Stereochemical Studies on Medicinal Agents. 23.[1] Synthesis and Biological Evaluation of 6-Amino Derivatives of Naloxone and Naltrexone

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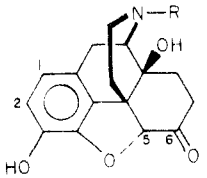
Epimeric 6-amino derivatives of naloxone and naltrexone have been synthesized and the configuration at the C-6 chiral center was determined from NMR studies. All of the derivatives possess narcotic antagonist activity in mice, with each of the 6β epimers having greater potency than the corresponding 6α epimers. In vitro binding experiments indicate that the affinities of these epimers parallel their in vivo potencies. Slight antinociceptic properties were observed with three of the four compounds. The naloxone derivatives **3a** and **3b** appear to be attractive candidates for investigation as long-acting narcotic antagonists in view of their fourfold greater duration of action relative to the other antagonists (1, 2, **4a**, and **4b**).

The past decade has witnessed a resurgence of interest in opioid antagonists.[2] This has been stimulated by the need for strong analgetics devoid of side effects and by the possible utility of pure antagonists in the management of narcotic addiction.

Molecular modification of the pure opioid antagonists, naloxone (1) and naltrexone (2), at the C-6 position has afforded agents which retain antagonist activity.[3] Some of these congeners exhibited longer duration of action and have exceeded the potencies of **1** and **2**.

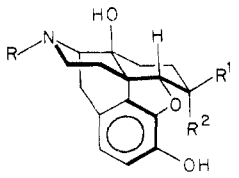


1, R = $CH_2CH{=}CH_2$
2, R = CH_2-c-C_3H_5

Of particular interest was the report that the 6-hydroxy derivatives[4] possess reduced antagonist potency but that the purity of the antagonism is related to the configuration at C-6.[5] In this regard it is of interest that only the α epimers possess antinociceptic activity while corresponding β epimers appear to be devoid of this effect and are pure antagonists.

In an effort to investigate this relationship further, we have synthesized the corresponding 6-amino epimers (**3** and **4**) and have evaluated their biological activity profiles.

Chemistry. The 6-amino diastereomers **3** and **4** were prepared from naloxone (1) and naltrexone (2) by reductive amination using $NaCNBH_3$[6] in the presence of NH_4OAc. The 6α and 6β epimers (**3a**, **4a** and **3b**, **4b**) were formed in a ratio of approximately 2:1, and separation of the isomers was achieved by fractional crystallization of the dihydrochloride salts.



3 a, R = $CH_2CH{=}CH_2$; R^1 = H; R^2 = NH_2
b, R = $CH_2CH{=}CH_2$; R^1 = NH_2; R^2 = H
4 a, R = CH_2-c-C_3H_5; R^1 = H; R^2 = NH_2
b, R = CH_2-c-C_3H_5; R^1 = NH_2; R^2 = H

The stereochemical assignment of the epimers was based on the relative magnitude of the NMR vicinal coupling constants, $J_{5,6}$ (Table I). The α and β epimers possessed

Table I. NMR Spectral Data for *N*-Allyl- and *N*-Cyclopropylmethyl-6-amino-14-hydroxynordesomorphine

Compd[a]	$J_{5,6}$, Hz	Chemical shift (δ)[b] H-1	H-2	Δδ[c]
1		6.47	6.66	0.19
2		6.47	6.67	0.20
3a	4.0	6.38	6.62	0.24
3b	7.4	6.42	6.55	0.13
4a	4.0	6.43	6.72	0.29
4b	7.4	6.42	6.58	0.16

[a] Determined in $CDCl_3$ using the base. [b] Determined at 60 MHz; $J_{1,2}$ = 8 Hz. [c] $\Delta\delta = \delta_{H\text{-}2} - \delta_{H\text{-}1}$.

J values of 7.4 and 4 Hz, respectively. These coupling constants are in the range of that corresponding to the 6-hydroxy analogues which contain the C ring in a distorted chair conformation.[5,7] It therefore is reasonable that a similar situation exists with epimers of **3** and **4**.

Additional evidence consistent with this assignment was obtained from the chemical shift differences (Δδ) of the aromatic protons (Table I). The α epimers **3a** and **4a** exhibit a greater chemical shift difference between H-1 and H-2, perhaps as a consequence of deshielding of H-2 by the axial 6-amino group. By comparison, the chemical shifts for H-1 in the epimers are very similar due to its greater distance from this group. Since the 6-amino group in both β epimers (**3b** and **4b**) is in a trans orientation relative to the aromatic ring, the chemical shift difference between the aromatic protons is less than that of the α isomer.

Experimental Section

Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed by MHW Laboratories, Garden City, Mich., and were within ±0.4% of the theoretical values. IR spectra were determined on a Perkin-Elmer 237B grating spectrophotometer and are consistent with the assigned structures. NMR data (δ) were obtained at ambient temperature using Me_4Si as internal standard using a T-60 and A-60D spectrometer.

6-Amino-14-hydroxy-17-allylnordesomorphine (3a,b). To a mixture of naloxone (3.3 g, 10 mmol) and ammonium acetate (7.7 g, 100 mmol) dissolved in methanol (25 mL) under nitrogen was added a methanolic solution (20 mL) of $NaCNBH_3$ (0.385 g, 6.3 mmol). The resulting solution was adjusted to pH 7 with concentrated HCl, stirred for 17 h, and acidified to pH 1 with additional concentrated HCl. After removal of the solvent and dissolution of the residue in water, the solution was extracted with chloroform to remove the water-insoluble material and was then adjusted to pH 9 with Na_2CO_3. This mixture was saturated with NaCl and extracted with $CHCl_3$, and the dried (Na_2SO_4) chlo-

Table II. Antagonistic Activities of 6-Amino Derivatives of Naloxone and Naltrexone in Mice

Antagonist	Dose mg/kg	Dose μmol/kg	Morphine ED_{50}, mg/kg (95% confidence limits)	Apparent pA_2
None			5.3 (4.3-6.6)	
Naloxone (1)[a]	0.04	0.1	12.3 (9.9-15.5)	7.06 (6.94-7.18)
	0.08	0.2	18.0 (14.4-22.4)	
	0.16	0.4	44.4 (35.7-55.5)	
	0.32	0.8	80.0 (64.2-99.7)	
3b[b]	1.6	4.9	16.0 (9.4-27.4)	
	6.4	19.5	29.6 (20.3-43.2)	
3a[b]	1.6	4.9	9.3 (6.1-14.2)	
Naltrexone (2)[a]	0.005	0.013	9.2 (7.4-11.3)	7.76 (7.61-7.91)
	0.02	0.053	32.4 (25.9-40.0)	
	0.08	0.212	134.4 (107.6-165.8)	
4b[b]	0.32	0.77	27.4 (22.8-32.2)	
	1.28	3.08	30.1 (25.3-35.3)	
4a[b]	0.32	0.77	8.6 (6.7-10.7)	
	1.28	3.08	21.6 (17.2-27.4)	
	5.12	12.3	31.6 (24.7-39.7)	

[a] As HCl salts. [b] As 2HCl salt.

roform extract was evaporated in vacuo. The oily residue was dissolved in absolute methanol, acidified to pH 1 with concentrated HCl, and maintained at 4 °C for 8 h. Fractional crystallization of the dihydrochloride salts **3a** and **3b** from aqueous acetone was monitored by TLC (silica gel, EtOAc–MeOH–NH_4OH 100:10:3). The first product, **3a**·2HCl, to crystallize was obtained in a yield of 50% (2.1 g): mp >270 °C; $[\alpha]^{25}_D$ −165.4° (*c* 2.37, H_2O); R_f 0.12; mass spectrum (70 eV) 330 [M^+]; NMR (deuterium-exchanged free base in $CDCl_3$) δ 6.62 and 6.38 (1 H each, two doublets, J = 8 Hz, aromatic H), 6.08–4.95 (3 H, multiplet, olefinic H), 4.55 (1 H, d, J = 4.0 Hz, C_5-H). Anal. ($C_{19}H_{26}N_2O_3Cl_2$) C, H, N. The second product was **3b**·2HCl (1.2 g): mp >270 °C; $[\alpha]^{25}_D$ −123.9° (*c* 1.24, H_2O); R_f 0.16; mass spectrum (70 eV) 330 [M^+]; NMR (deuterium-exchanged free base in $CDCl_3$) δ 6.55 and 6.42 (1 H each, two doublets, J = 8 Hz, aromatic H), 6.0–4.90 (3 H, multiplet, olefinic H), 4.18 (1 H, d, J = 7.4 Hz, C_5-H). Anal. ($C_{19}H_{26}N_2O_3Cl_2$) C, H, N.

6-Amino-14-hydroxy-17-cyclopropylmethylnordesomorphine (4a,b). The reductive amination procedure was identical with that described above and the dihydrochloride salts of **4a** and **4b** were separated in a fashion similar to that employed for the allyl analogues. Epimer **4a**·2HCl was obtained in a yield of 48% (2.0 g): mp >270 °C; $[\alpha]^{25}_D$ −166.3° (*c* 1, H_2O); R_f 0.14; mass spectrum (70 eV) 344 [M^+]; NMR (deuterium-exchanged free base in $CDCl_3$) δ 6.72 and 6.43 (1 H each, two doublets, J = 8 Hz, aromatic H), 4.68 (1 H, d, J = 4.0 Hz, C_5-H). Anal. ($C_{20}H_{28}N_3O_3Cl_2$) C, H, N. Epimer **4b**·2HCl (1.0 g): mp >270 °C; $[\alpha]^{25}_D$ −131.9° (*c* 1, H_2O); R_f 0.17; mass spectrum (70 eV) 344 [M^+]; NMR (deuterium-exchanged free base in $CDCl_3$) δ 6.58 and 6.42 (1 H each, two doublets, J = 8 Hz, aromatic H), 4.28 (1 H, J = 7.4 Hz, C_5-H). Anal. ($C_{20}H_{28}N_2O_3Cl_2$) C, H, N.

Pharmacology. Male, Swiss–Webster mice (Biolab, White Bear, Minn.) weighing 20–25 g were used in all experiments. Solutions of the test compounds were made in saline solution such that 10 mL/kg was administered sc to the animals at each dose level. The tail-flick assay of D'Amour and Smith[8] which was modified for mice[9] was used to assess the analgesic potency of morphine. At least 24 animals were used to determine each dose–response curve and ED_{50} value. The ED_{50} values with 95% confidence limits were estimated by the method of Litchfield and Wilcoxon.[10] For the assessment of the antagonistic activity, the derivatives of naltrexone were co-administered with morphine and the derivatives of naloxone were administered 1.5 h before the administration of morphine and the analgesic assay was conducted 30 min after the injection of morphine. These procedures ensured that the analgesic measurement was being performed at the peak effect of both morphine and the antagonists. The values for apparent pA_2 were estimated as described previously.[11]

Since the analgesic property of the test compounds was undetectable by the tail-flick assay, modification of the writhing assay[12] was used. Acetic acid solution[13] was used as the writhing agent and the method has been described in detail elsewhere.[14] Inhibition of the binding of [^{3}H]naloxone to putative narcotic receptors was performed by the method of Pert and Snyder[15] as modified by Pasternak et al.[16] The procedure included the preincubation step prior to the binding assay. Stereospecific binding was determined by incubating with and without 1×10^{-6} M levallorphan. The concentration of [^{3}H]naloxone used was 1.5×10^{-9} M. The binding was carried out in the absence and presence of 100 mM NaCl to assess the relative extent of the agonistic and antagonistic properties of the compounds.[16]

Pharmacological Results

Naloxone and naltrexone were used as standard antagonists to which their corresponding derivatives were compared after sc administration in mice. The duration of the antagonism of morphine analgesia by naloxone was 1 h with the peak antagonistic effect at about 20 min. The peak antagonistic effect of naltrexone was about 30 min and lasted slightly over 1 h. The antagonistic effects of **3a** and **3b** were not detected until well over 1 h after administration. The antagonistic effect peaked at 2–3 h and lasted a little over 4 h. The peak and duration of antagonistic action of **4a** and **4b** were similar to those of naltrexone.

The data for the antagonism of morphine analgesia by naloxone, naltrexone, and their derivatives are recorded in Table II. On the basis of the comparison of the apparent pA_2 values of naloxone–morphine and naltrexone–morphine naltrexone is about five times more potent than naloxone on a molar basis in its ability to antagonize morphine analgesia in mice. Also, the doses of **3a** and **3b** required to shift the morphine dose–response curve an equivalent amount was five times those of the corresponding isomers **4a** and **4b**. Whereas good proportional shifts of the morphine dose–response curves were observed with increasing doses of naloxone or naltrexone, proportional shifts were not obtained with any of the 6-amino epimers.

Among the derivatives, with equivalent doses the β isomer **3b** increased the ED_{50} of morphine by threefold whereas the 6α isomer **3a** increased it 1.75 times. Similarly the 6β isomer **4b** increased the ED_{50} of morphine by fivefold whereas the 6α isomer **4a** increased it only 1.6 times.

The amount of **3a** and **3b** required to raise the ED_{50} of morphine an equivalent amount as that caused by naloxone was about 50 and 25 times, respectively, the molar dose of naloxone. About 60–230 and 15–60 times the molar dose of **4a** and **4b**, respectively, were required to increase the ED_{50} of morphine the same amount as naltrexone.

No antinociceptive activity was observed for any of the 6-amino epimers using the tail-flick assay. Using the

Table III. Antinociceptive Activities of 6-Amino Derivatives of Naloxone and Naltrexone in Mice

Compd	ED_{50}, mg/kg (95% confidence limits)
Morphine[a]	0.33 (0.22–0.47)
3b[b]	4.9 (1.1–22.0)
3a[b]	No effect at 1.6 mg/kg
4b[b]	27% inhibn of writhes at 5.12 mg/kg
4a[b]	18% inhibn of writhes at 5.12 mg/kg

[a] HCl salt. [b] 2HCl salt.

Table IV. Inhibition of [^{3}H]Naloxone Binding by 6-Amino Derivatives of Naloxone and Naltrexone

	IC_{50} (−NaCl)[a]	IC_{50} (+NaCl)[b]	Binding ratio[c]
Naloxone	5.5×10^{-9}	4.3×10^{-9}	0.8
3b	8.6×10^{-8}	5.3×10^{-8}	0.6
3a	1.4×10^{-7}	1.1×10^{-7}	0.8
Naltrexone	5.5×10^{-10}	5.9×10^{-10}	1.1
4b	9.1×10^{-10}	7.0×10^{-10}	0.8
4a	2.9×10^{-9}	9.0×10^{-9}	3.1

[a] Molar concentration ($n = 2$) required to inhibit [^{3}H]-naloxone binding 50% in the absence of 100 mM NaCl. [b] Molar concentration ($n = 2$) required to inhibit [^{3}H]naloxone binding 50% in the presence of 100 mM NaCl. [c] $[IC_{50}$ (+NaCl)]/[IC_{50} (−NaCl)].

writhing assay, **3b** had slight antinociceptive properties with an ED_{50} of about ten times greater than that of morphine (Table III). The α isomer **3a** did not inhibit writhing at the dose employed. Both **4a** and **4b** also appeared to have slight antinociceptive properties. These studies were not pursued beyond the few dosages employed because of the scarcity of the compounds.

The stereospecific binding assay revealed that the binding ratios of all the derivatives were about one except for **4a** which had a binding ratio of 3.1 (Table IV). The inhibition of naloxone binding also revealed that the naltrexone derivatives **4a** and **4b** were more potent inhibitors than the naloxone derivatives **3a** and **3b** and the β isomers of the antagonists were more potent than the corresponding α isomers.

Discussion

The epimers of **3** and **4** are less potent than their parent compounds, naloxone (**1**) and naltrexone (**2**), in antagonizing the in vivo effects of morphine. A similar reduction of narcotic antagonist potency has been reported[4] for the corresponding 6-hydroxy epimers. Although the in vivo rank-order potencies of the 6-amino compounds and their parent ketones are qualitatively similar to the in vitro IC_{50} values, it is noteworthy that the differences between the parent compounds and their derivatives are less in the receptor binding assay. One explanation for this may be related to differences between the ability of the parent ketones and the 6-amino derivatives to enter the brain. Another factor contributing to this difference might be related to their weak antinociceptive activity.

The 6β epimers **3b** and **4b** are more potent antagonists than the corresponding α isomers **3a** and **4a**, but the fact that the isomeric potency ratios are not great either in vivo or in vitro suggests that the 6-amino group is not situated in a critical chiral environment during ligand–receptor association. It is of interest that the 6-hydroxy epimers do not appear to follow this pattern.[5]

The binding ratios of unity observed with **3a**, **3b**, and **4b** presumably suggest that these derivatives are relatively pure antagonists like naloxone. The fact that agonistic activities of **3b** and **4b** were not revealed by this binding assay might be related to their weak agonistic properties. For example, naltrexone which has a slight agonistic property when it is tested by the writhing assay[18] has a binding ratio of 1.0.[17] On the other hand, **4a**, which has equal or less antinociceptive activity compared with **4b**, has a binding ratio comparable to other narcotic-antagonist analgesics such as pentazocine.[17]

In view of the fourfold greater duration of action of **3a** and **3b** relative to its parent compound, naloxone, these derivatives appear to be attractive candidates for investigation as long-acting narcotic antagonists. By comparison, parenterally administered **2**, **4a**, and **4b** possessed durations of action which were similar to that of naloxone.

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