From nonpeptide toward noncarbon protease inhibitors: Metallacarboranes as specific and potent inhibitors of HIV protease

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HIV protease (PR) represents a prime target for rational drug design, and protease inhibitors (PI) are powerful antiviral drugs. Most of the current PIs are pseudopeptide compounds with limited bioavailability and stability, and their use is compromised by high costs, side effects, and development of resistant strains. In our search for novel PI structures, we have identified a group of inorganic compounds, icosahedral metallacarboranes, as candidates for a novel class of nonpeptidic PIs. Here, we report the potent, specific, and selective competitive inhibition of HIV PR by substituted metallacarboranes. The most active compound, sodium hydrogen butylimino bis-8,8-[5-(3-oxa-pentoxy)-3-cobalt bis(1,2-dicarbollide)]di-ate, exhibited a K_i value of 2.2 nM and a submicromolar EC_{50} in antiviral tests, showed no toxicity in tissue culture, weakly inhibited human cathepsin D and pepsin, and was inactive against trypsin, papain, and amylase. The structure of the parent cobalt bis(1,2-dicarbollide) in complex with HIV PR was determined at 2.15 Å resolution by protein crystallography and represents the first carborane–protein complex structure determined. It shows the following mode of PR inhibition: two molecules of the parent compound bind to the hydrophobic pockets in the flap-proximal region of the S3 and S3′ subsites of PR. We suggest, therefore, that these compounds block flap closure in addition to filling the corresponding binding pockets as conventional PIs. This type of binding and inhibition, chemical and biological stability, low toxicity, and the possibility to introduce various modifications make boron clusters attractive pharmacophores for potent and specific enzyme inhibition.

rational drug design | aspartic proteases | carboranes | x-ray structure analysis | virostatics

HIV protease (PR) is responsible for cleaving viral polyprotein precursors into mature, functional viral enzymes and structural proteins. This process, called viral maturation, is required for the progeny virion to become replication competent and infectious. Chemical inhibition or inactivation by mutation of PR blocks the infectivity of the virus (1). PR has thus become a prime target for therapeutic intervention in AIDS. Academic and industrial research has led to the rapid development of eight effective inhibitors that are currently in clinical use, with several others still in the pipeline (for review, see refs. 2 and 3).

Despite the considerable success of rational drug design, the need for effective PR inhibitors (PIs) is still urgent. Most of the current PIs are pseudopeptide compounds with limited bioavailability and stability. Moreover, their clinical use is compromised by high production cost, various side effects, and rapid development of resistant viral strains (4). Therefore, there is a continuing need for the design of new PIs with an emphasis on broad specificity against PI-resistant HIV mutants (5, 6).

Molecular modeling and/or random testing of compound libraries revealed several PR inhibitors with unexpected structures. Most of the first-generation PIs were pseudopeptides. Some recent compounds involve nonpeptidic structures, such as cyclic ureas, sulfonamides, etc. (2). However, even inorganic compounds, Nb-containing polyoxometalates, specifically inhibit HIV PR with submicromolar EC_{50} values in tissue cultures (7). In this case, the inhibitors were shown to be noncompetitive, and a model suggested binding to the cationic pocket on the outer surface of the flaps. There is also an evidence for compounds with unexpected chemistry capable to target the active site of the enzyme. The HIV PR-binding cleft was shown to accommodate C_{60} fullerenes, hydrophobic and electrophilic spheric compounds, and some fullerene derivatives are indeed weak inhibitors of HIV PR (8–11).

We searched for other types of unconventional chemical structures that would fit into the PR-binding cleft, would be biologically stable, and would enable facile chemical modification. When screening a number of structural motifs, we identified a group of inorganic compounds, icosahedral boranes, carboranes, and namely 12-vertex metal bis(dicarbollides), as promising frameworks for a novel class of nonpeptide PIs. These boron/carbon clusters are polyhedra based on a three-dimensional skeleton with triangular facets.

Boron-containing polyhedral compounds have been intensively studied because of their use in boron neutron capture therapy (12) and in radioimaging (13, 14). From the structural point of view, the variety of known structural types of boranes, heteroboranes, and metallaboranes represents an interesting counterpart to organic compounds, especially to aromates. With the icosahedral cage being only slightly larger than the phenyl ring rotation envelope, carboranes were used as stable hydrophobic pharmacophores (e.g., refs. 15–17). There is little information on the use of carboranes as

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Abbreviations: MIA, mouse intracisternal A particles; PR, protease; PI, HIV PR inhibitor; PDB, Protein Data Bank.

Data deposition: The atomic coordinates and structure factors have been deposited in the Protein Data Bank, www.rcsb.org/pdb (PDB ID code 1ZTZ).

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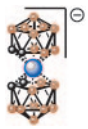
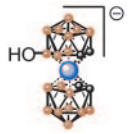
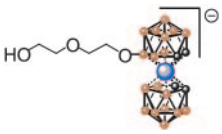
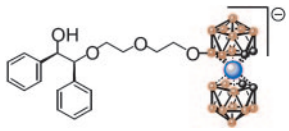
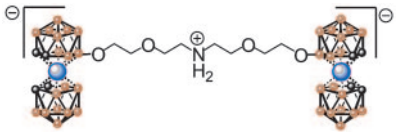
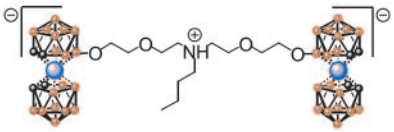
Table 1. Structures and activities of metallacarborane inhibitors

Compound no.	Structure	Molecular weight of anion	K_i, nM	EC_{50}, μM
1		323.74	66 ± 30	6
2		339.74	6,800 ± 500	20
3		427.85	2,500 ± 400	6
4		624.09	20 ± 5	13
5		837.71	4.9 ± 2.1	3
6		893.82	2.2 ± 1.2	0.25

All compounds were prepared as their sodium salts. Color coding: orange, BH groups; black, CH groups; blue, Co atom. For details see *Materials and Methods*.

enzyme inhibitors. Few examples reported in the literature involve benzolactams bearing dicarba-*closo*-dodecaborane (16, 18) or carborane substitution of the phenyl ring in the phenyl-phthalimido-imid, yielding a tumor necrosis factor-α modulator with the activity comparable with the parental compound (19). Porphyrins substituted with dicarba-*closo*-dodecaboranes were found as inhibitors of HIV PR, with IC_{50} in the submicromolar range (20).

Our main attention has been focused on ionic metal bis(dicarbollides) that consist of two dicarbollide subclusters sandwiching the central metal atom. In metal bis(dicarbollides), the equal 11-vertex dicarbollide subclusters are connected by a *commo* metal vertex, forming two 12-vertex metal dicarbollide subclusters. These *closo* 26-electron compounds with "peanut-like 12-vertex geometry" were described as early as 1965 by Hawthorne *et al*. (21) and thus form basic stones in metallacarborane chemistry.

Among other transition metal metallacarboranes (22), cobalt bis(1,2-dicarbollide) ion (23) shows certain unique features: synthetic availability, wide possibilities of *exo*-skeletal modifications, high stability, charge delocalization, low nucleophilicity, strong acidity of conjugated acids, and high hydrophobicity. These properties are reflected in unique solution properties and ion-pairing behavior of this ion, which in turn led to its known applications in extraction chemistry (24, 25) and in the development of lowest coordinating anions (26) and compounds for radioimaging (14). However, the metal bis(dicarbollides) have never been considered as biologically active compounds or pharmacophores.

In this article, we report the potent, specific, and selective inhibition of HIV PR by parental and substituted metallacarboranes, namely cobalt bis(1,2-dicarbollides). We provide evidence for the mechanism of action of these compounds, show their antiviral activity in tissue cultures, analyze their binding toward the enzyme by x-ray crystallography, and show the potential of this class of compounds to become a novel pharmacophore for enzyme inhibition.

Materials and Methods

Chemical Syntheses. Compound **1** (Table 1) was converted to

Table 2. Data collection and refinement statistics for crystallographic structure determination

Space group	C2
Unit cell	a = 85.3 Å, b = 67.2 Å, c = 42.5 Å, β = 95.0°
Data collection resolution, Å	52.7–2.14
Completeness, %	99.2 (99.2)*
Average $I/\sigma(I)$	9.3 (3.0)*
R_{merge},† %	5.6 (22.8)*
Refinement resolution, Å	30–2.15
R factor‡/R_{free} factor,§ %	17.59/23.25
No. of atoms (protein/water/others)	1,535/202/46
rms deviation bond length/angles, Å/°	0.009/1.92

*Values in parentheses refer to the highest resolution shell 2.19–2.14.
†$R_{merge} = \Sigma_{hkl} \mid I - \langle I \rangle \mid \Sigma_{hkl} I$.
‡$R = \Sigma \mid F_{calc} - F_{obs} \mid / \Sigma \mid F_{obs} \mid$.
§The R_{free} as defined in ref. 45 was calculated for 5% of reflections.

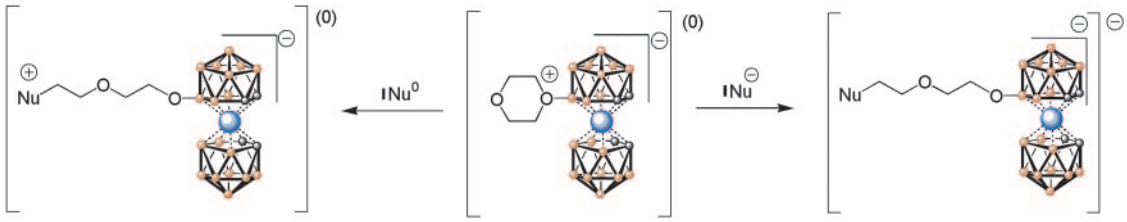


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Fig. 1. Ring opening reaction of 8-dioxane-3-cobalt bis(1,2-dicarbollide) **7** by different nucleofiles Nu^0 (e.g., NH_3) and Nu^- (e.g., RO^-) yielding zwitterionic and anionic compounds, respectively.

sodium salt from the commercially available cesium salt (Katchem, Rez u Prahy, Czech Republic) by using the extraction procedure described in ref. 27. Compound **2** was obtained by direct hydroxylation of **1** by using warm diluted sulfuric acid according to procedures in ref. 28. The starting dioxanate intermediate was prepared as described in ref. 29. The synthesis of compounds **3–6** is described in *Supporting Materials and Methods*, which is published as supporting information on the PNAS web site.

Enzymes. The expression, refolding, and purification of HIV-1 PR, HIV-2 PR, and the HIV-1 PR variant (Q7K, L33I, L63I) bearing three mutations that minimize the autoproteolytic cleavage (30) were performed as described (31). PRs from mouse intracisternal A particles (MIA14 PR) (32), human cathepsin D, and pepsin were prepared as described (33, 34). Porcine α-amylase, bovine trypsin, and papain were purchased from Sigma.

Inhibition Assays. ***Inhibition of HIV PRs.*** The IC_{50} and K_i values were determined by spectrophotometric assay with the chromogenic substrate KARVNleNphEANle-NH_2 as described (31). The inhibition constants were estimated by using a competitive inhibition equation according to ref. 35. The mechanisms of inhibition were derived from initial reaction rates versus concentrations of substrate in the presence of various concentrations of inhibitor by using a Lineweaver–Burk plot.

Inhibition of MIA14 protease. A spectrophotometric assay was used to determine inhibition characteristics by using chromogenic substrate DSAYNphVVS as described (32).

Inhibition of human cathepsin, pepsin, trypsin, papain, and α-amylase. For experimental details, see *Supporting Materials and Methods*.

Testing of Antiviral Activities in Tissue Cultures. Antiviral activity was analyzed by using PM-1 cells infected with HIV-1 strain NL4-3 modified from a published procedure (36). PM-1 cells were infected by coculture and washed 4 h after infection, and compounds **1–6** or the solvent DMSO, respectively, was added after the wash. Newly produced virus was harvested at 48 h postinfection and cleared by brief centrifugation, and infectious titer was determined on TZM cells, which express β-galactosidase from a Tat-responsive promoter. Viral titers and standard deviations are derived from three independent experiments.

Crystallization, Data Collection, and Structure Solution. The complex for crystallization was prepared by mixing HIV-1 PR (Q7K, L33I, L63I) with 3.7-fold molar excess of compound **1** dissolved in DMSO and concentrated by ultrafiltration to final concentration of 7.5 mg/ml. The crystals were grown by hanging drop vapor diffusion technique at 19°C by using 0.1 M Tris·HCl (pH 8.5) and 2.0 M ammonium dihydrogen phosphate as the precipitating solution. Diffraction data were collected at 100 K by using synchrotron radiation of wavelength 0.8 Å [X13 beamline, Deutsches Elektronen-Synchrotron (DESY) Hamburg, Germany] and were processed by using the HKL 2000 software package (37). The HIV PR structure was solved by molecular replacement by using protein coordinates from Protein Data Bank (PDB) structure 1NH0 (38). The structure solution and refinement were performed by using the CCP4 program suite.

Crystal parameters and data collection statistics are summarized in Table 2. Atomic coordinates and structure factors have been deposited to PDB: code 1ZTZ. The details of structure determination are found in Table 4, which is published as supporting information on the PNAS web site.

Results and Discussion

Inhibitor Design and Synthesis. Most of the HIV PIs currently used in clinics are pseudopeptide or peptide mimetics based on a limited number of structural building blocks. Our intention was to identify novel core structures and thus expand chemical space available for

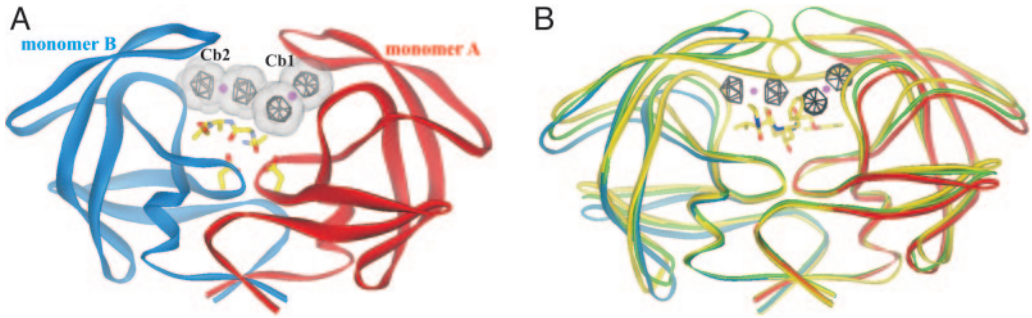

Fig. 2. X-ray structure analysis of the binding of compound **1** to HIV-PR. (*A*) Overall structure of the HIV PR–compound **1** complex. The PR dimer is in ribbon representation with the two catalytic aspartates shown in sticks. Two compound **1** molecules are represented by their van der Waals surfaces and gray stick model, with cobalt ions shown as magenta spheres. Autoproteolytic peptide product is represented as stick model. (*B*) Superposition of PR–compound **1** complex with PR–lopinavir complex and with the free PR structure. Protease complex with lopinavir (PDB ID code 1MUI) is represented in yellow ribbons, lopinavir is shown as a stick model, free PR structure (PDB ID code 1HHP) is shown in green ribbons, and color coding for PR–compound **1** complex is the same as in *A*.

the development of PIs with novel qualities. We have identified 12-vertex metallacarborane clusters as suitable hydrophobic, stable, nontoxic structural analogues of aromatic compounds. In our initial tests, substituted metallacarboranes showed the most promising results.

Our synthetic approach started from easily accessible parent cobalt bis(dicarbollide) ion **1** to yield either 8-hydroxyderivative **2** (28) or the 8-dioxane-3-cobalt bis(1,2-dicarbollide) (29) reagent **7** (see Fig. 1). This reagent was then reacted under mild conditions, giving rise to a series of *exo*-skeletally modified metallacarborane cluster anions **3**–**6** for testing. For synthesis of compounds **3**–**6**, ring cleavage reaction of **7** with *O*- and *N*-nucleophiles was used (see Fig. 1), thus producing ionic species if the ring opener is an anion, and betain-type zwitterions if the base is not charged.

The ring opening procedure of **7** zwitterion has already become a widely applicable method for attachment of the cobalt bis(dicarbollide) moiety to various organic substances (39–41). However, compounds **5** and **6** represent examples of zwitterionic-anionic structures containing two cobalt bis(1,2-dicarbollide) subunits bonded via flexible organic spacer chain.

Inhibition Constants and Antiviral Activities. Compounds **1**–**6** were tested as potential inhibitors of HIV PR *in vitro* and in tissue cultures. The corresponding inhibition constants (K_i values) and antiviral activities (EC_{50} values) are summarized in Table 1. All compounds exhibit classical competitive binding (data shown in *Supporting Materials and Methods* and Fig. 4, which is published as supporting information on the PNAS web site). This kinetic analysis suggests that tested cobalt bis(dicarbollide) competes with the peptide substrate and, therefore, binds to the active cleft of the enzyme. This suggestion has been confirmed by x-ray analysis of the complex of HIV PR with compound **1** (see below). Parent compound **1** shows tight inhibition *in vitro* and micromolar antiviral potency. Derivatization of compound **1** by hydroxyl and 2-(2-hydroxyethoxy) ethoxy groups yielded compounds **2** and **3**, exhibiting much weaker activity *in vitro* and comparable antiviral activities in tissue cultures. Simple visual inspection of the size of compounds **1**–**3** (Table 1) in comparison with the volume of the closed form of the HIV PR active cleft led to the notion that these compounds would not have sufficient contacts with the corresponding substrate-binding clefts. The solvent accessible area of compound **1** is more then two times lower when compared with a representative conventional pseudopeptide PI, lopinavir (LPV). However, the x-ray structure analysis solved this apparent contradiction, showing that two inhibitor units are needed for the efficient binding to the PR active cleft (see Figs. 2 and 3). Because the relative molecular weight of compound **1** is one of the lowest ever reported to inhibit HIV PR, it provides enough room for further improvement by means of structure-activity analyses, and therefore it was selected as the lead compound of our series of metallacarborane inhibitors of HIV PR.

Approximately a 100-fold improvement of the K_i value was achieved by enlarging the side chain of compound **3** in position 8 of the cage by the addition of a 1,2-diphenyl-2-hydroxy-ethoxy group, yielding compound **4** with 20 nM K_i. The binding of the compound was further improved four times by designing a symmetric compound **5** and, even further, by alkylating the secondary amino group with a butyl moiety (compound **6**), which represents the most active inhibitor of the compound series, with a K_i value of 2.2 nM and submicromolar antiviral activity in tissue culture. The mode of binding of this compound and its interaction with the enzyme-binding pockets could be inferred from the structure of the parent compound **1**.

It is striking that compound **6** showed an EC_{50} for inhibition of HIV-1 in tissue culture of 250 nM, which was ≈10-fold better than that observed for the structurally very similar compound **5**. In contrast, the K_i value for PR inhibition *in vitro* exhibited only a 2-fold difference. This result indicates that subtle differences in

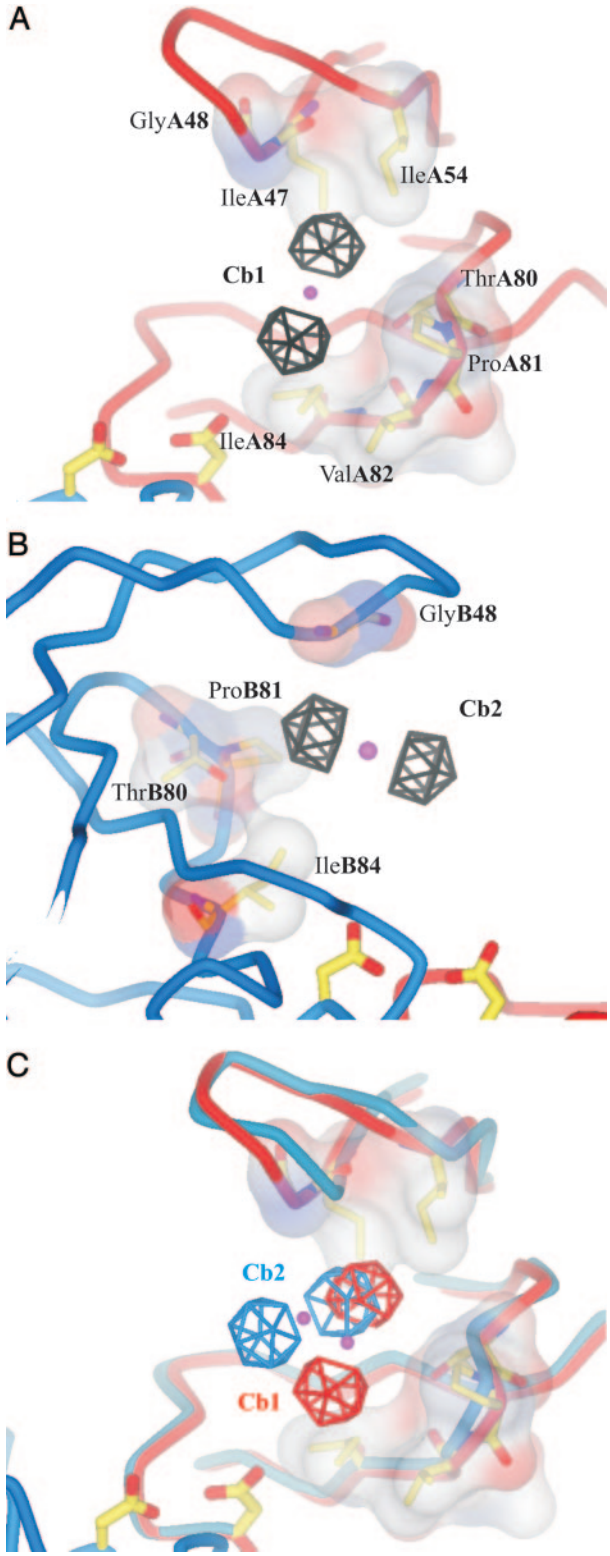

Fig. 3. Interactions of compound **1** with the amino acid residues in the corresponding PR-binding pocket. (*A*) Binding of compound **1** molecule Cb1 by PR monomer A (red tube). (*B*) Binding of compound **1** molecule Cb2 by PR monomer B (blue tube). Compound **1** is represented by a stick model in gray, with cobalt shown as a magenta sphere. PR residues in contact with compound **1** are represented by stick models, and their solvent-accessible surfaces are colored by atom charge (blue, positive; red, negative). (*C*) Superposition of the two compound **1**-binding modes. The color scheme and representation for PR is the same as in *A* and *B*, and atoms in compound **1** are colored with the color of the interacting PR chain.

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Table 3. The IC_{50} values that demonstrate specificity and selectivity of individual compounds as analyzed with other retroviral PRs, representatives of aspartic, serine and cystein PRs, and amylase

Enzyme	Compound 1	4	5	6
WT HIV-1 PR	1.4 μM (66 $\pm$ 30 nM)	0.13 μM (20 $\pm$ 5 nM)	0.14 μM (4.9 $\pm$ 2.1 nM)	0.10 μM (2.2 $\pm$ 1.2 nM)
WT HIV-2 PR	1.5 μM (220 $\pm$ 34 nM)	0.76 μM (140 $\pm$ 8 nM)	0.35 μM (110 $\pm$ 17 nM)	0.31 μM (39 $\pm$ 1 nM)
MIA14 PR	1.0 μM (85 $\pm$ 17 nM)	0.21 μM (22 $\pm$ 7 nM)	0.63 μM (60 $\pm$ 22 nM)	0.59 μM (85 $\pm$ 4 nM)
Human cathepsin D	2.1 μM (1,100 $\pm$ 100 nM)	1.3 μM (670 $\pm$ 30 nM)	1.9 μM (960 $\pm$ 30 nM)	0.50 μM (250 $\pm$ 30 nM)
Pepsin	1.5 μM (760 $\pm$ 90 nM)	0.86 μM (430 $\pm$ 40 nM)	1.3 μM (630 $\pm$ 160 nM)	0.73 μM (360 $\pm$ 50 nM)
Trypsin	$\gg$50 μM	$\gg$50 μM	10 μM (ND)	$\gg$50 μM
Papain	$\gg$50 μM	$\gg$50 μM	46 μM (ND)	$\gg$50 μM
Amylase	$\gg$50 μM	$\gg$50 μM	3 μM (ND)	17 μM (ND)

The experimental error in the IC_{50} determination is $<$10% of the given value. The inhibition constants K_i are shown in parentheses when applicable.

structure may lead to significant alterations in potency and suggests that further derivatization of this new group of PIs may significantly enhance their potential as antiretroviral drugs.

Analysis of the polyprotein processing by Western blotting shows a processing defect in the virus grown in the presence of active compounds (data not shown). No significant toxicity of tested compounds in tissue cultures was observed in the concentration range up to 50 μM.

Specifity and Selectivity Testing. The selectivities of the lead compound **1** and the more potent compounds **4–6** were tested on a panel of seven enzymes, including PR from the highly homologous HIV-2 virus, PR from more distantly related retrovirus MIA 14, prototype human aspartic PRs cathepsin D and pepsin, serine PR trypsin, cystein PR papain, and amylase as a representative of nonproteolytic enzymes with an anionic active-site cleft. The results are summarized in Table 3 in terms of IC_{50} values; the corresponding K_i values are shown in parentheses when appropriate.

All tested compounds inhibit homologous HIV-2 PR and MIA PR, although less tightly, suggesting that they might be active against mutated resistant PR species selected under the pressure of clinically used PIs in HIV-positive patients. The activity of tested compounds toward cathepsin D and pepsin is two orders of a magnitude lower in terms of K_i when compared with HIV-1 PR. The tested compounds do not significantly inhibit any other enzyme analyzed.

Crystal Structure of PR–Compound 1 Complex. Structure of HIV PR–compound **1** complex was determined at 2.15 Å resolution with R factor of 17.6% and R_{free} of 23.6%. The final model comprises a PR dimer (chains A and B) with two molecules of compound **1** bound in the active site (labeled Cb1 and Cb2 in Fig. 2*A*). Because compound **1** is highly symmetrical in shape, it is not, however, possible to distinguish unambiguously the positions of carbons and borons in electron density maps at 2.15 Å resolution.

With the two molecules of compound **1** bound, the overall conformation of the PR is similar to the open conformation typical for free PR. Most of the structures of substrate-based active site inhibitor complexes exhibit flaps closed over the active site. However, the PR complexed with **1** can be superimposed with the unliganded PR structure [PDB ID code 1HHP (42)] with an rms deviation in α-carbon positions of 0.99 Å (Fig. 2*B*). Flaps are obviously held in the open conformation by binding of the inhibitor molecules to the flap-proximal, "upper" part of the active site cleft (Fig. 2). The structure thus provides evidence that the hitherto unexplored class of inhibitors shows an unexpected mode of inhibition. So far, the open conformation of flap was reported only for unliganded PR, whereas all inhibitor-bound structures of wild-type HIV PR show closed flap conformation. Here, we describe the structure of a complex of PR with a relatively potent inhibitor bound uniquely in the enzyme open form. Because the conformation of flap in PR is functionally very important, this finding might suggest that inhibition mechanism of these compounds is blocking the flap closure rather than filling the specific bonding pockets in the active site cleft.

Compound **1** is bound in the hydrophobic pockets formed by side-chains of PR residues Pro-81, Ile-84, and Val-82 and covered by flap residues Ile-47, Gly-48, and Ile-54 (Fig. 3). These pockets correspond approximately to S3 and S3′ substrate-binding subsites. Although the inhibitor-binding site is identical in both monomers, the positions of the two compound **1** molecules are different (Fig. 3*C*). Inhibitor molecule Cb1 makes 21 van der Waals contacts with seven residues in monomer A (Fig. 3*A*) whereas inhibitor molecule Cb2 makes 12 van der Waals contacts with four monomer B residues (Fig. 3*B*). On average, 84% contacts are made with PR nonpolar atoms. The two Cb1 and Cb2 molecules contact each other with 3 van der Waals contacts. Compound **1** loses 89% of its total solvent-accessible surface upon complex formation, and good shape complementarity between both S3 and S3′ PR subsites and compound **1** molecules is illustrated by an average gap volume index of 0.45. This result is slightly lower than an average value for enzyme–inhibitor complexes (43).

The PR compound **1** complex crystallized in C2 crystal form, unique among all 169 HIV PR structures deposited in PDB. In the crystal, symmetrically related PR complexes are oriented head-to-head by their active sites. As a result of this crystal packing, symmetrical flaps are in contact with each other and molecules of compound **1** are in contact with their symmetry mates, as well as with flaps belonging to neighboring symmetry molecule. Thus, in addition to the above described contacts, interactions of compound **1** with PR based on crystal contacts can be observed with residues Gly-48, Gly-49, Ile-50, and Phe-53. However, formation of these interactions in solution seems unlikely because assembly of a complex consisting of two PR dimers and four inhibitor molecules is highly improbable.

Five of the PR residues that are in contact with compound **1** are often mutated in drug-resistant PR variants (Ile-47, Ile-48, Ile-54, Val-82, and Ile-84). The question whether the presence of these

mutations affects compound **1** binding needs to be answered by further biochemical and structural studies. Nevertheless, compound **1** binds by two different modes to two identical binding pockets formed by monomers A and B (Fig. 3*C*). Thus, we can expect that compound **1** is able to adapt its position so that it could bind into an appropriate pocket altered by mutations.

In addition to the two inhibitor molecules, a continuous electron density map at the bottom of the PR active site allowed modeling of the tetrapeptide Ala-Gly-Ala-Ala, which represents a product of PR autoproteolytic cleavage, often observed during cocrystallization of PR with weak active-site inhibitors. The product of PR degradation is then found in the active site instead of inhibitor (ref. 46; PDB ID code 1SP5). In the present structure, however, the peptide and compound **1** occupy different sites of the active cleft, and, therefore, they can bind simultaneously. Nevertheless, high temperature factors of the peptide main chain atoms point to high mobility and/or its probable lower occupancy. The lack of electron density for side chains further suggests that several peptides originating from three possible cleavage sites (44) are present in the structure. Therefore, we presume that the presence of peptide in the active site is not required for compound **1**-specific binding.

In conclusion, we have shown that boron clusters represent convenient building blocks that can create important interactions with hydrophobic patches of the HIV PR-binding site. X-ray structure analysis of the metallaborane–PR complex brings up compelling evidence about the inhibitor-binding site and type of interaction. *Exo*-skeletal substitution of the parent metallaborane cluster tecton introduces additional noncovalent interactions leading to the dramatic improvement in inhibition efficacy and selectivity. The combination of the hydrophobic interactions of the scaffold with substitutions allowing for specific H-bonding and coulombic interactions might further increase the potency of this class of nonpeptide PR inhibitors based on an inorganic framework. Chemical and biological stability, low toxicity, and the possibility to introduce heteroatoms into the cage or polar group modifications to the side chains make boron clusters very attractive pharmacophores.

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