DESIGN AND SYNTHESIS OF AN OPIOID RECEPTOR PROBE: MODE OF BINDING OF *S*-ACTIVATED (-)-6β-SULFHYDRYLDIHYDROMORPHINE WITH THE SH GROUP IN THE μ-OPIOID RECEPTOR

Ken KANEMATSU,*,[a] Ryo NAITO,[a] Yasuyuki SHIMOHIGASHI,[b] Motonori OHNO,[b] Tomio OGASAWARA,[c] Masayasu KURONO,[c] and Kunio YAGI*,[c]

Institute of Synthetic Organic Chemistry, Faculty of Pharmaceutical Sciences, Kyushu University 62,[a] Higashi-ku, Fukuoka 812, Japan, Laboratory of Biochemistry, Faculty of Science, Kyushu University 33,[b] Higashi-ku, Fukuoka 812, Japan, and Institute of Applied Biochemistry,[c] Yagi Memorial Park, Mitake, Gifu 505-01, Japan

An *S*-activated sulfhydrylmorphine derivative was synthesized, and its linking to the μ–opioid receptor through a disulfide bond was demonstrated.

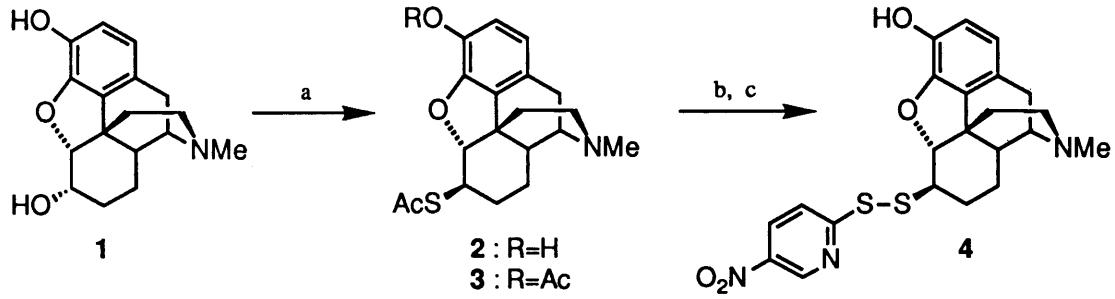
KEYWORDS opioid receptor; sulfhydryl group; sulfhydrylmorphine; thiol-disulfide exchange reaction; guinea pig ileum; opioid receptor probe

The existence of multiple opioid receptors in the brain and peripheral tissues has been documented in biochemical and pharmacological studies. The binding of opiate or opioid peptide agonists with receptors in rat brain is effectively inhibited by reagents for the sulfhydryl (SH) groups such as *N*-ethylmaleimide (NEM). One of the SH groups alkylated by NEM is the cysteine β-thiol in the GTP-binding regulatory protein G_i, which exists inside the plasma membrane and couples with the opioid receptor to let the receptor react with agonists.[1] Smith and Simon found by a modification experiment that the opioid receptor *per se* contains an SH group near its binding site.[2] Also, it was reported that disulfide bonds in the receptor are essential for opioid ligand binding and that reduction of one or more of these bonds to form the SH groups may play a role in opioid receptor activation by agonists.[3] Our recent examination on the binding of enkephalin analogs containing Cys-3-nitro-2-pyridinesulfenyl with the opioid receptor suggested that a distinct SH group labeled with this enkephalin analogs occurs near the enkephalin binding site of the μ-receptor.[4]

As a part of our research programs on the design of opioid receptor probes,[5] we describe here direct evidence for the interaction of *S*-activated (-)-6β-sulfhydryldihydromorphine with the SH group near the μ-opioid receptor binding site.

Requisite (4) was prepared by the route shown in Chart 1. Treatment of dihydromorphine (1) with thioacetic acid in the presence of triphenylphosphine and diisopropyl azodicarboxylate in dry THF at 0°C (Mitsunobu reaction)[5] produced the 6β-thioester (2) and 3-acetyl-6β-thioester (3) in 53% and 23% yields, respectively. Compound (2) or (3) was then hydrolyzed with 0.2 N potassium hydroxide under nitrogen bubbling to produce 6β-sulfhydryldihydromorphine, which readily reacted with 5-nitro-2-pyridinesulfenyl chloride in dichloromethane at 0°C to give 6β-(5'-nitro-2'-pyridyldithio)deoxydihydromorphine (4) as yellow needles (mp 141-142°C, $[\alpha]_D^{25}$ -411° (*c* = 1.0, $CHCl_3$), 84% yield).

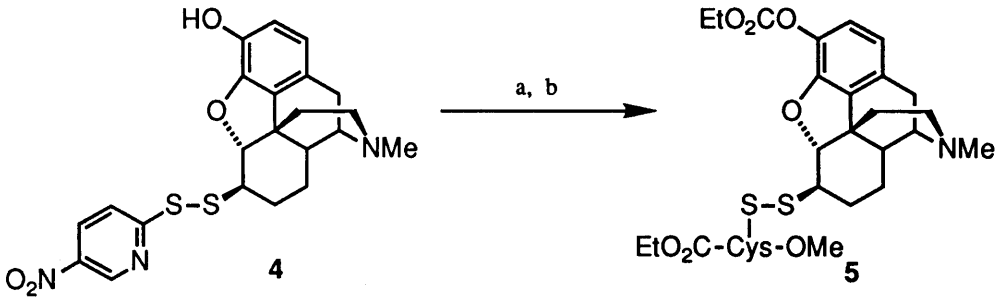
Analgesic activity of compound (4) was found by the inhibition of the electrically stimulated contraction of guinea pig ileum (GPI) and mouse vas deferens (MVD); the IC_{50} of compound (4) in GPI and MVD was 9.3 and 76 nM, respectively. Compound (4) showed about three times higher potentcy than morphine in mice.



(a) Ph_3P, diisopropyl azodicarboxylate (each of 5 eq), THF; AcSH (5 eq), 0°C; (b) 0.2 N KOH, EtOH, N_2; (c) 5-nitro-2-pyridinesulfenyl chloride, CH_2Cl_2, 0°C.

Chart 1

The thiol-disulfide exchange reaction of compound (**4**) with L-cysteine methyl ester in DMF smoothly afforded a 66% yield of the disulfide, identified as compound (**5**) by treatment with ethyl chloroformate and potassium carbonate in chloroform at 0°C (Chart 2).



(a) L-Cys-OMe•HCl, DMF; (b) $ClCO_2Et$ (3 eq), K_2CO_3 (anh.), $CHCl_3$, 0°C.

Chart 2

Since compound (**4**) was highly reactive with L-cysteine methyl ester *in vitro*, the thiol-disulfide exchange reaction with opioid receptor in GPI was also examined by the wash-out method.[4] When GPI was incubated with 1 μM compound (**4**) for 10 min, the activity was not reversed at all even by washing 50 times (Fig. 1A), indicating a continuous stimulation of the receptor by tight binding. With 1 μM naloxone, a μ-selective antagonist, the retained activity was completely reversed. Then washing 10 times elicited 95% of the activity of compound (**4**) again, suggesting that compound (**4**) can be replaced by naloxone at the binding site, and still be retained near the binding site (Fig. 1B).

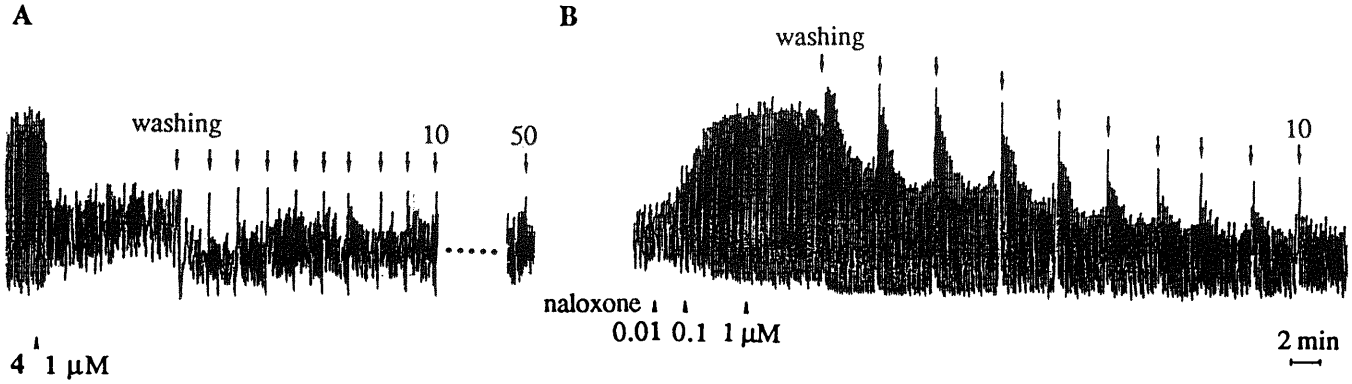


Fig. 1. Recording of the Electrically Stimulated Contraction of Guinea Pig Ileum (GPI)
A : Irreversibility of the activity of 1 μM compound (**4**) by washing; **B** : Reversibility of the activity of 1μM compound (**4**) by treatment with 1 μM naloxone and recovery of the original inhibitory activity by washing.

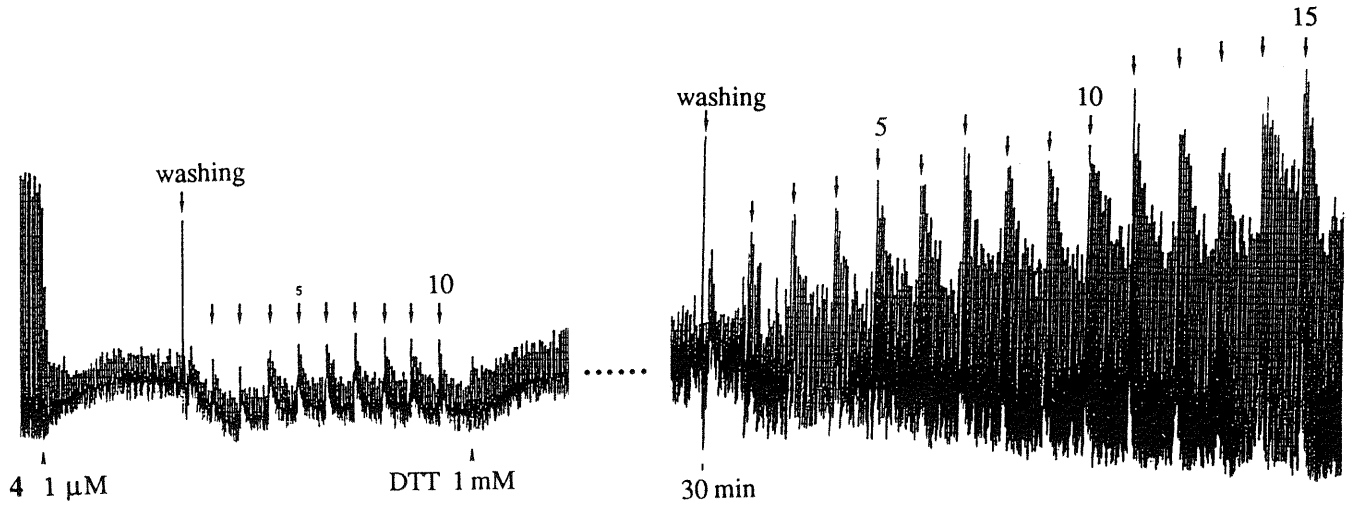


Fig. 2. Reversibility of the Activity of 1 μM Compound (4) by Treatment with 1 mM DTT and Washing

In contrast, incubation of GPI, of which contraction had been fully suppressed by compound (4), with 1 mM of dithiothreitol (DTT) for 30 min eliminated almost all of the activity after washing 15 times (Fig. 2). These results indicate that compound (4) reacted with a thiol in opioid receptor near the 6-position of opiate through a disulfide linkage and that this linkage was cleaved reductively by DTT as illustrated in Fig. 3.

This is evidence of opiate ligand linking to the μ-opioid receptor through the thiol-disulfide exchange. It is important to comfirm whether this sulfhydryl group is identical with that reactive with thiol-enkephalin,[4] and to clarify its role in the binding of the opiate ligand with the μ-opioid receptor.

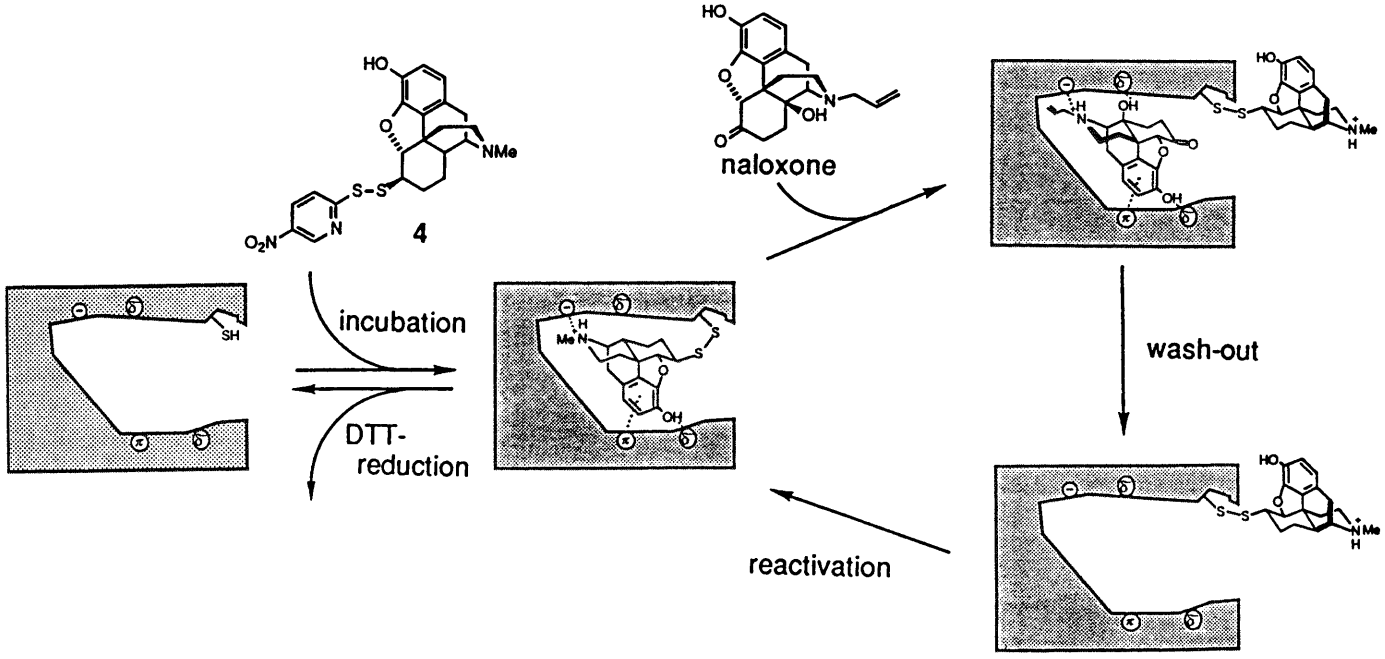


Fig. 3. Schematic Illustration of the Interaction of Compound (4) with an Opioid Receptor

REFERENCES

1) T. Asano, and N. Ogasawara, *Mol. Pharmacol.*, **29**, 244 (1986).
2) J. R. Smith, and E. J. Simon, *Proc. Natl. Acad. Sci. U.S.A.*, **77**, 281 (1980).
3) W. D. Bowen, S. Gentleman, M. Herkenham, and C. B. Pert, *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 4818 (1981).
4) H. Kodama, Y. Shimohigashi, T. Ogasawara, T. Koshizaka, M. Kurono, R. Matsueda, K. Soejima, M. Kondo, and K. Yagi, *Biochem. Int.*, **19**, 1159 (1989).
5) a) I. Fujii, H. Togame, M. Yamamoto, K. Kanematsu, I. Takayanagi, and F. Konno, *Chem. Pharm. Bull.*, **36**, 2282 (1988); b) K. Kanematsu, T. Yoshiyasu, and M. Yoshida, *Chem. Pharm. Bull.*, following paper in this issue.

(Received March 19, 1990)