inoculation. **B16 melanoma** was implanted ic in $B_6D_2F_1$ mice according to the standard protocol.[44] The drugs, formulated as described, were administered ip every other day for 17 days beginning 24 h after tumor implantation. Antitumor activity was determined by comparing the median survival time of treated animals (T) with that of a control group (C), which received only the drug vehicle, and is expressed as a percentage increase in life span (% ILS), where % ILS = $(T/C - 1) \times 100$. A % ILS of at least 25% was considered significant. The optimum dose shown in Tables II–IV is that which produced the maximum % ILS in the screen.

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Registry No. 1, 33330-89-1; 2, 36120-57-7; 3, 33330-91-5; 4, 35967-28-3; 5, 35972-50-0; 6, 29168-87-4; 7, 91860-71-8; 8, 52416-17-8; 9, 52416-18-9; 10, 20119-28-2; 11, 7203-91-0; 12, 20241-00-3; 13, 20241-05-8; 14, 7203-90-9; 15, 23456-94-2; 16, 7239-21-6; 17, 23456-93-1; 18, 52388-42-8; 19, 91860-72-9.

Notes

Opioid Agonists and Antagonists. 6,6-Hydrazi and 6-Oximino Derivatives of 14-Hydroxydihydromorphinones

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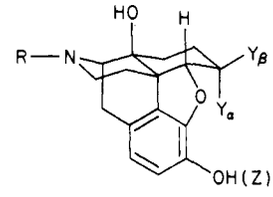
Naloxone (**1a**), naltrexone (**1b**), and oxymorphone (**1c**) were converted to the corresponding 6,6-diaziridines (**4a–c**), oximes (**5a–c**), and oxime *O*-methyl ethers (**6a–c**). The antagonist derivatives (R = $CH_2CH{=}CH_2$ and R = CH_2-c-C_3H_7) were less active than the parent ketones in the tail-flick assay vs. morphine, by 2–10-fold, except for **6a**, which was much less active. The agonist analogues (R = Me) were more active than morphine but less active than dihydromorphine in standard agonist assays. None were significantly longer in duration of action. Thus structural changes at the C-6 position to produce diaziridines, oximes, and oxime *O*-methyl ethers provide compounds retaining expected opioid activity.

The 14-hydroxy-7,8-dihydromorphinone nucleus has provided many pharmacologically interesting compounds that have been important in opiate research. Among them are the important opioid antagonists naloxone (**1a**) and naltrexone (**1b**) and the useful analgesics oxymorphone (**1c**) and oxycodone (**1d**). More recently, mixed agonist–antagonist analogues butorphanol (**1e**) and nalbuphine (**1f**) have been proven to be clinically useful analgesics.[1] A large number of derivatives with this nucleus have been made, which clearly indicates functional group changes may be made in the C ring, particularly at the 6-, 7-, and 8-positions while still retaining significant activity as opioid agonists or antagonists.[2,3] Among these are the 6-desoxy-6-methylene compounds and their corresponding 6α-epoxides, which are highly potent.[3]

The 6-position has been the locus of funtional group changes that have produced several potential alkylating



1a. R = $CH_2CH{=}CH_2$; Y = O
1b. R = CH_2-c-C_3H_7; Y = O
1c. R = Me; Y = O
1d. R = Me; Y = O; Z = Me
1e. R = CH_2-c-C_4H_9; Y = H_2
1f. R = CH_2-c-C_4H_9; Y_α = OH; Y_β = H
2a,b. R = CH_2-c-C_3H_7; Y_β or Y_α = $N(CH_2CH_2Cl)_2$
2c,d. R = CH_2-c-C_3H_7; Y_β or Y_α = $HNC(=O)CH{=}CHCOOMe$
2e,f. R = CH_2-c-C_3H_7; Y_β or Y_α = $N{=}C{=}S$
3a. R = $CH_2CH{=}CH_2$; Y = $N{=}N$- (dimeric)
3b. R = $CH_2CH{=}CH_2$; Y = NNH_2
4a–c. Y_α, Y_β = NHNH
5a–c. Y = NOH
6a–c. Y = NOMe
a. R = $CH_2CH{=}CH_2$
b. R = CH_2-c-C_3H_7
c. R = Me

agents that have been used to aid in characterization of opioid receptors. Among them are *N*,*N*-bis(β-chloroethyl) derivatives of 6α- and 6β-naltrexamine and -oxymorphamine (**2a,b**) and the fumaramide methyl ester (**2c,d**) and isothiocyanate derivatives (**2e,f**) of these amines.[4] Nal-

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Table I. Antagonist Activity Determined in the Tail-Flick Assay vs. Morphine[a]

compd	M_r	6-subst	TF vs. morphine: ED_{50}, mg/kg (95% CL)
4a (*N*-allyl)	341.4	HNNH	0.06 (0.04–0.10)
4b (*N*-CPM)[b]	355.4	HNNH	0.02 (0.01–0.03)
5a (*N*-allyl)	342.4	=NOH	0.06 (0.2–0.10)
5b (*N*-CPM)	356.4	=NOH	0.08 (0.03–0.20)
6a (*N*-allyl)	356.4	=NOMe	1.90 (0.40–7.8)
6b (*N*-CPM)	370.5	=NOMe	0.08 (0.02–0.30)
1a ·HCl	363.8	=O	0.031 (0.010–0.093)
1b ·HCl	377.8	=O	0.007 (0.002–0.02)

[a] Compounds were tested as previously described: Dewey, W. L.; Harris, L. S. *J. Pharmacol. Exp. Ther.* **1971**, *179*, 652. Dewey, W. L.; Harris, L. S.; Howes, J. F.; Nuite, J. A. *J. Pharmacol. Exp. Ther.* **1970**, *155*, 435. Jacobson, A. E.; May, E. L. *J. Med. Chem.* **1965**, *8*, 563. Perine, T. D.; Atwell, L.; Tice, I. B.; Jacobsen, A. E.; May, E. L. *J. Pharm. Sci.* **1972**, *61*, 86. Aceto, M. D.; Harris, L. S.; Dewey, W. L.; May, E. L. "Problems on Drug Dependence, 1980", Proceedings of 42nd Annual Scientific Meeting of the Committee on Problems in Drug Dependence, NIDA Monograph No. 34, L. S. Harris, ed., Feb 1981, pp 298–299. Compounds were administered subcutaneously in awueous solution of HCl salts. [b] CPM = CH_2-c-C_3H_7.

oxonazine (**3a**), the azine from naloxone, has also been shown to have irreversible effects on opioid ligand binding, thought initially to be due to the effects of naloxazone (**3b**), the hydrazone of naloxone.[5] More recently, **3a** has been used to examine opioid receptor multiplicity.[6] The activities of these compounds suggested to us preparation of other derivatives at the 6-position. In this paper we report preparation of the 6,6-hydrazi (diaziridine) derivatives **4a–c** and the corresponding ketone oximes **5a–c** and ketone oxime *O*-methyl ethers **6a–c**.

The synthesis of the 6,6-hydrazi derivatives **4a–c** was accomplished directly from the corresponding ketones **1a–c**, by reaction with hydroxylamine-*O*-sulfonic acid in the presence of ammonia.[7] Chromatography of the products provided the desired diaziridine and the oxime in a ratio of about 3:2. We attempted to convert the diaziridines to the corresponding diazirines, possible photoaffinity ligands;[8] however, we were unsuccessful.

The ready availability of the oxime derivatives **5a–c** from this process and from reaction of the parent ketone with hydroxylamine also suggested preparation of the oxime *O*-methyl ethers **6a–c** as compounds in which the polarity of the oximino group would be reduced. These oxime *O*-methyl ethers were prepared by reaction of the corresponding ketones with methoxyamine. Only single oximes and oxime *O*-methyl ethers were isolated, as demonstrated by single signals in the ^1H NMR for the C-5 methine proton and *O*-methyl groups, respectively. These compounds were tentatively assigned the *E* configuration on the basis of steric considerations. Naloxazone (**3b**) and the hydrazones of **1b** and **1c** have recently been shown to exist predominantly in the *E* configuration, based on ^{13}C NMR work.[9]

The compounds were tested in several in vivo assays (Tables I and II). The *N*-allyl and *N*-cyclopropylmethyl compounds were antagonists in the tail-flick assay using morphine as the agonist (Table I). The observed ED_{50}'s showed they were one-half to one-third as potent as corresponding parent ketones naloxone and naltrexone, except for oxime **5b**, which was 1 order of magnitude less potent than naltrexone. The compounds showed durations of action similar to their parent ketones. The *N*-methyl compounds were active in several agonist assays. Data from hot-plate, tail-flick, Nilsen, and phenylquinone writhing assays are given in Table II. The compounds were consistently more potent than morphine but less potent then dihydromorphine. Their potencies were within 1 order of magnitude expected for oxymorphone, which has been reported to be 12 times as potent as morphine when administered subcutaneously to mice.[10] An interesting observation was that oxime *O*-methyl ether **6a** was least potent in the antagonist assay, but the corresponding *N*-methyl oxime *O*-methyl ether **6c** was most potent in the rodent agonist assays. None of the *N*-cyclopropyl and *N*-alkyl analogues (**4a**, **4b**, **5a**, **5b**, **6a**, **6b**) showed dose–response curves in the agonist assays and thus were similar to naltrexone and naloxone, as expected.

Compounds **4a–c**, **5a–c**, **6a–c**, and parent ketones **1a–c** were assayed for mutagenic activity in the Ames *Salmonella typhimurium* reversion assay in strains TA-98 and TA-100, in the absence and presence of the S-9 liver fraction from Arochlor 1254 treated male rats and NADPH, as previously reported.[11] Marginally weak mutagenic activity was noted for naloxone derivatives **4a** and **6a**, which showed revertants 2–3 times background (about 150 revertants) at 500 μg/plate in tester strain TA-100, both in the absence and presence of NADPH. The other compounds were nonmutagenic.

In conclusion, the opioid activities of C-6 diaziridine, oxime, and oxime *O*-methyl ethers derivatives of naloxone, naltrexone, and oxymorphone resemble to a significant extent their parent ketones. Thus, we conclude such changes can be made while retaining opioid activity.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were recorded on a Beckman IR-5A spectrophotometer. NMR spectra were recorded on Varian T-60 and EM-360 spectrometers using Me_4Si as internal standard. Mass spectra recorded on a VG-7070H mass spectrometer operated in the CI mode were consistent with the assigned structures. Microanalyses were performed on Galbraith Laboratories, Knoxville, TN. Where indicated by the symbols of the elements, analyses were within ±0.4% of theoretical values.

6,6-Hydrazi- and 6-Oximinonaloxone (4a and 5a). A stirred, cold solution (0–2 °C) of 3.00 g (9.2 mmol) of naloxone in 100 mL of methanol was treated with dry gaseous NH_3 for 2 h (to saturation). Hydroxylamine-*O*-sulfonic acid (1.18 g, 10.1 mmol) was then added in small portions over a 20-min period. The reaction mixture was stirred at 0–10 °C for 1 h, adjusted to pH 8.5 with aqueous 10% HCl, and then extracted with $CHCl_3$ (4 × 50 mL). The combined $CHCl_3$ extracts were dried (Na_2SO_4), and the solvent was evaporated. The residue was chromatographed in

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Table II. Narcotic Agonist Activity of *N*-Methyl Analogues in Various Assays[a]

compd	M_r	6-subst	tail flick, mg/kg (95% CL)	PPQ writhing, mg/kg (95% CL)	hot plate, mg/kg (95% CL)	Nilsen, mg/kg (95% CL)
4c	397.3	NHNH	0.70 (0.30–1.7)	0.05 (0.01–0.20)	0.81 (0.67–0.97)	2.0 (0.4–2.9)
5c	315.4	=NOH	2.2 (1.4–3.6)	0.10 (0.03–0.40)	0.56 (0.40–0.77)	0.45 (0.31–0.65)
6c	348.4	=NOMe	0.08 (0.02–0.3)	0.02 (0.01–0.03)	0.22 (0.20–0.24)	not done
morphine sulfate			5.8 (5.68–5.92)	0.23 (0.204–0.256)	0.98 (0.83–1.1)	1.3 (1.0–19.7)
dihydromorphine hydrochloride					0.19 (0.15–1.25)	0.2 (0.15–0.30)

[a] Compounds were tested as previously described; see footnote *a* to Table I. All compounds were given subcutaneously as aqueous solutions of HCl salts.

silica gel (90 g, 70–325 mesh), eluting with EtOAc–EtOH–NH_3 (aqueous) (100:1:1). The gelatinous solid was dissolved in benzene and precipitated with petroleum ether (bp 30–60 °C), affording 1.06 g (34%) of oxime **5a** as a white solid. Alternatively, **5a** was prepared from equal to molar quantities of naloxone, NH_2OH·HCl, and aqueous 2 N NaOH in methanol at room temperature. Water was added and the oxime extracted into $CHCl_3$. Evaporation and crystallization afforded oxime **5a** in 90–95% yield: mp 118–119 °C; NMR ($CDCl_3$) δ 4.98 (s, C_5 H); $[\alpha]^{25}_D$ −270° (EtOH, c 1.0). Anal. **5a** ($C_{19}H_{22}N_2O_4$) C, H, N.

The diaziridine was eluted from the column using EtOAc–EtOH–NH_3 (aqueous) (100:10:1). Crystallization from EtOAc afforded 1.73 g (55%) of **4a** as a pale yellow solid: mp 159–160 °C; NMR ($CDCl_3$) δ 4.78 (s, C_5 H); $[\alpha]^{25}_D$ −149.6° (EtOH, c 1.0). Anal. **4a** ($C_{19}H_{23}N_3O_3$) C, H, N.

6,6-Hydrazi- and 6-Oximinonaltrexone (4b and 5b). The compounds were prepared by a procedure similar to that described for the preparation of **4a** and **5a**. From 3.0 g (8.8 mmol) of naltrexone (**1b**) and 1.13 g (9.7 mmol) of hydroxylamine-*O*-sulfonic acid was obtained 1.05 g (34%) of naltrexone oxime (**5b**) [mp 235–236 °C (THF–petroleum ether); NMR ($CDCl_3$) δ 5.00 (s, C_5 H); $[\alpha]^{25}_D$ −330° (EtOH, c 1.0)] and 1.68 g (54%) of the 6,6-diaziridine of naltrexone (**4b**) [mp 198–199.5 °C (EtOAc); NMR ($CDCl_3$) δ 4.80 (s, C_5 H); $[\alpha]^{25}_D$ −169.3° (EtOH, c 1.0)]. Anal. **5b** ($C_{20}H_{24}N_2O_4$) C, H, N. Anal. **4b** ($C_{20}H_{25}N_3O_3$) C, H, N.

6,6-Hydrazi- and 6-Oximinooxymorphone (4c and 5c). These compounds were prepared by a procedure similar to that described for the preparation of **4a** and **5a**. From 3.5 g (11.6 mmol) of oxymorphone and 1.5 g (12.8 mmol) of hydroxylamine-*O*-sulfonic acid was isolated 1.32 g (36%) of oxymorphone oxime (**5c**) [mp 270–271 °C (THF); NMR (acetone-d_6) δ 4.83 (s, C_5 H); $[\alpha]^{25}_D$ −350° (EtOH, c 1.0)] and 2.00 g (55%) of 6,6-hydrazioxymorphone (**4c**) [mp 215–216 °C (EtOAc); NMR ($CDCl_3$) δ 4.78 (s, C_5 H); $[\alpha]^{25}_D$ −126.0° (EtOH, c 1.0)]. Anal. **5c** ($C_{17}H_{20}N_2O_4$) C, H, N. Anal. **4c** ($C_{17}H_{21}N_3O_3$) C, H, N.

6-Oximinonaloxone *O*-Methyl Ether (6a). Naloxone (**1a**) (1.00 g, 3.05 mmol) was added in portions to a solution of $MeONH_2$·HCl (0.330 g, 3.9 mmol) in a mixture of 1.6 mL of 10% aqueous NaOH and 15 mL of MeOH. The mixture was refluxed for 5 h, cooled, diluted with 50 mL of H_2O, and extracted with $CHCl_3$ (3 × 15 mL). The combined $CHCl_3$ extracts were dried (Na_2SO_4) and evaporated. The yellow oil obtained after evaporation of the solvent solidified on standing at room temperature was crystallized from hexane–petroleum ether (bp 35–60 °C), affording 1.03 g (95%) of the ketoxime *O*-methyl ether: mp 128–129 °C; NMR ($CDCl_3$) δ 4.95 (s, C_5 H), 3.82 (s, OCH_3); $[\alpha]^{25}_D$ −229.6° (MeOH, c 0.25). Anal. ($C_{20}H_{24}N_2O_4$) C, H, N.

In a similar way the ketoxime *O*-methyl ethers of naltrexone and oxymorphone (**6b** and **6c**) were prepared in 90–95% yield. 6-Oximinonaltrexone *O*-methyl ether (**6b**): mp 172–173 °C; NMR ($CDCl_3$) δ 4.95 (s, C_5 H), 3.82 (s, OCH_3); $[\alpha]^{25}_D$ −212.8° (MeOH), c 0.25). Anal. **6b** ($C_{21}H_{26}N_2O_4$) C, H, N. 6-Oximinooxymorphone *O*-methyl ether (**6c**): mp 115–116 °C; NMR ($CDCl_3$) δ 4.92 (s, C_5 H), 3.85 (s, OCH_3); $[\alpha]^{25}_D$ −175.0° (MeOH, *c* 0.25). Anal. **6c** ($C_{18}H_{22}N_2O_4 \cdot H_2O$) C, H, N.

Mutagenesis Testing. The mutagenesis assays in bacterial tester strains TA-98 and TA-100 using the 9000*g* supernatant fraction from livers of male rats treated with Arochlor 1254 were performed as previously described.[11]

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Registry No. **1a**, 465-65-6; **1b**, 16590-41-3; **1c**, 76-41-5; **4a**, 92078-77-8; **4b**, 92078-78-9; **4c**, 92078-79-0; **5a**, 92078-80-3; **5b**, 92096-20-3; **5c**, 75659-97-1; **6a**, 92078-81-4; **6b**, 92078-82-5; **6c**, 92078-83-6; NH_2OH·HCl, 5470-11-1; $MeONH_2$·HCl, 593-56-6; hydroxylamine-*O*-sulfonic acid, 2950-43-8.

Synthesis and Binding to Tubulin of Colchicine Spin Probes

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Spin probes of deacetylcholchicine (1), 4-(hydroxymethyl)colchicine (2), and colchifoline (3) have been synthesized to study the binding site for colchicine on tubulin. Acylation of 1–3 with (±)-2,2,5,5-tetramethyl-1-pyrrolidinyloxy-3-carboxylic acid (4) afforded diastereomeric mixtures of the esters 5–8 and the amides 9 and 10. Pure diastereomers of 3 were synthesized with **4a** and **4b**, which inhibited the binding of colchicine by 60%. In the presence of calf brain microtubular protein, the colchifoline spin labels underwent reduction of the nitroxide group, which precluded their use to study the topography of the colchicine binding site.

The antimitotic activity of colchicine is thought to arise from its interaction with tubulin, a protein that polymerizes to form the microtubules of the mitotic spindle. Previous studies have shown that tubulin is a dimer containing nonidentical subunits, one of which has a single colchicine binding site.[1,2] There is currently great interest

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