(ED_{50} > 125 mg/kg per day). The implied ability of cyclobut-A and cyclobut-G to cross the blood–brain barrier may be important in treating the neurological manifestations of AIDS. Despite its lower in vitro activity against HSV, cyclobut-A was as effective as cyclobut-G in protecting mice from a systemic challenge with 10 LD_{50}'s of HSV-1 (Table IV). In this model, intraperitoneal (ip) therapy with either agent was initiated 7 h postinfection and then continued twice a day for 4 days. Both drugs produced 100% survival at 18.7 mg/kg per day and again appeared to be more potent than ara-A. In comparison, acyclovir produced only 40% survival at 200 mg/kg per day. Cyclobut-A and cyclobut-G were also effective in a murine CMV model (Table V). In this experiment, an ip dose of 16.7 mg/kg of either cyclobut-A, cyclobut-G, or ganciclovir twice daily for 5 days increased survival from 20% in the untreated controls to 93, 87, and 100% respectively.

The broad-spectrum activity of cyclobut-A and cyclobut-G against herpesviruses and HIV warrants further development of these compounds as possible agents for the treatment of AIDS. Preliminary studies have found neither cyclobut-A or cyclobut-G to be acutely toxic to mice at 1000 mg/kg ip. Ongoing investigations will address the mechanisms of action and pharmacodynamics of these promising agents, as well as the biological activities of the individual enantiomers.

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Hydroxyethylamine Analogues of the p17/p24 Substrate Cleavage Site Are Tight-Binding Inhibitors of HIV Protease

Sir:

A key step in the replication of the human immunodeficiency virus (HIV) occurs when HIV-1 protease, the proteolytic enzyme encoded by the retrovirus, cleaves specific amide bonds in precursor *gag* and *pol* proteins to form the mature proteins needed for production of infectious viral particles.[1] Replacement of the catalytically active residues in protein precursors by site-directed mutagenesis techniques leads to the formation of noninfective virions,[2] and for this reason HIV protease is regarded as a potential target for developing agents for the treatment of acquired immunodeficiency syndrome (AIDS) and related diseases. Several X-ray crystal structures have established that the mature HIV protease is an aspartic proteinase that is formed from two identical 99 amino acid peptides, each subunit contributing one Asp-Thr-Gly unit.[3-6] The active site so formed closely resembles the active sites found in other well-characterized aspartic proteinases[7-9] and confirms some of the properties of HIV protease that were predicted on the basis of sequence homology.[10,11]

The discovery that mature HIV protease is an aspartic proteinase suggested to us and others[12a-e] that the general design strategy of replacing the P_1–P_1' cleavage point in substrates with transition-state analogues could be used to design tight-binding inhibitors of HIV protease, in the same way this approach was used to prepare inhibitors of other aspartic proteinases, e.g. pepsin, penicillopepsin, renin, cathepsin D.[13] We report herein the synthesis of hydroxyethylamine (HEA) dipeptidyl isosteres **1** and **2** that were designed to mimic the tetrahedral intermediate (**3**) for hydrolysis of Tyr-Pro, one of the partial substrate sequences cleaved by HIV protease (Figure 1). Incorporation of hydroxyethylamines **1** and **2** in peptides related

(1) Krausslich, H.-G.; Wimmer, E. *Ann. Rev. Biochem.* **1988**, *57*, 701.
(2) Seelmeier, S.; Schmidt, H.; Turk, V.; von der Helm, K. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 6612. Mous, J.; Heimer, E. P.; LeGrice, S. F. J. *J. Virol.* **1988**, *62*, 1433.
(3) Wlodawer, A.; Miller, M.; Jaskolski, M.; Sathyanarayana, B. K.; Baldwin, E.; Weber, I. T.; Selk, L. M.; Clawson, L.; Schmeider, J.; Kent, S. B. H. *Science* **1989**, *245*, 616.
(4) Navia, M. A.; Fitzgerald, P.; McKeever, B.; Leu, C-. T.; Heimbach, J.; Herber, W.; Sigal, I.; Darke, P. *Nature* **1989**, *337*, 615.
(5) Lapatto, R.; Blundell, T.; Hemmings, A.; Overington, J.; Wilderspin, A.; Wood, S.; Merson, J. R.; Whittle, P. J.; Danley, D. E.; Geoghegan, K. F.; Hawrylik, S. J.; Lee, S. E.; Scheid, K. G.; Hobart, P. M. *Nature (London)* **1989**, *342*, 299–302.
(6) Miller, M.; Sathyanarayanba, B. K.; Toth, M. V.; Marshall, G. R.; Clawson, L.; Selk, L.; Schneider, J.; Kent, S. B. H.; Wlodawer, A. *Science* **1989**, *246*, 1149.
(7) Bott, R.; Subramanian, E.; Davies, D. R. *Biochemistry* **1982**, *21*, 6956.
(8) James, M. N. G.; Sielecki, A. R.; Salituro, F.; Rich, D. H.; Hofmann, T. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 6137.
(9) Blundell, T. L.; Cooper, J.; Foundling, S. I.; Jones, D. M.; Atrash, B.; Szelke, M. *Biochemistry* **1987**, *26*, 5585.
(10) Toh, H.; Ono, M.; Saigo, K.; Miyata, T. *Nature* **1985**, *315*, 691.
(11) Pearl, L. H.; Taylor, W. R. *Nature* **1987**, 328, 482; *Ibid* **1987**, *329*, 351.
(12) (a) Moore, M. L.; Bryan, W. M.; Fakhoury, S. A., Magaard, V. W.; Huffman, W. F.; Dayton, B. D.; Meek, T. D.; Hyland, L.; Dreyer, G. B.; Metcalf, B. W.; Strickler, J. E.; Gorniak, J. G.; Debouck, C. *Biochem. Biophys. Res. Commun.* **1989**, *159*, 420. (b) Dreyer, G. B.; Metcalf, B. W.; Tomaszek, T. A., Jr.; Carr, T. J.; Chandler, A. C., III; Hyland, L.; Fakhoury, S. A.; Magaard, V. W.; Moore, M. L.; Strickler, J. E.; Debouck, C.; Meek, T. D. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 9752–9756. (c) Sigal, I. S.; Huff, J. R.; Darke, P. L.; Vacca, J. P.; Young, S. D.; Desolms, J. S.; Thompson, W. J.; Lyle, T. A.; Graham, S. L.; Ghosh, A. K., European Patent Appln. #0337714, 1988. (d) McQuade, T. J.; Tomasselli, A. G.; Lui, L.; Karacostas, V.; Moss, B.; Sawyer, T. K.; Heinrikson, R. L.; Tarpley, W. G. *Science* **1990**, *247*, 454–456. (e) Billich, S.; Knoop, M.-T.; Hansen, J.; Strop, P.; Sedlacek, J.; Mertz, R.; Moelling, K. *J. Biol. Chem.* **1988**, *263*, 17905–17908.

0022-2623/90/1833-1285$02.50/0

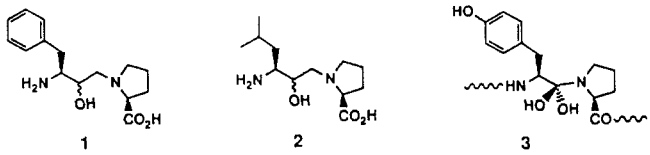
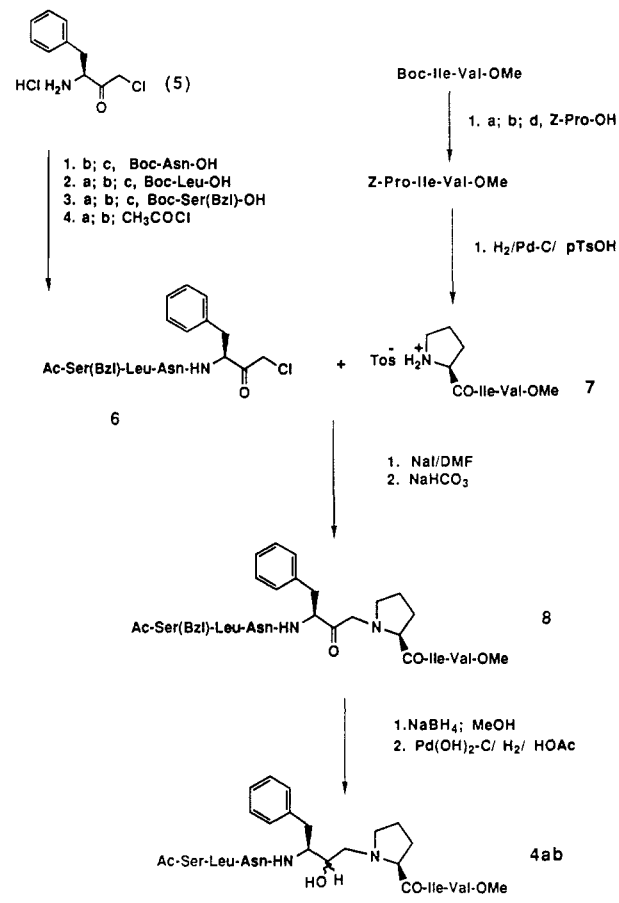


Figure 1. Structures of hydroxyethylamine analogues of p17/p27 Tyr-Pro substrate cleavage sites: **1**, hydroxyethylamine corresponding to Phe-Pro sequence; **2**, hydroxyethylamine corresponding to Leu-Pro sequence; **3**, structure of a tetrahedral intermediate for the enzyme-catalyzed cleavage of substrate Tyr-Pro bonds.

to the p17/p24 substrate sequence[14] produces tight-binding inhibitors of HIV protease. HEA inhibitor **4** has a K_i = 0.66 nM. A preliminary description of the X-ray crystal structure of inhibitor **4** bound to synthetic HIV protease has been presented.[15]

HEA inhibitor **4** was synthesized (Scheme I) by modification of the methods reported by Gordon and co-workers for the synthesis of other ketomethyleneamine and hydroxyethylamine inhibitors of ACE and renin.[16] Phenylalanine chloromethyl ketone (**5**)[17] was coupled stepwise with Boc-asparagine, Boc-leucine, Boc-(*O*-benzyl)serine, and acetyl chloride to give the corresponding protected tetrapeptide chloromethyl ketone **6**. Leucine, which occupies the P_3 site in the p66/p51 substrate site, was placed in P_3 in place of glutamine for synthetic convenience. Right-hand fragment **6** was prepared from Boc-Ile-Val-OMe[18] by standard methods.[19] In the key reaction leading to **4**, tetrapeptide chloromethyl ketone **6** was allowed to react with proline tripeptide **7** in DMF in the presence of NaI and $NaHCO_3$. Ketomethyleneamine (KMA) **8** obtained by this procedure was reasonably pure, but is not stable to prolonged storage. Attempts to purify **8** and related ketones by chromatography led to significant epimerization of the carbon center α to the ketone group. Consequently, crude KMA **8** was immediately reduced to hydroxyethylamines **4a,b** by reaction with excess $NaBH_4$. Purification of **4ab** by chromatography on silica gel, by eluting with methanol in chloroform, gave pure **4a,b** in 46% yield as a mixture of diastereomers. The diastereomeric alcohols **4a,b** did not separate upon chromatography, and their relative potencies have not yet been determined, although it is likely that the *S* diastereomer **4a** is more active since this is the diastereomer present in the X-ray structure.[15] The other new inhibitors (**9**–**18**) listed in Table I were prepared by closely related procedures. Full details for the preparation of **4** are provided as supplementary material.[32]

Scheme I[a]



[a] (a) 4 N HCl/dioxane; (b) *N*-methylmorpholine; (c) EDCl/HOBt;[19] (d) isobutyl chloroformate.

Inhibition of either synthetic HIV protease[20] (in which the two Cys residues, Cys^{67} and Cys^{95}, have been replaced by the isosteric L-α-aminobutyric acid) or cloned material expressed in *E. coli*[21] was measured by using the HPLC[22a] and spectrofluorometric assays[22b] described by Toth et al. In all cases examined, both preparations gave identical results.[22a] Ac-Thr-Ile-Met-Met-Gln-Arg-NH_2 (K_m = 1.4 mM, V_{max} = 725 nmol/min per mg) was used as the substrate. For the inhibitor assays, a solution of the inhibitor in DMSO (final concentration 1–2%) was added to the

(13) (a) For a discussion of the relationships between transition-state mimics and the structure of the tetrahedral intermediate, see: Rich, D. H.; Northrop, D. B. Enzyme Kinetics in Drug Design: Implications of Multiple Forms of Enzyme on Substrate and Inhibitor Structure–Activity Correlations. *Computer-Aided Drug Design*; Perun, T. J., Probst, C. L., Eds.; Marcel Dekker, Inc.: New York, 1989; pp 185–250. (b) Rich, D. H. Peptidase Inhibitors. *Comprehensive Medicinal Chemistry*; Sammes, P. G., Ed.; Pergamon Press: Oxford, Vol. 2, in press and references therein.

(14) For a review of retroviral proteases including substrate sequences, see: Skalka, A. M. *Cell* **1989**, *56*, 911.

(15) Miller, M.; Swain, A. L.; Jaskolski, M.; Sathyanarayana, B. K.; Marshall, G. R.; Rich, D. H.; Kent, S. B. H.; Wlodawer, A. In *Retroviral Proteases: Control of Maturation and Morphogenesis*; Pearl, L., Ed.; MacMillan Press: New York, in press.

(16) (a) The hydroxyethylamine (HEA) and ketomethyleneamine (KMA) units were originally designated amino alcohols and amino ketones. Natarajan, S.; Gordon, E. M.; Sabo, E. F.; Godfrey, J. D.; Weller, H. N.; Puscec, J.; Rom, M. B.; Cushman, D. W. *Biochem. Biophys. Res. Commun.* **1984**, *124*, 141. Gordon, E. M.; Natarajan, S.; Pluscec, J.; Weller, H. N.; Godfrey, J. D.; Rom, M. B.; Sabo, E. F.; Engebrecht, J.; Cushman, D. W. *Biochem. Biophys. Res. Commun.* **1984**, *124*, 148. Gordon, E. M.; Godfrey, J. D.; Pluscec, J.; Von langen, D.; Natarajan, S. *Biochem. Biophys. Res. Commun.* **1985**, *126*, 419. We suggest the present nomenclature since it is derived from nomenclature currently used in the peptide mimetic field. (b) Ryono, D. E.; Free, C. A.; Neubeck, R.; Samaniego, S. G.; Godfrey, J. D.; Petrillo, E. W. In *Peptides: Structure and Function. Proceedings of the Ninth American Peptide Symposium*; Deber, C. M., Hruby, V. J., Kopple, K. D., Eds.; Pierce Chemical Co., Rockford, IL, 1985; p 739.

(17) Fittkau, S. J. *Prakt. Chem.* **1973**, *315*, 1037.

(18) Lloyd, K.; Young, G. T. *J. Chem. Soc. C* **1971**, 2890.

(19) *The Peptides: Analysis, Synthesis, Biology*. Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1980; Vol. 1.

(20) Schneider, J.; Kent, S. B. H. *Cell* **1988**, *54*, 363.

(21) Recombinant enzyme provided by Dr. George Glover, Monsanto Co.

(22) (a) Toth, M. V.; Chiu, F.; Glover, G.; Kent, S. B. H.; Ratner, L.; Heyden, N. V.; Green, J.; Rich, D. H.; Marshall, G. R. In *Proceedings of the 11th American Peptide Symposium*; Rivier, J., Ed.; ESCOM: Leiden, in press. (b) Toth, M. V.; Marshall, G. R., submitted for publication.

Table I. Inhibition Constants for HIV Protease Inhibitors[a,b]

no.	structure	K_i, nM
4	Ac-Ser-Leu-Asn-[Phe-HEA-Pro]-Ile-Val-OMe	0.66[f]
9	Ac-Ser-Leu-Asn-[Phe-HEA-Pro]-Ile-OMe	420
10	Ac-Leu-Asn-[Phe-HEA-Pro]-Ile-Val-OMe	21
11	Ac-Leu-Asn-[Phe-HEA-Pro]-Ile-OMe	4520
12	Ac-Ser-Leu-Asn-[Leu-HEA-Pro]-Ile-Val-OMe	11[g]
13	Ac-Leu-Asn-[Leu-HEA-Pro]-Ile-Val-OMe	21
14	Ac-Ser-Leu-Asn-[Leu-HEA-Pro]-Ile-OME	980
15	Ac-Leu-Asn-[Leu-HEA-Pro]-Ile-OMe	>10000
16	Ac-Ser-Leu-Asn-[Sta-]-Ile-Val-Ome	1060
17	Boc-Ser-Leu-Asn-[AHPPA-]-Ile-Val-OMe	2640
18	Ac-Ser-Leu-Asn-[AHPPA-]-Ile-Val-OMe	8000
19	Ac-Ser-Gln-Asn-Phe[CH_2N]Pro-Val-Val-NH_2	13000
20	Ac-Thr-Ile-Nle[CH_2NH]Nle-Gln-Arg-NH_2	780
21	Ac-Ser-Gln-Asn-[AHPPA-]-Val-Val-OMe	39000
22	Boc-Phe-[CHOHCH$_2$]Phe-Ile-Phe-OMe	0.6[c]
23	Ala-Ala-Phe[CHOHCH$_2$]Gly-Val-Val-OMe	18[d]
24	^{t}BuAc-Cha[CHOHCH$_2$]Val-Ile-Amp	70[e]

[a] Initial rates were determined at pH = 6.4, 37 °C, as described previously[22] and by using the fluorometric assay of Toth and Marshall.[22b] Error estimates are derived from multiple independent determinations. Each K_i represents the average of three or more independent determinations. The substrate Ac-Thr-Ile-Met-Gln-Arg-NH_2 was varied from 0.5 to 10 mM. Inhibitor assays contained 2–3% DMSO final concentration. [b] The structures of [Phe-HEA-Pro] and [Leu-HEA-Pro] are given in Figure 1. [HEA] indicates the hydroxyethylamine isostere reported by Gordon et al[16]; [CH_2NH] indicates the reduced amide isostere; Sta = statine, (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid; AHPPA = (3*S*,4*S*)-4-amino-3-hydroxy-5-phenylpentanoic acid; Amp = 2-(aminomethyl)pyridine; Cha = cyclohexylalanine. [c] Taken from ref 12c. [d] Taken from ref 12b. [e] Taken from ref 12d. [f] Error = ±0.24 nM. [g] Error = ±5 nM.

protease, and the resulting solution was preincubated for 10 min prior to starting the reaction by the addition of substrate. Inhibition constants (K_i; Table I) were calculated by fitting the initial velocity data to the equation for competitive inhibition as previously described for another aspartic protease.[23]

Systematic variation of the structure of inhibitor **4** (Table I) established that for the sequence related to the p17/p24 cleavage sequence, maximal binding was achieved with peptides which span subsites P_4–$P_{3'}$. Replacement of the benzyl side chain in the P_1 position with an isobutyl group weakened binding to the enzyme. Incorporation of a $P_{3'}$ substituent is important for tight binding to the enzyme for both series of analogues. However, binding of hydroxyethylamines derived from Leu-Pro to HIV protease was not significantly affected by incorporation of serine at P_4. Other structure–activity results (data not shown) suggest that these related HEA derivatives probably bind to the HIV protease in slightly different modes in spite of their closely related structures. Ketomethyleneamine precursors of **4** and **9–15** (not shown) are weak inhibitors of HIV protease, suggesting that the hydroxyl group on the HEA unit, or a reasonable equivalent, will be needed for full inhibitory activity. Replacement of the HEA unit with statine[24] or the corresponding aromatic analogue of statine, AHPPA,[25] gave only moderately active HIV protease inhibitors (Table I). Analogues **16–18** and **21** are essentially equipotent with related inhibitors derived from other substrate sequences.[12,22] It is possible that any differences are due to slight variations in experimental procedures.[26]

Many potent inhibitors of aspartic proteinases[27] have been designed by application of the transition-state analogue hypothesis[28] or by modification of the naturally occurring aspartic proteinase inhibitors[29] or by systematic structure–activity studies.[13b] Much of this work has been stimulated by the desire to develop novel inhibitors of renin for use as antihypertensive drugs.[30] In all cases, the most potent aspartic proteinase inhibitors contain a critical hydroxyl group (or its equivalent) that binds to the catalytic aspartic acid carboxyl groups in the enzyme–inhibitor complex. This hydroxyl group and adjacent tetrahedral carbon atom (or equivalent) are thought to mimic a reaction pathway intermediate for enzyme-catalyzed hydrolysis of amide bonds, and analogues that lack the critical hydroxyl group are orders of magnitude weaker inhibitors.[13,24] Our results with hydroxyethylamine inhibitor **4** are consistent with this pattern in that hydroxyethylamine inhibitor **4** is over 10 000-fold more potent than the closely related aminomethylene inhibitor **19**, which lacks the hydroxyl group.[20] Furthermore, in the crystal structure of the HIV protease–inhibitor **4** complex described by Miller et al.[15] the hydroxyl group in **4** is hydrogen bonded to the two catalytically active aspartic acid residues, as is expected for an analogue that mimics a reaction pathway intermediate.[13a,24] We anticipate that the hydroxyl group in the hydroxyethylene isosteres[31] used to prepare HIV protease inhibitors **22–24**[12b,d,e] will be bound in this enzyme's active site in a closely related fashion.

The remarkable binding of the Phe-Pro hydroxyethylamine moiety in peptide **4** to HIV protease provides an additional structural unit that can be used to generate tight-binding inhibitors of HIV protease. Hydroxyethylamine **4** is a versatile starting point for developing analogues with the pharmacodynamic properties that are required to obtain a clinically useful anti-HIV drug. The convergent route employed to synthesize **4** can be readily modified to generate analogues that probe the enzyme's binding pockets and that lead to metabolically stable analogues. Most importantly, the availability of the high-resolution crystal structure of inhibitor **4** complexed with HIV protease[15] provides medicinal chemists with the rare opportunity to design improved inhibitors of a therapeutically important enzyme by analyzing the crystal structure of a tight-binding inhibitor bound to the clinically relevant enzyme.

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(23) Rich, D. H.; Sun, E. T. O. *Biochem. Pharmacol.* **1980**, *29*, 2205.
(24) Rich, D. H. *J. Med. Chem.* **1985**, *28*, 263.
(25) Rich, D. H.; Sun, E. T. O. *J. Med. Chem.* **1980**, *23*, 27.
(26) (a) Richards, A. D.; Broadhurst, A. V.; Ritchie, A. J.; Dunn, B. M.; Kay, J. *FEBS Lett.* **1989**, *253*, 214. (b) Richards, A. D.; Roberts, R. F.; Dunn, B. M.; Graves, M. C.; Kay, J. *FEBS Lett.* **1989**, *247*, 113.
(27) Rich, D. H. In *Proteinase Inhibitors: Research Monographs in Cell and Tissue Physiology*; Barrett, A. J., Salvesen, G., Eds.; Elsevier Science Publishers: Amsterdam, 1986; Vol 12, pp 179–217.
(28) Wolfenden, R. *Acc. Chem. Res.* **1972**, *5*, 10–18. *Ibid. Ann. Rev. Biophys. Bioeng.* **1976**, *5*, 271–306.
(29) Boger, J.; Lohr, N. S.; Ulm, E. H.; Poe, M.; Blaine, E. H.; Fanelli, G. M.; Lin, T.-Y.; Payne, L. S.; Schorn, T. W.; LaMonte, B. I.; Vassil, T. C.; Stabilito, I. I.; Veber, D. F.; Rich, D. H.; Boparai, A. S. *Nature (London)* **1983**, *303*, 81.
(30) Greenlee, W. *J. Pharmaceutical Res.* **1987**, *4*, 364 and references therein.
(31) Szelke, M.; Leckie, B.; Hallett, A.; Jones, D. M.; Sueiras, J.; Atrash, B.; Lever, A. F. Nature (*London*) **1982**, *299*, 555–557.
(32) All new compounds reported here were homogeneous by TLC or HPLC and were characterized by their satisfactory IR, NMR, and high-resolution FAB mass spectral data.

Supplementary Material Available: Experimental procedures for the synthesis of hydroxyethylamine inhibitors **4a,b** (20 pages). Ordering information is given on any current masthead page.

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Articles

Hypoxia-Selective Antitumor Agents. 4. Relationships between Structure, Physicochemical Properties, and Hypoxia-Selective Cytotoxicity for Nitracrine Analogues with Varying Side Chains: The "Iminoacridan Hypothesis"

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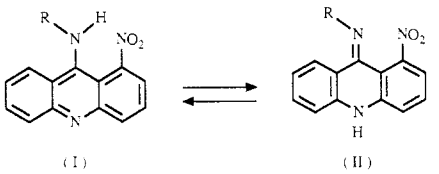
The nitroacridine derivative nitracrine is a potent hypoxia-selective cytotoxin for mammalian cells in culture. In an attempt to modulate the degree of hypoxia selectivity among this class of compounds, we have studied a series of side-chain analogues of nitracrine. Both the electronic and steric properties of the side chain are shown to be important in determining the hypoxia selectivity of the compounds, by controlling the degree of aminoacridine/iminoacridan tautomerism. Studies with the repair-defective Chinese hamster cell line UV4 indicate that the cytotoxicity of all the compounds is due to nitro group reduction and subsequent macromolecular adduct formation. However, compounds such as the 9-amino derivative, which exist totally as the aminoacridine tautomer, form much less lethal lesions than the 9-alkylamino derivatives, which exist to varying degrees in the iminoacridan conformation. For the whole set of compounds, the degree of hypoxia-selective cytotoxicity correlates well with the proportion of iminoacridan tautomer present.

Nitracrine (**2**) is a nitroacridine DNA-intercalating ligand that has been used clinically as an anticancer drug.[1,2] We have previously shown[3] that nitracrine has potent hypoxia-selective cytotoxicity against tumor cells in culture. This property is of considerable interest, since hypoxic cells are relatively resistant to ionizing radiation and to most chemotherapeutic agents. Nitracrine represents a novel approach to the selective targeting of hypoxic tumor cells since it combines a readily reduced nitro group (the source of hypoxia-selective cytotoxicity via reductive bioactivation[4]) with a DNA-intercalating chromophore, which may enhance cytotoxicity by targeting reduced metabolites to DNA.

Although the net metabolic reduction of nitracrine is partially inhibited by oxygen (due to back-oxidation of the initial radical anion[4]), absolute rates of metabolism are high even in well-oxygenated tissue.[4] This leads to two problems which appear to limit activity against hypoxic cells in solid tumors. Firstly, metabolic activation in normal tissues with high nitroreductase content, such as liver,[4] probably contributes to host toxicity. Secondly, studies with multicellular spheroids indicate that metabolic consumption of drug in relatively well-oxygenated tissue is one of the important determinants restricting diffusion to the target hypoxic microenvironments.[4] The development of nitroacridines with clinical utility against hypoxic tumor cells will require analogues with improved metabolic stability and/or more effective inhibition of metabolism (and cytotoxicity) by oxygen.

Scheme I



Improved metabolic stability has been sought by using a series of acridine-substituted analogues of nitracrine, where the nitro group reduction potentials are modulated

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(1) Radzikowski, C.; Ledochowski, A.; Hrabowska, M.; Stefanska, B.; Horowska, B.; Konopa, J. *Arch. Immunol. Ther. Exp.* **1969**, *17*, 99. *WHO Chron. (WHO, Geneva)* **1976**, *30*, No. 3 (Supplement), 11.
(2) Denny, W. A.; Baguley, B. C.; Cain, B. F.; Waring, M. J. In *Aspects of Anticancer Drug Action*; Neidle, S., Waring, M. J., Eds.; MacMillan: London, 1983; pp 1-34.
(3) Wilson, W. R.; Denny, W. A.; Twigden, S. J.; Baguley, B. C.; Probert, J. C. *B. J. Cancer* **1984**, *49*, 215.
(4) Wilson, W. R.; Denny, W. A.; Stewart, G. M.; Fenn, A.; Probert, J. C. *Int. J. Radiat. Oncol. Biol. Phys.* **1986**, *12*, 1235.

0022-2623/90/1833-1288$02.50/0