Mimetics of the Peptide β-Strand

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Abstract: Bioactive structures of peptides represent important clues for drug discovery and development although peptides themselves have substantial limitations as drugs. One promising approach to overcoming the limitations of peptides is to progressively replace amide bonds in peptides with non-peptidic constraints that bring drug-like properties like stability and bioavailability to the molecules. These constraints can also be used to mould molecules into shapes which mimic key elements of protein secondary structure that confer bioactivity to protein surfaces. Preorganizing a molecule into the shape recognized by a receptor results in high affinity binding though a considerable entropy saving and is an effective approach to engineering highly bioactive drug leads. One peptide structure, the extended beta strand, has only recently been identified as a fundamental recognition element in physiological processes. Relatively few molecules have been described as constrained mimics of extended peptide conformations. We now summarize some approaches to mimicking peptide beta strands, and illustrate these with examples of bioactive, stable and bioavailable molecules that are conformationally biased to mimic the extended peptide beta strand.

Keywords: Peptidomimetic, β-Strand, Extended, Conformation, Drug, Review

IMPORTANCE OF β-STRANDS

A common approach to drug design involves the examination of protein-protein interactions associated with disease, followed by the design of small molecules that can mimic or bind to one of the interacting proteins [1-7]. Often the bioactivity stems from only a small localised region of a protein surface created by secondary structural elements such as α-helices, β- or γ- turns, or β-strands that usually align to form β-sheets. The β-strand has usually been considered to be a random peptide conformation but, more recently, the β-strand has become recognized as a fundamental discrete element of protein structure that is recognized by a very wide range of biomolecular receptors.

For example, it has been convincingly demonstrated that all proteolytic enzymes (aspartic, serine, metallo and cysteine proteases) commonly bind their inhibitors/substrates in extended β-strand structures in which the peptide backbone or equivalent non-peptide molecule is drawn out in a linear conformational arrangment [8-10]. Proteases are involved in the synthesis and turnover of all proteins, are associated with most diseased states, and their selective inhibitors are showing very promising therapeutic uses [7]. Major Histocompatability Complex (MHC) proteins [11] also recognise their ligands exclusively in the extended β-strand conformation. In immune defence MHC proteins selectively bind peptides derived from intracellular processing of viral, bacterial and endogenous proteins and present them at the cell surface for recognition and immunological destruction [12]. This type of strand recognition is implicated in leukemia and inflammatory and neurological diseases [13]. Transferases such as farnesyl transferases [14], which are implicated in the development of cancers, also recognize peptide β strands.

In addition to such specific recognition of discrete β-strands, combinations of strands form β-sheets that not only act as important scaffolding elements to stabilise protein structure, but are sometimes key recognition motifs that bind to other proteins or DNA. Some proteins use β-sheets to bind to DNA, such as Arc repressor proteins and TATA box-binding proteins [15,16]. β-strand peptidomimetics could thus find important roles as competitive ligands for receptors that typically bind to β-sheets. In another context, the formation of β-sheets themselves is sometimes undesirable. For example Alzheimer's and prion diseases are thought to result from aggregated sheets and intervention in β-sheeet formation by strand mimetics could represent an important new therapeutic strategy that has not yet been realized.

NEED FOR β-STRAND MIMETICS

Short peptides do not tend to adopt discrete structures in aqueous solution [6,17,18]. Thus the arrangement of peptides into β strands for recognition by biomolecular targets such as those above is either a chance event in which the receptor captures the small percentage of peptide present in the strand conformation, or else the receptor plays an active role in contorting the peptide into the preferred strand shape. A central principle in medicinal chemistry is that molecules, which are conformationally pre-organized or fixed into a shape that is recognized by a receptor, will have

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higher affinity for that receptor due to the reduced entropy penalty for adopting the receptor-binding shape. It is therefore surprising that, unlike turns and helices, there are relatively few known conformationally restricted surrogates for the β-strand.

Another reason that strand mimetics are needed is that peptides have several properties which compromise their use as drugs [3]. They are very susceptible to degradation through amide bond cleavage by peptidases, have very low bioavailability particularly when orally administered, and they generally exhibit very poor pharmacological profiles due to a combination of these factors, other forms of metabolism, rapid clearance rates, and poor membrane permeability. Thus, while biomolecular interactions with peptides provide very useful clues for drug design, changes need to be made to create more pharmacologically acceptable drug candidates with higher stability, higher membrane permeability, longer plasma lifetimes, slower elimination, higher selectivity, and good bioavailability preferably by oral delivery. Peptidomimetics such as those described below are not only conformationally biased towards the β-strand shape, but also exhibit improved drug-like properties over peptides.

GENERAL APPROACHES TO β-STRAND MIMETICS

The peptide β-strand is a saw-toothed arrangement of amino acids where side chains alternate above and below the plane of the peptide backbone, ideally with torsional angles φ, ψ, and τ = -139, 135 and –177.2° respectively, and d = 8.0 Å (Fig. **1**) [19]. Nature frequently moulds peptides into turn shapes by replacing amino acid components with a wide variety of conformational constraints including disulfides, double bonds, N-methyl amino acids, D-amino acids, aromatic and heterocyclic rings, often in conjunction with cyclisation [2,6]. Similar conformational constraints can be used to synthetically mimic the peptide β-strand. For example one or more amino acids could be replaced within a linear peptide sequence by one or more rigid organic units that bridge between retained amino acid ends. Such replacement of amino acids leads to peptidomimetics with drug-like components and examples are presented ahead. Another minimalist approach would involve side chain to side chain, side chain to main chain, or main chain to main chain linkages to form macrocycles. Cyclic peptides have the advantages over linear peptides of being more resistant to amide bond cleavage by proteolytic enzymes and of being more conformationally restrained or less flexible. Examples are given in the next section of strand peptidomimetics created through macrocyclization (generally $C_i^{\alpha} \rightarrow C_i^{\alpha}+1$, $C_i^{\alpha}+2$ or $C_i^{\alpha}+3$), inter- or intra- residue cyclization, or ring fusion.

MACROCYCLIC PEPTIDOMIMETICS

Macrocycles formed through condensing peptide side chains to the main chain have been shown to be excellent restraints for structurally mimicking tri- and tetra- peptides in extended conformations, as demonstrated for example by crystal structures that reveal their interactions with protein residues in active sites of proteases [20-25]. This approach together with side chain to side chain cyclisation has produced a variety of macrocyclic peptidomimetics (e.g. **1**-**3**) as potent and selective inhibitors of HIV-1 protease [20-24,26-28]. This minimalist approach retains all the amide bonds and associated hydrogen bonding interactions made between peptide and receptor, while simultaneously pre-organising the extended conformation of the inhibitor. Similar macrocyclic peptidomimetics (e.g. **4**-**6**) have been designed and constructed as potent inhibitors of other aspartic, serine and metallo proteases [6,25,29,30]. Such macrocycles are not only resistant to peptidases of the gut, bloodstream or cells, but can also penetrate cell membranes and exhibit potent antiviral activity in cell culture [26,31].

There are also a number of macrocyclic natural products that were originally thought to be turn mimetics but are now known to present short extended peptide segments to proteases. Among these are K-13 (**7**) and OF4949-IV (**8**) [32-34], which are inhibitors of the metalloproteases ACE and aminopeptidase. They lack the metal binding functionality normally associated with inhibitors of metalloenzymes, suggesting an alternate mechanism of interaction. NMR and molecular mechanics calculations on the thioether analogue of **7** suggested an extended conformation for the peptide [35], and this is now supported by recent NMR and X-ray analyses on synthetic analogues [36].

Cyclotheonamide A (**9**) is a naturally occuring macrocyclic serine protease inhibitor isolated from the

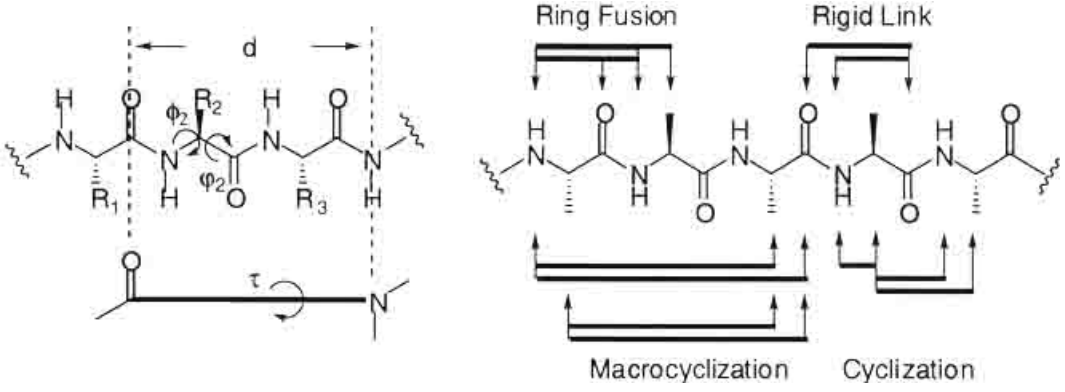
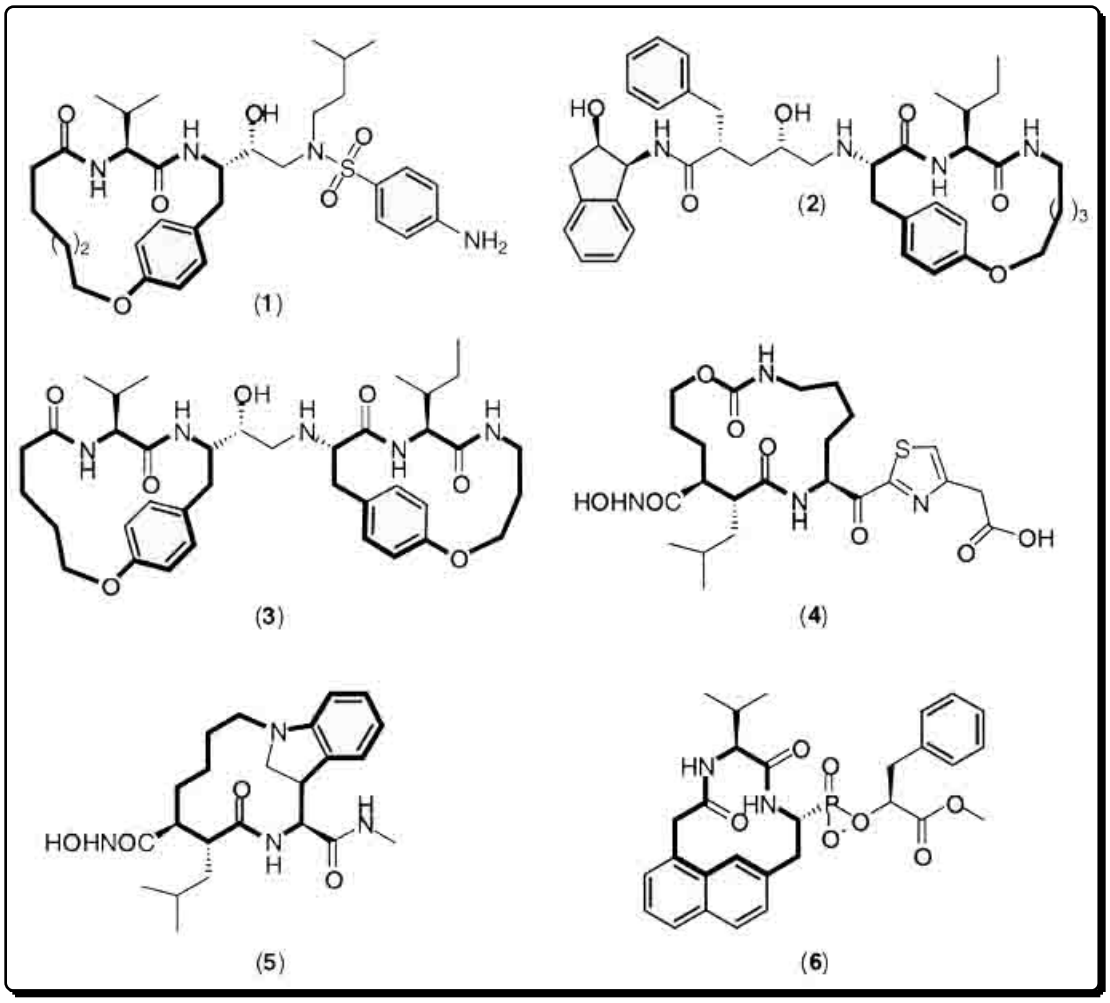
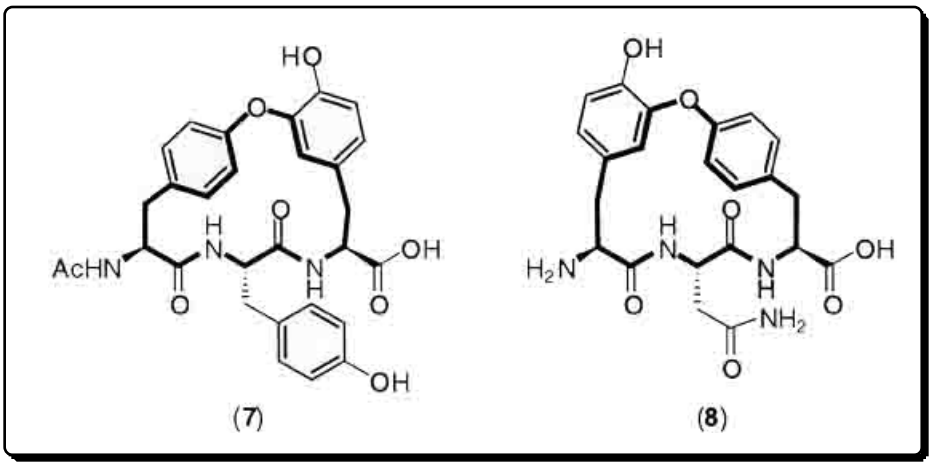


Fig (1). Peptide β-strand (left) defined by φ, ψ, τ angles and d distance and (right) approaches to cyclisation.



marine sponge *Theonella* sp [37]. It was originally thought to exert its potent thrombin- and trypsin- inhibiting properties by enforcing a bioactive turn conformation. However X-ray crystallographic studies of its complexes with these serine proteases have since identified an extended strand conformation for the protease-binding segment of this macrocycle (Fig. **2**) [38]. The NH-C-C(O)-Pro-Arg segment forms a hydrogen-bonded two strand antiparallel β-sheet with Ser214/Trp215/Gly216. Moreover, solution NMR studies of cyclotheonamide A in aqueous media reveal that the conformation of the protease-binding segment D-Phe-Arg-Pro is almost identical to that found in the solid state [39], indicating that cyclotheonamide pre-organizes this segment in the extended conformation needed for protease binding. The macrocycle does not undergo significant conformational change on binding to the active site of the protease. Subsequent studies of simplified cyclotheonamide analogues, which utilize an aliphatic tether, realized a number of potent thrombin inhibitors which were shown to maintain the tripeptide array in the extended conformation [40].



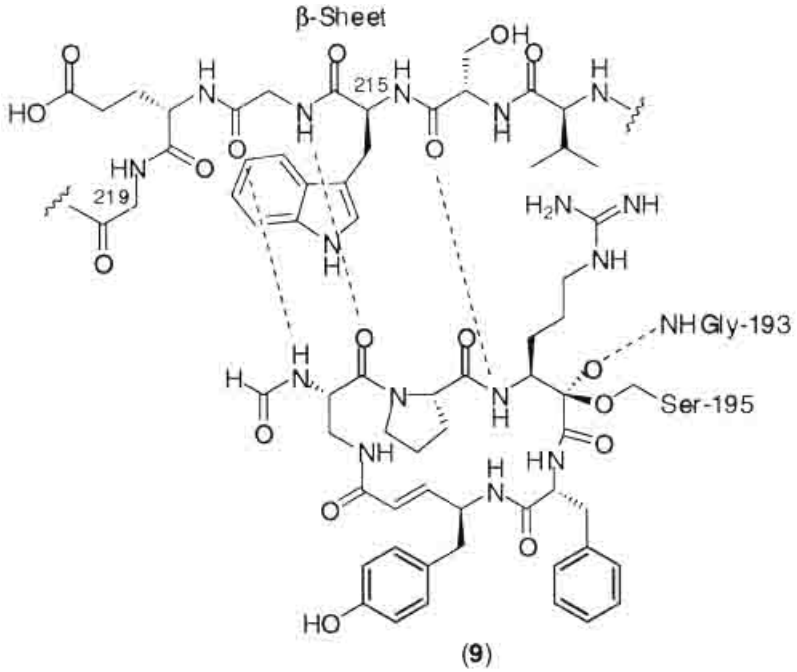


Fig (2). Schematic representation of the interactions between the β-strands of cyclotheonamide A (**9**) and thrombin.

CARBOCYCLES

Aromatic spacers have been incorporated into inhibitor design as extended dipeptide mimics, for example inhibitors of Ras farnesylation. Inhibition of this process was identified as an effective method for slowing the development of cancers in which oncogenic Ras proteins are active [41,42]. A series of lead tetrapeptide inhibitors of the general form CAAX (where A is an aliphatic amino acid and X is Met or Ser), were identified as potent peptide inhibitors of FTase *in vitro*, but proved to be inactive in cell based assays [43-46]. Replacement of the aliphatic AA unit with hydrophobic spacers identified strong selection for rigid extended systems as replacements for the dipeptide (Fig. **3**) [14,47]. In related work, replacement of Phe for the conformationally constrained Tic, resulted in a significant increase in potency that directly correlated to the proportion of extended conformation present [48].

5-Amino-2-methoxy- benzamide and benzoic hydrazides (**16**) (Fig. **4**) have been incorporated into novel β-strand mimetics to induce artificial sheet formation. While inhibitors of any particular processes based on these units have not yet been realized, the evidenced sheet formation by

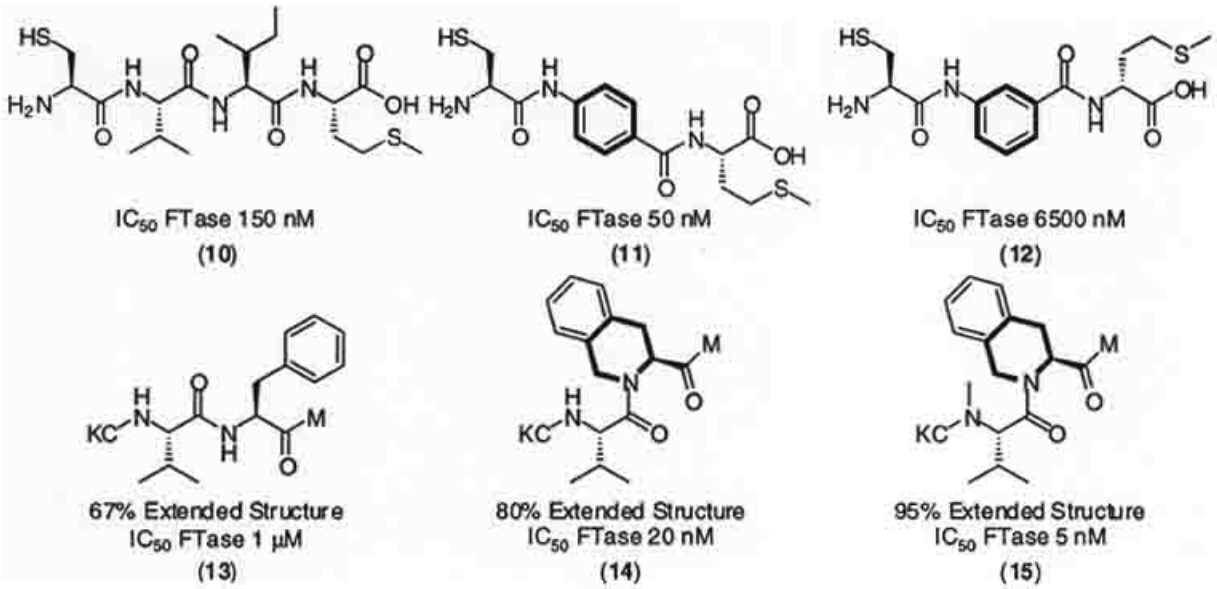


Fig (3). p21^{ras} CAAX peptides and peptidomimetics [14,48].

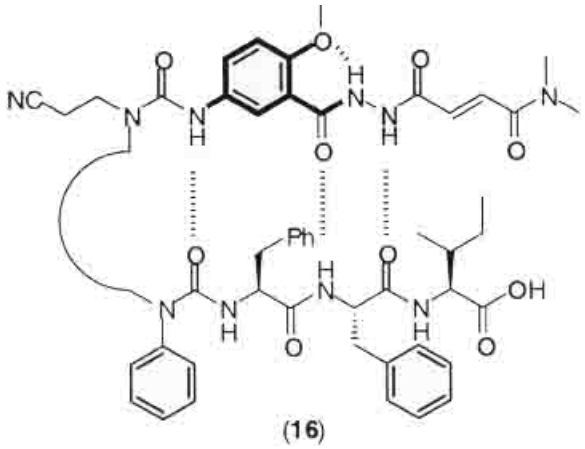
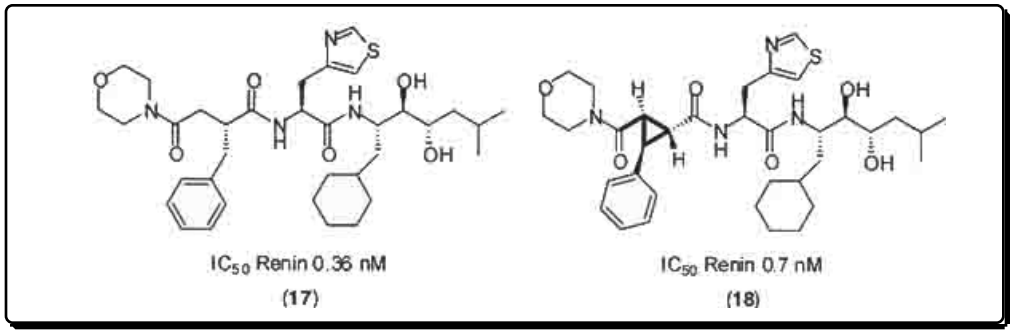


Fig (4). β-Strand template for artificial β-sheet formation [52].

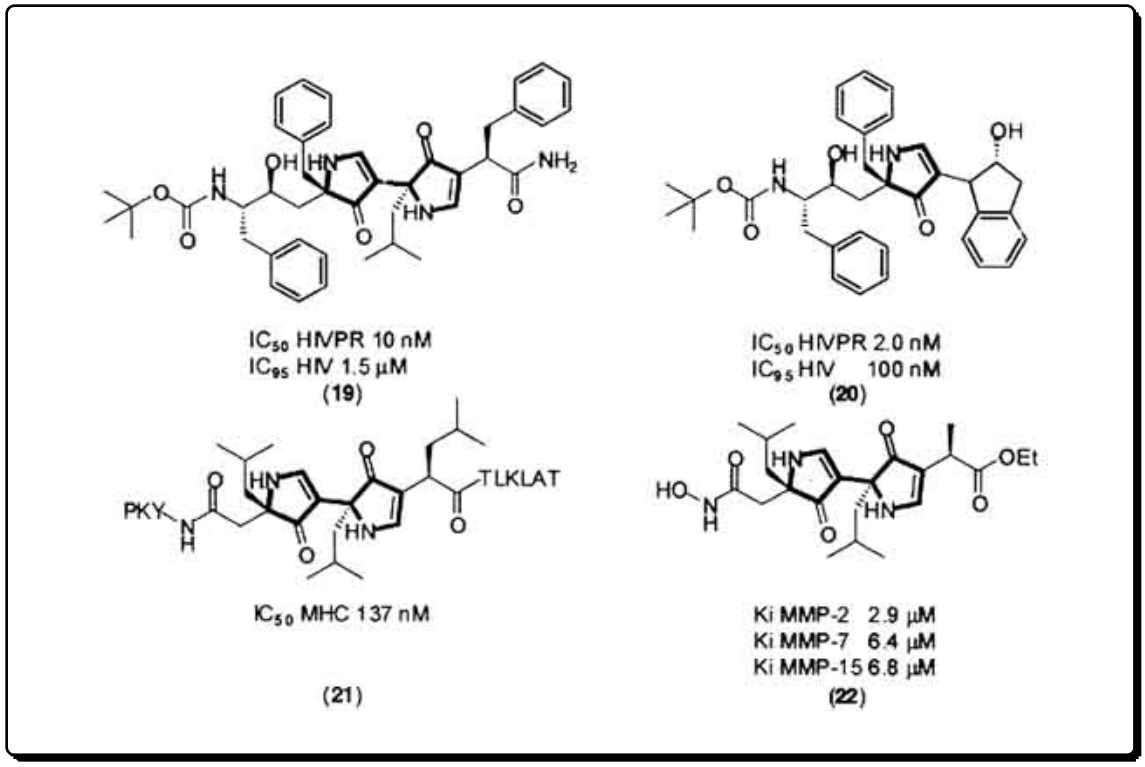
these templates confirms the extended nature of the scaffold [49-52] and suggests new approaches to β-strand mimetics. include cyclopropanes, the side chain being rigidly fixed to χ-angles corresponding to ±60° [53,54]. Potent inhibitors of the aspartic protease renin have been realized using this constraint (**17**, **18**).

PYRROLINONES

Strand mimetics have been developed using pyrrolinones (**19**-**22**) within novel scaffolds that replace the hydrolysable backbone of bioactive peptides. These mimetics maintain key side chain interactions and some of the intermolecular hydrogen bonds made with the complementary enzyme strand[55-57]. X-ray structural analyses reveal an extended conformation for this type of peptidomimetic, with the scaffold closely mimicking the positions and orientations of the backbone carbonyls and side chains [55,58]. Two different pyrrolinone systems have been developed, the 3,5- and 2,5-linked scaffold. The 3,5- system has an all carbon



Other carbocyclic conformational constraints which restrict the available φ-angle to closely approximate the β-strand backbone (**19**-**22**) while the 2,5- system has backbone NHs. It is envisaged that the 3,5- system would be employed



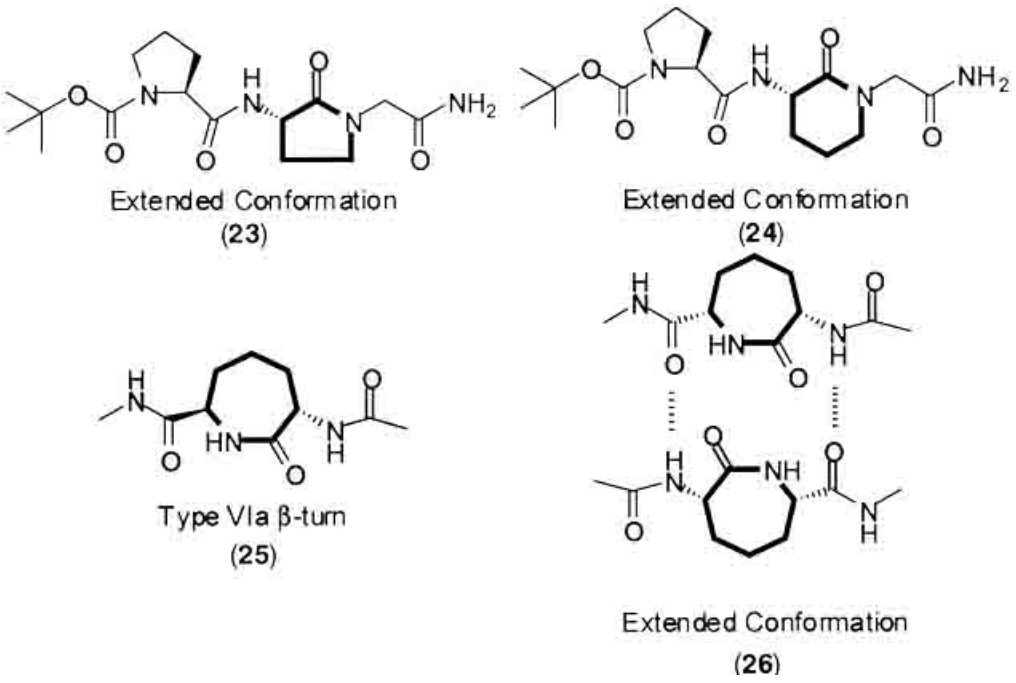


Fig (5). Lactams shown by X-ray structural analysis to form an extended conformation [68,69].

when hydrogen bonding to enzyme amide NHs is most important, while the 2,5- system would be more useful when H-bond donors are required.

The 3,5-linked pyrrolinone scaffold has been successfully implemented in creating inhibitors of HIV-1 protease [56,59,60], matrix metallo proteases (MMP) [61] and major histocompatibility complex (MHC) protein HLA-DR1 [62-64]. High potencies for enzyme inhibition are observed, in addition to enhanced cellular uptake and bio-availability [56,59]. Where X-ray crystallography of enzyme-bound pyrrolinone inhibitors has been possible, remarkably good mimicry with peptides has been observed [62].

LACTAMS

Cyclization ($C_i^{\alpha} \rightarrow N_{i+1}$) to form Freidinger lactams has been widely used to produce a peptidic backbone constraint that maintains *trans* amide bond configurations, significantly limiting the attainable ψ angles (-125±10°) [65]. Generally considered a type II' β-bend mimetic, conformational analyses [66,67] of lactams in some systems by NMR, X-ray and modelling, indicate a discrete extended motif in the lactam backbone (C_i^{α} - C_i^{CO} - N_{i+1}).

X-ray crystal structure studies [68] of this constraint in lactam derivatives of **23** and **24**, have clearly identified an extended conformation. Similar observations [69] were also made on the 7-membered lactams **25** and **26** (Fig 5). The *cis* diastereomer in this series was observed by X-ray analysis to have an extended conformation, while the *trans* isomer formed the expected β-turn. More interestingly, NMR solution studies of the *cis* isomer in non-coordinating solvents indicated head-to-tail self-association. This type of interaction was only weakly apparent for the *trans* isomer. These observations suggest that cyclic constraints of this type, although generally associated with turn structures, may be able to access extended conformations.

Appropriately functionalised lactams have also been found to be potent inhibitors of the metalloprotease angiotensin converting enzyme (ACE) [70-73], which has been proposed to select for a conformationally extended pharmacophore [74]. The use of these lactams as potential ACE inhibitors stemmed from attempts to improve the activity of captopril (**27**) (Fig 6), the first orally active ACE inhibitor, by restricting both the available ψ angles and the configuration of the amide bond [75,76]. Subsequent work identified a number of potent lactam-based inhibitors of both ACE and the related metalloprotease neutral endopeptidase (NEP) (**28**).

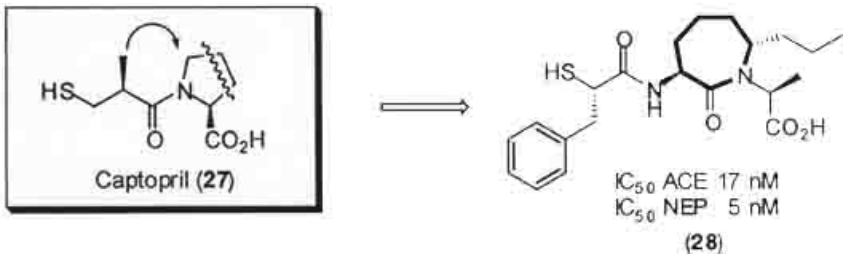


Fig (6). Design constraint of Freidinger lactams leading to ACE inhibitors [67,108].

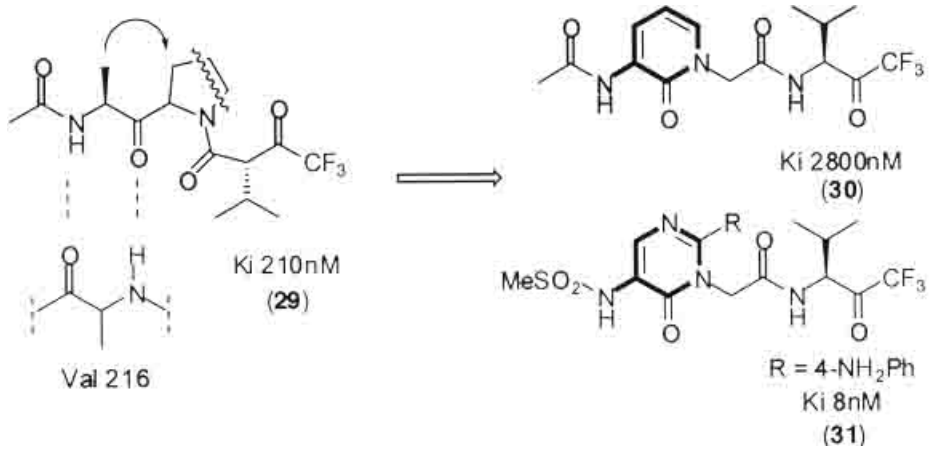


Fig (7). Designed peptidomimetic inhibitors of HLE [78,81].

PYRIDONES

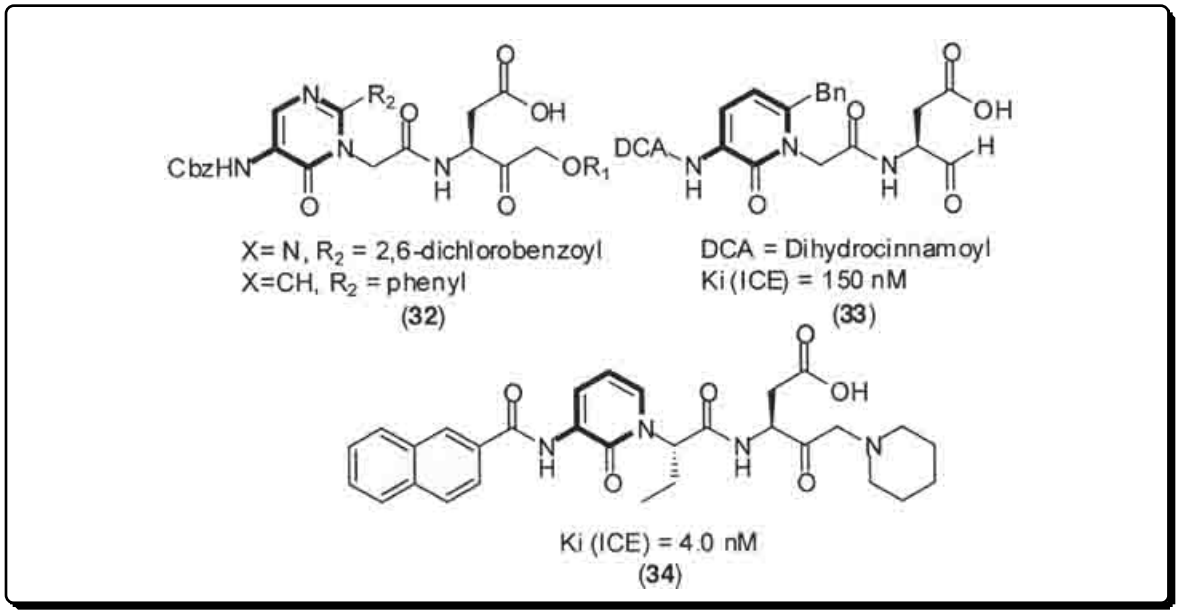
Peptidomimetic pyridone antagonists (Fig. 7) of human leukocyte elastase (HLE), a serine protease implicated in the etiology of adult respiratory distress syndrome (ARDS) as well as many other human inflammatory conditions, have been developed from X-ray crystallographic studies of the heptapeptide inhibitor AcAPV-TFMK (**29**) bound to the closely related enzyme porcine pancreatic elastase (PPE) [77]. Critical hydrogen bonding interactions between the β-sheet region of Val-216 and the inhibitor prompted the incorporation of a P3 to P2 conformational restraint [78]. A number of potent inhibitors of HLE based on this scaffold have now been developed [79,80], with subsequent X-ray crystallography studies of **31** bound to PPE demonstrating the successful retention of this hydrogen bonding pattern [81].

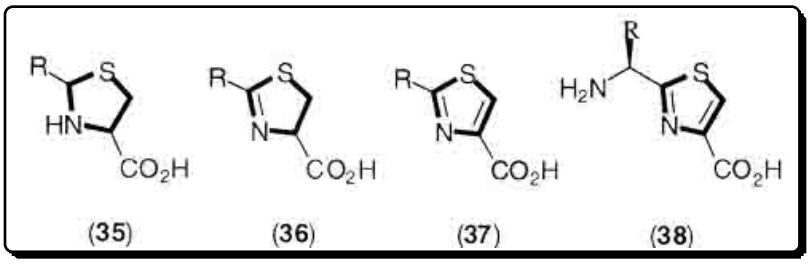
This structural constraint was subsequently adopted in the design of antagonists of interleukin-1β converting enzyme (ICE) [82], a cysteine protease which cleaves prointerleukin-1β to generate biologically active mature IL-1β [83]. A mimetic was sought to replace the P3-P2 Val-Ala dipeptide portion of lead antagonists but maintain the P3 carbonyl and NH, to ensure integrity of this β-sheet hydrogen-bonding motif (**32**-**34**) [84-87].

THIAZOLES AND THIAZOLIDONES

Alternative 5-membered heterocyclic rings used as proline replacements in medicinal chemistry include thiazoles (**35**), thiazolines (**36**), thiazolidines (**37**), their corresponding oxazoles and oxazolines, and their condensation products with other amino acids to form dipeptide surrogates (**38**).

For example the potent inhibitor of HIV-1 protease (**39**) [88,89], known as KNI-272, is representative of a class of compounds that possess an allophenylnorstatine transition



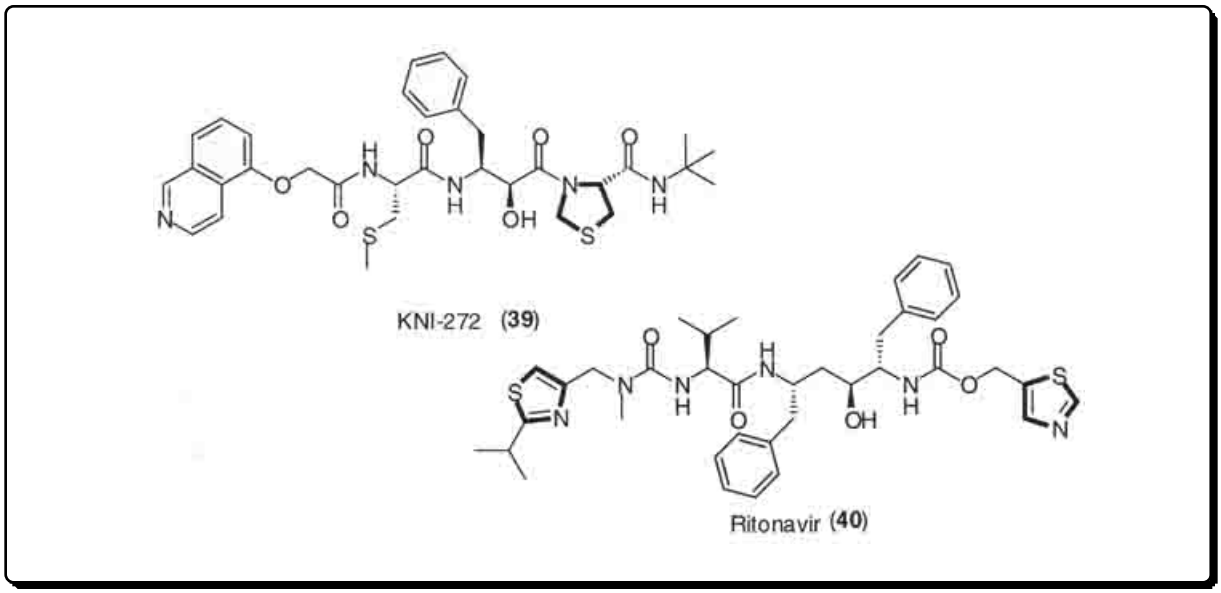


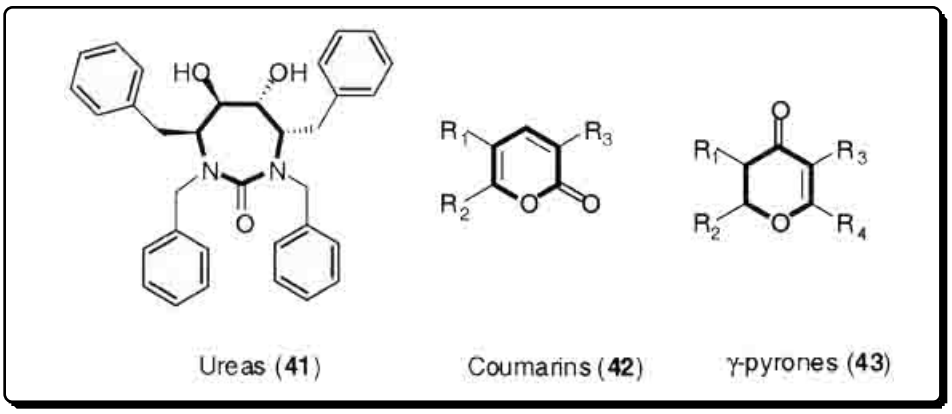
state isostere, a hydrophobic quinoline at P3, and a more bulky thiazoline instead of proline at P1'. The thiazoline has an important β-strand inducing role to play in this molecule, which has a solution structure that is almost identical to its conformation in its crystal structure with HIV-1 protease. This indicates that KNI-272 and analogues are pre-organized into β-strands for receptor binding.

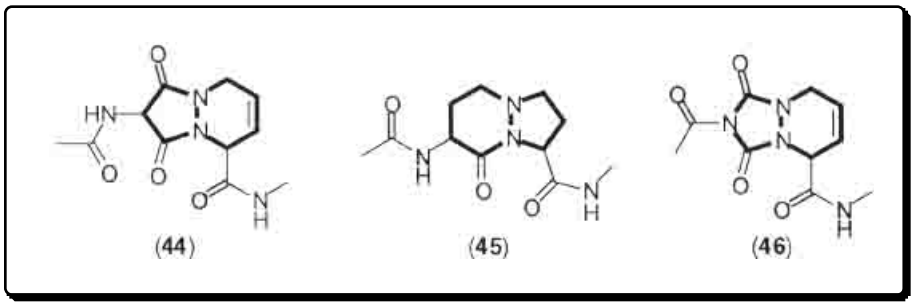
Another potent inhibitor of HIV-1 protease is ritonavir (**40**) which, although possessing rigidifying thiazoles at either end and a urea and carbamate, still has a conformationally flexible interior and thus pays an entropic penalty for reorganizing to the extended β-strand that binds to the protease [90].

CYCLIC UREAS AND PYRONES

An interesting series of cyclic urea inhibitors of HIV-1 protease have used 5-7 membered cyclic ureas (**41**) as scaffolds to project benzylic and related hydrophobic substitutents into appropriate P2, P1, P1' and P2 positions for interaction with the enzyme [91-93]. Although these compounds do not mimic all the protease-binding H-bond donors and acceptors of peptide strands they can still be considered as constrained peptidomimetic scaffolds in which the ring substitutents do occupy the same spaces within the protease active site as amino acid side chains of linear peptide β-strands. The central location of the cyclic urea template within the molecule makes these compounds







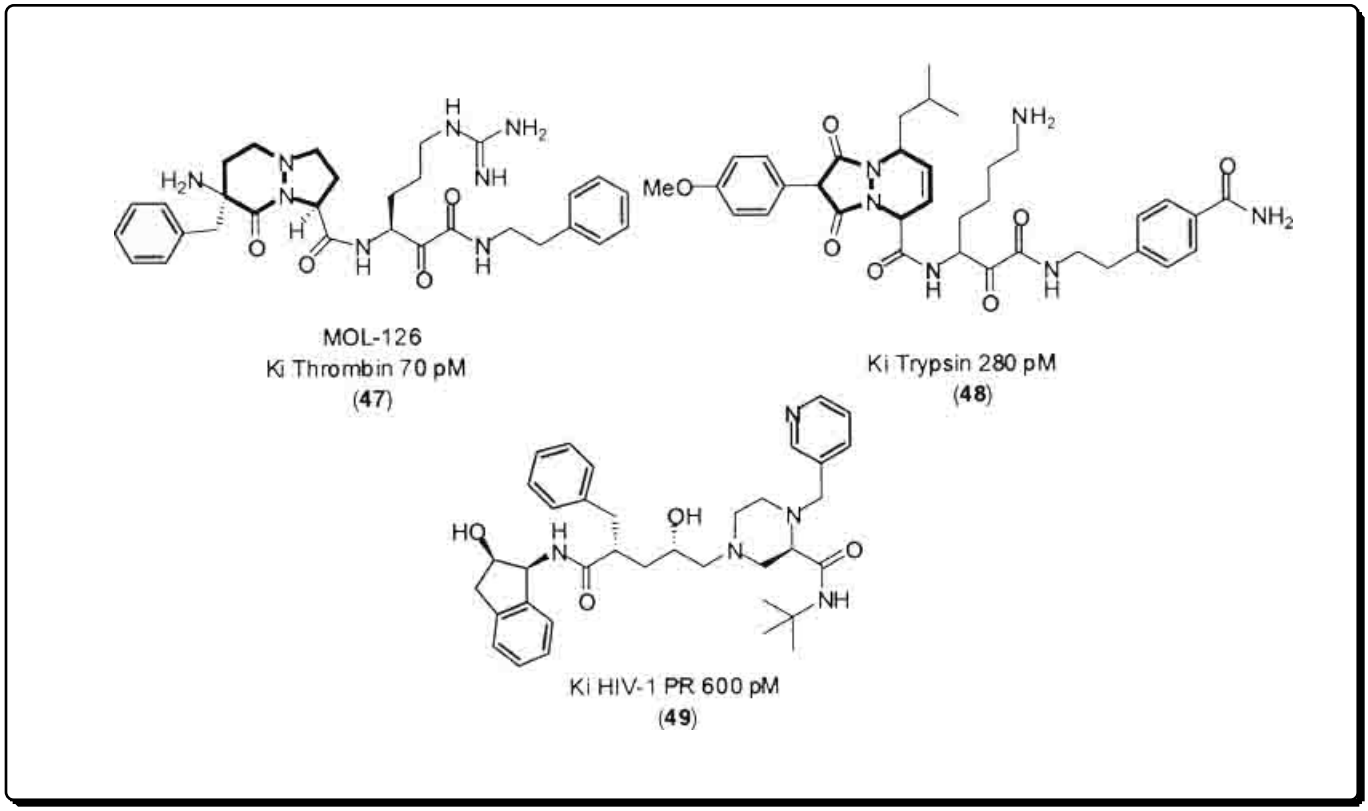
conformationally highly constrained. A similar idea is embodied in coumarin (**42**) and gamma-pyrone (**43**) inhibitors [94-97] of HIV-1 integrase and HIV-1 protease, the cycle serving as a central scaffold upon which substituents can be mounted to mimic the side chains of peptides.

FUSED BICYCLIC CONSTRAINTS

Fused bicycles have been studied extensively as dipeptide mimetics to induce turn conformations. With the realization that the extended conformation is a recognition element for biological function [8-10,98], a number of bicyclic scaffolds have now been developed to mimic the β-strand. Molecular modelling and conformational searches indicated that the 5,6- fused bicyclic templates **44**-**46** would meet the spatial requirements of a dipeptide β-strand mimetic, while facilitating versatile incorporation of substitutents [99].

Extremely potent enzyme inhibitors have been realized with these templates, two of which are shown as **47** and **48** [100-102]. An X-ray structural study of MOL-126 bound to thrombin revealed anti-parallel β-strand hydrogen bonded interactions between the bicyclic template carbonyl oxygen and its amino nitrogen, and the Ser-214 to Gly-216 main chain segment of thrombin [102,103]. The similar 5,6-fused bicyclic 2-hydroxyaminoindane (**49**) has been successfully used as a bulky P2 substitutent in crixivan, a potent inhibitor of HIV-1 protease that is currently used to treat humans with AIDS [104]

These templates have a number of similarities to the to the bicyclic peptidomimetic inhibitors developed for ICE, ACE and NEP, the monocyclic inhibitors of which have been discussed above. With respect to the ICE system, it was determined that good potency required a constrained peptide mimetic which retained the P3 NH, P3 C(O) and P1 NH hydrogen bonding functionality in the correct disposition for β-sheet hydrogen bonding with the enzyme,



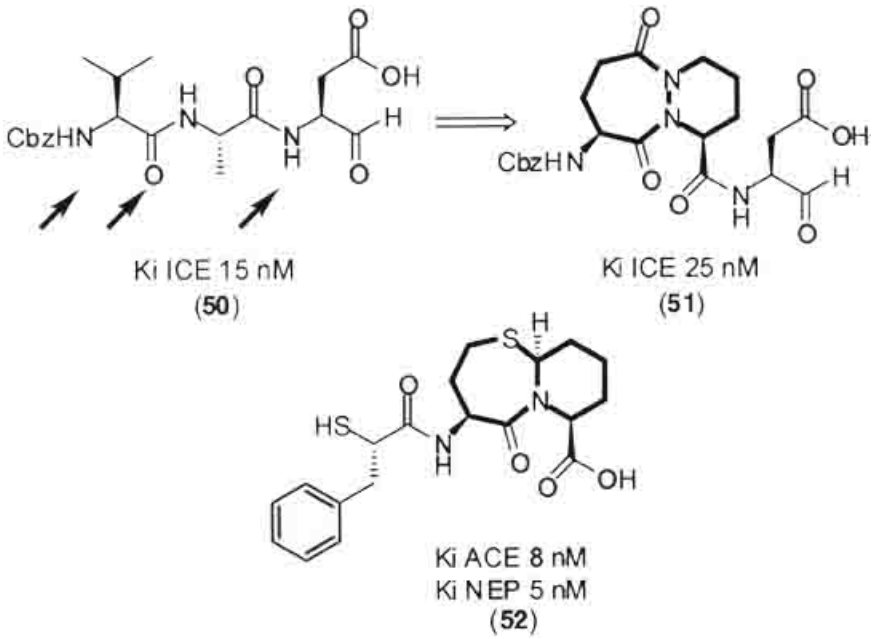
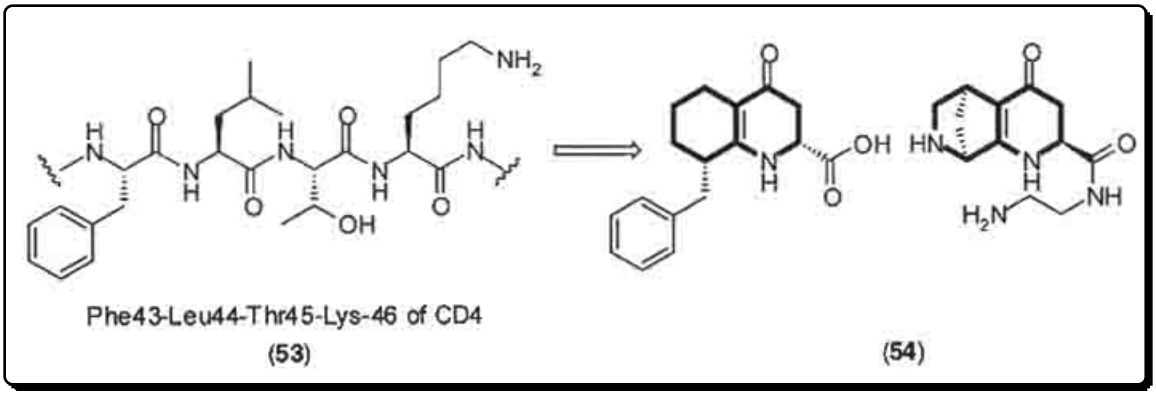


Fig (8) Top: Important hydrogen bonding interactions leading to development of β-strand constrained dipeptide mimetic for ICE [105]. Bottom: Dual metallo protease inhibitor of ACE and NEP [73].

and led to the development of the template shown in Figure 8 [105]. Similar bicyclic scaffolds have been developed as dual metalloprotease inhibitors of ACE and NEP [73].

Fused bicyclic dipeptide β-strand mimetics have also been developed to inhibit the binding of CD4, the cellular receptor for HIV, and the viral protein gp120, after X-ray studies indicated that the binding of these groups occurred through β-stranded regions (**53**). Mimetics were developed for the key binding residues, Phe43-Leu44 and Thr45-Lys46 (**54**), and the sections subsequently condensed to form a β-strand tetrapeptide mimetic [106,107]. The efficacy of these cycles is yet to be determined.



SUMMARY

It is only fairly recently that the extended peptide β-strand has been universally recognised as key recognition motif for biomolecular receptors. Relatively few methods currently exist for fixing β-strand conformations. Yet compounds that can be moulded around β-strand mimicking constraints can be expected to be pre-organized for binding to such biomolecular receptors and thus to have high receptor-binding affinities. The availability of new drug-like components for use as β-strand shaping templates will enable faster and more effective development of enzyme inhibitors and receptor antagonists, even for receptors where there is no three dimensional structural information available to guide drug design. We encourage further studies designed to create new β-strand mimetics and to identify the importance of the strand structure in other examples of protein recognition.

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