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# Inhibition of Human Immunodeficiency Virus-1 Protease by a $C_2$-symmetrical Phosphinic Acid Amide

**Anusch Peyman[#*], Konrad Wagner[#], Karl-Heinz Budt[#], Jörg Spanig[#], Dieter Ruppert[#], Christoph Meichsner[#] and Arno Paessens[+]**

[#] *Hoechst AG, PharmaResearch, 65926 Frankfurt, FRG*

[+] *Institute of Virology, Pharma Research Center, 42096 Wuppertal, FRG*

**Abstract:** The inhibition of HIV protease with $C_2$-symmetric phosphinic acid based inhibitors is independent of the dissociation grade of the phosphinic acid. The corresponding amides are not only good inhibitors of HIV protease but also inhibit HIV replication in vitro because of their increased lipophilicity.

The virally encoded protease of the Human Immunodeficiency Virus (HIV) is one of the most interesting targets for HIV/AIDS chemotherapy [1]. The HIV protease, a member of the aspartyl protease family, plays a key role in processing the HIV polyproteins produced directly after translation, especially the gag and gag-pol polyproteins [1]. Blockade of these processing steps leads to viral particles which are morphologically immature and noninfectious [2]. HIV protease functions as a $C_2$-symmetrical homodimeric protein [3]. This led to the synthesis of $C_2$-symmetric HIV protease inhibitors that match the symmetry of the enzyme [4,5]. We recently reported the synthesis of bis($\alpha$-aminoalkyl)phosphinic acids [6], which allows the placement of a phosphinic acid group into a $C_2$-symmetrical peptide environment, where it acts as a transition state analog of peptide bond hydrolysis. This approach lead to powerful inhibitors **1** of HIV protease (Table 1) with $IC_{50}$ values in the subnanomolar range. Despite good enzyme inhibition, compounds **1** have only weak antiviral activity in PBL-cells acutely infected with HIV, most likely due to poor cell penetration.

We now present evidence that the ionized form of the phosphinic acids **1** is not required for effective enzyme inhibition. Furthermore we report the synthesis of the corresponding phosphinic acid amides **2** which are good inhibitors of HIV protease, and in addition effectively prevent virus replication in cell culture due to their increased lipophilicity.

Grobelny et al.[7] demonstrated that there is a strong pH dependence of HIV protease inhibition for phosphinate transition state analogs, with lower $K_i$ values at pH 4.5 as compared to pH 6.5, suggesting that the neutral phosphinic acid has the highest affinity to the protease. This is supported by a recently published three-dimensional structure of a phosphinic acid based inhibitor bound to HIV-1 protease[8].

We measured the pH dependence of protease inhibition from pH 4.3 to pH 6.5 of inhibitor 1a and for comparison of a structurally related non ionic inhibitor containing a bishydroxyethylene-moiety instead of the phosphinic acid[5]. The results are shown in figure 1.

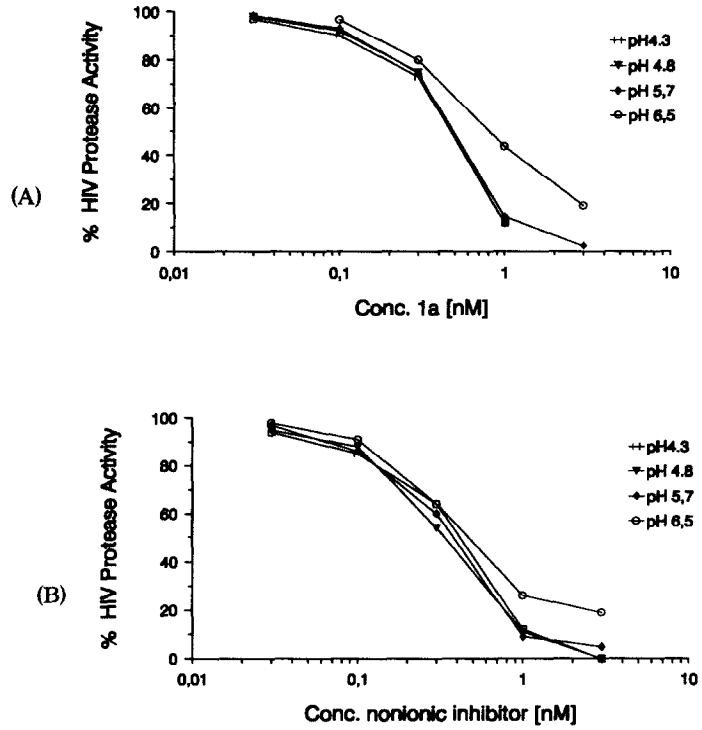
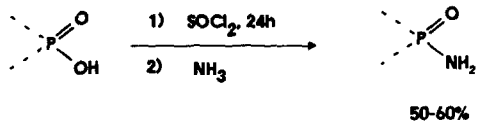


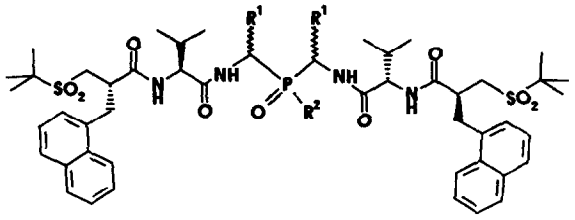

**Figure 1:** pH dependence of HIV protease inhibition (A) with inhibitor 1a; (B) with the nonionic inhibitor containing a bishydroxyethylene-moiety instead of the phosphinic acid[5].

Surprisingly, the diagrams show that inhibition of HIV protease with 1a is essentially not pH dependent. The slight increase of the $IC_{50}$ value observed at pH 6.5 is also found for the nonionic inhibitor. Obviously binding is only weakly influenced by the dissociation grade of the phosphinic acid. On the other hand the methylester 3 is 1000 fold less potent than the free acid 1c (table 1). This leads to the conclusion that the acidic hydroxyl group may be replaced by other groups which are capable of hydrogen bond formation and sterically non demanding. This requirement is met by phosphinic acid amides 2[9]. We found that the compounds 2 could be conveniently prepared by conversion of the acid to the acid chloride with $SOCl_2$ followed by ammonolysis with $NH_3$. The hydrazide 4 was prepared in the same way. Great care must be taken while preparing the acid chloride because of its extreme sensitivity towards hydrolysis. Best results were obtained when the chlorination was carried out for 24 h and an excess of $SOCl_2$ was removed by

repeated coevaporation with toluene. The amides 2 are stable towards hydrolysis under the conditions of the enzyme assay and the cell test.




**Scheme 1:** Preparation of phosphinic acid amides from phosphinic acids



| No. | $R^1, R^{1'}$ [Configuration] | $R^2$ | $IC_{50}$ [nM] (HIV-Protease) | $EC_{50}$ [µM] (PBL) |
|---|---|---|---|---|
| 1a | Ph-$CH_2$ [R,R] | OH | 0,5 | >10 |
| 1b | Ph-$CH_2$ [S,S] | OH | 85 | >10 |
| 1c | H | OH | 1,1 | >10 |
| 2a | Ph-$CH_2$ [R,R] | $NH_2$ | 1,0 | 0.1 |
| 2b | Ph-$CH_2$ [S,S] | $NH_2$ | 250 | >10 |
| 2c | H | $NH_2$ | 5,5 | > 10 |
| 3 | H | OMe | 950 | >10 |
| 4 | H | NH-$NH_2$ | 8,5 | > 10 |

**Table 1:** Phosphinic acid derivatives as inhibitors of HIV protease ($IC_{50}$)[10] and HIV replication ($EC_{50}$) in peripheral blood mononuclear cells (PBL)[11].

The phosphinic acid amides proved to be excellent inhibitors of HIV protease[12]. Table 1 contains a comparison of the $IC_{50}$ values of the free phosphinic acids and their amides, which are only increased by a factor of 5 in case of the unsubstituted amide 2c (in comparison to 1c) and by a factor of 2 in case of the [R,R]-configurated benzyl substituted amide 2a (in comparison to 1a). Even hydrazide 4, which has a comparable steric requirement as the methylester 3 is still a potent inhibitor of HIV protease. Moreover, the phosphinic acid amides are also capable of intervening with virus replication in cell culture, especially

2a, which has an $IC_{50}$ value of 100 nM in peripheral blood lymphocytes. The improved antiviral efficacy may be explained by the increased lipophilicity and therefore higher capability for cell penetration of the amides compared to the free phosphinic acids. The use of phosphinic acid amides instead of phosphinic acids could possibly turn out to be a general principle for application in transition state analog drug design.

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9) The use of a phosphinic acid amide as an enzyme inhibitor (of pepsin) has so far only been described once by Bartlett, P. A. and Acher, F. *Bull. Chem. Soc. France* **1986**, 771. They used a slightly different synthesis by first converting the phosphinic acid to phosphinic azides, followed by ammonolysis.

10) The enzyme inhibition assay was carried out as described in Henke, S., König, W., Breipohl, G., Mölling, K., (Hoechst AG), EP-A 0373576, **1989**; *Chem. Abstr.* **1990**, 114, P825535s.

11) pHA and IL-2 stimulated peripheral blood lymphocytes (PBL's) were infected wit HIV-1 D34 isolate by pellet infection and incubated in the presence of diluted test compound for 3-8 days at 37 °C. Viral replication was evaluated by light microscopiy for syncytia formation and detection of viral p24 capture assay (Vironostika HIV, Organon Teknika).

12) The phosphinic acid amides 2 are only pseudo-$C_2$-symmetric, however, they could be subject to pseudo-rotation (2c does show only one signal in $^{31}$P-NMR at 34,2 ppm).

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