JOURNAL OF
MEDICINAL
CHEMISTRY

Volume 37, Number 11 May 27, 1994

Communications to the Editor

2-(3,4-Dichlorophenyl)-*N*-methyl-*N*-[(1*S*)-1-(3-isothiocyanatophenyl)-2-(1-pyrrolidinyl)ethyl]acetamide: An Opioid Receptor Affinity Label That Produces Selective and Long-Lasting κ Antagonism in Mice

An-Chih Chang,† Akira E. Takemori,‡ and Philip S. Portoghese*,†

Department of Medicinal Chemistry, College of Pharmacy, and Department of Pharmacology, Medical School, University of Minnesota, Minneapolis, Minnesota 55455

Received March 8, 1994

The existence of at least three types of opioid receptors (μ, κ, δ) is now well established.[1] The κ-opioid receptor has been of special interest because its activation produces analgesia with minimum physical dependence and respiratory depression.[2] Moreover, the recent cloning and sequencing of the κ-opioid receptor have heightened the need for additional κ ligands, particularly antagonists, as probes.[3] In particular, a κ-selective affinity label would be useful because it could be employed as a biochemical probe to identify the binding locus on the κ receptor and also be used as a pharmacologic tool in vivo. While a number of κ-selective affinity labels have been reported,[4-8] as evidenced from receptor binding studies, no pharmacological activity has been published for such ligands. Here we report on the first documented example of an arylacetamide affinity label 1 (DIPPA) that possesses κ antagonist activity in vivo.

Because 2-(3,4-dichlorophenyl)-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]acetamide (U50,488)[9,10] is a highly selective κ-opioid receptor agonist, numerous structurally related arylacetamide analogues also have been studied. The design of target compound 1 as an affinity label was based on the report that arylacetamide 2 is a highly potent, κ-selective ligand.[11,12]

The synthesis (Scheme 1) of 1 involved the nitration of optically pure 3[11] to afford a mixture consisting of *m*- and *p*-nitro isomers which were coupled to 3,4-dichlorophenylacetyl chloride to yield enantio- and regioisomerically pure 5 after chromatography and crystallization. Raney nickel reduction of 5 followed by reaction with thiophosgene gave the target compound 1.

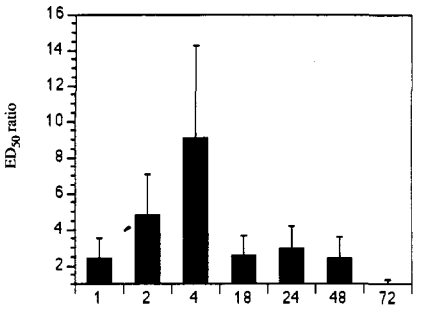
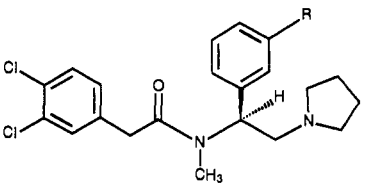


Figure 1. Time-course of antagonism of U50,488 antinociception by DIPPA (0.53 μmol/kg sc). Antinociception was measured by the tail-flick assay 20 min after sc administration of U50,488 in mice. The ED_{50} ratio is the ED_{50} of U50,488 in the presence of 1 divided by the control ED_{50}. Error bar indicates the upper limit of the 95% confidence intervals.



1, DIPPA, R = NCS
2, R = H

Using the mouse abdominal stretch assay,[13] the antinociceptive effect of both 1 and 2 peaked 30 min after sc administration and completely disappeared after 4 h. The weaker antinociceptive potency of 1 relative to that of 2 also was indicated by its inability to produce antinociception (2.11 μmol/kg sc)[14] in the tail-flick assay,[13,15] which is less sensitive for detecting κ receptor activation.[16] Compound 1 was selective for κ-opioid receptors, as reflected by the fact that norbinaltorphimine[17] (nor-BNI) significantly increased the ED_{50} of 1, while the δ and μ antagonists, naltrindole[18] (NTI) and β-funaltrexamine[19] (β-FNA), were ineffective in this regard (Table 1).

* To whom correspondence should be addressed.
† Department of Medicinal Chemistry.
‡ Department of Pharmacology.

0022-2623/94/1837-1547$04.50/0

Scheme 1

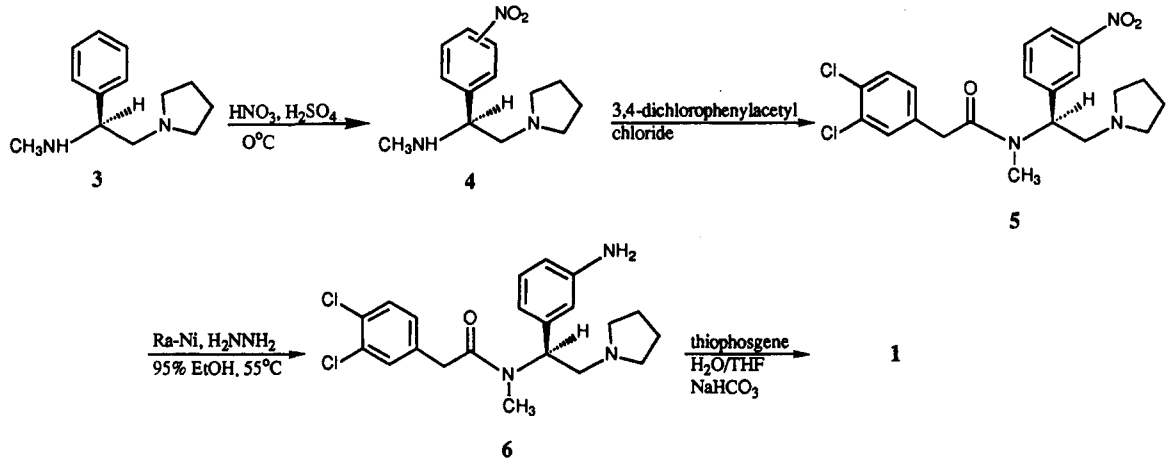


Table 1. Antinociceptive Potency and Selectivity in the Mouse Abdominal Stretch Assay

compd	ED_{50}, μmol/kg	ED_{50} ratio[a] nor-BNI (κ)	NTI (δ)	β-FNA (μ)
1	1.53 (1.11–2.06)	16.7 (10.0–25.0)[b]	1.25 (0.76–2.04)[c]	3.03 (1.89–5.26)[d]
2	0.017 (0.013–0.022)	2.31 (1.6–3.3)[e]	1.19 (0.55–2.77)[c]	1.72 (1.00–2.86)[f]

[a] The ED_{50} of the agonist (sc) in the antagonist-treated mice divided by the control ED_{50}; numbers in parentheses are 95% confidence lvels. [b] Nor-BNI (12.25 μmol/kg sc) was administered 3.5 h prior to agonist. [c] NTI (44.44 μmol/kg sc) was administered simultaneously with agonist. [d] β-FNA (10.18 μmol/kg sc) was administered 24 h prior to agonist. [e] Nor-BNI (12.25 μmol/kg sc) was administered 1.5 h prior to agonist.[f] β-FNA (20.36 μmol/kg sc) was administered 24 h prior to agonist.

Table 2. Antagonist Potency of DIPPA 4 h after Administration Using the Mouse Tail-Flick Assay[a]

agonist[b]	selectivity	control[c]	treated[c]	ED_{50} ratio[d]
U50,488	κ	26.7 (20.4–34.6)	254 (203–321)	9.1 (6.7–14.3)
morphine	μ	9.1 (6.3–38.4)	16.7 (11.6–22.9)	1.8 (0.8–2.9)
DPDPE	δ	8.1 (6.2–10.6)	10.5 (7.5–15.2)	1.3 (0.8–2.1)

[a] Antagonist dose of 1, 0.53 μmol/kg sc. [b] U50,488 and morphine were administered sc at 220 and 210 min after administration of antagonist, respectively. DPDPE was administered icv at 220 min after administration of antagonist. [c] ED_{50}'s are in micromoles per kilogram for U50,488 and morphine and nanomoles/mouse for DPDPE. [d] ED_{50} of agonist in the antagonist-treated mice divided by the control ED_{50}; numbers in parentheses are 95% confidence levels.

Table 3. Opioid Receptor Binding of DIPPA[a]

selectivity	IC_{50},[b] nM
κ[c]	2.21
μ[d]	1799
δ[e]	>1000[f]

[a] Conducted on guinea pig brain membranes using the procedure of Werling et al.[20] [b] Values are geometric means of three replicate experiments for κ and μ. [c] [^{3}H]U69593 or [^{3}H]-(5α,7α,8β)-(−)-*N*-methyl-*N*-(1-pyrrolidnyl-1-oxaspiro[4.5]dec-8-yl)benzeneacetamide (1 nM).[33] [d] [^{3}H]DAMGO or [^{3}H]-[D-Ala^2,MePhe^4,Gly-ol^5]-enkephalin (2 nM).[34] [e] [^{3}H]DPDPE or [^{3}H]-[D-Pen^2,D-Pen^5]enkephalin (5 nM).[35] [f] Value is based on two experiments.

Table 4. Agonist Potencies in Smooth Muscle Preparations

compd	IC_{50},[a] nM GPI	MVD	K_e,[b] nM GPI
1	23.8 ± 4.2	11.1 ± 4.4	0.3
2	0.199 ± 0.085	1.60 ± 1.65	0.05

[a] Values are means of at least three experiments; numbers in parentheses represent SEM values. [b] GPI was incubated with 5 and 20 nM nor-BNI for 15 min before 1 and 2 were tested, respectively. K_e (nM) = [nor-BNI]/(IC_{50} ratio − 1), where the IC_{50} ratio is the IC_{50} of the agonist in the presence of antagonist divided by the IC_{50} in the absence of antagonist.

In the mouse tail-flick assay,[13,15] 1 (0.53 μmol/kg sc) was found to be an antagonist with selectivity for κ-opioid receptors (Table 2). The antagonism peaked at 4 h postadministration and lasted at least 48 h (Figure 1). In contrast, the parent compound 2 (0.598 μmol/kg sc) was found to have no antagonist activity within the same time period. Thus, it appears that the electrophilic isothiocyanate group was responsible for the long-lasting antagonist activity of 1, presumably as a consequence of covalent binding to the receptor.

Receptor binding studies[20] indicate that 1 binds selectively and with high affinity to κ-opioid receptors (Table 3). Both 1 and 2 are full agonists in smooth muscle preparations,[21] although the parent compound 2 is 173-fold more potent than 1 in the guinea pig ileum (GPI) (Table 4). The strong antagonism of 1 (K_e = 0.3 nM) and 2 (K_e = 0.05 nM) by nor-BNI[18] indicate interaction with κ-opioid receptors. While 1 possessed antagonist activity in vivo, it did not produce antagonism in the GPI.[22] A possible explanation would be tissue or species differences in κ receptor subtypes. Additional studies would be needed to verify whether the differential activity of 1 in the GPI and mice is due to differences between κ receptors in peripheral tissue and those in the CNS, to species differences, or to some other mechanism.

There is increasing evidence that physically distinct agonist and antagonist binding domains may exist for G-protein coupled receptors (GPCR).[23–27] Because opioid receptors belong to the GPCR superfamily,[3,28–30] an analogous situation may be shared by the κ-opioid receptor. Accordingly, a possible explanation for the biphasic in vivo effects of 1 would be that it binds both agonist and antagonist sites and becomes covalently bound only at the antagonist site. The antagonist effect of 1 would be observed only after the agonist effect is dissipated due to

its reversible dissociation from the agonist site. Alternately, the reversibly bound ligand may act as an agonist and subsequently convert the receptor to an antagonist state after it becomes covalently bound at the same binding site. It is noteworthy that short-term agonism, followed by long-term antagonism, has also been reported for the opioid receptor affinity labels β-chlornaltrexamine[31] (β-CNA) and [D-Ala²,Leu⁵,Cys⁶]enkephalin[32] (DALCE).

In conclusion, 1 is the first in vivo κ-selective antagonist belonging to the arylacetamide class of ligands. Studies presently are in progress to determine the specific amino acid residue that is covalently bound by 1.

Acknowledgment. We thank Veronika Doty, Mary Lunzer, Joan Naeseth, Michael Powers, and Idalia Sanchez for in vitro and in vivo testing of the compounds.

Supplementary Material Available: Experimental conditions and elemental analysis data (3 pages). Ordering information is given on any current masthead page.

References

(1) Simon, E. J.; Gioannini, T. L. Opioid Receptor Multiplicity: Isolation, Purification, and Chemical Characterization of Binding Sites. In *Opioids I*; Herz, A., Ed.; Springer-Verlag: Berlin, 1993; pp 3–21.

(2) Millan, M. J. κ-Opioid Receptors and Analgesia. *Trends Pharmacol. Sci.* **1990**, *11*, 70–76.

(3) Yasuda, K.; Raynor, K.; Kong, H.; Breder, C. D.; Takeda, J.; Reisine, T.; Bell, G. I. Cloning and Functional Comparison of κ and δ Opioid Receptors from Mouse Brain. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 6736–6740.

(4) de Costa, B. R.; Rothman, R. B.; Bykov, V.; Jacobson, A. E.; Rice, K. C. Selective and Enantiospecific Acylation of κ Opioid Receptors by (1S,2S)-trans-2-Isothiocyanato-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide. Demonstration of κ Receptor Heterogeneity. *J. Med. Chem.* **1989**, *32*, 281–283.

(5) de Costa, B. R.; Band, L.; Rothman, R. B.; Jacobson, A. E.; Bykov, V.; Pert, A.; Rice, K. C. Synthesis of an Affinity Ligand ('UPHIT') for in Vivo Acylation of the κ-Opioid Receptor. *FEBS Lett.* **1989**, *249*, 178–182.

(6) de Costa, B. R.; Rothman, R. B.; Bykov, V.; Band, L.; Pert, A.; Jacobson, A. E.; Rice, K. C. Probes for Narcotic Receptor Mediated Phenomena. 17. Synthesis and Evaluation of a Series of trans-3,4-Dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide (U50,488) Related Isothiocyanate Derivatives as Opioid Receptor Affinity Ligands. *J. Med. Chem.* **1990**, *33*, 1171–1176.

(7) de Costa, B. R.; Thurkauf, A.; Rothman, R. B.; Jacobson, A. E.; Rice, K. C. Synthesis of High Specific Activity Tritium Labelled 1S,2S(−)-trans-2-isothiocyanato-*N*-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide, a Specific Irreversible Ligand for Kappa Opioid Receptors. *J. Labelled Compd. Radiopharm.* **1990**, *28*, 1257–1264.

(8) Cheng, C.-Y.; Wu, S.-C.; Hsin, L.-W.; Tam, S. W. Selective Reversible and Irreversible Ligands for the κ Opioid Receptor. *J. Med. Chem.* **1992**, *35*, 2243–2247.

(9) Szmuszkovicz, J.; Von Voigtlander, P. F. Benzeneacetamide Amines: Structurally Novel Non-mu Opioids. *J. Med. Chem.* **1982**, *25*, 1125–1126.

(10) Von Voigtlander, P. F.; Lahti, R. A.; Ludens, J. H. U-50,488: A Selective and Structurally Novel Non-Mu (Kappa) Opioid Agonist. *J. Pharmacol. Exp. Ther.* **1983**, *224*, 7–12.

(11) Costello, G. F.; James, R.; Shaw, J. S.; Slater, A. M.; Stutchbury, N.C. 2-(3,4-Dichlorophenyl)-N-methyl-N-[2-(1-pyrrolidinyl)-1-substituted-ethyl]-acetamides: The Use of Conformational Analysis in the Development of a Novel Series of Potent Opioid Kappa Agonists. *J. Med. Chem.* **1991**, *34*, 181–189.

(12) Barlow, J. J.; Blackburn, R. P.; Costello, G. F.; James, R.; Le Count, D. J.; Main, B. G.; Pearce, R. J.; Russell, K.; Shaw, J. S. Structure/Activity Studies Related to 2-(3,4-dichlorophenyl)-N-methyl-N-[2-(1-pyrrolidinyl)-1-substituted-ethyl]acetamides: A Novel Series of Potent and Selective Kappa-Opioid Agonists. *J. Med. Chem.* **1991**, *34*, 3149–3158.

(13) Hayashi, G.; Takemori, A. E. The Type of Analgesic-Receptor Interaction Involved in Certain Analgesic Assays. *Eur. J. Pharmacol.* **1971**, *16*, 63–66.

(14) The compound was tested at six doses ranging from 16.5 nmol/kg to 2.11 μmol/kg and at times ranging from 15 min to 6 h after injection. No antinociceptive activity was observed.

(15) D'Amour, F. E.; Smith, D. L. A Method of Determining Loss of Pain Sensation. *J. Pharmacol. Exp. Ther.* **1941**, *72*, 74–79.

(16) Hayes, A. G.; Sheehan, M. J.; Tyers, M. B. Differential Sensitivity of Models of Antinociception in the Rat, Mouse and Guinea-Pig to μ- and κ-Opioid Receptor Agonists. *Br. J. Pharmacol.* **1987**, *91*, 823–832.

(17) Portoghese, P. S.; Lipkowski, A. W.; Takemori, A. E. Binaltorphimine and Nor-Binaltorphimine, Potent and Selective κ-Opioid Receptor Antagonists. *Life Sci.* **1987**, *40*, 1287–1292.

(18) Portoghese, P. S.; Sultana, M.; Takemori, A. E. Design of Peptidoimimetic δ Opioid Receptor Antagonists Using the Message-Address Concept. *J. Med. Chem.* **1990**, *33*, 1714–1720.

(19) Takemori, A. E.; Larson, D. L.; Portoghese, P. S. The Irreversible Narcotic Antagonistic and Reversible Agonistic Properties of the Fumaramide Methyl Ester Derivative of naltrexone. *Eur. J. Pharmacol.* **1981**, *70*, 445–451.

(20) Werling, L. L.; Zarr, G. D.; Brown, S. R.; Cox, B. M. Opioid Binding to Rat and Guinea Pig Neural Membranes in the Presence of Physiological Cations at 37 °C. *J. Pharmacol. Exp. Ther.* **1985**, *233*, 722–728.

(21) (GPI) Rang, H. P. Stimulant Actions of Volatile Anaesthetics on Smooth Muscle. *Br. J. Pharmacol.* **1964**, *22*, 356–365. (MVD) Henderson, G.; Hughes, J.; Kosterlitz, H. W. A new Example of a Morphine-sensitive Neuro-effector Junction: Adrenergic Transmission in the Mouse Vas Deferens. *Br. J. Pharmacol.* **1972**, *46*, 764–766.

(22) Incubation of the GPI with 1 (1–0.001 nM) for 30 min followed by washing produced no significant antagonism of morphine, EK, or U50,488. Concentrations greater than 1 nM afforded agonism in the GPI which was not removed by washing.

(23) Sarne, Y.; Itzhak, Y.; Keren, O. Differential Effect of Humoral Endorphin on the Binding of Opiate Agonists and Antagonists. *Eur. J. Pharmacol.* **1982**, *81*, 227–235.

(24) Portoghese, P. S.; Takemori, A. E. Different Receptor Sites Mediate Opioid Agonism and Antagonism. *J. Med. Chem.* **1983**, *26*, 1341–1343.

(25) Fong, T. M.; Cascieri, M. A.; Yu, H.; Bansal, A.; Swain, C.; Strader, C. D. Amino-Aromatic Interaction Between Histidine 197 of the Neurokinin-1 Receptor and CP 96345. *Nature* **1993**, *362*, 350–353.

(26) Beinborn, M.; Lee, Y.-M.; McBride, E. W.; Quinn, S. M.; Kopin, A. S. A Single Amino Acid of the Cholecystokinin-β/Gastrin Receptor Determines Specificity for Non-Peptide Antagonists. *Nature* **1993**, *362*, 348–350.

(27) Gether, U.; Johansen, T. E.; Snider, R. M.; Lowe, J. A., III; Nakanishi, S.; Schwartz, T. Different Binding Epitopes on the NK_1 Receptor for Substance P and a Non-Peptide Antagonist. *Nature* **1993**, *362*, 345–348.

(28) Evans, C. J.; Keith, D. E., Jr.; Morrison, H.; Magendzo, K.; Edwards, R. H. Cloning of a Delta Opioid Receptor by Functional Expression. *Science* **1992**, *258*, 1952–1955.

(29) Offermanns, S.; Schultz, G.; Rosenthal, W. Evidence for Opioid Receptor-mediated Activation of the G-proteins, G_o and G_{i2}, in Membranes of Neuroblastoma x Glioma (NG108-15) Hybrid Cells. *J. Biol. Chem.* **1991**, *266*, 3365–3368.

(30) Misawa, H.; Ueda, H.; Satoh, M. κ-Opioid Agonist Inhibits Phospholipase C, Possibly via an Inhibition of G-protein Activity. *Neurosci. Lett.* **1990**, *112*, 324–327.

(31) Portoghese, P. S.; Larson, D. L.; Jiang, J. B.; Takemori, A. E.; Caruso, T. P. 6β-[N,N-Bis(2-chloroethyl)amino]-17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxymorphinan (Chlornaltrexamine), a Potent Opioid Receptor Alkylating Agent with Ultralong Narcotic Antagonist Activity. *J. Med. Chem.* **1978**, *21*, 598–599.

(32) Calcagnetti, D. J.; Helmstetter, F. J.; Fanselow, M. S.; Bowen, W. D. [D-Ala²,Leu⁵,Cys⁶]Enkephalin:Short-term Agonist Effects and Long-term Antagonism at Delta Opioid Receptors. *Peptides* **1989**, *10*, 319–326.

(33) Lahti, R. A.; Mickelson, M. M.; McCall, J. K.; von Voightlander, P. F. [³H]U-69593, A Highly Selective Ligand for the Opioid κ Receptor. *Eur. J. Pharmacol.* **1985**, *109*, 281–284.

(34) Handa, B. K.; Lane, A. C.; Lord, J. A. H.; Morgan, B. A.; Rance, M. J.; Smith, C. F. C. Analogs of β-LPH61-64 Possessing Selective Agonist Activity at Mu-Opiate Receptors. *Eur. J. Pharmacol.* **1981**, *70*, 531–540.

(35) Mosberg, H. I.; Hurst, R.; Hruby, V. J.; Gee, K.; Yamamura, H. I.; Galligan, J. J.; Burks, T. S. Bis-penicillamine Enkephalins Possess Highly Improved Specificity Toward δ Opioid Receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 5871–5874.