BIOLOGICAL EVALUATION OF COMPOUNDS FOR THEIR PHYSICAL DEPENDENCE POTENTIAL AND ABUSE LIABILITY. XXIV. DRUG EVALUATION COMMITTEE OF THE COLLEGE ON PROBLEMS OF DRUG DEPENDENCE (2000)

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THE DRUG EVALUATION COMMITTEE (DEC) AND ITS MEMBERSIHP

Dr. A. Coop replaced Dr. A. E. Jacobson, the Biological Coordinator of DEC, CPDD, from 1976 to 2000. Dr Coop is the fourth DEC Biological Coordinator (the initial two were Drs. N. Eddy and E. L. May). The other members of DEC remained unchanged this year; they are in DEC's two analgesic testing groups, at Virginia Commonwealth University (VCU, Drs. L. Harris, M. Aceto, E. Bowman, P. Beardsley) and the University of Michigan (UM, J. Woods (DEC Chair), J. Traynor), and three stimulant/depressant testing groups, at the University of Mississippi (UMs, W. Woolverton), Louisiana State University (LSU, C. France), and UM (G. Winger, J. Woods). Drs. T Cicero and A. E. Jacobson act as emeritus members. The DEC reports to the CPDD's Liaison Committee for Drug Testing and Evaluation (A. Young, Chair). Members of both that CPDD Committee, and the Industry Relations Committee (R. Mansbach, Chair), as well as NIDA, attend DEC's meeting held during the Annual Scientific Meeting of the CPDD. One or two other DEC meetings are held during the year to discuss the work which has been accomplished, and future plans. Separate meetings are held at VCU quarterly with the members of the VCU Analgesic Testing Group, as well as Drs. E. L. May and E. Bowman, the DEC Biological Coordinator, and a NIDA representative, to discuss the results obtained from the VCU testing and research program.

Data were released for publication this year on 34 different compounds evaluated by DEC's Analgesic Testing Program. Of these, 33 compounds were evaluated at VCU (antinociceptive assays in mice - tail flick, hot plate, and phenylquinone, and the tail-flick antagonist assay, as well as substitution for morphine and precipitated withdrawal assays in rhesus monkeys), and 26 at UM (binding affinity to the μ, δ, and κ opioid receptors, and monkey self- administration) came from disparate sources: 65% from universities (56% from US universities and 9% from foreign universities); 21% of the compounds, more than usual, came from pharmaceutical industry, and most of them (18%) were from US industry. Many of the remaining compounds (12%) came from governmental sources. A comparatively large number of compounds were released for publication this year (5 drugs) by the groups in the Stimulant/Depressant Testing Program. One of the compounds was examined to obtain data which the World Health Organization requested.

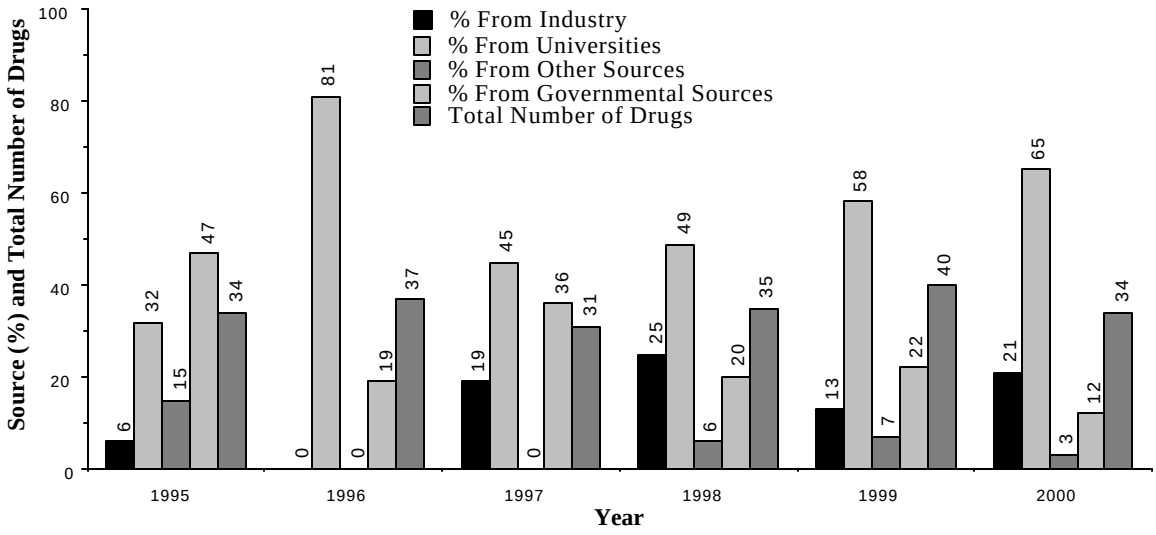
Two joint publications based on the data gathered under DEC auspices from MCV, UM and NIH, are in review or preparation (May et al., 2000a; May et al., 2000b).

EXPERIMENTAL OBSERVATIONS

The names of the compounds that were released for publication this year are listed in Table 1, and their molecular structures and a summary of their *in vivo* and *in vitro* data are in Tables 2 to 8. As in previous years (Jacobson, 2000), the examined compounds are classified according to their molecular structure, 4,5-epoxymorphinans in Table 2, morphinans in Table 3, and the 6,7-benzomorphans in Tables 4 and 5. Miscellaneous compounds (those which do not fall into the usual opioid classes) are listed in Tables 6 and 7, and the compounds evaluated by the Stimulant/Depressant testing groups are shown in Table 8. The more interesting compounds evaluated during the year are discussed below. For compounds that have been previously evaluated, the new data are discussed in relation to the published data.

STATISTICS

FIG. 1. DEC ANALGESIC PROGRAM: PERCENT, TOTAL NUMBER, AND SOURCE OF EXAMINED DRUGS (1995-2000)



NIH 10497 possesses an unusual *N*-1*R*-1-cyclopropylethyl substituent, similar to the μ-antagonist conferring *N*-cyclopropylmethyl. Previous reports (1989) showed that NIH 10497 is not active as a morphine antagonist in the mouse, and indeed can completely substitute for morphine in monkeys, indicating a μ-agonist profile. Side-effects seen in the monkeys (e.g. salivation) also suggested kappa agonist activity. Table 1 shows that NIH 10947 possesses high affinity at both μ and κ receptors, with somewhat lower affinity at δ receptors - thus the μ-agonist/κ-agonist activity seen in the monkey is consistent with the binding data. In contrast, GTPγS functional data show that NIH 10497 has low mu efficacy, which is not consistent with the *in vivo* substitution data. This is, however, consistent with the fact that NIH 10497 appears to be relatively free of μ-opioid dependence liability in the rat.

NIH 10924 (naltriben) is generally regarded as a δ_2-subtype selective antagonist, but we have reported that the pharmacology of natriben is complicated (Jacobson, 2000). Previous studies show that NIH 10924 is only moderately δ-opioid preferring (34- and 48-fold selective for δ- over μ- and κ-receptors, respectively). Indeed, potent morphine antagonism was seen in the mouse. Interestingly, NIH 10924 reversed DPDPE induced antinociception, even though DPDPE is considered a δ_1-subtype selective agonist. Lethal convulsions were also noted at high doses; these convulsions were not blocked by the δ-antagonist naltrindole, suggesting that they are not delta receptor mediated. Weak antinociception was reported in PPQ, but is probably not opioid receptor mediated, as Table 2 shows it is not reversed by nor-BNI or β-FNA. Thus, care must be taken when using naltriben as a δ_2-selective antagonist *in vivo*.

NIH 10968 in Table 2, 14-methoxymetapon, was found to be a potent and fairly selective μ-agonist. It was about fifty times more potent than morphine in the monkey single-dose-suppression assay, and had about that potency in antinociceptive assays, as well. This potency is somewhat less than noted in the literature as determined from an acetic acid writhing antinociceptive assay (Schmidhammer et al., 1990). The 14-methoxy substituent appears to greatly enhance potency; metapon was previously found to be about three times more potent than morphine (Deneau and Seevers, 1955). **NIH 10998** (heterocodeine), in Table 2, was found to be a typical μ-agonist, more potent than, but otherwise quite similar to morphine. The increased C-ring

hydrophobicity caused by blocking morphine's 6-hydroxy group apparently enhanced potency in a similar fashion to that seen for 6-acetylmorphine. Heterocodeine was initially examined in 1932 only in the hot plate assay (as NIH 00111), and it was found at that time to have an ED_{50} = 0.35 (0.31-0.39), when morphine's ED_{50} was about 2 mg/kg. NIH 00111 was seen to be a little more potent than NIH 10998, which was found to have an ED_{50} = 0.51 (0.3-0.87) in the hot plate assay (morphine = 0.85 (0.39-1.86)), but the difference is not extreme, especially considering the 70 year time span.

The unusual endoethenomorphinan in Table 3 (**NIH 10931**) was a very potent long-acting antinociceptive. Interestingly, this compound, which was up to 2000 times more potent than morphine in the monkey single-dose-suppression assay, had opioid antagonist actions following the disappearance of its agonist activity, and this antagonist effect lasted for more than 168 hours. **NIH 10984**, Table 3, possesses a phenyl group fixed in a similar orientation to that in NIH 10931, and is similarly very active as a μ-opioid agonist. Unlike NIH 10931, NIH 10984 exhibited only agonist activity, and was not selective for a subtype of opioid receptor. Morphinan **NIH 10965** (Table 3), with an unusual 4-benzyl ether, has only been evaluated in binding assays. It demonstrates relatively high μ-opioid affinity for a simple 3-methyl ether substituted morphinan, indicating that a 4-benzyl ether is not detrimental to μ-affinity. The poor activity of NIH 10965 in the GTPγS functional assay at mu receptors, suggests that this compound will have mu antagonist properties.

An *N*-pent-4-ynylnormetazocine, **NIH 10972** in Table 4, was notable in that it was nonselective and not particularly potent as a μ-agonist. It antagonized morphine in the mouse (tail-flick), yet, surprisingly, substituted for morphine in the monkey (single-dose-suppression). In contrast, *N*-but-3-ynylnormetazocine, **NIH 10974**, was a potent agonist with good affinity for κ-opioid receptors (1.4 nM) and fair affinity for the remaining subtypes. NIH 10974 was inactive as a morphine antagonist in the mouse, and, as expected, substituted for morphine in the monkey (SDS). The *N*-methoxyethyl analogue in Table 4 (**NIH 10980**) was a potent agonist with high affinity for μ- and κ-receptors, and good affinity for δ-receptors, as well. The agonist activity in the mouse tail-flick was reversed by β-FNA, but not nor-BNI nor naltrindole, demonstrating μ-agonism. Thus, the fact that it did not substitute for morphine in the monkey (single-dose-suppression) is unusual. The change in activity, from antagonist to agonist, from relatively poor potency to high antinociceptive activity by modification of the N-substituent in opioids is not well understood even for compounds that mainly interact with the μ-opioid receptor, and is certainly less understood for those that interact with the other opioid receptors. These compounds are among those discussed in an article by DEC-associated authors (May et al., 2000a). The corresponding (+)-isomers (which possess the unnatural opioid stereochemistry) are shown in Table 5. Most have very low affinity for opioid receptors, and the minor effects *in vivo* are probably non-opioid in nature.

Ketocyclazocine (**NIH 10964** in Table 5) was examined and found to be have potent antinociceptive activity. It was the prototypic κ-agonist (Iwamoto and Martin, 1981; Martin et al., 1976),. In the present study, β-FNA, but not nor-BNI (a κ-antagonist) or naltrindole (a δ-antagonist), antagonized its agonist activity in the mouse tail flick assay, indicating that the drug acts as a the μ-opioid receptor agonist in that antinociceptive assay, and not as κ agonist. In fact, *in vitro* studies showed that NIH 10964 was nonselective and had almost equally high affinity to all three opioid receptors (*Ki* at μ = 6 nM, δ = 7 nM, and κ = 4 nM). Ketocyclazocine was examined previously as NIH 8847 in 1972 (hot plate ED_{50} = 0.4; it neither substituted for morphine nor precipitated withdrawal in monkey substitution studies), and as NIH 10346 in 1984 (hot plate ED_{50} = 0.65; partial suppression of abstinence observed in monkeys). Thus, similar hot plate assay results were observed over a span of 30 years. It was most recently found to be six times more potent than morphine, compared with three times more potent in 1972, and two times more potent in 1984. The results from monkey single dose suppression studies were only a little different. No substitution was found in the earliest study, and partial substitution was found both in 1984 and most recently. In this most recent study, the compound was found to precipitate withdrawal in the monkey. Its antagonist activity was found to be about 0.25 x naloxone. Since ketocyclazocine was inactive in the tail flick vs. morphine assay in mice, an assay for μ-antagonists, NIH 10964 could be acting as a κ-receptor antagonist in the monkey. In contrast to these data, GTPγS assays indicated that it has good potency and efficacy as a κ-agonist. It possessed much lower potency and efficacy at μ and δ receptors. **NIH 10993** (Table 5) is a racemic benzomorphan with an unusual acetamide N-substituent. This

substituent removes almost all opioid activity; only weak binding to κ- and μ-receptors (304 and 677 nM, respectively) remains.

The notorious γ-hydroxybutyrate (GHB, **NIH 10947**) has unique properties as shown in Table 6. NIH 10947 has little opioid-like activity alone, but acts synergistically with morphine in PPQ. In addition, when NIH 10947 was given with morphine to morphine tolerant mice, antinociception was partially restored. These data suggest potential therapeutic uses for GHB in the treatment of pain in morphine tolerant patients, and potential safety issues for opioid abusers if they also administer GHB. Tramadol (**NIH 10969**, Table 6) and its symmetrical relative, **NIH 10970**, were examined and found to have little, if any, opioid-like activity. Tramadol is said to possess a μ-affinity of about 2 μM (c.f. 3 μM Table 6) and to have codeine-like potency in man; its analgesic effects are said to be produced through both opioid and non-opioid mechanisms (Raffa et al., 1992). The poor activity in the mouse, shown in Table 6, may be due to species differences, but it is in accord with our determined binding affinity.

NIH 10908 (Sameridine, Table 6), has been reported to possess local anaesthetic and analgesic effects, was found to be toxic in mice and to have relatively weak antinociceptive activity, probably mediated through μ-receptors (hot plate activity was antagonized by naloxone, AD_{50} = 0.07; completely substituted for morphine in the monkey). Carisoprodol (**NIH 10966**) (Table 6) was previously examined by the Stimulant/Depressant group (CPDD 0054). As expected, it had no opioid-like activity *in vivo* or *in vitro.* Further studies on NIH 10966 by the stimulant group are reported below. The ketobemidones (**NIH 11001** and **NIH 11002**) (Table 6) represent further examples of binding data that do not correlate with *in vivo* animal data, and underscore the importance for functional assays. NIH 11001 possesses about the same affinity for μ-receptors as, for example, heterocodeine (NIH 10998) (33 vs. 21 nM), yet NIH 11001 is completely inactive *in vivo*, both as an agonist and as an antagonist. NIH 11002 possesses a slightly lower affinity at μ-receptors (118 nM), and is also completely inactive *in vivo*.

The two enantiomers of norisonicotine (**NIH 10975** and **NIH 10976**) (Table 7) are under study for the treatment of cognitive dysfunction in conditions such as Alzheimer's disease (Levin et al., 1999). Table 7 shows that they have no opioid actions. **NIH 10977** is a coumarin-based cyclic prodrug of the peptidic δ-opioid agonist DADLE. As shown in Table 7, peripheral administration of NIH 10977 gave rise to only slight antinociceptive effects in PPQ. The effect was not reversed by naltrindole, indicating a non-δ-opioid mediated effect. It appears that NIH 10977 does not enter the CNS. **NIH 10991** is currently receiving a great deal of attention as a potentially potent analgesic agent that acts through nicotinic rather than opioid receptors. Table 7 shows that NIH 10991 is indeed a potent antinociceptive agent, yet unlike previous reports (Bannon et al., 1998), the nicotinic antagonist mecamylamine did not reverse the antinociceptive effects. When combined with the toxic effects seen with NIH 10991, it is obvious that this compound requires further study to fully understand its pharmacology. **NIH 11008, NIH 11009, and NIH 11010** have been studied by both the analgesic and stimulant/depressant groups. (Table 8 gives their CPDD numbers). These compounds are *S*-(+), *R*-(-), and racemic mecamylamine, respectively. All three gave weak antinociception in PPQ (interestingly, the racemic NIH 11010 was the most potent) which was not reversed by naloxone or mecamylamine, demonstrating that the antinociception is neither opioid receptor mediated nor a nicotinic agonist effect. Indeed, all three compounds effectively antagonized the antinociceptive (tail-flick) effects of nicotine. The toxicity of these ligands (at 10 and 30 mg/kg) should be noted. **CPDD 0057** (NIH 11008) and **CPDD 0058** (NIH 11009) in the stimulant program showed no reinforcing effects in methohexital trained monkeys.

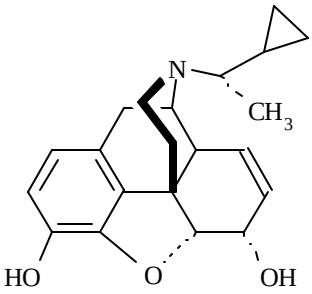
CPDD 0054 (NIH 10966) (Table 6) has been previously reported to possess no pentobarbital-like discriminative effects when administered i.g., however when administered i.v. full discrimination for pentobarbital was observed. CPDD 0054 may therefore have pentobarbital-like subjective effects in humans. **CPDD 0055** (Table 8) is a constituent in certain asthma medications; it displayed no stimulant effects. **CPDD 0056** (Table 8), a sulfur containing derivative of amphetamine, was inactive in the cocaine dependent monkey, and was not discriminated for the benzodiazepine midazolam at doses up to 3.2 mg/kg. Larger doses (10 mg/kg) of CPDD 0056 were lethal in the monkey. These data were generated at the request of the World Health Organization. Complete details on these drugs can be found in the Stimulant/Depressant Annual Report (France et al., in press).

TABLE 1. EVALUATED COMPOUNDS

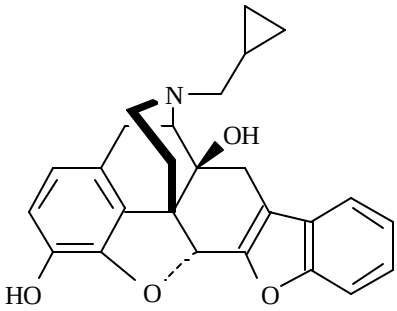
NIH#	COMPOUND NAME	TABLE #-Evaluator
10497	N-(1*R*-1-Cyclopropyl)ethylnormorphine hydrochloride	2-VCU
10908	Sameridine hydrochloride	6-VCU/UM
10924	Naltriben (NTB) methanesulfonate	2- VCU
10931	N-Methyl[5β,7β,3',5']pyrrolidino-2'-[*S*]-phenyl, 7α-methyl, 3-hydroxy, 6-methoxy-6,14-endoethenomorphinan dihydrochloride	3- VCU
10947	γ-Hydroxybutyric Acid, sodium salt	6-VCU/UM
10963	(±)-N-(But-3-ynyl)-N-normetazocine	4-VCU/UM
10964	(±)-8-Ketocyclazocine (also examined as NIH 8847 and 10346)	5-VCU/UM
10965	4-Benzyloxy-17-cyclopropylmethyl-14-hydroxy-17-nordihydrothebainone	3-UM
10966	Carisoprodol (also examined as CPDD 0054)	6-VCU/UM
10968	8-(Ethylmethylamino)-5,6,7,8-tetrahydroisoquinoline oxalate	2-VCU/UM
10969	Tramadol hydrochloride	6-VCU/UM
10970	2,6-Bis[(dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol dihydrochloride	6-VCU/UM
10971	(+)-(1*S*,5*S*,9*S*)-5,9-dimethyl-2'-hydroxy-2-(pent-4-ynyl)-6,7-benzomorphan	5- VCU/UM
10972	(-)-(1*R*,5*R*,9*R*)-5,9-dimethyl-2'-hydroxy-2-(pent-4-ynyl)-6,7-benzomorphan	4- VCU/UM
10973	(+)-(1*S*,5*S*,9*S*)-2-(But-3-ynyl)-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan .HCl	5-VCU/UM
10974	(-)-(1*R*,5*R*,9*R*)-2-(But-3-ynyl)- 5,9-dimethyl-2'-hydroxy-6,7-benzomorphan .HCl	4-VCU/UM
10975	(-)-N-Norisonicotine .di-l-tartrate	7-VCU/UM
10976	(+)-N-Norisonicotine .di-d-tartrate	7-VCU/UM
10977	Coumarin-based cyclic prodrug of DADLE	7-VCU/UM
10980	(-)-(1*R*,5*R*,9*R*)-5,9-Dimethyl-2'-hydroxy-2-(2-methoxyethyl)-6,7-benzomorphan.HCl	4- VCU/UM
10981	(+)-(1*S*,5*S*,9*S*)-5,9-Dimethyl-2'-hydroxy-2-(2-methoxyethyl)-6,7-benzomorphan.HCl	5-VCU/UM
10982	(+)-(1*S*,5*S*,9*S*)-5,9-Dimethyl-2'-hydroxy-2-(3-hydroxypropyl)-6,7-benzomorphan.HCl	5-VCU/UM
10983	(-)-(1*R*,5*R*,9*R*)-5,9-Dimethyl-2'-hydroxy-2-(3-hydroxypropyl)-6,7-benzomorphan.HCl	4- VCU/UM
10984	E-3-Methoxy-4-hydroxy-5,14-ethano-18-(1-methyl)benzylidene-6-oxo-N-methylmorphinan	3-VCU/UM
10991	(*R*)-5-(2-Azetidinylmethoxy)-2-chloropyridine .*p*-toluenesulfonate	7-VCU/UM
10993	(-)-(1*R*,5*R*,9*R*)-2-Acetamido-5,9-dimethyl-2'-hydroxy-6,7-benzomorphan	5-VCU/UM

10998	Heterocodeine hydrochloride	2-UM
10999	(-)-(1*R*,5*R*,9*R*)-5,9-Dimethyl-2'-hydroxy-2-(3-trifluoromethylbenzyl)-6,7-benzomorphan hydrochloride	4-VCU/UM
11000	(+)-(1*S*,5*S*,9*S*)-5,9-Dimethyl-2'-hydroxy-2-(3-trifluoromethylbenzyl)-6,7-benzomorphan hydrochloride	5-VCU/UM
11001	4-(3-Hydroxyphenyl)-4-(1-oxopropyl)-1-(4-trifluoromethylbenzyl)piperidine .HCl	6-VCU/UM
11002	4-(3-Hydroxyphenyl)-4-(1-oxopropyl)-1-(3-trifluoromethylbenzyl)piperidine .HCl	6-VCU/UM
11008	*S*-(+)-Mecamylamine hydrochloride (see CPDD 0057)	7-VCU/UM
11009	*R*-(-)-Mecamylamine hydrochloride (see CPDD 0058)	7-VCU/UM
11010	(±)Mecamylamine hydrochloride (see CPDD 0059)	7-VCU/UM
CPDD 0055	(-)-Phenylephrine hydrochloride	8-S/D Group
CPDD 0056	4-Methylthioamphetamine hydrochloride	8-S/D Group
CPDD 0057	*S*-(+)-Mecamylamine hydrochloride (see NIH 11008)	8-S/D Group
CPDD 0058	*R*-(-)-Mecamylamine hydrochloride (see NIH 11009)	8-S/D Group
CPDD 0059	(±)Mecamylamine hydrochloride (see NIH 11010)	8-S/D Group

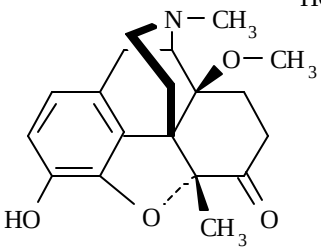
TABLE 2. 4,5-EPOXYMORPHINANS



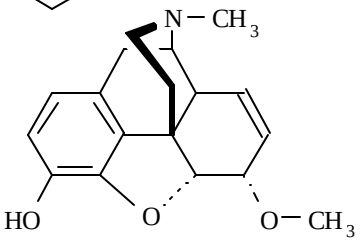
NIH 10497



NIH 10924



NIH 10968



NIH 10998

ANTINOCICEPTIVE/ANTAGONIST ASSAYS IN VITRO MONKEY (MOUSE ED50/AD50, s.c., mg/kg)

NIH #	Hot Plate	Phenylquinone	Tail Flick	Tail Flick Antagonist	Binding Affinity, nM	Substitution-for-Morphine (s.c., mg/kg)
10497	-	0.03[a]	2.0[a]	Inactive[a]	μ=0.1, δ=29, κ=1.3[b]	Complete substitution[a,c]
10924	Inactive[d]	4.2[d,e]	Inactive[d,e]	0.99[c]	μ=12.4, δ=0.36, κ=17.5[c]	-
10968	0.03	0.009	0.03	Inactive	μ=0.03, δ=41, κ=304	Complete substitution (50 x morphine)
10998	0.51	0.04	0.21[f]	Inactive	μ=21, δ=251, κ=271	Complete substitution (2 x morphine)

a) Previously reported 1989.

b) GTPγS assay: mu EC_{50} = 2191 ± 773 nM (18.7 ± 5.8% stimulation); delta EC_{50} = 72.2 ± 21.0 nM (11.7 ± 3.3% stimulation; kappa EC_{50} = 18.3 ± 4.1 nM (78.4 ± 3.8% stimulation).

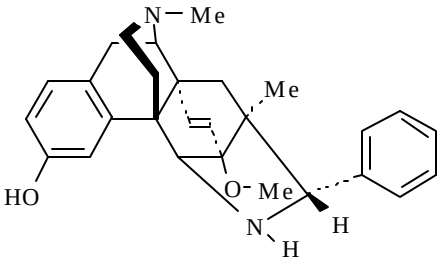
c) Monkey self-administration: maintained rates between saline and codeine; monkey drug discrimination: codeine like; thermal analgesia: μ + κ, more effective @ 50 than 55 °C; rat primary physical dependence: relatively free of μ-opioid dependence liability; naloxone AD50 (tail flick): 2.98; vas deferens[a]: κ-profile; rat brain homogenate binding[a]: 2.1 nM.

d) Previously reported 1998

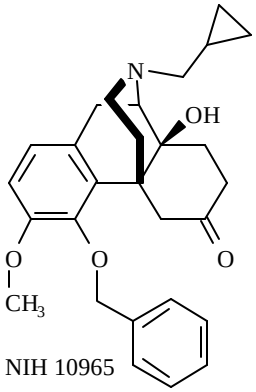
e) Convulsions, lethal @ 30 mg/kg. Naltrindole pretreatment did not abolish lethal effects[c]; 10924 vs. DPDPE (i.c.v., tail flick) AD_{50} = 3.2,[c] naltrindole (s.c., tail flick) vs 10924 = inactive[c]. μ- & δ-antagonist, agonist in PPQ[c] ; 10924 vs ED80 DPDPE (i.c.v., PPQ) AD50 = 3.2 (previously reported 1999); nor-BNI (s.c.) and β-FNA (i.c.v.) vs 10924 in PPQ: inactive.

f) Naloxone vs ED_{80} of 10998: AD_{50} = 0.04; opioid subtype: β-FNA (i.c.v.) vs ED_{80}: AD_{50} = 0.06 μg/brain; nor-BNI and naltrindole vs ED_{80}: inactive.

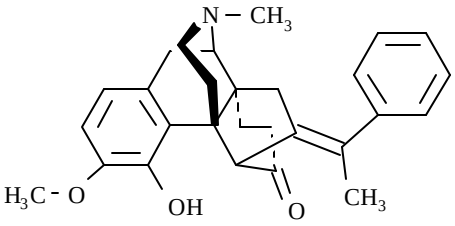
TABLE 3. MORPHINANS



NIH 10931



NIH 10965



NIH 10984

ANTINOCICEPTIVE/ANTAGONIST ASSAYS IN VITRO MONKEY (MOUSE ED50/AD50, s.c., mg/kg)

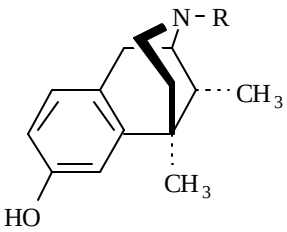
NIH #	Hot Plate	Phenylquinone	Tail Flick	Tail Flick Antagonist	Binding Affinity, nM	Substitution-for-Morphine (s.c., mg/kg)
10931	0.01	0.004	0.005[a]	Inactive	–	Complete suppression (potency 500-2000 x morphine)
10965	-	-	-	-	μ=10.4, δ= 6476, κ=202[b]	-
10984	0.1	0.012	0.05[c]	Inactive	μ=0.9, δ=6.7, κ=0.4	Complete suppression (potency 100 x morphine)

a) Naloxone AD50 = 0.02. Time course study (mice): Long duration, potent antinociceptive, followed by antagonist activity for > 168 hours. Drug naïve monkeys: μ- and κ-agonist, μ-antagonist, muscarinic effects on acute administration.

b) GTPγS assay: mu EC_{50} = 1254 ± 576 nM (21.4 ± 10.6% stimulation)

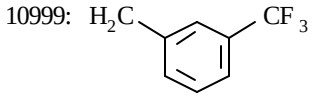
c) β-FNA (i.c.v., tail flick) vs ED_{80} 10984: AD_{50} = 1.2 (nor-BNI and naltrindole: inactive) - potent μ-agonist. No antagonism of morphine ED_{80} after 10984 pretreatment from 2 to 120 hours.

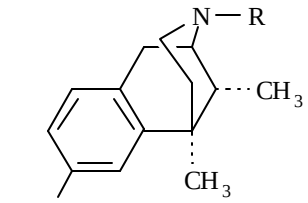
TABLE 4. 6,7-BENZOMORPHANS



(-)-(2*R*,5*R*,9*R*)

R =

10974: $CH_2CH_2C{\equiv}CH$
10972: $CH_2CH_2CH_2C{\equiv}CH$
10980: $CH_2CH_2OCH_3$
10983: $CH_2CH_2CH_2OH$
10999: 



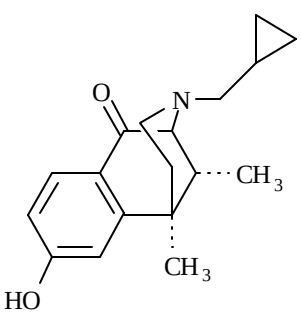
R = (±)-
10963: $CH_2CH_2C{\equiv}CH$

ANTINOCICEPTIVE/ANTAGONIST ASSAYS (MOUSE ED50/AD50, s.c., mg/kg) **IN VITRO** **MONKEY**

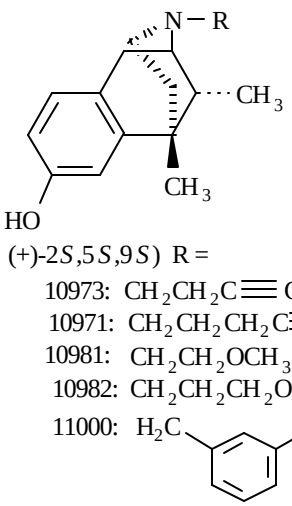
NIH #	Hot Plate	Phenylquinone	Tail Flick	Tail Flick Antagonist	Binding Affinity, nM	Substitution-for-Morphine (s.c., mg/kg)
10963	0.14	0.04	0.18[a]	Inactive	μ=0.8, δ=15, κ=3	Complete substitution (potency 6 x morphine)
10972	Inactive	11.8[b]	Inactive	2.0	μ=18, δ=137, κ=11	Complete substitution
10974	0.13	0.04[c]	0.05	Inactive	μ=4.6, δ=10, κ=1.4	Complete substitution
10980	0.15	0.01	0.07[d]	Inactive	μ=0.5, δ=9, κ=0.8	No sustitution[e]
10983	Inactive	11.6	Inactive	Inactive	μ=34, δ=124, κ=137	Exacerbated withdrawal. Weak agonist-antagonist.
10999	Inactive	Inactive	Inactive	Inactive	μ=314, δ=2904, κ=704	Partial substitution (brief)

a) Nor-BNI or naltrindole vs ED_{80} 10963 are inactive; β-FNA vs ED_{80}: AD_{50} = 0.03.
b) β-FNA (i.c.v.) vs ED_{80} of NIH 10963: 43% maximum @10 μg/brain; nor-BNI or naltrindole: inactive; 10972 vs ED80 DPDPE (i.c.v., tail flick): AD50 = 0.05. Weak μ-agonist, μ-, κ-, δ-antagonist.
c) Naltrindole, nor-BNI, and β-FNA (s.c., s.c., i.c.v., respectively, tail flick) vs ED_{80} 10974: AD50: 0.29, 3.1, 0.64, respectively.
d) β-FNA (i.c.v., tail flick) vs ED_{80} 10980: AD50: 3.1, and nor-BNI or naltrindole: inactive.
e) Non-dose related attenuation of withdrawal. Incomplete substitution even at doses inducing overt μ- or κ- behavioral effects.

TABLE 5. 6,7-BENZOMORPHANS (CONTINUED)



NIH 10964 (±)



(+)-2*S*,5*S*,9*S*) R =

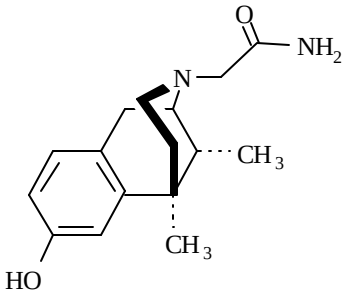
10973: $CH_2CH_2C{\equiv}CH$

10971: $CH_2CH_2CH_2C{\equiv}CH$

10981: $CH_2CH_2OCH_3$

10982: $CH_2CH_2CH_2OH$

11000:



NIH 10993 (±)

ANTINOCICEPTIVE/ANTAGONIST ASSAYS IN VITRO MONKEY (MOUSE ED50/AD50, s.c., mg/kg)

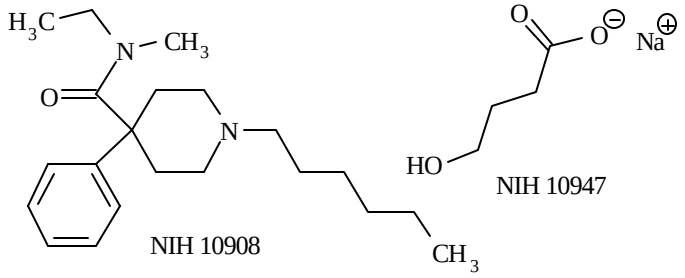
NIH #	Hot Plate	Phenylquinone	Tail Flick	Tail Flick Antagonist	Binding Affinity, nM	Substitution-for-Morphine (s.c., mg/kg)
10964	0.14	0.12	0.45[a]	Inactive	μ=6, δ=7, κ=4[b]	Partial suppression[c]
10971	Inactive	14.8	Inactive	Inactive	μ=3572, δ=>10000, κ=328	No substitution - exacerbates withdrawal
10973	Inactive	Inactive	Inactive	Inactive	μ=>10000, δ = >10000, κ=1726	Non-dose related attenuation of withdrawal
10981	Inactive	22.7	Inactive	Inactive	μ=778, δ=5712, κ=1158	Partial suppression
10982	Inactive	Inactive	Inactive	Inactive	μ=2102, δ=>10000, κ=915	Partial suppression. Non-dose related.
10993	Inactive	3.5	Inactive	Inactive	μ=677, δ=2005, κ=304	No substitution, no exacerbation of withdrawal.
11000	Inactive	Inactive	Inactive	Inactive	μ=598, δ=1644, κ=528	No substitution, no exacerbation of withdrawal

a) β-FNA (s.c.) vs. ED_{80} of NIH 10972: AD50 = 5.7; nor-BNI and naltrindole: inactive

b) GTPγS assay: mu EC_{50} = 273 ± 103 nM (19.1 ± 4.9% stimulation); delta EC_{50} = 122 ± 35 nM (45.9 ± 9.6% stimulation); kappa EC_{50} = 14.3 ± 1.6 nM (91.0 ± 4.3% stimulation).

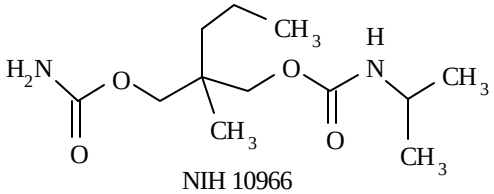
c) Precipitated withdrawal: antagonist, potency 0.25 x naloxone.

TABLE 6. MISCELLANEOUS

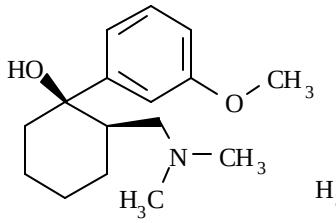


NIH 10908

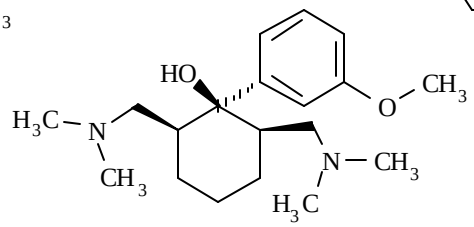
NIH 10947



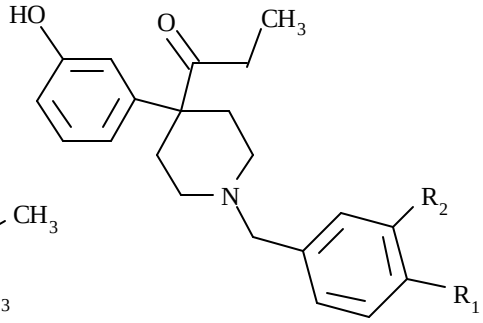
NIH 10966



NIH 10969



NIH 10970



	R_1	R_2
NIH 11001:	CF_3	H
NIH 11002:	H	CF_3

ANTINOCICEPTIVE/ANTAGONIST ASSAYS IN VITRO MONKEY (MOUSE ED50/AD50, s.c., mg/kg)

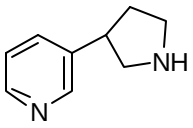
NIH #	Hot Plate	Phenylquinone	Tail Flick	Tail Flick Antagonist	Binding Affinity, nM	Substitution-for-Morphine (s.c., mg/kg)
10908	8.8[a]	2.5	7.4	Inactive	–	Complete substitution
10947	-	iv: 31[b]	i.v., s.c., p.o.: inactive	-	–	Inverse dose-response.
10966	Inactive	Inactive	Inactive	Inactive	μ, δ, κ =>10000	No effect.
10969	Inactive	6.1[c]	Inactive	Inactive	μ = 2995, δ, κ =>10000	-
10970	Inactive	Inactive	Inactive	Inactive	μ, δ, κ =>10000	-
11001	Inactive	Inactive	Inactive	Inactive	μ=33, δ=291, κ=118	No substitution
11002	Inactive	Inactive	Inactive	Inactive	μ=118, δ=316, κ=203	No substitution

a) Unusually toxic to mice. Naloxone AD_{50} (tail flick): 0.07.

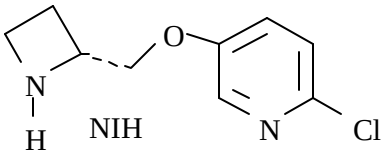
b) Co-administration (20-100 mg/kg, s.c.) with ED25 morphine: dose-related synergism. Morphine tolerant mice: 10947 (GHB) + morphine partially restored antinociception (abolished by naloxone).

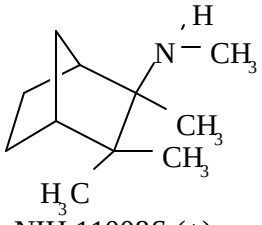
c) Naltrindole (s.c., PPQ) vs 10969 ED80: inactive.

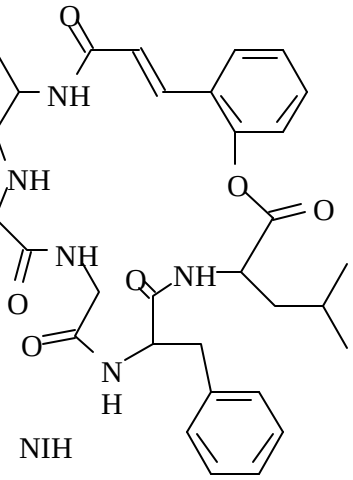
TABLE 7. MISCELLANEOUS (CONTINUED



NIH 10975:(-)
NIH 10976:(+)







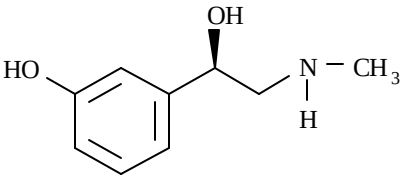
NIH 11008*S*-(+)
NIH 11009*R*-(-)
NIH 11010:

ANTINOCICEPTIVE/ANTAGONIST ASSAYS IN VITRO MONKEY (MOUSE ED50/AD50, s.c., mg/kg)

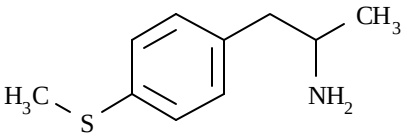
NIH #	Hot Plate	Phenylquinone	Tail Flick	Tail Flick Antagonist	Binding Affinity, nM	Substitution-for-Morphine (s.c., mg/kg)
10975	Inactive	Inactive	Inactive	Inactive	μ, δ, κ =>10000	Weak, non-dose related attenuation of withdrawal
10976	Inactive	Inactive	Inactive	Inactive	μ, δ, κ =>10000	No effect
10977	Inactive	32.5[a]	Inactive	Inactive	μ, δ, κ =>10000	-
10991	2.57	0.004[b]	Inactive	Inactive	-	-
11008[f]	-	11.4[c,d]	Inactive	-	-	-
11009[f]	-	9.5[c,e]	Inactive	-	-	-
11010[f]	-	4.2 [c,d]	Inactive	-	-	-

a) Naltrindole vs 10977 ED80 (PPQ): inactive. Tail flick (i.v. and s.c., 90 min pretreat): inactive.
b) Toxic, convulsions. β-FNA (i.c.v.), naltrindole (s.c.), or mecamylamine (s.c.) vs ED80 10991: <60%, and nor-BNI: AD50 = 11.3. Actions not nicotine-related.
c) Neither mecamylamine nor naloxone (pretreatment) antagonized ED80.
d) 6/6 Mice died @ 30 mg/kg (iv).
e) 4/6 Mice died @ 10 mg/kg (iv); immobile, tremors.
f) Special test: Effect vs. ED80 of nicotine in tail-flick: NIH 11008 AD50 = 0.03; NIH 11009 AD50 = 0.12; NIH 11010 AD50 = 0.36.

TABLE 8. EVALUATION OF STIMULANT/DEPRESSANT DRUGS



CPDD 0055



CPDD 0056

CPDD#	Discriminative Stimulus Effects in Monkeys. Comparison to Flumazenil & Midazolam (s.c.)	Monkey Self-Administration (iv)	Monkey Drug Discrimination (i.g.)
0054; NIH 10966[a]	No benzodiazepine discriminative stimulus effects[c]	-	Did not discriminate for amphetamine[c] Discriminated for pentobarbital i.v., (but not i.g.)[c]
0055	No benzodiazepine discriminative stimulus effects	No reinforcing effects in cocaine dependent monkey	No amphetamine discriminative effects at doses up to 10mg/kg
0056	Toxic, 10 mg/kg	No reinforcing effects in cocaine dependent monkey	-
0057; NIH 11008[b]	-	No reinforcing effects in methohexital trained monkeys	-
0058; NIH 11009[b]	-	No reinforcing effects in methohexital trained monkeys	-
0059; NIH 11010[b]	-	-	-

a) See Table 6 for molecular structure.
b) See Table 7 for molecular structure.
c) Previously reported 2000.

NOTES FOR TABLES 2 - 8

Rounded numbers are used; precise values and details of the procedures are given in the VCU and UM reports (Aceto et al., 2001; Woods et al., 2001).

1) Antinociceptive reference data:
Morphine ED_{50} (confidence limits): Hot Plate = 0.8 (0.3-1.8); Phenylquinone = 0.23 (0.20-0.25); Tail-Flick = 5.8 (5.7-5.9)
Tail-Flick Antagonism vs. morphine (naltrexone AD_{50}= 0.007 (0.002-0.02); naloxone AD_{50}= 0.035 (0.01-0.093)).
2) *In Vitro* - Subtype selective binding affinity using monkey brain cortex membranes. Selectivity for μ, δ, and κ-opioid receptors determined with [^{3}H]-DAMGO, [^{3}H]-*p*-Cl-DPDPE and [^{3}H]-U69,593, respectively. Affinities of labeled ligands: [^{3}H]DAMGO K_i = 0.57 nM, [^{3}H]*p*-Cl-DPDPE K_i = 1.2 nM, [^{3}H] U69,593 K_i = 0.95 nM. With C6 glioma cells, morphine K_i = 1.7 nM, DPDPE K_i = 8 nM.

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