Specific Inhibition of HIV-1 Protease by Boronated Porphyrins

The rapid spread of human immunodeficiency virus (HIV), the causative agent of acquired immunodeficiency syndrome (AIDS), throughout the world has prompted an intense search for antiretroviral therapeutics. An analysis of nonpeptide compounds with useful pharmacological properties led us to test the ability of porphyrins to inhibit HIV protease (HIV PR). Determination of the IC_{50}'s of natural porphyrins on purified recombinant HIV-1 and HIV-2 PRs revealed that they are micromolar inhibitors of these enzymes. Availability of a series of carboxyl carborane ester derivatives of porphyrins synthesized as experimental neutron-capture therapeutics led to the discovery that compound 1, the tetrakiscarborane carboxylate ester of 2,4-bis-(α,β-dihydroxyethyl)deuteroporphyrin IX[1,2] Table I), is a submicromolar inhibitor of HIV protease. This compound is also capable of inhibiting HIV-1 viral polyprotein processing in cultured mammalian cells (ex vivo).

The in vitro effect of the porphyrin derivatives on HIV-1 and HIV-2 PR activity was examined by monitoring the cleavage of a decapeptide substrate.[3] The IC_{50} values for a series of porphyrin-based compounds measured on HIV-1 and HIV-2 PRs are shown in Table I. Since many of the derivatives were insoluble in buffer alone, 5% DMSO was used to increase solubility and to allow a comparison of the binding affinities of the various compounds. The best inhibitor is 1, with an IC_{50} of 185 nM for HIV-1 PR and 700 nM for HIV-2 PR. The inhibitory potency of the porphyrin derivatives for HIV-2 PR is generally a factor of 2–5 less than for HIV-1 PR. Dipotassium salts of several of the inhibitors were soluble in aqueous solution and were assayed in the absence of DMSO. The IC_{50} values are shown in Table II.

The photosensitivity of 1 made the metalloporphyrin derivatives (3, 5, and 6) preferable for subsequent kinetic analysis. These were easily prepared in high yield by standard techniques, as described in the supplementary material. Initial enzyme rates were fit to the Michaelis–Menten equation and kinetic constants were calculated using a nonlinear regression program.[4] A Dixon plot for 5 is shown in Figure 1. The appearance of the intersecting inhibition curves is consistent with a competitive mode of inhibition, with an inhibition constant (K_i) of 140 ± 25 nM. Compound 3 also appears to be a competitive inhibitor, with a K_i of 85 ± 5 nM (data not shown). The effect of salt concentration on inhibition by 3 was examined. The IC_{50} in 0.3 M NaCl (85 nM) was similar to the value obtained with 1 M NaCl (100 nM). This low ionic strength dependence of inhibition is important for in vivo applications.

The compounds assayed can be divided into three

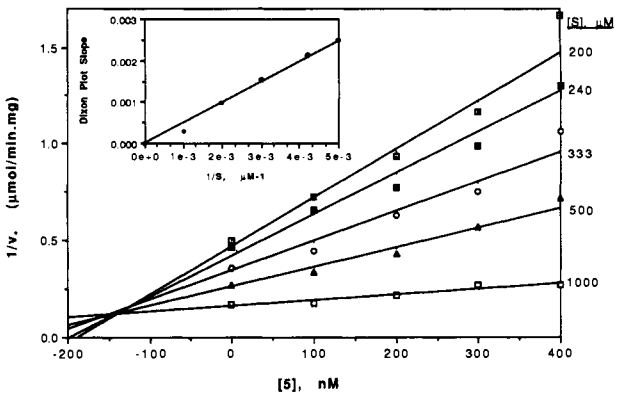

Figure 1. Dixon plot of 5 inhibition of HIV-1 PR. Purified HIV-1 PR (6×10^{-4} mg/mL) was incubated with 5 in 50 mM sodium acetate buffer, pH 5.5, containing 1 mM dithiothreitol, 1 mM EDTA, and 1 M NaCl. After 1 min, the substrate peptide was added to give the final substrate concentrations shown. The assay solutions were incubated for 30–45 min at 37 °C and enzyme activity was determined by quantitation of the hydrolysis products on HPLC.

classes: (1) carborane esters, (2) noncarborane esters, and (3) protoporphyrin IX. The best inhibitor in vitro is 1, which is esterified with four molecules of 1,2-dicarba-*closo*-dodecaboranecarboxylic acid. Although complexation with Co(II) or Cu(II) weakens binding about 10-fold, addition of Mn(III) has only 2-fold effect on inhibition, indicating that the hexacoordinate Mn(III) may be able to make favorable ionic interactions with the enzyme. Removal of all four carborane moieties, as in 12, substantially reduces inhibition. Removal of only two cages, as in 2, has little effect on binding. This suggests that only two of the four *closo*-carborane cages are responsible for most of the binding interaction. The metacarborane isomer, 9, binds approximately 60-fold less tightly, indicating that not only the presence of the carborane groups but also their isomeric conformation is important. Adding a methyl group to the unsubstituted carborane cage CH, as in 4, also substantially decreases the binding affinity. The carborane cages thus appear to have a specific interaction with HIV PR which results in high affinity between the molecule and enzyme. Replacement of the carborane cages with similarly sized but less hydrophobic groups such as benzoyl (10), adamantoyl (7), or even β-napthoyl (11) groups gives inhibitors with IC_{50} values in the low micromolar range, suggesting the importance of supplying hydrophobic groups at these positions. The effect of the porphyrin derivatives on HIV-2 PR generally follows the same trend, with most of the IC_{50} values being several-fold higher.

The cytotoxicity of compounds (LD_{50}) and their ability to inhibit capsid protein processing ex vivo during 4-h incubations (IC_{50}) are shown in Table I. A plasmid which encodes the HIV-1 proviral genome, with the exception of the gp 160 envelope protein,[5] was stably introduced into the monkey cell line COS 7. Cloned progeny, COS A6 cells, constitutively release viral capsids into the media. Inhibition of polyprotein processing was determined by measuring the amount of p24 present in the viral capsid samples with an ELISA assay. The decrease in the amount of detectable p24 antigen correlated with a specific inhibition of HIV PR activity, as judged by the accumulation of capsid precursor in conjunction with a disappearance of the p24 mature protein band in Western blots (data not

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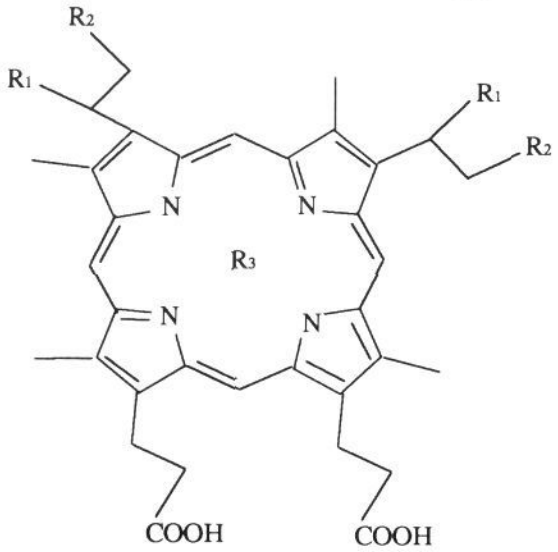
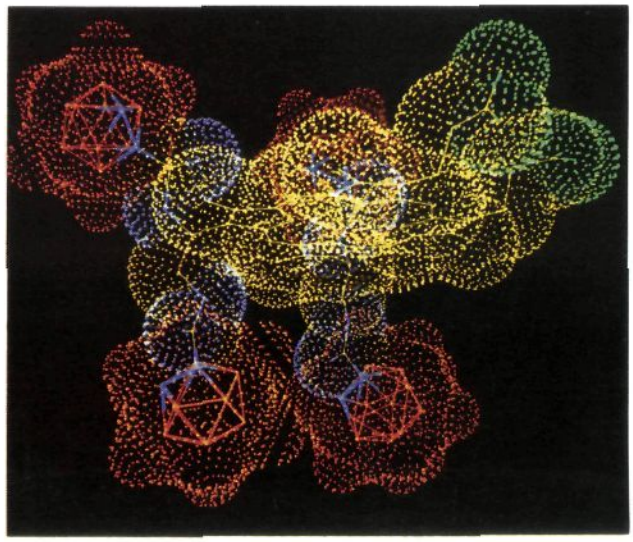
Table I. In Vitro and Cell Culture Potencies of Porphyrin Derivatives





entry	$R_{1,2}$	R_3	IC_{50}, in vitro,[a] μM HIV-1	IC_{50}, in vitro,[a] μM HIV-2	LD_{50},[b] μM	IC_{50}, ex vivo,[c] μM
1	$OCOB_{10}H_{11}C_2$	H_2	0.185	0.70	25	25
2	R_1 = H R_2 = $OCOB_{10}H_{11}C_2$	H_2	0.275	1.0	75	<50
3	$OCOB_{10}H_{11}C_2$	Mn(III)	0.40	1.2	70	65
4	$OCOB_{10}H_{14}C_3$	H_2	0.70	1.55	25	10
5	$OCOB_{10}H_{11}C_2$	Cu(II)	0.975	2.2	80	>70
6	$OCOB_{10}H_{11}C_2$	Co(II)	2.25	1.5	150	25
7	OCO(adamantoyl)	H_2	5	13	250	ND[d]
8	OCO-*p*-[$(CH_3)_2$N]benzoyl	H_2	7	18	>250	75
9	*m*-$OCOB_{10}H_{11}C_2$	H_2	12	11	250	>150
10	$OCOC_6H_5$	H_2	14	30	250	>100
11	OCO(β-naphthoyl)	H_2	14	23	250	>150
12	OH	H_2	280	480	250	>200
Protoporphyrin IX			300	500	<50	>200
U-75875[e]			<0.001	0.03	45	0.75

[a] Determined in the presence of 5% DMSO. [b] Cytotoxicity toward COS A6 cells cultured in the absence of FCS. [c] Cells were incubated with inhibitor in 2.5 mL of Dulbecco's phosphate-buffered saline. After 15 min, 2.5 mL of media containing 10% FCS as well as inhibitor was added. Cells were incubated for an additional 3.75 h, after which viral capsids were isolated from the culture supernatant. [d] Not determined. [e] Reference 7.

Table II. IC_{50} for Porphyrin Derivatives on HIV PR in the Absence of DMSO

compd	IC_{50}, μM HIV-1	IC_{50}, μM HIV-2	compd	IC_{50}, μM HIV-1	IC_{50}, μM HIV-2
1	0.05	0.230	4	0.90	0.550
3	0.10	0.70	5	0.725	0.470

Table III. IC_{50} for 1 on Viral and Cellular Aspartyl Proteases

protease	IC_{50}, μM	protease	IC_{50}, μM
renin	3	HIV-1	0.05
cathepsin D	10	HIV-2	0.23
pepsin	4	SIV	0.25

shown). The MTT stain assay was used to obtain LD_{50} values for all the compounds tested.[6] The peptidomimetic inhibitor U-75875 was used as a standard for HIV PR inhibition.[7]

We have observed that the presence of albumin prevents the ability of the compounds to inhibit HIV-1 PR during short-term incubations (0.25–4 h); therefore ex vivo IC_{50} values shown in Table I were determined in the presence of a reduced concentration of fetal calf serum (FCS). However, COS A6 cells require 10% FCS for optimal growth and viability and certain porphyrin derivatives show cytotoxicity toward COS A6 cells at <100 μM concentrations when cultured in the absence of FCS (Table I, LD_{50}). Cytotoxicity is reduced by culturing COS A6 cells in 10% FCS. Under those conditions, the LD_{50} for **1**–**12** is >250 μM (data not shown). Compound **1** has similar LD_{50} values in C6 glioma and V79 CHO cells (100–125 μM and ≥150 μM, respectively) in the presence of FCS, as measured by standard colony survival techniques (J. H. Hill and B. H. Laster, personal communication). Moreover, **1** is tolerated in mice at doses as high as 200 mg/kg, with no apparent signs of morbidity or mortality of animals.[1] Since mice receiving **1** at 100 mg/kg by iv bolus are exposed to peak plasma concentrations of approximately 900 μM, the ex vivo LD_{50} values determined here may not be representative of the in vivo tolerance of compound **1** or its metallo derivatives. A more serious consideration is the potential usefulness in clinical therapy of drugs which are inactivated by albumin. The antagonistic effect of albumin in cell culture may not reflect the situation in

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whole animals, where serum proteins can bind porphyrins and deliver them to various tissues rather than sequestering them.[8]

To address selectivity, the effect of 1 on other aspartyl proteases was evaluated. IC_{50} values of the water-soluble dipotassium salt of 1 measured in the absence of DMSO are shown in Table III. Compound 1 is approximately 5-fold more effective for HIV-1 PR than for the closely related HIV-2 and SIV PRs, and at least 60-fold more inhibitory for HIV-1 PR compared to cellular aspartyl proteases.

We have found that boronated porphyrins are inhibitors of HIV-1 PR and HIV-2 PR. Compound 1 is a submicromolar inhibitor in vitro and inhibits polyprotein processing in cell culture at micromolar concentrations. It selectively inhibits HIV PR over cellular aspartyl proteases. Kinetic studies on metalloporphyrin derivatives suggest that tetracarboranyl porphyrins are competitive inhibitors of HIV-1 PR. Compound 1 represents a new class of HIV PR inhibitors which are promising candidates for anti-AIDS therapeutics.

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Supplementary Material Available: Experimental procedures for the synthesis of carborane derivatives and in vitro and ex vivo assays are provided (6 pages). Ordering information is given on any current masthead page.

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