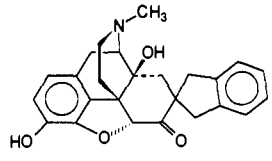
A Selective δ_1 Opioid Receptor Agonist Derived from Oxymorphone. Evidence for Separate Recognition Sites for δ_1 Opioid Receptor Agonists and Antagonists

P. S. Portoghese,*,† S. T. Moe,† and A. E. Takemori‡

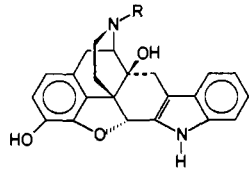
Department of Medicinal Chemistry, College of Pharmacy, and Department of Pharmacology, Medical School, University of Minnesota, Minneapolis, Minnesota 55455

Received May 21, 1993

It is now well-established that there are three families of opioid peptide precursors. Proopiomelanocortin, proenkephalin, and prodynorphin give rise to a variety of opioid peptides[1] which exert their physiologic effects through at least three types of opioid receptors (μ, δ, κ).[2] Because the endogenous opioid peptides exhibit relatively low pharmacologic selectivity for these receptor types and are metabolically unstable, a number of more selective ligands have been developed.[3] The armamentarium of selective agonists for δ opioid receptors has been comprised of peptide ligands related to the enkephalins.[4,5] These include δ_1 subtype-selective peptides, such as [D-Pen^2,D-Pen^5]enkephalin[6] (DPDPE) and [D-Ala^2,D-Leu^5]enkephalin[7] (DADLE), and the δ_2 subtype-selective peptides [D-Ala^2,Glu^4]deltorphin[8] and [D-Ser^2,Leu^5]enkephalin-Thr$^{6\,7}$ (DSLET).[9-12] Here we report on the design and pharmacologic evaluation of the first selective nonpeptide δ_1 opioid agonist, 7-spiroindanyloxymorphone 1 (SIOM).



1

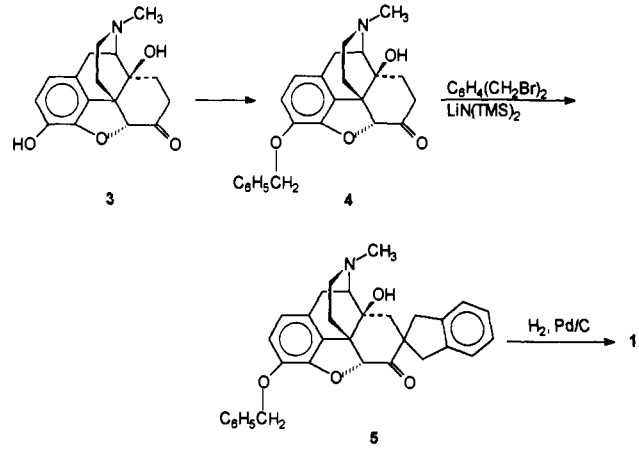


2a, R = $CH(CH_2)_2$
2b, R = CH_3
2c, R = $CH_2CH_2C_6H_5$

The design of 1 was based in part on structure–activity relationship studies of the prototypical δ opioid antagonist naltrindole, **2a** (NTI).[13] Inasmuch as the δ antagonist activity and high affinity of NTI is due to its indolic benzene moiety, which is thought[13] to function as a putative "address"[14] mimic of the Phe^4 phenyl group of enkephalin, we had attempted to transform **2a** into a δ agonist by replacing its cyclopropylmethyl group with N-substituents (**2b,c**) that usually confer agonist activity to opiates.[15,16] These studies revealed that such substitutions afforded ligands that retained δ antagonist activity in vivo without conferring selective δ agonist activity. In this connection, it has been found that **2b** is 10-fold more potent as a δ antagonist than **2a**.[16]

† Department of Chemistry.
‡ Department of Pharmacology.

Scheme I



3 4 5

Table I. Binding of SIOM[a]

radioligand	selectivity	K_i, nM[b]	radioligand	selectivity	K_i, nM[b]
[^{3}H]DPDPE	δ_1	1.4	[^{3}H]DAMGO[c]	μ	10.6
[^{3}H]DSLET	δ_2	3.3	[^{3}H]U69593[d]	κ	588

[a] Conducted on guinea pig brain using the procedure of Werling et al.[21] [b] Values are geometric means of at least three replicate experiments. [c] [^{3}H]-[D-Ala^2,MePhe^4,Gly-ol^5]enkephalin.[35] [d] [^{3}H]-(5α,7α,8β)-(−)-*N*-methyl-*N*-1-pyrrolidinyl-1-oxaspiro[4.5]dec-8-yl-benzeneacetamide.[36]

As it was conceivable that the conformation of the putative δ address component in these morphindoles **2** might stabilize an antagonist state of the δ receptors in the CNS, we have synthesized the opiate SIOM (1), whose address is in a conformation similar to that of the Phe^4 phenyl group of DPDPE reported[17-19] from NMR analysis and energy-minimization studies.

The spiroindane 1 was obtained from oxymorphone **3** (Scheme I); this involved formation of the 3-*O*-benzyl derivative **4** and alkylation with α,a′-dibromo-*o*-xylene in the presence of hexamethyldisilazane to afford **5**, followed by hydrogenolysis of the *O*-benzyl group.

Pharmacologic evaluation (n = 3) in smooth muscle preparations[20] revealed SIOM to be a full agonist in the mouse vas deferens (IC_{50} = 19 nM) and a partial agonist (55% maximal response at 1 μM) in the guinea pig ileum. The fact that naltrindole **2a** antagonized the full agonist effect (K_e = 1.5 nM) of SIOM while naloxone afforded only feeble antagonism (K_e = 68 nM), suggests that the full agonist effect is mediated by δ opioid receptors. Thus, the in vitro pharmacologic data are consistent with 1 as a δ-selective agonist.

The binding data[21] also support in vitro pharmacology in that the greatest affinity of SIOM was for δ opioid receptor (Table I). The order of affinities was $\delta_1 > \delta_2 > \mu \gg \kappa$, suggesting that SIOM is δ_1-selective.

Antinociceptive testing[22] in mice using the tail-flick procedure revealed SIOM to be nearly 7 times more potent than the standard δ_1 agonist, DPDPE, on icv administration (Table II). SIOM also was active (ED_{50} = 16.3 μmol/kg) when administered sc. It is noteworthy that the δ_1 opioid receptor antagonist 7-benzylidenenaltrexone[23] (BNTX) was considerably more effective than the antagonists naltriben[24] (NTB), β-funaltrexamine[25] (β-FNA), and norbinaltorphimine[26] (norBNI), in blocking the antinociceptive effect of SIOM (Table II).

When SIOM was administered at a dose (0.5 nmol icv)

0022-2623/93/1836-2572$04.00/0

Table II. Antinociceptive Potency and Selectivity of SIOM

compd	ED_{50}, nmol/mouse[b]	ED_{50} ratio[a] BNTX (δ_1)[c]	NTB (δ_2)[c]	β-FNA (μ)[d]	norBNI (κ)[e]
1 (SIOM)	1.7 (1.66–1.73)	10.6 (5.5–21.3)	2.5 (1.3–5.7)	1.5 (1.2–2.0)	1.6 (1.3–2.1)
DPDPE	11.5 (9.1–14.6)	5.0 (3.6–7.4)	1.4 (0.8–2.2)	1.4 (1.0–1.9)	1.0 (0.5–2.1)

[a] The ED_{50} of the agonist in the antagonist-treated mice divided by the control ED_{50}. [b] Administered icv. [c] Antagonist dose, 1.3 μmol/kg sc 5 min prior to administration of agonist. [d] Antagonist dose, 10 μmol/kg sc 24 h prior to administration of agonist. [e] Antagonist dose, 12 μmol/kg sc 3.5 h prior to administration of agonist.

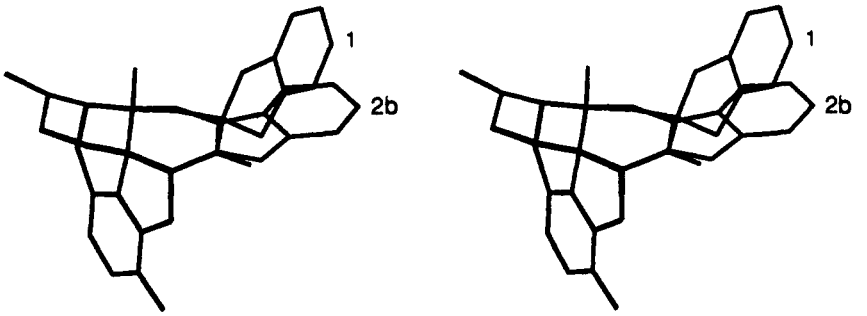


Figure 1. A stereoreview of the superposition of SIOM 1 and OMI **2b**. Note that the aromatic group in 1 is perpendicular to the indolic benzene moiety in **2b**.

Table III. Antagonist Selectivity of SIOM

agonist[a]	selectivity	ED_{50} ratio (95% confidence limits)[b]
DPDPE	δ_1	3.8 (3.4–4.3)
DSLET	δ_2	0.8 (0.5–1.2)
morphine	μ	3.0 (2.2–4.0)
U50488	κ	0.8 (0.5–1.4)

[a] Administered either icv (DPDPE and DSLET) or sc (morphine and U50488) to mice. [b] ED_{50} of agonist in the presence of SIOM (0.5 nmol icv).

that did not produce antinociception, antagonism of the agonist effect of DPDPE and morphine, but not DSLET and U50488, was observed (Table III). These data indicate that SIOM possesses the unusual feature of acting as a δ_1 antagonist at low dose and as a δ_1 agonist at higher dose. Also, SIOM appears to be an antagonist at μ receptors, but does not appear to act as δ_2 or κ opioid receptors at the dose employed.

One possible explanation for both the agonist and antagonist effects of SIOM at δ_1 receptors is that there are two recognition sites on this receptor system. Thus, SIOM may interact with an antagonist site at lower concentration and an agonist site at higher concentration. If the agonist site is allosterically coupled in a vectorial mode[27] to the antagonist site, this could account for the observed results. Such a model has been discussed previously in connection with the interaction of agonist and antagonist ligands with opioid receptors.[28,29] In view of the fact that the δ opioid receptor is a member of the G protein-coupled receptor superfamily[30] in which there is precedent for multiple binding sites for agonists and antagonists,[31-33] this appears to be a possibility.

In conclusion, these data are consistent with the idea that the agonist activity of the δ_1-selective opioid peptides may involve pharmacophoric conformations of message and address elements that are common with those of SIOM when bound to the δ_1 agonist recognition site. Moreover, the fact that OMI[16] **2b** is not a selective δ agonist suggests that the coplanarity of its aromatic group with ring C of the opiate may decrease the binding to the δ_1 agonist site and enhance its affinity for the antagonist site. In this connection, it is noteworthy that the putative address moiety of SIOM is oriented approximately perpendicular to ring C of the morphinan nucleus in the OMI (Figure 1).[34] Whether the agonist and antagonist sites are on a single receptor or receptors associated with opposing neural pathways[37,38] remains to be clarified.

Acknowledgment. This research was supported by the National Institute on Drug Abuse. We thank Mary Lunzer, for capable technical assistance, and Dr. Dennis L. Larson for generating the graphics in Figure 1.

References

(1) Höllt, V. Regulation of Opioid Peptide Gene Expression. In *Opioids I*; Herz, A., Ed.; Springer-Verlag: Berlin, 1993; pp 307–346.
(2) 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.
(3) Zimmerman, D. M.; Leander, J. D. Selective Opioid Receptor Agonists and Antagonists: Research Tools and Potential Therapeutic Agents. *J. Med. Chem.* **1990**, *33*, 895–902.
(4) Hruby, V. J.; Gehrig, C. A. Recent Developments in the Design of Receptor Specific Opioid Peptides. *Med. Res. Rev.* **1989**, *9*, 343–401.
(5) Schiller, P. W. Development of Receptor-Specific Opioid Peptide Analogues. *Prog. Med. Chem.* **1991**, *28*, 301–340.
(6) 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.
(7) Fournie-Zaluski, M. C. Gacel, G.; Maigret, B.; Premilat, S.; Roques, B. Structural Requirements for Specific Recognition of μ or δ Opioid Receptors. *Mol. Pharmacol.* **1981**, *20*, 484–491.
(8) Erspamer, V.; Melchiorri, P.; Falconieri-Erspamer, G.; Negri, L.; Corsi, R.; Severini, C.; Barra, D.; Simmaco, M.; Dreal, G. Deltorphins: A Family of Naturally Occurring Peptides with High Affinity and Selectivity for δ Opioid Binding Sites. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 5188–5192.
(9) Sofuoglu, M.; Portoghese, P. S.; Takemori, A. E. Differential Antagonism of Delta Opioid Agonists by Naltrindole and its Benzofuran Analogue (NTB) in Mice: Evidence for Delta Opioid Receptor Types. *J. Pharmacol. Exp. Ther.* **1991**, *257*, 676–680.
(10) Sofuoglu, M.; Portoghese, P. S.; Takemori, A. E. Cross-Tolerance Studies in the Spinal Cord of β-FNA-Treated Mice Provides Further Evidence for Delta Opioid Receptor Subtypes. *Life Sci.* **1991**, *49*, PL-153–PL-156.
(11) Jiang, Q.; Takemori, A. E.; Sultana, M.; Portoghese, P. S.; Bowen, W. D.; Mosberg, H. I.; Porreca, F. Differential Antagonism of Opioid Delta Antinociception by [D-Ala^2,Leu^5,Cys^6]Enkephalin and Naltrindole 5′-Isothiocyanate: Evidence for Delta Receptor Subtypes. *J. Pharmacol. Exp. Ther.* **1991**, *257*, 1069–1075.
(12) Mattia, A.; Vanderah, T.; Mosberg, H. I.; Porreca, F. Lack of Antinociceptive Cross-Tolerance Between [D-Pen^2,D-Pen^5]Enkephalin and [D-Ala^2]Deltorphin II in Mice: Evidence for Delta Receptor Subtypes. *J. Pharmacol. Exp. Ther.* **1991**, *258*, 583–587.

(13) Portoghese, P. S. An Approach to the Design of Non-peptide Receptor-type Selective Opioid Antagonists of Peptidergic Receptors: δ Opioid Receptor Antagonists. *J. Med. Chem.* **1991**, *34*, 1757–1762.
(14) Schwyzer, R. ACTH: A Short Introductory Review. *Ann. N.Y. Acad. Sci.* **1977**, *297*, 3–26.
(15) Portoghese, P. S.; Larson, D. L.; Sultana, M.; Takemori, A. E. Opioid Agonist and Antagonist Activities of Morphindoles Related to Naltrindole. *J. Med. Chem.* **1992**, *35*, 4325–4329.
(16) Takemori, A. E.; Sultana, M.; Nagase, H.; Portoghese, P. S. Agonist and Antagonist Activities of Ligands Derived from Naltrexone and Oxymorphone. *Life Sci.* **1992**, *50*, 1491–1495.
(17) Hruby, V. J.; Kao, L. F.; Pettit, B. M.; Karplus, M. The Conformational Properties of the Delta Opioid Peptide [D-Pen^2,D-Pen^5]-Enkephalin in Aqueous Solution Determined by NMR and Energy Minimization Calculations. *J. Am. Chem. Soc.* **1988**, *110*, 3351–3359.
(18) Schiller, P. W.; Weltrowska, G.; Nguyen, T. M.-D.; Lemieux, C.; Chung, N. N.; Marsden, B. J.; Wilkes, B. C. Conformational Restriction of the Phenylalanine Residue in a Cyclic Opioid Peptide Analogue: Effects on Receptor Selectivity and Stereospecificity. *J. Med. Chem.* **1991**, *34*, 3125–3132.
(19) Mosberg, H. I.; Sobczyk-Kojiro, K.; Pullachipatti, S.; Crippen, G. M.; Ramalingam, K.; Woodard, R. W. Combined Use of Stereospecific Deuteration, NMR, Distance Geometry, and Energy Minimization for the Conformational Analysis of the Highly Delta Opioid Receptor Selective Peptide [D-Pen^2,D-Pen^5]enkephalin. *J. Am. Chem. Soc.* **1990**, *112*, 822–829.
(20) Portoghese, P. S.; Takemori, A. E. TENA, A Selective Kappa Opioid Receptor Antagonist. *Life Sci.* **1985**, *36*, 801–805.
(21) 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.* **195**, *233*, 722–728.
(22) Tulunay, F. C.; Takemori, A. E. The Increased Efficacy of Narcotic Antagonists Induced by Various Narcotic Analgesics. *J. Pharmacol. Exp. Ther.* **1974**, *190*, 395–400.
(23) Portoghese, P. S.; Sultana, M.; Nagase, H.; Takemori, A. E. A Highly Selective δ_1-Opioid Receptor Antagonist: 7-Benzylidenenaltrexone. *Eur. J. Pharmacol.* **1992**, *218*, 195–196.
(24) Portoghese, P. S.; Nagase, H.; MaloneyHuss, K. E.; Lin, C.-E.; Takemori, A. E. Role of Spacer and Address Components in Peptidomimetic δ Opioid Receptor Antagonists Related to Naltrindole. *J. Med. Chem.* **1991**, *34*, 1715–1720.
(25) Takemori, A. E.; Larson, D. L.; Portoghese, P. S. The Irreversible Narcotic Antagonistic and Reversible Agonistic Properties of the Fumaramate Methyl Ester Derivative of Naltrexone. *Eur. J. Pharmacol.* **1981**, *70*, 445–451.
(26) Portoghese, P. S.; Lipowski, A. W.; Takemori, A. E. Binaltorphimine and Norbinaltorphimine, Potent and Selective κ Opioid Receptor Antagonists. *Life Sci.* **1987**, *40*, 1287–92.
(27) The term vectorial is meant to imply that binding to an agonist site will allosterically reduce the affinity of the antagonist side, and that this effect is unidirectional.
(28) 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.
(29) Portoghese, P. S.; Takemori, A. E. Different Receptor Sites Mediate Opioid Agonism and Antagonism. *J. Med. Chem.* **1983**, *26*, 1341–1343.
(30) Evans, C. J.; Keith, D. E., Jr.; Morrison, H.; Magendzo, K.; Edward, R. H. Cloning of a Delta Receptor by Functional Expression. *Science* **1992**, *258*, 1952–1955.
(31) Gether, U.; Johansen, T. E.; Snider, R. M.; Lowe, J. A., III; Nakanishi, S.; Schwartz, T. W. Different Binding Epitopes on the NK_1 Receptor for Substance P and a Non-Peptide Antagonist. *Nature* **1993**, *362*, 345–348.
(32) Beinborn, M.; Lee, Y.-M.; McBride, E. W.; Quinn, S. M.; Kopin, A. S. A Single Amino Acid of the Cholecystokinin-B/Gastrin Receptor Determines Specificity for Non-Peptide Antagonists. *Nature* **1993**, *362*, 348–350.
(33) Fong, T. M.; Cascieri, M. A.; Yu, H.; Bansai, A.; Swain, C.; Strader, C. D. Amino-Aromatic Interaction Between Histidine 197 of Neurokinin-1 Receptor and CP 96345. *Nature* **1993**, *362*, 350–353.
(34) Computer-assisted molecular overlay studies were collected for compounds using Biosym software (Insight II v 2.0.0 and Discover v 2.7) operating in an IRIX 3.3.2 environment on a 35/TG Personal Iris Workstation. All chemical structures were minimized using a quasi-Newton-Raphson algorithm (VA09A). Each minimized structure was subjected to high-temperature molecular dynamics (700 K) for 1 ps. The resulting structure was then reminimized using va09a, and the minumum-energy conformation was compared to the initial minimized structure. Discover calculations used a consistent valence force field (cvff) supplied with the Biosym package.
(35) 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.
(36) Lahti, R. A.; Mickelson, M. M.; McCall, J. M.; von Voightlander, P. F. [^{3}H]U-69593, A Highly Selective Ligand for the Opioid κ Receptor. *Eur. J. Pharmacol.* **1985**, *109*, 281–284.
(37) Crain, S. M.; Shen, K.-F. Opioids Can Evoke Direct Receptor-Mediated Excitatory Effects on Sensory Neurons. *Trends Pharmacol. Sci.* **1990**, *11*, 77–81.
(38) Fields, H. L.; Heinricher, M. M.; Mason, P. Neurotransmitters in Nociceptive Modulatory Circuits. *Annu. Rev. Neurosci.* **1991**, *14*, 219–245.