EVALUATION OF NEW COMPOUNDS FOR OPIOID ACTIVITY (2004)

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This report contains information on compounds that have been submitted to the Drug Evaluation Committee of the College and released for publication by the submitters. The information obtained usually involves *in vitro* evaluation in opioid-binding assays. In addition, the compounds may be evaluated for discriminative and reinforcing effects. Analgesic and respiratory function assays are also possible. These behavioral assessments are conducted in rhesus monkeys.

The evaluation of new compounds by the programs at the University of Michigan and the Medical College of Virginia is currently administered by the Biological Coordinator, Dr. A. Coop, University of Maryland. The compounds come originally from pharmaceutical companies, universities, government laboratories, or international organizations.

At the UM and MCV laboratories, drug samples arrive from the Biological Coordinator with only the following information: (1) an identifying NIH number, (2) molecular weight, (3) solubility information. After the evaluation is complete and the report sent to Dr. Coop, the submitter of the compound(s) is requested to release the chemical structure to include with the evaluation data in the ANNUAL REPORT. The submitter can withhold the structure for up to three years. When the structure is released all of the data on the compound are reported herein.

SUMMARY OF TESTS PERFORMED

The compounds that were evaluated at the University of Michigan during the past year are shown in the following Table. Also shown are dates of Reports to the Biological Coordinator, Dr. Coop.

NIH #	Date Submitted to Biological Coordinator	NIH #	Date Submitted to Biological Coordinator	NIH #	Date Submitted to Biological Coordinator
10945	09 September 2003	11067	15 January 2002	11128	10 April 2003
11001	04 August 2003	11068	15 January 2002	11140	14 March 2003
11027	31 July 2003	11072	15 January 2002	11161	31 January 2003
11031	31 July 2003	11073	14 March 2002	11163	10 April 2003
11041	04 August 2003	11074	14 March 2002	11164	10 April 2003
11044	31 July 2003	11075	14 March 2002	11176	31 July 2003
11045	04 August 2003	11076	14 March 2002	11177	31 July 2003
11050	09 September 2003	11077	14 March 2002	11178	31 July 2003
11053	24 October 2001	11078	23 October 2001	11179	31 July 2003
11054	24 October 2001	11079	23 October 2001	11180	31 July 2003
11055	24 October 2001	11081	04 August 2003	11181	31 July 2003
11057	01 November 2001	11082	01 August 2003	11182	31 July 2003
11058	01 November 2001	11088	01 August 2003	11183	31 July 2003
11059	20 November 2001	11093	04 August 2003	11185	31 July 2003
11060	20 November 2001	11095	04 August 2003	11186	31 July 2003
11061	20 November 2001	11096	09 September 2003	11187	31 July 2003
11062	05 December 2001	11097	04 August 2003		
11063	25 November 2001	11098	09 September 2003		
11065	03 December 2001	11100	31 July 2003		
11066	03 December 2001	11101	04 August 2003		

METHODS
Opioid Receptor Binding and In Vitro Efficacy Assessment

Details of the binding assay have been described previously (Lee *et al*., 1999). Briefly, aliquots of a membrane preparation are incubated with [^{3}H]diprenorphine (0.3 nM) in the presence of different concentrations of the drug under investigation at 25° C for 1 hr. Specific, *i.e*., opioid-receptor-related binding is determined as the difference in binding

obtained in the absence and presence of 10:M naloxone. The potency of the drugs in displacing the specific binding of [3H]-ligand is determined from data using Graphpad Prism (GraphPAD, San Diego, CA) and converted to Ki values by the method of Cheng and Prussoff (1973). Opioid binding is performed in membranes from C_6 rat glioma cells expressing recombinant μ (rat; Emmerson *et al*., 1994) or δ (rat; Clark *et al*., 1997) and CHO cells expressing the recombinant κ (human, Zhu *et al*., 1997). The affinity (Kd) values of [3H]diprenorphine at the receptors are: μ (0.15 nM); δ (0.45 nM); κ (0.25 nM).

The results of the selective binding assays are given as means ± SEM from three separate experiments, each performed in duplicate. Ki values for standard compounds using recombinant receptors and [3H]diprenorphine as radioligand are: μ (DAMGO, 7.6 nM; morphine, 11.2 nM), δ (SNC80, 0.8 nM) and κ (U69593, 0.3 nM). If less than 50% displacement of [3H]diprenorphine is seen at 10 μM, it is reported as > 10 μM and the percent displacement given in parentheses.

[35S]GTP(S assays are carried out using membranes from C6 cells expressing either μ (Emmerson *et al*., 1996) or δ (Clark et al., 1997) receptors or CHO cells expressing 6 receptors (Zhu *et al*., 1997). Assays are performed as described by Traynor and Nahorski (1995). Values are given as EC_{50} with % effect compared to a standard agonist (DAMGO, SNC80, or U69593) or as maximal stimulation achieved at 10 μM. EC_{50} values (nM) for standard compounds are as follows: mu receptor (morphine, 65 nM; DAMGO, 34 nM; fentanyl, 13 nM), delta receptor (SNC80, 9 nM; DPDPE 8.3 nM), and kappa receptor (U69593, 31.0 nM; bremazocine, 0.5 nM)

DPDPE (60%) and bremazocine (86%) are partial agonists compared with the standards SNC80 and U69593. Morphine and DAMGO give equivalent responses.

Antagonist activity is given as AD_{50} values or as pK_B values. AD_{50} refers to the concentration of test compound that reduces [35S]GTPγS binding stimulated by an ED_{80} concentration of appropriate agonist (DAMGO,μ; DPDPE, δ; U69593, κ) by 50%. pK_B is the concentration of antagonist required to shift the dose-effect curve for appropriate agonist by 2-fold. It is a measure of the affinity of the antagonist for a receptor.

Self-Administration by Monkeys

Tests of self-administration determine the ability of the drug to maintain responding in monkeys trained to self-inject alfentanil. Each of at least three monkeys is studied with saline as a negative control and a number of doses of the test compound until a maximum rate of responding was obtained or until, in the absence of evidence of a reinforcing effect, observable changes in behavior are produced by the compound.

The schedule of intravenous drug delivery is a fixed-ratio 30; when a light above a lever is illuminated, the 30th response produce an intravenous drug injection accompanied by another light that is illuminated during drug delivery. After each injection, a 45 sec timeout period occurs. A component of the session ends after 20 injections have been received or 25 min have passed, whichever occurs first. Different doses of the drug are available during each of four components of a session. Other procedural details are given in Winger *et al*. (1989 and 1992).

NIH 10945   (±)-(5S,8S,9R)-8-Amino-3-hydroxy-5,9-methano-9-(methoxymethyl)-5-methylbenzocyclooctene
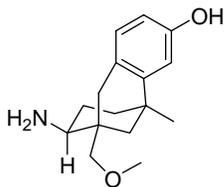
OPIOID RECEPTOR BINDING (nM) †

μ-receptor: 3.7 ± 0.2
δ-receptor: 156.0 ± 25.0
κ-receptor: 6.3 ± 0.4

[^{35}S]GTPγASSAY

Agonist Activity

μ-receptor: maximal stimulation = 59.6 ± 5.9% with EC_{50} = 151 ± 49 nM
κ-receptor: maximal stimulation = 74.4 ± 1.9% with EC_{50} = 66.1 ± 10.1 nM

SUMMARY

NIH 10945 has high affinity for both μ and κ opioid receptors. Affinity at δ receptors is over 20-fold less. NIH 10945 is also a partial agonist at μ and κ receptors that likely explains its activity in the PPQ assay in the mouse and its ability to partially substitute for morphine in the morphine-dependent monkey. ††

† Binding data previously reported in NIDA Monograph 182:141, 2002.
†† PPQ data reported previously in NIDA Monograph 182:168, 2002.

* * *

NIH 11001   4-(3-Hydroxyphenyl)-4-(1-oxopropyl)-1-(4-trifluoromethylbenzyl)piperidine.HCl
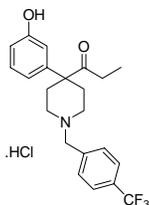
OPIOID RECEPTOR BINDING (nM) †

μ-receptor**:**   32.9 ± 1.1
δ-receptor: 291 ± 83
κ-receptor: 118 ± 28

[^{35}S]GTPγS ASSAY

Agonist Activity

No significant stimulation of [^{35}S]GTPγS binding in C6 cells expressing the rat μ (C6μ) cells.

Antagonist Activity

Ke (μ) = 516 ± 121 nM

NIH 11001 (continued)

SUMMARY

NIH 11001 has affinity for $\mu > \kappa = \delta$, but showed no activity *in vivo* or *in vitro* as an agonist or μ antagonist in the mouse or monkey††. The present findings show that the compound has only very weak μ antagonist activity in spite of a reasonably high binding affinity. Note: repeat of the binding results in a buffer containing guanine nucleotides and Na^+ ions afforded a Ki at the μ receptor of 221 ± 71 nM, in line with the functional affinity measure.

† Binding data previously reported in NIDA Monograph 181:151, 2001
†† NIDA Monograph 181:197, 2001

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NIH 11027   (-)-(1*R*,5*R*,9*R*)-2-(3-Chlorobenzyl)-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan.HCl
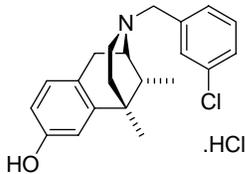
OPIOID RECEPTOR BINDING (nM) †

μ-receptor:	25.0 ± 9.9
δ-receptor:	1362 ± 53
κ-receptor:	11.1 ± 2.3

[^{35}S]GTPγS ASSAY

Agonist Activity

No stimulation of [^{35}S]GTPγS binding was observed.

Antagonist Activity

Ke (μ) 34.5 ± 17.5 nM
Ke (κ) 23.2 ± 6.2 nM

SUMMARY

NIH 11027 has affinity for κ and μ receptors with high selectivity ($\delta/\kappa = 123$; $\delta/\mu = 55$) for both of these over δ receptors. However, no activity as agonist or antagonist was seen in the mouse, although a non-dose-dependant exacerbation of withdrawal was observed in morphine-dependent monkeys†. The present findings show that the compound is an antagonist at both μ and κ receptors. However, the affinity (Ke) at μ receptors is approximately 10-fold less than that of naloxone. This may explain the *in vivo* results. Alternatively, the lack of *in vivo* activity may relate to the pharmacokinetic profile of this compound.

† Binding data previously reported in NIDA Monograph 183:175, 2003

NIH 11031   17-Cyclopropylmethyl-[5β,7β,3',5']-pyrrolidino-2'-[*S*]-phenyl-7α-methyl-6,14-endo-ethenomorphinan.HCl
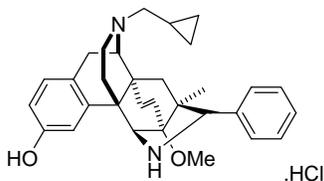
OPIOID RECEPTOR BINDING (nM) †

μ-receptor = 0.35 ± 0.40
δ-receptor = 0.95 ± 0.08
κ-receptor = 0.08 ± 0.01

[^{35}S]GTPγS ASSAY

μ-receptor: <5% up to 10 :M
δ-receptor: maximal stimulation = 35.0 ± 5.7% with EC$_{50}$ = 1.8 ± 0.5 nM
κ-receptor: maximal stimulation = 76.3 ± 11.0 with EC$_{50}$ = 0.36 ± 0.03 nM

SUMMARY

The binding affinity (Ki) of NIH 11031 is very high at all three receptors. The opioid effects in the mouse and monkey†† may be explained by the highly potent agonist activity at 6 receptors.

† Binding data previously reported in NIDA Monograph 194:155. 2004
†† NIDA Monograph 184:181-183, 2004

* * *

NIH 11041   (-)-(1*R*,5*R*,9*R*)-2-(3-Bromobenzyl)-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan.HCl
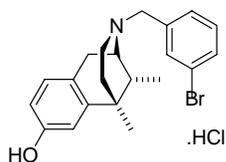
OPIOID RECEPTOR BINDING (nM) †

μ-receptor: 47.7 ± 21.1
δ-receptor: 1326 ± 53.3
κ-receptor: 9.9 ± 1.4

[^{35}S]GTPγS ASSAY

No significant stimulation of [^{35}S]GTPγS binding was observed up to a concentration of 10 μM.

SUMMARY

NIH 11041 has affinity for κ receptors and has 5-times lesser affinity at μ receptors and 26 times lesser at δ receptors. However, it has no activity in vivo or in vitro as an agonist or μ antagonist in the mouse or monkey. †† The present findings show that the compound has no agonist action and is likely to be a κ/μ antagonist. The affinity values suggest that it will be approximately 20-fold less potent as a : antagonist than naloxone. This may explain the lack of observed antagonist activity in vivo.

† Binding data previously reported in NIDA Monograph 183:177, 2003
†† Monkey data previously reported in NIDA Monograph 183:211, 2003

NIH 11044   (-)-(1*R*,5*R*,9*R*)-2'-Acetoxy-2-(3-*cis*-chloro-2-propenyl)-5,9-dimethyl-6,7-benzomorphan.oxalate
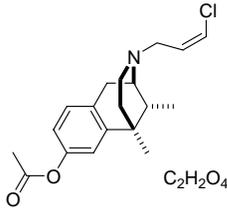
OPIOID RECEPTOR BINDING (nM) †

μ-receptor: 14.9 ± 2.5
δ-receptor: 17.4 ± 2.3
κ-receptor: 3.0 ± 0.1

[^{35}S]GTPγS ASSAY

μ-receptor: no stimulation up to 10 μM
δ-receptor: 5% stimulation at 10 μM
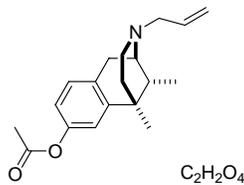κ-receptor: maximal stimulation = $56.1 \pm 10.0\%$ with $EC_{50} = 22.7 \pm 7.3$ nM

SUMMARY

NIH 11044 has high affinity for κopioid receptors > μ = δ receptors, with a selectivity of 5-fold for κ receptors over the other types. It acts as a partial agonist at κ opioid receptors. Its binding affinity (Ki) is high at all three receptors (see above), suggesting that it is also a potent μ and δ antagonist. This agrees with its *in vivo* profile in the mouse that it as μ antagonist activity but is antinociceptive in the PPQ†† test and that it has μ antagonist activity with some κ agonist properties in the monkey.

† Binding data previously reported in NIDA Monograph 183:178, 2003
†† NIDA Monograph 183:214, 2003

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NIH 11045   (-)-(1*R*,5*R*,9*R*)-2'-Acetoxy-5,9-dimethyl-2-(propenyl)-6,7-benzomorphan.oxalate

OPIOID RECEPTOR BINDING (nM) †

μ-receptor: 136 ± 34
δ-receptor: 96.2 ± 8.1
κ-receptor: 29.2 ± 1.6

[^{35}S]GTP(S ASSAY

μ-receptor: no stimulation up to 10 μM
δ-receptor: no stimulation up to 10 μM
κ-receptor: maximal stimulation = $45.5 \pm 5.6\%$ with $EC_{50} = 127 \pm 2.3$ nM

NIH 11045 (continued)

SUMMARY

NIH 11045 has affinity forκ opioid receptors but is only 3-4-fold selective for the κ over δ and μ receptors. In vivo, the compound is a μ and κ antagonist (versus morphine and enadoline, respectively) in the mouse but in the monkey is a μ antagonist with signs of κ agonism.†† The present findings show that the compound has partial agonist action at 6 receptors, which would explain the *in vivo* observation.

† Binding data previously reported in NIDA 183:179, 2003
†† See NIDA Monograph 183:215, 2003.

* * *

NIH 11050   17-Methyl-6,7-didehydro-3,14-dihydroxy-4,5α-epoxy-[(2-methyl)-pyrazolo-[6,7]]morphinan.2HCl

2HCl
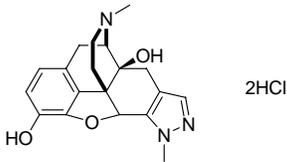
OPIOID RECEPTOR BINDING (nM)⊥

μ-receptor**:** 23.9 ± 10.1
δ -receptor: 22.7 ± 3.3
κ-receptor: 157 ± 53

[^{35}S]GTP(S ASSAY

μ-receptor: maximal stimulation = $89 \pm 3\%$ with $EC_{50} = 154 \pm 58$ nM
δ-receptor: maximal stimulation = $27 \pm 2\%$ with $EC_{50} = 302 \pm 78$ nM
κ-receptor: maximal stimulation = $19 \pm 7\%$ with $EC_{50} = 245 \pm 54$ nM

SUMMARY

NIH 11050 has the same affinity for μ and δ receptors, but with no selectivity. It is approximately 7 times weaker at κ receptors. It is a relatively high efficacy agonist at μ receptors but has only low efficacy at δ and κ receptors. There is little selectivity in potency across the three receptors.

† Binding data previously reported in NIDA Monograph 183:179, 2003.

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NIH 11052   (-)-(1*R*,5*R*,9*R*)-5,9-Dimethyl-2-(propenyl)-2'-proprionoxy-6,7-benzomorphan.HCl

HCl
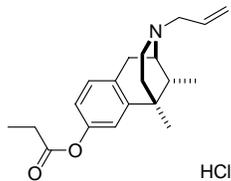
OPIOID RECEPTOR BINDING (nM)⊥

μ-receptor: 107 ± 42
δ-receptor: 90.7 ± 15.4
κ-receptor: 7.9 ± 0.7

NIH 11052 (continued)

[^{35}S]GTPγS ASSAY

Agonist Activity

μ-receptor: no stimulation up to 10 μM
δ-receptor: no stimulation up to 10 μM
κ-receptor: maximal stimulation = 37.3 ± 3.1% with EC_{50} = 147 ± 67nM
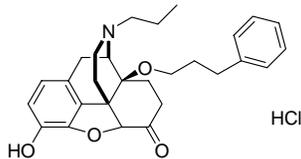
SUMMARY

NIH 11052 has high affinity for κ opioid receptors and is 12 times more selective for κ over μ or δ receptors. In the mouse and monkey, NIH 11052 was a μ antagonist, with weak antinociceptive properties in the mouse.†† The present findings show that the compound is likely to be a μ and δ antagonist with a partial agonist action at κ receptors, which would explain the weak antinociceptive properties observed in the mouse.

† Binding data previously reported in NIDA Monograph 183:180, 2003
†† NIDA Monograph 183:217, 2003

* * *

NIH 11053   17-Propyl-4,5α-epoxy-3-hydroxy-14-β-(3-phenylpropyloxy)morphinan-6-one.HCl

OPIOID RECEPTOR BINDING (nM)

μ-receptor: 0.09 ± 0.05
δ-receptor: 0.93 ± 0.18
κ-receptor: 0.37 ± 0.17

SUMMARY

NIH 11053 has very high affinity for all opioid receptors with selectivity ratios of κ/μ = 4 and δ/μ = 10.

* * *

NIH 11054   17-([2-*R*,*S*-Tetrahydrofuranyl)methyl)-4,5α-epoxy-3-hydroxy-14β-(3-phenylpropyloxy)morphinan-6-one.HCl
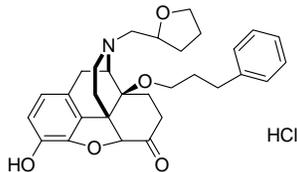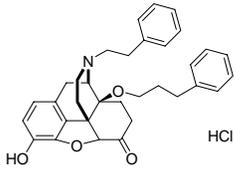

OPIOID RECEPTOR BINDING (nM)

μ-receptor: 0.20 ± 0.04
δ-receptor: 0.09 ± 0.02
κ-receptor: 0.08 ± 0.02

SUMMARY

NIH 11054 has very high affinity for all opioid receptors, but with no selectivity.

NIH 11055   17-Phenethyl-4,5α-epoxy-3-hydroxy-14β-(3-phenylpropyloxy)morphinan-6-one.HCl
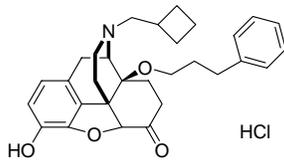

OPIOID RECEPTOR BINDING (nM)

μ-receptor: 1.1 ± 0.4
δ-receptor: 1.25 ± 0.5
κ-receptor: 0.60 ± 0.2

SUMMARY

NIH 11055 has high affinity for all opioid receptors, but with no selectivity.

* * *

NIH 11057   17-Cyclobutylmethyl-4,5α-epoxy-3-hydroxy-14β-(3-phenylpropyloxy)morphinan-6-one.HCl
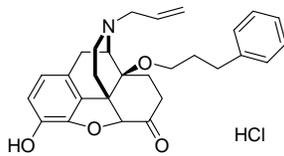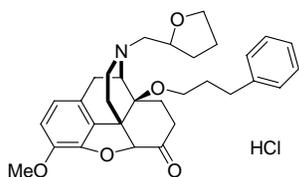

OPIOID RECEPTOR BINDING (nM)

μ-receptor: 0.25 ± 0.07
δ-receptor: 0.46 ± 0.16
κ-receptor: 0.49 ± 0.25

SUMMARY

NIH 11057 has very high affinity for all three opioid receptors.

* * *

NIH 11058   17-Cyclobutylmethyl-4,5α-epoxy-3-hydroxy-14β-(3-phenylpropyloxy)morphinan-6-one.HCl

OPIOID RECEPTOR BINDING (nM)

μ-receptor: 0.20 ± 0.01
δ-receptor: 0.26 ± 0.07
κ-receptor: 0.11 ± 0.05

SUMMARY

NIH 11058 has very high affinity for all three opioid receptors, but with no selectivity.

NIH 11059   17-[(2-*R*,*S*-Tetrahydrofuranyl)methyl]-4,5α-epoxy-3-methoxy-14β-(3-phenylpropyloxy) morphinan-6-one.HCl
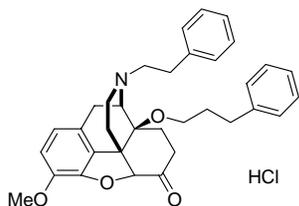

OPIOID RECEPTOR BINDING (nM)

μ-receptor: 1.9 ± 0.38
δ-receptor: 5.4 ± 1.2
κ-receptor: 1.4 ± 0.67

SUMMARY

NIH 11059 has high affinity for all three opioid receptors.

* * *

NIH 11060   4,5α-Epoxy-3-methoxy-17-(2-phenyletheyl)-14β-(3-phenylpropyloxy)morphinan-6-one.HCl
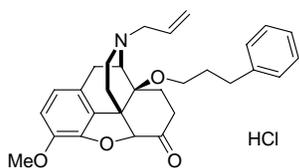

OPIOID RECEPTOR BINDING (nM)

μ-receptor: 3.8 ± 1.3
δ-receptor: 6.2 ± 0.2
κ-receptor: 61.4 ± 1.9

SUMMARY

NIH 11060 has high affinity for μ and δ receptors with 10- to 16-fold lower affinity for κ receptors.

* * *

NIH 11061   17-Allyl-4,5α-epoxy-3-methoxy-14β-(3-phenylpropyloxy)morphinan-6-one.HCl
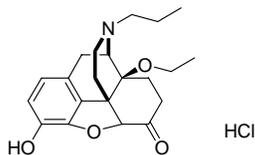

OPIOID RECEPTOR BINDING (nM)

μ-receptor: 1.7 ± 0.9
δ-receptor: 16.4 ± 5.2
κ-receptor: 4.1 ± 0.6

SUMMARY

NIH 11061 has high affinity for the three opioid receptors in the order μ > κ > δ.

NIH 11062   17-Allyl-4,5α-epoxy-3-methoxy-14β-(3-phenylpropyloxy)morphinan-6-one.HCl
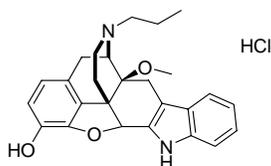

OPIOID RECEPTOR BINDING (nM)

μ-receptor: 1.6 ± 0.6
δ-receptor: 69.8 ± 21.2
κ-receptor: 6.13 ± 1.9

SUMMARY

NIH 11062 has high affinity for μ > κ receptors and δ receptor affinity. Its selectivity for κ receptors is 4-fold over κ and 44-fold over δ.

* * *

NIH 11063   4,5α-Epoxy-14β-ethoxy-3-hydroxy-17-(propyl)morphinan-6-one.HCl

OPIOID RECEPTOR BINDING (nM)⊥

μ-receptor: 270 ± 20.3
δ-receptor: 1.07 ± 0.18
κ-receptor: 108 ± 5.3

[^{35}S]GTPγS ASSAY

Agonist Activity

No stimulation of [^{35}S]GTPγS binding was observed up to 10 μM.
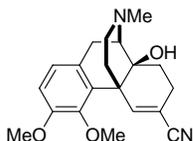
Antagonist Activity

Ke (δ) = 0.24 ± 0.05 nM

SUMMARY

NIH 11063 is a high affinity δ-antagonist. It is 100-fold selective for δ over κ and 250-fold selective for δ over μ in binding assays.

† Binding data previously reported in NIDA Monograph 183:180, 2003.

NIH 11065   5,6-Didehydro-14β-hydroxy-3,4-dimethoxy-17-methylmorphinan-6-carbonitrile

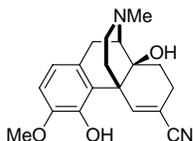**OPIOID RECEPTOR BINDING (nM)**

μ-receptor: 11.7 ± 1.0
δ-receptor: 342 ± 68
κ-receptor: 383 ± 89

SUMMARY

NIH 11065 has affinity for μ opioid receptors. It is 30-fold selective for μ receptors over κ receptors and δ receptors.

*   *   *

NIH 11066   5,6-Didehydro-4,14β-dihydroxy-3-methoxy-17-methylmorphinan-6-carbonitrile

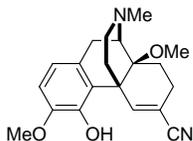**OPIOID RECEPTOR BINDING (nM)**

μ-receptor: 261 ± 30.4
δ-receptor: 3386 ± 138
κ-receptor: 4179 ± 629

SUMMARY

NIH 11066 has low affinity for the μ opioid receptor, but is 16-fold selective for μ over κ and 13-fold selective for μ over δ.

*   *   *

NIH 11067   5,6-Didehydro-4-hydroxy-3,14β-dimethoxy-17-methylmorphinan-6-carbonitrile
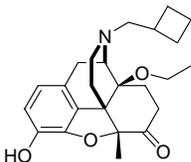
OPIOID RECEPTOR BINDING (nM)

μ-receptor: 22.3 ± 8.5
δ-receptor: 1006 ± 316
κ-receptor: 1480 ± 369

SUMMARY

NIH 11067 has μ receptor affinity with > 40-fold selectivity for the μ receptor compared with δ and κ receptors.

NIH 11068   17-Cyclobutylmethyl-4,5α-epoxy-14β-ethoxy-3-hydroxy-5β-methymorphinan-6-one

OPIOID RECEPTOR BINDING (nM)

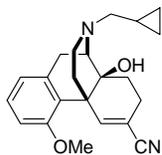μ-receptor: 0.47 ± 0.03
δ-receptor: 31.3 ± 9.3
κ-receptor: 6.1 ± 2.0

SUMMARY

NIH 11068 has very high μ receptor affinity with some (12-fold) selectivity over the κ receptor and 60-fold selectivity over the δ receptor.

*   *   *

NIH 11072   17-Cyclopropylmethyl-5,6-didehydro-14β-hydroxy-4-methoxymorphinan-6-carbonitrile

OPIOID RECEPTOR BINDING (nM)

μ-receptor: 68.7 ± 21.6
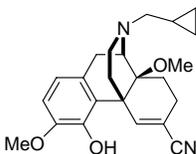δ-receptor: 2108 ± 434
κ-receptor: 93 ± 20

SUMMARY

NIH 11072 has approximately equal affinity for μ and κ receptors and 20-fold lower affinity for the δ receptor.

*   *   *

NIH 11073   17-Cyclopropylmethyl-5,6-didehydro-4-hydroxy-3,14β-dimethoxymorphinan-6-carbonitrile

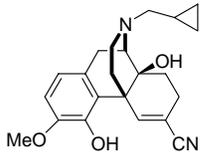**OPIOID RECEPTOR BINDING (nM)**

μ-receptor: 236 ± 14.6
δ-receptor: 1613 ± 370
κ-receptor: 411 ± 127

SUMMARY

NIH 11073 has low affinity for μ and κ receptors, with even lower affinity for δ receptors.

NIH 11074   17-Cyclopropylmethyl-5,6-didehydro-4,14β-dihydroxy-3-methoxymorphinan-6-carbonitrile

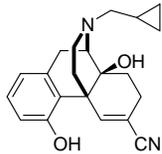**OPIOID RECEPTOR BINDING (nM)**

μ-receptor: 382 ± 25.5
δ-receptor: 4880 ± 881
κ-receptor: 368 ± 27

SUMMARY

NIH 11074 has low affinity for μ and κ receptors, with very low δ receptor affinity.

* * *

NIH 11075   17-Cyclopropylmethyl-5,6-didehydro-4,14β-dihydroxymorphinan-6-carbonitrile
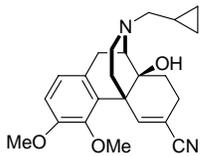

OPIOID RECEPTOR BINDING (nM)

μ-receptor: 23.1 ± 6.1
δ-receptor: 405 ± 121
κ-receptor: 12.2 ± 5.5

SUMMARY

NIH 11075 has affinity for μ and κ receptors, with lower δ receptor affinity.

* * *

NIH 11076   17-Cyclopropylmethyl-5,6-didehydro-14β-hydroxy-3,4-dimethoxymorphinan-6-carbonitrile
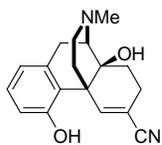

OPIOID RECEPTOR BINDING (nM)

μ-receptor: 40.9 ± 11.4
δ-receptor: 1138 ± 138
κ-receptor: 49 ± 15

SUMMARY

NIH 11076 has affinity for μ and κ receptors with low δ receptor affinity.

NIH 11077   5,6-didehydro-4,14β-dihydroxy-17-methylmorphinan-6-carbonitrile

OPIOID RECEPTOR BINDING (nM)
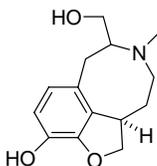
μ-receptor: 3.8 ± 1.1
δ-receptor: 419 ± 92
κ-receptor: 408 ± 30

SUMMARY

NIH 11077 has high μ receptor affinity with 100-fold selectivity over the κ- and δ-opioid receptors.

* * *

NIH 11078   7-Hydroxymethyl-8-methyl-6,7,8,9,10,10a-hexahydro-1H-2-oxa-8-aza-cycloocta[c,d]inden-3-ol

OPIOID RECEPTOR BINDING (nM)

μ-receptor: 22.4 ± 4.3% inhibition at 10 μM
δ-receptor: 22.6 ± 2.4% inhibition at 10 μM
κ-receptor: 27.0 ± 6.4% inhibition at 10 μM

SUMMARY

NIH 11078 has no affinity for opioid receptors.

* * *

NIH 11079   8-Methyl-6,7,8,9,10,10a-hexahydro-1H-2-oxa-8-aza-cycloocta[c,d]inden-3-ol
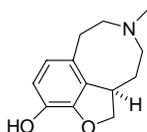
OPIOID RECEPTOR BINDING (nM)

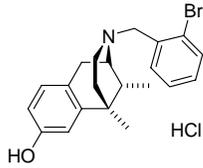μ-receptor: 25.0 ± 2.5% inhibition at 10 μM
δ-receptor: 18.0 ± 9.0% inhibition at 10 μM
κ-receptor: 12.0 ± 2.5% inhibition at 10 μM

SUMMARY

NIH 11079 has no affinity for opioid receptors.

NIH 11081   (-)-(1*R*,5*R*,9*R*)- 2-(2-bromobenzyl)-5,9-Dimethyl-2'-hydroxy-6,7-benzomorphan.HCl

OPIOID RECEPTOR BINDING (nM)⊥

μ-receptor: 40.2 ± 4.4
δ-receptor: 1227 ± 138
κ-receptor: 13.5 ± 2.0

[^{35}S]GTPγS ASSAY

Agonist Activity

No stimulation of [^{35}S]GTPγS binding was observed up to 10 μM.

SUMMARY

In binding assays, NIH 11081 has affinity for μ and κ receptors, but has no opioid effects in the mouse††. The present results suggest the compound to be a κ/μ antagonist with μ affinity 20-fold less than naloxone. This may explain the lack of observed in vivo activity.

†Binding data previously reported in NIDA Monograph 183:182, 2003.
†† NIDA Monograph 183:220, 2003

* * *

NIH 11082   (-)-(1*R*,5*R*,9*R*)-5,9-Dimethyl-2'-hydroxy-2-(6-hydroxyhexyl)-6,7-benzomorphan.HCl
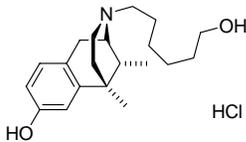
OPIOID RECEPTOR BINDING (nM)†

μ-receptor: 10.2 ± 0.73
δ-receptor: 140 ± 15.8
κ-receptor: 28.6 ± 4.5

[^{35}S]GTPγS ASSAY

Agonist Activity

μ-receptor: maximal stimulation = 50.5 ± 6.7% with EC$_{50}$ = 303 ± 57
δ-receptor   maximal stimulation = 9.3 ± 4.7% with EC$_{50}$ = 555 ± 149
κ-receptor: maximal stimulation = 21.7 ± 4.1% with EC$_{50}$ = 1346 ± 514

SUMMARY

These data show that 11082 is a partial agonist at μ and κ receptors, but with low potency. It has almost no efficacy at δ receptors. These data help to explain why the compound is only active in the phenylquinone writing assay in mice††, but not in nociceptive tests using heat, even through the compound has high binding affinity.

† Binding data previously reported in NIDA Monograph 183:182, 2003
††   NIDA Monograph 183:221, 2003

NIH 11088   (+)-(1*S*,5*S*,9*S*)-2-(2-Chlorobenzyl)-5,9-dimethyl-2'-hydroxy--6,7-benzomorphan.HCl
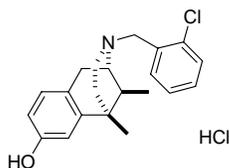
OPIOID RECEPTOR BINDING (nM) †

μ-receptor: 139 ± 33
δ-receptor: 3565 ± 1191
κ-receptor: 23.3 ± 2.4

[³⁵S]GTPγS ASSAY

Agonist Activity

NIH 11088 has no agonist activity at μ, δ, or κ opioid receptors.

SUMMARY

NIH 11088 has binding affinity for κ > μ >δ, with selectivity for κ over μ of 12-fold, but with no effects *in vivo* in the mouse††. It shows no agonism in the [³⁵S]GTPγS assay and may therefore be a κ antagonist with some selectivity.

† See NIDA Monograph 183:222, 2003)
†† See NIDA Monograph 184:190, 2004

* * *

NIH 11093   (-)-(1*R*,5*R*,9*R*)-2-(2-Chlorobenzyl)-5,9-dimethyl-2'-hydroxy--6,7-benzomorphan.HCl
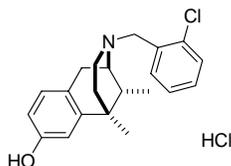
OPIOID RECEPTOR BINDING (nM) †

μ-receptor: 16.8 ± 2.1
δ-receptor: 600 ± 93
κ-receptor: 17.5 ± 5.4

[³⁵S]GTPγS ASSAY

Agonist Activity

No significant stimulation of [³⁵S]GTPγS binding was observed up to a concentration of 10 μM.

SUMMARY

In binding assays, NIH 11093 showed equal affinity for κ and μ, with low affinity for δ receptors. However, no activity as agonist or μ-antagonist was seen in the mouse or monkey. The present findings show that the compound has no agonist activity and is likely to be a μ/κ nonselective antagonist. However, the affinity values suggest that it will be approximately 10-fold less potent as a μ antagonist than naloxone. This may explain the lack of observed antagonist activity in vivo.

† Binding data previously reported in NIDA Monograph 183:184, 2003
†† NIDA Monograph 183:223, 2003

NIH 11095   (+)-(1*S*,5*S*,9*S*)- 5,9-Dimethyl-2-(2-fluorobenzyl)-2'-hydroxy--6,7-benzomorphan.Oxalate

OPIOID RECEPTOR BINDING (nM) †

μ-receptor: 560 ± 92
δ-receptor: 4129 ± 867
κ-receptor: 47 ± 10.5

[^{35}S]GTPγS ASSAY

Agonist Activity

No significant stimulation of [^{35}S]GTPγS binding was observed up to a concentration of 10 μM.

SUMMARY

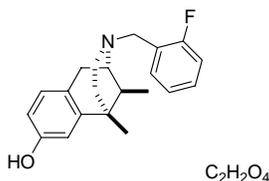In binding assays, NIH 11095 showed a 12-fold selectivity for κ over μ, with very low affinity for δ receptors. However, no activity as agonist or μ antagonist was seen in the mouse. †† The present findings show that the compound has no agonist activity and is likely to be an antagonist with a preference for the κ receptor. However, the affinity values suggest that it will be a weak antagonist.

† Binding data previously reported in NIDA Monograph 183:184, 2003.
††NIDA Monograph 183:223, 2003.

* * *

NIH 11096   (-)-(1*R*,5*R*,9*R*)- 2'-Butyroxy-5,9-Dimethyl-2-(2-propenyl)-6,7-benzomorphan.HCl
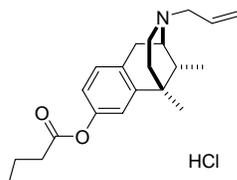
OPIOID RECEPTOR BINDING (nM)

μ receptor: 30.2 ± 13.5
δ receptor: 37.0 ± 1.7
κ receptor: 0.9 ± 0.05

[^{35}S]GTPγS ASSAY

Agonist Activity

μ-receptor: maximal stimulation = 11 ± 7% with EC$_{50}$ = 919 ± 685
κ-receptor: maximal stimulation = 74.4 ± 1.9% with EC$_{50}$ = 66.1 ± 10.1

SUMMARY

NIH 11096 is a partial κ agonist with no significant μ efficacy. The binding affinity at the μ receptor suggests the compound would be a μ antagonist. These results are in keeping with the *in vivo* findings†† that the compound has μ and κ antagonist properties against high efficacy agonists.

†   Binding data previously reported in NIDA Monograph 183:185, 2003
††   NIDA Monograph 184:193-194, 2004

NIH 11097   (-)-(1*R*,5*R*,9*R*)-5,9-Dimethyl-2-(2-fluorobenzyl)-2'-hydroxy-6,7-benzomorphan.Oxalate
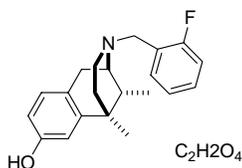
OPIOID RECEPTOR BINDING (nM)⊥

μ-receptor: 23.3 ± 4.5
δ-receptor: 326 ± 40
κ-receptor: 2.1 ± 0.9

[^{35}S]GTPγS ASSAY

Agonist Activity

No stimulation of [^{35}S]GTPγS binding in C6 cells was observed.

Antagonist Activity

Ke (μ)   96.5 ± 17.4
Ke (κ)   17.4 ± 3.3

SUMMARY

In binding assays, NIH 11097 showed a 10-fold selectivity for κ over μ. However, no activity as agonist or μ-antagonist was seen in the mouse. The present findings show that the compound is an antagonist at both μ and κ receptor with a small (~5-fold) preference for the κ receptor. However, the affinity (Ke) at μ receptors is approximately 10-fold less than that of naloxone, which may explain the previously reported lack of μ antagonist activity *in vivo*††. Alternatively, the lack of *in vivo* activity may relate to the pharmacokinetic profiles of the compound.

† Binding data previously reported in NIDA Monograph 183:185, 2003
 †† NIDA Monograph 183:224, 2003

* * *

NIH 11098   (+)-(1*S*,5*S*,9*S*)-2'-Butryoxy-5,9-dimethyl-2-(2-propenyl)-6,7-benzomorphan.HCl
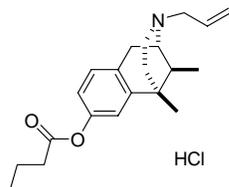
OPIOID RECEPTOR BINDING (nM) †

μ-receptor: 601 ± 10.8
δ-receptor: 3099 ± 405
κ-receptor: 1712 ± 167

[^{35}S]GTPγS ASSAY

Agonist Activity

μ-receptor: maximal stimulation = 17.8% with EC50 = 950 ± 307 nM
κ-receptor: no stimulation up to 10 μM

NIH 11098 (continued)

SUMMARY

NIH 11098 is a weak, very low efficacy μ agonist with no agonist activity at the κ receptor. The weak action in the PPQ assay in the mouse†† may be due to this μ activity, but could also be non-opioid.

† Binding data previously reported in NIDA Monograph 183:185, 2003.
†† NIDA Monograph 183: 224, 2003

* * *

NIH 11100 18-(*E*)-benzylidene-4-hydroxy-3-methoxy-17-methyl-[6,7:2',3']-indolomorphinan.oxalate

$C_2H_2O_2$
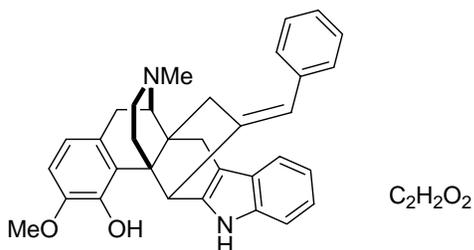
OPIOID RECEPTOR BINDING (nM)⊥

μ-receptor: 41.8 ± 17.2
δ-receptor: 30.0 ± 3.6
κ-receptor: 60.0 ± 9.3

Agonist Activity

μ-receptor: no stimulation up to 10 μM
δ-receptor: maximal stimulation = $40.6 \pm 6.2\%$ with $EC_{50} = 463 \pm 38$ nM
κ-receptor: maximal stimulation = $64.0 \pm 18.7\%$ with $EC_{50} = 2063 \pm 324$ nM

SUMMARY

The binding affinity (Ki) of NIH 11100 is similar at all three receptors. However, its efficacy at the three receptors is different such that NIH 11100 is a partial agonist with low potency at δ receptors and very low potency at κ receptors. The results indicate that it would be a weak μ antagonist.

† See NIDA Monograph 184:156, 2004

* * *

NIH 11101 18-Isopropylidene-4-hydroxy-3-methoxy-17-methyl-[6,7:2',3']-indolomorphinan.oxalate

$C_2H_2O_2$
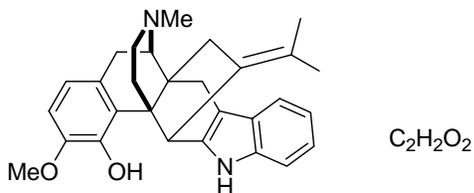
OPIOID RECEPTOR BINDING (nM) †

μ-receptor: 267 ± 18
δ-receptor: 148 ± 33
κ-receptor: 158 ± 51

NIH 11101 (continue)

[^{35}S]GTPγS ASSAY

Agonist Activity

μ-receptor: maximal stimulation = 63.6 ± 3.1 with EC$_{50}$ = 2126 ± 587 nM (n=2)
δ-receptor: no stimulation up to 10 μM
κ-receptor: no stimulation up to 10 μM
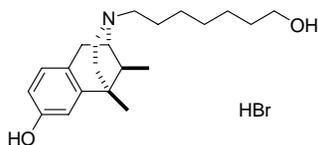
SUMMARY

NIH 11101 is a weak partial agonist at μ receptors. From its binding profile, it is also likely to be a low affinity δ/κ antagonist.

† Binding data previously reported in NIDA Monograph 184:156, 2004

* * *

NIH 11128   (+)-(1*S*,5*S*,9*S*)-5,9-dimethyl-2'-Hydroxy-2-(7-hydroxyheptyl)- 6,7-benzomorphan.HBr

OPIOID RECEPTOR BINDING (nM)

μ-receptor: 907 ± 112
δ-receptor: 5822 ± 3500
κ-receptor: 1789 ± 379

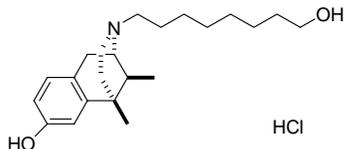**SUMMARY**

NIH 11128 has very low affinity for μ, δ, and κ receptors.

* * *

NIH 11140   (+)-(1*S*,5*S*,9*S*)-5,9-Dimethyl-2'-hydroxy-2-(8-hydroxyoctyl)6,7-benzomorphan.HCl

OPIOID RECEPTOR BINDING (nM)
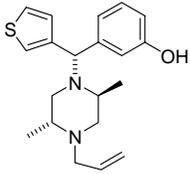
μ-receptor: 136 ± 13.5
δ-receptor: 204 ± 33
κ-receptor: 449 ± 104

SUMMARY

NIH 11140 has low affinity for μ, δ, and κ opioid receptors nor is there evidence of selectivity.

NIH 11161   (-)-3-{(S)-[(2S,5R)-4-Allyl-2,5-dimethyl-1-piperazinyl](3-thienyl)methyl}phenol

Four doses of NIH 11161 were evaluated in four rhesus monkeys. Each animal was tested at least twice per dose. This compound generated an inverted-U shaped dose-response curve (see rates in Table 1), and was self-administered by all four animals studied.

Table 1 shows absolute response rates (± SEM) for alfentanil and NIH 11161 self-administration, as well as their appropriate vehicles, aggregated across all four animals. Rates of response for NIH 11161 were high across a dose range approximately 30-fold higher than that required to engender contingent responding for alfentanil. The maximal rate of responding for NIH 11161 (at 0.01 mg/kg/inj) peaked at approximately 70% of alfentanil control, although rates for all doses tested were higher than those engendered by contingent saline or the NIH 11161 vehicle. By way of comparison, NIH 11661 is thus 30-fold less potent and 30% less effective than alfentanil in terms of reinforcing effects.

Dose (mg/kg/inj)	0.00003	0.0001	0.0003	0.001
Alfentanil	0.27 ± 0.13	0.95 ± 0.22	2.23 ± 0.48	2.06 ± 0.61
Saline	0.21 ± 0.11	0.20 ± 0.09	0.22 ± 0.14	0.14 ± 0.10

Dose (mg/kg/inj)	0.001	0.003	0.01	0.03
NIH11161	0.58 ± 0.34	1.33 ± 0.54	1.69 ± 0.47	0.45 ± 0.12
Vehicle	0.08 ± 0.03	0.14 ± 0.06	0.11 ± 0.08	0.08 ± 0.05

Table 1 – Response rates (responses per second) for alfentanil, NIH11161, and their infusion volume control vehicles across four doses (in mg/kg/inj) and expressed as mean ± SEM. Data were aggregated across four experimental subjects, and each dose condition was studied at least twice.

* * *

NIH 11163   (+)-(1S,5S,9S)-5,9-Dimethyl-2'-hydroxy-2-(5-hydroxypentyl)-6,7-benzomorphan.HCl
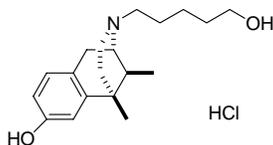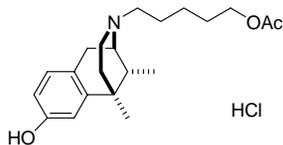

OPIOID RECEPTOR BINDING (nM)

μ-receptor: 45 ± 2.0% displacement at 10 μM
δ-receptor: 10.3 ± 1.2% displacement at 10 μM
κ-receptor: 13.5 ± 6.5% displacement at 10 μM

SUMMARY

NIH 11163 has no affinity for opioid receptors.

NIH 11164   (-)-(1*R*,5*R*,9*R*) -2-(5-Acetoxypentyl)-5,9-Dimethyl-2'-hydroxy-6,7-benzomorphan.HCl

OPIOID RECEPTOR BINDING (nM)

μ-receptor: 7.3 ± 2.9
δ-receptor: 140 ± 27
κ-receptor: 55.3 ± 4.2

SUMMARY

NIH 11164 has high affinity for μ receptors and some selectivity for μ over κ (7-fold) and δ (20-fold) receptors.

* * *

NIH 11176   (-)-(1*R*,5*R*,9*R*)- 5,9-Dimethyl-2-(1,3-dioxalanylethyl)- 2'-hydroxy-6,7-benzomorphan.HCl
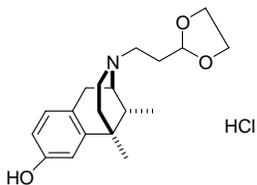
OPIOID RECEPTOR BINDING (nM)

μ-receptor: 26.6 ± 9.5
δ-receptor: 53.4 ± 5.9
κ-receptor: 41.9 ± 15.6

SUMMARY

NIH 11176 has affinity for all three opioid receptors with no selectivity.

* * *

NIH 11177   (+)-(1*S*,5*S*,9*S*)- 5,9-Dimethyl-2-(1,3-dioxalanylethyl)- 2'-hydroxy-6,7-benzomorphan.HCl

OPIOID RECEPTOR BINDING (nM)

μ-receptor: 673 ± 95
δ-receptor: 8734 ± 2570
κ-receptor: 642 ± 70

SUMMARY

NIH 11177 has low, but equivalent, affinity for μ and κ opioid receptors and very low affinity for δ opioid receptors.

NIH 11178   (+)-(1*S*,5*S*,9*S*)- 5,9-Dimethyl-2'-hydroxy-2-(4-methylpentyl)-6,7-benzomorphan.HCl

OPIOID RECEPTOR BINDING (nM)

μ-receptor: 613 ± 163
δ-receptor: 6072 ± 4320
κ-receptor: 260 ± 83

SUMMARY

NIH 11178 has low affinity for μ and κ opioid receptors. It has very low affinity for δ opioid receptors.

* * *

NIH 11179   (-)-(1*R*,5*R*,9*R*)- 5,9-Dimethyl-2'-hydroxy-2-(4-methylpentyl)-6,7-benzomorphan.HCl
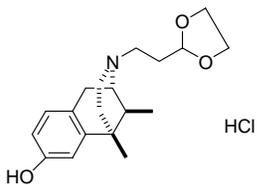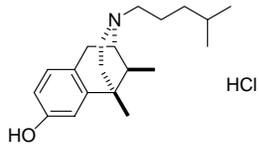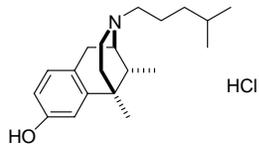

OPIOID RECEPTOR BINDING (nM)

μ-receptor: 6.0 ± 1.2
δ-receptor: 59. ± 5.1
κ-receptor: 7.3 ± 2.2

SUMMARY

NIH 11179 has high affinity for μ and κ opioid receptors, with 8- to 10-fold selectivity for these receptors over δ.

* * *

NIH 11180   (-)-(1*R*,5*R*,9*R*)- 2-(3-Acetoxypropyl)-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan.oxalate
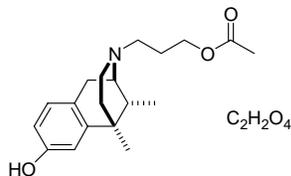
OPIOID RECEPTOR BINDING (nM)

μ-receptor: 33.7 ± 1.8
δ-receptor: 285 ± 14
κ-receptor: 24.9 ± 3.7

SUMMARY

NIH 11180 has similar affinity for μ and κ opioid receptors with approximately 10-fold selectivity for these receptors over δ.

NIH 11181   (+)-(1*S*,5*S*,9*S*)- 2-(3-Acetoxypropyl)-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan.oxalate

C₂H₂O₄

OPIOID RECEPTOR BINDING (nM)

μ-receptor: 4478 ± 358
δ-receptor: 5% inhibition at 10 :M
κ-receptor: 4141 ± 778

SUMMARY

NIH 11181 has similar, very low affinity for μ and κ opioid receptors with no affinity for δ receptors.

* * *

NIH 11182   (+)-(1*S*,5*S*,9*S*)-2-(3-Acetoxyethyl)-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan.oxalate

C₂H₂O₄
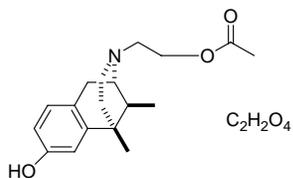
OPIOID RECEPTOR BINDING (nM)

μ-receptor: 1951 ± 198
δ-receptor: 22% inhibition at 10 μM
κ-receptor: 1830 ± 269

SUMMARY

NIH 11182 has similar, very low affinity for μ and κ opioid receptors. It shows no appreciable binding to δ receptors.

* * *

NIH 11183   (-)-(1*R*,5*R*,9*R*)- 2-(3-Acetoxyethyl)-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan.oxalate

C₂H₂O₄

OPIOID RECEPTOR BINDING (nM)
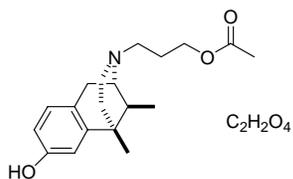
μ-receptor: 42.6 ± 6.9
δ-receptor: 421 ± 41
κ-receptor: 60.1 ± 17

SUMMARY

NIH 11183 has similar affinity for μ and κ opioid receptors, with low affinity for δ receptors.

NIH 11185   (-)-(1*R*,5*R*,9*R*)-2-(2-Cyclohexylethyl)-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan.HCl
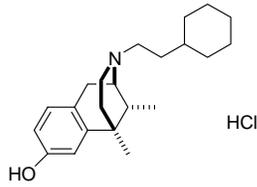
OPIOID RECEPTOR BINDING (nM)

µ-receptor: 4.3 ± 0.8
δ-receptor: 42.8 ± 5.1
κ-receptor: 51.0 ± 15.5

SUMMARY

NIH 11185 has high affinity for µ opioid receptors and is 10- to 12-fold selective over δ and κ receptors.

* * *

NIH 11186   (+)-(1*S*,5*S*,9*S*)-2-(2-Cyclohexylethyl)-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan.HCl

OPIOID RECEPTOR BINDING (nM)

µ-receptor: 142 ± 10.5
δ-receptor: 2793 ± 430
κ-receptor: 215 ± 246

SUMMARY

NIH 11186 has similar, low affinity for µ and κ opioid receptors and very low affinity for δ receptors and very low affinity for δ receptors.

* * *

NIH 11187   (-)-(1*R*,5*R*,9*R*)-2-(2-Ethylbutyl)-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan.HCl

OPIOID RECEPTOR BINDING (nM)
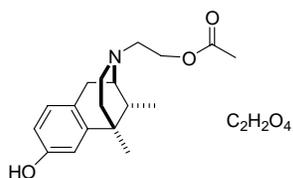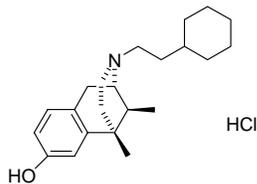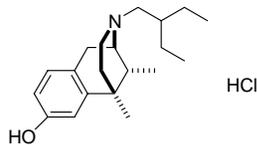
µ-receptor: 9.2 ± 0.8
δ-receptor: 58.2 ± 4.7
κ-receptor: 5.9 ± 0.3

SUMMARY

NIH 11187 has high affinity for µ and κ opioid receptors and low affinity for δ receptors. It has no selectivity between µ andκ.

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ACKNOWLEDGMENTS

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APPENDIX

The University of Michigan laboratories also offer the following tests under the auspices of the Drug Evaluation Committee:

DRUG DISCRIMINATION IN RHESUS MONKEYS

We currently use three groups of monkeys to test the discriminative stimulus effects of submitted drugs: one of these groups discriminates the administration of the κ agonist ethylketazocine (EKC); a second group discriminates the μ agonist alfentanil or fentanyl; a third group is treated daily with morphine and discriminates the opioid antagonist naltrexone.

The procedures used with the EKC-trained monkeys have been described by Bertalmio et al. (1982). The monkeys are removed from their home cages each day and seated in primate restraining chairs. These chairs are placed in chambers equipped with two response levers, several stimulus lights and a cup to receive Noyes, banana-flavored pellets. These monkeys are required to make 100 consecutive responses on the correct one of the two levers and receive ten 300-mg food pellets. The right lever is correct if they were given a subcutaneous injection of 0.0032 mg/kg EKC immediately prior to the start of the cycle. The left lever is designated correct if they were given a sham injection before the start of the cycle. Each cycle lasts l5-min and consists of an initial l0-min black out period followed by a period of as long as 5 min, during which a blue light is illuminated in the chamber and the monkey can respond for food. If the food pellets are delivered before the 5 min period is completed, the lights are extinguished for the remainder of this time. Typically, a daily session consists of several 15 min cycles. During a training session, if EKC is given, it is given on the penultimate cycle of that session. Responding on the drug-appropriate lever is reinforced during that cycle and on the subsequent, final cycle of the day. These last two cycles may be preceded by from zero to four sham cycles on a training day. A training session of six sham cycles is also scheduled from time to time.

With this type of multiple, discrete-cycle training, the animals can be tested with a cumulative dosing procedure. On a test session, the first cycle is preceded by an injection of saline, and prior to subsequent cycles, increasing, cumulative doses of the test drug are administered. One hundred consecutive responses on either lever are reinforced throughout the test session. The test drug is administered in increasing doses until the monkey either responds on the drug-appropriate lever, the response rate falls to less than half of the saline-control rate, or six cycles are given. In the latter situation, it is assumed that the selected dose range is too low, and the test is continued at higher doses on the next test session. Each test session is preceded and followed by a training session. The criterion for satisfactory performance must be met on each training session that is followed by a test session. This criterion is that at least 90% of the responses during each cycle of a training session must be on the injection-appropriate lever, either sham or EKC.

The procedure for the alfentanil-trained monkeys is similar, but not identical. These animals are also trained and tested in a discrete, multiple-cycle procedure. The main difference between the alfentanil procedure and the EKC procedure is that the alfentanil monkeys are required to make 20 rather than l00 responses, and they receive a single pellet for correct responses. They can receive as many as 10 pellets during the 5-min, food-availability period of each cycle, but each pellet is delivered after 20 responses. Because in this procedure, monkeys can switch from one lever to another following the delivery of food, an additional criterion is added for satisfactory performance. In addition to making 90% or more of their responses on the correct lever, the monkeys must make fewer than 20 responses on the incorrect lever prior to delivery of the first food pellet of each cycle. Tests of the discriminative stimulus effects of submitted drugs in the alfentanil-trained monkeys are also done using a cumulative dosing procedure with dosing criteria identical to those used in the EKC-trained monkeys.

The procedure for studying discriminative stimulus effects in morphine-treated monkeys has been described previously (France and Woods, 1989). Daily sessions are comprised of a 10-min time out during which lever presses have no programmed consequence and a 5-min response period during which green stimulus lights are illuminated and signal the activation of a schedule of stimulus-shock termination. Sessions consist of between two and six discrete, 15-min cycles with each cycle. Under these experimental conditions, electric shock is scheduled to be delivered to the subject's feet every 15 seconds; monkeys can terminate the lights and postpone scheduled shocks for 30 seconds by pressing five

times consecutively (*i.e.*, fixed-ratio 5) the lever appropriate for the solution administered during the first minute of the time out (left lever, saline; right lever, naltrexone). Monkeys receive an injection of saline (0.1 ml/kg) or drug (0.01 mg/kg naltrexone) during the first minute of each time out. On drug-training days a single injection of naltrexone is administered during one time out and for that cycle and all subsequent cycles on that day only responding on the right lever postpones shocks. A variable number of saline cycles (0-5) precede the naltrexone cycle and on some days saline is administered during the time out of all cycles. Under these conditions monkeys switch their response choice from the saline lever to the naltrexone lever with complete generalization occurring in all three subjects at a dose of 0.01 mg/kg. Responding on the naltrexone lever is accompanied by other behavioral effects indicative of opioid withdrawal (*e.g.*, irritability, miosis, salivation). Moreover, when saline is substituted for the daily injection of 3.2 mg/kg of morphine monkeys respond predominantly on the naltrexone lever and show directly observable signs of withdrawal; the discriminative stimulus and other effects produced by morphine abstinence are reversed by some opioid agonists (*e.g.*, alfentanil; France and Woods, 1989; France *et al.*, 1990).

For test sessions increasing doses of drug are administered during the first minute of consecutive time outs and five consecutive responses on either lever postpone shocks. In monkeys that receive 3.2 mg/kg of morphine 3 hours earlier, increasing doses of a test compound are administered up to doses that produce an average of at least 80% responding on the naltrexone lever or to doses that disrupt responding and result in the delivery of electric shock. Drugs that do not substitute for naltrexone (*i.e.*, precipitate withdrawal) are also studied for their ability to reverse responding on the naltrexone lever in morphine-abstinent (*i.e.*, withdrawn) subjects. Test compounds are studied using a cumulative-dosing procedure in morphine-abstinent monkeys up to doses that reverse completely responding on the naltrexone lever (<20%) or to doses that disrupt responding. Some compounds that substitute for naltrexone also are studied for their capacity to prevent the effects of cumulative doses of opioid agonists. Monkeys that receive saline three hours earlier, rather than the daily injection of morphine, receive saline (control) or a single injection of test compound during the first cycle and increasing doses of agonist (alfentanil or morphine) during subsequent cycles. Agonists are administered up to doses that produce a switch from the naltrexone lever to the saline lever or to doses that disrupt responding and result in the delivery of electric shock.

THERMAL ANALGESIA IN RHESUS MONKEYS

The tail-withdrawal procedure used to study analgesic effects of test compounds in rhesus monkeys has been described previously (Dykstra and Woods, 1986). Monkeys are restrained loosely at the neck and arms while seated in Plexiglas primate chairs. For tests of tail-withdrawal latency, the lower 10-12 cm of the shaved tail is immersed in a thermos containing water at 40°, 50°, or 55° C and the latency until the tail is withdrawn from the thermos is recorded for each monkey at each temperature. When the tail is not withdrawn within 20 seconds (cut-off latency) the experimenter removes the thermos and a latency of 20 seconds is recorded. Experimental sessions begin with several exposures to 40°C water. Four or five monkeys are tested consecutively and the time between tail immersions for individual monkeys is 5 minutes. Generally, 40°C water does not produce tail withdrawal in rhesus monkeys (Dykstra and Woods, 1986); however, if a monkey fails to keep its tail in 40° C water for 20 seconds on at least 3 of 4 immersions, that animal is not tested further for that particular session. In a subsequent pre-test component, tails are immersed in 40°, 50°, and 55° C water. The order in which the three temperatures are presented is varied among subjects. If the latencies for tail withdrawal in the pre-test component are at or near 20 seconds for 40° C water and less than 5 seconds for 55° C water, monkeys receive the test compound. The test is identical to the pre-test, except that monkeys receive s.c. injections of drug 10 minutes prior to tail immersion. The time between immersions for individual subjects is 5 minutes or less and the order in which temperatures are presented varies among subjects and across cycles. The interinjection interval typically is 30 minutes and between four and six doses are studied in a single experiment using the cumulative dosing procedure. For some studies a single dose of an opioid antagonist is administered prior to the test compound and for other studies a single dose of test compound is administered prior to increasing doses of a μ (*e.g.*, alfentanil) or κ (*e.g.*, U-50,488) opioid agonist.

RESPIRATORY STUDIES IN RHESUS MONKEYS

The effects of test compounds on ventilatory function are studied in rhesus monkeys breathing air or 5% CO_2 in air (France and Woods, 1990; Howell *et al.*, 1988). Monkeys are restrained at the neck and waist while seated in a

Plexiglas primate chair. Normal air or 5% CO_2 in air is delivered at a rate of 10 1/min into a sealed helmet placed over the subject's head. Changes in pressure within the helmet are measured and recorded by a transducer and a microprocessor, and are transformed according to known standards to frequency of respiration (f) in breaths/minute and to tidal volume (V_T) in ml/inspiration. Data are recorded continuously during 23-minute exposures to air alternating with 7-minute exposures to CO_2. The last 3 minutes of exposure to CO_2 are used for data analyses and are compared to the last 3 minutes of exposure to air only. Increasing doses of drug are administered during the first minute of consecutive time outs so that the interinjection interval is 30 minutes. For some studies a single injection of an opioid antagonist is administered prior to increasing doses of a test compound and for other studies a single injection of test compound is administered prior to cumulative doses of a standard compound (*e.g.*, alfentanil).