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ARTICLES

■ Supplementary material for this paper is available separately (consult the masthead page for ordering information); all supplementary material except structure factor tables will also appear following the paper in the microfilm edition of this journal.

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AUTHOR INDEX

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

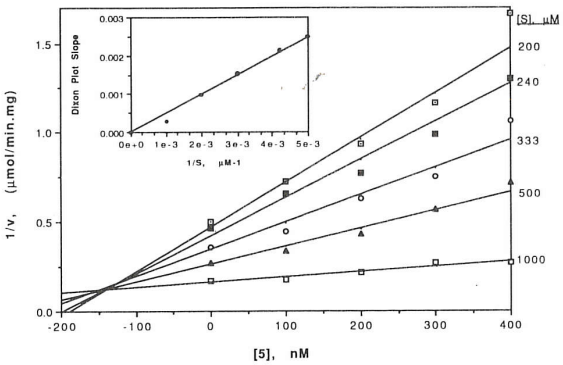

Figure 1. Dixon plot of **5** inhibition of HIV-1 PR. Purified HIV-1 PR (6×10^{-4} mg/mL) was incubated with **5** in 50 mM sodium acetate buffer, pH 5.5, containing 1 mM dithiothreitol, 1 mM EDTA, and 1 M NaCl. After 1 min, the substrate peptide was added to give the final substrate concentrations shown. 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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