3-Alkyl Ethers of Clocinnamox: Delayed Long-Term μ-Antagonists with Variable μ Efficacy

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In recent years there has been considerable interest in the relationship between clocinnamox (C-CAM) and its methyl ether methoclocinnamox (MC-CAM). While C-CAM appears to be an insurmountable μ-antagonist, MC-CAM has been shown to be a potent partial agonist at μ-opioid receptors. To further investigate this relationship we prepared other ethers of C-CAM and evaluated these in opioid receptor binding assays and in vivo in mouse antinociceptive assays and in morphine-dependent monkeys. In opioid binding assays, the ethers were generally μ-selective with affinity equivalent to that of C-CAM itself. Although they displayed little or no efficacy in vitro, some of the ethers showed substantial agonist activity in the in vivo antinociceptive tests. Two of the ethers, the propargyl ether **7** and the cyclopropylmethyl ether **5**, were chosen for more detailed analysis in vivo. **7** was shown to have significant μ-agonist character and was able to substitute for morphine in morphine-dependent monkeys. Interestingly, when this agonist effect abated, **7** displayed long-lasting μ-antagonism. In contrast, **5** displayed little agonist activity in vivo and was characterized as a potent, long-acting μ antagonist. Although further work is needed to determine whether metabolism is a crucial factor in determining the pharmacological profile of these ethers, it is clear that 3-O-alkylation is a useful means of varying the μ efficacy displayed by this class of acyl-substituted 14-aminomorphinones. MC-CAM itself has generated considerable interest as a potential pharmacotherapy for opiate abuse. These analogues with differing μ efficacy but retaining the long-lasting μ-antagonist effects provide further opportunities for the development of treatment drugs.

In recent years, we have studied acyl derivatives in the 14-aminomorphinone and 14-aminocodeinone series. The cinnamoyl derivatives, particularly clocinnamox (C-CAM, **1**) and methoclocinnamox (MC-CAM, **2**) have aroused substantial interest.[1a,b,2] In a variety of assays, C-CAM acts as an insurmountable antagonist of μ receptors.[3a−f] In contrast to the irreversible μ antagonist β-FNA,[4a−b] C-CAM is devoid of any opioid agonist activity,[4b,5] and it has greater potency than β-FNA when injected systemically.[6] MC-CAM was shown to be a potent opioid agonist in the phenylquinone-induced antiabdominal stretch assay in mice but was inactive in the tail flick and hot plate tests.[3a] The antiabdominal stretch activity of MC-CAM could not be reversed by administering naltrexone 2 h after MC-CAM, although it was prevented by pretreatment with the antagonist.[1b] When administered to withdrawn, morphine-dependent rhesus monkeys, MC-CAM suppressed abstinence, i.e., it substituted for morphine; however, when given to nonwithdrawn morphine-dependent monkeys, it precipitated a delayed but long-lasting morphine withdrawal syndrome. Thus, MC-CAM was characterized as a μ partial agonist. Since the μ-antagonist effect was delayed but long-lasting, metabolism of the codeinone by 3-O-demethylation to the morphinone (C-CAM) was suspected. This is analogous to the metabolism of codeine to morphine that has been held responsible for the in vivo activity of codeine.[7a−c] This view is supported by the disparity between the very low receptor affinity of codeine (less than 10^{-3} times that of morphine) and its in vivo activity (~0.1 times that of morphine).[8] However, evidence exists which suggests that the efficacy of codeine in vivo is greater than that of morphine despite codeine having much lower potency.[9] This would require that much of the activity of codeine is independent of its metabolic conversion to morphine. Studies of the in vitro metabolism of [^{3}H]-MC-CAM in rat, cynomolgus monkeys and human liver homogenates showed that O-demethylation to C-CAM predominated in the monkey, whereas N-dealkylation was the primary process in rats. In human liver homogenates, MC-CAM was transformed to both C-CAM and its N-dealkylated analogue. Preliminary in vivo studies in rat and cynomolgus monkeys suggested that in both species C-CAM was the principal metabolite.[1b]

The work described in this paper aimed to shed some light on the relationship of C-CAM to its 3-alkyl ethers, that is, to investigate the effect of the ether function on agonist/antagonist activity. A series of ethers was prepared and evaluated in opioid receptor binding assays and in vivo in mouse antinociceptive assays and in morphine dependent rhesus monkeys. The results

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Table 1. Binding Affinities[a] of 3-Substituted C-CAM Derivatives at μ, δ, and κ Opioid Receptors

compd	μ (DAMGO)	δ (DPDPE)	κ (U69593)
1	9.0 ± 2.1	12.5 ± 2.2	5.4 ± 1.1
3	8.9 ± 1.6	65.4 ± 3.1	17.9 ± 2.2
4	12.4 ± 1.9	89.5 ± 2.6	18.0 ± 2.1
5	7.6 ± 1.2	56.5 ± 2.1	131.2 ± 4.2
6	24.6 ± 2.4	82.6 ± 2.5	19.1 ± 0.9
7	1.6 ± 0.3	21.3 ± 1.0	19.1 ± 0.9
8	7.6 ± 1.3	24.8 ± 1.3	95.4 ± 4.6
9	59.8 ± 4.2	96.4 ± 2.2	14.9 ± 1.8

[a] K_i (nM). Values are means from 3 experiments.

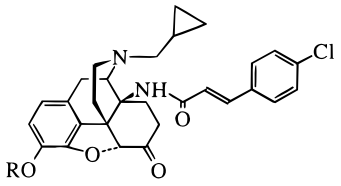
show that the ethers differ in affinity for the individual types of opioid receptor and in efficacy at μ receptors.

Chemistry

The compounds were prepared from C-CAM (**1**)[1a] by reaction with 3 equiv of alkyl halide in the presence of potassium carbonate. The more reactive halides gave good yields at ambient temperature, the others requiring reflux. The yields quoted are for material isolated after recrystallization.

Results

In Vitro Assays. In the opioid binding assays in rat brain homogenates, the ethers showed unexpectedly high affinity, particularly for the μ receptor (Table 1). The K_i values for **3**, **4**, **5**, and **6** were all about equal to that of the parent phenol, C-CAM, as has been previously found with MC-CAM.[3d,16] The propargyl ether **7** showed μ affinity significantly higher than that of the phenol, whereas the *n*- and isopropyl ethers (**6** and **9**)



1: R = H, C-CAM
2: R = CH_3, MC-CAM
3: R = $CH_2CH{=}CH_2$
4: R = CH_2CN
5: R = CH_2–cyclopropyl
6: R = $CH_2CH_2CH_3$
7: R = CH_2CCH
8: R = $CH_2CO_2CH_3$
9: R = $CH(CH_3)_2$

had lower affinity. The affinity of the ethers for δ and κ receptors was in all cases lower than that of the phenol so that the ethers were generally μ-selective. However, this did not apply to the isopropyl derivative **9** which had the lowest μ-affinity and highest κ-affinity among the ethers and was somewhat κ-selective. The ethers were tested up to a concentration of 10 μM in the guinea pig ileum assay yet showed only weak agonist responses (data not shown). This is a sensitive assay[10] in which μ- and κ-opioid agonists typically give a robust response. **5** showed the highest level of inhibition, but this was only 30% at a concentration of 100 nM. At higher concentrations, the inhibition was reduced so that the dose−response curve was bell-shaped.

Table 2. Antinociceptive Potencies of CCAM Ethers in the Mouse Tail Withdrawal Assay

compd	ED_{50}[a] (mg/kg)	% MPE
3	11.1 ± 5.4	100
4	20.8 ± 10.4	78 ± 16
6	nc[b]	35% at 32 mg/kg
7	6.3 ± 1.8	100
morphine	17.9 ± 3.6	100

[a] Values are means ± SEM from 6 animals. [b] Not calculated.

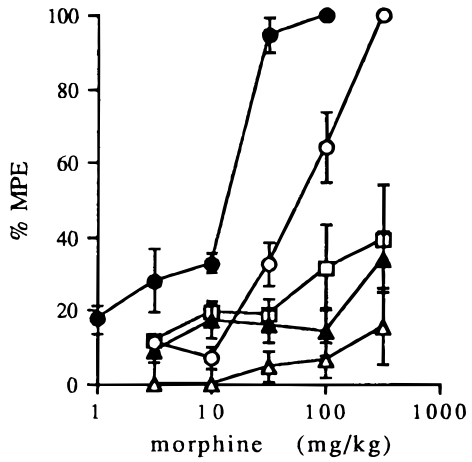


Figure 1. Antagonism of morphine antinociception in the mouse tail withdrawal test using water at 50 °C. Control (●), control + **5** with pretreatment time of 30 min (△), 24 h (▲), 2 days (□) and 6 days (○).

In Vivo Studies. In preliminary studies in the mouse tail withdrawal test (in warm (50 °C) water), ethers **3**, **4**, **6**, and **7** showed agonist effects (Table 2), whereas **5** showed no agonist effects in this test or in the acetic acid-induced abdominal stretch test in which less efficacious opioid agonists are active. At a dose of 32 mg/kg, **5** caused flattening of the morphine dose−response curve which lasted for at least 6 days (Figure 1). In addition, 24 h after administration when the agonist actions of the agonist ethers had waned, they also inhibited the activity of morphine in the tail withdrawal assay (data not shown).

The most efficacious ether (**7**) together with the antagonist (**5**) was selected for detailed in vivo evaluation in a battery of mouse antinociceptive tests and in morphine-dependent rhesus monkeys. Preliminary reports of these evaluations have been published.[11]

The propargyl ether (**7**) was active in all three antinociceptive tests (tail flick (TF), hotplate, and phenylquinone-abdominal stretch) with potency about equal to that of morphine. The cyclopropylmethyl (CPM) ether (**5**) was moderately active in the abdominal stretch test but inactive in the other two tests (Table 3).

Morphine antagonist activity was assessed in the TF assay. Both ethers were inactive when the test compound was administered the standard 30 min before morphine. However, in a more extensive time-course study, they were shown to be quite active but with slow onset. The peak effect for **5** was at about 6 h, whereas for **7** it was between 24 and 48 h, and they were both active up to 72 h (Table 4).

The opioid receptor types responsible for the agonist and antagonist actions of **5** and **7** were investigated in the acetic acid-induced abdominal stretch test in mice, in which standard agonists selective for μ, κ, and δ

Table 3. Comparison of the Antinociceptive Agonist/Antagonist Profiles of Activity of C-CAM Ethers[a]

	tail flick	tail flick vs morphine	abdominal stretch (phenylquinone)	hot plate
2	I[b]	6.0 (2.1−17.4)	0.2 (0.05−0.6)	I[c]
5	I[b]	I[b]	6.7 (2.2−20.7)	I[c]
7	1.2 (0.6−2.9)	I[b]	0.3 (0.09−0.82)	0.3 (0.08−0.70)
morphine	1.9 (0.9−4.1)	I[b]	0.4 (0.2−0.8)	0.9 (0.4−1.9)

[a] ED_{50} or AD_{50} (95% confidence limits) expressed as mg/kg sc. [b] Inactive up to 30 mg/kg. [c] Inactive up to 20 mg/kg.

Table 4. Antagonism of Morphine Antinociceptive ED_{50} by C-CAM Ethers. Onset and Duration of Morphine Antagonist Action in the Mouse Tail Flick Assay

pretreatment time (h)	AD_{50}[a]	AD_{50}[a]
	compd **5**	compd **7**
2	57% at 30	17% at 30
6	0.97 (0.39−2.4)	14.11 (5.99−33.23)
24	3.41 (1.37−8.50)	4.09 (4.86−9.03)
36	6.82 (3.13−12.76)	3.02 (1.12−8.10)
72	11.39 (4.14−31.36)	36% at 30

[a] Expressed as 95% confidence limits or % antagonism (mg/kg sc).

Table 5. Effect of Antagonists on the Inhibition of Acetic Acid -Induced Abdominal Stretches by **7** (10 mg/kg sc)

antagonist	pretreatment time	% antagonism
β-FNA, 32 mg/kg	24 h	100 ± 15
Naltrindole, 10 mg/kg	15 min	3.9 ± 2.1
nor-BNI, 32 mg/kg	24 h	4.8 ± 3.4

Table 6. Antagonist Selectivity of **5** and **7** (32 mg/kg sc) after a 24-h Pretreatment Period, in the Mouse Abdominal Stretch Assay

	% antagonism of agonist effect	
agonist[a]	agonist + **5**	agonist + **7**
morphine 3.2 mg/kg	100 ± 16	96 ± 6
BW373U86 10 mg/kg	0	38 ± 8
bremazocine	19 ± 7	7 ± 5

[a] Agonists were given sc 15 min before the induction of stretches with ip acetic acid. **5** alone did not alter the number of stretches induced by acetic acid. The antagonist action of **5** was not evident until 2 h after administration.

opioid receptors are active. The antiabdominal stretch effect of **7** (10 mg/kg) was totally suppressed by the selective μ antagonist β-FNA, but other selective antagonists, naltrindole (δ) and nor-BNI (κ), had no effect (Table 5). Thus, the agonist effect of **7** is produced by selective activation of μ opioid receptors. The antinociceptive action of **7** was gone by about 8 h. The receptor type responsible for the long-term opioid receptor antagonism of **7** was determined with 24 h pretreatment of the test compound. Under these conditions, **7** totally inhibited the agonist action of morphine, partially inhibited the δ agonist BW 373U86, but had virtually no effect on bremazocine (κ) (Table 6). The O-CPM derivative (**5**) had very little agonist action in the acetic acid abdominal stretch assay, but the onset of the morphine antagonist actions was delayed by up to 2 h; it was still fully effective at 24 h (Table 6). At this time, there was no antagonism of BW 373U86 and very little effect against bremazocine. Thus, the antagonist actions of **5** and **7** were selective for the μ receptor type.

In single-dose suppression tests in withdrawn, morphine-dependent rhesus monkeys, **7** suppressed the withdrawal effects for the first 2-h observation period (Table 7). Later, the monkeys showed long-lasting signs of withdrawal that could be reversed on day 2 by the administration of morphine. Thus, **7** behaved initially as a μ agonist but then showed delayed, long-lasting μ antagonism. **5** did not suppress morphine withdrawal but exacerbated these effects which could then not be reversed by the administration of morphine in the first 24 h.

Discussion

The ethers were chosen to have potentially different levels of metabolic stability. Thus straight (**6**) and branched (**9**) chain alkyl ethers were targeted along with ethers containing different levels of saturation (allyl, **3** and propargyl, **7**). Compound **5** was synthesized since the CPM group is considered to be chemically similar to the allyl (**3**) but obviously has greater steric bulk which again would be expected to influence metabolic stability. Additionally two ethers (**4** and **8**) with electron-withdrawing substituents were synthesized.

The new C-CAM ethers, like the methyl ether MC-CAM, had high affinity for opioid receptors particularly the μ type. The μ receptor affinity was generally similar to that of C-CAM, but the affinity of the propargyl ether (**7**) was actually 5 times higher than that of the phenol. This is a very unusual relationship and contrasts with the usual situation in morphinan series where the methyl ethers have very much lower opioid receptor affinity than the phenols. In C-CAM and its ethers, it appears that the 14β-cinnamoylamino group is more important for μ binding than the phenolic hydroxyl group. Interestingly the lowest μ affinity and highest κ affinity was found in the 2-propyl ether (**9**) suggesting quite subtly different steric requirements for binding to the two types of opioid receptor.

The data from the in vitro functional assay and the in vivo antinociceptive tests showed disparities. In the GPI assay that provides a measure of μ and κ opioid receptor efficacy, none of the ethers was particularly active, whereas in vivo, most of them showed substantial agonist activity. However, in the antinociceptive assays, there were some clear differences, particularly between the propargyl (**7**) and the CPM (**5**) ethers. The agonist activity of **7** in the writhing test was shown to be mediated by μ opioid receptors, and this was confirmed in the single dose substitution test in withdrawn morphine-dependent rhesus monkeys. When the agonist effect had abated, a long-term μ-antagonist effect for **7** was shown in the abdominal stretch test and in the monkeys. In this battery of in vivo tests, the CPM ether (**5**) showed only a low level of agonist activity. It was however a potent long-lasting μ antagonist. The flattening of the morphine dose−response curve in the tail withdrawal test is characteristic of an irreversible antagonist. Thus, the profile of **5** was quite similar to that of the phenol C-CAM.

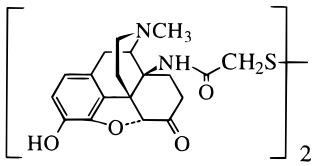
In the College on Problems of Drug Dependence evaluation, the propargyl ether (**7**) and the CPM ether

Table 7. Substitution of C-CAM Ethers for Morphine in Morphine-Dependent Monkeys

MC-CAM	**5**	**7**
Dose-related substitution in the range of 0.05−0.8 mg/kg.	Exacerbated withdrawal in 2 monkeys receiving 0.75 or 3.0 mg/kg, respectively. Morphine did not attenuate withdrawal for at least 24 h.	Substituted for morphine in the dose range of 0.05−2.1 mg/kg during first 2 h. Later exacerbated withdrawal. Morphine attenuated withdrawal on day 2.

(**5**) can be compared with the previously reported methyl ether MC-CAM (**2**) (Tables 3 and 7). In the antinociceptive tests, MC-CAM is closer in profile to the CPM ether (**5**) than to **7**, whereas in the monkey test, the reverse is the case. This species difference may be due to different rates and/or routes of metabolism. If metabolism of the ethers to the phenol C-CAM is the explanation for their antagonist activity, different rates of metabolism could account for the differences of μ efficacy observed in vivo. The propargyl ether (**7**) would be least readily metabolized, allowing the full agonist effects to be manifested and delaying and attenuating the appearance of C-CAM antagonist effects. MC-CAM which has a μ partial agonist profile[1b,3a] would be intermediate in the rate of O-dealkylation with the appearance of C-CAM preventing the development of full agonist effects. The CPM ether (**5**) would have to be the most easily metabolized, giving the highest concentrations of C-CAM in brain and behaving like C-CAM as an irreversible antagonist. However, the facile O-dealkylation of CPM ethers is not a general phenomenon in the epoxymorphinans since the CPM ethers of naltrexone and diprenorphine, unlike **5**, did not have in vivo profiles comparable to that of the parent phenols.[12]

Although there is evidence both in vitro and in vivo for the O-demethylation of MC-CAM to C-CAM, there is no proof that this metabolism is responsible for the delayed μ antagonism of MC-CAM or, by analogy, that of the other C-CAM ethers. If it were, MC-CAM would be expected to be the most easily metabolized and, therefore, the most antagonist.[13] The profile of agonism followed by delayed, long-lasting antagonism has also been found in derivatives of 14-aminomorphinones in which metabolism to an antagonist can be discounted. The bisthioglycolamido derivative TAMO (**10**)[14a,b] was



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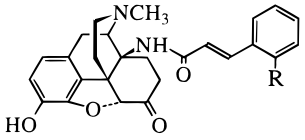
antinociceptive in the tail flick test when administered intracerebroventricularly, with a potency about 10 times greater than that of morphine and a duration of about 2 h. It was also able to antagonize the antinociceptive effect of morphine; the onset of this effect was about 8 h following administration, and its duration was at least 48 h. The antagonist effect of TAMO was differentiated from a cross-tolerance effect by its ability to suppress the development of morphine dependence in mice.[14a] In our own unpublished studies of analogues of C-CAM, the ortho-substituted morphinones (**11** and **12**) were high-potency full agonists when administered subcutaneously in the tail withdrawal assay, but with 48 h pretreatment they showed the characteristics of irreversible μ antagonists. Thus, the delayed antagonism of morphine antinociceptive effect by the C-CAM ethers could be largely attributable to the action of the ethers themselves. On the basis of the present data, it is not possible to conclude the extent of the contribution of the metabolite C-CAM to the μ-antagonist actions of the ethers.



11: R = Me
12: R = Cl

Conclusions

3-O-Alkylation of the μ-selective irreversible antagonist clocinamox produced a series of ethers with affinity for opioid receptors comparable to that of the parent. They were μ-selective in vivo; they were initially μ agonists and later showed pronounced morphine antagonist activity. The cyclopropylmethyl ether had low μ efficacy in vivo and behaved like clocinnamox as an irreversible antagonist of morphine. Further work is needed to determine the extent to which metabolism to clocinnamox is responsible for the μ antagonist activity of the ethers. MC-CAM has generated substantial interest as a potential treatment for opiate abuse. We have shown that it is possible to modify the μ efficacy by preparing other ethers of C-CAM, while still retaining the long-lasting μ-antagonist properties. These new ligands could provide further opportunities for the development of potential pharmacotherapies for opiate abuse.

Experimental Section

Chemistry. Infrared spectra were obtained on a Perkin-Elmer 881 spectrophotometer. The proton and carbon-13 NMR spectra were obtained on a JEOL JNM-GX 270 (67.5) spectrometer at 20 °C in CD_2Cl_2 unless otherwise stated. Tetramethylsilane was used as the internal standard. Mass spectra were obtained on a Fisons autosampler instrument with electron impact ionization (70 eV). Melting points were determined on a Reicher hot-stage microscope and are uncorrected. Elemental analyses were obtained on a Perkin-Elmer 240C analyzer. All reagents were used as supplied by Aldrich.

C-CAM-3-allyl Ether (3). Allylbromide (0.52 mL, 6.00 mmol) was added to a stirred mixture of C-CAM (1.00 g, 1.98 mmol) and potassium carbonate (0.82 g, 5.94 mmol) in a 0.5% water in acetone mix (60 mL). The mixture was stirred at room temperature for 60 h before the solid was filtered off and the filtrate evaporated to dryness. The off-white solid was recrystallized from methanol to give the product as white crystals (928 mg, 86%): mp 141−142.5 °C: IR (KBr) 3529 (NH), 1713 (CO, ketone), 1672 (CO, amide) cm^{-1}; ^1H NMR δ 4.60 (2H, dd, J = 5.7, 1.5, OCH_2), 4.78 (1H, s, H-5), 5.24 (1H, dd, J = 10.4, 1.5, allyl), 5.37 (1H, m, J = 17.2, 1.7, 1.5, allyl), 6.05 (1H, m, J = 17.2, 10.4, 5.7, allyl), 6.65 (1H, d, H-1), 6.76 (1H, d, H-2); ^{13}C NMR δ 4.0, 4.2, 9.9, 21.7, 30.5, 31.0, 37.1, 44.2, 48.5, 56.0, 59.8, 60.8, 70.9, 89.9, 117.3, 117.8, 119.5, 122.3, 126.7, 129.4, 129.5, 129.6, 133.9, 134.1, 135.7, 139.8, 141.6,

145.8, 166.5, 206.4; *m/z* 544 (M^+, 93), 503($M^+ - CH_2CH{=}CH_2$, 87); Anal. ($C_{32}H_{33}N_2O_4Cl$) CHNCl.

C-CAM-3-cyanomethyl Ether (4). The compound was synthesized as above but using bromoacetonitrile at room temperature for 16 h to give the product as white crystals (80%): mp 188−189 °C (MeOH); IR (KBr) 3284 (NH), 1725 (CO, ketone), 1665 (CO, amide) cm^{-1}; ^1H NMR δ 4.93 (2H, s, H-5), 4.94 (2H, s, OCH2), 6.72 (1H, d, H-1); 6.88 (1H, d, H-2); ^{13}C NMR δ 4.0, 4.3, 9.9, 21.8, 30.7, 30.8, 37.2, 44.2, 48.7, 56.1, 56.5, 59.6, 60.8, 90.8, 116.1, 119.9, 120.9, 122.2, 129.4, 129.5, 129.7, 130.5, 133.8, 135.8, 134.0, 139.3, 146.3, 166.7, 206.3; *m/z* 543 (M^+, 55), 503($M^+ - CH_2CN$, 51); Anal. ($C_{31}H_{30}N_3O_4Cl$) CHNCl.

C-CAM-3-cyclopropylmethyl Ether (5). The compound was synthesized as above but using (bromomethyl)cyclopropane at reflux for 48 h to give the product as white crystals (79%): mp 129.5−131.5 °C (MeOH); IR (KBr) 3344 (NH), 1705 (CO, ketone), 1669 (CO, amide) cm^{-1}; ^1H NMR δ 0.31 (2H, m, cyclopropyl), 0.60 (2H, m, cyclopropyl), 1.25 (1H, m, cyclopropyl), 3.86 (2H, m, J = 13.9, 7.1, OCH_2), 4.77 (1H, s, H-5), 6.65 (1H, d, H-1), 6.73 (1H, d, H-2); ^{13}C NMR δ 3.3, 3.4, 4.0, 4.3, 9.9, 10.8, 21.7, 30.6, 31.1, 37.1, 44.4, 48.5, 55.6, 59.6, 61.0, 74.9, 89.8, 116.6, 119.5, 122.3, 126.2, 129.4, 129.5, 129.6, 133.9, 135.8, 139.9, 142.3, 145.7, 166.3, 206.5; *m/z* 558(M^+, 70), 503($M^+ - CH_2CH(CH_2)_2$, 16); Anal. ($C_{33}H_{35}N_2O_4Cl{\cdot}H_2O$) CHNCl.

C-CAM-3-(1-propyl) Ether (6). The compound was prepared as above, but using 1-iodopropane at reflux for 60 h to give the product as white crystals (61%): mp 137−139 °C (MeOH); IR (KBr) 3343(NH), 1705 (CO, ketone), 1669 (CO, amide) cm^{-1}; ^1H NMR δ 1.00 (3H, t, J = 7.4, CH_3), 1.77 (2H, m, J = 7.4, 6.7, CH_2), 3.98 (2H, t, J = 6.7, OCH_2), 4.77 (1H, s, H-5), 6.66 (1H, d, H-1), 6.75 (1H, d, H-2); ^{13}C NMR δ 4.0, 4.2, 9.9, 10.6, 21.7, 23.2, 30.5, 31.1, 37.1, 44.3, 48.5, 56.0, 59.6, 61.0, 71.5, 89.8, 116.4, 119.5, 122.3, 126.1, 129.3, 129.4, 129.5, 133.9, 135.7, 139.8, 142.3, 145.7, 166.6, 206.5; *m/z* 546 (M^+, 100), 503($M^+ - CH_2CH_2CH_3$, 7); Anal. ($C_{32}H_{35}N_2O_4Cl$) CHNCl.

C-CAM-3-propargyl Ether (7). The compound was prepared as above but using propargyl bromide at reflux for 48 h to give the product as white crystals (75%): mp 146−148 °C (cyclohexane); IR (KBr) 3300 (CCH), 3266 (NH), 2121(CC), 1712 (CO, ketone), 1671 (CO, amide) cm^{-1}; ^1H NMR δ 2.55 (1H, t, J = 2.4, CC−H), 4.75 (2H, d, J = 12, OCH_2), 4.81 (1H, s, H-5), 6.68 (1H, d, H-1), 6.85 (1H, d, H-2); ^{13}C NMR δ 3.8, 4.1, 9.7, 21.5, 30.4, 30.5, 36.8, 44.0, 48.4, 55.9, 58.1, 59.3, 60.6, 75.4, 79.1, 89.9, 118.8, 119.2, 121.6, 126.9, 129.0, 129.1, 129.3, 133.2, 135.7, 140.0, 140.4, 146.0, 166.4, 206.0; *m/z* 542 (M^+, 100), 503($M^+ - CH_2CCH$, 98); Anal. ($C_{32}H_{31}N_2O_4Cl$) CHNCl.

C-CAM-3-methoxycarbonyl Methyl Ether (8). The compound was prepared as above but using methyl bromoacetate at room temperature overnight to give the product as white crystals (85%): mp 179−181 °C (MeOH); IR (KBr) 3267 (NH), 1756 (CO_2Et), 1724 (CO, ketone), 1667 (CO, amide) cm^{-1}; ^1H NMR δ 3.77 (3H, s, CH_3O_2C), 4.75 (2H, d, J = 16.3, OCH_2), 4.80 (1H, s, H-5), 6.66 (1H, d, H-1), 6.76 (1H, d, H-2); ^{13}C NMR δ 3.6, 36.6, 48.3, 52.1, 58.9, 67.3, 76.6, 89.8, 119.7, 121.6, 128.9, 129.2, 133.1, 135.5, 139.9, 145.1, 169.8, 205.8; *m/z* 576 (M^+, 90), 517 ($M^+ - CH_3O_2C$, 20); Anal. ($C_{32}H_{33}N_2O_6Cl$) CHNCl.

C-CAM-3-(2-propyl) Ether (9). The compound was prepared as above but using 2-iodopropane at reflux over 6 days to yield the product as a white solid (45%): mp 191−193 °C (MeOH); IR (KBr) 3357 (NH), 1708 (CO, ketone), 1670 (CO, amide) cm^{-1}; ^1H NMR δ 1.27 (6H, dd, J = 6.1, 2 × CH_3), 4.62 (1H, sept, J = 6.1, $CH(CH_3)_2$), 4.74 (1H, s, H-5), 6.65 (1H, d, H-1), 6.74 (1H, d, H-2); ^{13}C NMR δ 3.7, 22.3, 22.4, 37.8, 48.3, 72.3, 89.3, 118.4, 119.1, 121.7, 129.0, 129.2, 133.2, 134.7, 139.9, 146.2, 206.6; *m/z* 546 (M+, 100); Anal. ($C_{32}H_{35}N_2O_4Cl$) CHNCl.

Pharmacological Assays. Ligand Binding Assays. Brains (- cerebellum) were removed and homogenized in Tris buffer (50 mM, pH 7.4), and crude membranes were prepared as described previously.[15] Ligand binding assays were performed using [^{3}H]DAMGO (2 nM), [^{3}H]DPDPE (1.8 nM), and [^{3}H]U69,593 (1.1 nM) to define μ, δ, and κ opioid receptor binding sites, respectively. Test compounds were dissolved in DMSO and then diluted with distilled water to the required concentrations. The final DMSO concentration never exceeded 0.1%. Nonspecific binding was defined using 10 μM naloxone.

Antinociceptive Assays. The methods for the tail flick, hot plate, and phenylquinone abdominal stretching assays in mice were used as previously reported.[5] In these assays, the test compounds were dissolved in distilled water, in 5% aqueous DMSO, or in 5% hydroxypropyl-β-cyclodextrin and injected subcutaneously (sc). For the tail withdrawal and acetic acid-induced abdominal stretching assays, the ethers were dissolved in sterile water containing 16% MeOH and 1% HCl, and the assay was carried out according to published procedures.[1b]

Dependence−Liability Studies in Rhesus Monkeys. Single-Dose Substitution (SDS). Procedures for these studies have been reported previously.[5] Compounds were dissolved in distilled water or 10% aqueous hydroxypropyl-β-cyclodextrin or 5% aqueous DMSO and injected sc. At least three monkeys per dose were used.

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