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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 4291–4295

Opiate receptor binding properties of morphine-, dihydromorphine-, and codeine 6-*O*-sulfate ester congeners

Peter A. Crooks,[a,*] Santosh G. Kottayil,[b] Abeer M. Al-Ghananeem,[a] Stephen R. Byrn[c] and D. Allan Butterfield[b]

[a]*Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, Lexington, KY 40536-0082, USA*
[b]*Department of Chemistry, University of Kentucky, Lexington, KY 40536-0082, USA*
[c]*Department of Industrial and Physical Pharmacy, School of Pharmacy and Pharmaceutical Sciences, Purdue University, 575 Stadium Mall Drive, West Lafayette, IN 47907-2091, USA*

Received 14 March 2006; revised 13 May 2006; accepted 18 May 2006
Available online 13 June 2006

Abstract—A series of 3-*O*-acyl-6-*O*-sulfate esters of morphine, dihydromorphine, *N*-methylmorphinium iodide, codeine, and dihydrocodeine were prepared and evaluated for their ability to bind to μ-, δ-, κ_1-, κ_2-, and κ_3-opiate receptors. Several compounds exhibited good affinity for the μ-opiate receptor. Morphine-3-*O*-propionyl-6-*O*-sulfate had four times greater affinity than morphine at the μ-opiate receptor and was the most selective compound at this receptor subtype.

Opium contains a number of alkaloids, of which only a few, that is, morphine, codeine, noscapine, and papaverine, have found clinical use.[1] Metabolites of codeine, other than CYP2D6-dependent formation of morphine, have also been reported to have central nervous system effects.[2]

Metabolic conjugation (phase II metabolism) usually terminates the pharmacological action of a drug; however, in the case of morphine it has been observed that the metabolite morphine-6β-glucuronide (M6G) is almost 100-fold more potent than morphine when administered via the intracerebroventricular (icv) route in animals.[3] The 3-*O*- and 6-*O*-sulfate conjugates of morphine (**1**) have been known for many years.[4–6] Morphine-3-*O*-sulfate (**2**, M3S) is a known phase II metabolite of morphine,[7] and has been detected in rat tissues after administration of morphine.[8] However, morphine-6-*O*-sulfate (**3a**, M6S) has not been detected as a biotransformation product of morphine.[7,9] While M3S has been reported by several groups[4–6] to have little or no in vivo analgesic potency, Brown et al.[10] have reported this compound to be three times more potent than morphine as an analgesic when both compounds are administered by the icv route in mice.

Structural analogs of morphine that contain a 3-hydroxy group in their structure are generally 30- to 100-fold more potent than their corresponding 3-methoxy analogs at the μ-receptor, and these compounds generally exhibit greater efficacy (e.g., morphine produced 2-fold greater maximal stimulation than its 3-methoxy analog, codeine).[11] Furthermore, analgesic potency has been shown to increase by sulfation of the 6-hydroxy group of morphine.[6,10] Morphine-6-*O*-sulfate (**3a**, M6S) has been shown to displace [^{3}H]-morphine and [^{3}H]-leucine enkephalin binding in rat brain membranes with K_i values of 1.8 and 4.8 nM, respectively;[12] this study indicated that 6-O-sulfation of morphine results in reduced affinity for the μ-receptor but enhanced affinity for the κ-receptor.

M6S is an effective analgesic, with a 30-fold greater potency than morphine in the mouse radiant heat tail-flick assay, and is similar in potency to the active morphine metabolite, morphine-6β-glucuronide (M6G).[13] 3-O-acylation of the M6S molecule affords more lipophilic derivatives that are considered to be prodrugs of M6S.[14,15] These O-acylated derivatives were found to be relatively stable in phosphate-buffered saline over the pH range 6–8, and were slowly hydrolyzed in blood and brain homogenate to M6S.[16] It has been demon-

Keywords: Morphine analogs; Sulfate conjugates; Opiate receptors.
* Corresponding author. Tel.: +1 859 257 1718; fax: +1 859 257 7585; e-mail: pcrooks@email.uky.edu

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doi:10.1016/j.bmcl.2006.05.060

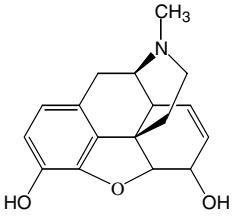
strated that the 3-*O*-acetyl derivative of M6S (M3A6S, **3b**) exhibits enhanced in vivo duration of antinociceptive activity compared to both M6S and morphine, when given subcutaneously (sc) to rats, and has greater potency and duration of action than morphine when given to rats via the icv route.[16,17]

The relative stability and interesting antinociceptive profile of M3A6S and other structurally related *O*-acyl analogs of M6S prompted us to undertake a comprehensive structure–affinity study of a number of 3-*O*-acyl derivatives of M6S and other congeners (Fig. 1)[18] to determine the affinity of these compounds for opiate receptors.

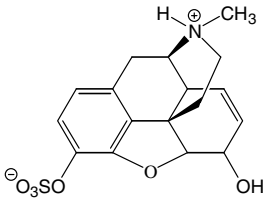
Compounds **3a**–**i** were prepared from 6-O-sulfation of codeine or the appropriate 3-*O*-acyl analog of morphine, with pyridine-SO_3 reagent (Scheme 1).

The dihydro analogs **4a**–**d** were synthesized in a similar manner from dihydrocodeine or the appropriate 3-*O*-acyl analog of dihydromorphine. The betaines **3g** and **3h** were obtained from pyridine-SO_3 sulfation of 3-*O*-acetyl-*N*-methylmorphinium iodide and 3-*O*-benzoyl-*N*-methylmorphinium iodide, respectively. M3S (**2**) was prepared from direct sulfation of morphine, and M6S (**3a**) and DM6S (**4a**) were prepared from deacetylation of 3-*O*-acetylmorphine-6-*O*-sulfate and 3-*O*-acetyl-dihydromorphine-6-*O*-sulfate, respectively, in MeOH–NaOH. X-ray crystallographic studies on M3A6S demonstrated the presence of two independent zwitter-ionic molecules per asymmetric unit.[17] The stereotopic crystal structures of the two molecules indicated that they differ from one another in the relative conformation of the C- and D-rings. Similar to the parent morphine molecule,[19] one of the structures incorporates the C- and D-rings in their boat and chair conformations, respectively. However, in the second structure, rings C and D exist in the skew-boat and skew-chair forms. The presence of two independent molecules in the crystal structure of M3A6S provides an explanation for the observation of the presence of several doublets in the solid state ^{13}C NMR spectrum of this molecule.[17]

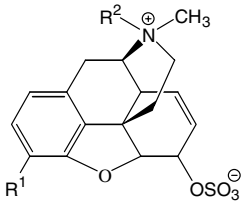
Hartley guinea pigs were decapitated and their brains were quickly removed and weighed. The brains were then homogenized in 50 mM Tris–HCl buffer, pH 7.7 (about 25 ml/brain), using a Polytron. The homogenate was centrifuged at 40,000*g* for 15 min, re-homogenized, and centrifuged. The final pellet was suspended in Tris–HCl, pH 7.7, at a final concentration of 6.67 mg original wet weight of tissue per milliliter, except for tissue prepared for NalBzOH (naloxone benzoylhydrazone) binding, which was suspended in buffer containing 5 mM EDTA. The 6-*O*-sulfate congeners of morphine, dihydromorphine, codeine, and dihydrocodeine in Table 1 were evaluated for their binding affinities for μ-, δ-, κ-1, κ-2, and



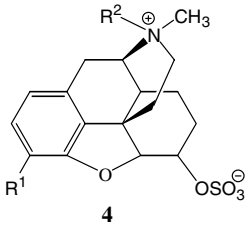
1
Morphine



2
M3S



3

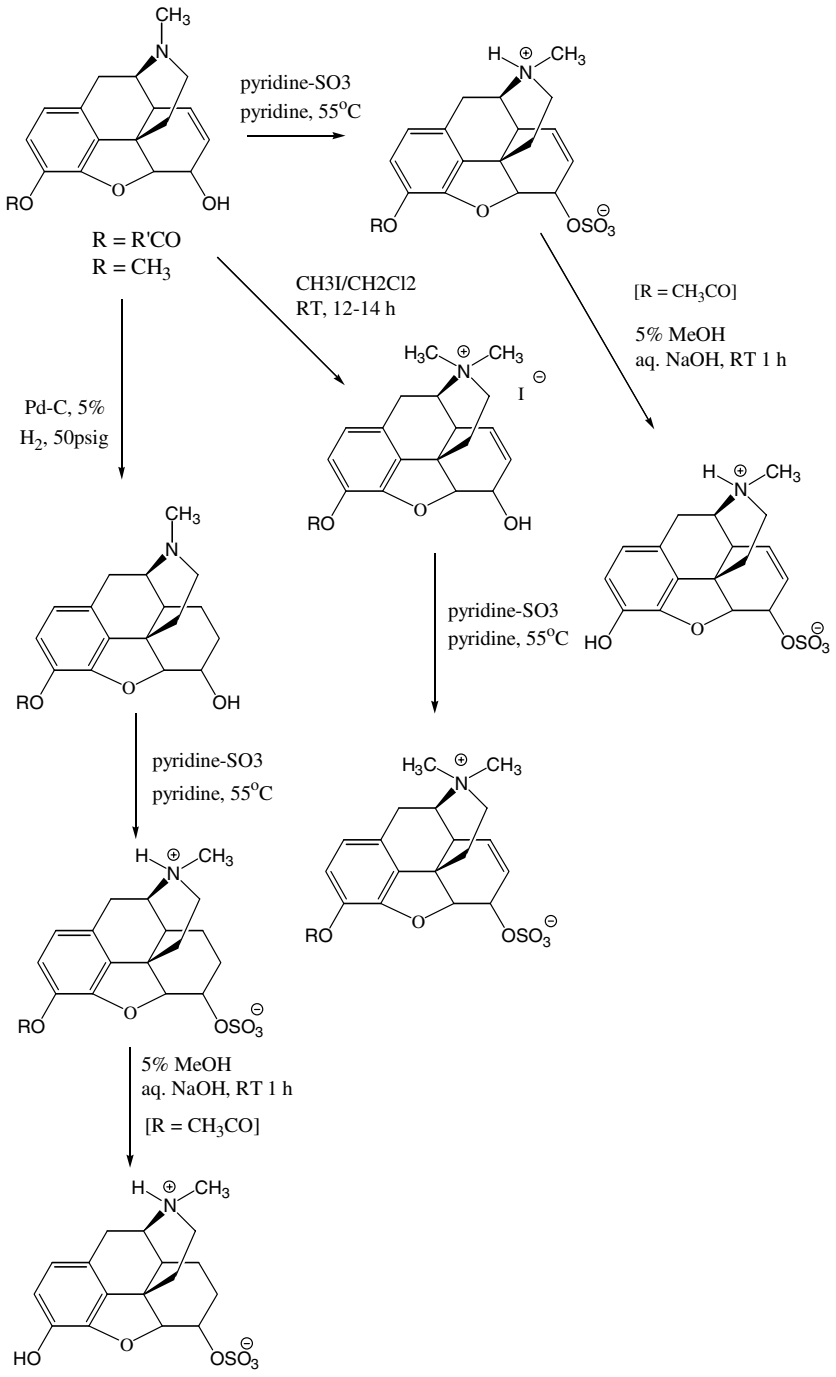


4

a) R^1 = OH, R^2 = H (M6S)
b) R^1 = $OCOCH_3$, R^2 = H (M3A6S)
c) R^1 = $OCOCH_2CH_3$, R^2 = H (M3Pr6S)
d) R^1 = $OCOCH(CH_3)_2$, R^2 = H (M3IBu6S)
e) R^1 = $OCOC(CH_3)_3$, R^2 = H (M3P6S)
f) R^1 = $OCOC_6H_5$, R^2 = H (M3B6S)
g) R^1 = $OCOCH_3$, R^2 = CH_3 (MM3A6S)
h) R^1 = $OCOC_6H_5$, R^2 = CH_3 (MM3B6S)
i) R^1 = OCH_3, R^2 = H (C_6S)

a) R^1 = OH, R^2 = H (DM6S)
b) R^1 = $OCOCH_3$, R^2 = H (DM3A6S)
c) R^1 = $OCOC_6H_5$, R^2 = H (DM3B6S)
d) R^1 = OCH_3, R^2 = H (DC6S)

Figure 1. Structure of morphine, dihydromorphine, and codeine 6-*O*-sulfate ester congeners.



Scheme 1. Synthesis of 3-*O*-acyl derivatives of morphine, dihydromorphine, and codeine 6-*O*-sulfate ester congeners.

κ-3 opiate receptors utilizing guinea pig brain homogenate preparations. Around 1 nM of morphine, Met-enkephalin, DAMGO (a μ-selective agonist), DPDPE-Cl (a δ-selective agonist), U69,593 (a κ-1-selective agonist), and NalBzOH (a κ-3-selective agonist) were utilized as standard radioligands at these opiate receptors. Affinities at κ-2 receptors were determined utilizing [^{3}H]-bremazocine in the presence of 100 nM DAMGO, U69,593, and DSLET.

The guinea pig brain suspension (1.8 ml) was incubated in 50 mM Tris–HCl. Nonspecific binding was determined by incubating in the presence of 1 μM of the 'cold' unlabeled counterpart of each labeled ligand, except that 10 μM NalBzOH was used for the κ-3 receptor assay. The samples were then filtered through glass fiber filters on a 48-well Brandel cell harvester. The filters were washed three times with 3 ml of buffer. Filters were incubated overnight with 5 ml of scintillation cocktail before counting.

Results are reported in terms of IC_{50} (concentration of test compound that produces 50% inhibition of labeled ligand binding). K_i values (inhibitory dissociation

Table 1. Inhibitory effects (K_i values) of opiate receptor ligands and morphine-6-*O*-sulfate congeners on the binding of titrated ligands to μ-, δ-, and κ-receptors in guinea pig brain homogenates

Compound	K_i; nM ± SEM (Hill slope)			
	μ	δ	κ-1	κ-3
Morphine (**1**)	2.5 ± 0.3 (1)	58 ± 3.8 (0.9)	33.9 ± 4.1 (0.8)	13.9 ± 4.1 (0.7)
DAMGO	1.1 ± 0.2 (1)	127 ± 13.9 (0.9)	1841 ± 230 (0.8)	26.9 ± 6.3 (0.8)
DAMGO	1.1 ± 0.2 (1)	127 ± 13.9 (0.9)	1841 ± 230 (0.8)	26.9 ± 6.3 (0.8)
DPDPE-C	180 ± 1.2 (1)	0.3 ± 0.07 (1)	>10,000	1028 ± 219 (0.8)
U69	692 ± 97 (0.9)	1358 ± 118 (0.8)	0.7 ± 0.05 (0.9)	1099 ± 0.2 (0.9)
NalBzOH	0.2 ± 0.01 (1)	1.4 ± 0.13 (0.8)	0.4 ± 0.1 (0.9)	0.3 ± 0.07 (1)
Met-Enkephalin	6.9 ± 1.5 (0.9)	1.1 ± 0.2 (0.9)	4467 ± 865 (0.8)	277 ± 29 (0.9)
M3S (**2**)	101 ± 0.7 (1)	>10,000	>10,000	1694 ± 287 (0.7)
M6S (**3a**)	0.9 ± 0.01 (1)	18 ± 0.4 (1)	1192 ± 697 (0.8)	6.3 ± 0.3 (0.9)
M3A6S (**3b**)	0.8 ± 0.01 (1)	154 ± 16.4 (0.4)	1165 ± 185 (0.6)	5.5 ± 1.2 (0.8)
M3Pr6S (**3c**)	0.6 ± 0.15 (1)	40 ± 1.9 (0.9)	252 ± 38.6 (0.9)	19.9 ± 16 (0.8)
M3IBu6S (**3d**)	19.8 ± 0.6 (1)	584 ± 28.8 (0.6)	4558 ± 229 (0.8)	266 ± 68.5 (0.7)
M3P6S (**3e**)	0.7 ± 0.15 (1)	15.2 ± 4.25 (0.9)	1940 ± 198 (0.8)	11.2 ± 3.16 (0.7)
M3B6S (**3f**)	36.9 ± 1.3 (1)	600 ± 150 (1.2)	>10,000	513 ± 14.7 (0.8)
DM6S (**4a**)	1.5 ± 0.25 (0.9)	18.8 ± 3.35 (0.9)	3129 ± 368 (1.3)	7 ± 0.2 (0.7)
DM3A6S (**4b**)	13.7 ± 0.55 (0.8)	325 ± 71.2 (0.9)	>10,000	838 ± 744 (0.8)
DM3B6S (**4c**)	61.8 ± 13.15 (0.9)	1598 ± 422 (0.8)	>10,000	822 ± 83.1 (0.9)
MM3A6S (**3g**)	34.2 ± 12.1 (0.9)	842 ± 189 (1)	>10,000	400 ± 58.4 (0.8)
MM3B6S (**3h**)	444 ± 169 (1)	>10,000	>10,000	>10,000
C6S (**3i**)	38.7 ± 7.95 (0.9)	414 ± 140 (0.8)	>10,000	455 ± 224 (0.8)
DC6S (**4d**)	196 ± 57.7 (0.8)	1152 ± 184 (0.9)	>10,000	760 ± 83.4 (1.2)

constant) are derived from the following equation: $K_i = IC_{50}/1 + [L]/K_d$. The K_d values were obtained by computer analysis of detailed self-inhibition curves for each of the labeled ligands (L), using the curve-fitting program LIGAND.

The compounds listed in Table 1 exhibited no affinity for κ-2 receptors (data not shown) and only weak affinity at κ-1 receptors was observed. Sulfation of the 6-hydroxy group of morphine afforded the compound M6S, which exhibited slightly improved affinity over morphine for μ-, δ-, and κ-3-receptors, and reduced affinity at κ-1-receptors. The 7,8-dihydro analog, DM6S, had similar activity to M6S.

Sulfation of the 3-hydroxy group of morphine afforded M3S, which had little or no affinity at any of the opiate receptors examined. Both codeine 6-*O*-sulfate (C6S) and its dihydro derivative, DC6S, exhibited a significant loss in affinity compared to M6S at all opiate receptors. However, M3A6S, the 3-O-acetylated derivative of M6S, exhibited comparable K_i values to M6S at μ- and κ-3-receptors, but showed decreased affinity for δ-receptors, whereas the dihydro derivative, DM3A6S, and the quaternary ammonium analog, MM3A6S both had reduced affinity compared to M3A6S in all the opiate receptor assays employed. The 3-*O*-propionyl (M3Pr6S), 3-*O*-isobutyryl (M3IBu6S), and 3-*O*-pivaloyl (M3P6S) analogs of M6S all showed high affinity for μ-receptors and low affinity for κ-1 receptors. The sterically hindered ester, M3P6S, exhibited a very similar receptor affinity profile to the parent compound, M6S. Both the benzyl ester, M3B6S, and its dihydro derivative, DM3B6S, were less potent than their respective parent compounds, M6S and DM6S, at all the opiate receptors examined. Interestingly, the betaines, MM3A6S and MM3B6S, had low affinity in all the opiate receptor assays employed.

The above data clearly demonstrate that 3-O-acylation of M6S and related compounds gives rise to molecules with high affinity for the μ-receptor. The 3-*O*-propionyl analog, M3Pr6S, was the most potent μ-receptor ligand in the series, exhibiting a K_i of 600 nM. M3Pr6S exhibited a profile similar to morphine, but was slightly more potent at μ-receptors. It is unlikely that these compounds are hydrolyzing to M6S in the binding assay buffer, since previous studies have shown that M3A6S, the compound likely to be the most labile 3-*O*-acyl analog of M6S, hydrolyzes relatively slowly to M6S in phosphate-buffered saline over the pH range 6.0–8.0 and is not converted to morphine under these conditions.[16] Interestingly, the rates of enzymatic hydrolysis of M3A6S in blood and brain were relatively faster than in buffer, and hydrolysis in rat brain was considerably faster than in blood.[16] Thus, these 3-*O*-acyl derivatives of M6S, although originally considered to be prodrugs, also exhibit high affinity for μ-receptors. This may explain the relatively prolonged duration of analgesia produced by these compounds in in vivo experiments in the rat when compared to morphine. This is likely due to these active 3-*O*-acyl compounds having plasma half-lives greater than 1 h, and to the fact that subsequent metabolism by plasma esterases will afford the 3-O-deacylated active parent compound.

Acknowledgment

This research was supported by a Grant from National Institute on Drug Abuse (Medications Development Division Contract No. 271-89-8159).

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18. Of the 13 compounds listed in Figure 1, M3S,[5] M6S,[16] M3A6S,[16] M3Pr6S,[16] DM6S,[20] C6S,[13] and DC6S[13] have been previously characterized in the literature. The characterization data for the following compounds: M3IBu6S, M3P6S, M3B6S, DM3A6S, DM3B6S, MM3A6S, and MM3B6S are reported in Ref. 21.
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