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Review Article

Dihydropyrone sulfonamides as a promising new class of HIV protease inhibitors

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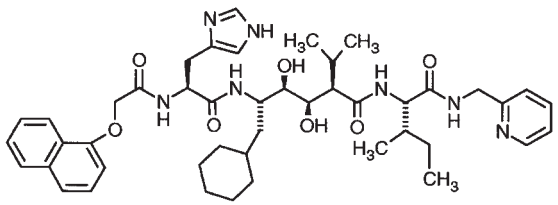
Introduction

The AIDS pandemic continues to be a medical, social and economic problem of staggering dimensions. A major therapeutic advance has been recently realized by the introduction of a number of HIV protease inhibitors into clinical practice (1). The initial excitement engendered by this potent and new type of anti-AIDS therapy has been tempered by the realization that significant levels of resistance are still occurring with the first-generation protease inhibitors and that even triple combination therapy (*e.g.*, simultaneous treatment with two different reverse transcriptase inhibitors and a protease inhibitor) has serious limitations (2). The increasing number of treatment failures with the regimens utilizing the compounds already on the market clearly indicate that novel drugs are needed which are better tolerated and not cross-resistant to the currently approved antiviral agents. Thus, there continues to be intensive efforts directed towards the discovery of new anti-HIV agents in general, and novel protease inhibitors in particular (3).

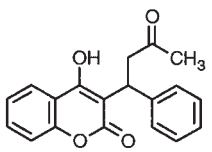
PNU-96988, the first-generation clinical candidate

The first-generation of HIV protease inhibitors are peptidomimetic compounds containing transition-state inserts in place of the dipeptide cleavage sites of the natural substrates for the viral enzyme (1). Similarly, the initial efforts at Pharmacia & Upjohn in this area involved the optimization of peptide leads to produce potent protease inhibitors such as PNU-75875 (Fig. 1) (4). However, these compounds had extremely poor pharmaceutic properties (very rapid clearance and low oral bioavailability), and it was going to be an uphill battle to turn these templates into a suitable drug candidate (5). Thus, an alternate approach was selected to find a lead template with overall more suitable properties. Broad screening for HIV protease activity of a dissimilarity subset of the company's internal storehouse of compounds generated relatively few hits; however, one interesting active noted was the well-known anticoagulant warfarin (Fig. 1) (6). While warfarin had only weak enzyme inhibitory activity, similarity searching identified a related compound, phenprocoumon (compound **1**), which turned out to have improved potency (K_i = 1 μM) (6).

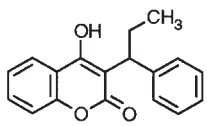
Compound **1** had only weak activity against the virus in cell culture (ED_{50} = 100-300 μM); however, the pharmacokinetic properties of both phenprocoumon and warfarin are excellent. They have both been extensively studied in the clinic and shown to have very high bioavailabilities and long half-lives. Furthermore, kinetic analysis of phenprocoumon indicated that it was a competitive inhibitor of HIV protease (6). Thus, it appeared that compound **1** was indeed binding in the active site of the enzyme, and this was confirmed by the x-ray structure of phenprocoumon bound to HIV-1 protease. The x-ray structure indicated that the 4-hydroxy group of the coumarin ring forms hydrogen bonds with the two key catalytic aspartic acid residues (Asp25 and Asp25'), and that



PNU--75875



Warfarin



1, phenprocoumon

Fig. 1. Structures of PNU-75875, warfarin and phenprocoumon.

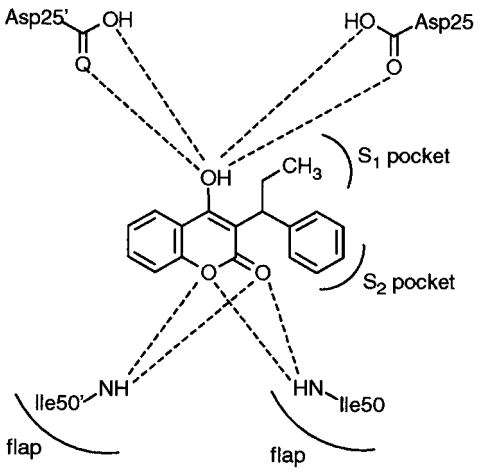


Fig. 2. Representation of compound **1** bound at the active site of HIV protease showing hydrogen bonds to Asp25, Asp25', Ile50 and Ile50'residues of the enzyme.

the two oxygens of the lactone portion of the ring form hydrogen bonds with the two isoleucine residues (Ile50 and Ile50') on the flaps of the enzyme (Fig. 2). The lactone ring thus replaces the water molecule that is found in x-ray structures of peptide type inhibitors bound to HIV protease, and likely provides an important favorable entropic effect upon binding (7).

Thus, while compound **1** was several orders of magnitude less potent than the peptidic leads, it clearly had superior pharmaceutic properties and was binding in the desired manner. The task was then to improve the potency, both at the enzyme level and in cell culture, while maintaining the desired pharmaceutic attributes of the original template. Lead optimization was greatly facilitated by the use of x-ray structures and modeling hypotheses. Overlays with the x-ray structures of the peptidic inhibitor PNU-75875 suggested that the phenyl group and ethyl group on compound **1** each occupied a peptide binding pocket but that the rigid coumarin ring prevented access to good binding to additional sites that were effectively occupied by PNU-75875. Thus, substituted pyrone analogs were investigated ultimately leading to the first-generation clinical candidate PNU-96988 (Fig. 3) (6). PNU-96988, prepared as a mixture of diastereomers in only two to three steps from commercially available sources, was an effective enzyme inhibitor of HIV-1 (K_i = 38 nM) and HIV-2 (K_i = 32 nM), but was only weakly inhibitory, if at all, against a variety of other human aspartyl proteases. It was also active against HIV in cell culture (ED_{50} = 3-4 μM), including against clinical isolates (6).

The pharmacokinetics of PNU-96988 proved to be very promising with oral bioavailabilities of 76% and 45% in rats and dogs, respectively, with 4- to 6-hour half-lives after dosing in animals. The compound was well tolerated in preclinical safety studies and entered phase I clinical trials as the first-generation pyrone-based HIV protease inhibitor (6). PNU-96988 was also well tolerated in humans during the phase I trials, and the predicted blood levels safely achieved; however, clinical studies with this drug candidate were terminated when candidates with much superior antiviral activity were identified (8).

PNU-103017, the second-generation clinical candidate

While PNU-96988 was at least 25 times more potent than the original lead template phenprocoumon, it was still significantly less potent than most of the peptidic type inhibitors in development. Because of this, and concerns about the development of resistance with suboptimal candidates, analogs with improved potency but which would still maintain superior pharmacokinetics were continually sought. One significant discovery, based on the modeling hypothesis that increasing the flexibility of the coumarin nucleus in compound **1** (by saturating the bonds in one of the benzene rings) would provide additional binding interaction, ultimately led to the cyclooctylpyrone analogs as exemplified by compound **2** (Fig. 3) (9). Subsequent x-ray structures of this type of compound bound to HIV protease verified that the analogs did now occupy three of the pockets around the active site of the enzyme. As predicted by the modeling, compound **2** did indeed have increased potency as an enzyme inhibitor (K_i = 15 nM) though it was considerably less potent in cell culture (ED_{50} = 57 μM). However, what was most encouraging about compound **2** was its excellent pharmacokinetics (*e.g.*, > 90% oral bioavailability in rats) (9).

Structure-based drug design based on overlays of compound **1** with PNU-75875 also suggested that an additional binding site could be realized by attaching an amide group at the *meta* position of the C-3 phenyl group. Indeed, compound **3** (K_i = 160 nM) did lead to an increase in potency over phenprocoumon (K_i = 1000 nM) (10). This principle was effectively carried over into the pyrone and cyclooctylpyrone templates; however, generally there was no corresponding increase in antiviral activity with the amide substituted analogs (11). Further modeling based on the structure of compound **3** bound to HIV

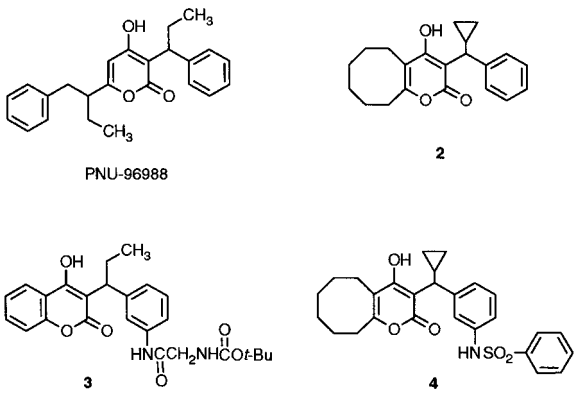


Fig. 3. Structures of PNU-96988 and compounds **2-4**.

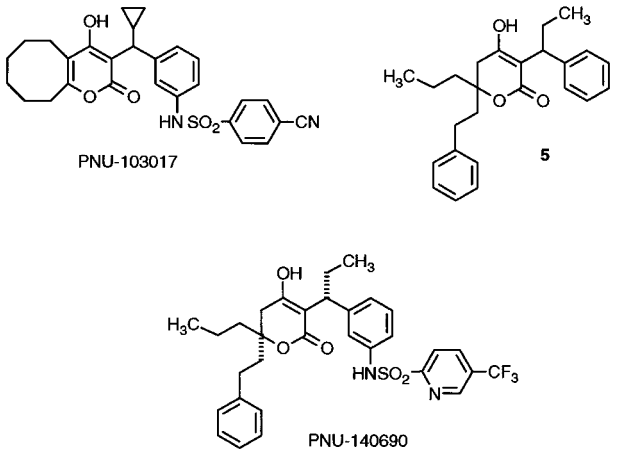


Fig. 4. Structures of PNU-103017, dihydropyrone **5** and PNU-140690.

protease suggested that replacement of the amide carbonyl with an SO_2 group could result in an additional hydrogen bond to the enzyme backbone which was not present in structures such as **3**.

Indeed, when this idea was applied in the cyclooctylpyrone template, enhanced enzymatic potency was observed, particularly when benzenesulfonamides such as compound **4** (K_i = 3 nM) were prepared (12). X-ray studies of compound **4** bound to HIV-1 protease showed the binding of the phenyl group on the sulfonamide into the predicted pocket as well as the proposed hydrogen bonding of the sulfonamide itself to Gly48 and Asp29. Subsequent optimization of the sulfonamide moiety led to the clinical candidate PNU-103017 (Fig. 4) (12, 13). Structural characterization of PNU-103017 bound in the active site of HIV-1 protease revealed that there was a small pocket in the enzyme that permitted the favorable insertion of the *para*-cyano group, a small electron withdrawing moiety (Fig. 5).

An important characteristic of these pyronesulfonamides was that not only did they have increased enzyme inhibitory activity, they also had significant levels of antiviral activity in cell culture. Thus, PNU-103017 was a potent inhibitor of both HIV-1 and HIV-2 (K_1 = 0.8 nM and 3.2 nM, respectively) but was devoid of significant activity against a large number of other aspartyl proteases (12). In cell culture, PNU-103017 was effective against a range of clinical isolates (ED_{50} = 5 μM), and the pharmacokinetic properties were particularly encouraging. For example, in dogs, PNU-103017 had an oral bioavailability of 77% and a half-life of 6 hours (12). PNU-103017 proved to have a good safety margin and entered phase I clinical trials as the second-generation pyrone-based protease inhibitor.

PNU-140690, the third-generation clinical candidate

Despite the now subnanomolar protease inhibitory activity achieved with the second-generation candidate PNU-103017, the level of HIV inhibitory activity in cell culture was still modest when compared to many of the peptidic inhibitors in development. Furthermore, it was also discovered that the cellular activity of PNU-103017 was greatly reduced when human serum protein was added to the cell culture. Thus, the high protein binding exhibited by PNU-103017 was likely to limit its effectiveness in the clinic. Therefore, the search continued for increasingly potent inhibitors, particularly those that would not be significantly affected by protein binding. For example, sulfonamides of the pyrone class were investigated and found also to have good enzyme inhibitory and antiviral activity (14).

Another class of potent inhibitors that was discovered via the structure-based drug design approach was the dihydropyrone class of inhibitors as illustrated by compound **5** (K_i = 35 nM) (15). Attachment of the sulfonamide sidechain at the *meta* position of the dihydropyrone type analogs resulted in compounds with exceedingly interesting properties. Further optimization of the sulfonamide moiety eventually resulted in the trifluoromethylpyridyl sulfonamide PNU-140690 (Fig. 4) (16). PNU-140690 was impressively effective against HIV-1 protease, with a K_i = 8 picomolar! Furthermore, a marked increase in antiviral effect was noted with an ED_{50} = 30 nM (16). A major advance was the discovery that in the dihydropyrone sulfonamide class of protease inhibitors, the cell culture activity was relatively unaffected by protein binding. For example, with PNU-140690 in a cell culture assay with 10% fetal bovine serum and 33% human plasma, the antiviral ED_{90} was still less than 1 μM (17). Furthermore, PNU-140690 was synergistic in combination with AZT against the virus in cell culture.

As noted previously, one of the most important aspects in the success or failure of anti-AIDS therapy is the development of resistance. Resistance has been noted with the first-generation protease inhibitors, both in the laboratory and in the clinic. For example, ritonavir-resistant isolates have been described which are highly

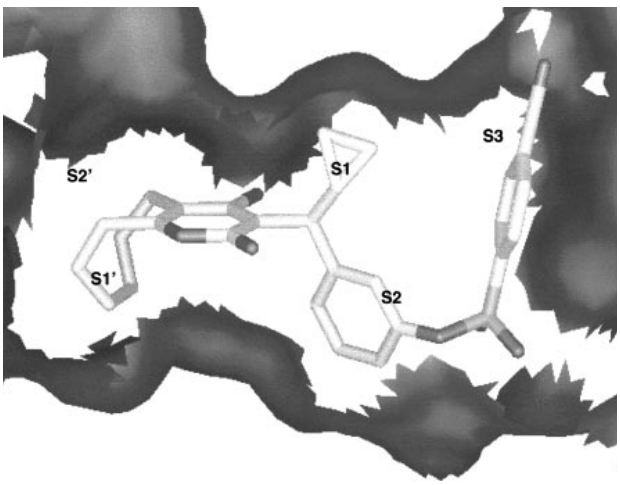


Fig. 5. Crystal structure of PNU-103017 bound to HIV-1 protease.

Table I: PNU-140690 potently blocks replication of HIV-1 broadly cross-resistant to marketed protease inhibitors (17).

Protease inhibitor	IC_{90} (μM)* *vs.* Parental HIV-1	Resistant HIV-1	Fold increase (IC_{90})
PNU-140690	0.07	0.45	6
Ritonavir	0.08	6.4	80
Indinavir	0.03	1.4	47
Nelfinavir	0.025	>3.1	>125
Saquinavir	0.03	3.75	125

*Compound concentration required to inhibit HIV-1 p24 antigen production in MT-4 cell culture by 90% relative to that in no-drug controls.

cross-resistant (47- to more than 125-fold decrease in sensitivity to the virus) to saquinavir, indinavir and nelfinavir (Table I) (18). These isolates remained quite sensitive to PNU-140690, showing only a 2- to 6-fold decrease in sensitivity to the highly ritonavir-resistant isolates *versus* wild-type isolates (Fig. 6) (17, 19). In fact, PNU-140690 proved to be additive or moderately synergistic with ritonavir against a ritonavir-sensitive isolate, and synergistic with ritonavir against a ritonavir-resistant isolate (19). This lack of cross-resistance to the peptide derived marketed agents is not too surprising given the very different structural motif of the pyrone sulfonamides. Intense efforts have been directed towards deriving resistance to PNU-140690 in the laboratory; however, even

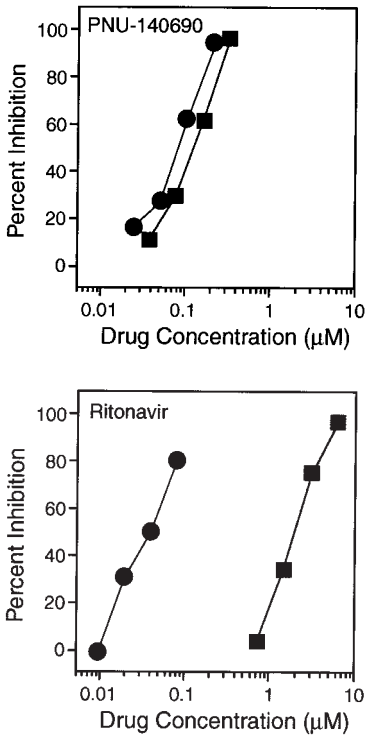


Fig. 6. Dose response of PNU-140690 and ritonavir against ritonavir-sensitive (●) and ritonavir-resistant (■) clinical isolates (19).

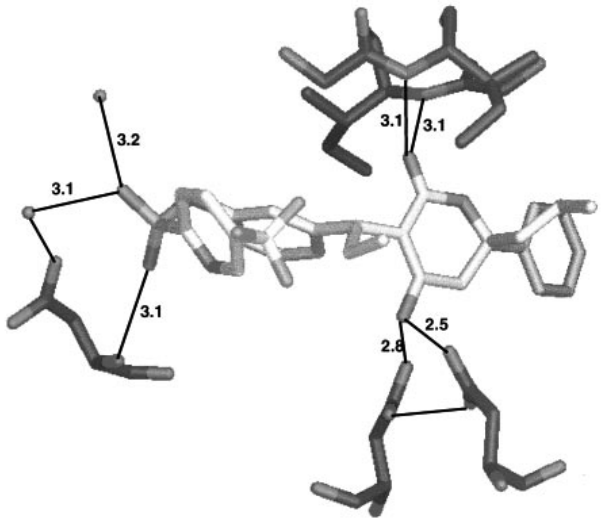


Fig. 7. Structure of PNU-140690 bound to HIV-1 protease showing two 3.1 Å hydrogen bonds between the lactone oxygen atom and the two-flap region isoleucine NH residues in blue (Ile50/50'), the 4-hydroxy group binding to the two catalytic aspartate residues (Asp25/25') at distances of 2.5 and 2.8 Å, and the sulfonyl oxygen atom binding to Asp30 (3.1 Å).

employing the same techniques that led to the highly ritonavir-resistant isolate (18) only gave (after 30 passages in cell culture) less than a 3-fold increase in resistance to PNU-140690 (17). Thus, resistance to PNU-140690 in the clinical situation will likely require multiple amino acid substitutions and possibly lead to reduced enzymatic (and viral) activity.

PNU-140690 contains only two chiral centers (as opposed to the four or more chiral centers present in other currently approved HIV protease inhibitors), and an efficient asymmetric synthesis of the compound has been developed (20). The x-ray crystal structure of PNU-140690 bound to HIV-1 protease showed binding into four pockets of enzyme as well as favorable hydrogen binding interactions at numerous positions (Fig. 7). Like the earlier pyrone-based development candidates PNU-96988 and PNU-103017, PNU-140690 had good pharmacokinetic and safety properties in animals and has been entered into clinical trials. In phase I trials,

PNU-140690 was very well tolerated with the primary adverse events being mild diarrhea or mild nausea at the high doses (21). Furthermore, PNU-140690 concentrations in excess of 1 μM were observed at 8 hours following a single 500-mg dose of the compound (21).

Conclusions

Using structure-based drug design techniques, a weakly potent HIV protease inhibitor, discovered through broad screening, has been developed into a series of three clinical candidates for the treatment of AIDS. All three compounds retain the excellent pharmacokinetic properties of the original lead. The third- generation candidate, PNU-140690, is particularly promising given its high potency at the enzyme level and in cell culture, its relative insensitivity to protein binding, and, in particular, the lack of cross-resistance to marketed HIV protease inhibitors. PNU-140690 (tipranavir) is currently undergoing investigation in clinical trials in AIDS patients.

Acknowledgements

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