93. Mixed Azines of Naloxone with Dihydromorphinone Derivatives

by **Helmut Schmidhammer*** and **Felizia Kaspar**

Institute of Pharmaceutical Chemistry, University of Innsbruck, Innrain 52a, A-6020 Innsbruck

and **Arpad Marki** and **Anna Borsodi**

Institute of Biochemistry, Biological Research Center Szeged, H-6701 Szeged

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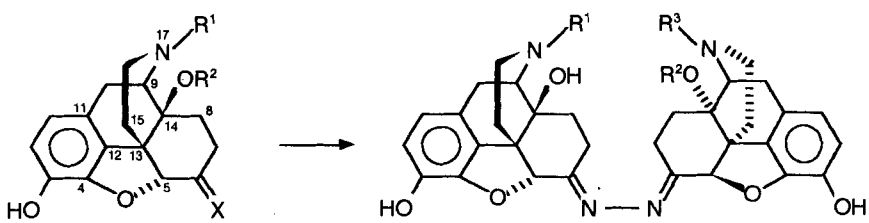
The mixed azines **4** and **5** were prepared by reaction of naloxazone (**2**) with either oxymorphone (**6**) or 14-*O*-methyloxymorphone (**7**) and tested *in vitro* using opioid receptor binding assays and *in vivo* using the AcOH-writhing test in mice. Compound **4** was found to be a partial agonist, while compound **5** was a potent opioid agonist with higher potency than morphine.

Introduction. – Naloxonazine (**1**) is an opioid antagonist which blocks high affinity binding sites (μ_1 receptors) selectively and irreversibly [1–3]. Also some hydrazone derivatives of morphinan-6-ones (*e.g.* naloxazone (**2**) and oxymorphazone (**3**) [4]) block μ_1 receptors selectively and irreversibly. The pharmacological significance of μ_1 sites was evaluated extensively using naloxonazine. Treatment of either rats or mice with **1** eliminated the high affinity binding of a series of radiolabelled opioids. This loss of binding was associated with a dramatic shift of the analgesic dose-response curve to the right, implying that μ_1 sites mediate analgesia. On the other hand, μ_1 blockade did not alter the respiratory depression of morphine or most of the signs associated with morphine dependence [5]. In consideration of the μ_1 selectivity of naloxonazine and its pharmacological action, it was of interest to prepare 'mixed' azines of the opioid antagonist naloxone with opioid agonists of the dihydromorphinone series and to test these compounds biochemically and pharmacologically. We prepared the 'mixed' azine **4** of naloxone and oxymorphone and the 'mixed' azine **5** of naloxone and the highly potent opioid agonist 14-*O*-methyloxymorphone [6].



2 $R^1 = CH_2{=}CHCH_2$, $R^2 = H$, $X = NNH_2$
3 $R^1 = Me$, $R^2 = H$, $X = NNH_2$
6 $R^1 = Me$, $R^2 = H$, $X = O$
7 $R^1 = Me$, $R^2 = Me$, $X = O$

1 $R^1 = R^3 = CH_2{=}CHCH_2$, $R^2 = H$
4 $R^1 = CH_2{=}CHCH_2$, $R^2 = H$, $R^3 = Me$
5 $R^1 = CH_2{=}CHCH_2$, $R^2 = R^3{=}Me$

Chemistry. – Azines **4** and **5** were synthesized by refluxing naloxazone (**2**; prepared essentially as described in [1]) with either oxymorphone (**6**) or 14-*O*-methyloxymorphone (**7**) [6] in MeOH (*Scheme*). The novel azines were not examined regarding their *cis*- and *trans*-isomers. Such azines exist as mixtures of isomers [7] [8].

Biochemical and Pharmacological Evaluation. – Compounds **4** and **5** were evaluated *in vitro* in opioid receptor binding studies [9–11] (*Table 1*). [^{3}H]DAGO (= [D-Ala^2-MePhe^4-Gly^5-ol]enkephaline; μ-selective agonist), [^{3}H]U-69,593 (= (−)-5α,7α,8β -*N*-methyl,*N*-[7-(pyrrolidin-1-yl)cyclohexyl]benzacetamide; κ-selective agonist), and [^{3}H]deltorphin (= Tyr-D-Ala-Phe-Glu-Val-Val-Gly-NH_2; δ-selective agonist) were used as ligands. For *in vivo* evaluation, the AcOH-writhing test in mice[1]) was performed [17–19] (*Table 2*).

Table 1. *Opioid Receptor Binding Assays*[a])

	[^{3}H]DAGO (μ)	[^{3}H]U-69,593 (κ)	[^{3}H]Deltorphin (δ)
4	1.23 ± 0.43	17.50 ± 3.21	49.70 ± 0.46
5	0.55 ± 0.29	8.83 ± 0.74	8.48 ± 2.74
Cyprodime [12] [13]	1.14 ± 0.23	1070 ± 159	1186 ± 181
Naloxone	3[b])	40[c])	20[d])
Morphine	1[b])	80[c])	70[d])

[a]) The values are K_d in nM. [b]) [^{3}H]DHM (= dihydromorphine) was used; values from [14]. [c]) [^{3}H]EKC (= ethylketocyclazocine) was used; values from [15]. [d]) [^{3}H]DALE (= [D-Ala^2-Leu]enkephaline) was used; values from [16].

Table 2. *AcOH Writhing Test in Mice*

	Agonism test ED_{50} [μg/kg; *s.c.*][a])	Antagonism test	
		Morphine (μ) (1.25 mg/kg; *s.c.*) AD_{50} [mg/kg; *s.c.*][b])	U-50,488 (κ) (2.5 mg/kg; *s.c.*) AD_{50} [mg/kg; *s.c.*][b])
4	WP[c])	1.97	2.51
5	84	–	–
Oxymorphone (**6**)	31	–	–
6·HBr	0.48	–	–
Morphine sulfate	389	–	–
Naloxone	–	0.08	1.12

[a]) The ED_{50} values represent the effective dose at which 50% of the animals showed an analgesic response. [b]) The AD_{50} value is defined as the dose at which the antinociceptive effect of the agonist was antagonized in 50% of the animals. [c]) Weak potency: 30% inhibition of writhing at 5 mg/kg.

In opioid receptor binding, **4** and **5** did not block any of the opioid receptors irreversibly like naloxonazine. The compounds showed preference for μ receptors. The following selectivity ratios were found: compound **4**: δ/μ 40 and κ/μ 14; compound **5**: δ/μ 15 and κ/μ 16.

[1]) The tests were carried out for us at the Lilly Research Laboratories, *Eli Lilly and Company*, Lilly Corporate Center, Indianapolis, IN 46285, USA, through the courtesy of Dr. *J. D. Leander*.

In the AcOH-writhing test, compound **5** possessed considerable antinociceptive potency (*ca.* 5 times more active than morphine), while compound **4** showed only weak antinociceptive potency. Compound **4** exhibited antagonism against both morphine- and U-50,488-induced antinociception, thus representing a partial agonist.

In conclusion, the azine containing the opioid agonist with higher potency (compound **5**) showed considerable opioid agonism, while the azine with the weaker opioid agonist (compound **4**) was a partial agonist.

Experimental Part

General. M.p.: *Kofler* melting-point microscope; uncorrected. IR Spectra: in cm^{-1}; *Beckman-Accu-Lab-2* apparatus. ^{1}H-NMR Spectra: *Jeol-JNM-PMX-60* spectrometer; δ in ppm rel. to $SiMe_4$ as internal reference; J in Hz. EI-MS: *Finnigan-MAT-44S* apparatus. Elemental analyses were performed at the Analytical Department of *F. Hoffmann-La Roche AG*, Basel [^{3}H]DAGO and [^{3}H]U-69,593 were purchased from *Amersham*. [^{3}H]Deltorphin was prepared at the Biological Research Center in Szeged.

Mixed Naloxone-Oxymorphone Azine (= 4,5α-Epoxy-3,14-dihydroxy-17-(prop-2-enyl)morphinan-6-one (= 4,5α-Epoxy-3,14-dihydroxy-17-methylmorphinan-6-ylidene)hydrazone; **4**). A soln. of **2** (460 mg, 1.35 mmol) and **6** (405 mg, 1.34 mmol) in anh. MeOH (5 ml) was refluxed for 3 h. After *ca.* 45 min, **4** began to precipitate. After cooling the mixture to +4°, 732 mg (87%) of **4** were isolated. A small amount was recrystallized from EtOH for analysis. M.p. > 300° (dec.). IR (KBr): 3200 (OH), 1635 (C=N). ^{1}H-NMR ($CDCl_3$): 6.67 (*d*, J = 8, 2 arom. H); 6.52 (*d*, J = 8, 2 arom. H); 5.78 (*m*, 1 olef. H); 5.17 (*m*, 2 olef. H); 4.95 (*s*, H–C(5), H–C(5′)); 2.34 (*s*, MeN). EI-MS: 624 (M^+). Anal. calc. for $C_{36}H_{40}N_4O_6$ (624.74): C 69.21, H 6.45, N 8.97; found: C 69.04, H 6.79, N 8.66.

Mixed Naloxone-(14-O-Methyloxymorphone) Azine (= 4,5α-Epoxy-3,14-dihydroxy-17-(prop-2-enyl)morphinan-6-one (4,5α-Epoxy-3-hydroxy-14-methoxy-17-methylmorphinan-6-ylidene)hydrazone; **5**). A soln. of **2** (350 mg, 1.11 mmol) and **7** (379 mg, 1.11 mmol) in anh. MeOH (4 ml) was refluxed for 4.5 h. Compound **5** began to precipitate after *ca.* 1.5 h. After cooling the mixture to +4°, 590 mg (63%) of **5** were collected. An anal. sample was prepared by recrystallization of a small portion from EtOH. M.p. > 210° (dec.). IR (KBr): 3400 (OH), 1630 (C=N). ^{1}H-NMR ($CDCl_3$): 6.56 (*d*, J = 8, 2 arom. H); 6.40 (*d*, J = 8, 2 arom. H); 5.65 (*m*, 1 olef. H); 5.10 (*m*, 2 olef. H); 4.82 (*s*, H–C(5), H–C(5′)); 3.21 (*s*, MeO); 2.31 (s, MeN). Anal. calc. for $C_{37}H_{42}N_4O_6 \cdot 2\,H_2O \cdot 0.5$ EtOH (697.83): C 65.41, H 7.08, N 8.03; found: C 65.06, H 6.98, N 7.85.

Pharmacology. For AcOH-writhing tests, see [17–19]. Opioid receptor binding assays were performed using homogenates of rat brain as described in [9–11].

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