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Recent advances in the design of HIV proteinase inhibitors

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Summary

Inhibition of HIV proteinase is currently one of the most widely studied approaches for chemotherapeutic intervention in the treatment of AIDS. A range of inhibitors of this essential enzyme has been designed from detailed knowledge of its mechanism of action and cleavage sites. These inhibitors have been classified according to their derivation. All are transition-state analogues and contain a hydroxyethylene, hydroxyethylamine, phosphinate or symmetrical moiety. Many of these inhibitors have high selectivity for the viral enzyme and significant antiviral activity. Advances in the design of HIV proteinase inhibitors that have been reported in the past year are reviewed.

HIV proteinase; Enzyme inhibition; Transition-state analog; Peptide mimetic

Introduction

The acquired immunodeficiency syndrome (AIDS) was first described in 1981, but it took two years before a group of human retroviruses known as human immunodeficiency viruses (HIV) were identified as the etiological agents responsible for this disease (Gallo et al., 1983; Barré-Sinoussi et al., 1983). During the next few years extensive studies on the molecular biology of retroviruses and especially HIV greatly enhanced the understanding of processes involved in the replication of these viruses. In addition, these studies unveiled a range of new targets that have enormous potential for chemotherapeutic intervention in this disease. One such target is the HIV-

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encoded proteinase, which is responsible for the post-translational processing of the polyprotein gene products of *gag* and *gag/pol* to afford the structural proteins of the virus core, together with the essential enzymes including proteinase itself. In fact, even before its isolation this enzyme had been suggested as a potential target for AIDS chemotherapy (Kramer et al., 1986). Based on the primary amino acid sequence (Ratner et al., 1985), its inhibition by pepstatin (Richards et al., 1989) and crystal structure (Wlodawer et al., 1989), HIV proteinase was characterized as an aspartic proteinase which functions as a homodimer (Pearl and Taylor, 1987). The vast body of knowledge accumulated from studies with other aspartic proteinases, especially renin, facilitated the design and discovery of potent inhibitors of HIV proteinase. Thus far, most inhibitors of aspartic proteinases have been based on the concept of transition-state mimetics. A range of transition-state mimetics has now been incorporated into inhibitors of HIV proteinase, which include reduced amides (Dreyer et al., 1989; Moore et al., 1989), hydroxyethylene isosteres (Dreyer et al., 1989; Vacca et al., 1991; Tomasselli et al., 1990), phosphinic acid derivatives (Grobelny et al., 1990), statine analogues (Dreyer et al., 1989) and difluoroketone derivatives (Dreyer et al., 1989; Sham et al., 1991). In this paper advances in the design of inhibitors of HIV proteinase that have been reported in the past year are reviewed together with studies in molecular modelling which have potential to aid the development of even more potent inhibitors and, more importantly, the design of non-peptide based inhibitors.

Design of inhibitors

At present HIV proteinase is known to be responsible for the specific cleavage at eight different sites in the *gag* and *gag/pol* gene products (Schneider et al., 1988; Darke et al., 1988; Billich et al., 1988; Krausslich et al., 1989). These cleavage sites can be grouped into three distinct types (Fig. 1). In the first type, which is exemplified by cleavage sites 1, 5 and 6, the scission occurs between tyrosine and proline or phenylalanine and proline. Since these amide bonds are not susceptible to cleavage by mammalian endopeptidases it is reasonable to expect that inhibitors based on these dipeptide sequences are likely to be highly specific for the viral enzyme. In the second type, illustrated by cleavage site 2, the scission occurs between leucine and alanine and is very reminiscent of the hydrolysis of a Leu-Val bond by the enzyme renin. Therefore, it is reasonable to expect that carefully selected inhibitors of renin could prevent the proteolytic action of HIV proteinase. Finally, in the third type where cleavage occurs at sites 3, 7, 4 and 8 there is good reason to believe that inhibitors could be designed to exploit the symmetrical nature of both the enzyme and that of the amino acid sequences being cleaved in the natural substrates. In fact, during the past year a range of inhibitors of HIV proteinase has been described which exemplify each of the three classes.

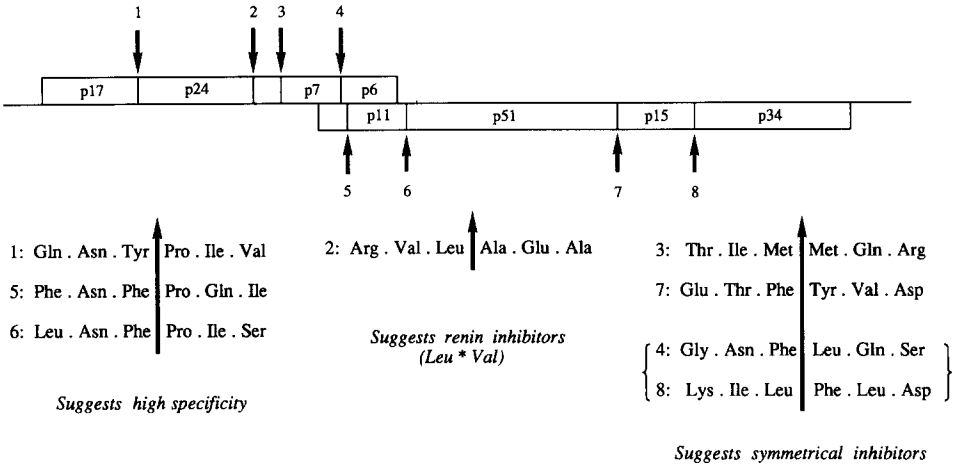


Fig. 1. Display of sites cleaved by HIV proteinase within *gag* and *gag/pol* polyproteins.

Type 1 inhibitors

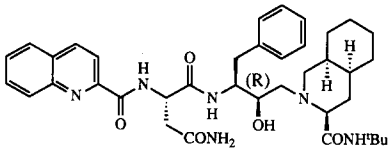
Last year we reported (Martin et al., 1990; Roberts et al., 1990) the design of very potent and selective inhibitors of HIV proteinase containing a hydroxyethylamine transition-state mimetic. We reasoned that this chemical entity readily accommodates the imino acid moiety characteristic of the proline residue contained in the type 1 cleavage site in *gag* and *gag/pol*. Our design was primarily based on the *pol* fragment Leu^{165}-Ile^{169} by substituting a hydroxyethylamine transition-state mimetic in place of the Phe^{167}-Pro^{168} scissile bond. The lead structure in this work was a protected tripeptide

TABLE 1

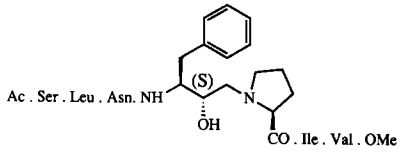
Structure and HIV proteinase inhibitory activity of hydroxyethylamine derivatives

	Compound		IC_{50}/K_i (nM)
(1)	$PhCH_2OCO$-Asn-Phe Ψ	[$CH(OH)CH_2N$] Pro-O^{t}Bu	140[a]
(3)	Ac-Leu-Asn-Phe Ψ	[$CH(OH)CH_2N$] Pro-Ile-OMe	4520[b]
(4)	Ac-Ser-Leu-Asn-Phe Ψ	[$CH(OH)CH_2N$] Pro-Ile-OMe	420[b]
(5)	Ac-Leu-Asn-Phe Ψ	[$CH(OH)CH_2N$] Pro-Ile-Val-OMe	21[b]
(6)	Ac-Ser-Leu-Asn-Phe Ψ	[$CH(OH)CH_2N$] Pro-Ile-Val-OMe	0.66[b]

[a]IC_{50}; [b]K_i.



(2) Ro 31-8959



(6) JG-365

TABLE 2

Inhibitory activity of Ro 31-8959 against HIV-1, HIV-2 and a range of mammalian proteinases

Proteinase	Class	IC_{50} (nM)
HIV-1	Aspartic	$<0.37^a$ (K_i = 0.1)
HIV-2	Aspartic	$<0.80^a$ (K_i = 0.12)
Human renin	Aspartic	>10000
Human pepsin	Aspartic	>10000
Human gastricsin	Aspartic	>10000
Human cathepsin D	Aspartic	>10000
Human cathepsin E	Aspartic	>10000
Human leucocyte elastase	Serine	>10000
Bovine cathepsin B	Cysteine	>10000
Human synovial fibroblast collagenase	Metallo	>10000
Prolidase	Dipeptidase	>10000

[a]IC_{50} values limited by mutual depletion.

derivative (1). Structural modification of each residue was explored systematically and gave information regarding preferred substituents for optimal binding to the enzyme. The most important findings were that both the P_3 and P_1' subsites had a preference for large lipophilic substituents and also there was a marked preference for R stereochemistry of the hydroxyl group in the transition-state mimetic. By incorporating all of the individually optimized side chains and stereochemical preferences into the same molecule the very potent inhibitor (2), Ro 31-8959, was obtained (Table 1). The selectivity of this compound was measured against a range of mammalian enzymes as an indicator of potential toxicity. At a concentration of 10 μM less than 50% inhibition of the human aspartic proteinases renin, pepsin, gastricsin, cathepsin D and cathepsin E was observed (Table 2). At the same concentration the compound had no effect on representative proteinases from the serine, cysteine and metallo classes; furthermore, it did not inhibit the dipeptidase prolidase. These data support the notion that this class of inhibitor should have little or no toxicity to mammalian cells.

In the latter half of 1990 a research team at the University of Wisconsin disclosed a series of inhibitors using the same design principle (Rich et al., 1990). Their lead compound (3) was also derived from the p11-p51 cleavage site in *pol*. Extension of the lead compound at either the N-terminus (compound 4) or the C-terminus (compound 5) by only one amino acid significantly enhanced the enzyme inhibitory activity and when both modifications were incorporated into the same molecule sub-nanomolar activity was obtained (compound 6, JG-365), thereby achieving a greater than 6000-fold increase in activity over the lead compound (Table 1). In this study all compounds were prepared as mixtures of diastereoisomers about the hydroxyl group of the hydroxyethylamine moiety, therefore any stereochemical preference that existed could not be determined. However, when compound 6 was co-crystallized with HIV-1

proteinase, only the S diastereoisomer was present in the complex and it was inferred that the more potent diastereoisomer had been selected. Subsequently, the individual diastereoisomers of compound 6 were synthesized from chiral precursors (Rich et al., 1991), confirming the strong preference for the hydroxyl group in the S configuration which also applied to C-terminally elongated members of this series. This result is in sharp contrast to the preferred R configuration in the related series exemplified by Ro 31-8959.

The difference between JG-365 and Ro 31-8959 appears to be related to both the overall length and the different amino acid residues in each compound. In an attempt to understand these differences the University of Wisconsin group compared the X-ray crystal structure of JG-365 with a molecular model of Ro 31-8959. These studies suggested differences in the mode of binding of each compound but they concluded that X-ray crystallographic studies on Ro 31-8959 were needed to elucidate the precise molecular details of the binding mode.

Ro 31-8959 has been co-crystallized with HIV-1 proteinase and the partially refined crystal structure is compared with that of JG-365 in Fig. 2. Both inhibitors bind in an extended conformation with the corresponding side chains of the asparagine and phenylalanine residues occupying the same subsites. The hydroxyl group in the transition-state mimetic of each inhibitor binds to the aspartate residues in the catalytic centre. In order to accommodate the opposite stereochemical configuration of the hydroxyl group in JG-365 and Ro 31-8959 there is a divergence in structure after the hydroxymethylene group in the transition-state mimetic. Interestingly, the *tert*-butyl group in Ro 31-8959 occupies the S_2' subsite in the enzyme which in the case of JG-365 is occupied by the isoleucine side chain. In addition, the carbonyl group of the decahydroisoquinoline in Ro 31-8959 and the carbonyl group of the proline residue in JG-365 have similar orientations and both form a hydrogen bond to the water molecule connecting the inhibitor to the flap region of the enzyme. The proline and decahydroisoquinoline moieties occupy the same subsite with the imino-nitrogen atoms slightly displaced relative to each other. Of key importance is the all-S configuration of the decahydroisoquinoline residue. Although these inhibitors are very similar in structure, especially at the N-terminal region, differences are observed at the C-terminus where Ro 31-8959 terminates at the S_2' subsite, whereas JG-365 extends as far as the S_4' binding site of the enzyme.

Type 2 inhibitors

In an approach to identify type 2 inhibitors we screened a selection of renin inhibitors containing a hydroxyethylene transition-state mimetic, derived from the dipeptide moiety Leu-Val, which we reasoned is a very close analogue of the Leu-Ala hydrolysis mediated by HIV proteinase. From that screening programme the protected pentapeptide (7) was identified as a lead structure. The systematic modification of each amino acid residue was studied, and when

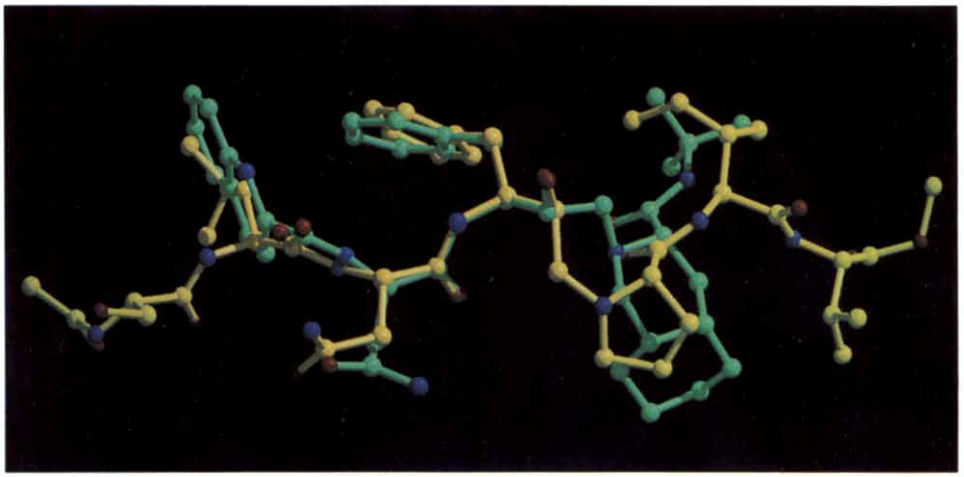


Fig. 2. Graphical representation of X-ray crystal structure of Ro 31-8959 (green) and JG-365 (yellow).

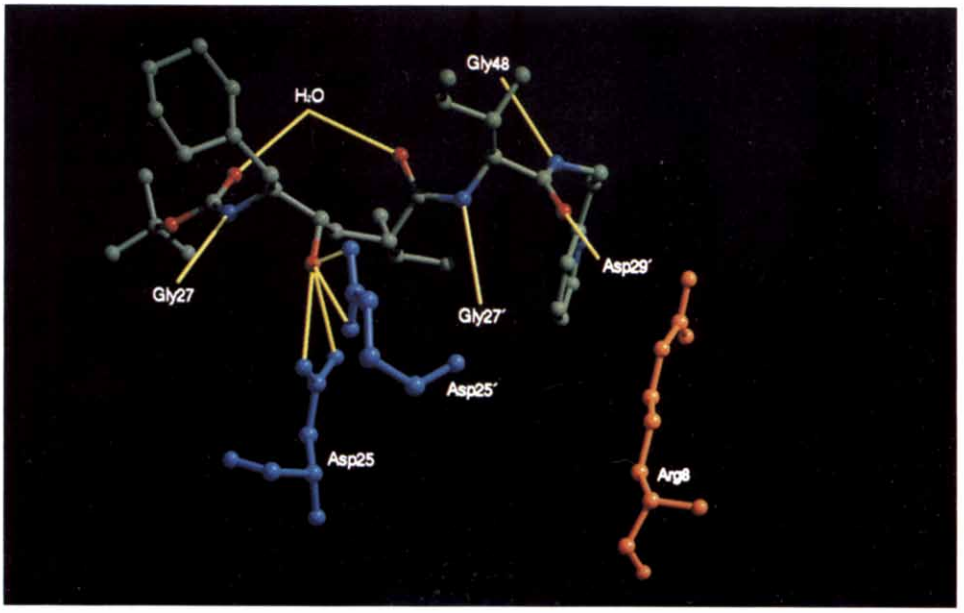


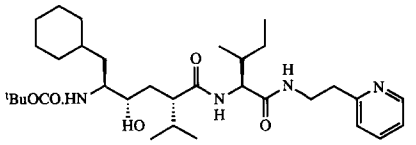
Fig. 3. Graphical representation of the X-ray crystal structure of Ro 31-8588 (green); hydrogen bonds are displayed in yellow.

TABLE 3

Structure and HIV proteinase inhibitory activity of hydroxyethylene isosteres

	Compound		Enzyme IC_{50}/K_i (nM)
(7)	$PhCH_2OCO$-Gln-Leu Ψ	[$CH(OH)CH_2$] Val-Ile-His-OMe	27[a]
(8)	^tBuOCO-Cha Ψ	[$CH(OH)CH_2$] Val-Ile-$NHCH_2CH_2Ph$	4.5[a]
(9)	$^tBuCH_2CO$-Cha Ψ	[$CH(OH)CH_2$] Val-Ile-$NHCH_2CH_2Ph$	7.7[a]
(10)	^tBuOCO-Cha Ψ	[$CH(OH)CH_2$] Val-Ile-$NHCH_2CH_2$-2-Py	<2.5[a]
(11)	$^tBuCH_2CO$-Cha Ψ	[$CH(OH)CH_2$] Val-Ile-$NHCH_2$-2-Py	70[b]

[a]IC_{50}; [b]K_i; Cha, cyclohexylalanine; Py, pyridyl.



(10) Ro 31-8588

the individually optimized groups were incorporated into the same molecule, the potent inhibitor (10) Ro 31-8588 was obtained. In this molecule only the valine and isoleucine residues at P_1' and P_2' respectively in the lead structure are retained (Table 3). It is not surprising that a very similar exercise had probably been undertaken by research workers at the Upjohn company, who quite independently reported compound 11, U-81749, to be a potent inhibitor of HIV proteinase (McQuade et al., 1990). The only differences between Ro 31-8588 and U-81749 are the N-terminal protecting groups in which an oxygen atom is replaced by a methylene group and at the C-terminus where there is one less methylene group in the side chain. During structure-activity relationship studies we found no difference with regard to enzyme inhibition between the *tert*-butoxycarbonyl and the *tert*-butylacetyl N-protecting groups (compound 8 vs. compound 9). Therefore, we reasoned that the marked difference in potency between Ro 31-8588 and U-81749 must reside in the C-terminal group. Fortunately, Ro 31-8588 co-crystallized with HIV-1 proteinase and from X-ray diffraction studies (Graves et al., 1990) the crystal structure was determined (Fig. 3). In the crystal structure, Ro 31-8588 exists in an extended conformation with the expected hydrogen bonding network associated with the amide groups along the backbone of the inhibitor. The aspartic acid residues in the catalytic centre form a hydrogen bond to the hydroxyl group in the transition-state mimetic. Interestingly, the C-terminal 2-pyridyl function participates in an optimized face-stacking interaction with the guanidine moiety of Arg^8. From molecular modelling studies we have determined that the 2-pyridyl moiety in U-81749 cannot participate in an equally favourable face-stacking interaction with Arg^8 of the enzyme, which we believe is responsible for the significant difference in the enzyme inhibiting properties of these two very similar compounds.

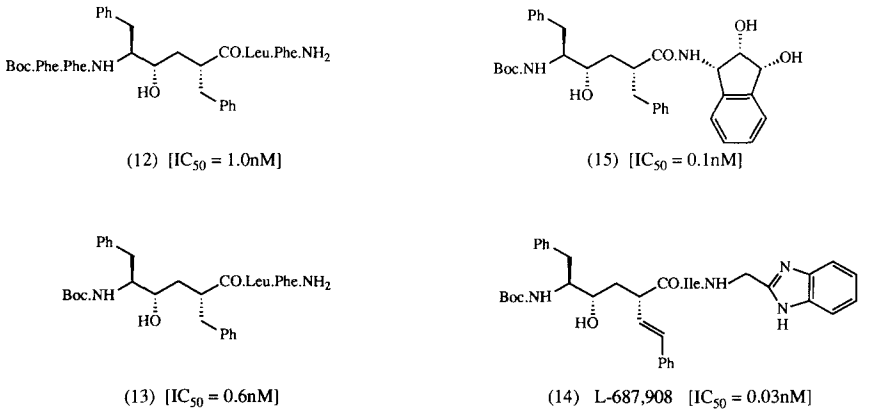


Fig. 4. Structure and HIV proteinase inhibitory activity of Phe-Phe hydroxyethylene isosteres.

Other examples of potent inhibitors of HIV proteinase which contain a hydroxyethylene moiety have been reported (Vacca et al., 1991). The protected hexapeptide (12) which contains a Phe-Phe transition-state mimetic (Fig. 4) was the starting point for this work. Two phenylalanine residues from the N-terminus were removed which afforded a slightly more active inhibitor (13). When the benzyl side chain at P_1' was replaced by a styrene residue and the C-terminal residues by an isoleucyl aminomethylbenzimidazole function the very potent derivative compound 14 was obtained, which is one of the most potent inhibitors of HIV proteinase reported so far. Concurrently, another team reported compound 15 in which the P_2' and P_3' substituents in compound 13 were replaced by a dihydroxyaminoindane group (Lyle et al., 1991). In this compound none of the peptide bonds in the original lead structure remain. Antiviral activity in the 10–100 nM range has been reported for this class of compound.

A series of phosphinate derivatives based on the Phe-Phe dipeptide has been described (Grobelny et al., 1990). The lead structure (16) had a K_i of 0.6 nM (Table 4). Replacement of the benzoyl N-protecting group with Boc-Val-Val gave compound 17 which had enhanced activity, whereas N-terminal extension with Z-Ile-His as in compound 18 had no effect on potency. The most potent compound in the series (17) has modest specificity with a selectivity index of

TABLE 4

Structure and HIV proteinase inhibitory activity of phosphinate derivatives

	Compound		K_i (nM)
(16)	PhCO-Phe Ψ	$[PO(OH)CH_2]$ Phe-Val-Val-NH_2	0.6
(17)	^{t}BuOCO-Val-Val-Phe Ψ	$[PO(OH)CH_2]$ Phe-Val-Val-NH_2	0.04
(18)	$PhCH_2OCO$-Ile-His-Phe Ψ	$[PO(OH)CH_2]$ Phe-Val-Val-NH_2	0.4

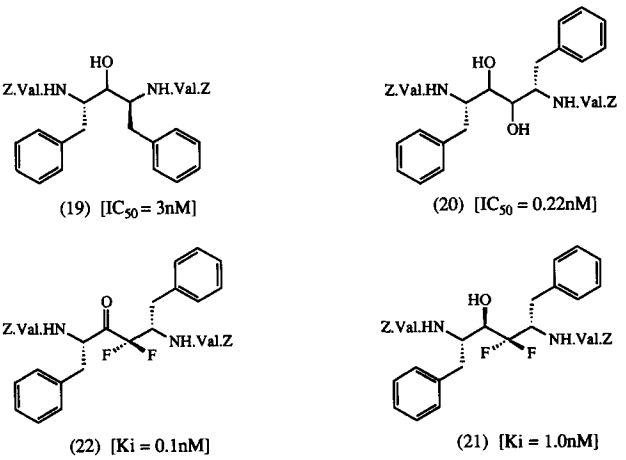
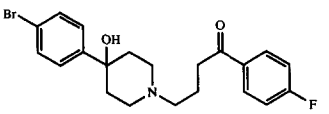


Fig. 5. Structure and HIV proteinase inhibitory activity of symmetrical inhibitors.

224 against cathepsin D. No antiviral activity has been reported for these compounds so far.

Type 3 inhibitors

In an elegant piece of work (Kempf et al., 1990) the Abbott group has developed symmetrical inhibitors based primarily on the rationale that HIV protease acts as a homodimer with C_2 symmetry. In the design process they started from the Phe-Pro transition-state and deleted the P_1' region. The remaining half of the molecule (the Phe residue at P_1) was rotated about the C_2 axis of the enzyme near to the amide bond being cleaved to afford a symmetrical transition-state mimetic. From this design process two series of compounds, exemplified by the disubstituted methanol (19) and the 1,2-ethanediol (20), were developed with potent enzyme inhibitory activity (Fig. 5). Following on from these initial studies the related difluoroalcohol (21) and the corresponding difluoroketone (22) were developed (Sham et al., 1991). The superior potency of compound 22 over all other compounds in this series is probably due to the fact that it exists as the hydrate which more closely resembles the transition-state of hydrolysis than any of the other analogues. Interestingly, these symmetrical inhibitors have antiviral activity in the 10–100 nM range, depending on the cell line and virus strain used.



(23) bromperidol [Ki ~ 100μM]

Fig. 6. Structure and HIV proteinase inhibitory activity of bromperidol.

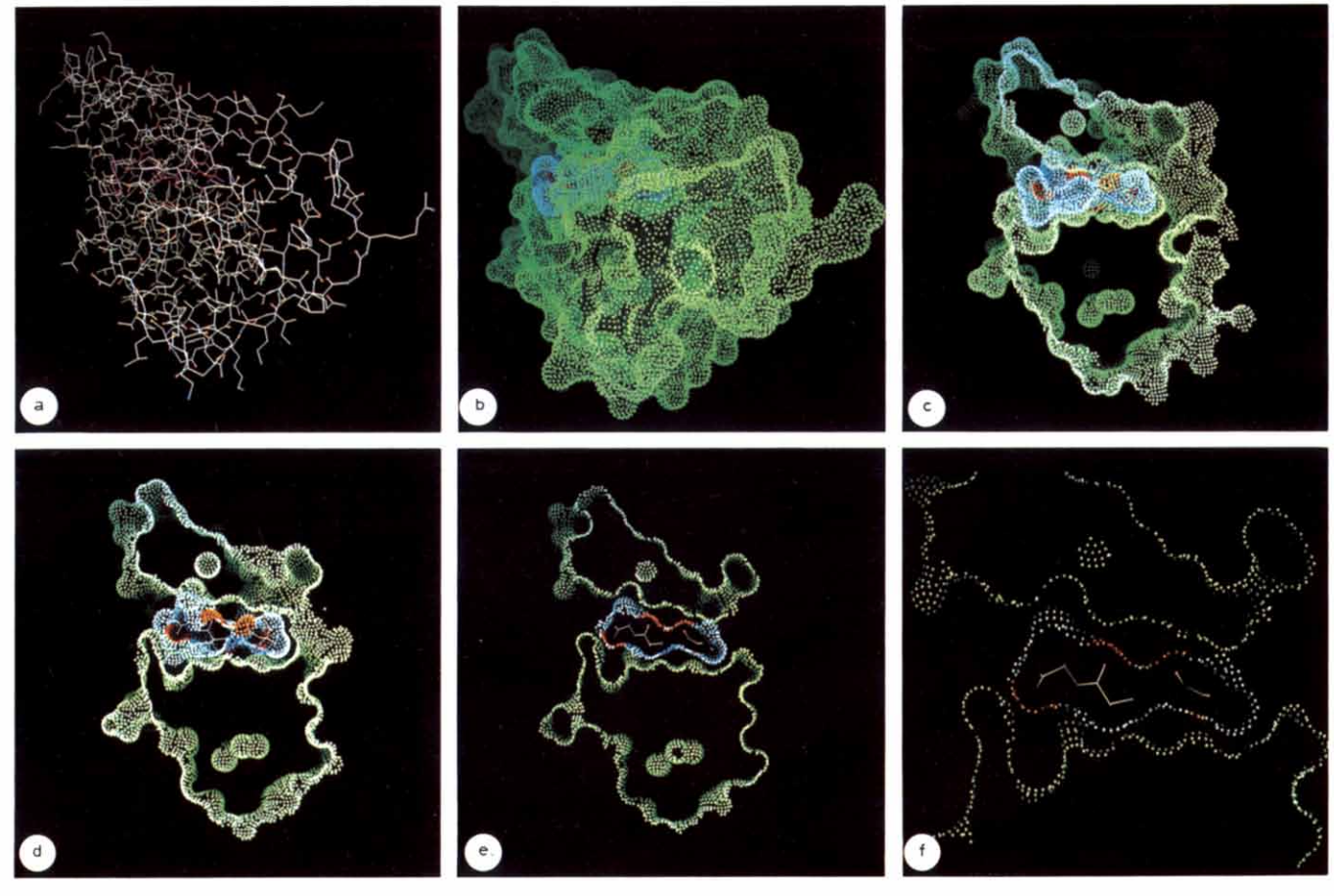


Fig. 7. (a) Graphical representation of X-ray crystal structure of HIV proteinase complexed with Ro 31-8588; (b) graphical representation of X-ray crystal structure of HIV proteinase complexed with Ro 31-8588 with van der Waals surface added; (c,d,e) sections taken through enzyme-inhibitor complex; (f) magnification of section through catalytic centre.

Future prospects

In most drug discovery programmes the involvement of medicinal chemistry escalates following the identification of a lead structure which may be an existing drug, may arise from random screening, or as in the case of enzyme inhibitors evolve from a detailed knowledge of the enzyme mechanism and substrate specificity. Lead structures can be optimized by modifying portions of the molecule in a systematic way, a process aided by structure-activity relationships generated in the process. The majority of inhibitors of HIV proteinase reported so far have been developed using this kind of approach. Not surprisingly, this strategy has afforded inhibitors which retain many peptide-like features and often the associated liabilities of poor oral bioavailability, rapid elimination and short duration of action. The translation of a peptide-based structure into a small organic molecule with minimal resemblance to the starting peptide remains a major challenge in medicinal chemistry.

In an attempt to design non-peptide inhibitors of HIV proteinase a group at the University of California modelled the X-ray crystal structure of uncomplexed enzyme from which they constructed a negative image of the active site (DesJarlais et al., 1990). The cylindrical image obtained was compared with 10 000 compounds from the Cambridge Crystallographic data base to find a molecule that would fit into the active site and in addition have potential to provide some of the important hydrogen bonding interactions for strong binding to the enzyme. From modelling studies, bromperidol (23) was suggested as a lead structure (Fig. 6), based on a reasonable steric fit and the potential for the hydroxyl group to be orientated between the active-site aspartate residues. There were some concerns with bromperidol as a lead since the long axis of the crystal structure was not aligned with the backbone of known peptide-based inhibitors. Nevertheless, bromperidol was reported to inhibit HIV proteinase in a dose-dependent manner, albeit with low potency. It remains to be seen whether more potent and useful inhibitors will evolve from this novel approach.

We have also been studying approaches that are directed towards the design of non-peptide-based enzyme inhibitors. In order to make smaller molecules we are working to maximize the binding of inhibitors at or around the catalytic centre of the enzyme which could be achieved through an extended medicinal chemistry programme, but this could turn out to be a lengthy process. In an attempt to facilitate the design of second-generation inhibitors with enhanced potency we are using information derived from X-ray crystal structures and molecular modelling techniques. The crystal structure of HIV proteinase complexed with Ro 31-8588 is shown in Fig. 7a with the location of the inhibitor in the body of the enzyme clearly displayed. In another representation of the same data (Fig. 7b) van der Waals surface has been added which reveals that the pyridyl residue at the C-terminus of the inhibitor is just visible at the surface of the enzyme. We have taken sections through the enzyme-inhibitor

complex progressively until the catalytic centre is reached (Fig. 7c, d and e). During this process regions of good contact between the inhibitor and enzyme are identified as well as areas where contact is sub-optimal (Fig. 7f). Currently, we are using molecular graphics to design molecules where contact between the inhibitor and enzyme is improved, especially in the region close to the active centre, which we expect to markedly enhance potency. Then, we aim to remove adjacent appendages, reducing the size of the molecule significantly, affording a structure with minimal peptide-like features.

Whether these approaches are successful or not, it is almost certain that novel, non-peptide-based inhibitors of HIV proteinase will be discovered. Meanwhile, there is every expectation that the utility of proteinase inhibitors against clinical HIV infection will be demonstrated during the next year, which will stimulate further efforts to identify the next generation of HIV proteinase inhibitors.

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