Paradoxical Effects of *N*-Cyanoalkyl Substituents upon the Activities of Several Classes of Opioids

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The pharmacological effect of the *N*-(β-cyanoethyl) moiety is dependent on the opioid on which it is substituted. It caused a large increase in antinociceptive potency, in (−)-3-hydroxymorphinan and (−)-normetazocine, as compared with the *N*-methyl opioid. These cyanoethyl compounds do not substitute for morphine in morphine-dependent monkeys. This moiety also appears to greatly increase the ability of the opiate receptor to differentiate enantiomers. An ca. 100 000-fold difference in binding was noted between the epimeric *N*-(β-cyanoethyl)-3-hydroxymorphinans and the normetazocines. The levo enantiomers have little acute toxicity and showed excellent therapeutic ratios. In contrast, the *N*-(β-cyanoethyl) moiety on normorphine, norcodeine, and noroxymorphone did not appear to improve their pharmacological properties. Homologous *N*-cyanoalkyl opioids were less potent antinociceptives.

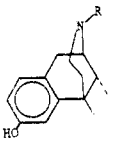
In the course of preparing various *N*-substituted normetazocines and 3-hydroxymorphinans for other purposes, we noted that the *N*-(β-cyanoethyl) intermediates were extremely potent antinociceptives with excellent therapeutic ratios (Table II). We decided, then, to examine the effect of cyanoalkyl moieties on various opioids.

We prepared the *N*-(β-cyanoethyl) derivative of racemic normetazocine and its enantiomers, the (+) and (−) enantiomers of *N*-(β-cyanoethyl)-3-hydroxymorphinan, and the derivative of noroxymorphone, norcodeine, and normorphine. Since the parent compounds were all rigid multicyclic opioids, we presumed that the effect of the cyanoethyl group would be similar in each. An *N*-cyanomethyl derivative was prepared in the (±)-normetazocine and (−)-3-hydroxymorphinan series, and an *N*-(γ-cyanopropyl) derivative of (±)-normetazocine was also prepared.

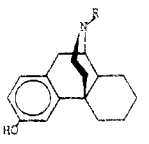
These compounds were examined for their antinociceptive effect in mice and receptor binding affinity to rat brain homogenates, and some of them were examined for acute toxicity (in mice), in single-dose suppression studies (SDS) and in precipitated withdrawal (NW) tests in monkeys.

The *N*-(β-cyanoethyl) and *N*-(γ-cyanopropyl) (±)-normetazocines have previously been noted to have antinociceptive activity.[1] The former was found to be 50% more potent than morphine and the latter was stated as being about half as active in an acetic acid induced writhing test in mice, by interperitoneal injection.[1] Neither compound was noted to produce an abstinence syndrome in rats, during withdrawal, after administration of 20 (mg/kg)/day for 4 weeks.[1] Also, *N*-(β-cyanoethyl)normorphine was prepared ca. 30 years ago and found to have little antinociceptive activity and no narcotic antagonist activity;[2] its affinity for the opiate receptors could not be obtained at that time, of course.

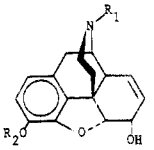
Chemistry. Using the general procedure outlined under the Experimental Section, the *N*-(β-cyanoethyl) compounds [(±)-**1b**, (+)-**1b**, (−)-**1b**, (+)-**2b**, (−)-**2b**, (−)-**3c**, (−)-**3d**, and (−)-**4d**] were prepared by the addition of acrylonitrile to the appropriate *N*-nor base. Alkylation of the *N*-nor bases with chloroacetonitrile or 4-bromobutyronitrile (see Experimental Section) gave the cyanomethyl compounds (±)-**1c** and (−)-**2c** and the *N*-(γ-cyanopropyl) derivative (±)-**1d**, respectively. Physical data are listed in Table I for the bases and their salts.



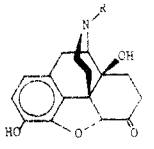
(±)-**1a**, (+)-**1a**, (−)-**1a**: R = H
(±)-**1b**, (+)-**1b**, (−)-**1b**: R = CH_2CH_2CN
(±)-**1c**: R = CH_2CN
(±)-**1d**: R = $CH_2CH_2CH_2CN$



(−)-**2a**: R = H
(−)-**2b**: R = CH_2CH_2CN
(−)-**2c**: R = CH_2CN
(+)-**2a** and (+)-**2b** are the antipodes of (−)-**2a** and (−)-**2b**



(−)-**3a**: R_1 = R_2 = H
(−)-**3b**: R_1 = H, R_2 = CH_3
(−)-**3c**: R_1 = CH_2CH_2CN, R_2 = H
(−)-**3d**: R_1 = CH_2CH_2CN, R_2 = CH_3



(−)-**4a**: R = H
(−)-**4b**: R = CH_2CH_2CN

Experimental Section

Melting points were determined in open capillary tubes using a Thomas-Hoover melting point apparatus and are corrected. Elemental analyses, listed in Table I, were performed by the Section on Microanalytical Services and Instrumentation of this laboratory. IR and mass spectra were obtained on a Perkin-Elmer 257 and Hitachi Perkin-Elmer RMU-6E spectrometer, respectively. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter.

***N*-Nor Compounds.** (±)-Normetazacine [(±)-**1a**] was resolved as previously described,[3] to give the enantiomers (+)- and (−)-**1a**. *N*-Demethylation of (−)-morphine and (−)-codeine by our refined procedure[4] gave (−)-**3a** and (−)-**3b**, respectively.

Addition of Acrylonitrile to *N*-Nor Compounds. (−)-*N*-(Cyanoethyl)normetazacine [(−)-**1b**]. The following procedure for the preparation of (−)-**1b** is representative. A mixture of 4.0 g (18.4 mmol) of (−)-**1a**, 1.08 g (1.34 mL, 20.3 mmol) of acrylonitrile, 7.08 g (9.75 mL, 70 mmol) of $(Et)_3N$, and 75 mL of EtOH was stirred under reflux until no (−)-**1a** remained by TLC analysis ($CHCl_3$–MeOH–concentrated NH_4OH (89:10:1), silica gel GF, I_2). The mixture was cooled, evaporated to an oil, and dissolved in Et_2O. The Et_2O solution was washed with H_2O, dried (Na_2SO_4), and evaporated, and the residue was crystallized from

Table I. Cyanoalkyl Opioids and Salts

compd	mp, °C	recrystn solvent	yield, %	formula	$[\alpha]^{23}_D$[a]	anal.
(±)-1b	165	Et_2O	85	$C_{17}H_{22}N_2O$		
(±)-1b·HBr	146-148	*i*-PrOH-H_2O	85[b]	$C_{17}H_{23}BrN_2O$		C, H, N
(+)-1b	109	Et_2O-pet. ether	88	$C_{17}H_{22}N_2O$		
(+)-1b·HBr	222-223	*i*-PrOH-H_2O	91[b]	$C_{17}H_{23}BrN_2O$	+77.1°	C, H, N
(-)-1b	108	Et_2O-pet. ether	84.5	$C_{17}H_{22}N_2O$		
(-)-1b·HBr	221.5-223	*i*-PrOH-H_2O	82[b]	$C_{17}H_{23}BrN_2O$	-77.4°	C, H, N
(±)-1c	oil		78	$C_{16}H_{20}N_2O$		
(±)-1c·HCl·0.5H_2O	168 (dec)	*i*-PrOH	55.5[c]	$C_{16}H_{21}ClN_2O·0.5H_2O$		C, H, N
(±)-1d	156	$CHCl_3$-pet. ether	94	$C_{18}H_{24}N_2O$		
(±)-1d·HBr	232-233	*i*-PrOH	74[b]	$C_{18}H_{25}BrN_2O$		C, H, N
(+)-2b	oil		90	$C_{19}H_{24}N_2O$		
(+)-2b·HCl·0.5H_2O	189[h]	*i*-PrOH-Et_2O	67[d]	$C_{19}H_{25}ClN_2O·0.5H_2O$	+58.5°	C, H, N
(-)-2b	oil		95	$C_{19}H_{24}N_2O$		
(-)-2b·HCl·H_2O	188[h]	*i*-PrOH-Et_2O	74[d]	$C_{19}H_{25}ClN_2O·H_2O$	-58.7°	C, H, N
(-)-2c	oil		81	$C_{18}H_{23}N_2O$		
(-)-2c·HCl·CH_3COCH_3	174 (dec)[i]	acetone	69	$C_{18}H_{22}N_2O·HCl$	-52.8°	C, H, N
(-)-3c	223-224[e,f]		94	$C_{19}H_{20}N_2O_3$		
(-)-3c·HCl	233-236	*i*-PrOH	98[b]	$C_{19}H_{21}ClN_2O_3$	-107.0°	C, H, N
(-)-3d	178-179[e]		85	$C_{20}H_{22}N_2O_3$		
(-)-3d·CH_3SO_3H·2H_2O	129-130 (dec)	EtOH	88	$C_{21}H_{26}N_2O_6S·2H_2O$	-95.8°	C, H, N
(-)-4b	176-178[g]	Et_2O-pet. ether	96	$C_{19}H_{20}N_2O_4$		
(-)-4b·HBr·H_2O	218-222	85% AcOH	76[b]	$C_{19}H_{21}BrN_2O_4$	-138.7°	C, H, N

[a] Determined in MeOH, $c \sim 1.0$. [b] Based on free base. [c] Based on (+)-1a. [d] Based on (+)- or (-)-2a. [e] Crude after washing with Et_2O. [f] Lit.[16] mp 220-221 °C. [g] Phase change at 114-115 °C. [h] Softening at 160 °C. [i] Turned brown at 159 °C.

Table II. Biological Activities of *N*-(Cyanoalkyl) Opioids

compd	ED_{50}[a]	ED_{50}[b]	TR[c]	EC_{50}[d]	EC_{50}[e]	PDC[f] (SDS/NW)
(±)-1b	0.26 (0.20-0.34)	0.31 (0.20-0.46)		3		none[g]
(-)-1b	0.11 (0.09-0.14)	0.03 (0.02-0.05)	13 000	3	0.08	none[h]
(+)-1b	inactive[i]			>100 000		none[j]
(±)-1c	22.2 (14.9-32.8)	39.1 (26.2-58.6)		20		none[k]
(±)-1d	2.4 (1.8-3.2)	1.5 (1.2-2.1)		4		none[l]
(-)-2b	0.07 (0.05-0.10)	0.14 (0.09-0.21)	23 000	1.5	0.015	none[m]
(+)-2b	inactive[i]			~100 000		
(-)-2c	5.3 (3.8-7.8)	13.2 (9.4-18.8)		7		
(-)-3c	8.3 (6.1-11.4)	33.0 (22.4-48.2)		20	1.0[n]	none[o]
(-)-3d	inactive[p]			700		none[q]
(-)-4b	inactive[r]	inactive[i]		60		none[s]
morphine sulfate	3.3 (2.5-4.4)	4.1 (2.9-5.6)	900	3	1.5	high
(-)-metazocine hydrobromide	2.0 (1.4-2.7)	1.5 (1.0-2.2)		4	0.4	none
levorphanol tartrate	0.49 (0.25-0.74)	0.54 (0.39-0.74)		0.7	0.2	intermed
codeine phosphate	14.6 (11.1-18.9)	20.4 (13.8-29.7)		800		low[t]
oxymorphone hydrochloride	~0.27 (0.25-0.31)			0.25		high

[a] Eddy hot-plate assay for antinociceptive activity, in μmol/kg. Parentheses indicate probit analysis confidence interval (± 2 SE), subcutaneous injection, in mice.[5-7] [b] Nilsen assay for antinociceptive activity, in μmol/kg.[8] Parenthesized numbers and conditions are as in footnote a. [c] Therapeutic ratio = acute toxicity by subcutaneous injection, in mice, divided by ED_{50} from Eddy hot plate assay. [d] Binding constant from rat brain homogenates, in μmol/L.[13] [e] Binding constant from neuroblastoma × glioma (NG 108-15) cells, in μmol/L.[15] [f] Physical dependence capacity, in rhesus monkeys (SDS = single dose suppression; NW = precipitated withdrawal). [g] Salivation at all doses, and head tremors, convulsions, and ataxia at highest dose, in SDS from 0.25 to 2.0 mg/kg.[9] [h] No suppression noted in SDS from 0.16 to 2.56 mg/kg.[10b] Ataxia was noted at 0.32 to 2.56 mg/kg, tremors at 0.64 to 2.56 mg/kg, and rigidity at 2.56 mg/kg. Narcotic antagonist in NW (0.32 to 1.28 mg/kg). [i] None of the eight mice were effected at 100 mg/kg. [j] Minimal CNS depression in SDS from 1.5 to 12.0 mg/kg.[10b] [k] Caused CNS depression, not reversed by naloxone, in SDS from 0.625 to 5.0 mg/kg.[10a] [l] Exhibited narcotic antagonist activity in NW from 1.0 to 2.0 mg/kg. Ataxia and mild stupor were noted in SDS from 1.0 to 2.0 mg/kg.[10b] [m] May exacerbate withdrawal in SDS from 0.005 to 0.04 mg/kg. Salivation noted at 0.005 to 0.02 mg/kg. [n] Showed narcotic antagonist activity in adenylate cyclase assay[15] comparable to nalorphine. [o] Minimal CNS depression at some doses in SDS from 1.5 to 36.0 mg/kg. Some tremors noted at 36.0 mg/kg.[12] [p] Only four (out of ten) mice were effected at 100 mg/kg. [q] Some signs of withdrawal noted at 96.0 mg/kg, in NW from 24.0 to 96.0 mg/kg.[11] [r] Although five (out of ten) mice were effected at 50 mg/kg, a dose-effect relationship could not be obtained. [s] No effect in SDS from 10.0 to 40.0 mg/kg.[11] [t] Substituted completely for morphine at 12.0 mg/kg, in SDS from 3.0-12.0 mg/kg.[9]

Et_2O–petroleum ether to give 4.2 g (84.5%) of (-)-**1b** base; mp 108 °C. A solution of 4.0 g (14.8 mmol) of the base was dissolved in 17 mL of *i*-PrOH, and 2.6 mL of 48% HBr was added. After cooling, the crystalline salt was filtered, washed with *i*-PrOH, and dried to give 4.26 g of (-)-**1b**·HBr, mp 219–220 °C. Recrystallization from *i*-PrOH containing a few drops of H_2O gave pure material.

Alkylation of *N*-Nor Compounds. (±)-*N*-(γ-Cyanopropyl)normetazacine [(±)-1d]. Alkylation of the *N*-nor compounds was accomplished using the procedure described below for the preparation of (±)-**1d**. A mixture of 3.25 g (15.0 mmol) of (±)-**1a**, 2.29 g (15.5 mmol) of 4-bromobutyronitrile, 5.0 g (36.2 mmol) of anhydrous K_2CO_3, and 50 mL of dry DMF was heated at 110 °C for 2.5 h while stirring. After cooling the mixture, the inorganic material was filtered and washed with $CHCl_3$. The combined filtrate and washings were evaporated, dissolved in $CHCl_3$, washed with H_2O, and dried (Na_2SO_4). Evaporation of the solvent left a residue, which was crystallized from $CHCl_3$–

petroleum ether to yield 4.0 g (94%) of the base (±)-**1d**. A solution of 1.20 g (4.2 mmol) of (±)-**1d** in 25 mL of *i*-PrOH was treated with 0.8 mL of 48% HBr to give, after crystallization and recrystallization, 1.1 g (74%) of pure (±)-**1d**·HBr.

Pharmacology. In contrast to the former work,[1] we found that racemic *N*-(β-cyanoethyl)normetazocine [(±)-**1b**·HBr] was at least 12 times as potent as morphine in the hot-plate[5-7] or Nilsen[8] assays (in mice, subcutaneous injection; see Table II) and did not substitute for morphine in single-dose suppression (SDS) tests in rhesus monkeys.[9] To test the effect of the length of the side-chain vs. antinociceptive activity, we examined (±)-*N*-(cyanomethyl)- [(±)-**1c**·HCl·0.5H_2O] and (±)-*N*-(γ-cyanopropyl)normetazocine [(±)-**1d**·HBr]. Although the *N*-cyanomethyl group could be considered most similar, structurally, to an allyl moiety known to confer antagonist activity to *N*-nor multicyclic opioids, the compound did not appear to have narcotic antagonist activity. It neither suppressed nor precipitated the withdrawal syndrome in SDS tests.[10a] It had about one-seventh the potency of morphine. However, the (±)-*N*-(γ-cyanopropyl) derivative [(±)-**1d**·HBr] was morphine-like in antinociceptive potency. It was an atypical drug in SDS and NW studies in monkeys, appearing to have narcotic antagonist and CNS depressant activities.[10a,b] In the normetazocine series, the antinociceptive potency was greatest with the (−)-*N*-(β-cyanoethyl) compound [(−)-**1b**·HBr], which was found to be 30 times as potent as morphine in the hot-plate assay. Over a wide range of doses, this compound did not suppress morphine abstinence in monkeys.[10a]

The *N*-(β-cyanoethyl)normorphine [(−)-**3c**·HCl] was about one-third as potent as morphine as an antinociceptive agent, and the noroxymorphone and norcodeine derivatives, (−)-**4b**·HBr·H_2O and (−)-**3d**·HCl, respectively, were essentially inactive. Biological data for the corresponding *N*-methyl compounds are listed in Table II. None of these three cyano compounds substituted for morphine in SDS tests or appeared to have narcotic antagonist activity.[11,12]

The most unusual of the *N*-cyanoalkylated compounds was the (−)-3-hydroxy-*N*-(β-cyanoethyl)morphinan [(−)-**2b**·HCl·H_2O]. It was about 50 times more potent than morphine as an antinociceptive in the hot-plate assay, and its binding affinity to the opiate receptor, although high, was comparable to levorphanol. The antinociceptive activity of (−)-**2b**·HCl·H_2O was found to be readily reversed by naloxone. In the tail-flick assay (in Taconic Farm Swiss-Webster mice, sc injection) a 1.6 μmol/kg dose, which gave 100% antinociceptive activity, was completely antagonized by 8.5 μmol/kg of naloxone, 80% antagonism was obtained with 0.8 μmol/kg of naloxone and 46% antagonism was obtained with 0.08 μmol/kg of naloxone. Its potency in the neuroblastoma x glioma adenylate cyclase assay was comparable to that of etorphine, however. An SDS study indicated that the compound would not substitute for morphine, and it appeared to exacerbate the withdrawal syndrome.[9]

The (−)-3-hydroxy-*N*-(cyanomethyl)morphinan [(−)-**2c**·HCl], like the comparably substituted normetazocine, lost most of the antinociceptive activity. It was about half as potent as morphine.

The enantiomeric (+) compounds in the morphinan and benzomorphan series were inactive as antinociceptive agents.

Receptor Binding Affinity. The affinities of the various compounds for the opiate receptor from rat brain homogenates were surprising in that optimal binding, measured under standard conditions (i.e., 0.32 M sucrose–0.01 M Tris, pH 8.0, at 37 °C),[13] did not occur with the compounds which displayed the highest antinociceptive potency (Table II) in the homologous *N*-(cyanoalkyl)normetazocines. The (±)-*N*-(γ-cyanopropyl) homologue [(±)-**1d**·HBr] had an EC_{50} comparable with the (−)-cyanoethyl compound [(−)-**1b**·HBr]. It can, thus, be assumed that we may not have reached optimal receptor binding in this series. Further, we noted a greater stereospecificity in interaction with the opiate receptors between the (+)- and (−)-*N*-(β-cyanoethyl)morphinans and benzomorphans than we have ever observed heretofore, with any other enantiomeric pair. There was about five orders of magnitude difference in their binding affinities, compared with the ca. 100-fold difference between levorphanol and dextrorphan, and the 10 000-fold difference between (+)- and (−)-naloxone.[14]

Narcotic agonists have been found to exert an inhibiting effect on the opiate-sensitive adenylate cyclase of neuroblastoma x glioma hybrid (NG 108-15) cells.[15] The (−)-3-hydroxy-*N*-(cyanoethyl)morphinan was about 100 times as effective as morphine in this cyclase inhibition and was almost as effective as etorphine. However, the compound did not exhibit any effect which could be attributed to narcotic antagonist activity in this system. It appeared to have only agonist activity.

Therapeutic Ratio. The acute toxicity (LD_{50}, sc injection, mice) of (−)-(β-cyanoethyl)normetazocine [(−)-**1b**·HBr] was 489 mg/kg, which gave a therapeutic ratio (LD_{50}/ED_{50}) of ca. 13 000 (Table II). Thus, the β-cyanoethyl moiety appeared to confer little acute toxicity to the base. Morphine, a relatively nontoxic analgesic, has been found to have a therapeutic ratio of ca. 900 under these conditions. Similarly, the therapeutic ratio of the (−)-*N*-(β-cyanoethyl)morphinan [(−)-**2b**·HCl·0.5H_2O] (LD_{50} = 534 mg/kg) was found to be ca. 23 000. It was a remarkably nontoxic compound in mice.

Discussion

The *N*-(cyanoethyl) moiety causes an increase in antinociceptive activity in certain opioids (the *N*-norbenzomorphan and 3-hydroxymorphinan) and enhances their binding to the opiate receptor from rat brain homogenate, without increasing acute toxicity. The moiety apparently prevents these opioids from displaying the morphine-like property of sustaining the withdrawal syndrome in single-dose suppression tests in monkeys.

Although increased antinociceptive activity in the benzomorphan series was associated with increased receptor binding, that in the morphinan series was not, and binding decreased in the morphine series (Table II). The results with the adenylate cyclase assay may be better correlated with the in vivo data, since both (−)-*N*-(β-cyanoethyl)normetazocine and (−)-*N*-(β-cyanoethyl)-3-hydroxymorphinan are potent agonists in this test.

However, the *N*-(β-cyanoethyl) moiety does not effect all opioids in the same manner. It decreases antinociceptive activity in morphine and its derivatives and in 14-hydroxydihydromorphinone. One possible explanation for the disparate results from the various families of rigid multicyclic opioids could be their different metabolic transformations. Further work in this area will be explored, as well as further pharmacological work in other in vivo systems, to examine the narcotic antagonist activity of the morphinan and benzomorphan.

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Conformations, DNA Binding Parameters, and Antileukemic Activity of Certain Cytotoxic Protoberberine Alkaloids

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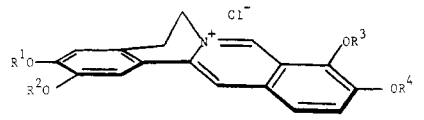
The tetrahydroprotoberberine alkaloids **5** and **7** possessing a *trans*-quinolizidine conformation display in vitro KB cytotoxicities in contrast to their corresponding diastereomers **4** and **6** which exist in the *cis*-quinolizidine conformation and are much less toxic. The DNA-binding parameters of these compounds as well as the protoberberine salts **1**, **8**, and **9** have been examined by equilibrium dialysis. Only the quaternary salts bind to DNA. The alcohol **5** showed low in vivo activity against leukemia P388 systems, while the quaternary salts **8** and **9** proved to be toxic to the host.

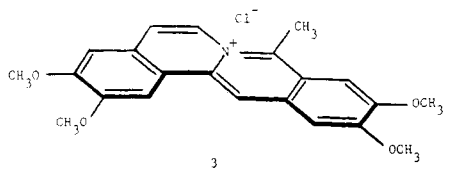
Berberine chloride (**1**) belongs to the protoberberine



1, $R^1R^2 = -CH_2-$; $R^3 = R^4 = CH_3$

2, $R^1 = R^2 = R^3 = R^4 = CH_3$

class of isoquinoline alkaloids found in a variety of plant tissues.[1,2] It antagonizes cholera toxin[3] and possesses broad spectrum antibiotic activity against Gram-positive and Gram-negative bacteria, fungi, and protozoa.[4-7] Berberine chloride (**1**) also causes the elimination of certain bacterial R factors by a hypothetical mechanism involving intercalation into closed circular superhelical plasmid DNA resulting in the formation of unnatural left-handed supercoils which cannot be replicated,[8-11] and its mutagenic action in yeast mitochondria may involve a similar mechanism.[12] Both berberine (**1**) and the structurally related natural product palmatine (**2**) are effective against experimental tumors,[13] and the synthetic dehydroprotoberberine coralyne (**3**) exhibits inhibitory activity against



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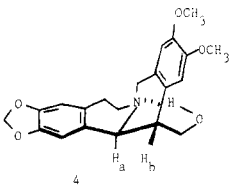
both leukemias L1210 and P388 in mice.[14-16]

A number of novel alkaloids recently became available to us during a program directed toward the development of new protoberberine syntheses.[17,18] The present communication describes observations on the in vitro cytotoxicities, conformations, DNA-binding properties, and in vivo antileukemic activities of several of these compounds.

The cytotoxicities of compounds **4**–**7** were determined



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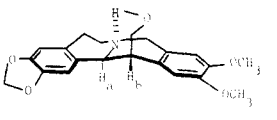

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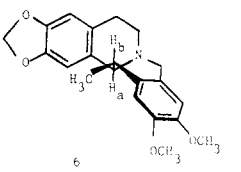

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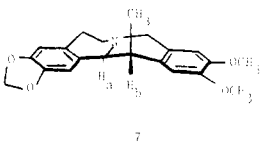

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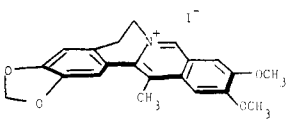

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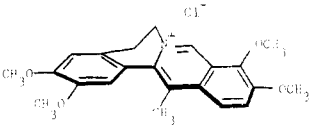

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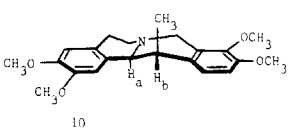

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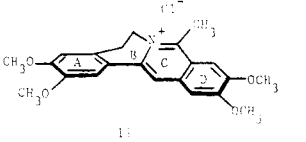

in KB cell culture (Table I). In searching for an explanation for the dramatic difference in cytotoxicities between the active compounds **5** and **7** and the inactive compounds

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