# The influence of esters and carboxylic acids as the N-substituent of opioids. Part 1: Benzomorphans

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Abstract—To investigate the effects of carboxylic ester and acid moieties as the N-substituent of opioids, a short series of racemic N-substituted normetazocines was prepared. The introduction of both groups as the normetazocine N-substituent produced compounds which displayed low potency in vitro and in vivo, with the esters displaying the greater activity. The pharmacology of the compounds is discussed with implications resulting from potential in vivo metabolic hydrolysis.
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## 1. Introduction

Modification and replacement of the functional group attached to the basic nitrogen in opioids has been the subject of much exploration in opioid chemistry. The nature of the N-substituent determines, to a large extent, the pharmacological profile in a majority of classes of opioids, affecting affinity, potency, and efficacy at the three opioid receptor types mu (μ), kappa (κ), and delta (δ).[1] The naturally occurring archetypal opiates morphine and codeine each have an N-methyl substituent and, in general, an N-methyl substituent produces a compound with opioid agonist activity. Changing from an N-methyl group (oxymorphone) to an N-allyl group (naloxone) converts a compound which exhibits μ opioid agonist activity to a compound which exerts μ opioid antagonism.[1] Changing the N-substituent from an N-phenethyl to an N-benzyl, in the benzomorphan class of compounds, transforms a compound with high agonist potency into a compound without opioid activity.[2] While not the sole determinant the N-substituent is very important for opioid pharmacology, yet the molecular

interactions responsible for such an important role are not clear.[3]

The lipophilicity and hydrophilicity of the N-substituent play a large role in the activity of opioids. Generally, lipophilic substituents are required for opioid activity and hydrophilic substituents are not well tolerated.[1] The classic example of the effects of lipophilic and hydrophilic N-substituent replacement is the development of remifentanil (N-ester) from fentanyl (carfentanil) (both, N-phenethyl) in the 4-anilidopiperidine class of opioids.[4] When remifentanil, an N-ester, is compared to the corresponding N-acid congener, opioid activity is greatly diminished. The change from lipophilic ester to hydrophilic acid results in changes in $ED_{50}$ from 3.55 nM (N-ester, remifentanil) to 1.95 μM (N-acid, metabolite) in the guinea pig ileum (GPI) assay, and from 4.4 μg/kg to 1.6 mg/kg in the rat tail withdraw (RTW) assay.[4] Outside of this series (remifentanil) of compounds, few opioid N-carboxylic ester or acid substituted compounds have been reported in the literature, fewer still reported with pharmacology. A literature report of an N-ester and acid pair (NME-1 and NMA-1) in the N-normetazocine (1) (benzomorphan) series presented limited data for the two compounds.[5] The report was investigating the possible metabolites of pentazocine and included both the ester and acid. The data presented showed NME-1 inactive as an

agonist but weakly antagonized the effects of meperidine with an $AD_{50}$ of 66 (pentazocine $AD_{50}$ 3.9 from the same study) in the rat tail flick (RTF) assay. The corresponding *N*-acid (**NMA-1**) was devoid of both agonist and antagonist activity in this study.[5] To further study the effects of *N*-esters and *N*-acids, and the effects of changing the lipophilicity/hydrophilicity of the N-substituent, we synthesized a series of *N*-substituted-*N*-normetazocine analogs, with the aim of improving the understanding of molecular interactions between the N-substituent and the opioid receptors.

## 2. Results and discussion

The racemic (±)-*N*-normetazocine-*N*-ester (**NME-1-3**) and –*N*-acid (**NMA-1-3**) analogs (Scheme 1) in this series displayed low potency in both the in vitro assays and in vivo antinociceptive assays (Table 1). Overall the compounds examined in this series required high (micromolar) concentrations to display significant effects. Since this series of compounds was tested as racemates the activity profiles may display decreased potency, as the greater opioid potency is known to reside in the (−)-isomers.[1] Despite their racemic nature, the compounds in this series present an intriguing profile of N-substituent effects.

The compounds in this series displayed activity in only one of the four in vivo assays for activity, the phenylquinone antiwrithing assay (PPQ) (Table 1). Since the PPQ is an antinociceptive assay which is non-selective for narcotic analgesia (opioid) and can also detect antiinflammatory (NSAID type) analgesia, no conclusions can be made from this assay alone as to the type of analgesia detected.[6] In both the *N*-ester series and the *N*-acid series there is a gain of antinociceptive activity in the PPQ with increasing chain length. This increased potency with increasing chain length is similar to the increase in potency reported for the remifentanil ester series of compounds.[4] In the remifentanil series of esters potency increased from the methyl acetate 39 mg/kg $ED_{50}$ to the methyl propionate 4.4 μg/kg (remifentanil), to the methyl butyrate 1.6 μg/kg in the RTW.[4] The corresponding propionic acid analog of remifentanil had an $ED_{50}$ of 1.6 mg/kg in the RTW, no data were presented on the acetic or butyric acid congeners.[4] While interesting to note this similarity of in vivo activity with increasing spacing chain lengths, no direct comparisons can be made due to the differing assay types. Further, the activity of **NMA-2** and **NMA-3** is likely due to non-opioid effects, suggested by the lack of both binding and opioid agonist activity in the in vitro experiments.

Another result from the in vivo studies presented here which bears comment is the failure of the compound **NME-1** to show antagonist activity in the mouse tail flick antagonism of morphine (TFA) assay. This compound was previously reported to be active, though weak ($AD_{50}$ of 66) as an antagonist.[5,7] The differences in studies may have contributed to the lack of in vivo activity reported here. The use of rats instead of mice, freebase instead of oxalate salt, meperidine instead of morphine, and higher versus lower tested dosage used in the original versus experiments presented here could all factor into the lack of antagonist activity reported. However, the in vitro experiments presented here argue for the opioid antagonistic effect of **NME-1**.
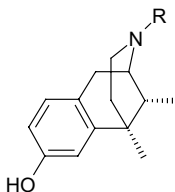
The ethyl acetate analog (**NME-1**) displayed micromolar binding affinity and weak opioid antagonist activity in the MVD assay. The opioid antagonist activity of **NME-1** in the MVD was, though weaker, similar to that of naltrexone. **NME-1** produced 12-fold, 11.6-fold, and 2.8-fold right shifts in the concentration-effect curves of sufentanil (μ), U50,488 (κ), and DSLET (δ), respectively, without altering the maximum response of any of the agonists (a similar profile as naltrexone in this assay). For a comparison of antagonist potency, if the 2.8-fold shift for **NME-1** versus DSLET is used as a basis for calculation, $pA_2$ value of 4.52 was obtained.[8] Naltrexone has a $pA_2$ value of 7.41 for DSLET, and the comparison of values indicates that **NME-1** is three orders of magnitude less potent than naltrexone.[9] These results support the previously reported evidence of the compound's weak in vivo antagonist effect on meperidine induced antinociception.[5] The corresponding acetic acid analog (**NMA-1**), previously reported to be devoid of agonist opioid activity in vivo in the RTW or antagonism of meperidine's effects in the RTW, here displayed opioid agonist activity in the in vitro MVD assay.

In the in vitro tests **NMA-1** displayed μ opioid agonist activity in the MVD, but failed to show binding activity. In the MVD, **NMA-1** exhibited a profile consistent with (mu) opioid agonists (inhibition of the twitch, and reversal of its effects by both naltrexone and β-fulnaltrexamine but not ICI 174864).[10] At no concentration did **NMA-1** inhibit the effects of sufentanil in the MVD.
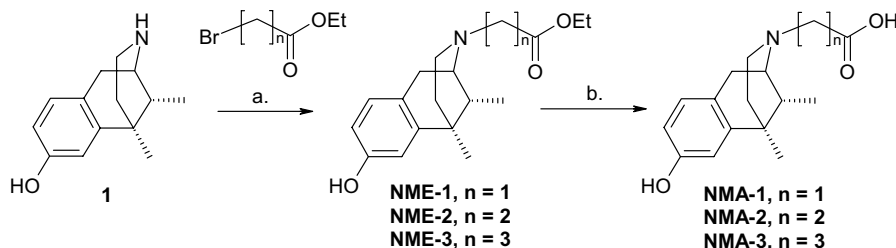


**Scheme 1.** Reagents and conditions: (a) DMF/THF, $K_2CO_3$, reflux 3–6 h; (b) EtOH, $H_2O$, KOH, reflux 1–2 h.

**Table 1.** The pharmacological effects of N-ester and N-acid substituted-N-normetazocines



| Compound | R | In vivo ED$_{50}$ (mg/kg, sc) | | | | In vitro EC$_{50}$ (M) | |
|---|---|---|---|---|---|---|---|
| | | HP | PPQ | TF | TFA | Binding | MVD |
| **NME-1** | CH$_2$COOEt | I | I | I | I[k] | 2.08 μM | NE, ANT[a] |
| **NME-2** | CH$_2$CH$_2$COOEt | I | I | I | I | NT | NT |
| **NME-3** | CH$_2$CH$_2$CH$_2$COOEt | I | 7.1 (2.4–21.4) | I | I | 1.02 μM | 17.9 μM[b,c] |
| **NMA-1** | CH$_2$COOH | I | I | I | I | >6 μM[d] | 9.79 μM[b,c] |
| **NMA-2** | CH$_2$CH$_2$COOH | I | 11.4 (2.9–44.6) | I | I | >6 μM[e] | NE[f] |
| **NMA-3** | CH$_2$CH$_2$CH$_2$COOH | I | 1.4 (0.3–6.4) | I | I | >6 μM[g] | NE[f] |
| Morphine | — | 0.98 | 0.23 | 5.8 | — | 23.6 nM | 395 nM |
| (−)Metazocine | CH$_3$ | 0.62 | 0.3 | 0.81 | I | | |
| Nalorphine | — | 9.9 | 0.6 | I | 2.6 | 20.0 nM[h] | |
| Naloxone | — | I | I | I | 0.03 | 6.26 nM | ANT[i] |
| Naltrexone | — | I | I | I | 0.007 | 0.63 nM | ANT[j] |

[a] Weak antagonist [30 μM] of sufentanil (μ), U50,488 (κ), and DSLET (δ).
[b] Neither altered or reversed the effects of sufentanil.
[c] Antagonized by $10^{-7}$ M naltrexone and $10^{-7}$ M β-fulnaltrexamine but not $10^{-7}$M ICI-174864.
[d] 7.0% inhibition at 6 μM.
[e] 26% inhibition at 6 μM.
[f] Did not block or reverse the effects of sufentanil (μ), U50,488 (κ), or DSLET (δ).
[g] 27% inhibition at 6 μM.
[h] Concentration of etorphine for displacement of this ligand was 3.0 nM (compare to codeine 34.7 μM, morphine 142 nM, naloxone 9.1 nM, and naltrexone 2.0 nM when etorphine employed at 3.0 nM).
[i] pA$_2$ values 7.99, 6.90, 7.35 for sufentanil, U50,488, and DSLET.
[j] pA$_2$ values 8.76, 7.74, 7.41 for sufentanil, U50,488, and DSLET. I, inactive, did not reach 50% at highest dose ($\geqslant$30 mg); NE, no effect at any concentration tested ($10^{-7}$ to $10^{-5}$ M); NT, not tested; ANT, antagonist; MVD, Mouse Vas Deferens, Inhibition of electrically driven twitch, Inhibitory EC$_{50}$ (M); Binding = Displacement of specific [$^3$H]etorphine binding (0.5 nM) from rat cerebrum homogenates, in presence of 150 mM NaCl.
[k] Previous report showed activity as an antagonist of meperidine in rats.[4]

These results suggest **NMA-1** has weak opioid agonist effects in the MVD. The lack of binding activity of **NMA-1** should be noted, and argues against an opioid classification. The binding activity may have been revealed if higher concentrations were tested. Of the remaining analogs, only the ethyl butyrate analog (**NME-3**) exhibited significant binding and either agonist or antagonist activity at the doses tested. **NME-3** displayed micromolar (μ) opioid agonist activity in the MVD (its effects were reversed by naltrexone and β-fulnaltrexamine, but not ICI 174864) and micromolar binding affinity. Though both compounds (**NMA-1** and **NME-3**) have very weak opioid activity in the MVD, and only **NME-3** displayed significant opioid receptor binding, their profiles are consistent with that of opioid agonists.[10]

The compounds presented here can be examined for comparisons of the effects of esters and acids. One comparison of the compounds, examined as two series of different functional group types, N-esters and N-acids, indicates varying activity by chain length. In vitro, the N-ester series ethyl acetate analog (**NME-1**) displayed an opioid antagonist profile and the ethyl butyrate analog (**NME-3**) displayed an opioid agonist profile. In vitro, the N-acetic acid analog (**NMA-1**) was the only N-acid compound which displayed any opioid efficacy or activity (opioid agonist activity) while the other two acids (**NMA-2** and **NMA-3**) lost all opioid activity. Both N-esters and N-acids show a change in in vitro activity with increasing spacing chain length. In vivo both the N-ester series and the N-acid series gain antinociceptive activity with increasing chain length. While in vitro assays show the N-esters change from antagonist activity to agonist activity, and the N-acids lose activity with increasing chain length. This observation is consistent with other benzomorphans we have studied previously where in vitro and in vivo activities do not always correlate.[2,3] The broad picture gleaned from this in vitro based comparison is N-esters tend to retain opioid activity (though switching efficacy from antagonism to agonist activity) with increasing chain length, and N-acids lost opioid activity with increasing chain length. Nonspecific antinociception (in vivo PPQ activity) was revealed in both the N-ester and the N-acid series as chain length increased.

Another comparison examines the compounds in pairs with identical spacing chain lengths, representing the before and after effects of hydrolysis of the ester functional

**Table 2.** Effects of N-substituents on lipophilicity

| Compound | N-substituent | Opioid class | $C\log P$ |
|---|---|---|---|
| Fentanyl[a] | Phenylethyl | Anilidopiperidine | 3.621 |
| Carfentanil[a] | Phenylethyl | Anilidopiperidine | 3.686 |
| Remifentanil[a] | Ethylproprionate (carbethoxyethyl) | Anilidopiperidine | 1.956 |
| Hydrolyzed remifentanil[a] | Propionic acid (carboxyethyl) | Anilidopiperidine | −0.777 |
| Metazocine | Methyl | Benzomorphan | 2.965 |
| **NME-1** | Ethylacetate (carbethoxymethyl) | Benzomorphan | 3.751 |
| **NME-2** | Ethylpropionate (carbethoxyethyl) | Benzomorphan | 3.992 |
| **NME-3** | Ethylbutyrate (carbethoxypropyl) | Benzomorphan | 4.229 |
| **NMA-1** | Acetic acid (carboxymethyl) | Benzomorphan | 0.459 |
| **NMA-2** | Propionic acid (carboxyethyl) | Benzomorphan | 0.598 |
| **NMA-3** | Butyric acid (carboxypropyl) | Benzomorphan | 0.852 |

[a] Compound data from Feldman et al.[19]; Calculated $\log P$ values ($C\log P$) were calculated using ChemDraw Ultra 10.0, CambridgeSoft.

group. An examination of calculated $\log P$ ($C\log P$) values (a model for comparison of lipophilicity) shows a significant decrease in lipophilicity when *N*-esters are compared to their *N*-acid congeners (hydrolysis product) (Table 2).[11] Though each analog pair shows a significant decrease in lipophilicity resulting from the modeled hydrolysis, no consistent effect other than change in activity is seen between the pairs. The ethyl acetate (**NME-1**) acetic acid (**NMA-1**) pair shows an opioid antagonist (**NME-1**) transformed into an opioid agonist (**NMA-1**) in the MVD. In this pair, in vitro functional activity is reversed when the before (ester, lipophilic) and after (acid, hydrophilic) components of hydrolysis are compared. The ethyl butyrate (**NME-3**) butyric acid (**NMA-3**) pair shows an opioid agonist (**NME-3**, ester, lipophilic) transformed into a compound devoid of opioid activity (**NMA-3**, acid, hydrophilic) in the MVD. Both pairs show a change in activity when hydrolysis is simulated in vitro. However, the in vivo data (non-opioid specific antinociception) show little change in activity when comparing the before (**NME-3**) and after (**NMA-3**) components of hydrolysis. These results are again similar to previous observations where in vitro and in vivo activities do not correlate.[2,3]

The broad picture from comparing *N*-ester, *N*-acid congener pairs indicates change in in vitro activity. The **NME-3, NMA-3** pair compares similarly to the change observed in the remifentanil series of compounds where an opioid agonist is reduced in potency when changed from an ester to an acid. The **NME-1, NMA-1** pair also displays a change in activity but the change is in efficacy instead of potency. It is interesting to speculate that this change may be due to the physical properties of the short chain compounds interacting with receptor.

Overall the results indicate that a combination of both the functional group character (lipophilicity and electronic effects) and the spacing carbon chain *length* between the basic nitrogen and the carbonyl carbon appeared to determine the compound's activity (or lack thereof), and not solely the functional group itself. The opioid antagonist activity of **NME-1** reported here supports the previous report of the antagonist activity of this compound. The confirmation of antagonist activity for an opioid *N*-ester, an efficacy profile opposite the

profile of the *N*-ester remifentanil, suggests that the ester functional group's effects on efficacy may be dependent upon opioid class. This observation is supported by the analysis of other opioid classes where antagonist activity is dependent upon binding mode, and not solely on the nature of the N-substituent.[12,13] The potential of this observation is interesting, however additional compounds and data are required to make generalizations.

## 3. Conclusions

The compounds presented here expand upon the current knowledge of functional group tolerance for opioid carboxylic ester and acid N-substituents. Although displaying significantly lower potency than alkyl substituents, esters appear to be tolerated in the benzomorphan class of opioids, and produced both opioid agonist and an opioid antagonist. Acids in the benzomorphan series appeared less well tolerated as they are extended out from the basic nitrogen leading to loss of opioid activity with extension beyond one methylene spacing unit. The data presented in this study show that an *N*-ester can produce opioid antagonism, which is the opposite efficacy of the remifentanil series of *N*-esters. This finding suggests both an expanded profile of *N*-ester substituent effects upon opioid efficacy, and also supports the observation that *N*-substituent efficacy effects are also dependent upon opioid class. These observations will be incorporated into future models of efficacy at opioid receptors underway in this laboratory.

## 4. Experimental

### 4.1. Chemistry

The *N*-ester-normetazocines (**NME-1-3**) were prepared from racemic normetazocine (**1**) and the appropriate halogenated esters, in a suspension of DMF/THF, in the presence of sodium carbonate. The *N*-acid-normetazocine compounds (**NMA-1-3**) were prepared from the corresponding *N*-ester-normetazocine analogs by hydrolysis in potassium hydroxide in water, followed by neutralization with 10% hydrochloric acid (Scheme 1).

All compounds were converted into oxalate or HCl salts.[14]

### 4.1.1. Typical procedures

**4.1.1.1. (±)-2-Carbethoxymethyl-5,9-α-dimethyl-2′hydroxy-6,7-benzomorphan oxalate = N-carboethoxymethyl-α-N-normetazocine oxalate $C_{18}H_{25}NO_3 \cdot (COOH)_2 \cdot (H_2O)_{0.5}$ (NME-1).** (±)-*N*-Normetazocine (**1**) (2.0 g), ethylbromoacetate (1.5 g), and $K_2CO_3$ (2.0 g) were added to a mixture of tetrahydrofuran (6 mL) and dimethylformamide (2 mL), and the resulting mixture stirred under reflux for 5 h. Solvents were evaporated in vacuo. The residue was diluted with water and extracted three times with diethyl ether. The resulting extracts were combined, washed with brine, dried with magnesium sulfate, and concentrated under vacuum which gave 2.8 g of base. The acidification of the free-base with oxalic acid in acetone gave the hemihydrated oxalate salt mp 116–118 °C.

**4.1.1.2. α(±)-2-Carboxymethyl-5,9-α-dimethyl-2′hydroxy-6,7-benzomorphan oxalate = N-carboxymethyl-α-N-normetazocine oxalate $C_{16}H_{21}NO_3 \cdot (HCl)_1 \cdot (H_2O)_{0.25}$ (NMA-1).** NME-1 (1.0 g, freebase), ethanol (0.1 g) and KOH (1.0 g), were refluxed in water (2.5 mL) for 1.5 h. The ethanol was evaporated in vacuo and the residue neutralized to pH 7 with 10% HCl. The resulting white solid was collected by filtration, washed with cold water yielding 0.65 g of free acid (mp 195–200). The free acid was dissolved in a mixture of methanol and ethylacetate, and precipitated as the hydrochloride salt by bubbling HCl gas through the solution mp 232–233 °C.

### 4.2. Pharmacology

Hot-plate (HP), tail flick (TF), antagonism of morphine in the tail flick (TFA), and phenylquinone antiwrithing (PPQ) assays were carried out in mice as previously described at doses of 0.25, 1, 10, and 30 mg/Kg.[15,16] Binding was determined in rat cerebrum membrane preparations, and $EC_{50}$ determinations by displacement of 0.5 nM [$^3$H]etorphine from rat brain homogenates using the standard procedures of the Drug Evaluation Committee (DEC), College on Problems of Drug Dependence (CPDD).[17,18] This assay measures binding at all three opioid receptors and does not differentiate between the opioid receptor types (μ, κ, δ). Functional activity in the Mouse Vas Deferens (MVD) was determined by suppression of electrically stimulated twitch using 25–30 g NIH Swiss mice, in the presence and absence of antagonists, to determine the specific receptor responsible for the actions, again following standard procedures of DEC.[17] Briefly, in the MVD assay, opioid agonists are characterized by the suppression of twitch height and inhibition by naltrexone. If the compound suppresses twitch height but is not inhibited by naltrexone the compound's effect is non-opioid in nature. Compounds are evaluated for opioid antagonist activity in the MVD by their ability to reverse the effects of morphine's suppression of twitch height. A compound, opioid agonist or antagonist, can be further evaluated for receptor specific effects with the use of receptor specific agonists and antagonists replacing naltrexone (for agonists) or morphine (for antagonists). Full details for these standard protocols and procedures are presented in the work of Smith.[10] The in vitro experiments (binding and MVD assays) were carried out with concentrations ranging from $10^{-7}$ to $10^{-5}$ M. Results are listed in Table 1.

### Acknowledgments

### References and notes

1. Casy, A. F.; Parfitt, R. T. *Opioid Analgesics*; New York and London: Plenum Press, 1986.
2. May, E. L.; Jacobson, A. E.; Mattson, M. V.; Coop, A.; Aceto, M. D.; Bowman, E. R.; Traynor, J. R.; Woods, J. H.; Harris, L. S. *Med. Chem. Res.* **1998**, *8*, 311.
3. May, E. L.; Coop, A.; Woods, J. H.; Aceto, M. D.; Bowman, E. R.; Harris, L. S.; Traynor, J. R. *Bioorg. Med. Chem.* **2003**, *11*, 31.
4. Feldman, P. L.; James, M. K.; Brackeen, M. F.; Bilotta, J. M.; Schuster, S. V.; Lahey, A. P.; Lutz, M. W.; Johnson, M. R.; Leighton, H. J. *J. Med. Chem.* **1991**, *34*, 2202.
5. Albertson, N. F.; McKay, F. C. *J. Med. Chem.* **1977**, *20*, 602.
6. Taber, R. I. *Adv. Biochem. Psychopharmacol.* **1973**, *8*, 191.
7. Defined by the authors of the study (Ref. 5) as the dose causing a 50% decrease in effect of an approximate ED80 dose of meperidine.
8. pA2 = −log[antagonist] which causes a 2-fold shift to the right in dose–response curve of agonist.
9. Due to testing protocols employed; pA2 values for NME-1 for sufentanil and U50,488 were not calculated.
10. Smith, C. B. *NIDA Res. Monogr.* **1986**, *76*, 288.
11. Clog P values calculated from structures using ChemDraw Ultra 10.0, CambridgeSoft.
12. Carroll, F. I.; Melvin, M. S.; Nuckols, M. C.; Mascarella, S. W.; Navarro, H. A.; Thomas, J. B. *J. Med. Chem.* **2006**, *49*, 1781.
13. Carroll, F. I. *J. Med. Chem.* **2003**, *46*, 1775.
14. Spectra were consistent with the assigned structures; the salts gave satisfactory microanalyses (±0.4%).
15. May, E. L.; Jacobson, A. E.; Mattson, M. V.; Traynor, J. R.; Woods, J. H.; Harris, L. S.; Bowman, E. R.; Aceto, M. D. *J. Med. Chem.* **2000**, *43*, 5030.
16. Pearl, J.; Harris, L. S. *J. Pharmacol. Exp. Ther.* **1966**, *154*, 319.
17. Woods, J. H.; Traynor, J. R. *Problems of Drug Dependence 2000, NIDA Res. Monogr.* **2001**, *181*, 141.
18. Negus, S. S. *Drug Alcohol Depend.* **2006**, *82*, 182.
19. Feldman, P. L.; James, M. K.; Brackeen, M. F.; Bilotta, J. M.; Schuster, S. V.; Lahey, A. P.; Lutz, M. W.; Johnson, M. R.; Leighton, H. J. *J. Med. Chem.* **1991**, *34*, 2202.