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Peptidomimetic Therapeutic Agents Targeting the Protease Enzyme of the Human Immunodeficiency Virus and Hepatitis C Virus

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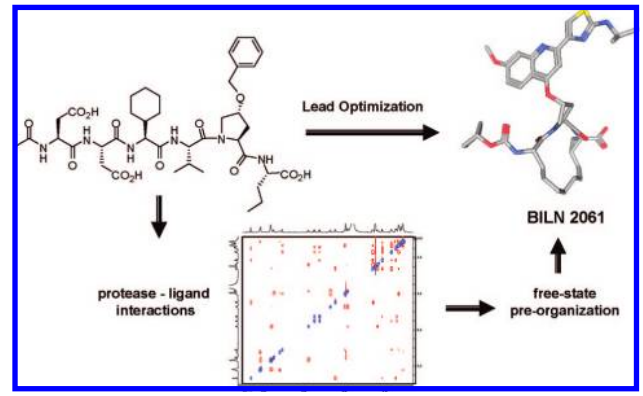
CONSPECTUS

During the past two decades, great strides have been made in the design of peptidomimetic drugs for the treatment of viral infections, despite the stigma of poor drug-like properties, low oral absorption, and high clearance associated with such compounds. This Account summarizes the progress made toward overcoming such liabilities and highlights the drug discovery efforts that have focused specifically on human immunodeficiency virus (HIV) and hepatitis C virus (HCV) protease inhibitors.



The arsenal against the incurable disease AIDS, which is caused by HIV infection, includes peptidomimetic compounds that target the virally encoded aspartic protease enzyme. This enzyme is essential to the production of mature HIV particles and plays a key role in maintaining infectivity. However, because of the rapid genomic evolution of viruses, an inevitable consequence in the treatment of all viral infections is the emergence of resistance to the drugs. Therefore, the incomplete suppression of HIV in treatment-experienced AIDS patients will continue to drive the search for more effective therapeutic agents that exhibit efficacy against the mutants raised by the earlier generation of protease inhibitors.

Currently, a number of substrate-based peptidomimetic agents that target the virally encoded HCV NS3/4A protease are in clinical development. Mechanistically, these inhibitors can be generally divided into activated carbonyls that are transition-state mimics or compounds that tap into the feedback mode of enzyme−product inhibition. In the HCV field, there is justified optimism that a number of these compounds will soon reach commercialization as therapeutic agents for the treatment of HCV infections.

Structural research has guided the successful design of both HIV and HCV protease inhibitors. X-ray crystallography, NMR, and computational studies have provided valuable insight in to the free-state preorganization of peptidomimetic ligands and their enzyme-bound conformation. Researchers have designed a variety of novel bioisosteric replacements of amino acids and short peptides that contain all of the required pharmacophore moieties and play a key role in inducing conformational changes to the overall molecule. The knowledge gained from these studies will undoubtedly guide the future design of therapeutic agents and further contribute to the success of this field.

Introduction

Current statistics from the WHO indicate that approximately 200 million people around the world are infected with the hepatitis C virus (HCV) and an additional 40 million are living with an incurable infection caused by the human immunodeficiency virus (HIV). In recent years, great progress was made in managing HIV/AIDS, transforming what once was a "death sentence" to a manageable chronic disease.[1] However, drug discovery efforts have yet to produce a small-molecule therapeutic agent for the treatment of HCV

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infections.[2] Currently, treatment of HCV infection is limited to interferon-based therapies that are effective in only ~50% of the patients and are often associated with severe side effects.

HIV or HCV infections can remain latent for many years before leading to disease symptoms. In the case of HIV infections, during that initial disease-free period, the immune system is progressively weakened to the point where the emergence of multiple infections becomes common, leading to tumors and, ultimately, death. In the case of HCV, the majority of infected individuals (>80%) can remain asymptomatic for as long as 20 years and some (~20%) are even able to clear the virus. However, those that become chronically infected usually progress to end-stage liver diseases, and some (~2−4%) eventually develop hepatocellular carcinomas.

HCV/HIV coinfections are also highly prevalent. In the United States and Europe, the prevalence of coinfection is estimated to average 35%. Coinfection accelerates the natural course of liver cirrhosis, hepatocellular carcinoma, or AIDS-related deaths.[3] In addition, coinfection may alter the effectiveness of the antiretroviral agents used to treat AIDS, possibly due to the liver toxicity associated with many of the current drugs. Therefore, the discovery of new, effective, and safe therapeutics for the treatment of HIV, HCV infections, and coinfections is a complex medical problem that needs urgent attention.

To date, a number of virally encoded enzymes are the focus of drug discovery efforts. Among these targets are the proteolytic enzymes HIV aspartic protease (HIV-PR) and HCV NS3/4A serine protease. Historically, the design of protease inhibitors has focused on the optimization of substrate-based peptides or transition-state analogs of the cleavage reaction. Experts in drug discovery would often advocate against these approaches, on the basis of the low oral bioavailability and rapid excretion of large peptidic molecules. Nonetheless, there are nine peptidomimetic drugs on the market for the treatment of AIDS and at least four in clinical development for treatment of HCV infections. The main objective of this Account is to provide a summary of the progress made toward overcoming the liabilities associated with peptidomimetic compounds and highlight the drug discovery efforts that have focused specifically on HIV and HCV protease inhibitors.

The Human Immunodeficiency Virus

The HIV viruses infect T cells via a process that initially involves fusion of the viral envelope glycoproteins (gp120, gp41) with the CD4 receptors and coreceptors (CCR5 and CXCR4) to allow entry of the virus into the host cell. The genome of the virus consists of ten open reading frames encoding structural (*gag, pol, env*), regulatory (*tat, rev, nef, vpr*), and accessory (*vif, vpu*) proteins.[4] Products of the *pol* gene encode all catalytic proteins essential for the viral life cycle. The reverse transcriptase (RT) is required for transcription of the viral ssRNA to dsDNA, which is then transported into the cell nucleus and incorporated into the host's chromosomal DNA under the catalytic control of the viral integrase. The host is then permanently infected with the *provirus* and the cellular machinery is used for viral replication. Viral mRNAs are spliced and transported into the cytoplasm where viral protein expression occurs. In the final stages of replication, the *gag* and *gag−pol* precursor polyproteins, along with two viral RNA strands, assemble with the envelope protein to form an immature virion. At this stage the HIV-1 aspartic protease (HIV-PR) is autocatalytically released from the *pol* precursor protein and proceeds to cleave the remaining precursor protein to produce a new, infectious viral particle that is subsequently released from the cell. Therefore, the function of HIV-PR is essential for proper virion assembly and maturation.[4]

Structure-Based Inhibitors of HIV-1 Protease (HIV-PR)

Mechanistically, protease enzymes can be broadly classified as (a) those using an activated water molecule as the nucleophile that attacks the scissile amide bond (e.g., zinc metalloproteases and aspartate proteases) or (b) those using the nucleophilic side chain of an amino acid residue in order to initiate amide bond hydrolysis (e.g., serine or cysteine proteases). The HIV-PR belongs to the former class of enzymes and specifically to the aspartic proteases.[5]

HIV-PR is a C_2 symmetrical homodimer (Figure 1) with 99 residues per monomer.[6] X-ray crystallography[6,7] and NMR[8] of unliganded and inhibitor-bound enzyme complexes have provided insight into the interactions associated with catalysis and inhibition. The biologically relevant interdomain interactions, such as the role of two flexible β hairpin loops (the flaps) that participate in the binding of both substrates and inhibitors, have also been investigated. Structural data suggests that the flap regions of the unliganded enzyme are in dynamic motion and open significantly to allow segments of the polyprotein substrates to access the active site residues, Asp 25/25′. However, following binding of the substrate, the flaps become highly ordered and close, placing the Ile-50/50′ residues over the cleavage site (Figure 1). Stabilization of the complex is then mediated by hydrogen bonding interactions between Ile-50/50′, a key water molecule (the flap water), and the two carbonyl oxygen atoms of the substrate near the scissile bond.

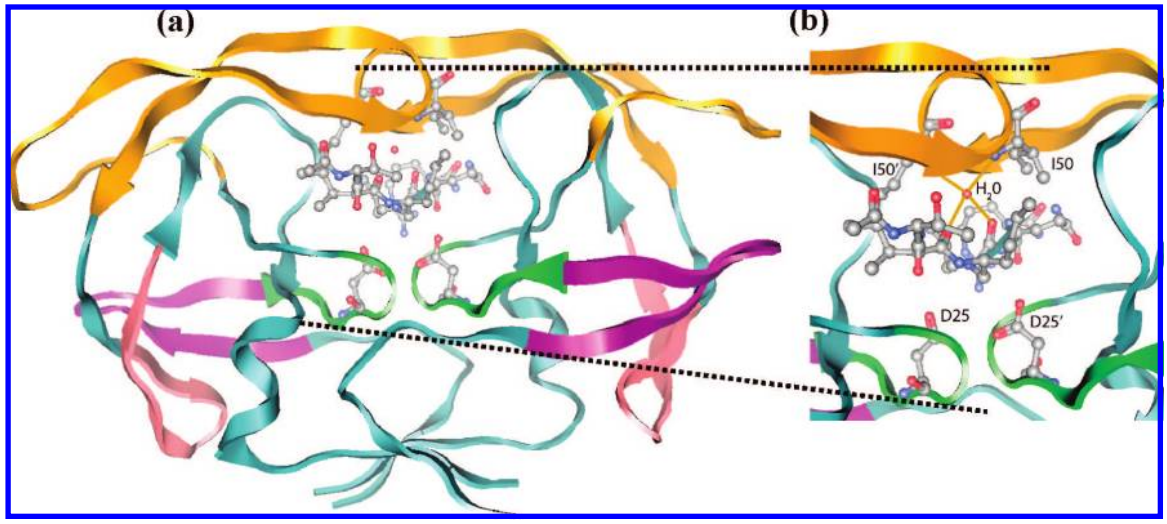


FIGURE 1. Structure of HIV-1 PR in complex with the hexapeptide *N*-acetyl-Thr-Ile-Nle-Ψ[CH_2NH]-Nle-Gln-Arg-$CONH_2$:[12] (a) The two subunits of the homodimer are similarly color coded to highlight the key domains (flaps residues 34−59 and 34′−59′ in orange). The catalytic residues Asp 25/25′ and the flap tips Ile 50/50′ are highlighted in ball-and-stick. (b) The flap water molecule and its interactions with both the ligand and the closed flaps are indicated.

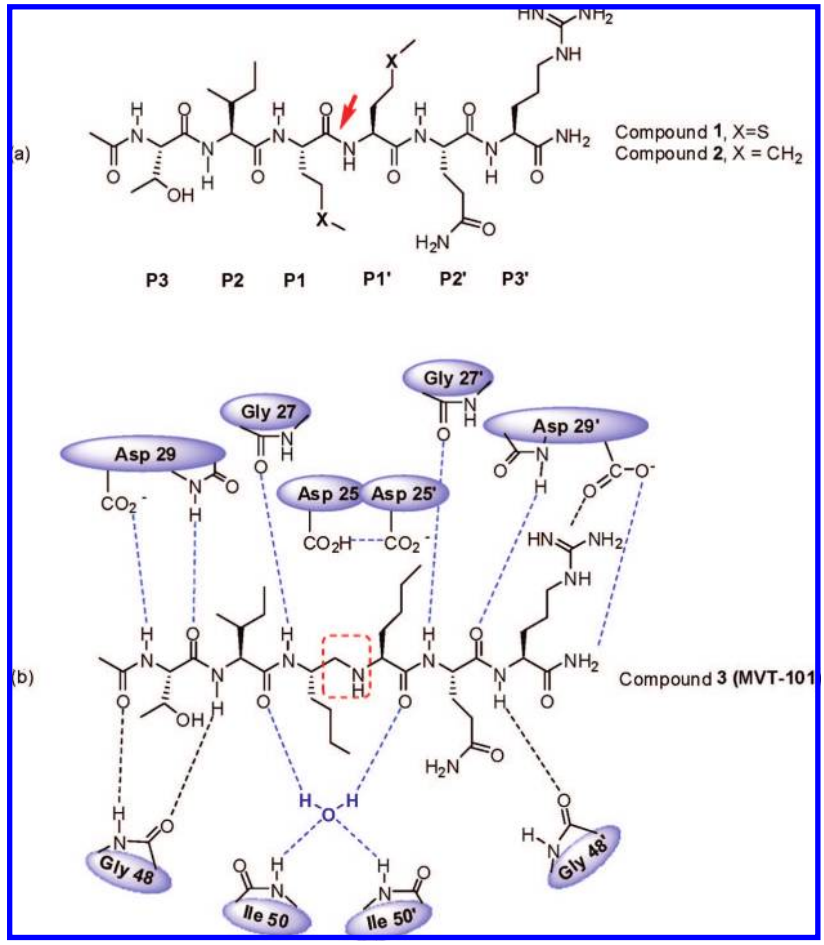


FIGURE 2. Substrate-based hexapeptide inhibitor MVT-101: (a) precursor hexapeptide ligands **1** and **2**, scissile bond indicated by red arrow; labeling of P3, P2, P1, P1′, P2′, and P3′ is based on the protease subsite nomenclature; (b) schematic representation of the hydrogen bond interactions between inhibitor **3** and the enzyme; the reduced amide bond is highlighted by the red box

Molecular dynamics simulations suggest that the catalytic power of this enzyme does not arise from the presence of a rigid/preorganized active site but from its ability to modulate both its shape and isoelectric surface near the active site.[8−10]

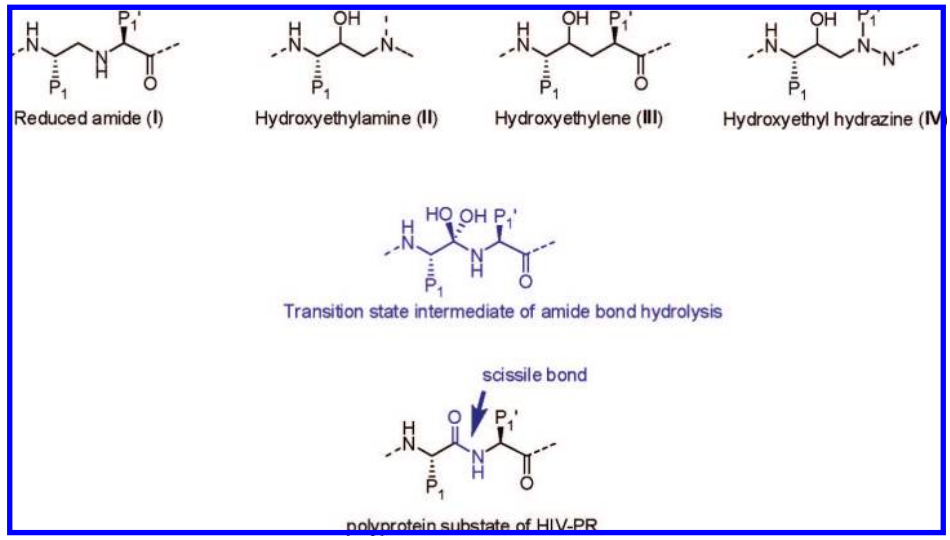
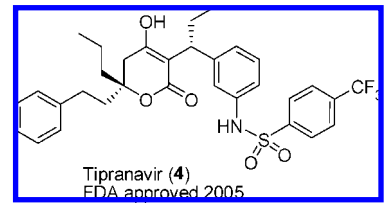


FIGURE 3. Transition-state mimics commonly used in the design of HIV protease inhibitors.

Development of the first drug targeting the HIV-PR was preceded by investigations into the design of peptidimimetic ligands for a variety of other aspartic proteases. Natural products such as pepstatin, a typical transition-state analog,[11] also contributed to the accumulated knowledge of peptidomimetic inhibitors. More importantly, in the early 1990s, an explosion of X-ray data became available that aimed to reveal the enzyme structure and its interactions with ligands.[5] The first X-ray structure of the unliganded enzyme was reported by Merck[6] and was followed by numerous other structures of both the unliganded enzyme and the enzyme in a complex with ligands. One such example is the complex of HIV-PR with compound **3** (Figure 2, MVT-101).[12] Compound **3** represents an early example of a peptidomimetic inhibitor that was based on the sequence homology of the substrate cleave site at the *gag*−*pol* viral polyprotein. Modifications of the methionine residues of ligand **1** to an isosteric norleucine resulted in the hexapeptide **2**, having comparable affinity for HIV-PR. These modifications were followed by the introduction of a reduced amide bond, leading to the discovery of inhibitor **3** (K_i of 780 nM).[12] An extensive network of interactions between **3** and the enzyme were observed in the X-ray structure (Figure 2b).[12] The backbone of **3** adopts a β-strand conformation and forms three direct hydrogen bonds with each of the two main chains of the protease, involving the amide NHs of Asp-29/29′ and the carbonyl oxygens of Gly-27/27′ and Gly-48/48′ (Figure 2b). In addition, two hydrogen bonds between the Ile-50/50′ and the two carbonyl oxygens of P1 and P1′ are mediated by the flap water. A number of other direct hydrogen bond interactions were also proposed based on the crystallographic data and are indicated in Figure 2b. Extensive van der Waals interactions between the enzyme and the P1/1′ and P2/2′ hydrophobic side chains of this inhibitor were also identified. Optimization of the ligand/inhibitor−enzyme interactions that were observed using such prototypical peptidomimerics as MVT-101 (**3**) became the focus of subsequent lead optimization efforts.

To date, there are ten commercially available drugs that target the HIV-PR; nine of these collectively represent a success story in rational molecular design of peptidomimetics (**5**−**14**). Although there is some debate as to the definition of a "peptidomimetic" inhibitor, the prevailing view is that all inhibitors tailored after substrate-based peptide of an enzyme should be referred to as peptidomimetics, even if they do not formally contain an amide bond. Based on that definition, the only nonpeptidomimetic inhibitor of HIV-PR is tipranavir (**4**), which bears no structural resemblance to a substate-based

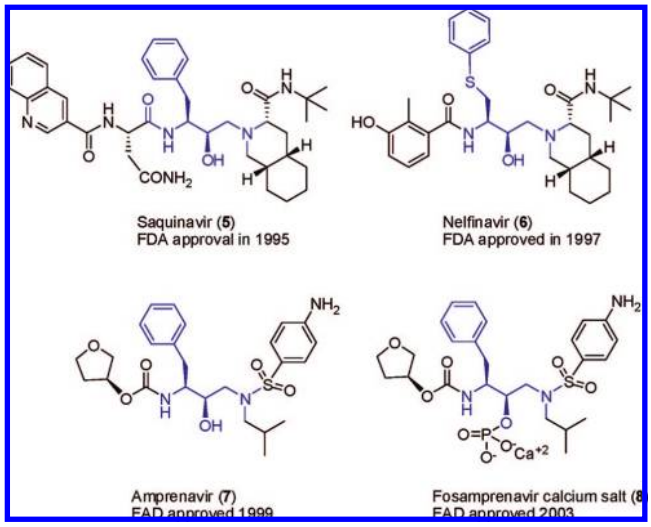


peptide and was discovered through lead optimization of 4-hydroxycoumarin and 4-hydroxy-2-pyrone leads (identified from screening).[13]

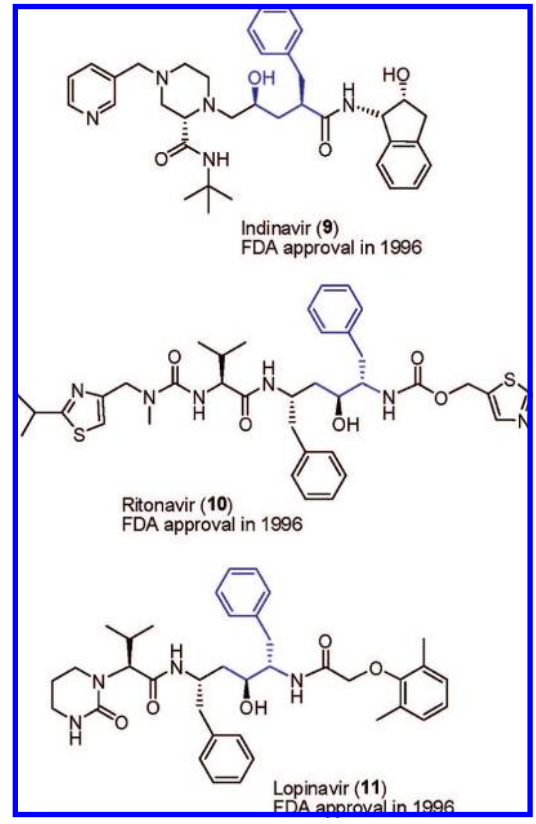
Saquinavir (**5**, K_i = 0.12 nM) was the first peptidomimetic to be approved by the FDA for the treatment of AIDS. The design strategy of the Roche team was to focus on the HIV *pol* substrate fragment Leu^{165}−Ile^{169}, which contains the scissile bond Phe^{167}−Pro^{168}.[14] The HIV-PR is unusual in its capacity to cleave Phe−Pro and Tyr−Pro bonds, whereas these bonds are not susceptible to cleavage by mammalian endopepti-

dases. Optimization of this peptide was achieved by implementing the reduced amide (**I**) and hydroxyethylamine (**II**) transition-state mimetics (Figure 3); however, this initial commercial product (Invirase) exhibits very poor oral bioavailability.

Saquinavir was followed by two other hydroxyethylamine isosteres, nelfinavir (**6**, $K_i = 2$ nM)[15] by Agouron (Pfizer)/Lilly and amprenavir (**7**, $K_i = 0.6$ nM) by Vertex Pharmaceuticals.[16] Nelfinavir (**6**) is the outcome of efforts that were focused on truncating the N-terminal moiety of a saquinavir (**5**) and replacing it with the less peptidic P2 moiety 3-hydroxy-2-methylbenzoyl fragment, thus achieving better oral bioavailability than that of **5**. Similarly, reduction of the peptidic nature and molecular weight guided the design of amprenavir (**7**), which exhibits acceptable binding affinity ($K_i = 0.6$ nM), good oral bioavailability and a long half-life in humans (~10 h) that permits therapeutic dosing of patients twice daily. In addition, a prodrug of **7** (fosamprenavir **8**) was developed that gets rapidly hydrolyzed at the intestinal brush border to deliver amprenavir (**7**) and can be formulated into higher-strength tablets, thus further reducing the pill number required to deliver a therapeutic dose. The central hydroxyl group (i.e., the reduced P1 carbonyl moiety) of these inhibitors is crucial for their tight binding with HIV-PR. This moiety mimics the activated water molecule that is normally hydrogen bonded to the carbonyl oxygens of the catalytic residues (Asp-25/25′) and acts as the nucleophile during the catalytic cycle.[5]



Indinavir (**9**, $K_i = \sim 0.6$ nM) brought new structural elements to the design of HIV-PR inhibitors.[17] Replacement of both the P2′−P3′ and P1−P2 fragments of a lead peptide with an aminohydroxyindan and a pyridylmethylpiperazine moiety, respectively, lead to compound **9**, which exhibits good solubility and bioavailability.[24] Two other hydroxyethylamine analogs bearing the PheΨ[CHOHCH$_2$]Phe transition state insert, ritonavir (**10**) and lopinavir (**11**) were developed by Abbott, where the *C*2 symmetry of the HIV-PR enzyme was exploited.[18] Ironically, most of the therapeutic value of ritonavir (**10**) is currently derived from its side effect as a potent inhibitor of cytochrome P450 3A4, and it is used extensively as a codrug for boosting plasma exposure and half-life of other coadministered pharmaceuticals.[1]



As is the case with all antivirals, ritonavir (**10**) therapy leads to mutations conferring viral resistance to treatment. The predominant mutations raised by compound **10** are on residue Val-82, which interacts primarily with the P3 moiety. A strategy that was employed by Abbott in designing their second generation drug was to select compounds with a smaller P3 than the isopropylthiazolyl moiety of **10**, hence diminishing the role of the inhibitor interactions with Val-82.[19] This lead to the design of lopinavir (**11**), which is 10-fold more potent than **10** and preserves its activity against the Val-82 mutants. Superposition of the X-ray structures of **10** and **11** in the active site of HIV-PR is consistent with significantly reduced hydrophobic interactions between inhibitor **11** and Val-82 compared with those of inhibitor **10** (Figure 4).[19]

The more recently approved drug atazanavir (**13**) has its roots in hydroxyethyl hydrazine aza-peptides, such as **12** initially explored at Ciba-Geigy.[20] The aza modification elimi-

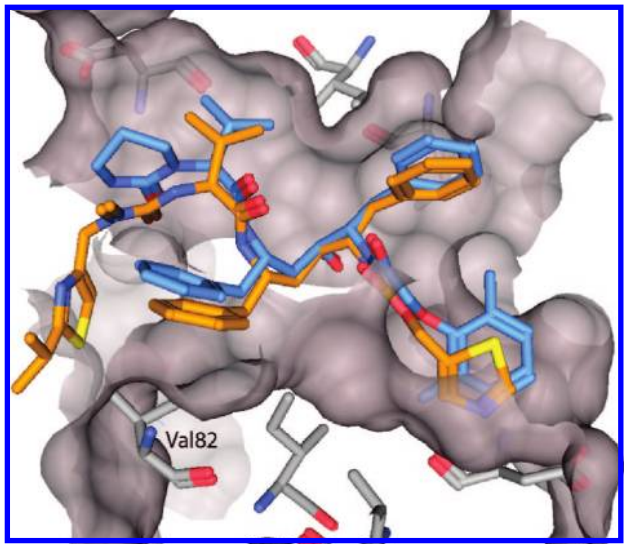
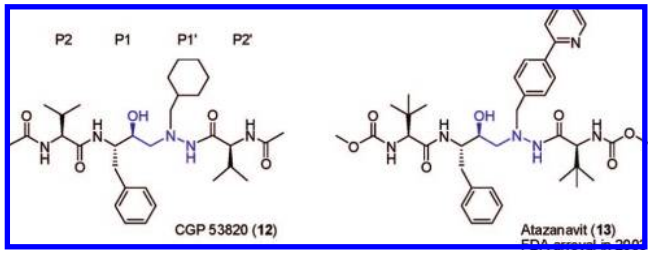
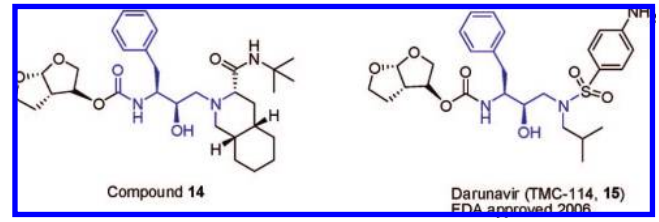


FIGURE 4. Superposition of X-ray structures of analogs **10** (orange) and **11** (blue) bound to the active site of HIV-PR.[19]



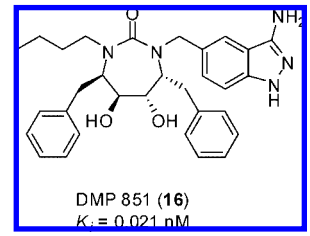
nates one of the chiral centers, decreasing the challenges associated with large-scale synthesis of these compounds. Atazanavir (**13**) compares favorably to the earlier marketed protease inhibitors in that it provides good serum adjusted antiviral potency, provides high oral exposure in man, and can support once per day dosing for effective treatment.

The latest entry to the arsenal against AIDS is the compound darunavir (TMC114, **15**). Development of this molecule has involved both industrial and academic research.[21] A unique structural feature of compound **15** is the bis-tetrahydrofuranyl moiety (a surrogate for an asparagine side chains) that was initially used by Merck as a high-affinity P2 moiety (later also introduced by Vertex in the development of amprenavir). Further substitution of the P2−P3 asparagine−quinaldic amide fragment of **5** with a bis-tetrahydrofuranyl moiety produced a lower molecular weight inhibitor (i.e., analog **14**) that was equipotent to **5**. Comparison of the crystallographic data of analogs **5** and **14** bound to the HIV-1 protease provided strong evidence that the fused bicyclic tetrahydrofuran could effectively offset the loss of binding affinity provided by the large lipophilic P3-quinoline of **5** by forming hydrogen bond interactions with the backbone NH's of Asp-29 and Asp-30.[21] Furthermore, the polar bicyclic tetrahydrofuran moiety provided an improvement in solubility.



From this class of analogs, darunavir (**15**, K_i = 16 pM) was prioritized for clinical development and found to outperform most other protease inhibitors in blocking the replication of HIV-1 mutants isolated from heavily drug experienced AIDS patients.

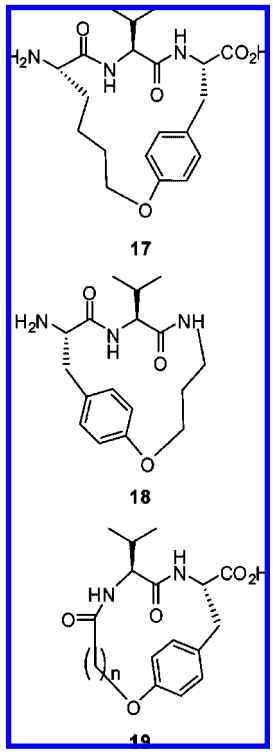
Numerous other investigations in the HIV protease field have contributed to the overall drug discovery process. Examples include the seven-membered cyclic ureas (e.g., compound **16**) of DuPont Merck that were identified through structure-



based *de novo* design.[22] A key feature of these analogs is the displacement of the flap water by the carbonyl oxygen of the urea and the diol fragment that interacts with the catalytic Asp-25/25′ residues.[22] Although this design is intellectually appealing, development of such compounds proved to be very challenging, largely due to their unfavorable solubility, high protein binding, and overall poor pharmacokinetic parameters.

A crucial structural element recognized by many proteases is the β-strand arrangement of their substrates. Most of the inhibitors already mentioned are essentially β-strand/β-sheet mimics of the natural substrates of HIV-PR.[23] However, there are enormous challenges that must be overcome in designing small peptide mimics that are locked in their free-state into a β-strand conformation. One approach that has been explored as a means of achieving this goal is macrocyclization of the peptidomimetic backbone. The design of HIV-PR macrocyclic inhibitors was primarily based on tyrosine derivatives having the *para*-phenoxy group covalently attached via a linker to NH of the backbone or to the side chain of another amino acid residue.[23] Novel tripeptide fragments, such as **17**−**19**, were designed having a rigid β-strand conformation and introduced into peptidomimetic inhibitors of HIV-PR.[32]

In conclusion, HIV-PR inhibitors constitute an indispensable component of the highly active antiretroviral therapy



(HAART) used for the treatment of AIDS. The majority of these compounds represent the successful outcome of structure-based drug design starting from a substate-based peptide. However, knowledge gained from these efforts can be also applied to the optimization of nonpeptidic leads, as was the case for tipranavir (**4**). Although the binding interactions of **4** with the HIV-PR are similar to those of peptidomimetic analogs, compound **4** is the only drug that does not require the flap water in order to interact with the HIV-RP.[24] The distinct nature of the interactions formed between tipranavir (**4**) and the enzyme gives rise to a unique and superior resistance profile compared with other inhibitors of HIV-PR.[25] A number of other strategies toward the design of new effective inhibitors of HIV-PR are underway that were recently reviewed by Turner.[26]

The Hepatitis C Virus (HCV)

Although the genome of the hepatitis C virus was first reported in 1989, unraveling the biology of this pathogen has proven to be an extremely challenging endeavor.[27] Currently, there are six major genotypes of the virus known (>50 subtypes), of which genotype 1a/b accounts for approximately 70% of all infections in the industrialized nations. The viral genome is a positive single-stranded RNA of ~9600 nucleotides that encode a precursor polypeptide of approximately 3000 amino acids. This polyprotein is processed both co- and post-translationally to produce structural (C, E1, E2, p7) and nonstructural (NS2, NS3, NS4A, NS4B, NS5A, NS5B) proteins.[27] Key nonstructural proteins include the protease enzymes (NS2 and the N-terminal domain of the NS3), the helicase/ATPase (C-terminal domain of the NS3) and an RNA-dependent RNA polymerase enzyme (NS5B). Initially, each of these enzymes was validated as a target for drug discovery by abrogating its catalytic function and demonstrating that the resulting HCV clone had lost its infectivity.

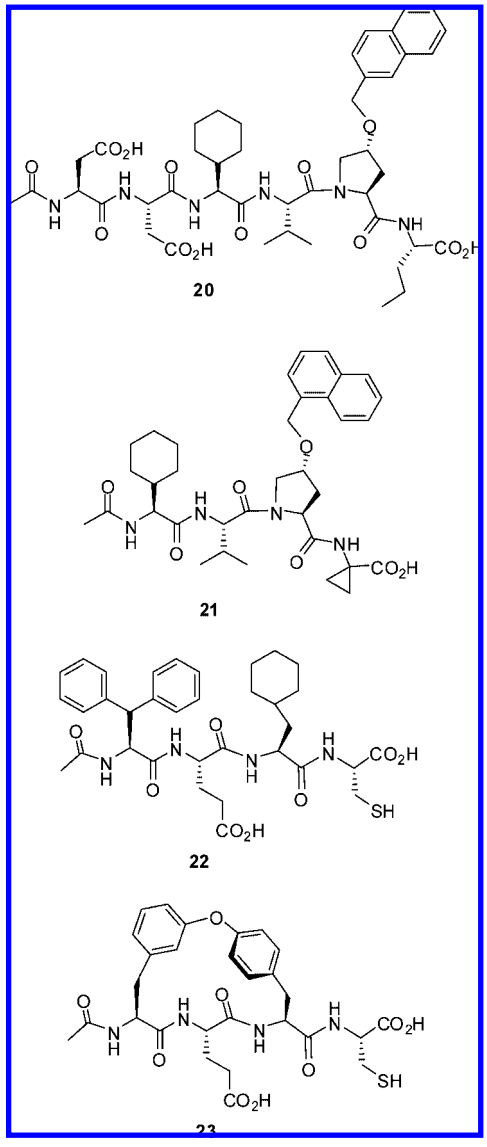
The early identification of therapeutics for the treatment of HCV was hampered by the inability to effectively replicate the virus in culture and the absence of a small animal model for evaluating the clinical relevance of potential new drugs. A major advance in the field was achieved by Bartenschlager and co-workers when they developed a cell-based HCV subgenomic replicon assay that allows evaluation of potential drug candidates against replicating HCV RNA in cell cultures *ex vivo*.[28] It was only in 2005 that the production of infectious hepatitis C viral particles in tissue culture was finally achieved.[27] Nonetheless, a number of inhibitors of the NS3 serine protease and the NS5B polymerase had entered clinical development prior to this achievement.[2]

Structure-Based Inhibitors of HCV NS3/4A Serine Protease

The HCV NS3 serine protease is a heterodimeric enzyme requiring a noncovalent association with the NS4A cofactor peptide for optimal catalytic function.[2,27] It is responsible for the proteolytic processing of four out of five junctions between the nonstructural protein regions along the HCV polyprotein and plays an essential role in viral replication. This enzyme is also involved in silencing the host's antiviral immune response, which normally signals interferon production as a natural defense against infection.[29] Consequently, the HCV NS3/4A protease has been a primary target for drug discovery for over 15 years.

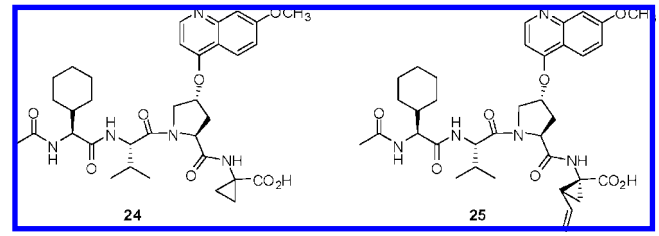
The successful paradigm established by the design of substrate-based HIV-PR inhibitors fueled the quest for a peptidomimetic inhibitor that targeted the HCV NS3/4A. Unfortunately, the latter enzyme proved to be a far more challenging target than the HIV-PR, mainly due to its shallow and solvent-exposed active site. In the early 1990s, Boehringer Ingelheim and the Istitute di Ricerche di Biologia Molecolare independently discovered that the N-terminal peptide products derived from substrates of the HCV NS3 protease act as competitive inhibitors of the enzyme (feedback inhibition).[2] These cleavage products became the initial "leads" for drug discovery.[30,31] Similar peptides were also used as tools for exploring the interactions between peptidic ligands and the

NS3 protease domain by NMR and molecular modeling.[32–34] Although the inherent conformational flexibility of these peptides magnified the challenges that had to be overcome, scientists pushed forward in exploring the structure–activity relationship of hexapeptides such as **20** and tetrapeptides such as **21**–**23**.[2,35] These early studies revealed some of the



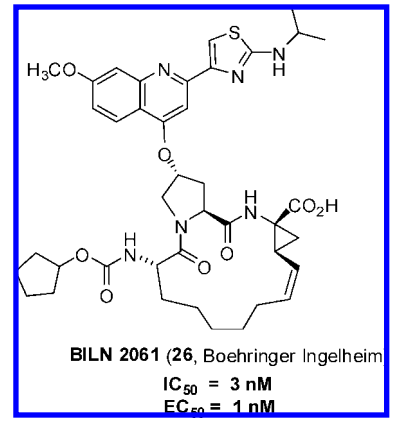
essential elements for favorable ligand–NS3 interactions that include a large lypophilic/aromatic residue at P2 and β-branching of the P3 and P4 side chains or macrocyclization of the peptide,[35] in order to stabilize the preferred β-strand conformation of the peptidic backbone.

Optimization of the dipole–quadrupole interactions between the P2 proline residue and the enzyme,[36] as well as the hydrophobic/electronic interaction of the P1 with the Phe154 residue defining the "floor" of the shallow NS3/4A S1 binding site,[37] lead to the identification of inhibitors, such as **24** and **25**, exhibiting enzymatic potencies of 800 nM and 30



nM, respectively. Unfortunately, despite these significant improvements in enzymatic potency and the reduction of size/peptidic nature of the inhibitors, these analogs did not exhibit any appreciable antiviral potency in the cell-based replicon assay. In parallel to these efforts, activated carbonyl derivatives that act as reversible covalent inhibitors, such as α-ketoacids, pentafluoroethyl ketones, and α-ketoamides were also under investigation.[2] Based on the mechanism of action of activated carbonyl inhibitors, it is not surprising that reduced selectivity toward some mammalian serine and cysteine proteases has been observed with analogs from this class.[38]

The first molecule to enter clinical trials and demonstrate unprecedented viral load reduction in patients infected with the hepatitis C virus, after only hours of treatment, was BILN 2061 (**26**), developed by Boehringer Ingelheim.[39] The design



of this class of macrocyclic inhibitors was guided by early NMR data on the enzyme-bound conformation of hexapeptides indicating that the acyclic P3 side chain lies on the solvent-exposed surface of the protein and in close proximity to the P1 side chain.[32] Armed with this insight, macrocyclic β-strand tripeptides were designed that adopt in the *free state* the enzyme-bound secondary structure of the acyclic hexapeptides and exhibit high affinity for the HCV NS3/4A protease.[40] The 15-membered ring scaffold forces the P2–P3 amide bond to adopt exclusively the *trans* geometry that is preferred for binding; [40] this is in contrast to all linear proline-containing peptides which, in the free-state, exist as mixtures of *cis*- and

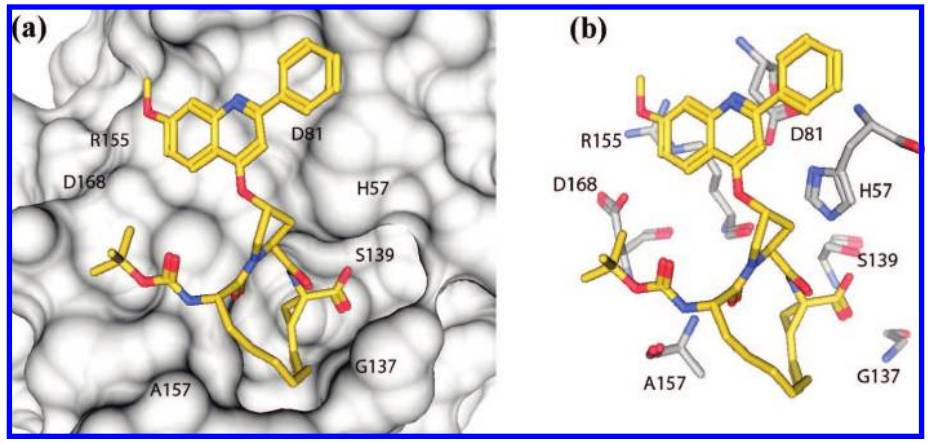
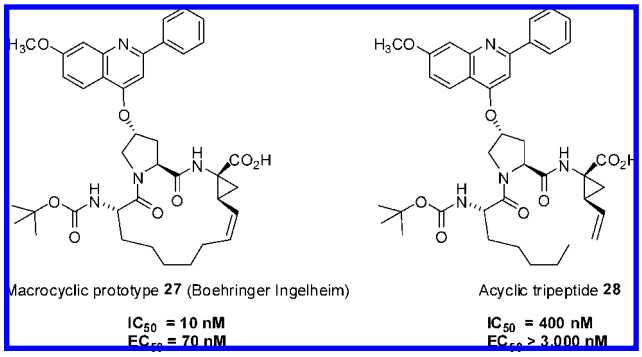


FIGURE 5. Structure of inhibitor **27** bound to the active site of HCV NS3/4A$_{peptide}$: (a) inhibitor bound to the protein surface reveals excellent shape and size complementarily between **27** and the shallow S1−S3 pocket; (b) the key NS3 residues that interact with the inhibitor are indicated.[40]

trans-rotamers. The anticipated reduction of conformational entropic penalty paid upon binding of the macrocyclic peptide to the enzyme leads to a significant increase in the overall binding energy, as can be deduced by comparing compound **27** to the corresponding acyclic tripeptide **28**.[40]

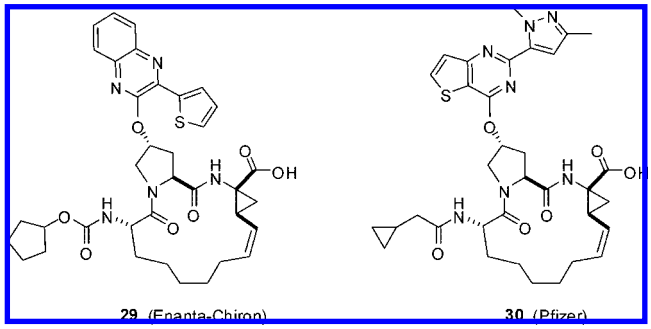


Binding of the macrocyclic inhibitors to the intended biological target was also confirmed by crystallography, providing the first structural analysis of a small-molecule carboxylic acid inhibitor bound to the active site of the HCV NS3/4A$_{peptide}$ (Figure 5a).[40] This structure revealed that the carboxylate moiety (e.g., compound **27**−NS3/4A$_{peptide}$ complex) interacts with the oxyanion hole of the NS3 (NH of Gly-137 and Ser-139) and the catalytic His-57 (Nδ) residue (Figure 5b). Formation of canonical hydrogen bonds between the inhibitor's amide moieties and the NS3 main chain residues Arg-155 and Ala-157 were also observed. The inhibitor's aliphatic linker (i.e., linker between P1 and P3) was clearly fixed within the S1−S3 pocket and likely involved in van der Waals interactions, thus contributing to the overall binding energy in the inhibitor−enzyme complex (Figure 5a). Inhibitor-induced conformational changes of the Arg-155 and Asp-168 side chains were observed that lead to the formation of interactions between Asp-168 and Arg-155 and between the guanidine group of Arg-155 and the methoxy moiety of the quinoline (Figure 5b). Some of these interactions were later also reported for other inhibitors of the NS3 protease.[2,45] Furthermore, preclinical (observed in a cell-based HCV replicon system) and clinical emergence of resistance at residues Ala-156, Arg-155, and Asp-168 have been reported.[2] Interestingly, polymorphisms at residue Asp-168 in some HCV genotypes (e.g., genotype 3) may be responsible for the variable activity and clinical efficacy observed with some of the NS3 protease inhibitors against different HCV strains.[41]

In addition to the design of effective macrocyclic inhibitors, the chemical manufacturing of such structurally complex molecules is often a challenge that may prohibit their commercialization. Initially, the development of a synthetic process that would be economically feasible and could support the production of multikilogram quantities of a macrocyclic therapeutic was a daunting task. Intensive investigations into the synthesis of each building block and evaluation of the factors that influence the stereochemical integrity of the product during the key macrocyclization step lead to the development of a protocol that could support the clinical trials of BILN 2061.[42] A 400 kg scale-up of this compound represents the first application of Ru-induced ring-closing metathesis chemistry in an industrial setting for the preparation of a potential therapeutic agents.[43]

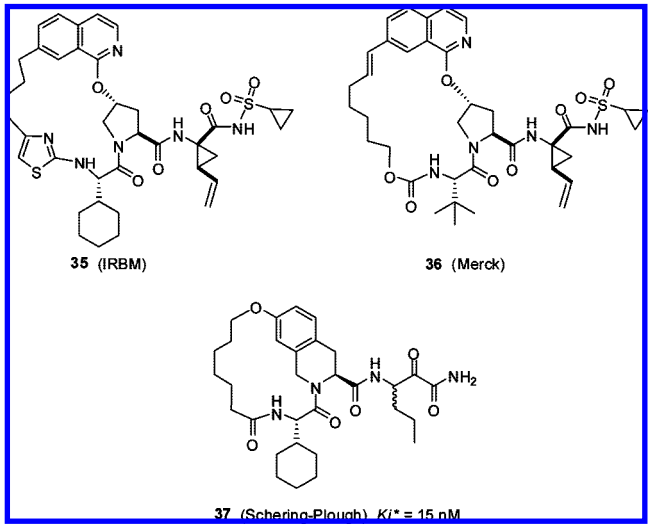
Since the disclosure of the first series of macrocyclic inhibitors[40] and in particular the phase I clinical data of BILN 2061 (**26**),[39] many compounds characterized by the same 15-membered ring peptidomimetic scaffold have appeared in the drug-discovery pipelines of different pharmaceutical companies; these include derivatives such as compounds **29** and **30**.

In addition, analogs having an acyl sulfonamide replacement of the carboxylic acid moiety, such compounds **31** and



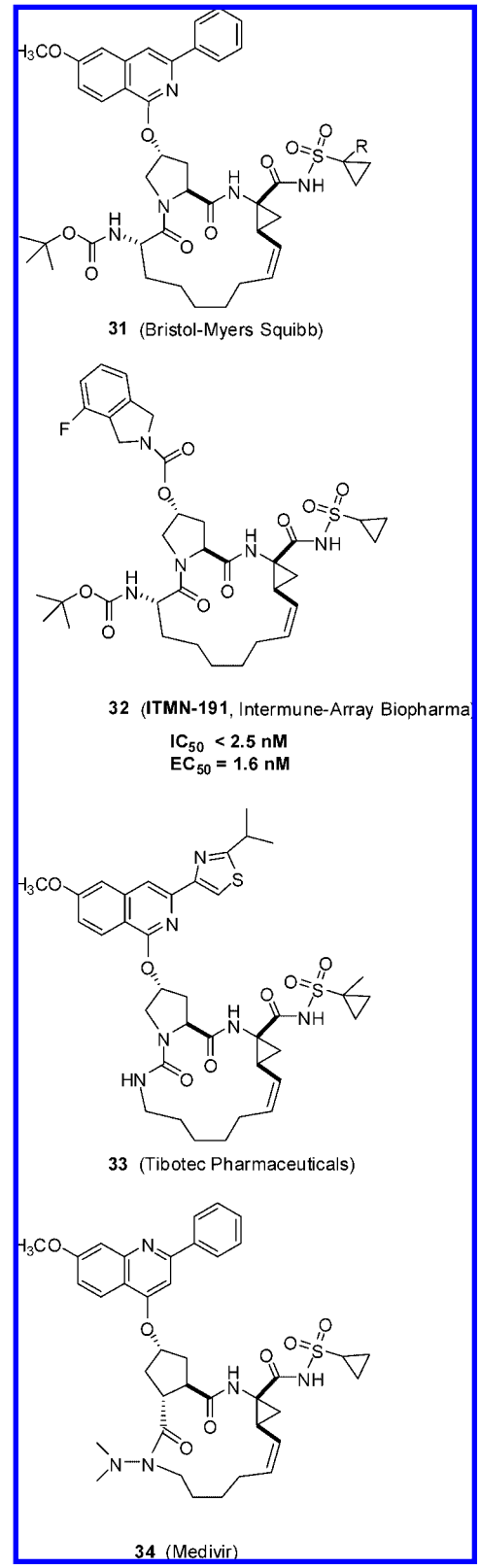
32 (ITMN-191) or those having combined modifications to the ring scaffold and replacement of the P1 acid, such as **33** and **34** have also entered research and development. ITMN-191 (**32**) is currently in clinical phase I and TMC-435, believed to be structurally related to **34** (having a cyclopentyl P2 core instead of a proline), is also reported to have entered clinical trials.[2]

Meanwhile, the IRBM/Merck team has recently returned to the design of their earlier macrocyclic inhibitors of NS3 protease,[35] which are covalently linked between the P2 aromatic moiety and the P3 capping group of a tripeptide. Analogs of this class (e.g., compounds **35** and **36**) can benefit from all the



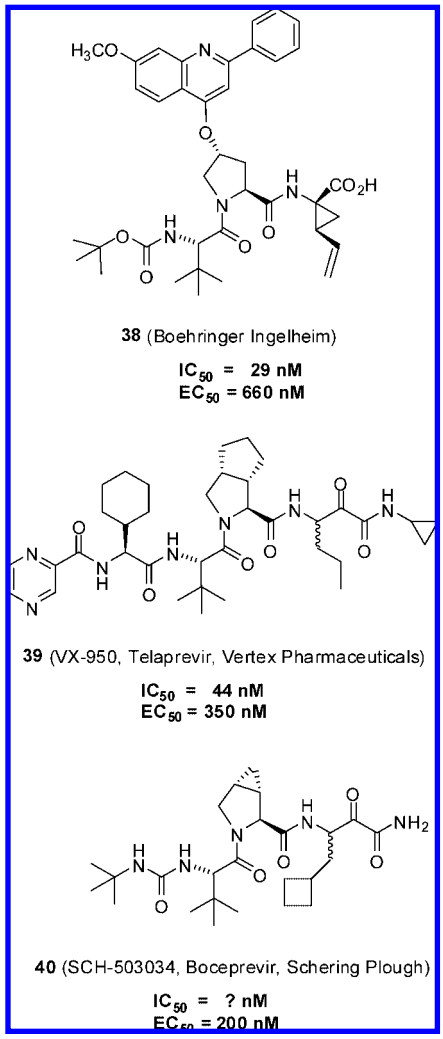
optimized P1, P2, and P3 moieties that are now in the public domain. Furthermore, Schering-Plough has added to the current plethora of macrocyclic inhibitors by combining the IRBM/Merck design with activated carbonyls (e.g., analog **37**).[44]

In parallel to all of the above efforts, acyclic peptides with a carboxylic acid moiety at P1 (e.g., **38**)[45] or a activated carbonyls were also under extensive investigation; from the latter class, compounds VX-950 (**39**)[2,46] and SCH 503034 (**40**)[47] are currently in phase II clinical development. Stability of the acyclic peptidic backbone to the preferred β-strand conformation was achieved by the incorporation of a *t*-butyl side chain at the P3 moiety that significantly reduces the population of



the *cis*-proline rotamer (other sterically bulky P3 side chains can produce the same effect).

In summary, although the stigma of the poor drug-like properties of peptidomimetics is unlikely to be dispelled



any time soon, the current therapeutic agents targeting the HIV-PR represent a remarkable success story in peptidomimetic research. The incomplete viral suppression observed in treatment-experienced AIDS patients will continue to drive medicinal chemistry toward the search for novel effective drugs with efficacy against the mutants raised by the previous generations of protease inhibitors. In the HCV field, there is a justified optimism that a drug targeting the NS3/4A protease, is on the horizon. Insight gained from structural research on the factors that influence free-state preorganization of a peptidomimetic ligand, and the impact of the free-state conformation on its affinity for the target, as well as the design of bioisostere replacements of peptide fragments, should collectively be recognized as important achievements. As for the professional rivalry between scientists in different industrial or academic settings for the discovery of the best therapeutic agents, there is no doubt that there is a great deal of knowledge to be shared that would ultimately benefit the patients and society.

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BIOGRAPHICAL INFORMATION

Youla S. Tsantrizos obtained her Ph.D. at McGill University under the supervision of Professor Kelvin K. Ogilvie and then pursued postdoctoral studies at Brown University under the supervision of Professor David E. Cane. Prior to joining Boehringer Ingelheim (Canada) Ltd., where she is currently a Distinguished Scientist in Chemistry, she was Associate Professor of Chemistry and Biochemistry at Concordia University in Montreal.

FOOTNOTES

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