics from the CNS.[9] Two commonly employed opioid analgesics, morphine (**1**) and oxycodone (**2**) (Fig. 1), are known substrates for this transporter and rats tolerant to both morphine[8] and oxycodone[7] show up-regulation in P-gp level at the BBB. Thus, on 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 are available and offer a useful model to study the effects of P-gp on opioids,[10] but an alternative approach in wild-type animals is the development of mu opioid receptor agonists which are not substrates for P-gp. 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 with no P-gp substrate activity would not be anticipated to occur, and also potentially be developed into opioid analgesics with lower degrees of tolerance.

The mu opioid analgesic meperidine (**3**) has been shown to possess low activity as a P-gp substrate,[11] but only moderate antinociceptive activity in vivo,[12,13] thus initial investigations described herein are focused on delineating the structure–activity relationships (SAR) of the

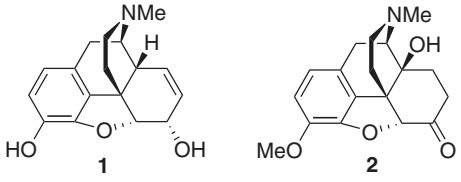


**Figure 1.** Morphine (**1**) and oxycodone (**2**).

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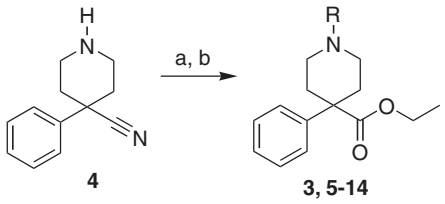


**Scheme 1.** Reagents and condition: (a) RX, $K_2CO_3$, DMF; (b) $H_2SO_4$, EtOH, reflux.

N-substituent in this class for low P-gp substrate activity, while increasing mu opioid potency based on known SAR for potency in this series.[13]

A range of previously reported and novel N-substituted analogs of meperidine were prepared from nitrile **4** (obtained from Sigma–Aldrich, Inc.), via alkylation with alkyl halides in DMF in the presence of $K_2CO_3$, followed by hydrolysis of the nitrile to the ethyl esters (**5**–**14**) through treatment with $H_2SO_4$ and EtOH[14] (Scheme 1). The alkyl substituents were chosen based on known active meperidine analogs and also following a series as previously described for other classes of opioids,[15] and include arylalky, alkyl, and branched alkyl groups (Table 1).

All esters were converted to oxalate salts (Scheme 1). Drug stimulated P-gp ATPase activity was estimated by Pgp-Glo assay system[20] (Promega, Madison, WI) and the results are shown in Figure 2. 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. Test compounds (all tested at 200 μM) which are significantly

**Table 1.** Compounds prepared, salt form, yield, and melting points

| R | Ester | Salt, yield (%), mp (°C) |
|---|---|---|
| $CH_3$ | **3**[14] | Oxalate, 7, 190–192 |
| $(CH_2)_2(C_6H_5)$ | **5**[16] | Oxalate, 33, 205–206 |
| $(CH_2)_3(C_6H_5)$ | **6**[17] | Oxalate, 14, 225 |
| $(CH_2)_4(C_6H_5)$ | **7**[17] | Oxalate, 25, 170 |
| $CH_2(C_6H_5)$ | **8**[14] | Oxalate, 46, 204–205 |
| $CH_2CH{=}CH_2$ | **9**[18] | Oxalate, 40, 213–214 |
| $(CH_2)_2CH_3$ | **10**[19] | Oxalate, 57, 215–216 |
| $CH_2CH{=}CHCH_3$ | **11** | Oxalate, 67, 173–177 |
| $(CH_2)_3CH_3$ | **12**[19] | Oxalate, 24, 190–192 |
| $CH_2C(CH_3){=}CH_2$ | **13** | Oxalate, 35, 180–181 |
| $CH_2CH(CH_3)_2$ | **14** | Oxalate, 55, 165–167 |

Citations reference of previously known compounds.

lower than the control (NT) are P-gp substrates, whereas test compounds significantly higher than the NT are P-gp inhibitors. Compounds which are not significantly different from the NT are neither P-gp substrates nor inhibitors.

The P-gp substrate activity of the esters showed differences depending on the nature of the N-substituent. Most analogs were substrates for P-gp like verapamil, with the exception of meperidine itself and *N*-phenylbutyl-*N*-normeperidine (**7**). These results show a distinct SAR for P-gp substrate activity in this series as *N*-phenylalkyl analogs of shorter length (phenethyl (**5**), phenylpropyl (**6**)) were substrates.

Previous studies have shown that **7** has twice the antinociceptive activity as meperidine.[13] Thus, **7** has the profile of low P-gp substrate activity and greater potency than meperidine required for use as a tool to study the contribution of P-gp upregulation to the development of opioid tolerance and cross-tolerance between opioids with P-gp substrate activity and opioids that are not P-gp substrates. Studies on this compound are currently underway and will be reported in due course.

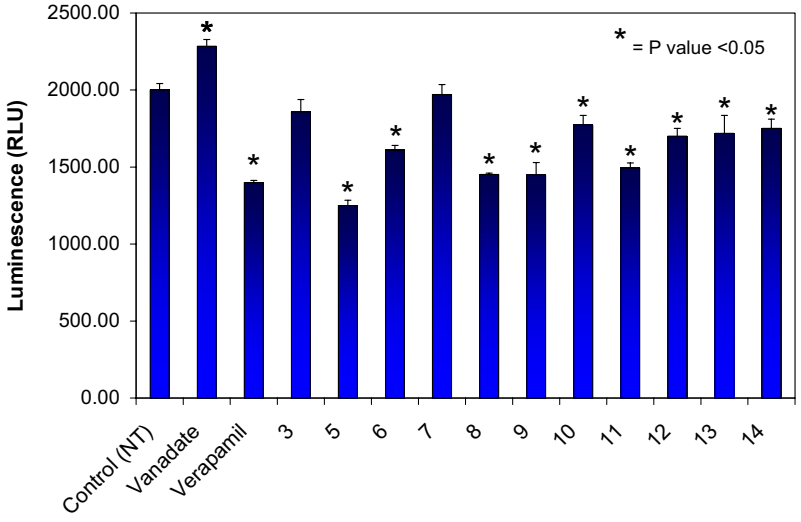


**Figure 2.** Results of compounds and standards in the Pgp-Glo assay system; all compounds assayed at 200 μM. Data are represented as means ± SEM ($n = 4$). * Indicates significant difference from the control at $p < 0.05$ as indicated by *t*-test.

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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.2006.12.042.

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