CHAPTER FIFTEEN

Peptidomimetics for Drug Design

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1 INTRODUCTION

Protein–protein interactions are central to biology and provide one mechanism to convert genomic information into regulated biological responses. Important examples of protein-peptide interactions include the binding of peptide ligands to proteases, the binding of peptide hormones to peptide receptors, the recruitment of proteins to effect signal transduction, and apoptosis. Peptides also act as neurotransmitters, neuromodulators, hormones, and autocrine and paracrine factors. Unfortunately, their use as pharmaceutical drugs is made difficult by their poor pharmacokinetic profiles; they are easily proteolyzed, poorly transported, and rapidly excreted. Although modern formulation techniques have improved delivery of peptides (e.g., inhalation of insulin), there remains a need for small potent molecules that can be administered orally.

For these reasons, much effort has been expended to find ways to replace portions of peptides with non-peptide structures, called peptidomimetics, in the hope of obtaining orally bioavailable entities. Several types of peptidomimetics have been developed, and the field has emerged as one of the important approaches to drug design and discovery. This review will describe the various methods developed to design peptidomimetics. Due to space limitations, the biological rationale for each peptidomimetic and its chemical synthesis can not be covered. Selected examples of the strategies employed to obtain peptidomimetics are provided to illustrate the breadth of research in this field.

2 CLASSIFICATION OF PEPTIDOMIMETICS

The term peptidomimetic is often used in the literature to indicate a multitude of structural types that differ in fundamental ways. Comparisons between peptidomimetics suffer from the lack of accepted definitions of what a peptidomimetic is (1). The term is often applied to highly modified analogs of peptides without distinguishing how these differ from classical analogs of peptides. For example, peptide (2) is derived from the decapeptide LH-RH (1); (2) contains only five amino acids, none of which is present in the parent compound, yet it is a powerful antagonist of the LH-RH receptor (Fig. 15.1) (2). Is (2) a peptide analog or a peptidomimetic?

In the 1970s, Hughes et al. were the first to show that two very different chemical structures have similar agonist properties (3). The opioid natural product, morphine (3), was found to resemble the N-terminal structure of the endogenous opioid peptides, enkephalins, (4a) and (4b), and β-endorphin (5) (Fig. 15.2). The remarkable similarity between the morphine phenol system and the *N*-terminal tyrosine residue in the peptide opioids implied that these units reacted with opioid receptors in a similar fashion to elicit comparable responses (4–6).

The realization that a non-peptide natural product was mimicking the action of a natural peptide effector led Farmer to postulate that other non-peptide structures might be found that would mimic other peptide effectors (7). His concept of "peptide mimetic," which later was called "peptidomimetic," proposed that

*p*Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2

LH-RH

(1)

↓

(4-fluorophenyl)propionyl-*D*1Nal-*N*MeTyr-*D*Lys(Nic)-Lys(Isp)-*D*Ala-NH_2

A-76154 ED_{50} = 10.3 μg/ml

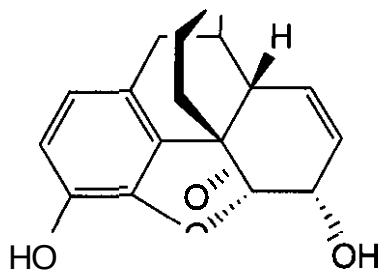
(2)

Figure 15.1. Reduced-size antagonist of LH-RH.

Met-enkephalin Tyr-Gly-Gly-Phe-Met
(4a)

Leu-Enkephalin Tyr-Gly-Gly-Phe-Leu
(4b)

β-Endorphin Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Phe-Lys-Asn-Ala-Ile-Ile-Lys-Asn-Ala-Tyr-Lys-Lys-Gly-Glu
(5)



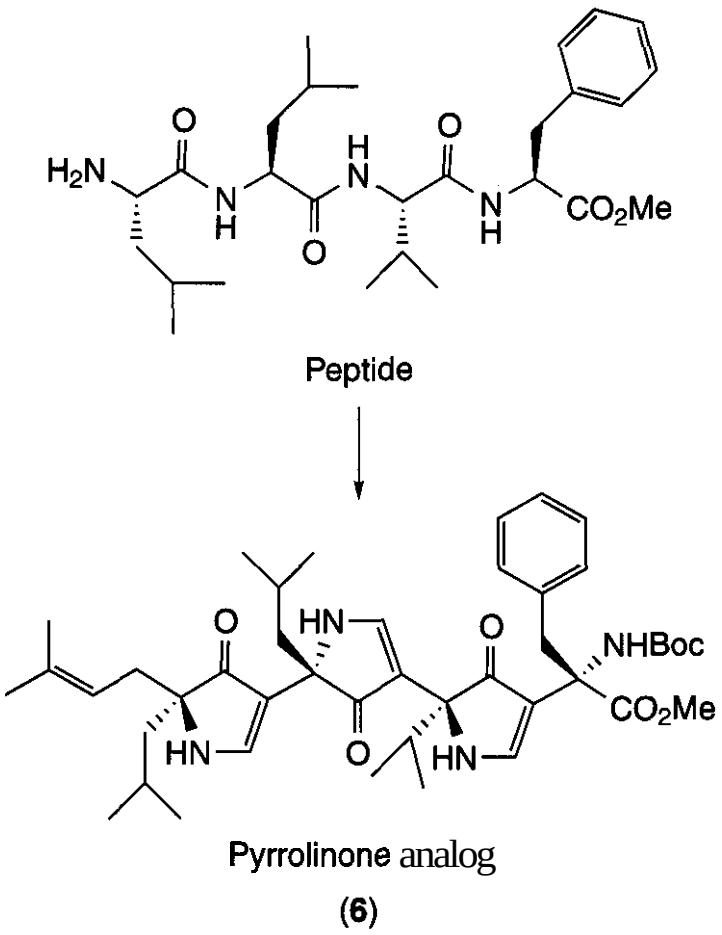
Morphine
(3)

Figure 15.2. Examples of peptidic and non-peptidic opioid receptor ligands.

novel scaffolds could be designed to replace the entire peptide backbone while retaining isosteric topography of the enzyme-bound peptide (or assumed receptor-bound) conformation. Farmer's definition went beyond simple replacement of amide bonds and the concept of stringing together conformationally restricted amino acid derivatives to mimic the native peptide structure. In the intervening years, many non-peptide and partially peptide structures have been found that mimic (or antagonize) the action of the peptide ligand at its receptor; this is particularly true with substances active at G-protein–coupled receptors.

The pyrrolinone unit (6) designed by Smith and Hirschmann illustrates a modern use of these two concepts (Fig. 15.3) (8). Pyrrolinones constrain the peptide-like side-chains into an extended β-structure topography that fits the active sites of most peptidases; pyrrolinones are resistant to normal proteolysis because no a-amino acid units remain, and the units impart sophisticated partitioning properties to the final inhibitor. Pyrrolinones, like many peptide-derived peptidomimetics, retain an atom-to-atom correspondence to the parent peptide, especially with respect to the peptide backbone structure. Most of these structures contain elements that accomplish one of two objectives: they replace amide bonds with metabolically stable units, and they affect a conformational constraint on peptides or on the peptide replacement. In contrast, heterocyclic natural products or screening leads that bind to peptide receptors also have been called peptidomimetics by virtue of their mimicking (or antagonizing) the function of the natural peptide. Although structural data confirming mimicry of the designed mimetics are rarely available for receptor bound ligands, ample evidence is available from X-ray crystallography that heterocyclic inhibitors are mimicking the extended β-strand of enzyme-bound substrate-derived inhibitors (vide *infra*).

Based on these considerations, four distinct types of peptidomimetics have been identified to date (9, 10). The first invented were structures that contain one or more mimics of the local topography about an amide bond (amide bond isosteres). Strictly speaking, these are properly classified as pseudopeptides (11), but in recent years, they have been called peptidomimetics on occasion. For historical reasons, we classify the peptide backbone mimetics as type I mimetics (Table 15.1). These



(6)

Figure 15.3. Correlation of pyrrolinone-based peptidomimetics and the parent peptide.

Table 15.1 Peptidomimetic *Types*

Peptidomimetic			Examples
Type I	Peptide backbone mimetics	Substrate-based design	Pseudopeptides
Type II	Functional mimetics	Molecular modeling, HTS	GPCR antagonists
Type III	Topographical mimetics	Structure-based design	Non-peptide protease inhibitors
Type IV	Non-peptide peptidomimetics	Group Replacement Assisted Binding	Piperidine inhibitors

mimetics often match the peptide backbone atom-for-atom while retaining functionality that makes important contacts with binding sites. Some units mimic short portions of secondary structure (e.g., β-turns) and have been used to generate lead compounds. Many early protease inhibitors were designed from transition state analog mimetics or from collected substrate/product mimetics, each designed to mimic reaction pathway intermediates of the enzyme-catalyzed reaction. These are mimics of the peptide bond in a putative transition state or product state and will be classified here as peptidomimetics.

The second type of mimetic to emerge was the functional mimetic, or type *II* mimetic, which is a small non-peptide molecule that binds to a peptide receptor. Morphine was the first well-characterized example of this type of peptidomimetic. Initially, type II mimetics were presumed to be direct structural analogs of the natural peptide, but characterization of both the endogenous peptide and antagonist's binding sites by site-directed mutagenesis has raised doubts about this interpretation (12). The mutagenesis data indicate that antagonists for a large number of receptors seem to bind to receptor subsites different than those used by the parent peptide. Consequently, functional mimetics may not mimic the structure of the parent hormone; this remains to be determined. Despite this uncertainty, the approach has been quite successful and produced a number of potential drug lead structures.

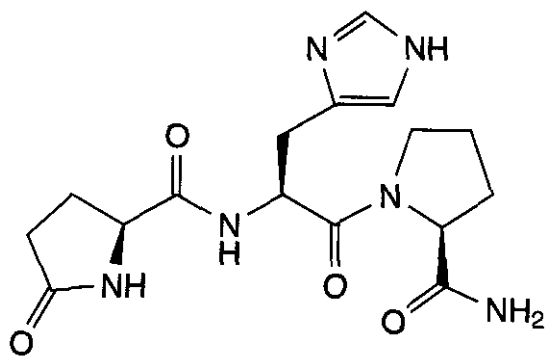
Type *III* mimetics represent the Farmer definition of peptidomimetics in that they *possess* novel templates, which appear unrelated to the original *peptides* but contain the essential groups, positioned on a novel non-peptide scaffold to serve as topographical mimetics. Several type III peptidomimetic protease inhibitors have been characterized where direct X-ray structural determination of both the peptide-derived inhibitor and the heterocyclic non-peptide inhibitor complexes have been compared. These examples demonstrate that alternate scaffolds can display side-chains so that they interact with proteins in fashion closely related to that of the parent peptide.

Recently, a fourth type of peptidomimetic called a GRAB-peptidomimetic (group replacement–assisted binding) has been identified (10). These structures might share structural-functional features of type I peptidomimetics, but they bind to an enzyme form not accessible with type I peptidomimetics.

Previous reviews on peptidomimetics have addressed pseudopeptides (11), macrocyclic mimetics (13), natural product mimetics (14), cyclic protease inhibitors (15), mimetics for receptor ligands (16–22), and earlier general overviews (23–29). This review will focus on the design process itself. Novel peptidomimetics in which the structural relationship between parent peptide and the peptidomimetic has been established by biophysical methods are used to clarify the principles. Successful approaches are highlighted to illustrate how these concepts are currently used.

3 DESIGN OF CONFORMATIONALLY RESTRICTED PEPTIDES

Peptide derivatives that contain conformationally restricting amino acid units or other conformational constraints were first called conformationally constrained (or restricted) peptide analogs. The use of steric hindrance or cyclization to limit rotational degrees of freedom in biologically active molecules has a long history and was originally applied to non-peptide neurotransmitters (30). Subsequently, it was applied to amino acid substituents and to



TRH
(7)

Figure 15.4. Structure of **TRH** tripeptide.

cyclic peptides (31,32) and to control secondary structure in model proteins.

Conformational restriction is a very powerful method for probing the bioactive conformations of peptides. Small peptides have many flexible torsion angles so that enormous numbers of conformations are possible in solution. For example, a simple tripeptide such as thyrotropin-releasing hormone (TRH; 7) (Fig. 15.4) with six flexible bonds could have over 65,000 possible conformations. The number of potential conformers for larger peptides is enormous, and some method is needed to exclude potential conformers. Modern biophysical methods, e.g., X-ray crystallography or isotope edited nuclear magnetic resonance (NMR), (33) can be used to characterize peptide–protein interactions for soluble proteins, but most biophysical methods cannot yet determine the conformation of a ligand bound to constitutive receptors, e.g., G-protein–coupled receptors (34, 35).

Cyclization is one of the earliest techniques applied to design peptidomimetics. Cyclic peptides are more stable to amide bond hydrolysis and allow less conformational flexibility; consequently, the resulting analogs are anticipated to be more selective and less toxic. Methods for restricting conformations include peptide backbone cyclization, disulfide bond formation, side-chain cyclization, and metal ion chelation.

The first successful application of conformational restriction to peptide chemistry was carried out by Veber et al. at Merck, (36), who were trying to simplify the structure of somatostatin (8) (Fig. 15.5) to produce an orally active derivative. Their approach was to introduce conformational restraints into the macrocyclic peptide ring system to reduce the number of conformations available to the analog. Not all substitutions were expected to produce biologically active products, but those that retained activity were assumed to be able to adopt conformations close to the normal bioactive conformation. This work began from the earlier discovery by Rivier et al. (37) that replacement of L-tryptophan in the position-8 of somatostatin by D-tryptophan produced an analog that retained biological activity. This unusual biological result is possible when a D,L-sequence (D-Trp-Lys) replaces an L,L-sequence (Trp-Lys) in a peptide at a type II p-turn, because the topography of the amino acid side-chains at these positions is essentially identical in these turns (38). These results led Veber et al. to postulate that the amino acid sequence Phe-Trp-Lys-Thr might be part of a type II β-turn, and that this tetrapeptide sequence might comprise the active pharmacophore. Although this hypothesis was highly speculative for its time, it was shown to be essentially correct by applying the principle of conformational restriction (Fig. 15.5). Deletion of the N-terminal dipeptide, followed by insertion of the D-Trp at position-8, and replacement of the disulfide sulfurs with carbons produced analog (9). NMR and other data suggested that the two Phe side-chains were clustered, thus they were replaced by a transannular disulfide bond limiting the available conformation, as in compound (10). After several iterations of this process, a biologically active cyclic hexapeptide (11) was discovered that retained only 6 of the original 14 amino acids in somatostatin yet produced a fully active derivative (31).

The work of Veber et al. established that valuable information about the bioactive conformation of a flexible peptide could be obtained by applying the principles of conformational restriction, and several additional examples soon were reported that followed this strategy. Conformationally restricted enkephalin analogs, e.g., 02–13), were formed by cyclizing between positions 2 and 5 of enkephalins (4a-b) (39). Cyclization of α-melanotropin (14) gave the unusually active analog (15) (40). Small cyclic analogs of endothelin (16) (41) have been discovered by applying these methods, as illustrated by (17) (Fig.

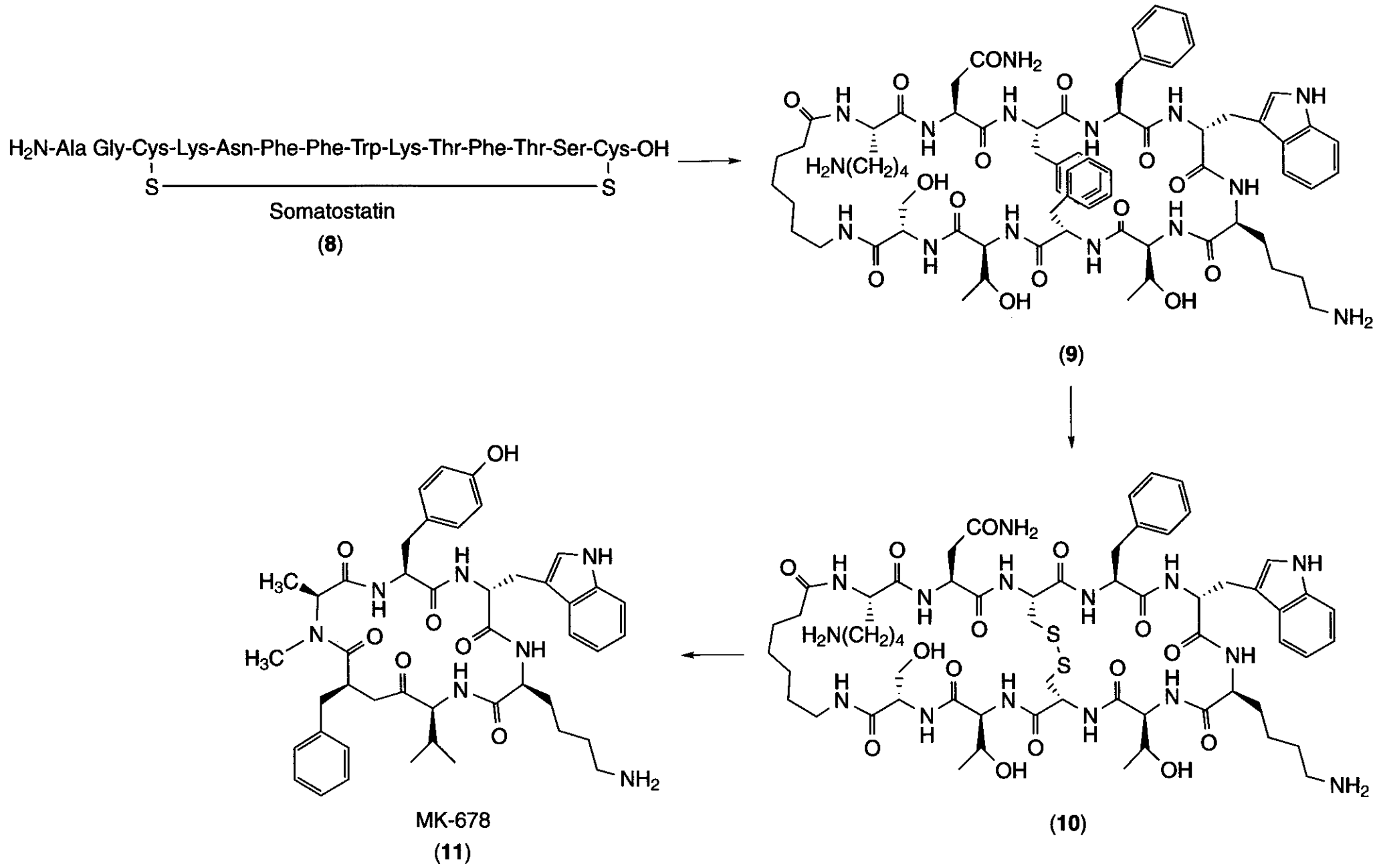
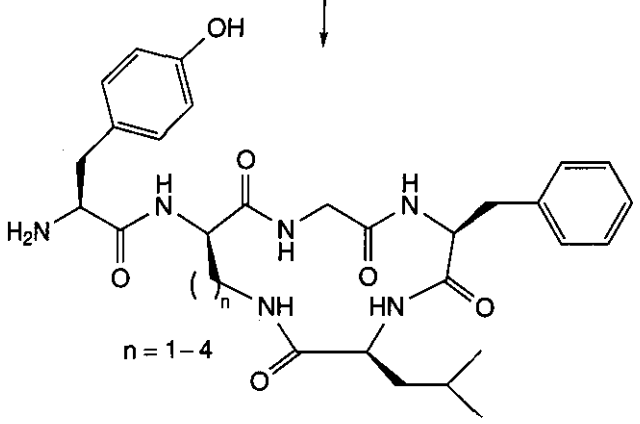


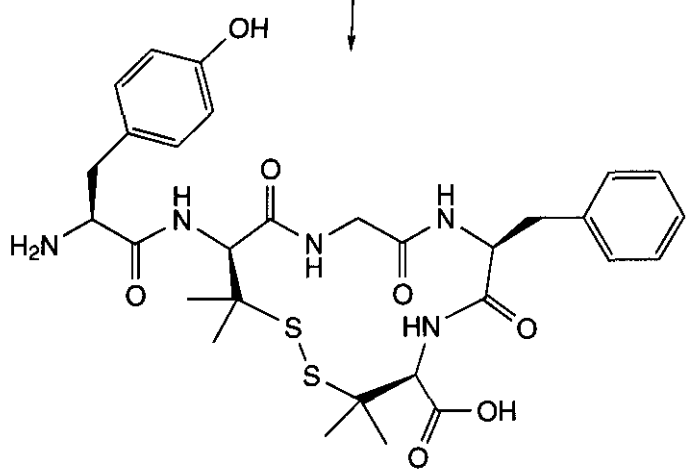
Figure 15.5. Conformationally restricted somatostatin analogs.

H_2N-Tyr-Gly-Gly-Phe-Leu-OH
Leu-enkephalin
(**4a**)



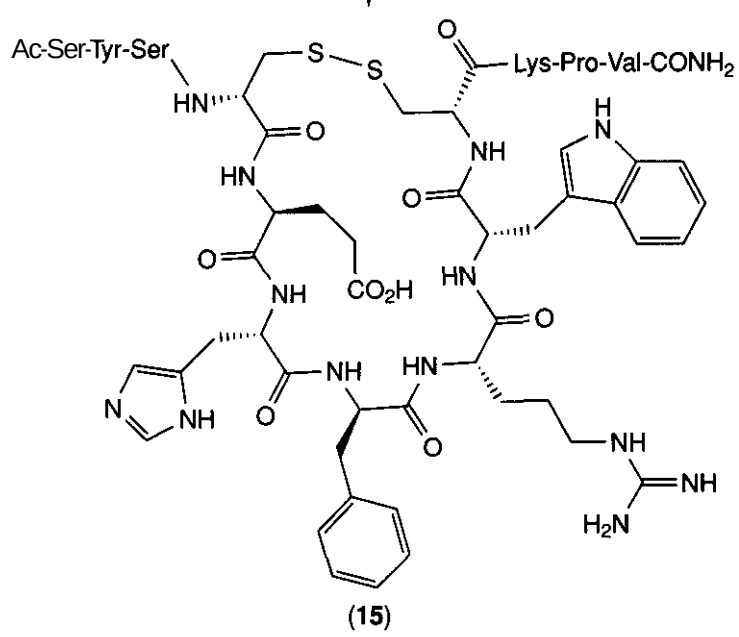
(**12**)

H_2N-Tyr-Gly-Gly-Phe-Met-OH
Met-enkephalin
(**4b**)



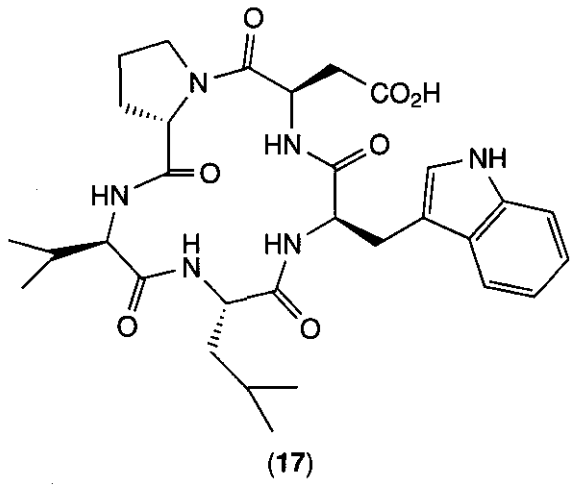
(**13**)

Ac-HN-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-$CONH_2$
α-Melanotropin
(**14**)



(**15**)

H_2N-Cys-Ser-Cys-Ser-Ser-Leu-Met
HO-Trp-Ile-Ile-Asp-Leu-His-Cys-Phe-Tyr-Val-Cys-Glu-Lys-Asp
Endothelin
(**16**)



(**17**)

Figure 15.6. Cyclic hormone peptide analogs.

15.6). Peptide chemists routinely apply conformational restriction in their attempts to determine possible bioactive conformations.

Flexible peptides can be conformationally restricted by a variety of methods other than macrocyclization of the peptide. For example, Marshall et al. introduced a-methyl amino acid substituents into peptides as a way to decrease the conformational space available to the resulting peptide (42); these types of approaches led to his "Active Analog" approach for determining bioactive conformations of flexible molecules (43). Some other traditional modifications of the peptide substrate are the replacement of the amino acids of the P_1-P_1' cleavage site by D-amino acids or the employment of *a-C* or a-N alkylated amino acids and cyclic or β-amino acids (Fig. 15.7).

Mimicking the secondary structure of peptides has become one of the most important tools for rational drug design (44–47). These methods induce the synthetic analog to adopt a set of target conformations, which are designed to mimic the bioactive conformation predicted in the native substrate from biophysical techniques. Molecular surrogates

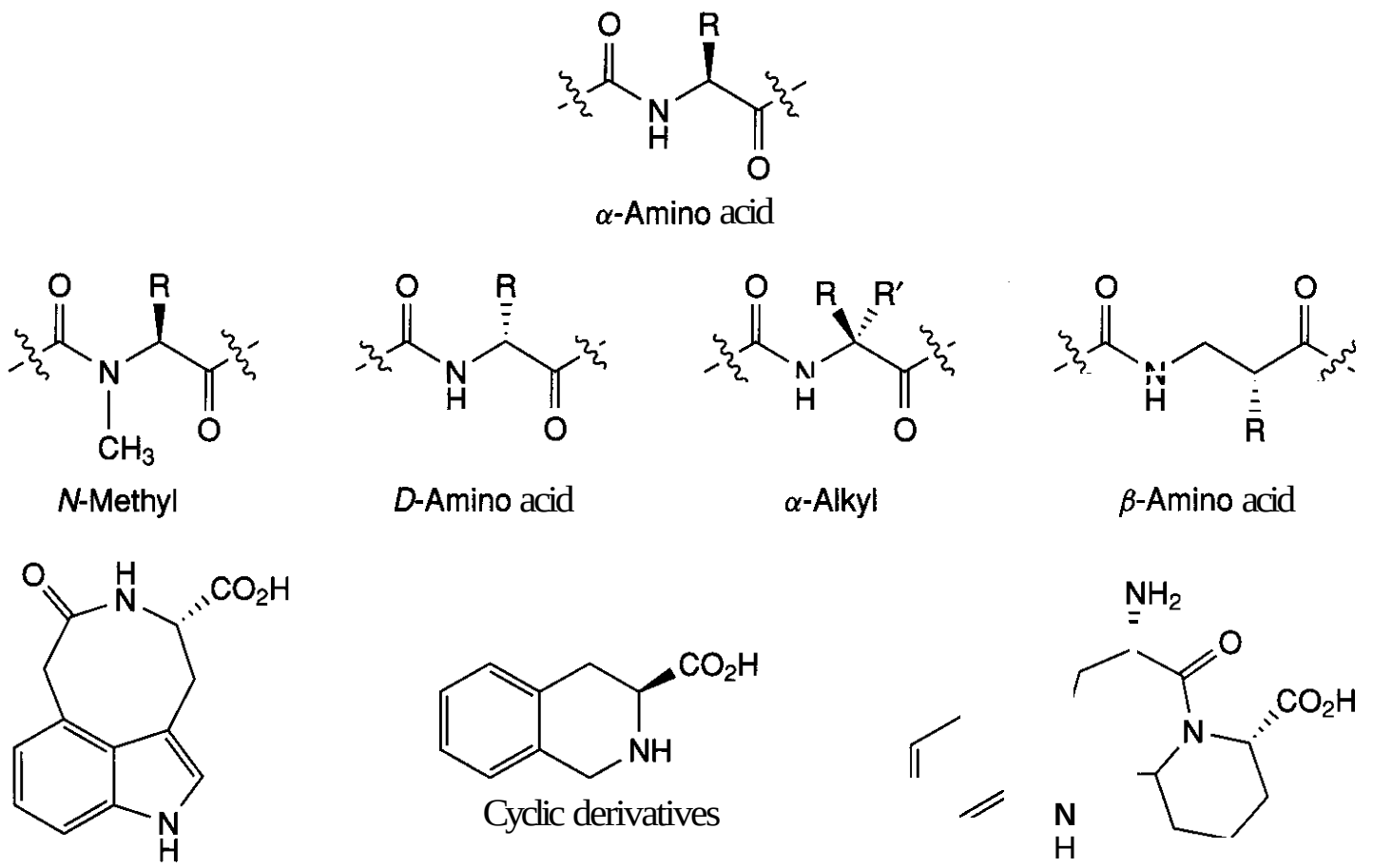


Figure 15.7. Representative amino acid mimetics.

have been found that efficiently mimic turns, strands, sheets, and helices. By far, the major efforts have focused on the design of β-turn mimetic~Some of the templates used to constrain the conformational torsion angles of the peptide chain are summarized in Figs. 15.8–15.14.

In a very early example, Freidinger et al. developed a series of cyclic lactams that stabilized β- and γ-turn structures in linear peptides (Fig. 15.8). This strategy was applied to determine conformations of LH-RH that are consistent with the turn structure permitted by the constraint. For example, the 3-aminolactam (18)was used to mimic a p-turn conformation. When inserted in LH-RH, compound (19) retained good biological activity so that the bioactive conformation of LH-RH probably contains a β-turn around residues 6 and 7 (48).

Conformational restriction has also been used to determine the bioactive conformation of enzyme-inhibitor systems for which no X-ray crystal structure is available. Thorsett et al. (49) synthesized conformationally restricted bicyclic lactam derivatives of the angiotensin converting enzyme (ACE)inhibitors enalapril (20) and enalaprilat (21) (Fig. 15.9) to characterize torsion angles in the bioactive conformation. Analog (22) was used to constrain the torsion angle psi (Ψ). Flynn et al.

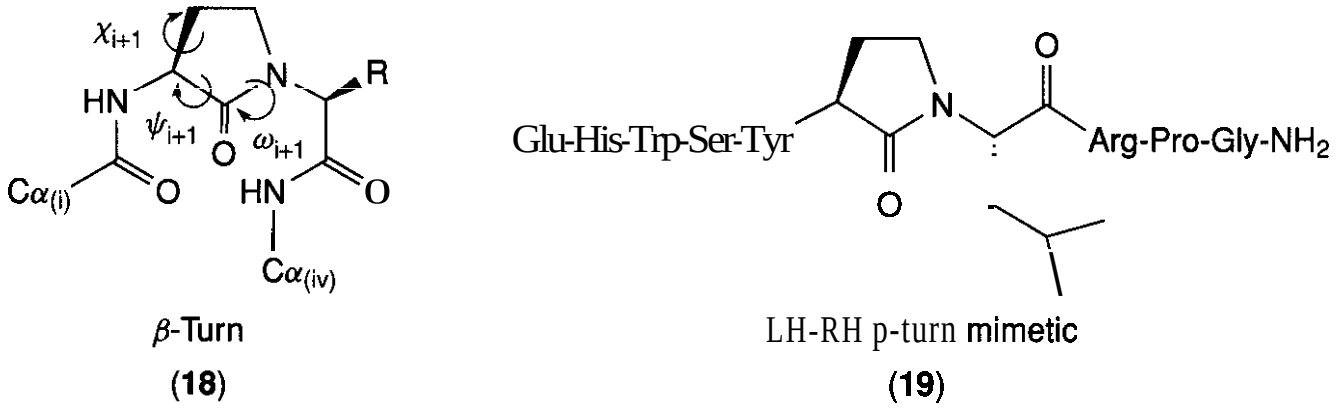
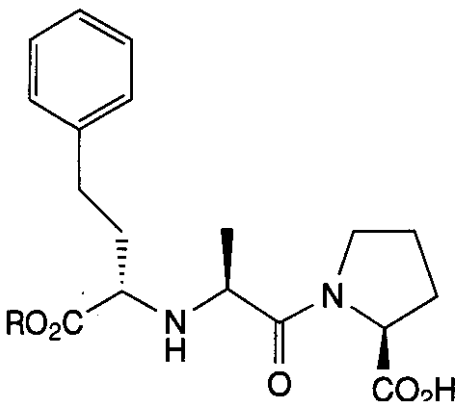
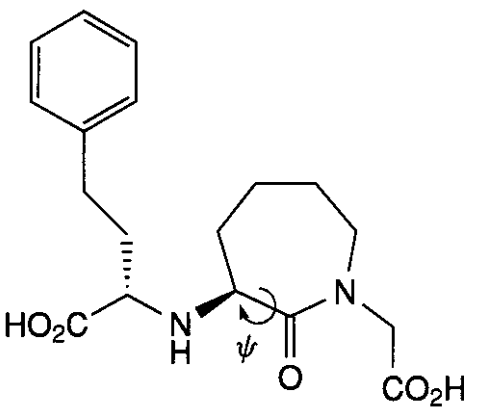


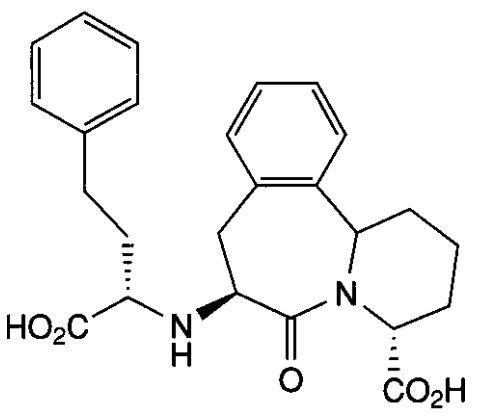
Figure 15.8. γ-Lactam analog of LH-RH. A β-turn mimetic.



Enalapril, R = Et, **(20)**
Enalaprilat, **R** = H, **(21)**



(22)

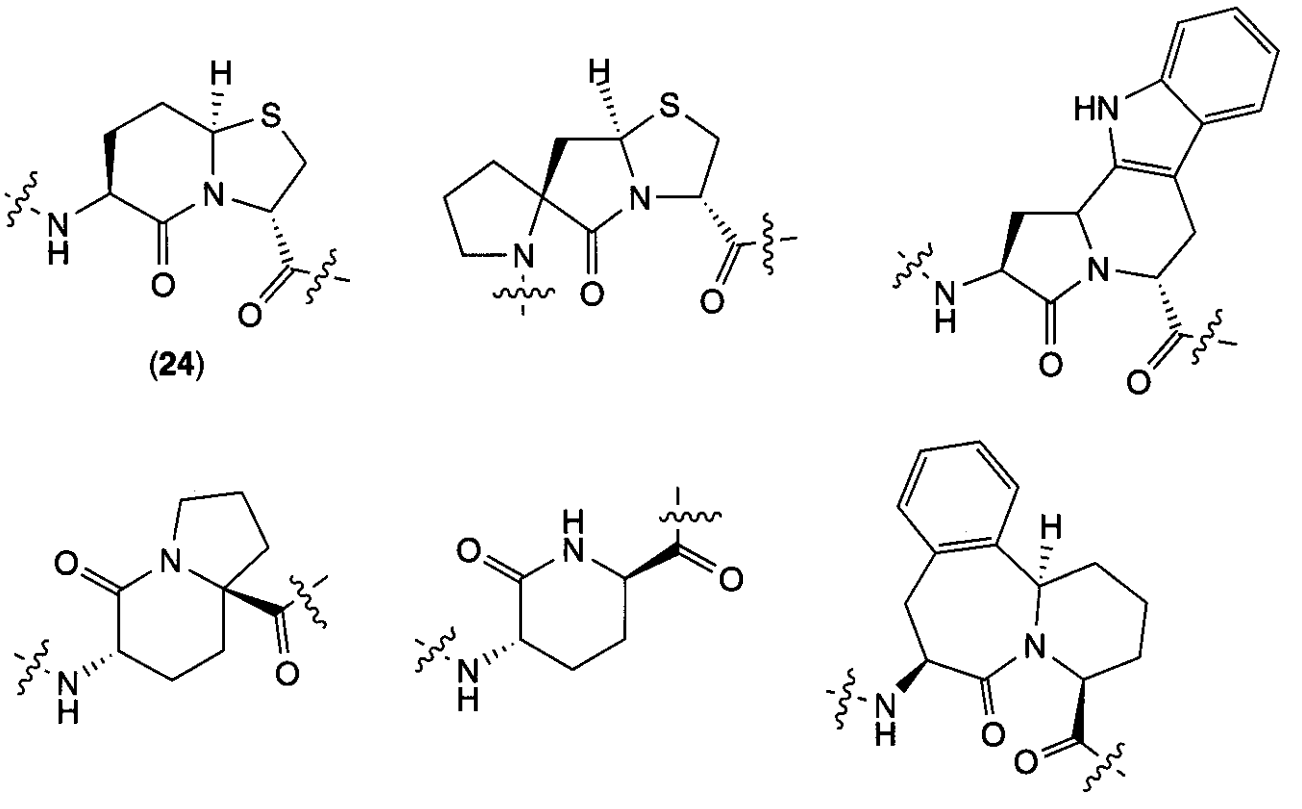


(23)

Figure 15.9. Conformationally restricted ACE inhibitors.

(50) extended this principle to prepare the very tight-binding tricyclic ACE inhibitor (23) (Fig. 15.9).

Several other y-, δ-, and ε-lactam derivatives have been prepared and inserted into receptor antagonists or agonists. For instance, the thiazolidine lactam (24) (Fig. 15.10) has been shown to induce the desired secondary structure in a gramicidine S analog. Later, it was used to prepare a conformationally restricted cyclosporin A analog (51). Several β-turn and y-turn mimetics are shown in Figs. 15.10–15.12, and many other examples are available in the recent literature (52–54).



(24)

Figure 15.10. Lactams as β-turn mimetics.

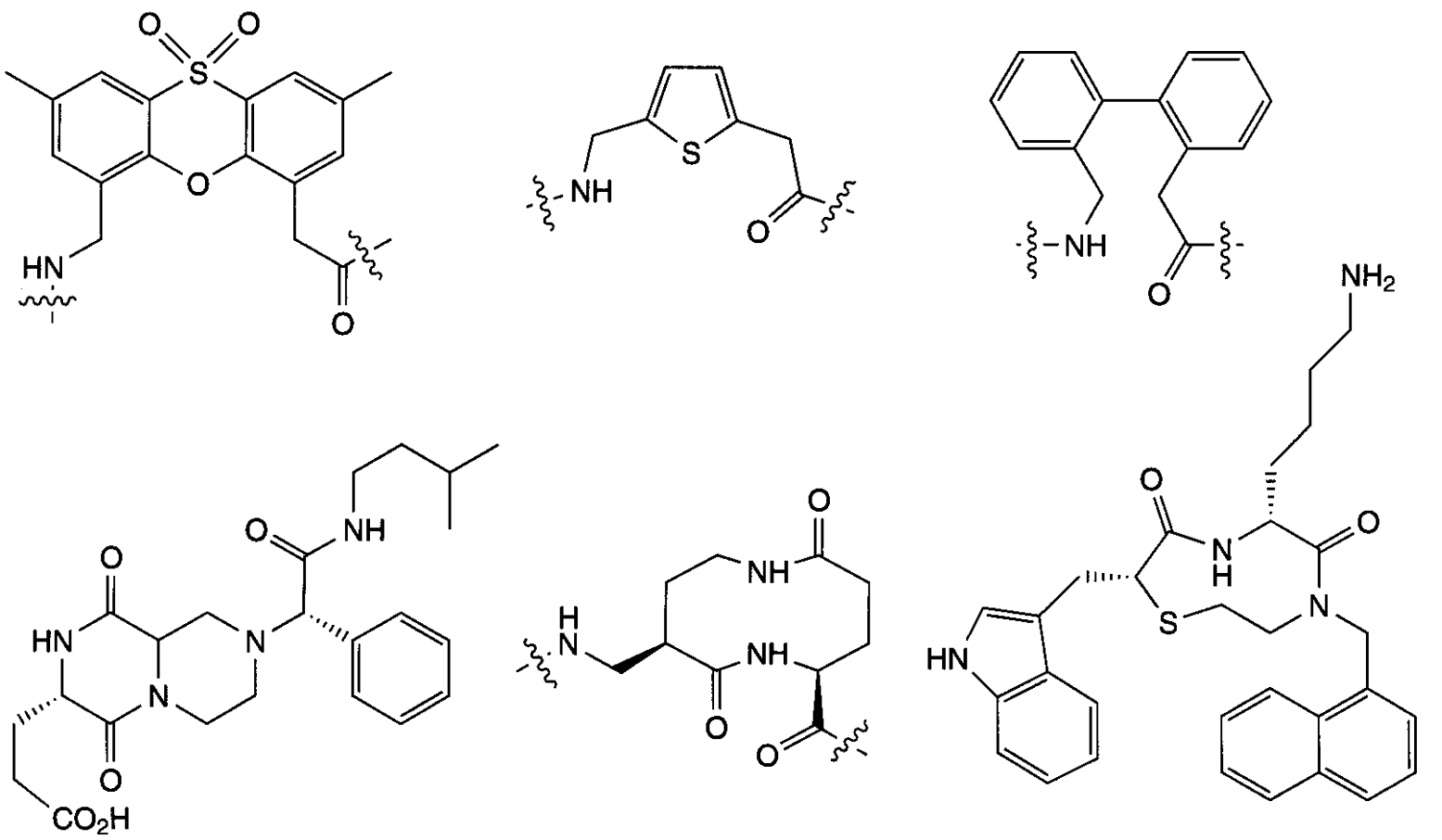


Figure 15.11. Other β-turn mimetic scaffolds.

The β-sheet is an important, biologically relevant secondary structure. As noted earlier, the pyrrolinones invented by Smith et al. (Fig. 15.3) adopt a β-strand conformation, which was corroborated by computer modeling and by X-ray crystallography (55). The diacylaminoeindolidinone (**25**), the dibenzofuran (**26**), and the *N,N*-linked oligourea (27) illustrate other molecular templates designed to stabilize peptides in a β-strand conformation (Fig. 15.13) (56, 57).

Peptidomimetic structures that support a-helixes (**28–30**) (Fig. 15.14) and loops have been reported less frequently because of the difficulty in presenting the side-chains correctly. However, newer approaches have pro-

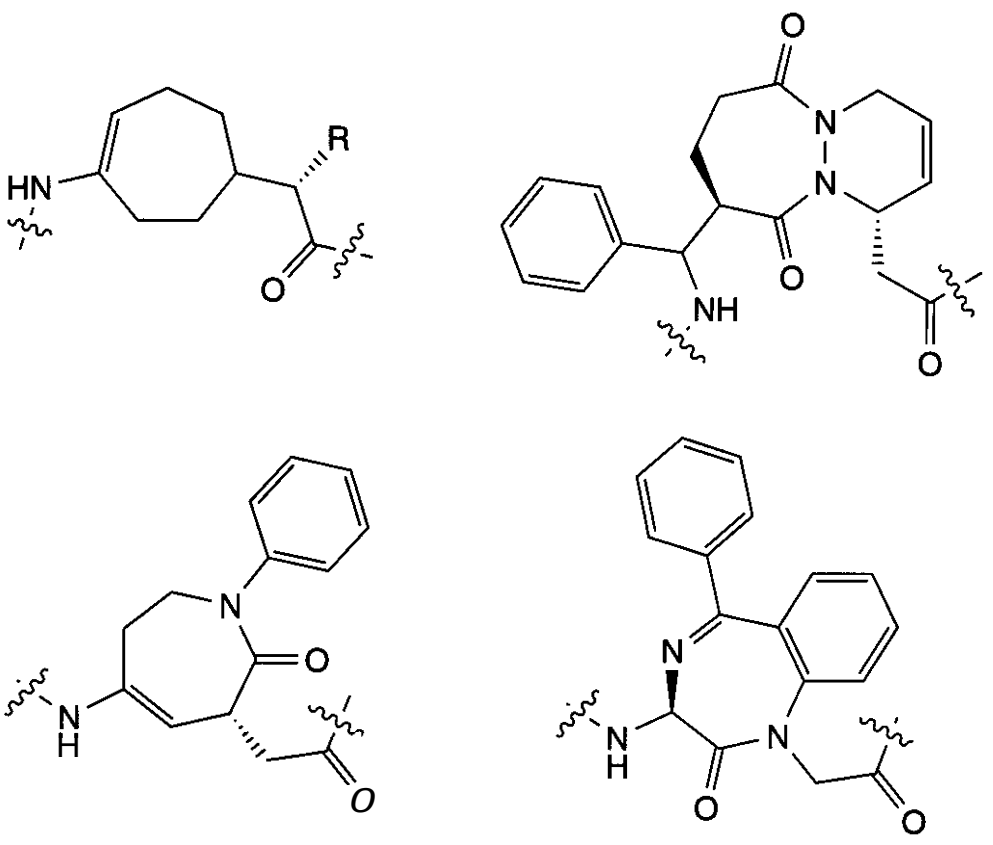
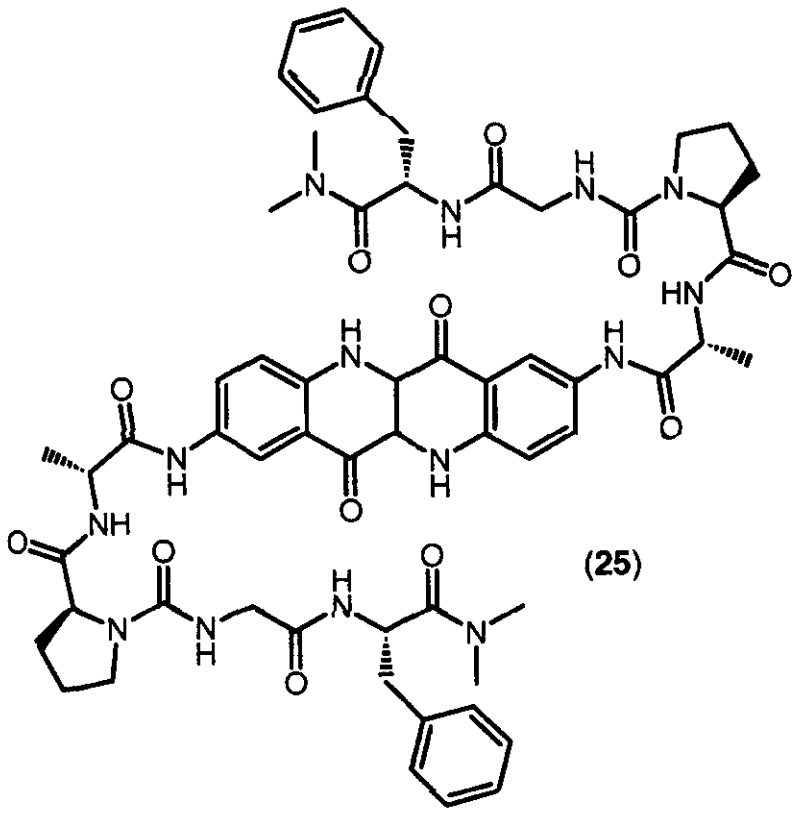
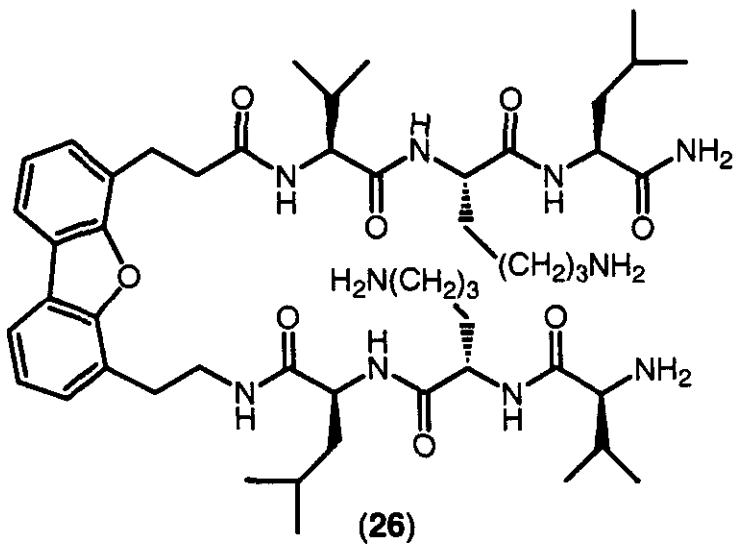


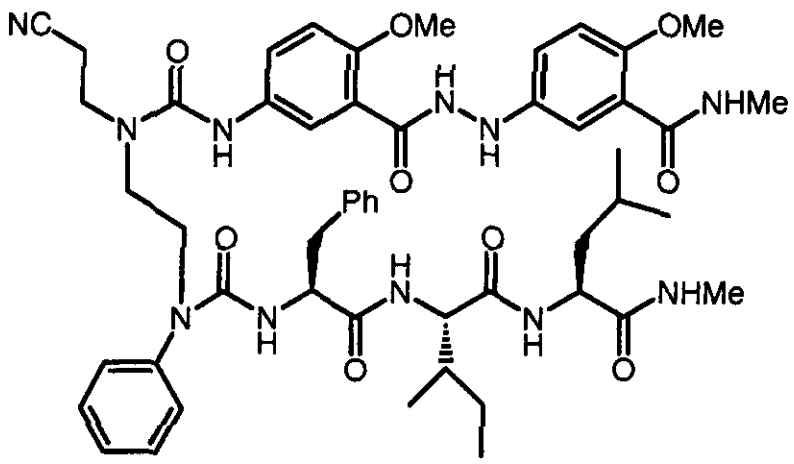
Figure 15.12. Some γ-turn mimetic scaffolds.



(25)



(26)



(27)

Figure 15.13. Structures of β-sheet mimetics.

vided mimetics of multiple discontinuous protein surfaces (56). Over the last few years, the Gellman, Seebach, and Hanessian research groups have invented novel helical structures (e.g., **31, 32**) by use of β-, γ-, and Gpeptides (58).

It is important to stress that even a small change in the structure or in a single torsional angle can be sufficient to dramatically modify the conformation of the resulting peptide. Numerous additional conformational constraints have been developed, and the reader is encouraged to consult these reviews for additional examples (32, 59–63).

4 TEMPLATE MIMETICS

Highly functionalized molecular scaffolds have proven to be very successful in mimicking specific protein–protein interactions. Insertion of the key pharmacophoric groups into a nonpeptidic framework has provided good inhibitors of a variety of biological targets.

This technique has been successfully applied in those biological targets where the key structural amino acids of the native peptide for peptide recognition are known. Miscellaneous examples are found in glycoprotein GbIIb/IIIa inhibitors (33) that mimic the RGD sequence (64) or in Ras-farnesyltransferase inhibitors (34) that mimic the CAAX sequence (Fig. 15.15) (65).

An early example of this concept was developed by Hirschmann et al. in the design of a somatostatin analog (Fig. 15.15) (55). Three of the four crucial amino acid side-chains of the parent peptide (Tyr, Trp, and Lys) were posi-

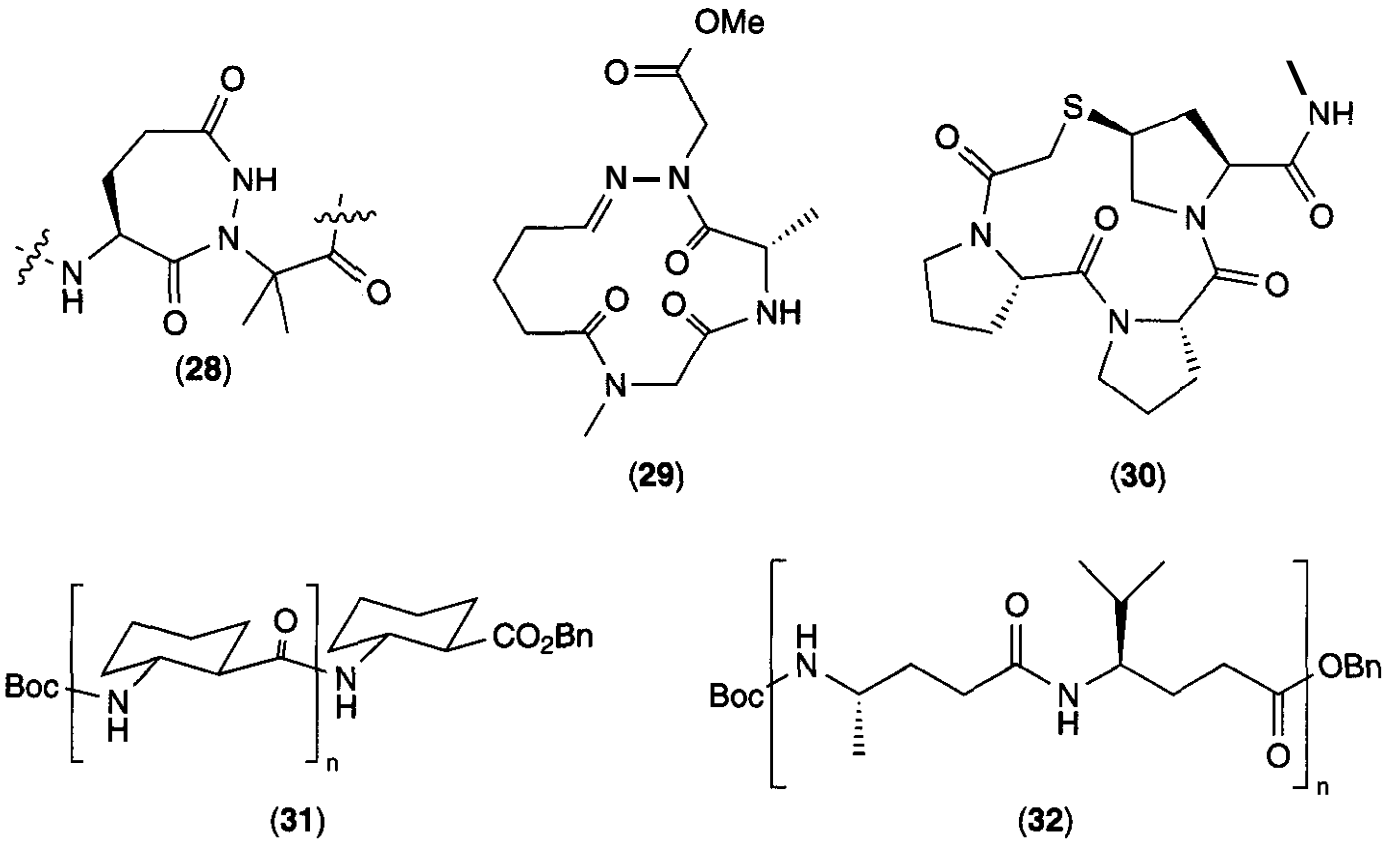


Figure 15.14. Newer templates found in helical or loop structures.

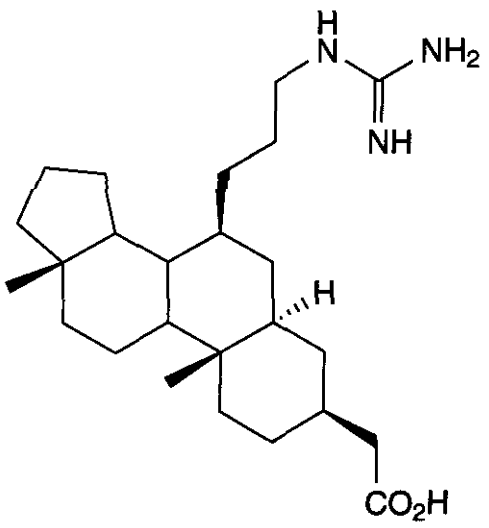
tioned on a sugar template (35). Although originally designed as a somatotropin release inhibitory factor (SRIF) antagonist, compound (35)also proved to be a good Substance P antagonist. These sugar derivatives, as well as the benzodiazepine, diphenylmethane, and spiropiperidine scaffolds, are elements found in a variety of inhibitors of receptors, and have been designated as "privileged structures" (66). Thus, these common scaffolds **can** often provide a template for further optimization of a desired activity. Evans et al. have noted that the essential surface area of biologically active peptides is similar to the surface area of benzodiazepines, one type of non-peptide scaffold known to bind to Gprotein–coupled receptors (67).

The quest for functionalized lead structures that effectively mimic the "hot spots" within the biological ligand is not easy (68). Molecular modeling and high-throughput screening (HTS) are techniques that are currently used for this purpose and have been summarized elsewhere.

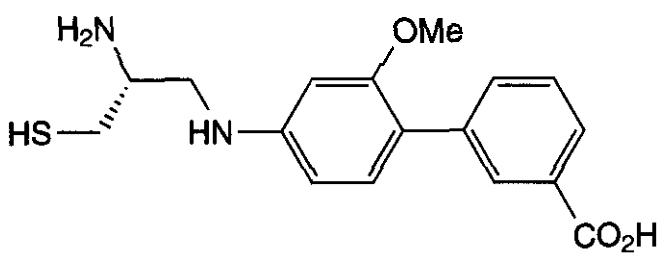
The design and synthesis of antifungal analogs of the cyclic peptide rhodopeptin (36) (Fig. 15.16) illustrate a recent application of peptidomimetic scaffolding, where the structure of the biological target is not known. After structure-activity relationship (SAR) studies, the important side-chains of the peptide ligand were identified; then, NMR and molecular modeling techniques were used to model these side-chains onto known scaffolds and to compare with the original three-dimensional (3D) structure of the native peptide. Compound (37) (Fig. 15.16) is a potent peptidomimetic derivative with improved solubility in water that functions the same as the cyolic tetrapeptide (69, 70).

5 PEPTIDE BOND ISOSTERES

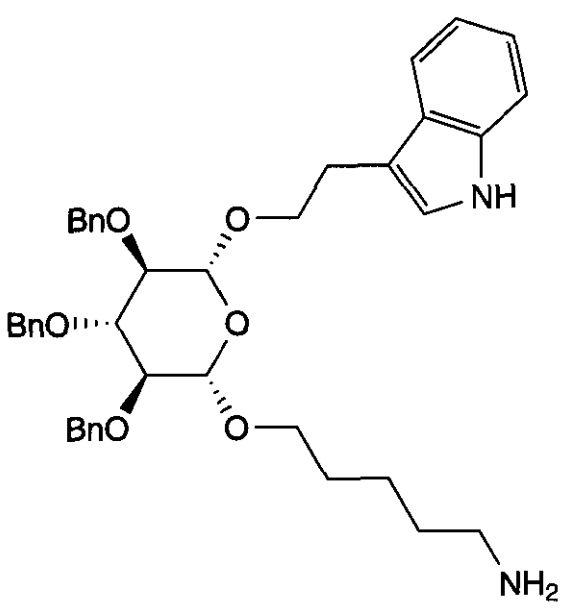
The replacement of amide bonds by retro-inverso amide replacements (71, 72) and other amide bond isosteres generates pseudopeptides (11). This process was first used to stabilize peptide hormones in *vivo*, and later to prepare transition state analog (TSA) inhibitors. Systematic efforts to convert good in vitro inhibitors into good in *vivo* inhibitors became the driving force for further development of peptidomimetics. Figure 15.17 illustrates some of the peptide backbone modifications that have been made in an effort to increase bioavailability. Replacement of scissile amide (CONH) bonds with groups insensitive to hydrolysis (e.g., CH_2NH) has been extensively practiced. Reviews of this work have appeared (11, 73). Removal of the proton donors and



GpIIb/IIIa
(**33**)



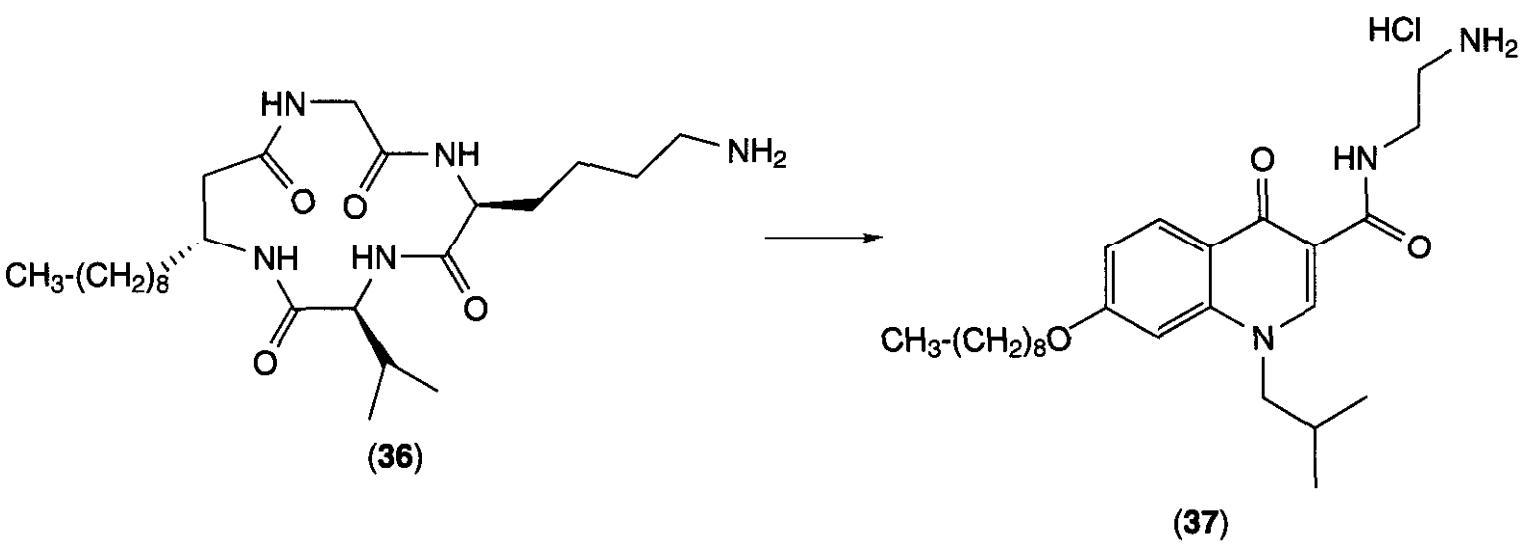
Ras-farnesyltransferase
(**34**)



Somatostatin
(**35**)

Figure 15.15. Biologically active template mimetics.

acceptors in an amide bond also reduces hydration, which improves the ability of the compounds to penetrate lipid membranes (74). These approaches represent important first steps in development of peptidomimetics. However, removal of an amide bond also affects the geometry and increases the flexibility of the molecule at this position, which decreases ligand binding. Effective analogs have been obtained when conformational restriction, transition-state analog design, and amide bond replacements have been applied to



(**36**)

(**37**)

Figure 15.16. Rhodopeptin analogs. Representative example of scaffolding methodology.

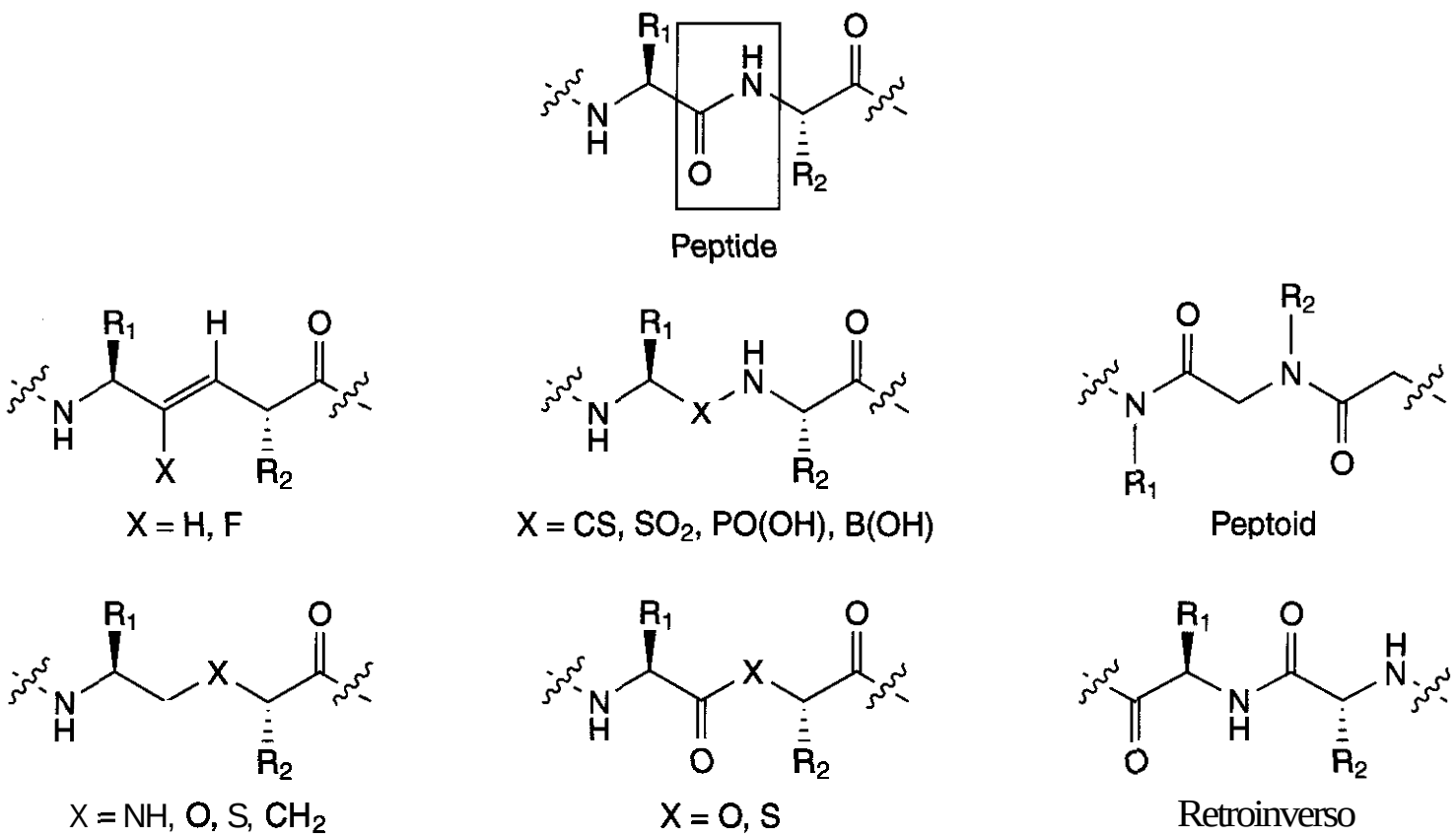


Figure 15.17. Isosteres that replace peptide backbone amide groups to generate pseudopeptides.

scaffolds with molecular weights below 500–600 (75, 76), but at present this process is very labor intensive.

6 FROM TRANSITION-STATE ANALOG INHIBITORS TO NON-PEPTIDE INHIBITORS: EXAMPLES IN PROTEASE INHIBITORS

Many peptidomimetics derived from the design of TSA inhibitors, molecules designed according to the hypothesis provided by Pauling (77) and implemented by Wolfenden (78, 79). TSA protease inhibitors are stable analogs of the tetrahedral intermediate for peptide bond hydrolysis that inhibit the enzyme (Fig. 15.18). The first successful commercial application was the development of captopril (38) by Ondetti et al. (80), and many applications have been reported over the past quarter century.

Figures 15.19–15.32 list examples of analogs of peptidyl transition states that have been employed to develop inhibitors of four classes of peptidases (81, 82). These units are used to replace the scissile amide bond in a substrate sequence with either an amino acid or dipeptide isostere, or with a chelating moiety in the case of metallo peptidases. The

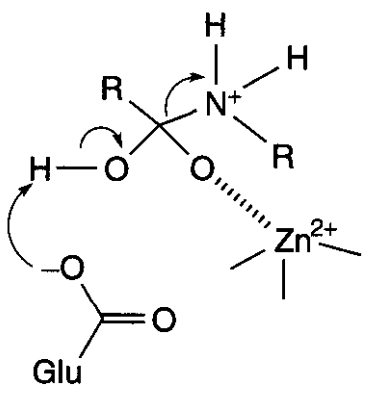


Figure 15.18. TSA inhibit peptide bond hydrolisis.

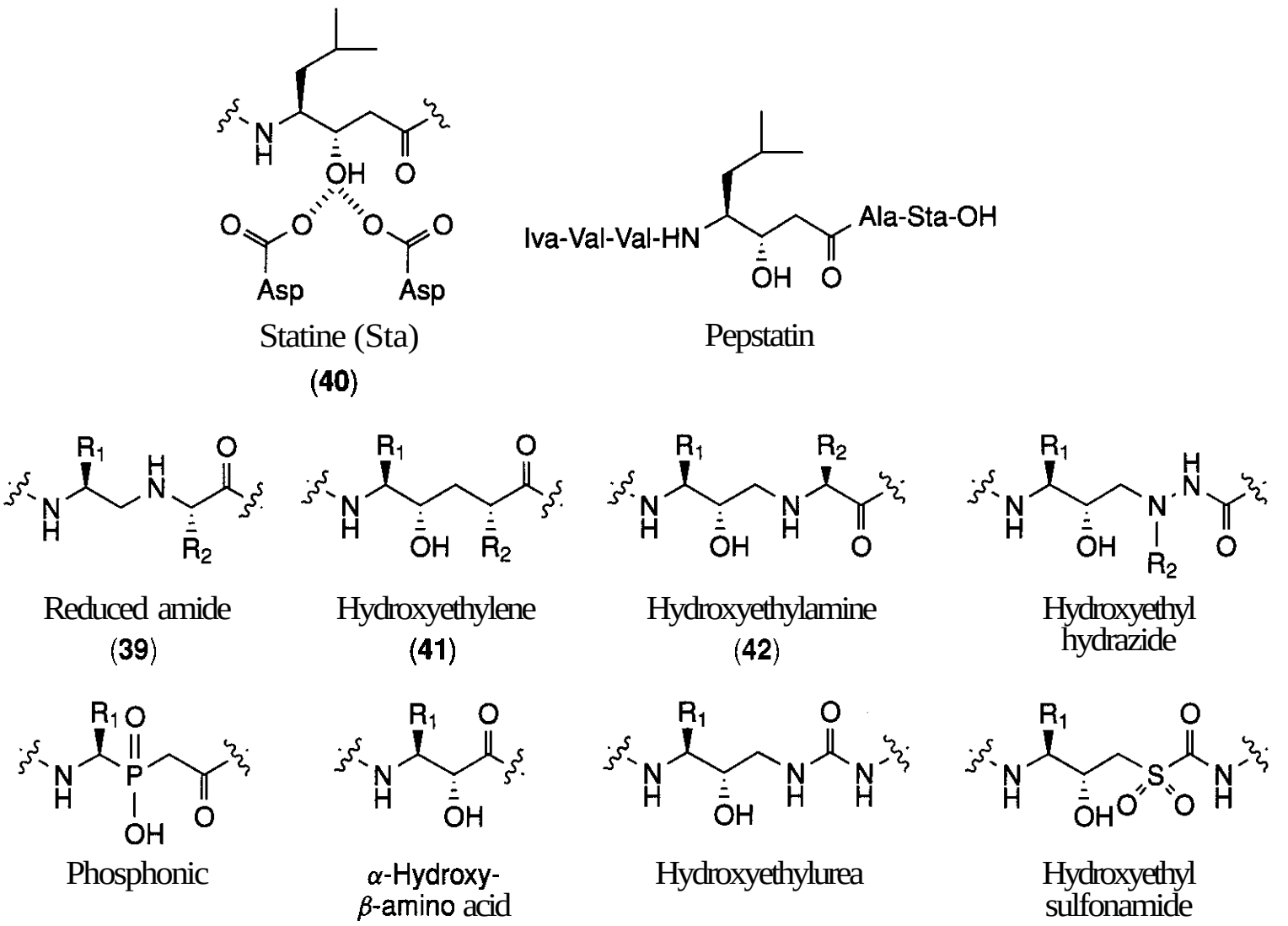


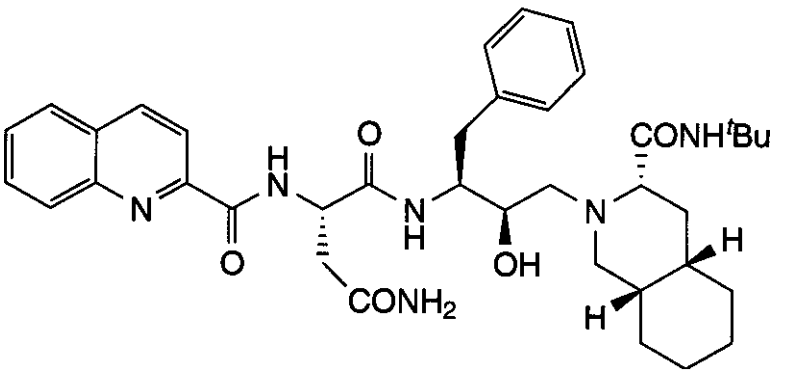
Figure 15.19. TSA used to inhibit aspartic peptidases.

dipeptide TSA provides the functionality that interacts tightly with the enzyme catalytic groups while the amino acid sequence up- and downstream on the peptide chain provides interactions that lead to selective inhibition of the target enzyme. The enzyme active site typically is buried in a cleft capable of accommodating up to three to nine amino acid residues of the substrate/inhibitor depending on the minimum amino acid sequence necessary for hydrolysis. The inhibitor's exquisite selectivity derives from the interactions of the ligand's p,–P,' residues with the enzyme binding sites (S_6–S_3') (83). Recently, some aspartic and serine peptidase inhibitors have been found that access an additional binding site sub-pocket (S_3^{sp}) to increase both inhibitor potency and selectivity (84–86).

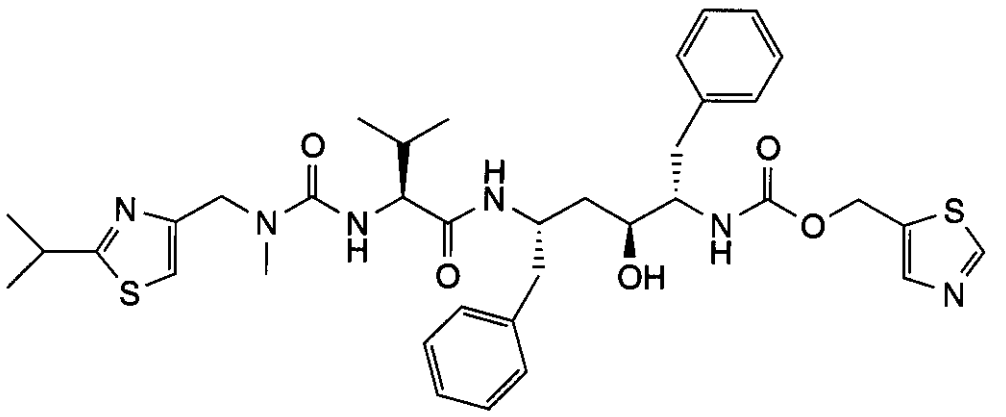
6.1 TSA in Aspartic Peptidase Inhibitors

The reduced amide isostere (39), developed by Szelke, and the statine (hydroxylmethylene) isostere (40) were early transition-state analogs used to design inhibitors of various aspartic proteases, (87–89), and their success led to other tetrahedral intermediate mimics such as the hydroxylethylene (41) and hydroxyethylamine (42) isosteres (Fig. 15.19) (90–92). The statine subunit, which mimics the tetrahedral intermediate, represents one of the earlier examples of TSA, although statine is one atom short in backbone length to be a true dipeptide or two atoms too long to be an isosteric replacement for a single amino acid.

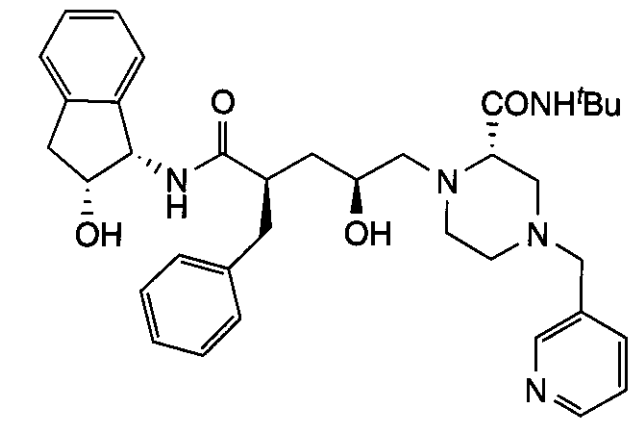
Early work focused on developing inhibitors of renin as potential antihypertensive agents, but these compounds failed to become drugs primarily because of difficulties in obtaining orally active drugs. As a result, the first pharmaceutical attempts to develop renin inhibitors for treatment of hypertension through TSA-based inhibitors failed (93). It was eventually realized after extensive modifications to the ancillary peptide functionality that developing bioavailable peptide-derived inhibitors critically depended on the molecular weight of the inhibitor. Developing inhibitors for HIV protease was substantially easier



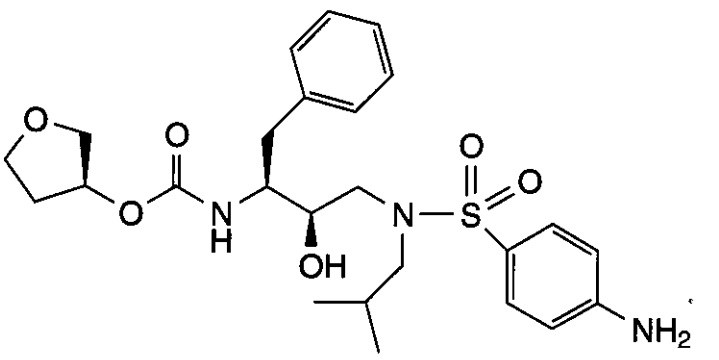
Saquinavir (Roche)



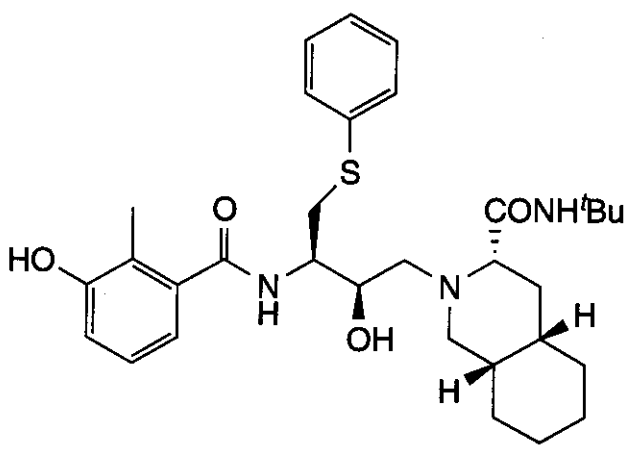
Ritonavir (Abbott)



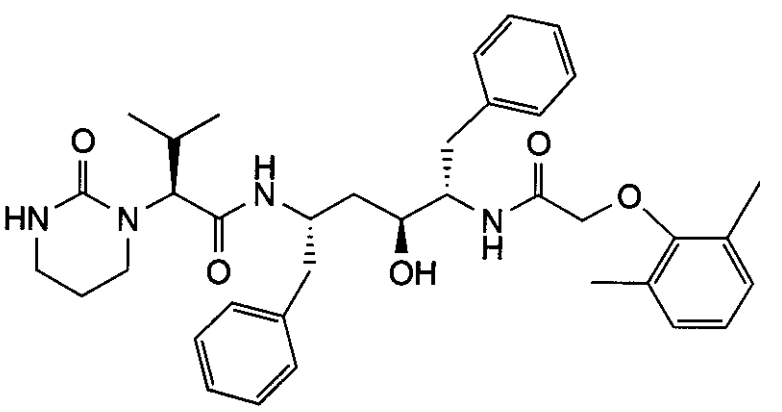
Indinavir (Merck)



Amprenavir (Vertex)



Nelfinavir (Agouron)



Lopinavir (Abbott)

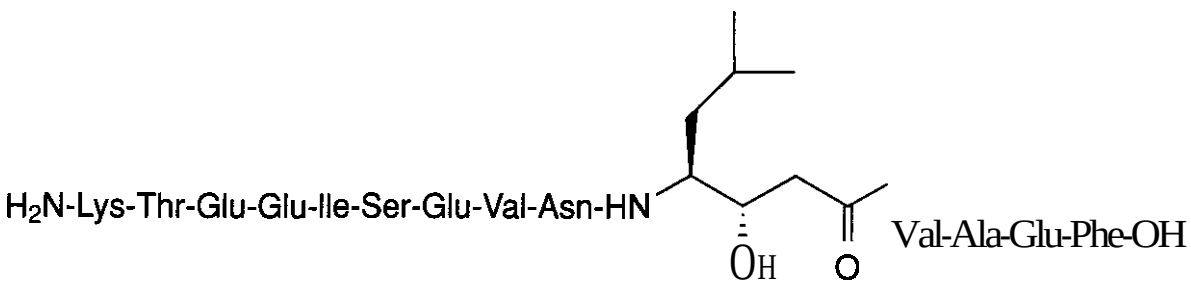
Figure 16.20. Peptide-derived TSA inhibitors used clinically in HIV protease-based AIDS therapies.

than for renin because HIV protease requires a significantly smaller minimum substrate sequence (94). In addition, the principles elucidated to develop renin inhibitors were known and could be applied to the development of HIV protease inhibitors. Variations on the hydroxyethyl amine moiety proved to be very successful. Some of the highly modified HIV

β-Secretase cleaves APP at:

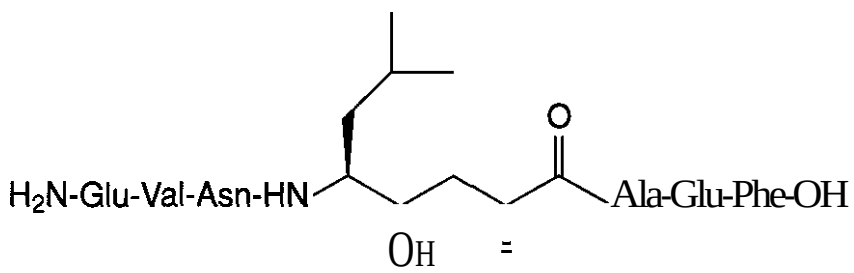
-Ser-Glu-Val-Lys-Met-¦--Asp-Ala-Glu-Phe-Arg-

-Ser-Glu-Val-Asn-Leu¦--Asp-Ala-Glu-Phe-Arg-



IC_{50} = 30 nM

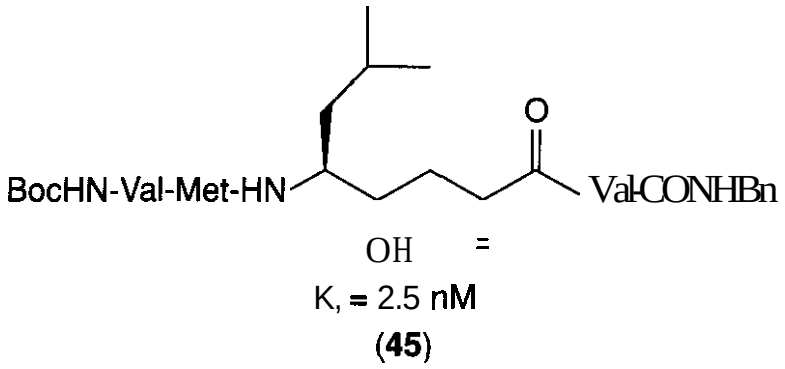
(**43**)



=

K_i = 1.6 nM

(**44**)



=

K_i = 2.5 nM

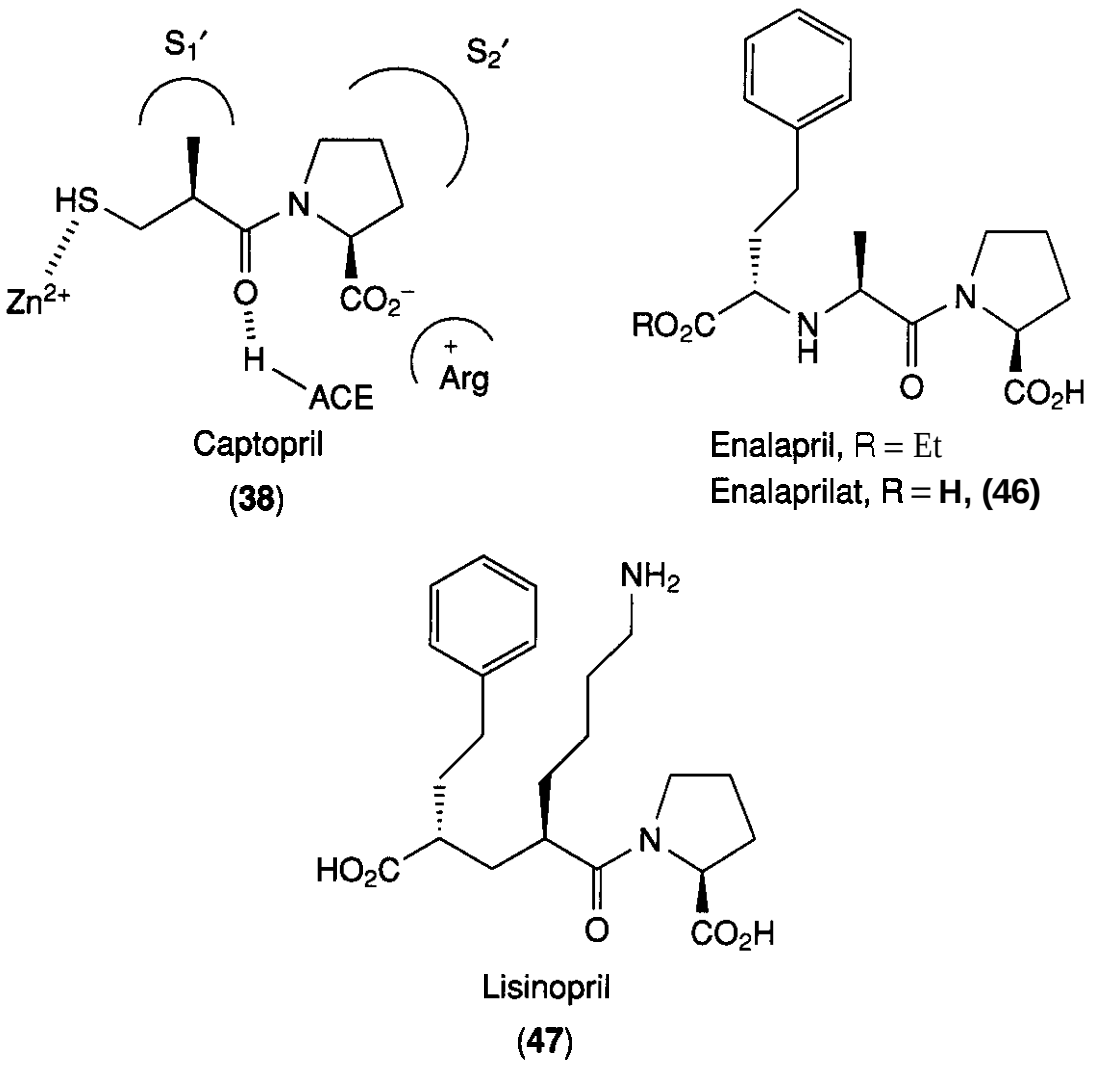
(**45**)

Figure 15.21. Peptide-derived TSA inhibitors as β-secretase inhibitors.

protease inhibitors now in clinical use (Fig. 15.20) have excellent oral bioavailability and establish that application of the transition state analog design process can be very successful in favorable cases.

More recently, the principles for designing inhibitors of aspartic proteases have been applied to the design of inhibitors of β-secretase (BACE or Memapsin-2) as potential agents for treating or preventing Alzheimer's disease (95, 96). Both statine-derived inhibitors (43) and hydroxyethylene-derived BACE inhibitors have been reported (Fig. 15.21) (97, 98). A crystal structure of (44) bound to β-secretase has been reported (99). As expected, the hydroxyl group is hydrogen bonded to Asp32 and Asp228, like in other hydroxyethylene derivatives, and the inhibitor binds in an extended conformation. Because the target β-secretase is within the CNS, successful inhibitors have to penetrate the brain blood barrier readily, a property not yet achieved with any of the peptidomimetic inhibitors currently available.

With the crystal structure in hand, structure-based modification of the parent lead compound has just started to provide new peptidomimetic structures with lower molecular weight and fewer hydrogen bonds (e.g., **45**) (Fig. 15.211, opening further avenues to pharmacologically useful compounds (100).



Captopril
(**38**)

Enalapril, R = Et
Enalaprilat, R = **H, (46)**

Lisinopril
(**47**)

Figure 15.22. Examples of TSA as ACE inhibitors.

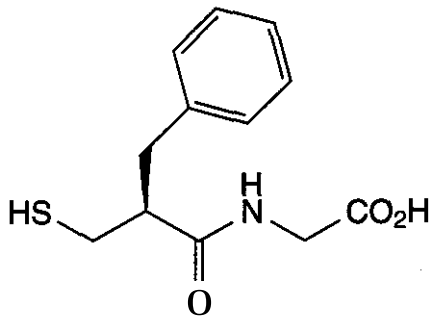
6.2 TSA in Metallo Peptidase Inhibitors

The discovery of the angiotensin converting enzyme inhibitors in the middle 1970s constitutes one of the major advances in the rational design of drugs, the consequences of which are still being realized. The discovery of these metallo peptidase inhibitors was carried out by Ondetti et al. as part of a long-term study to develop antihypertensive drugs (80); in 1999 they received the Lasker Prize in Clinical Medicine for their work.

Angiotensin converting enzyme (ACE) is a carboxy zinc metallo dipeptidase that cleaves His-Leu from the C-terminus of angiotensin-I. Ondetti et al. reasoned that the product of normal reaction, the carboxyl group, could bind to the active site zinc ion, and that the carboxyl group of a collected-product inhibitor also could bind weakly. To improve the interaction between inhibitor and enzyme zinc ion, they replaced the carboxyl group with a sulfhydryl group, which binds zinc about 1000 times more tightly. This led to captopril (Capoten) **(38)** (Fig. 15.22) (80). Later developments by other companies led to many ACE inhibitors. Some are illustrated by enalaprilat (**46**) and lisinopril (47) (Fig. 15.22) (101, 102).

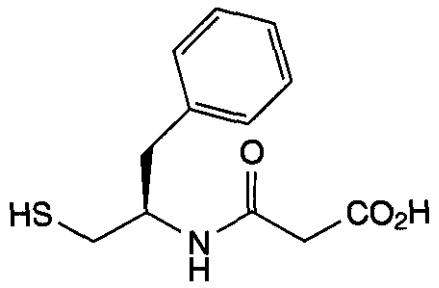
Most metallopeptidase inhibitors append a zinc chelating functionality to a peptide or peptidomimetic that is recognized by the S1′–S3′ subsites in the target enzyme. Successful clinical candidates invariably contain groups that replace the initial di- and tri-peptide moieties to achieve selectivity and orally activity. For example, neutral endopeptidase (NEP), another endopeptidase involved in degrading the larger opioid peptides dynorphan and/or endorphan, is inhibited by thiorphan (48) (103) and a variety of NEP inhibitors: retrothiorphan (49) (104) and kelatorphan (50) (Fig. 15.23) (105). The hydroxamic acid moiety is used in many inhibitors of metallopeptidases.

Inhibition of NEP also prevents the degradation of atrial natriuretic factor (ANF), a natural hypotensive peptide. Dual inhibitors of NEP and ACE have been designed successfully because both enzymes share significant structural homology, particularly in their active sites. Simultaneous inhibition of both peptidases produces a more powerful hypoten-



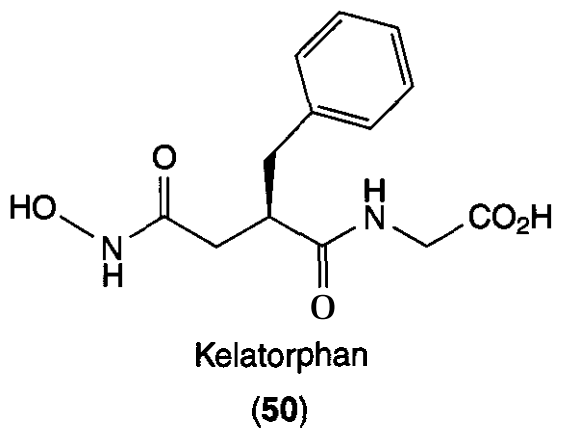
Thiorphan

(48)



Retrothiorphan

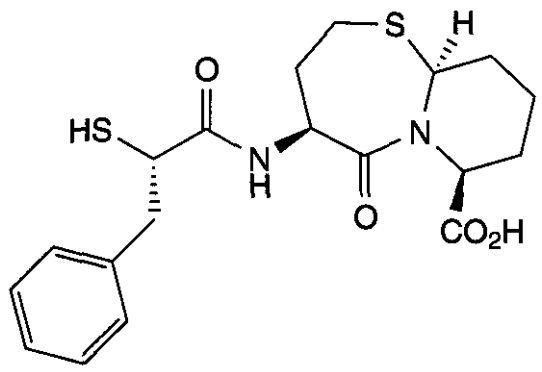
(49)



Kelatorphan

(50)

Figure 15.23. Examples of TSA as NEP inhibitors.

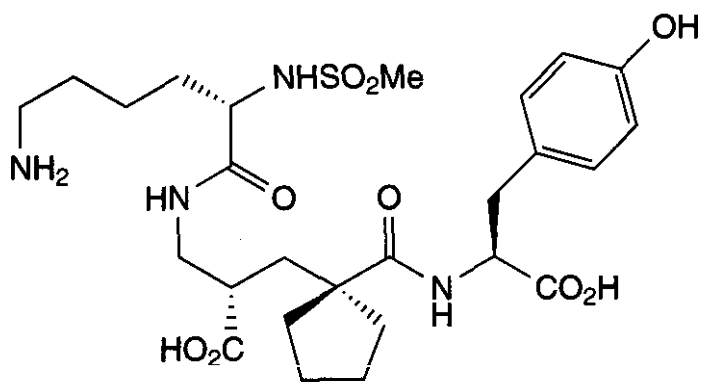


Omapatrilat

ACE $IC_{50} = 6$ nM

NEP $IC_{50} = 9$ nM

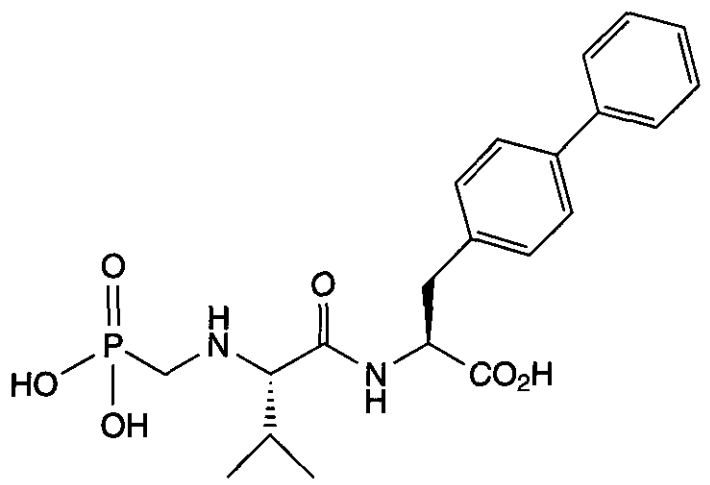
(51)



Sampatrilat

ACE $IC_{50} = 7$ nM

NEP $IC_{50} = 20$ nM



ACE $IC_{50} = 25$ nM

NEP $IC_{50} = 3$ nM

Figure 15.24. Examples of TSA as dual ACE/NEP inhibitors.

sive response (106, 107). Several dual inhibitors are in phase III clinical trial for treating hypertension (Fig. 15.24). Omapatrilat (51, BMS-189921) is the furthest along as of late 2001 (105).

Matrix metalloproteases (MMP) are also inhibited by hydroxamic acids and/or thiols. Over 25 variants of these enzymes are known, and some are involved in diseases ranging from inflammation to metastatic cancer (108). MMPs contain a zinc ion in the active site and function through the metallopeptidases catalytic mechanism already discussed. However, subtle differences between enzymes enable selective inhibitors to be developed (109). Fig. 15.25 lists some of the reported MMP inhibitors that use carboxylic acid (**52–53**), a hydroxamic acid (**54–55**), or thiol groups (56)as metal chelators.

Other reported zinc binding chelators used in matrix metalloproteinase inhibitors are summarized in Fig. 15.26. For instance, one of the oxygens in the phosphonamide (57) binds strongly to the zinc ion, whereas the other one coordinates weakly with the metal (110). More recently, "suicide substrate" MMP inhibitors have appeared (58)(Fig. 15.26) (111). The se-

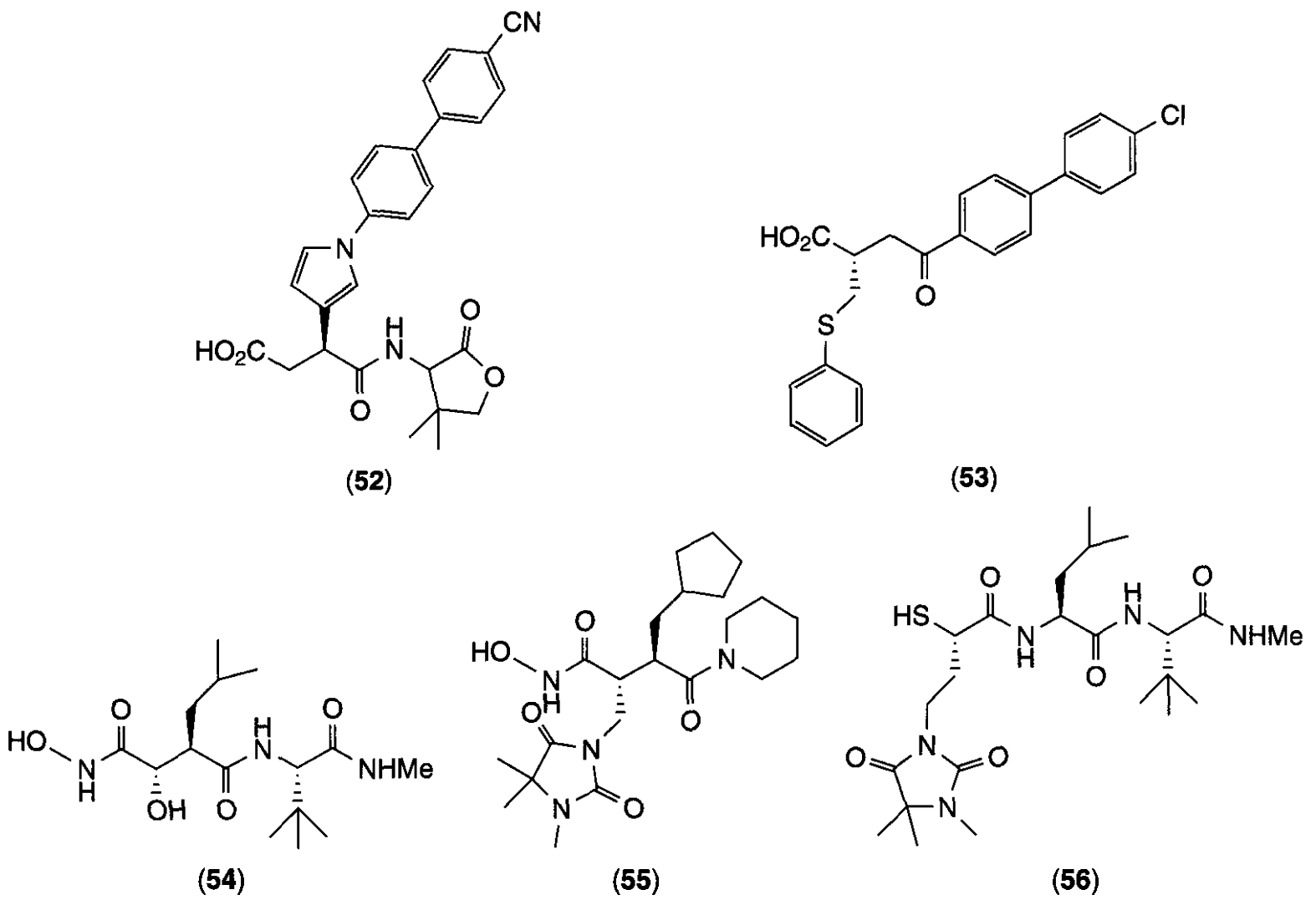


Figure 15.25. Traditional TSA used to inhibit metallopeptidases.

lectivity of this type of compound arises from the specific coordination of the thiirane with the active-site zinc ion, which facilitates thiirane ring opening by nucleophilic attack by neighboring Glu-404. This novel mode of binding was assessed by X-ray absorption studies because of the difficulty to obtain a suitable crystal structure (111,112).

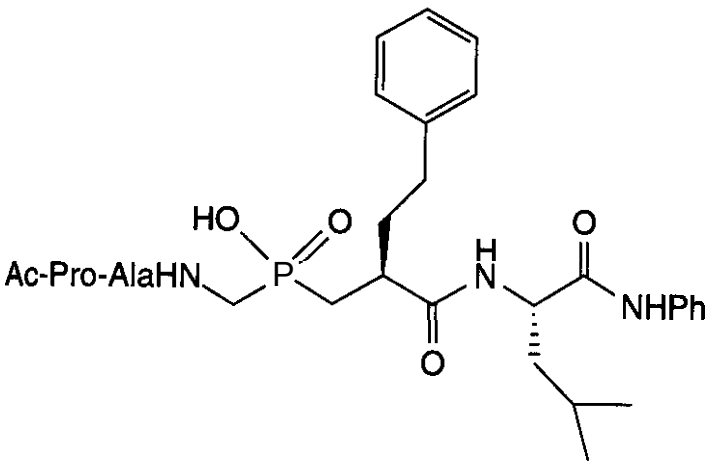
ADAMs are membrane proteins that contain *a* disintegrin and *a* metalloprotease domain. Disintegrins are RGD-containing proteins that inhibit cell/matrix interactions (adhesion) and cell/cell interactions (aggregation) through the integrin receptors. In addition, ADAMs have two other domains that are involved in signaling and transport (113).

There are more than 25 ADAMs proteases identified so far. ADAM 17 has been shown to be TNF-a converting enzyme (TACE) (114). Inhibition of TACE slows the production of TNF-a, a potent cytokine involved in inflammatory responses to infection. Normally TNF-a produces a useful response, but in some cases, too much TNF-a is released and inhibition of TNF-a production would be therapeutically useful. Synthetic analogs have been synthesized that inhibit this enzyme. Clinical candidates like Ro-32,7315 (59) (Fig. 15.27) are starting to emerge, and more are expected in the near future (115,116).

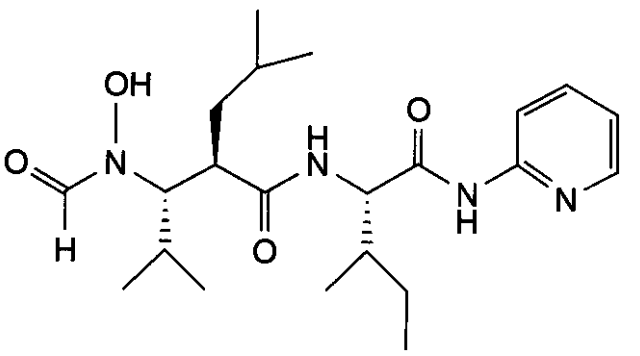
Aminopeptidases, enzymes that cleave off the N-terminal amino acid from a peptide chain, are bismetallo peptidases, a class of metallopeptidase that contain two metals ions in the catalytic site (117, 118). These can be inhibited by compounds related to bestatin (60) (Fig. 15.28), which contains the *N*-terminal α-hydroxy-β-amino acid residue, sometimes referred to as norstatine. In leucine amino peptidase, chelation occurs between both the amide carbonyl group and the adjacent hydroxyl and the hydroxyl and the *N*-terminal amino group (119,120).

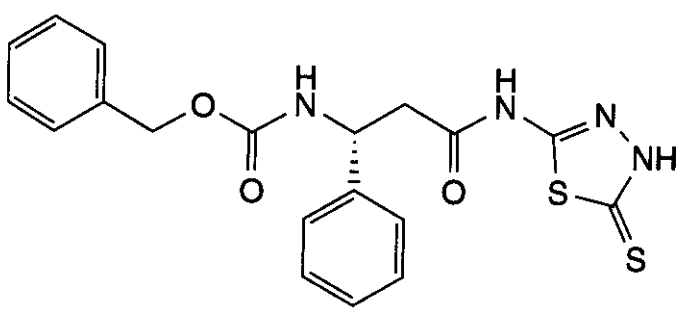
6.3 TSA-Derived Cysteine and Serine Peptidase Inhibitors

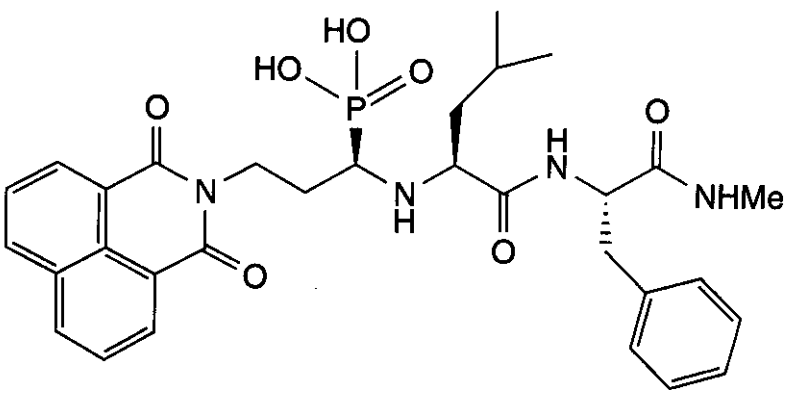
Classical TSA inhibitors of cysteine and serine proteases differ from the metallo- and aspartic protease inhibitors in that they mimic the tet-

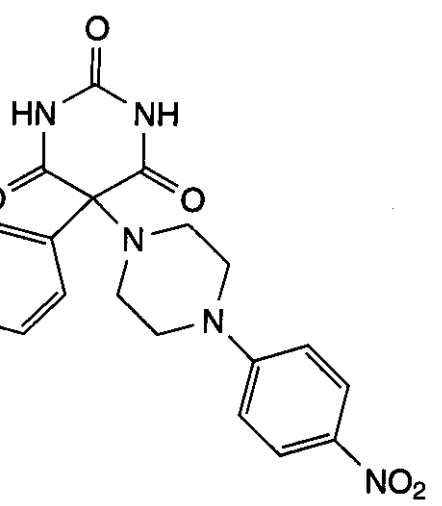


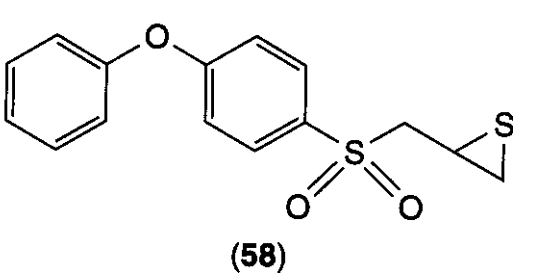
(57)





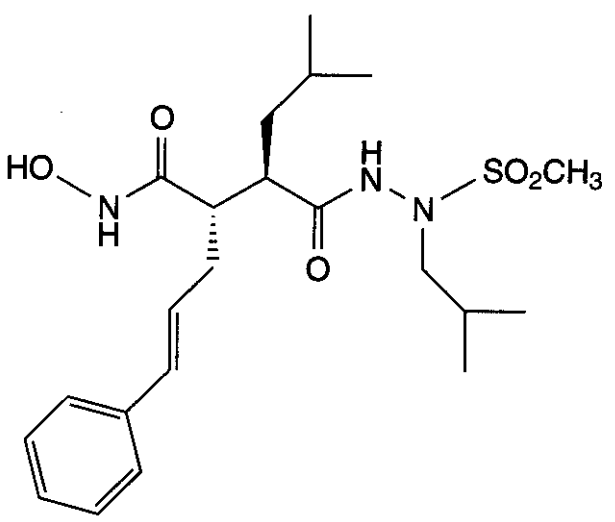






(58)

Figure 15.26. Novel **TSA** used to inhibit metallopeptidases.



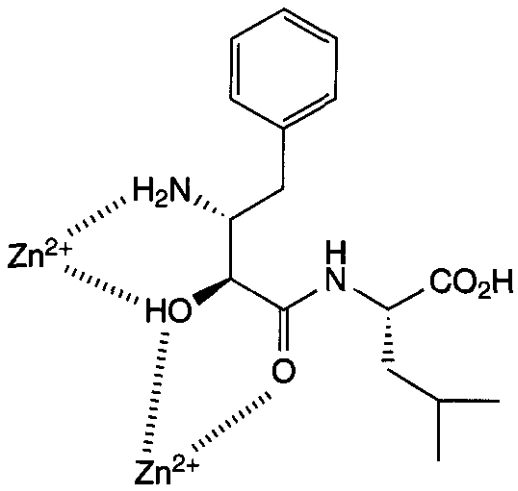
Ro-32-7315

(59)

Figure 15.27. Example of TSA as an TNF-α inhibitor.

rahedral intermediates for enzyme-catalyzed amide bond hydrolysis only after a reversible chemical reaction between enzyme and inhibitor takes place. Usually this involves the addition of the enzyme catalytic nucleophile (the serine protease hydroxyl group or the cysteine protease thiol group) to an electrophilic group in the inhibitor to generate ketal-like species (121).

Some of the serine and cysteine TSA moieties are shown in Fig. 15.29. Selective inhibition between these two classes of protease can be achieved easily. For example, trifluoromethylketones (**61**) and peptidyl boronic acids (62) do not efficiently inhibit cysteine proteases. However, selective inhibition of enzymes within each class can be very difficult.



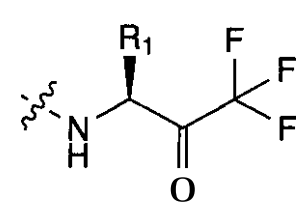
Bestatin
(60)

Figure 15.28. Proposed binding mode of bestatin.

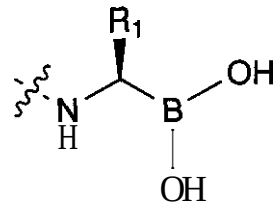
Many cysteine peptidases are involved in the biosynthesis and degradation of biologically important peptides. Most early work was done with papain, a cysteine peptidase isolated from the papaya fruit and used in meat tenderizer many years ago. The readily available source of this enzyme led to one of the very first X-ray crystal structures of any peptidase (122, 123), despite the fact that no cysteine peptidase was then known to be important in human pathology. Since then, cathepsins B, H, L, and S were discovered to be involved in biosynthetic steps in human immune response, inflammation, and other biologies. For example, cathepsin B is clearly involved in the metastatic process and must act at some stage to permit transformed tumor cells to migrate to other parts of the body; for 20 years, people have sought inhibitors of cathepsin B as potential anti-metastatic drugs (124). Cathepsin K was recently discovered and shown to be involved in osteoporosis and bone regulation (125).

Inhibitors of cathepsin K illustrate the principles developed to inhibit this class of enzyme. This enzyme sequence was detected in 1994 by sequencing of human DNA for the human genome project (126). Cathepsin K was found to be inhibited by leupeptin (63) and by compound (**64**), which surprisingly binds "backwards" to the active site (Fig. 15.30). A hypothesis to develop symmetrical inhibitors of cathepsin K derived from the superposition of both aldehydes on the carbonyl carbon; this led to the diamino ketone TSA (65). The diamino ketone moiety seems to work in several classes of cysteine proteases (127).

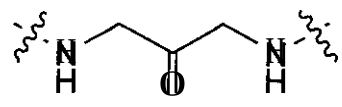
Based on these results, Marquis et al. have recently described the design and synthesis of conformationally constrained cyclic ketones as highly potent and selective cathepsin K inhibitors (**66–67**) (Fig. 15.31) (128). The labile stereogenic group in position α of the ketone was shown to be important for the binding mode and pharmacokinetic profile of these type of inhibitors. The crystal structure of the two epimers showed two alternate directions of binding to the enzyme active site. In both structures, the primed region of the enzyme was occupied by these inhibitors. Further investigation, resulted in the azepanone derivative (68) as a configurationally stable template for the selective inhibition of this cysteine protease ($K_i = 4.8\ pM$) (129).



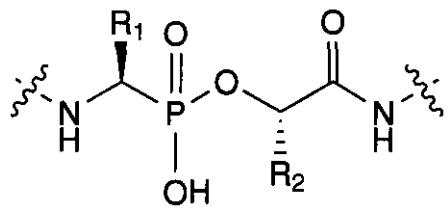
Trifluoromethylketone
(61)



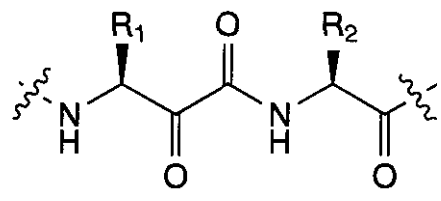
Boronic acid
(62)



Diaminoketone



Phosphonic acid



α-Ketoamide

Figure 15.29. TSA used to inhibit serine or cysteine peptidases.

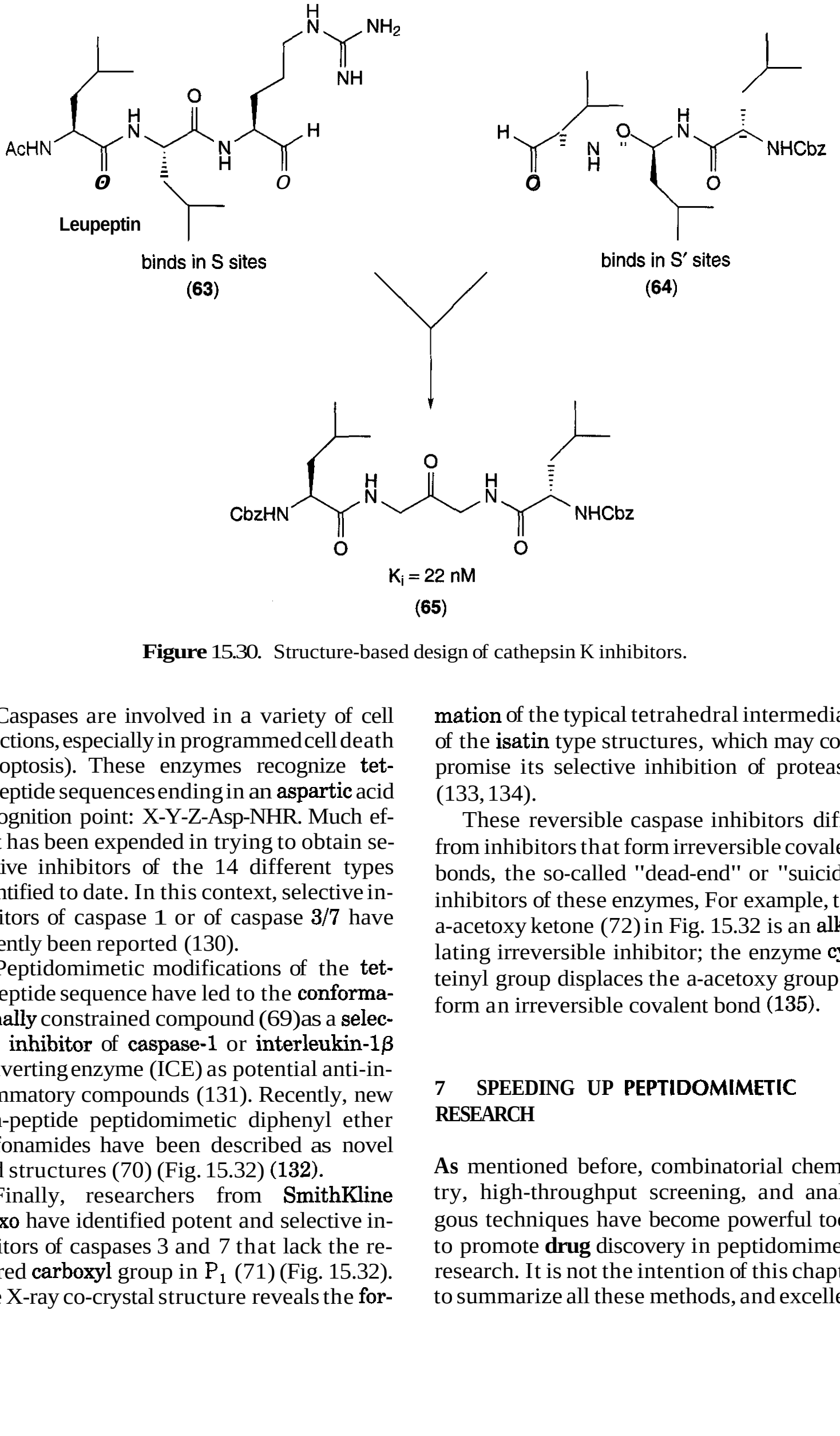


Figure 15.30. Structure-based design of cathepsin K inhibitors.

Caspases are involved in a variety of cell functions, especially in programmed cell death (apoptosis). These enzymes recognize tetrapeptide sequences ending in an aspartic acid recognition point: X-Y-Z-Asp-NHR. Much effort has been expended in trying to obtain selective inhibitors of the 14 different types identified to date. In this context, selective inhibitors of caspase 1 or of caspase 3/7 have recently been reported (130).

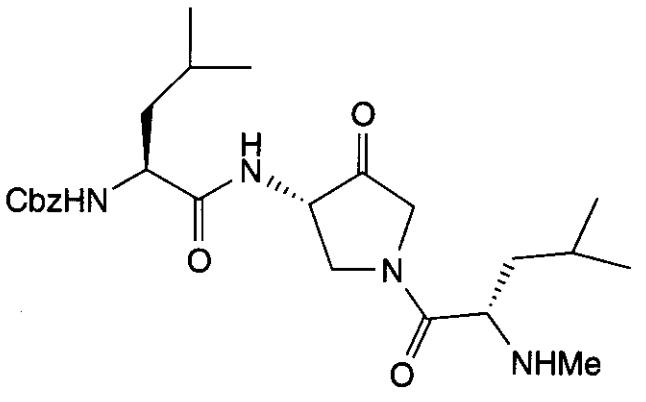
Peptidomimetic modifications of the tetrapeptide sequence have led to the conformationally constrained compound (69)as a selective inhibitor of caspase-1 or interleukin-1β converting enzyme (ICE) as potential anti-inflammatory compounds (131). Recently, new non-peptide peptidomimetic diphenyl ether sulfonamides have been described as novel lead structures (70) (Fig. 15.32) (132).

Finally, researchers from SmithKline Glaxo have identified potent and selective inhibitors of caspases 3 and 7 that lack the required carboxyl group in P_1 (71) (Fig. 15.32). The X-ray co-crystal structure reveals the formation of the typical tetrahedral intermediate of the isatin type structures, which may compromise its selective inhibition of proteases (133, 134).

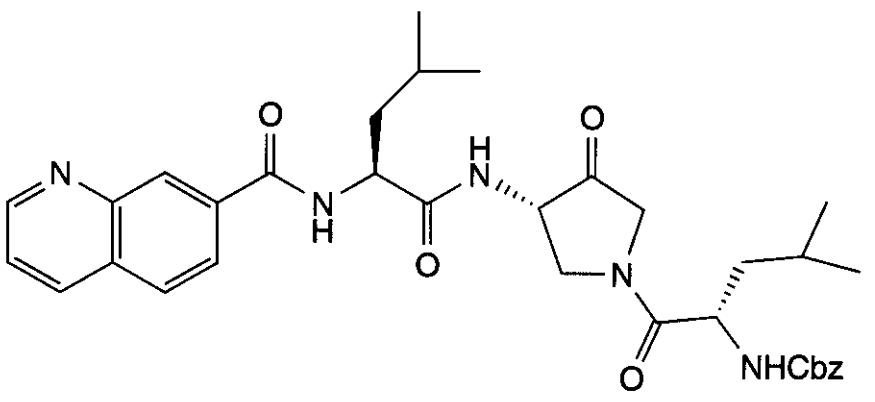
These reversible caspase inhibitors differ from inhibitors that form irreversible covalent bonds, the so-called "dead-end" or "suicide" inhibitors of these enzymes, For example, the a-acetoxy ketone (72) in Fig. 15.32 is an alkylating irreversible inhibitor; the enzyme cysteinyl group displaces the a-acetoxy group to form an irreversible covalent bond (135).

7 SPEEDING UP PEPTIDOMIMETIC RESEARCH

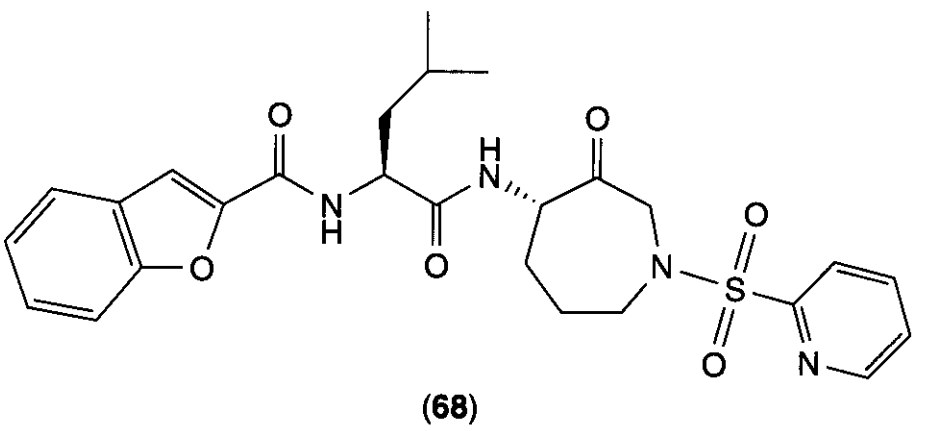
As mentioned before, combinatorial chemistry, high-throughput screening, and analogous techniques have become powerful tools to promote drug discovery in peptidomimetic research. It is not the intention of this chapter to summarize all these methods, and excellent



(66)

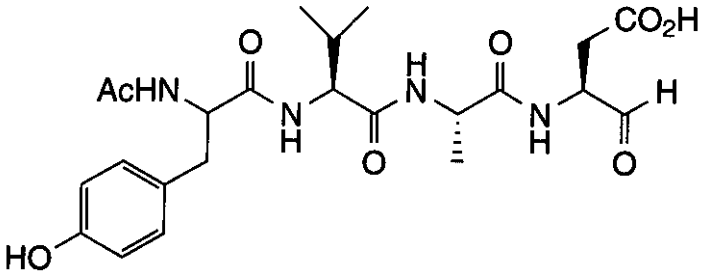


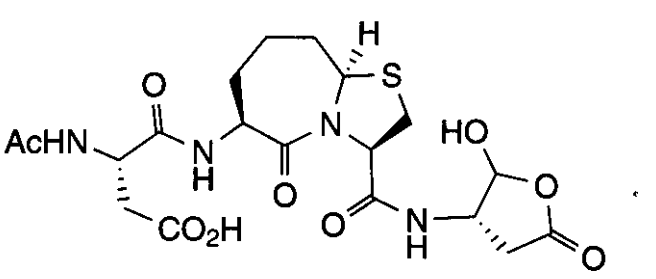
(67)



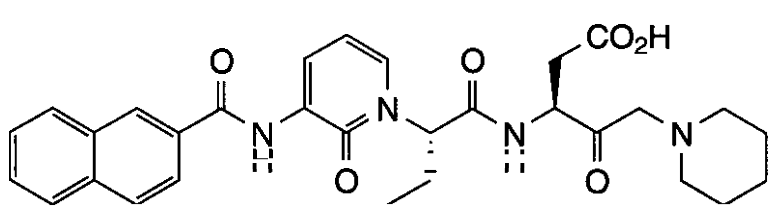
(68)

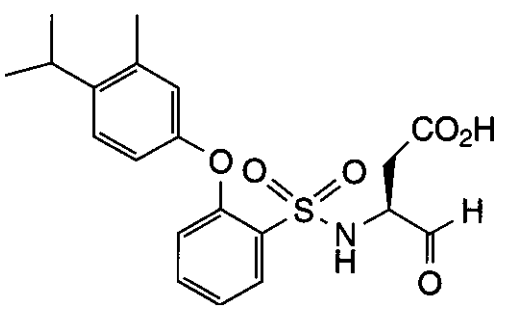
Figure 15.31. Cyclic ketones in novel cathepsin K inhibitors.



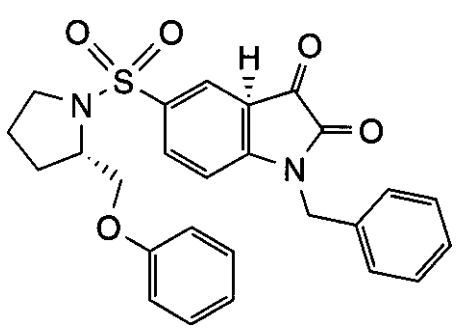


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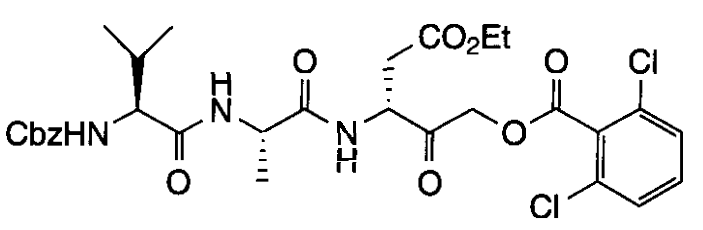




(70)

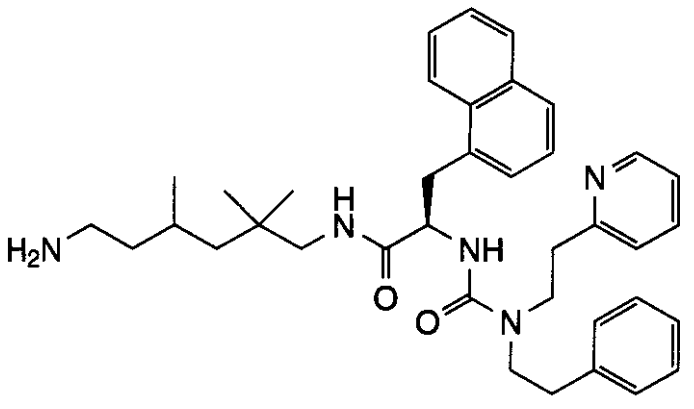


(71)



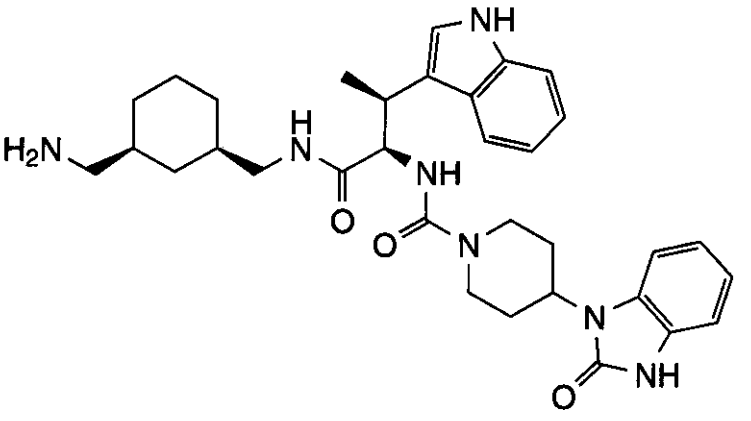
(72)

Figure 15.32. Examples of TSA as caspase inhibitors.



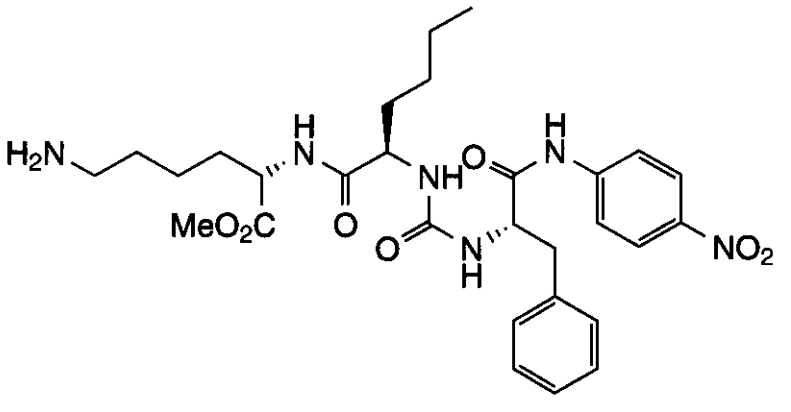
L-797, 591

(73)



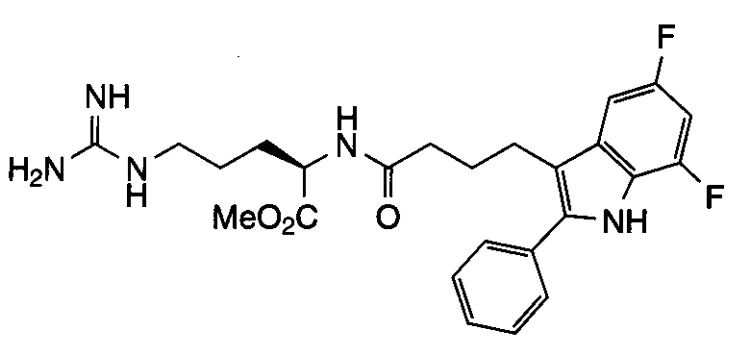
L-779, 976

(74)



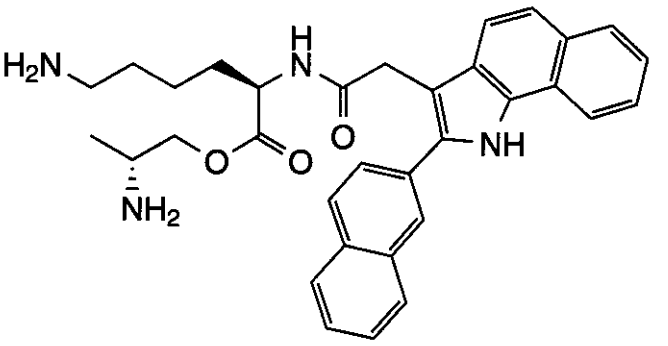
L-796, 778

(75)



L-803, 087

(76)



L-817, 818

(77)

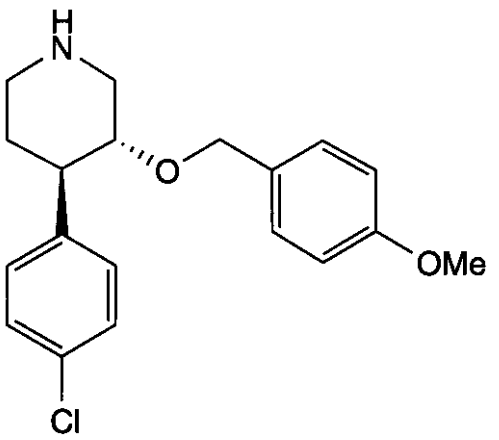
Figure 15.33. Somatostatin receptor agonists found through combinatorial chemistry.

reviews are available in the literature (136–140). However, one successful approach developed at Merck for the rapid identification of selective agonists of the somatostatin receptor through combinatorial chemistry should be highlighted, because it illustrates the evolution of a constrained peptide into a non-peptide peptidomimetic structure (141).

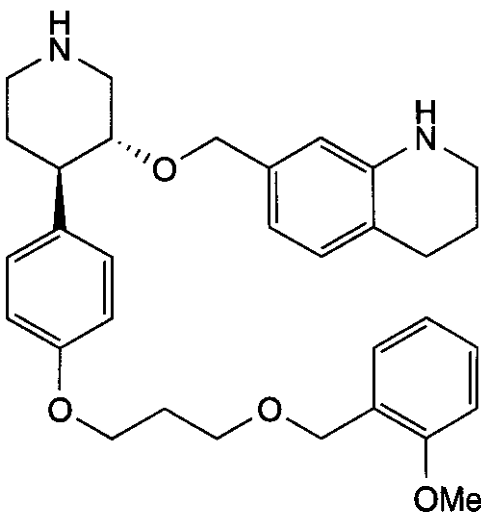
A series of combinatorial libraries were constructed on the basis of molecular modeling of known peptide agonists like MK-678 and ocreotide. A chemical collection of 200,000 compounds was screened, giving priority to the residues Tyr-Trp-Lys, important pharmacophores in somatostatin determined first by Veber et al. (31) This approach yielded five compounds (73–77) (Fig. 15.33), each being selective for one of the somatostatin receptor subtypes: sstl (73), sst2 (74), sst3 (75), sst4 (76), and sst5 (77).

8 TOWARD RATIONAL DRUG DESIGN: DISCOVERY OF NOVEL NON-PEPTIDE PEPTIDOMIMETICS

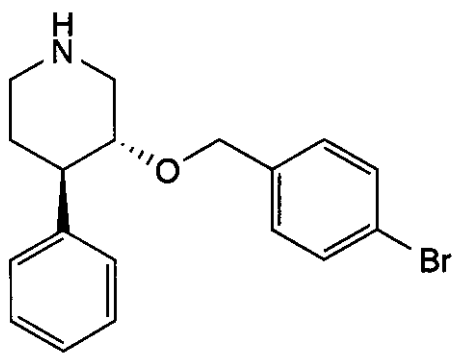
Current pharmaceutical research has taken advantage of newer computational methods, the so-called computer-aided drug design, and other physicochemical techniques such as X-



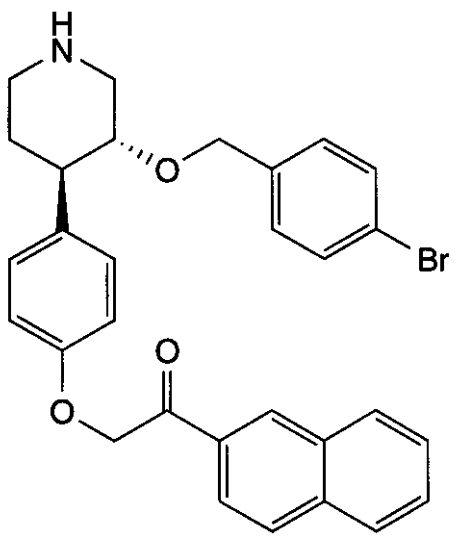
Roche
(78)



(79)



Rich et al.
(80)



(81)

Figure 15.34. Examples of GRAB peptidomimetics.

ray crystallography and NMR (142). The main goal in rational drug design is to translate the structural information in the native peptide into low molecular weight non-peptidic molecules. Over the past years, many 3D structures of biological targets have been solved and have been successfully used to design new, pharmacologically useful compounds (vide *infra*). Different computer-aided design methods, e.g., 3D pharmacophore model, 3D quantitative SAR (QSAR), docking, and de novo design, have been extensively reviewed elsewhere (75, 143–146).

Recently, the importance of generating inhibitors that target receptor conformational ensembles has been pointed out (10). This method goes beyond the current docking of known structures to known active site conformers and can lead to type III and GRAB peptidomimetics.

The concept of Group Replacement Assisted Binding (GRAB) peptidomimetics derives from the discovery at Roche of the piperidine class of renin inhibitors. The non-peptide inhibitors of renin (78) (K_i = 26 μM) and (79) (K_i = 31 nM) (Fig. 15.34)(84, 147–149) stabilize an enzyme active site conformation different than the β-strand binding enzyme conformation typical for other peptidase inhibitors. A close analysis of the X-ray crystal structure of the enzyme inhibitor complex shows that the piperidine C4-phenyl group binds to the enzyme to replace Tyr^{75} that has rotated to another position. Interestingly, Leu^{73} also rotates to fill some of the vacated Tyr^{75} pocket, and this in turn allows Trp^{39} to occupy a new site formed in part by the vacated Leu^{73} (Fig. 15.35). This cascade of conformational transitions in the renin active site allows the optimized inhibitor to stabilize an enzyme conformation not observed when the classic peptide-derived peptidomimetics bind. This stabilization process is defined as group replacement process, and the piperidine inhibitors constitute a new type of peptidomimetic: GRAB peptidomimetics.

Comparison of (78) and (79) with the structures of other peptide-derived inhibitors re-

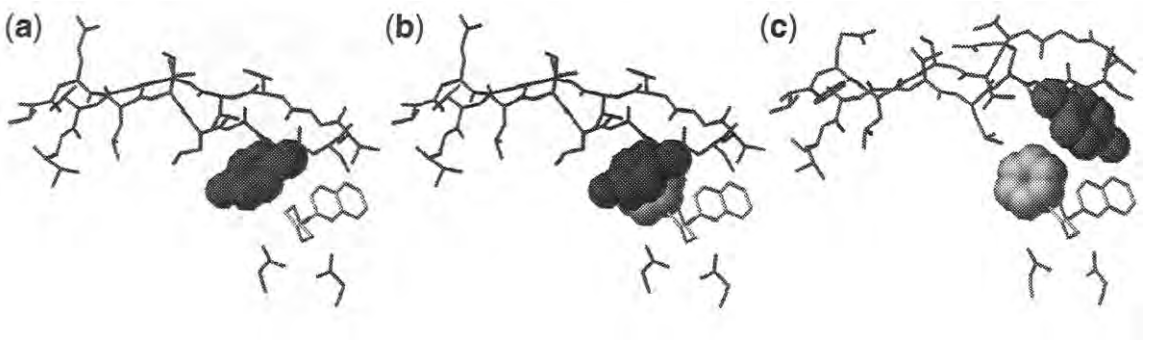


Figure 15.35. GRAB peptidomimetics in action. See color insert.

veiled how the different enzyme active site conformation were found. Bursavich et al. have successfully extended the initial renin modeling to the design of inhibitors of two other aspartic peptidases: pepsin and R. *chinensis* pepsin (80) (K_i = 2 *p&)* and (81)(K_i = 0.2 μM) (Fig. 15.34) (150).

The extended β-strand binding conformation could be changed into the piperidine binding conformation by a series of low-energy, mechanistically related conformational changes in active site side-chains. The discovery of the Roche inhibitors and the correlation of these structures with peptide-derived inhibitors are analogous to a peptidomimetic "Rosetta Stone." This design strategy has the potential for designing novel types of peptidomimetic structures.

9 HISTORICAL DEVELOPMENT OF IMPORTANT NON-PEPTIDE PEPTIDOMIMETICS

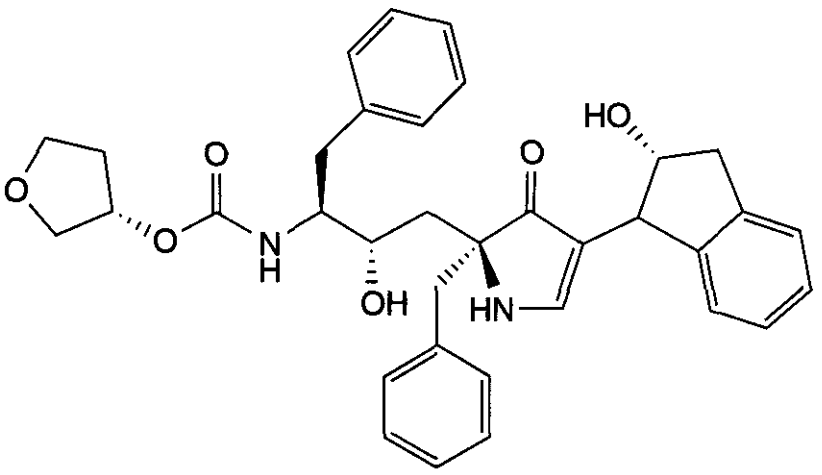
9.1 HIV Protease

Type-I HIV-1 protease inhibitors, Saquinavir, Ritonavir, Indinavir, Amprenavir, Viracept (neflinavir mesilate), and Lopinavir (Fig. 15.20) are established drugs for the treatment of AIDS. All these inhibitors employ the central hydroxyl transition state mimetic as a scaffold on which varying functionality was systematically added until the required balance between potency, *in uiuo* activity and oral absorption was achieved. In general, the binding interactions were optimized through iternative synthesis and co-crystallization of inhibitor with enzyme, molecular modeling, and re-designing the inhibitor side-chains. Pharmacokinetic properties were addressed only after the initial inhibitor was identified and optimized. Compounds (82–83) (Fig. 15.36) are highly modified peptidic structures that stabilize the enzyme-bound extended β-conformation (151, 152).

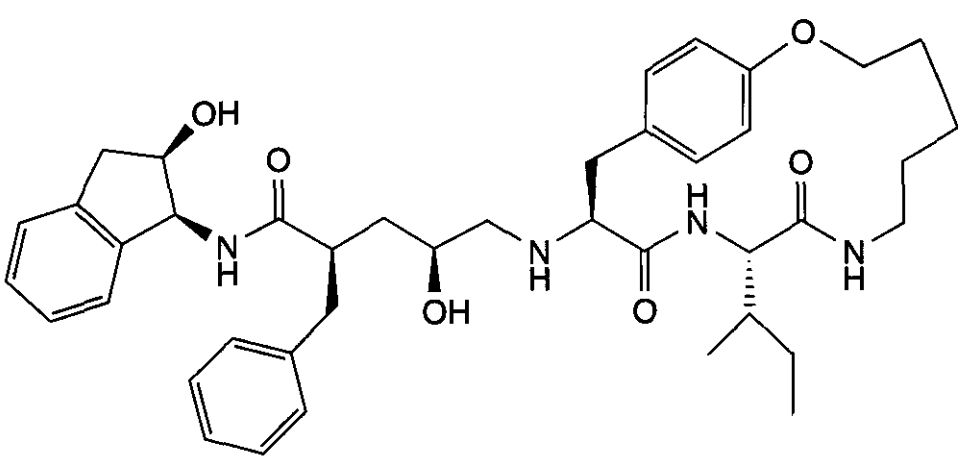
Another approach to achieve greater *in vivo* activity is to start with a molecular template with proven useful pharmacokinetics and oral bioavailability and to selectively modify it to achieve the desired activity. Identification of the orally active anticoagulant warfarin (84) (Fig. 15.37) as a weak inhibitor (IC_{50} = 18 μM) of HIV protease was followed by two reports of 4-hydroxycoumarins as possible type III HIV inhibitors. Subsequent SAR studies led to the more potent 5,6-dihydro-4-hydroxy-3-pyrone inhibitor (85)(IC_{50} = 2.7 nM), which has good anti-viral activity (EC_{50} = 0.5 μM) and is orally bioavailable (153). Upjohn researchers also used a structure-based design approach based on warfarin to obtain (**86**), their clinical candidate PNU-140690 (154). It should be noted that both inhibitors bind to the extended β-strand binding active site conformation.

Workers at DuPont used a pharmacophore model and database search to develop the first type III mimetic inhibitor of HIV protease, DuP 450 (87) (Fig. 15.38). This evolved from a 3D pharnacophore that retained two key interactions: replacement of the flap-bound water and a hydroxyl transition-state isostere (155). Molecular modeling led to a cyclohexanone as a better spacer between these groups, and finally the seven-membered cyclic urea (87) was created (Fig. 15.38). The development of these inhibitors illustrates the importance of conformational analysis in the design of constrained analogs.

Surprisingly, the symmetric cyclic sulfonyl-urea derivative analog (88) (Fig. 15.38, K_i = 3 nM) binds differently in the active site and adopts a flipped conformation (156).



(82)



(83)

Figure 16.36. β-Strand HIV protease inhibitors.

Moreover, SAR of the cyclic urea and cyclic sulfamide inhibitors do not follow a straightforward pattern. These contradictory results clearly illustrate the structural diversity created by a subtle structural modification in two otherwise related peptidomimetic protease inhibitors.

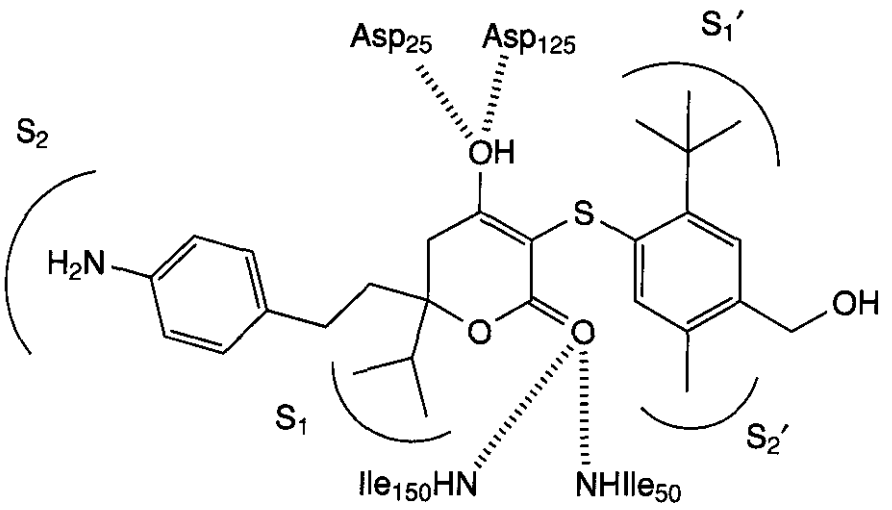
The peptidase inhibitors, (82) and (**83**), are actually amino acid and transition-state mimics pieced together to emulate the typical ligand-bound extended p-strand inhibitor conformation. The structurally distinct heterocyclic aspartic protease inhibitors (**85–86**) and (87–88) are non-peptide peptidomimetics because of their remote structural relationship to native peptide substrates. Yet these two distinct peptidomimetic classes bind to the same active site topography. These structurally distinct peptidomimetics selectively stabilize closely related enzyme conformations.

9.2 Thrombin

Thrombin and Factor Xa are both serine proteases involved in the blood coagulation cascade. Inhibition of these two enzymes is providing novel anticoagulants (**157–159**).

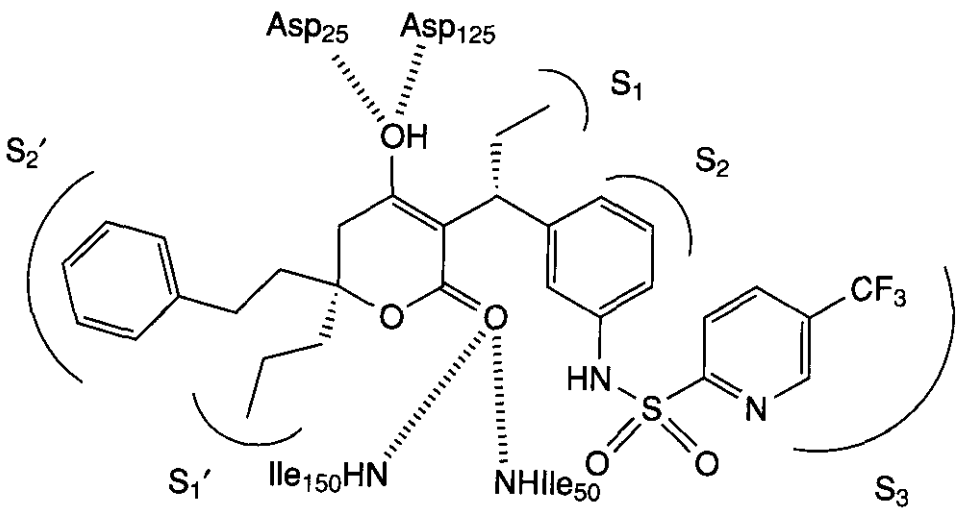
The development of thrombin inhibitors that lack the functionalized TSA highlights a major new approach to type I peptidomimetics. In 1995, a Lilly group found that D-Phe-Pro-Agmatine analogs showed increased selectivity for thrombin over other fibrinolytic enzymes despite a 100-fold loss in potency caused by the removal of the aldehyde group (160). These studies paved the way for Merck's development of picomolar thrombin inhibitors (161, **162**), which use a similar motif. Removal of an a-ketoamide transition state mimic from L-370,518 (**89**) (Fig. 15.39, $K_i = 0.09\ nM$) led to an expected 100-fold drop in potency for (90)($K_i = 5\ nM$). However, systematic modification of the P_3 position restored potency and led to an inhibitor (91) with a $K_i = 2.5\ pM$. Interestingly, potency seems to be enhanced by a fortuitous hydrophobic collapse into a favorable binding conformation.

Thrombin inhibitors (92) and (93) illustrate a novel type III peptidomimetic. Most protease inhibitors bind in an extended β-strand conformation that is stabilized by multiple enzyme ligand hydrogen bonds.



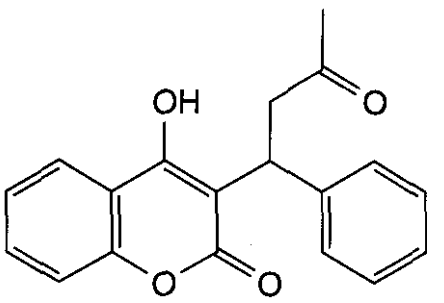
Parke-Davis X-ray structure

(**85**)



Pharmacia-Upjohn X-ray structure

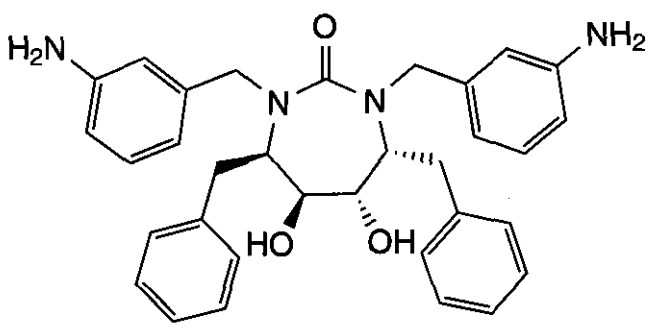
(**86**)



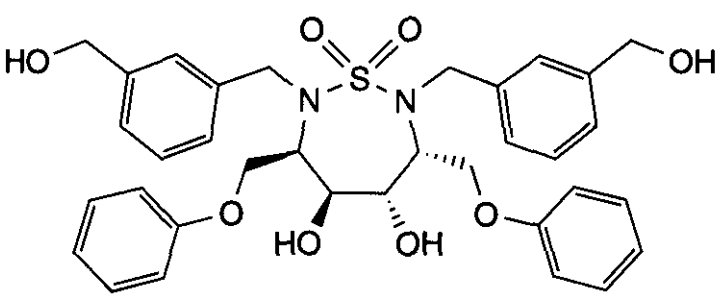
Warfarin

(**84**)

Figure 15.37. Warfarin analogs as non-peptide HIV protease inhibitors.



(**87**)

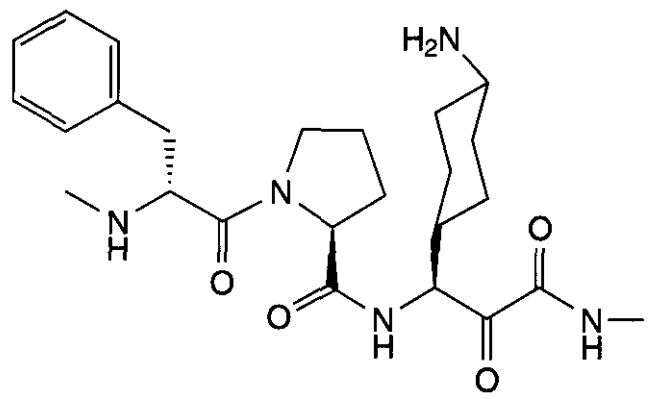


(**88**)

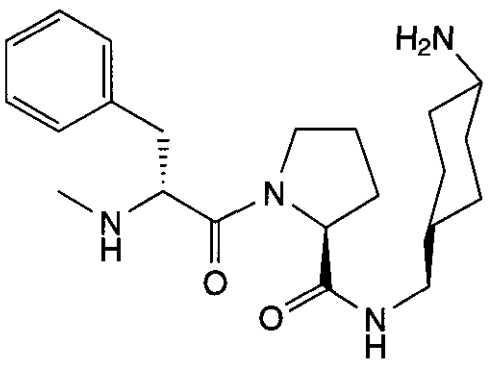
Figure 15.38. Cyclic ureas as non-peptide HIV protease inhibitors.

However, Boehringer Mannheim developed thrombin inhibitors (92) (Fig. 15.40) that lack these H-bonds (163). This idea was exploited by researchers at 3D Pharmaceuticals, who were able to crystallize (93) in the active site (164, 165). In this example, the benzene ring acts as a scaffold to display the three different substituents to fill the three principal binding pockets.

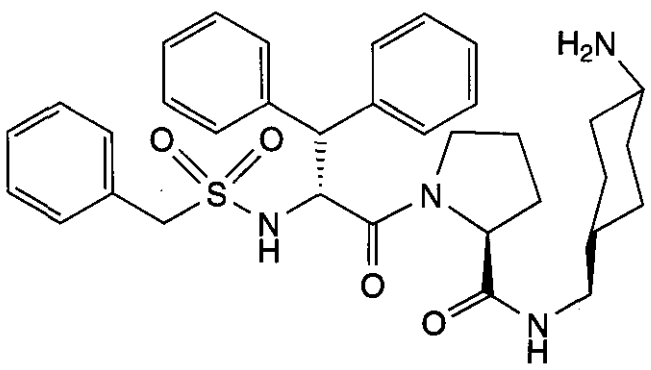
Other type III peptidomimetic inhibitors of thrombin have been developed from screening leads (166, 167) such as inhibitor (94) (Fig. 15.40). SAR led to the design of (95). Inhibitor (96) was derived from docking studies with the 5-amidino indole nucleus, followed by addition of a lipophilic side-chain to interact with the important S_3 subsite of thrombin. The crystal structures of both (95) and (96) in the active site of thrombin shows that the aromatic core, binds in the S_1 site as expected, but



(89)



(90)



(91)

Figure 15.39. Non-TSA thrombin inhibitors.

does not pick up hydrogen bonding from the important active site sequence Ser214–Gly216 (168). Both crystal structures showed a similar binding mode; where interaction of the C-2 side-chain with Trp60D might explain the high thrombin selectivity observed for this series (169).

Another type III peptidomimetic inhibitor was derived from the crystal structure of a bicyclic [3.1.3] inhibitor (170) complexed to thrombin (**97**) (Fig. 15.41). The X-ray structure revealed that one of the carbonyls was oriented towards the hydrophobic P-pocket (S_2). The desolvation necessary to place a carbonyl in a hydrophobic pocket is unfavorable and various alkyl groups were used as possible replacements. This led to the potent ($K_i = 13$ nM) and selective (>760 for thrombin over trypsin) inhibitor (**98**).

One of the major drawbacks in thrombin inhibitor design was the requirement for a basic side-chain in P_1 needed to form a salt bridge to enzyme Asp189. However, the other amino acid side-chains in S_1 are largely lipophilic and neutral. This feature suggested that the strongly basic group in P_1 could be replaced by a weaker base or even with hydrogen-bonding groups. Compounds (99–100) are representative of this strategy (Fig. 15.40) (171). An X-ray crystal structure of (99) shows a new binding mode in which the formamide group points out of the S_1 pocket and forms new hydrogen bonds with Gly219 (172). The ability to obtain crystal structures of thrombin inhibitors complexes for many of the inhibitors shown in Figs. 15.40–15.41 establishes that most are type III peptidomimetics.

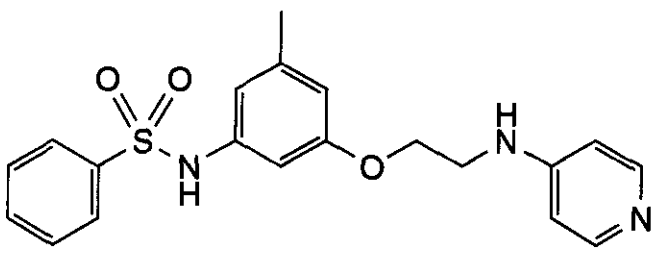
9.3 Factor Xa

New approaches to design inhibitors of Factor Xa as potential anticoagulants have been reviewed (173), and important type III mimetics have been described (Fig. 15.42). All inhibitors contain amidine or basic groups that bind in the enzyme's S_1 site; none of the inhibitors contains a classical electrophilic center of the type employed in TSA inhibitors (174–180).

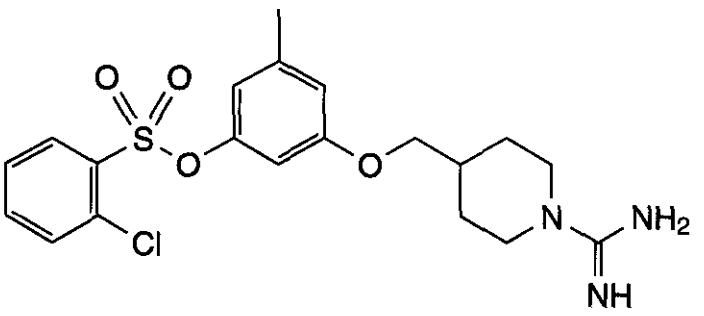
Compound (**101**) (Fig. 15.42) was designed from a strategy involving connection of a three-point pharmacophore derived from molecular modeling. Beginning with the X-ray structure of the Factor Xa dimer, Gong et al. (176) envisioned three important enzymatic contact points: a phenylamidine in the S_1 subsite, a phenylamidine in the S_4 site, and a carboxylate moiety postulated by a group at Daiichi to confer selectivity over thrombin through an interaction with Gln192 of Factor Xa. Systematic iterative modifications led to the potent inhibitor (101)($K_i = 9$ nM), which has 350-fold selectivity over thrombin. This approach highlights a truly de *novo* method where fragments were docked into the active site and an appropriate spacer was chosen to connect them. Further SAR data led to modifications that improved both potency and selectivity (176).

9.4 Clycoprotein IIb/IIIa (GP IIb/IIIa)

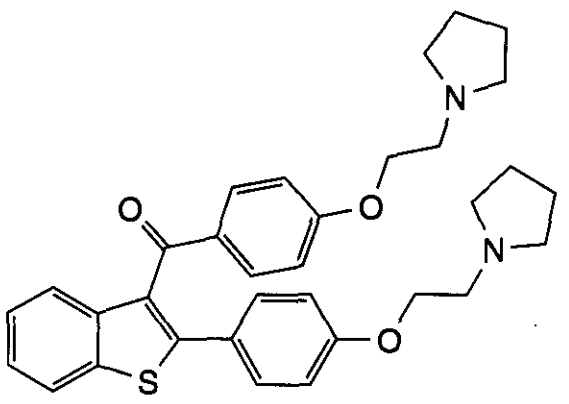
Some outstanding examples of the use of conformational restriction to characterize the



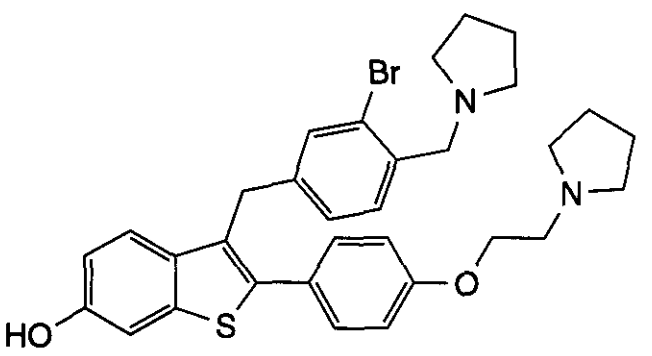
(92)



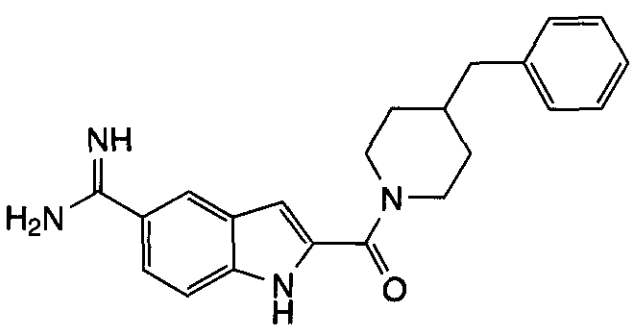
(93)



(94)



(95)

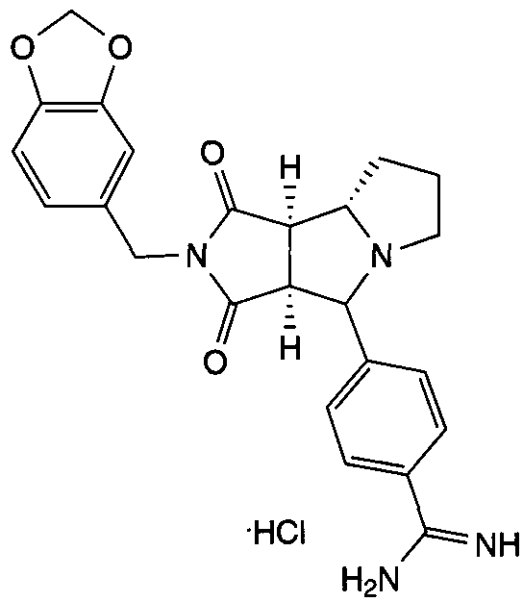


(96)

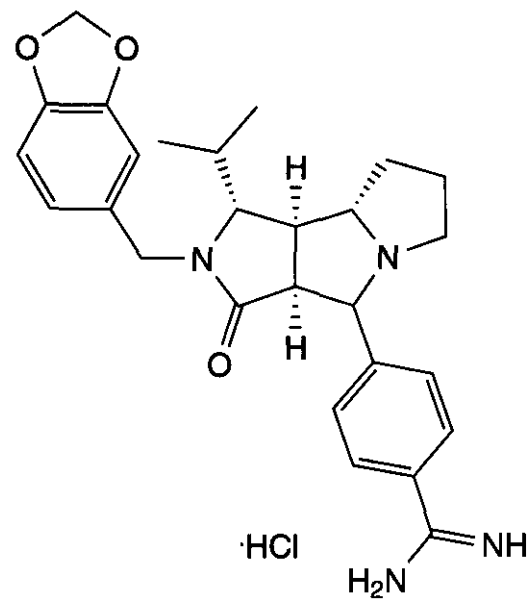
Figure 15.40. Non–H-bonding–based thrombin inhibitors.

bioactive conformations of Arg-Gly-Asp peptidometic antagonists illustrate the present state-of-the-art. Members of the integrin family of receptors recognize and bind the peptide sequence, Arg-Gly-Asp, as an important step in platelet aggregation and other physiological processes (181), and competitive antagonists for this process could serve as potential drug candidates. Much effort has been directed toward identifying small ligands that might mimic the RGD peptide sequence (182). This drug design concept was supported by the fact that protein antagonists of integrin receptors are known that contain the RGD sequence (183) and that small peptide sequences containing the RGD moiety weakly antagonize the endogenous ligand (184). Consequently, several groups synthesized conformationally restricted derivatives of small peptides as starting points for developing metabolically stable peptides or peptidemimetics. Ali et al. (185) svnthesized a series of disulfide derivatives of the RGD sequence, which were designed by analogy with the somatostatin work *(vide supra).* Excellent antagonists related to (102) were obtained. Further constraint of the peptide system by use of the o-thiol benzene derivatives led to the novel antagonist SKF 107260 (103) (Fig. 15.43), a good inhibitor of both platelet aggregation and binding to GPIIb/IIIa. Barker et al. (186) followed a similar strategy but used cyclic sulfides as an additional conformationally restricting element. These derivatives had the advantage of being rapidly synthesized by solid phase methods. Systematic structure-activity studies with respect to the amino acid preceding the RGD sequence and the chirality of sulfoxide derivatives led to the discovery of G-4120 (104), a potent, biologically active derivative.

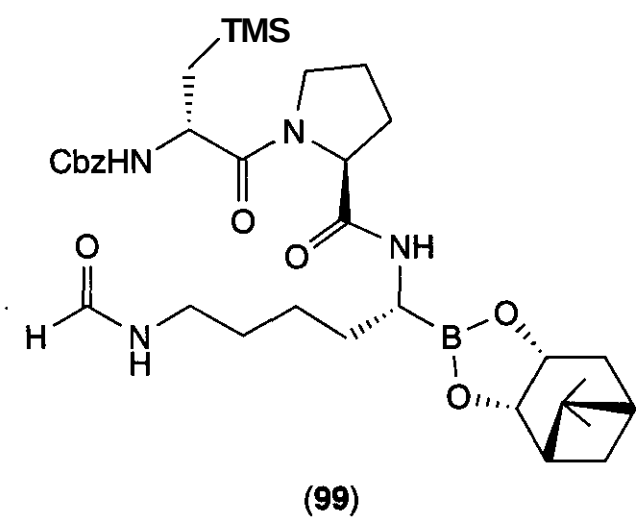
The conformation of both (103) and (104) in water was found to be highly constrained, and a single predominant conformation could be characterized in aqueous solution by use of



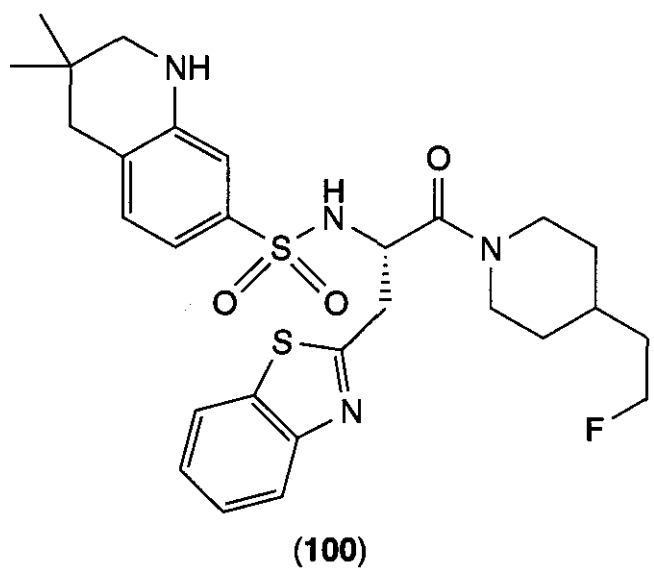
(97)



(98)



(99)



(100)

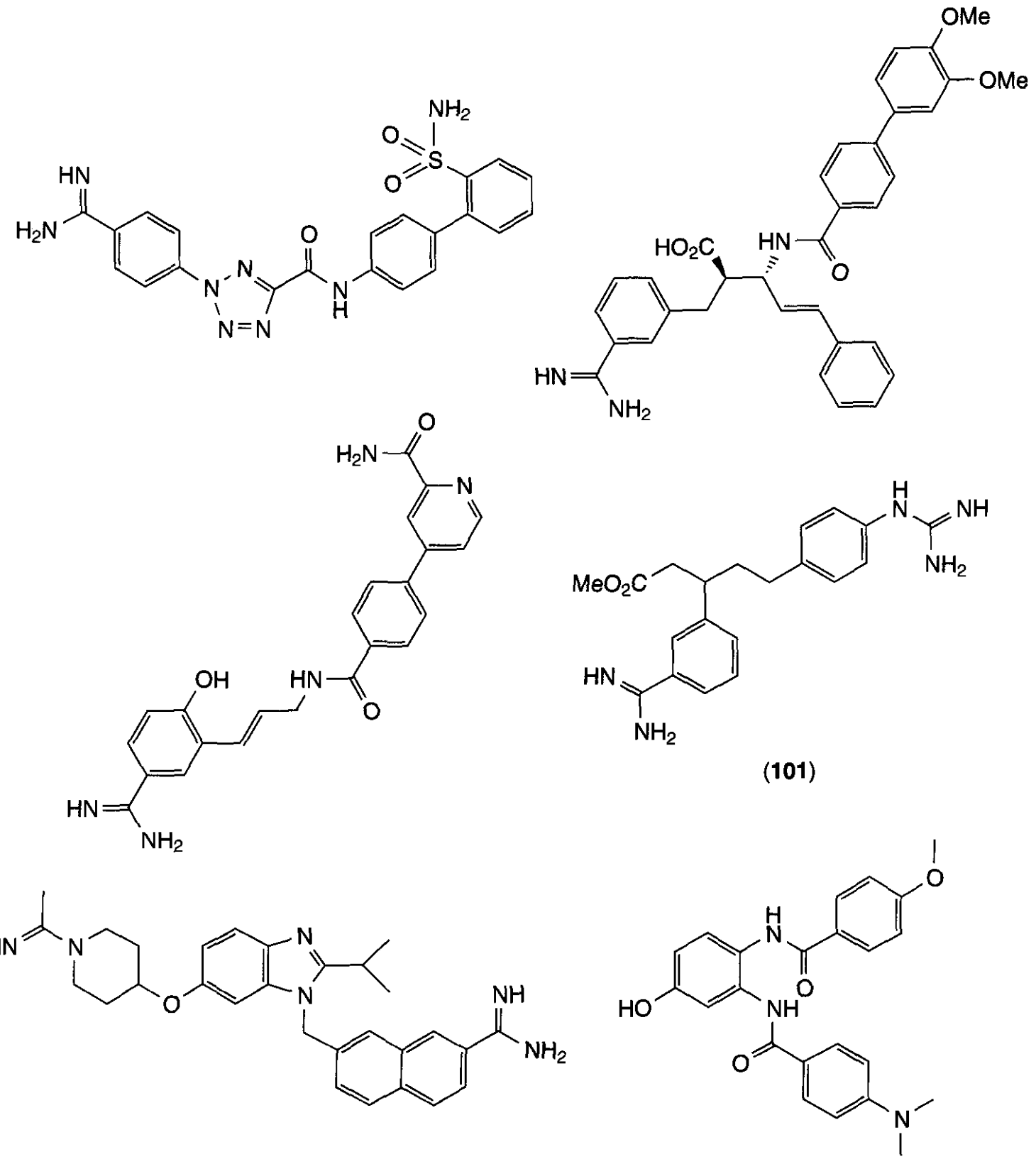
Figure 15.41. Optimized P_1 and P_2 thrombin inhibitors.

NMR methods and computational chemistry (185, 187). This bioactive conformation defined the topographical placement of the arginine guanidine group and the aspartic carboxyl group, and was superimposed onto a conformationally restricted template of a class of compounds with generally suitable pharmacodynamic properties. In this case, the benzodiazepine ring system was used, and the strategy generated the low molecular non-peptide RGD receptor antagonist (105–107) (Fig. 15.44), which contain at least two conformational restrictions, the bicyclic heterocycle and the acetylene linker. The compounds shown in Fig. 15.44 represent what can be achieved by applying the principles of conformational restriction to peptides when no X-ray or NMR structural information are available for the complex between ligand and receptor. Benzodiazepines (105–107) represent the first type III peptidomimetics designed de *novo* by systematically modifying a natural receptor-binding peptide (187–189).

A variety of other scaffolds have been developed by exploiting the idea that glycine represents a spacer between the two important recognition residues Arg and Asp. This template-based approach positions the key side functionality, a basic function and an acid one within a distance of 11–17 Å, required for presentation to the receptor. Several examples of these scaffolds are shown in Fig. 15.45 (190–195).

Recent results suggest that the RGD tripeptide can adopt multiple conformations that allow tight binding to the receptor. This theory is supported by the fact that nonpeptide RGD peptidomimetics can adopt a range of different topographies such as found in cupped, turn-extended-turn, or β-turn conformations (196).

RGD type I peptidomimetics are usually poorly bioavailable compounds because of the presence of multiple hydrogen-bonding sites



(101)

Figure 15.42. Examples of FXa protease inhibitors.

plus the charged polar functional groups at both ends. Esters or coumarin (197) linkers have been used to provide orally available prodrugs, and bioisosteric replacements of the guanidiniurn group by a pyridine, (198) tetrahydronaphtyridine, (199), or aminobenzimidazole (200) moieties provided more bioavailable analogs.

9.5 Ras-Farnesyltransferase

Inhibitors of Ras-farnesyltransferase have been developed by mimicking the C-terminal CAAX motif (where C is a cysteine residue, A is any aliphatic amino acid, and X is usually Met, Ser, or Ala). This tetrapeptide is the signal for farnesylation of Ras proteins. Ras-farnesyltransferase is one of the most promising targets for novel anti-cancer drugs, because at least 30% of the human cancers contain mutated Ras (201,202).

Two types of peptidomimetic structures have been used to develop inhibitors (203). Some typical type I inhibitors were generated by replacing the amide backbone with differ-

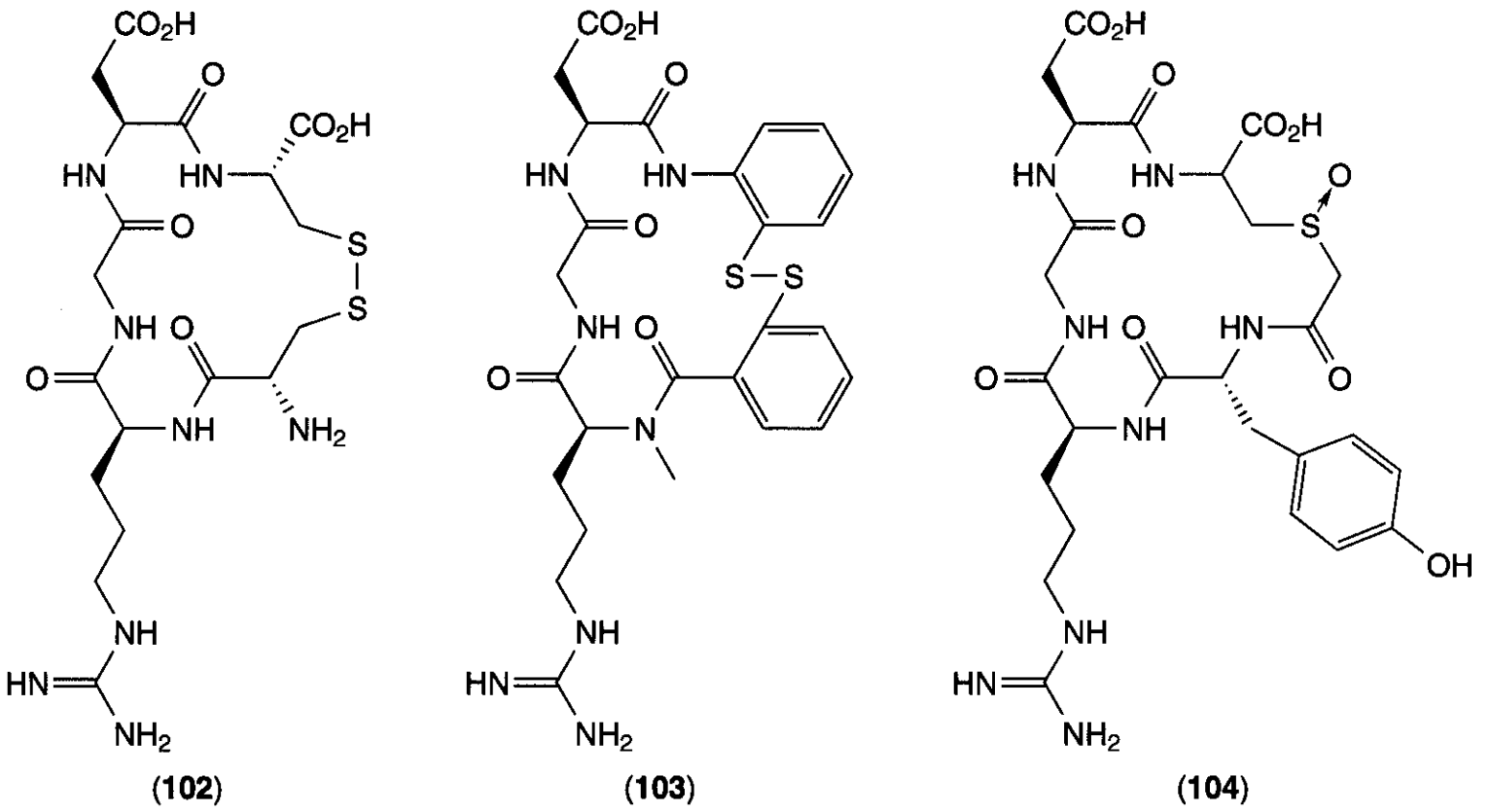


Figure 16.43. Conformationally restricted RGD cyclic peptides.

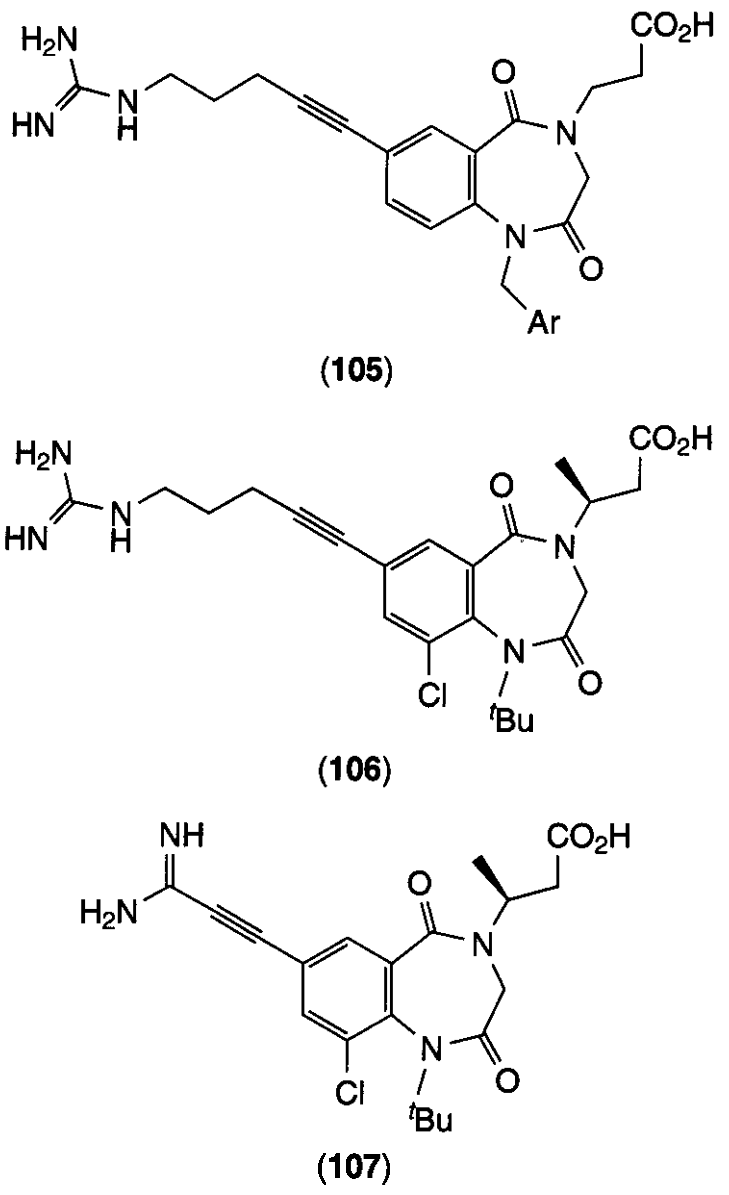


Figure 15.44. Benzodiazepines RGD analogs.

ent isosteres like the oxymethylene amide bond in (108)(Fig. 15.46, IC_{50} = 60 n*M*) (204). The central dipeptide segment of CA_1A_2X has been replaced with rigid linkers like the 3-aminomethylbenzoic acid (AMBA) in (109)(205). This novel inhibitor was not farnesylated, showing that the two amino acids in the middle of the CAAX tetrapeptide are required for farnesylation. An imidazole group has been used to replace the thiol group of the CAAX motif to produce compound (**110**) (206).

An outstanding example in peptidomimetic design evolved from these studies. Truncation and conformational restriction of a reduced isostere of the parent peptide substrate, followed by systematic replacement of the peptide-like side-chains provided the potent nonpeptidic inhibitor (**111**) (Fig. 15.46) (207). This approach highlights the transition from a peptide-derived structure to a compound with no apparent resemblance to the original peptide.

Recently, crystal structures of farnesyltransferase complexed with a farnesyl group donor and the native substrate or a type I peptidomimetic show the structural basis for inhibition of this enzyme. The X-ray data show that the CAAX motif adopts an extended conformation rather than a β-turn, which is the conformation observed by transferred nuclear

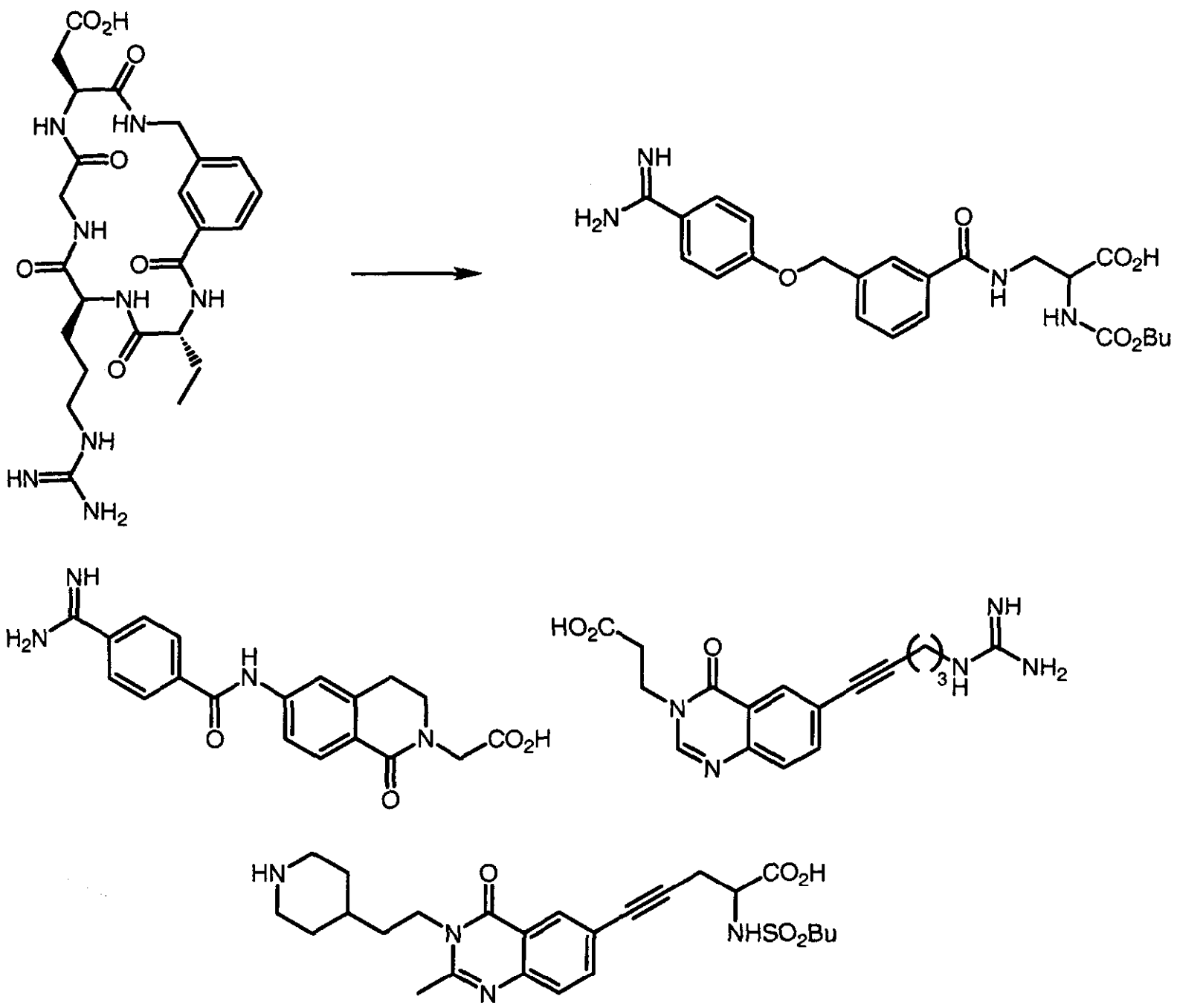


Figure 15.45. Different scaffolds used in RGD mimetics.

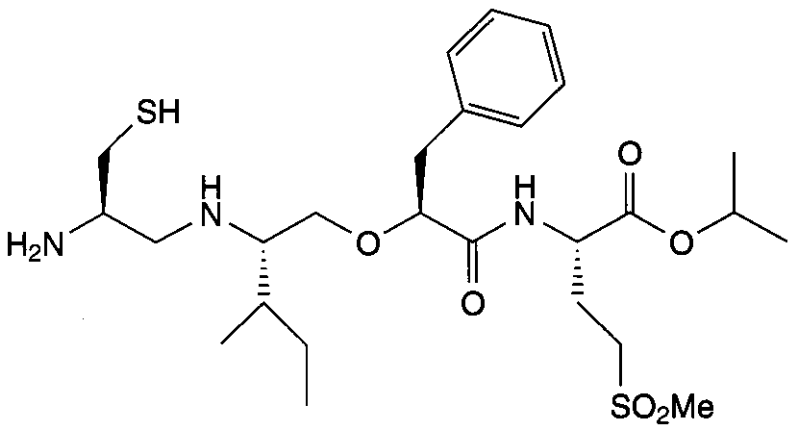
Overhauser effect experiments; coordination of the cysteine side-chain to the Zn ion promotes the conformational change in the peptide backbone. Moreover, differences in the conformation binding mode of peptides and peptidomimetics is one of the bases for selective farnesylation (208).

Other type III peptidomimetic inhibitors of this enzyme have also been reported. Inhibitor (112) (Fig. 15.47) was developed by replacing the A_1A_2 dipeptidyl sequence with a benzodiazepine scaffold (209). Later, SAR modifications of the benzodiazepine nucleus that included a hydrophilic 7-cyano group and a 4-sulfonyl group provided the potent, orally available and in *vivo* active (**113**) (210).

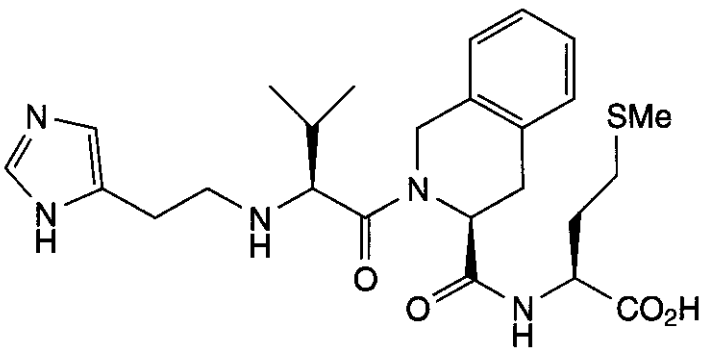
HTS also produced several non-peptide leads typified by inhibitor SCH **47307** (**114**) (Fig. 15.47) (**211**). Subsequent SAR work led to the potent inhibitor SCH 66701 (115)(K_i = 1.7 μM), which was crystallized within the enzyme active site (212). This series of compounds is completely non-peptidic and also lacks the free sulfhydryl or imidazole seen in the other inhibitors discussed here. This is a breakthrough that shows that potency can be achieved even without the "essential" cysteine or sulfhydryl mimic.

9.6 Non-Peptidic Ligands for Peptide Receptors

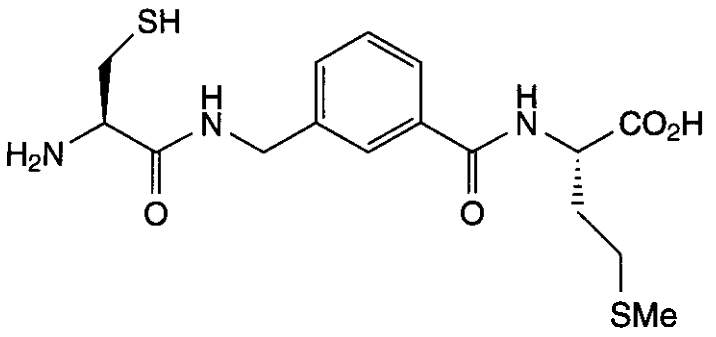
This section illustrates the successful development of non-peptide peptidomimetics from a screening lead by assuming the inhibitor binds to the receptor in the same way as does the native peptide hormone. These assump-



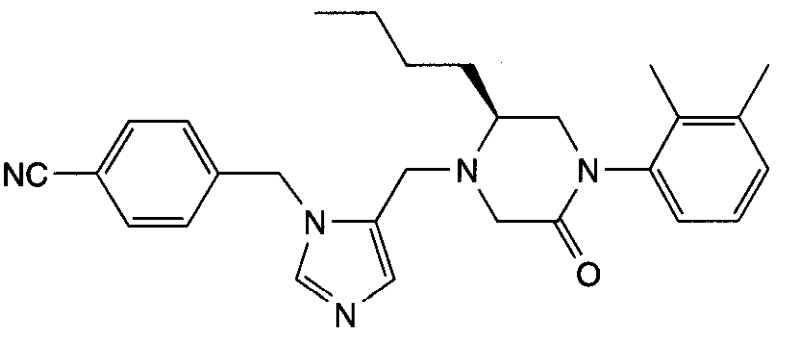
(108)



(110)



(109)



(111)

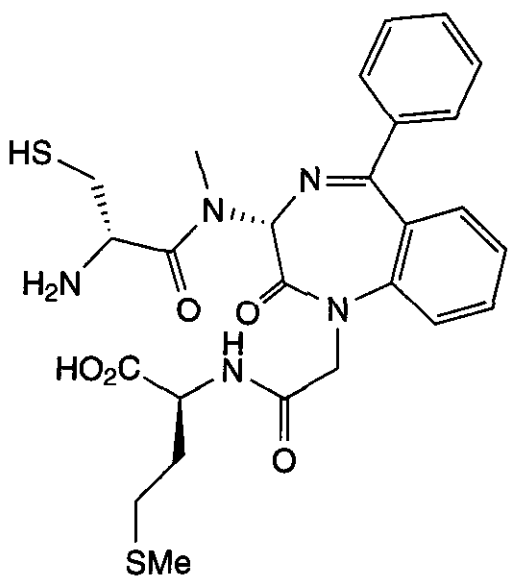
Figure 15.46. Peptide-like Ras-farnesyltransferase inhibitors.

tions actually led to effective inhibitors of the receptor. Later, site-directed mutagenesis of target receptors suggested that for many of these compounds, the mimetic was binding to the receptor at ancillary, perhaps overlapping, sites on the receptor. Later still, pharmacological studies indicated that peptide receptors adopted multiple states, suggesting that different antagonists might bind to different receptor forms. Of course, if compounds do not bind to the same receptor site as the endogenous hormone, SAR data collected on the natural peptide substrate is not applicable to these antagonists. Most of these peptidomimetics are probably type II or functional mimetic~Yet the success of this approach suggests that at least for some non-peptide antagonists, there may be some congruent structure that interacts with the receptor. These issues will only be determined unambiguously when high-resolution structures of the G-protein–coupled receptors (213) and other constitutive receptor systems are determined.

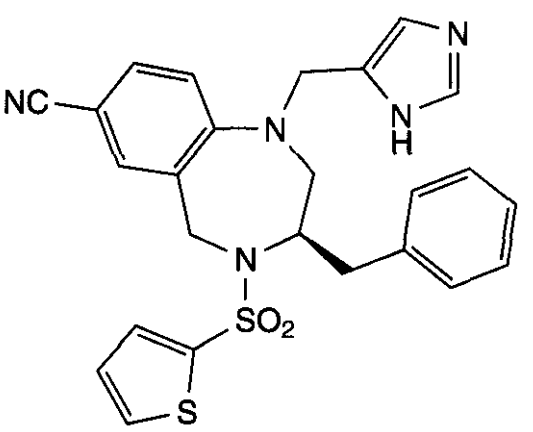
9.6.1 **Angiotensin II.** The first non-peptide antagonists of the AT1 receptor were found by HTS. The imidazole (116) (Fig. 15.48) is a weak (IC_{50} = 43 μM) but quite selective A-I1 receptor antagonist (214). Using this as a lead compound, DuPont and SmithKline Glaxo researchers independently developed potent small molecule A-I1 receptor antagonists. The DuPont group used the conformation sug-, gested by Smeby and Fernandjian to guide the design (215). It was speculated that the carboxyl group and the imidazol group of (**116**) were bound to the A-I1 terminal carboxyl group and to the imidazole group, respectively. This rationalization culminated in the synthesis of nanomolar inhibitors, with compound (117) as a clear representative (216).

Although workers at SmithKline Glaxo used the same conformation as starting point, they postulated other binding modes to the receptor. One of their alternative hypothesis considered compound (116) as a constrained analog in which the benzyl and the carboxyl groups corresponded to the Tyr side-chain and the C-terminal carboxyl group of A-11. Following this hypothesis, modification of lead compound (116) eventually led to compound (118) (Fig. 15.48) with an IC_{50} = 1.45 nM and oral activity of 30% (217).

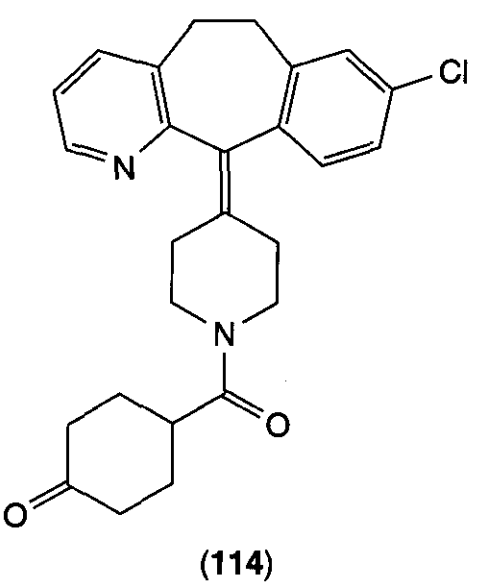
Site-directed mutagenesis studies on the AT1 receptor revealed differences in the bind-



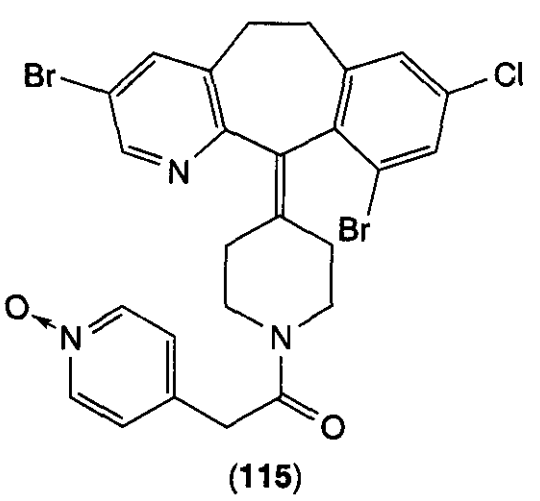
(112)



(113)



(114)



(115)

Figure 15.47. Non-peptidic Ras-farnesyltransferase inhibitors.

ing site of angiotensin and the small molecule non-peptide compounds (119–120) (Fig. 15.49). There is no evidence that the single residues involved in inhibitor binding overlap with endogenous peptide binding.

Some other non-peptide agonists have also appeared in the literature. Surprisingly, their binding mode differs from the binding mode of the peptide agonist (**121**), as well as that of the structurally similar non-peptide antagonist (122) (Fig. 15.49) (218). However, angiotensin and L-162,313 (122) require common critical residues for angiotensin AT1 receptor activation (219).

9.6.2 **Substance P.** The tachykinin receptors (NK-1, NK-2, and NK-3) and their endogenous ligands, the tachykinins, and neurokinins are important neurotransmitters (220–222). Antagonists of tachykinin receptors produce beneficial effects in several CNS disease states such as pain, asthma, emesis, and depression.

A general approach for converting a variety of peptide structures into small, type II peptidomimetic antagonists was devised by Horwell and colleagues and is illustrated here for antagonists to Substance P. An alanine scan of the parent undecapeptide revealed that the Phe^4–Phe^8 sequence was required for binding. Replacement of one these residues by Trp, followed by introduction of conformational constraints by a-alkylation, provided the subnanomolar inhibitor (123) (Fig. 15.50) (223). Improved brain penetration was achieved by amine (**124**) (224).

Chemical screening of corporate compound libraries resulted in the discovery of another

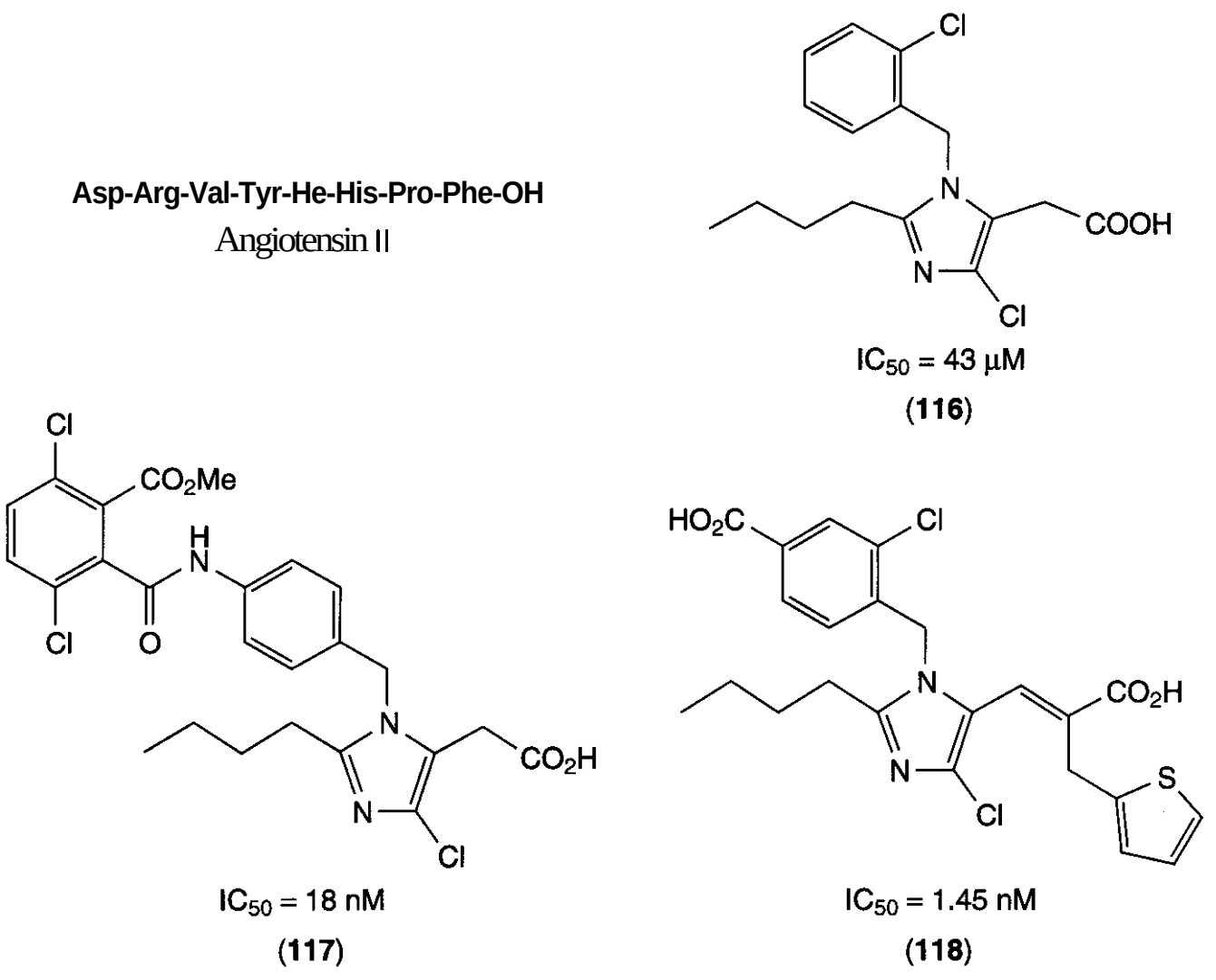


Figure 15.48. Angiotensin II inhibitors derived from a HTS lead.

type of non-peptidic NK-1 antagonist, CP-96,345 (125) (K_i = 0.66 n*M*, Fig. 15.51). This compound heralded a breakthrough in the design of these potential drugs (225, 226). Replacement of the basic quinuclidine ring with a morpholine core improves duration of action and insertion of an amino triazole unit confers excellent solubility and CNS penetration (126) (K_i = 0.19 n*M*, Fig. 15.51) (227).

Dual NK-1/NK-2 inhibitors, e.g., (127) (Fig. 15.52), have recently been designed by determining the important sites for maintaining NK-2 selectivity of the lead compound SR-48968 and introducing NK-1 pharmacophore groups (228–230).

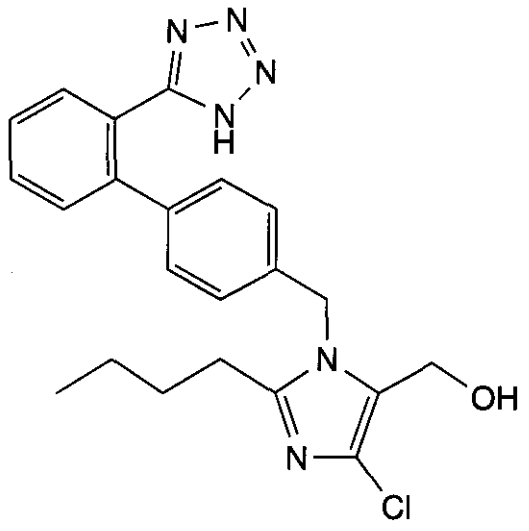
Fewer NK-3 selective receptor antagonists have been described, but a quinoline scaffold previously reported to be a selective NK-3 receptor antagonist, has been converted to a dual NK-2/NK-3 inhibitor (128) (K_i = 0.8 n*M* NK-2 and 0.8 n*M* NK-3). The lead optimization was carried out by docking potential structures into a novel receptor model. The theoretical model compares closely with the recently published crystal structure of rhodopsin (231).

9.6.3 Neuropeptide Y. Neuropeptide Y (NPY) is a 36 amino acid polypeptide that is involved in hormonal, sexual, and cardiac effects (232, 233). In 1994, two "first generation" type I NY-l selective antagonists, BIBP 3226 (129) (234) and SR120107A (130)(235) were reported (Fig. 15.53). BIBP 3236 (129) corresponds to a truncated and modified peptide in which the D-Arg is assumed to correspond with Arg^{33} in Neuropeptide Y.

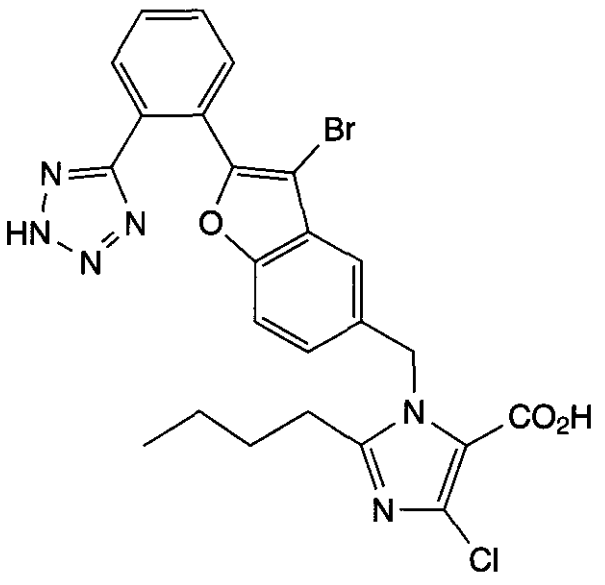
More recently, a series of indole Y1 antagonists discovered by screening (236) led to the benzimidazole (131)(K_i = 0.052 n*M*). In this type of compound, the diamino moieties are postulated to mimic the two C-terminal arginines of NPY (237).

Selective NPY-Y5 inhibitors have been shown to inhibit food intake activity *in vivo*. Most inhibitors found by HTS and lead optimization gave nanomolar and selective antagonists. It is not known whether these are functional or topological mimetics (Fig. 15.54) (238–240).

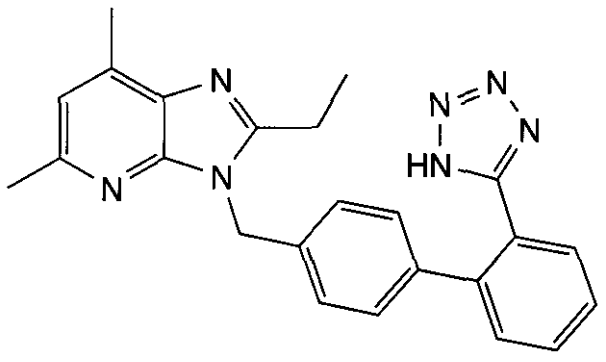
9.6.4 Growth Hormone Secretagogues. Growth hormone (GH) releasing peptide mimetics have become attractive alternatives to



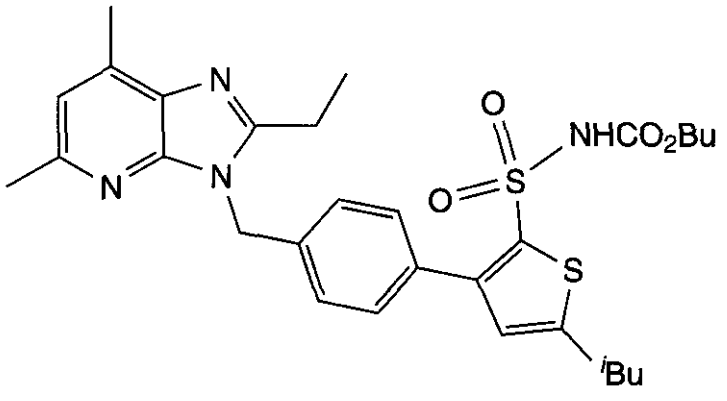
DuP753
(**119**)



GR 117289
(**120**)



L 158809
(**121**)



L 162313
(**122**)

Figure 15.49. Examples of angiotensin II inhibitors.

GH replacement therapy (241). The peptidyl GH secretagogue GHRP-6 (242) was used to develop the clinical candidate MK-0677 (**132**) (Fig. 15.55, EC_{50} = 1.3 nM) (243, 244). After identifying the important residues for bioactivity in GHPR-6, the Merck group began searching other receptor libraries for known "privileged structures" in a combinatorial synthetic fashion (see Section 4) (66). The more active derivative contained a spiropiperidine moiety attached to an indoline ring.

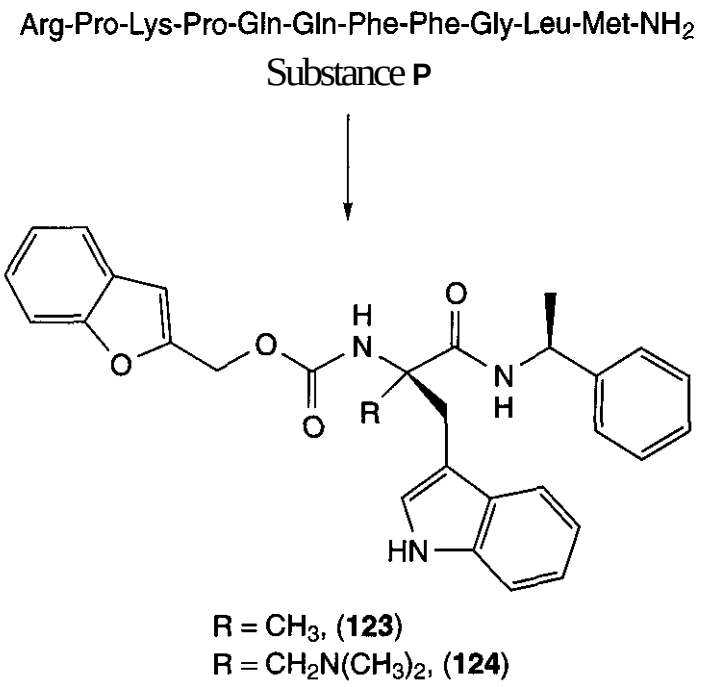
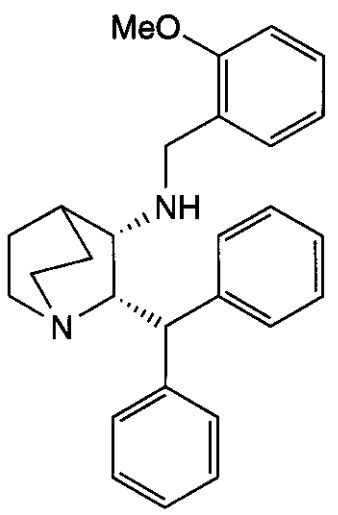


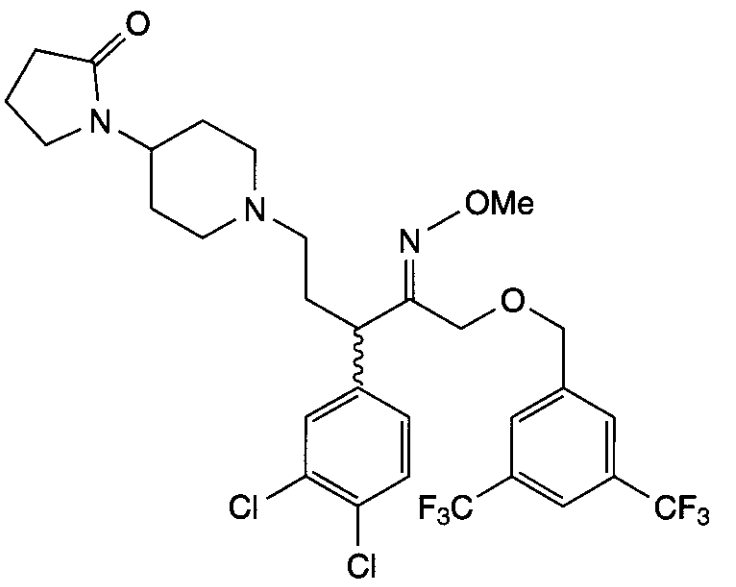
Figure 15.50. Development of a substance P inhibitor.

More recently, ghrelin has been isolated and identified as an endogenous ligand of the GHS receptor and some new peptidomimetic structures [e.g., 133 (Fig. 15.55)] have started to appear (245).

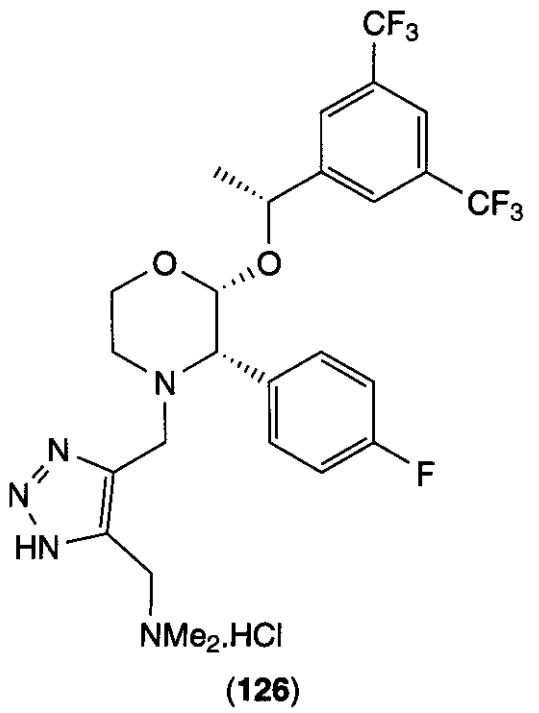
In another approach, SAR studies and systematic simplification of GHPR-6 at Novo Nordisk produced the orally bioavailable derivative NN-703 (134). Molecular modeling overlapping of NN703 (134) (Fig. 15.56) and MK-0677 (132) (Fig. 15.55) showed structural similarities between both compounds. Highly potent hybrids of Ipamorelin and NN-703 (e.g., 135) (Fig. 15.56) have also been described (246, 247).



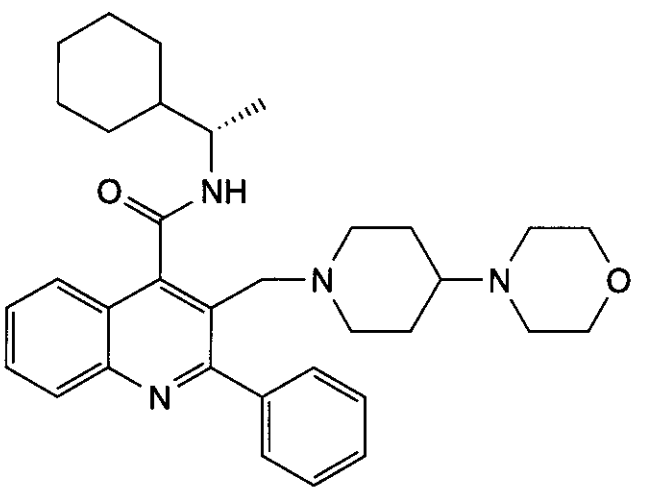
CP-96345
(**125**)



(**127**)



(**126**)



(**128**)

Figure 15.51. Examples of **NK-1** antagonists.

Figure 15.52. Dual NK-1/NK-2 and NK-1/NK-2 inhibitors.

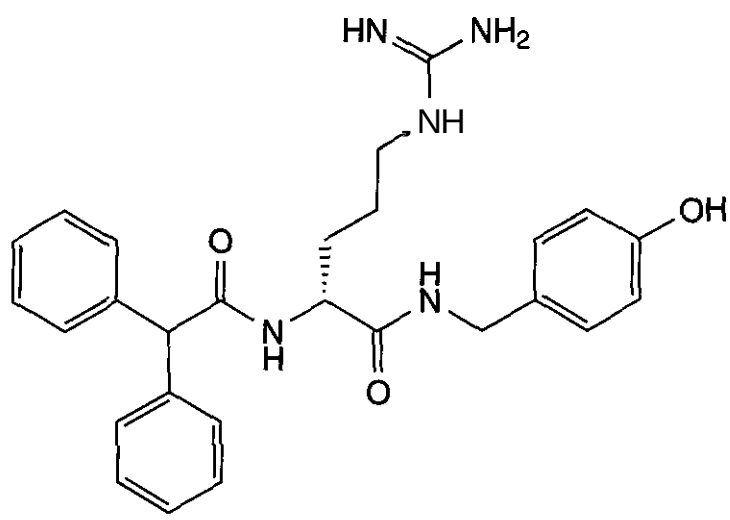
A common 3D pharmacophore was recently described for peptidic and non-peptidic GH secretagogues by means of computational chemistry. After QSAR analysis, four **pharma**cophoric sites were found: two aromatic rings, a proton acceptor, and a protonated amine. Using these strategies, some nanomolar antagonists **[e.g.,** 136 (Fig. **15.56)]** were discovered (248).

9.6.5 **Endothelin.** The first report of **endo**thelin in 1988 stimulated a huge effort to develop selective and non-selective endothelin receptor ($\mathbf{ET_A}$ and $\mathbf{ET_B}$) antagonists (249, **250**). One successful approach derived from the postulate that the phenyl groups of the screening lead might mimic two of the aromatic side-chains ($\mathbf{Tyr^{13}}$, $\mathbf{Phe^{14}}$, or $\mathbf{Trp^{21}}$) of ET-1 (251,252). Knowing that the carboxylic acid was also necessary for good activity, researchers at **SmithKline** overlaid their inhibitor with the aromatic groups $\mathbf{Tyr^{13}}$, $\mathbf{Phe^{14}}$, and $\mathbf{Asp^{18}}$ in ET-1. After using a conformationally constrained analog of ET-1 to further define their NMR-derived structure of **ET-1**, the final overlay suggested that a carboxylic acid attached with a linker of two to three atoms on the 2-position of the phenyl ring would provide further binding interaction by mimicking the C-terminal carboxylic acid. This led to compound (**137**) (Fig. 15.571, a potent antagonist of both the $\mathbf{ET_A}$ and $\mathbf{ET_B}$ receptors with K_i = 0.43 and 15.7 n*M*, respectively. **Analogs** based on a pyrrolidine scaffold are also effective **(e.g.,** 138) (Fig. 15.57) (253).

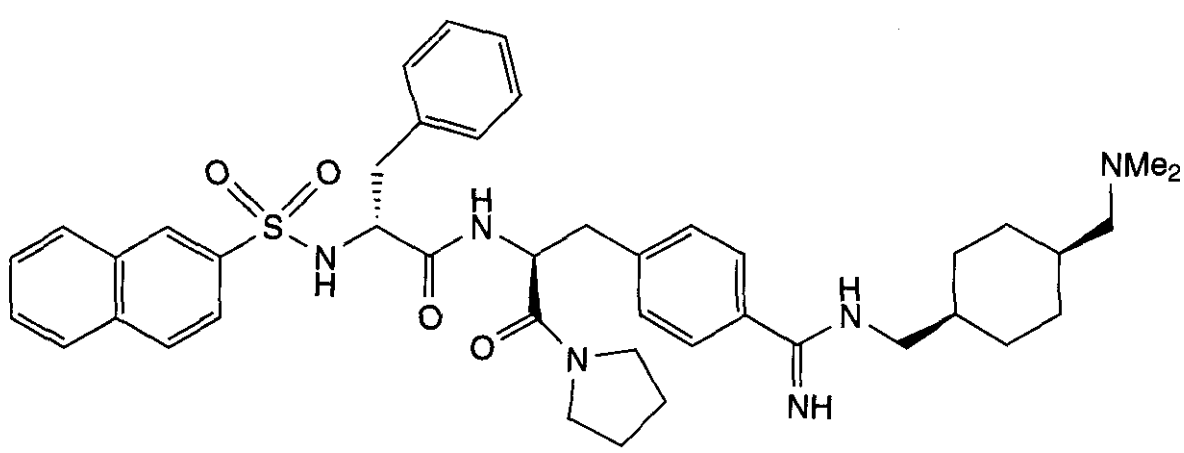
The Kohonen neural network has been used to develop bioisosteres of the **methylen**dioxyphenyl group found in a variety of **antag-**

Tyr-Pro-Ser-Lys-Pro-Asp-Asn-Pro-Gly-Glu-Asp-Ala-Pro-Ala-Glu-Asp-Leu-Ala
Arg-Tyr-Tyr-Ser-Ala-Leu-Arg-His-Tyr-Ile-Asn-Leu-Ile-Thr-Arg-Gln-Arg-Tyr-NH_2

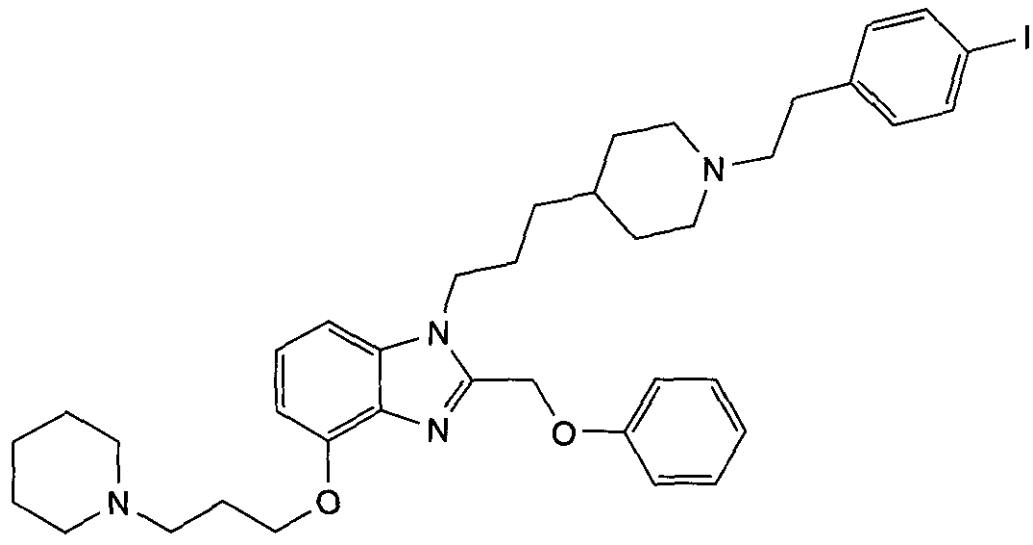
Neuropeptide Y



BIBP3226
(**129**)



SR 120107A
(**130**)



(**131**)

Figure 15.53. Examples of neuropeptide Y1 inhibitors.

onists [e.g., 139 (Fig. 15.58)]. The benzothiadiazole (140) functions as a bioisostere that retains and sometimes improves binding to the ET_A receptor (254–256).

Since the discovery of Ro46–2005 (**141**) (Fig. 15.58), the first orally active ET inhibitor, major efforts have been made to modify arylsulfonamide derivatives. An isoxazole as

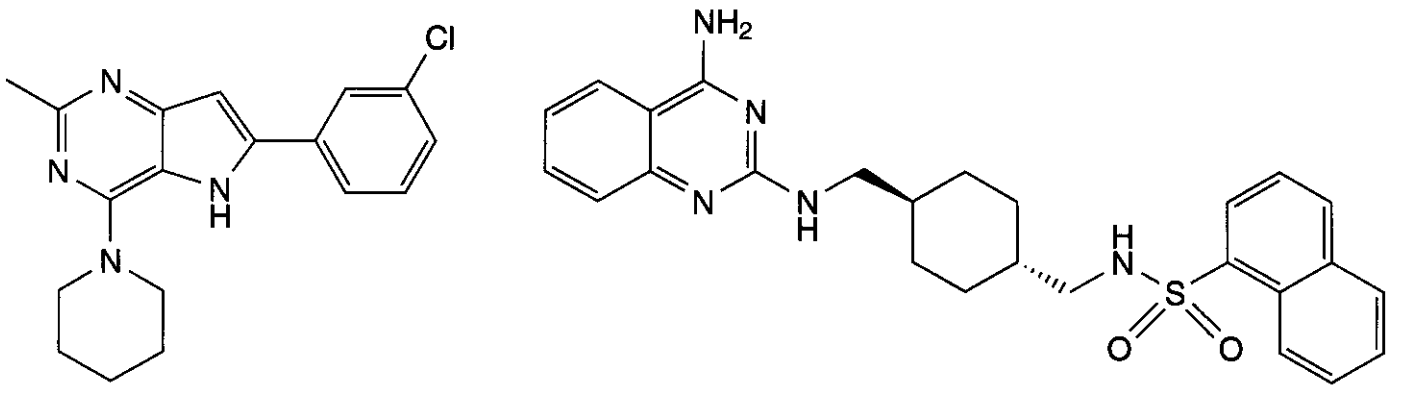


Figure 15.54. Examples of neuropeptide Y5 inhibitors.

the heterocycle attached to the amino functionality provided selectivity against ET_A receptor (257) and led to BMS 193884 (142)($K_i = 1.4$ nM) (258) and others, e.g., TBC 3214 (143)($K_i = 0.04$ nM) (259), which are potent, selective, and orally available ET_A antagonists.

Different binding modes have been proposed for ET antagonists. The acid or sulfonamido groups are needed to interact with a cationic site in the receptor, and an aromatic interaction with Tyr^{129} is postulated to be responsible for ET_A selectivity. However, because all these receptors are members of the GPCR, there is no assurance that any bind as modeled. Thus, they must be classified as type II peptidomimetics until structural data can resolve the issue.

10 SUMMARY AND FUTURE DIRECTIONS

The "Holy Grail" of peptidomimetic research in drug discovery has been to find ways to transform the structural information contained in peptides into non-peptide structures that have drug-like pharmacodynamic properties. Many different strategies have been

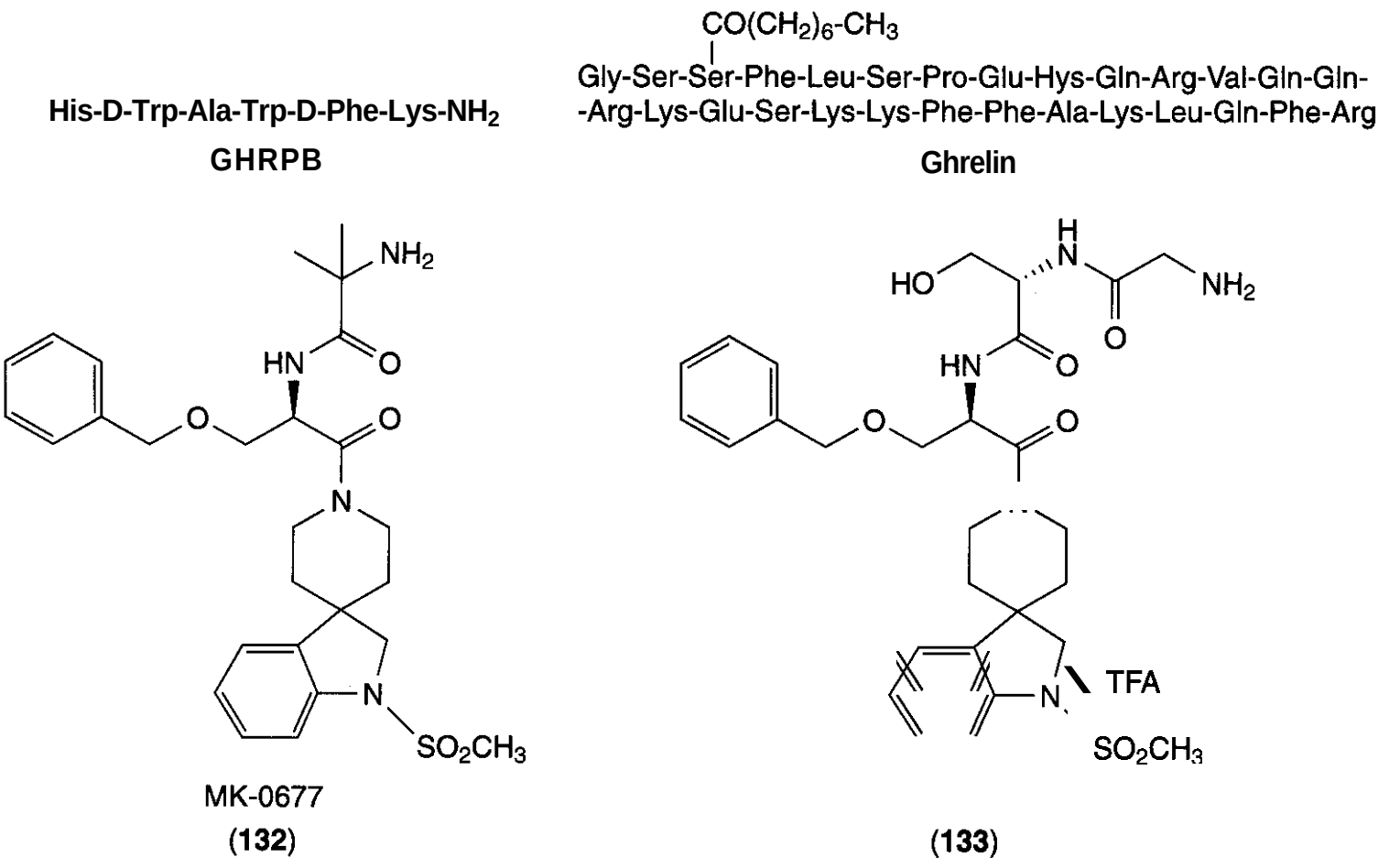
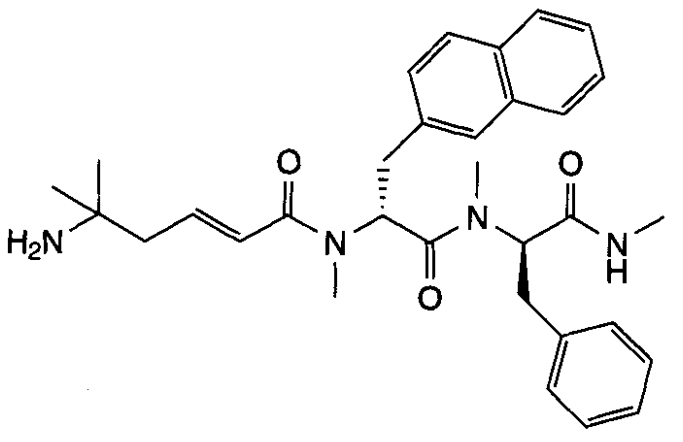
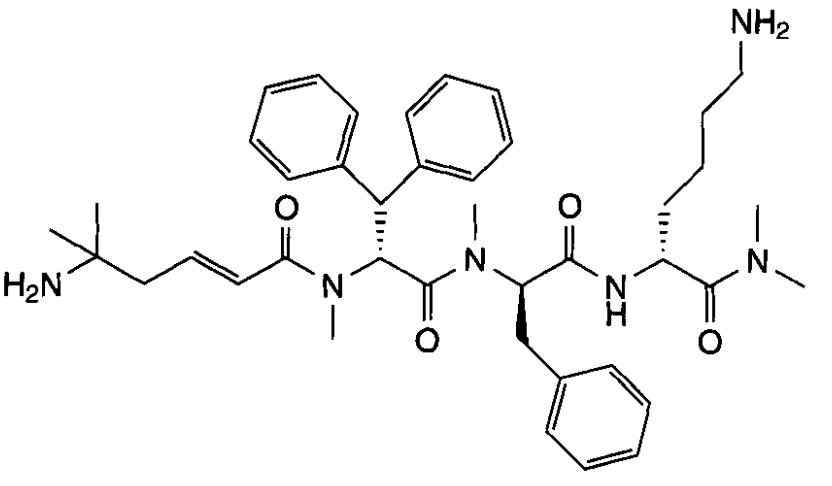


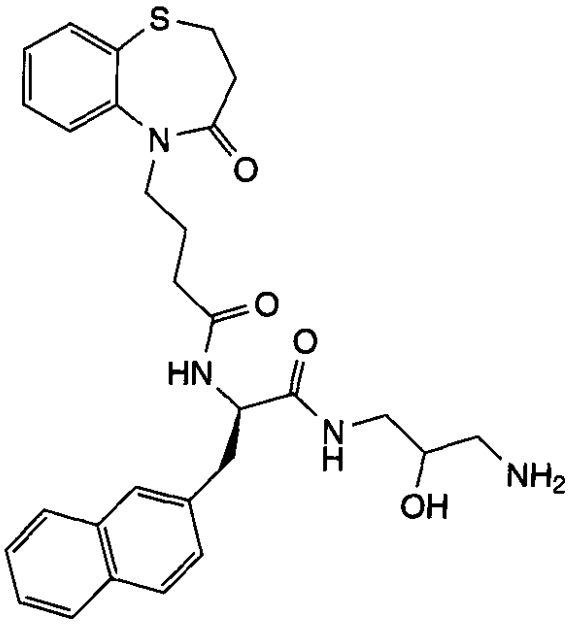
Figure 15.55. GHRP-6 and ghrelin non-peptide derivatives as growth hormone secretagogues inhibitors.



NN-703
(**134**)

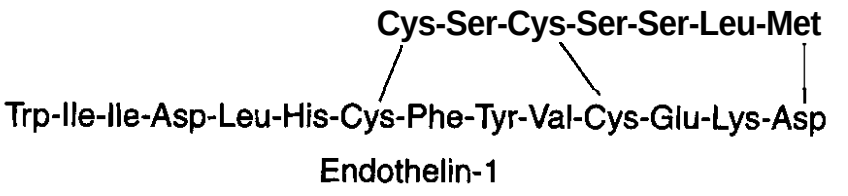


(**135**)

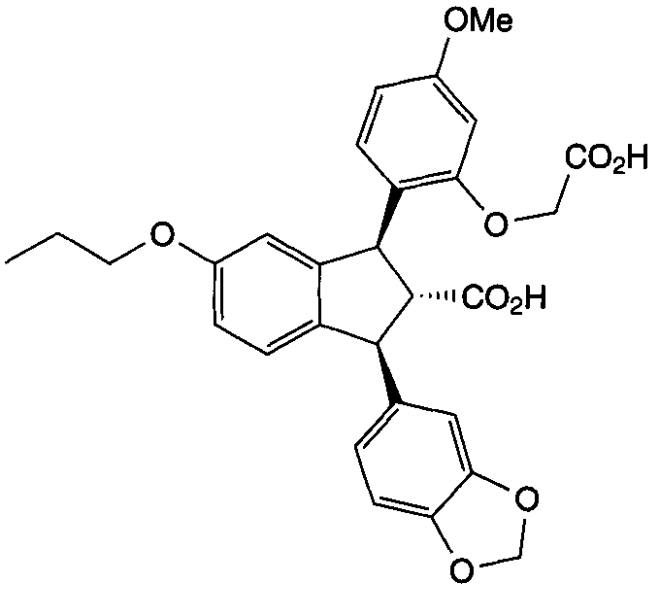


S-37435
(**136**)

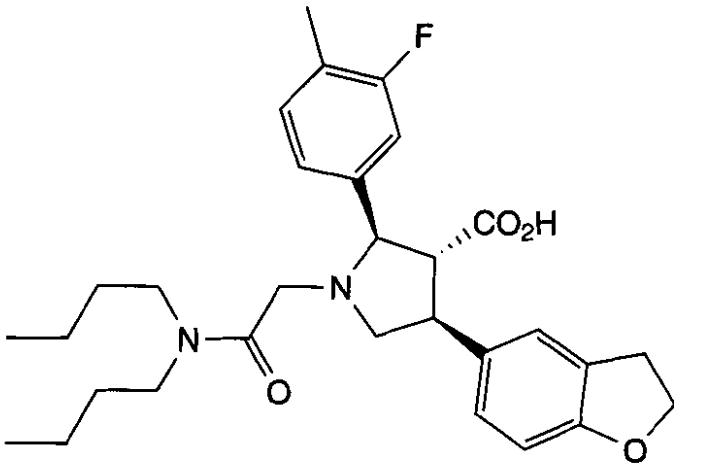
Figure 16.56. Newer approaches to growth hormone secretagogues inhibitors.



Endothelin-1

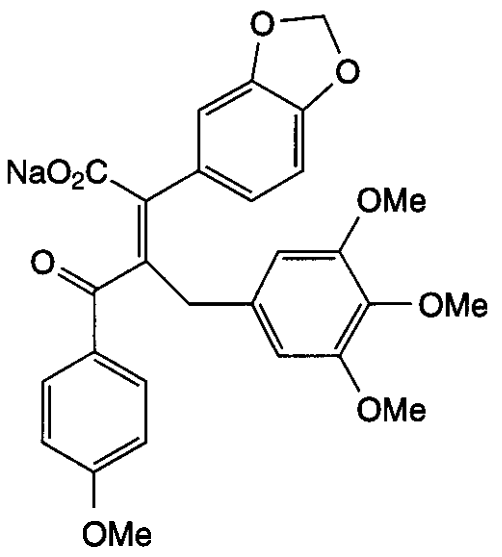


SB 209670
(**137**)

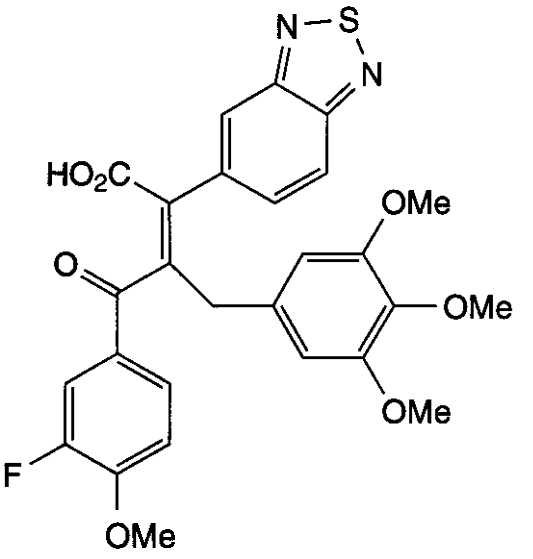


A-306552
(**138**)

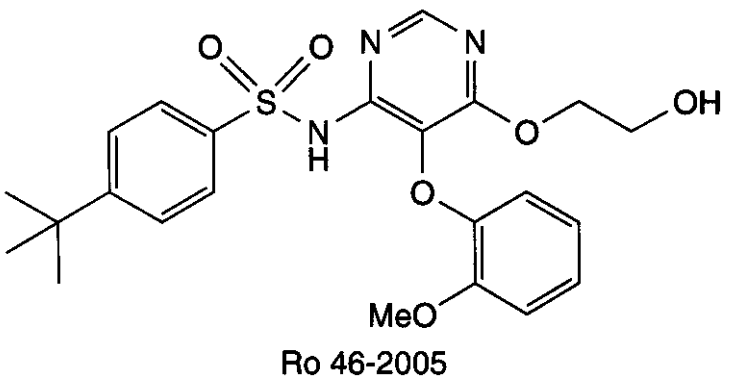
Figure 15.57. Non-peptide endothelin analogs.



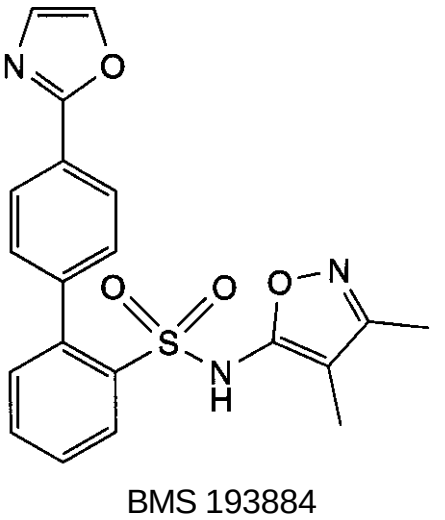
PD156707
(**139**)



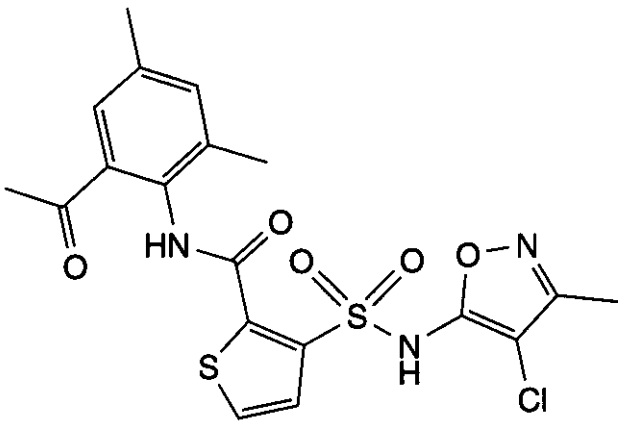
EMD 122946
(**140**)



Ro 46-2005
(**141**)



BMS 193884
(**142**)



TBC3214
(**143**)

Figure 15.58. Examples of ET_A inhibitors.

employed in the search for useful peptidomimetics—rational design of amide bond replacements, mimics of turn structures, and the like, as well as both designed and discovered scaffolds that replace the amide bond core of peptides. The field has a long way to go before rational design of type III peptidomimetics can be achieved routinely. However, the progress made to date suggests that this goal will be achieved. We know that some nonpeptide scaffolds are topographical mimetics of the extended β-strand of enzyme-bound protease inhibitors because we have the biophysical methods for characterizing both types of enzyme-inhibitor complexes. Type III peptidomimetic inhibitors of peptidases have

been designed from the substrate sequences and they have been revealed by HTS processes and optimized by application of structural biology. At this point, we have learned more about the design of inhibitors by studying how screening leads inhibit enzymes than from the design of inhibitors from our current, limited knowledge of enzyme catalysis. Probably the most important recent discovery is that some screening leads inhibit proteases by binding to a different enzyme active site conformation that is related mechanistically to the well-characterized extended β-strand of enzyme-bound protease inhibitors. This result emphasizes the importance of considering the entire ensemble of protein conformations when designing inhibitors of peptide-protein interactions.

Our understanding of peptide mimicry for ligands of constitutive receptors, such as G-protein–coupled receptors (GPCR), is much more primitive because high resolution structural data for agonist- and/or antagonist-receptor complexes are not yet available. For this reason, all attempts to rationalize the interactions between ligand and receptor contain a considerable element of speculation. It is too early to know whether small non-peptide structures that bind to GPCR are functional or topographical mimetics. However, based on the results obtained by studying peptidase inhibitors, it seem likely that at least some of the known functional peptidomimetics receptors ligands will be shown to be topographical mimetics. Others may be found to act more like GRAB-peptidomimetics in that they bind to receptor conformations closely related in energy and mechanism to native conformations. Still others will no doubt be found that inhibit or stimulate the receptor system by allosteric mechanisms or by interfering with some multi-step binding process preceding the formation of the active ligand-receptor complex. In any case, it is clear that successful design of functional mimetics by assuming some structural relationship between a screening lead and the parent peptide can work (see Section 9.6), as can the systematic modification of the parent peptide. The application of the principles of peptidomimetic research has become very important to drug discovery. Although our present knowledge about protein–protein interactions is still quite limited, the rapid growth of structural information and methods will eventually allow us to design rationally peptidomimetic compounds suitable for use in human therapy.

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