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Direct Renin Inhibitors as a New Therapy for Hypertension

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Introduction

The history of the renin angiotensin system (RAS[a]) traces its roots to the seminal experiments of Tigerstedt and Bergman describing a pressor response in the rabbit following injection of a rabbit kidney homogenate.[1] These studies were the first to describe the hypertensive effect of renin and laid the foundation for future investigations into the RAS pathway. Several crucial studies followed over the next 50 years that further elaborated additional key components of the RAS, including the identification of angiotensin II (AngII) as the principal substance mediating the activity of this system (Figure 1).[2] Angiotensinogen, the endogenous renin substrate, was first described in an elegant series of experiments in which the serial components of the system were clearly delineated.[3] Skeggs went on to explain how therapeutic benefit could be achieved by interfering with this system at several distinct points: by blocking the receptor for AngII, by inhibiting angiotensin converting enzyme (ACE) as the enzymatic step required for generation of AngII, or by preventing the formation of AngI through direct inhibition of renin. Interestingly, these investigators realized early on that "Since renin is the initial and rate-limiting substance in the renin−hypertensin system, it would seem that this last approach would be the most likely to succeed."

It has been shown consistently that blockade of the RAS, either with an ACE inhibitor (ACEi) or with an AngII AT1 receptor blocker (ARB) reduces blood pressure (BP). However, a powerful counter-regulatory mechanism is activated during RAS blockade and is in part responsible for the "flat" dose−response relationship observed with the use of these inhibitors.[4] Under normal physiologic conditions, AngII inhibits renin release through stimulation of the AT1 receptor. Therapeutic interventions that either reduce AngII (ACEi) or attenuate AT1 signaling (ARB) ultimately result in enhanced release of renin. Consequently, the historical depiction of this system as a linear cascade is an inappropriate representation of the pathway. The RAS is now better portrayed as a physiological circuit encompassing a negative feedback "loop" whereby renin levels are controlled by the amount of AngII present in plasma and tissues (Figure 1).[5] This renders renin a highly attractive target from a therapeutic standpoint, as it would provide the only tactic to reduce plasma renin activity and thus offers a novel approach for the management of hypertension. While a compensatory rise in renin occurs as a result of RAS blockade with an ACEi, ARB, or a renin inhibitor, renin is rendered ineffective only by renin inhibition. Renin inhibitors bind to the active site of renin, a member of the aspartic protease family with high specificity for its endogenous substrate angiotensinogen, and are now commonly referred to as "direct renin inhibitors" (DRI), as opposed to drugs interfering with other components of the RAS, like ACE inhibitors, ARBs, or β-adrenoceptor antagonists.[6] Despite many treatment options available to the clinician, hypertension remains an important public health issue. Hypertension affected more than 65 million Americans and more than 25% of adults worldwide and is a key risk factor for myocardial infarction, stroke, and heart and renal failure.[7] Its prevalence is expected to increase by 60% to >1.5 billion people worldwide by 2025.[8]

Academic institutions and major pharmaceutical companies have committed substantial resources over the past 50 years in an effort to discover the best therapeutic tactic for modulating the RAS. Initial therapeutic success was achieved with the introduction of ACEi[9] and then with the identification of ARBs a decade later.[10] While it was widely recognized that direct renin inhibition held great therapeutic potential, the design of small nonpeptidic molecules that effectively interact with the catalytic domain of renin and that demonstrate oral efficacy in humans remained elusive for more than 2 decades.[4] Compound **1** (aliskiren, CGP60536B, SPP100; Figure 2) represents a new generation of orally highly effective DRIs of a unique structural class designed by molecular modeling and X-ray crystallography.[11−13] This drug was introduced in the United States as Tekturna and in Europe as Rasilez in 2007[14] and hence became the first new therapy for the treatment of hypertension in more than 10 years.

Since the late 1990s, a resurgence of interest in DRIs in the industry became increasingly evident and was driven by several major advancements.[15] Emerging new insights at the molecular level related to human renin−inhibitor active site

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[a] Abbreviations: ADME, absorption, distribution, metabolism, and excretion; ACE(i), angiotensin converting enzyme (inhibitor); Ang, angiotensin; ARB, angiotensin II receptor blocker; BACE, β-site of β-amyloid precursor protein cleaving enzyme; BP, blood pressure; CYP, cytochrome P450; DRI, direct renin inhibitor(s); dTGM, double-transgenic mouse; dTGR, double-transgenic rat; GRAB, group replacement assisted binding; H-bond, hydrogen bond; HTS, high-throughput screening; ITC, isothermal titration calorimetry; PK, pharmacokinetics; PRA, plasma renin activity; PRC, plasma renin concentration; (P)RR, (pro)renin receptor; RAS, renin angiotensin system; rh-renin, recombinant human renin; SAR, structure−activity relationship; SHR, spontaneously hypertensive rat; TS, transition state; TSA, transition-state analogue.

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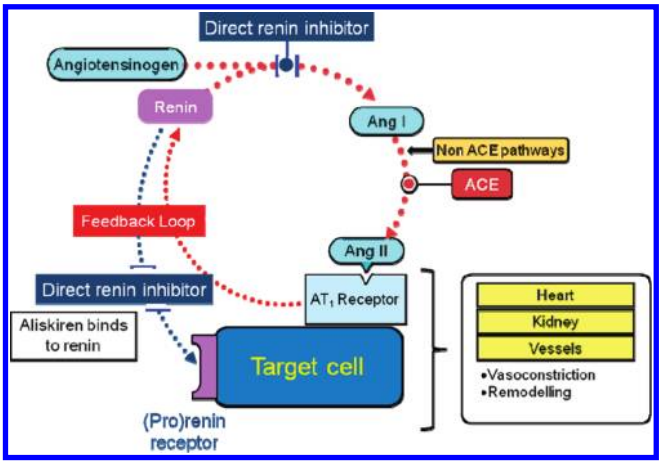


Figure 1. Renin−angiotensin system (RAS).[5]

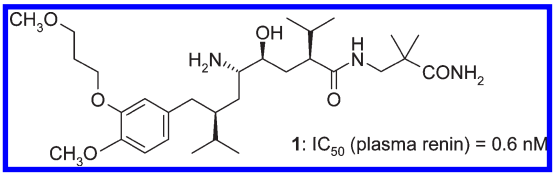


Figure 2. Compound **1** (aliskiren), the first marketed direct renin inhibitor (DRI).

interactions have paved new avenues for designing distinct chemotype inhibitors. The introduction of transgenic rodent models provided the capability to assess biological actions in small animals and thus reduced reliance on the use of non-human primates.[16] Also, the advanced clinical testing of **1** offered promise that this long sought after therapeutic approach might finally become a reality. Most importantly, extensive clinical trial results are now available and demonstrate its benefit.[5] Previous DRIs had been evaluated in limited clinical testing with short-term administration.

The objective of this review is to highlight key events and recent advances in the evolution of non-peptide peptidomimetic DRIs.[17] Special emphasis will be placed on the successful structure-based strategies that enabled the discovery of several novel classes of DRIs.[18,19] The potential limitations associated with these inhibitors and opportunities for future directions of renin inhibitor design will be discussed from a chemical and biological perspective. Lessons learned will also be discussed in an effort to provide a unique perspective on the development of **1**. With its introduction, it is now very important to distinguish this mechanism of action from indirect means of inhibiting renin. In this regard, ongoing clinical trials designed to demonstrate a clear benefit, beyond an effect on blood pressure, by reduction in morbidity and mortality are of paramount importance.

Medicinal Chemistry of DRIs: A Historical View

The search for clinically efficacious DRIs with druglike properties has a remarkable and long history. It has required tremendous investments in research and development in industry and has spanned more than 3 decades.[20−23] The first potent inhibitor of renin in plasma, reported as early as 1980, was completely peptidic in nature.[20] This decapeptide substrate analogue, labeled "RIP" (for renin inhibitor peptide), allowed the first in vivo pharmacology studies of a DRI in monkeys. The design of orally efficacious non-peptide DRIs remained a formidable task with incremental progress made by the early 1990s, when several peptide-based peptidomimetic DRIs (**2**−**6**, Figure 3) had entered into clinical trials as investigational drugs.[21b,22,23b]

Structure-based drug design (SBDD) involving X-ray crystallography and computer-assisted molecular modeling emerged as one of the most preeminent paradigms in drug discovery[24a,b] in the 1980s, and the successful design of highly potent and selective DRIs represents one hallmark example. Numerous X-ray crystal structures of native aspartic proteases and enzyme−inhibitor complexes in combination with elegant mechanistic studies have provided detailed insight into fundamental aspects of the three-dimensional architecture and conformational dynamics of this class of endoproteases, as well as into the general acid−general base mechanism of enzyme-catalyzed peptide bond hydrolysis.[20,25−29] This information, together with computational modeling methods, has been of invaluable importance for developing a rational design approach toward potent DRIs, initially by the use of renin homology models.[30] Notably, the X-ray structure of human apo-renin was reported only in 1989,[31] and the first renin−inhibitor complexes became available shortly thereafter.[30,32]

Since the early reports describing angiotensinogen substrate-derived peptides as low affinity inhibitors of renin in the mid-1970s, the design of highly potent and selective DRIs as potentially useful drug candidates has witnessed a remarkable transition through various stages of structure-guided medicinal chemistry approaches. On the basis of the increasing knowledge about the three-dimensional structure and the enzymatic mechanism of substrate cleavage, several generations of DRIs have evolved over the years. These range from modified peptide inhibitors as target-validating pharmacology tools followed by more druglike second generation peptidic inhibitors to the more recent discovery of diverse classes of non-peptide DRIs.

The incorporation of mimetics of the putative transition-state of the enzyme-catalyzed amide bond hydrolysis as non-hydrolyzable replacements of the scissile cleavage site represents a general inhibitor design principle originating in the postulates by Pauling and Wolfenden.[20,33] The application of this approach to N-terminal angiotensinogen substrate-derived peptide sequences has been instrumental in generating potent and proteolytically stable inhibitors of renin. Key structural features of these transition-state analogues (TSAs) are hydroxyl or (pro)hydroxyl groups that interact via hydrogen bonding with the catalytic Asp_{32} and Asp_{215} carboxylates by extrusion of the enzyme-bound cosubstrate water of the catalytic center in native renin. The entropically favored water displacement by the TSA hydroxyl and further evidence would suggest that these analogues function at least in part as "collected-substrate" inhibitors rather than as tetrahedral intermediates of enzyme-catalyzed peptide cleavage.[25,27,29] Most prominent early representatives are the nonproteinogenic amino acid statine, identified from the pepstatin family of natural product aspartic protease inhibitors,[34] and the hydroxyethylene (HE) dipeptide isostere first introduced by Szelke et al. (Chart 1B and Chart 1A, respectively).[20] Key strategies of extensive structural modifications of first generation DRIs included the elimination of their peptidic nature, optimization of critical binding interactions to the renin specificity pockets spanning S_3/S_4 to S_2' of the extended enzyme cleft, and variation of the central TSA moiety. These concepts aimed to accomplish a minimal molecular size sufficient for strong binding affinity.

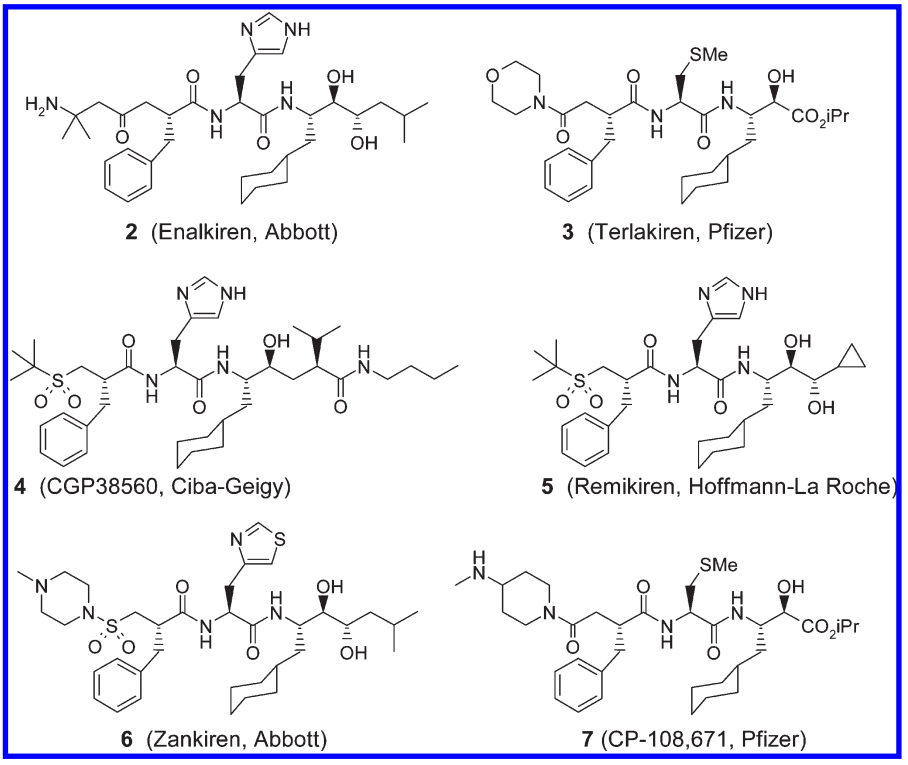
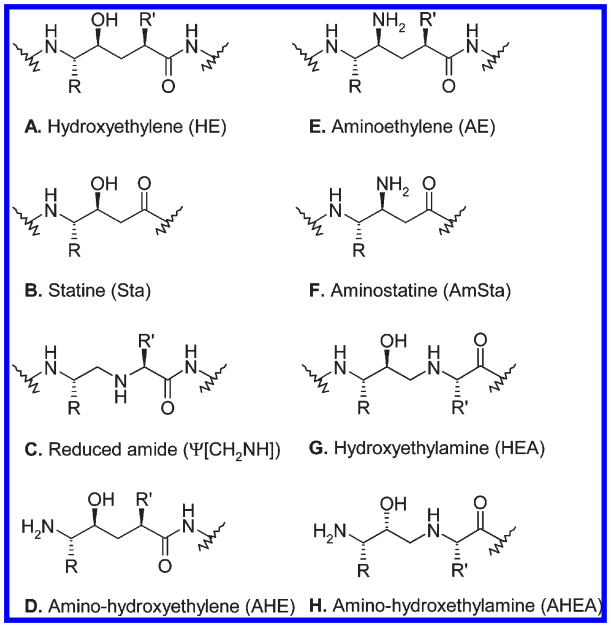


Figure 3. Second generation peptide-based peptidomimetic DRIs.

Chart 1. Aspartic Protease TSAs and Basic Amine TS Surrogates (Preferred Absolute Configurations Shown)



The vast majority of these potent peptide-derived peptidomimetics had limited druglike properties and hence multiple drawbacks, such as poor intestinal absorption and high liver first-pass metabolic inactivation resulting in low oral bioavailability, as well as high cost of goods due to synthetic complexity and high clinically effective doses. Excellent review articles[21−23] and historical cases, including the discovery of **7** (CP-108,671) exhibiting uniformly high oral bioavailability in dog and marmoset,[35] provide a comprehensive overview on these design efforts, the characterization of second generation DRIs, and their failure in clinical development.

A New Era of Non-Peptide DRI Design

Several key developments during recent years have contributed to a rapid and fascinating evolution of medicinal chemistry approaches aimed at the design of promising new classes of DRIs. Both the druggable topographical space at the enzyme protein level and, in part as a direct consequence, the chemical diversity space at the ligand level have dramatically expanded. This has evidently opened ample avenues toward the discovery of new structural classes of potent and selective non-peptide peptidomimetic DRIs. The early notion that the large contiguous S_3-S_1 pocket constitutes a key hydrophobic "hot spot" for ligand binding has been successfully employed for a topological inhibitor design concept. Ligand binding sites in the renin active site have been identified, which are distinct from the substrate specificity pockets or even fundamentally different from the extended β-strand substrate binding topography resulting from major conformational movements adjacent to the catalytic center.[29,36] Most notably, the classical dipeptide TSA concept, the dominating theme of inhibitor design over many years, has vanished. Novel privileged basic amine scaffolds that act as TS surrogates by a combination of electrostatic and H-bonding interactions to the catalytic aspartates have been uncovered and provide versatile templates for decoration with recognition site pharmacophores.

These major scientific advances are best illustrated by the discoveries of the orally active topological peptidomimetic **1** (Figure 2), an aminohydroxyethylene dipeptide isostere (AHE, Chart 1D) of a unique chemical class described by Ciba-Geigy (now Novartis),[11−13] and the new type of 3,4-disubstituted piperidine-derived DRIs reported by Hoffmann-La Roche.[36] The DRI **1** resulted from an entirely structure-based design concept using a renin homology model, by addressing the $S_3/S_3^{sp}-S_1$ active site topography in an unprecedented way (Figure 4). In contrast, the novel structural principle of

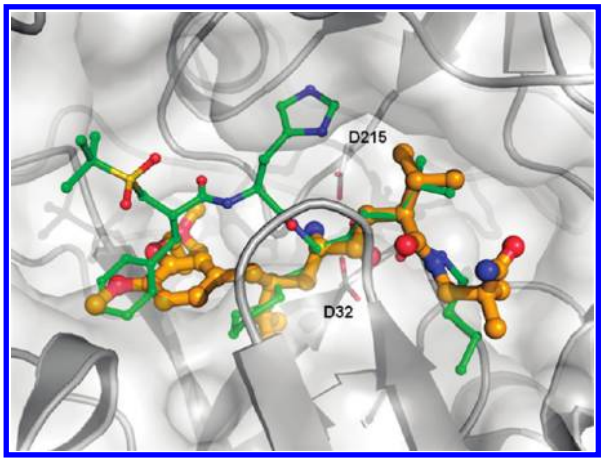


Figure 4. Structure of rh-renin in complex with inhibitor **1** (orange). Renin is shown in gray (ribbon diagram and surface) with the side chains of the catalytic aspartates Asp_{32} and Asp_{215} with gray carbon atoms (flap residues Ser_{76} and Thr_{77} were omitted from the surface calculation). The peptide-based peptidomimetic **4** (CGP38560) is shown superimposed (green).

4-arylpiperidine peptidomimetics emerged from high-throughput screening (HTS), followed by hit-to-lead elaboration based on crystallographic elucidation of renin−inhibitor complex structures. These inhibitors target a fundamentally different conformational topography of the renin active site. Both critical accomplishments and the positive outcome of late stage clinical trials with **1**[5] have triggered intense efforts to identify "best-in-class" DRIs and have provided several new clinical development candidates.[15] The structural diversity of non-peptide DRIs is anticipated to further expand in the future with the application of evolving state-of-the art drug discovery concepts.

Major advances in renin inhibitor design will first be reviewed in the following sections by focusing, on one hand, on the identification of core scaffolds binding to the catalytic aspartates and, on the other hand, on specific approaches taken to address the individual binding pockets of the active site, i.e., the S_3−S_1 "superpocket", the S_3^{sp} and other non-substrate binding pockets, as well as the S_2 and S_1'−S_2' sites. Subsequently, considerations regarding the optimization of ADME and safety properties of different novel chemotype DRIs and their preclinical pharmacology will be addressed, and finally aspects of the clinical pharmacology of **1** are reviewed.

Novel Lead Discovery Approaches

The discovery and optimization of DRIs have for many years relied mainly on rational substrate-based design by targeting the extended β-strand binding active site topography. Since the mid-1990s, HTS of large historical corporate collections, often containing more than 1 million compounds, has been used as one of the standard methods for identifying starting points for medicinal chemistry against a drug target of interest. In the case of aspartic proteases, as well as for other protease families, HTS has been perceived to suffer from low success rates in the identification of hits having a good potential for further optimization.[13,37] This is certainly related to the fact that the active sites of these enzymes are designed to recognize peptide sequences in an extended conformation and therefore are generally characterized by rather large van der Waals volumes. Remarkably, several pharmaceutical companies have been successful in their search for new chemotype

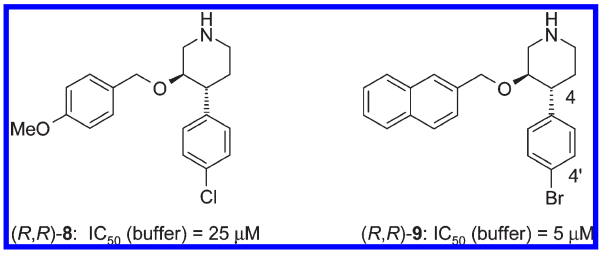


Figure 5. 4-Phenylpiperidine DRIs discovered by high-throughput screening (HTS).

scaffolds inhibiting renin, by performing large-scale HTS campaigns based on biochemical assays.[36,38] More recently, alternative technologies based mainly on biophysical methods (nuclear magnetic resonance (NMR), surface plasmon resonance, X-ray crystallography, mass spectrometry) and used in large part for fragment-based screening, as well as in silico computational approaches, were also successfully applied to identify novel active site binding motifs for aspartic proteases, including renin[38d,39] and BACE-1.[40]

In 1999, researchers at Hoffmann-La Roche described the discovery by HTS of the first non-peptide small molecule DRI.[36a] The original hit, *rac*-**8** (IC_{50} = 50 μM), showed only weak affinity against the purified enzyme, with the *R*,*R*-enantiomer accounting for the activity (Figure 5).[36b] The low resolution X-ray structures obtained for *R*,*R*-**8** and its close analogue *R*,*R*-**9** revealed the piperidine nitrogen atom to be positioned between the catalytic aspartates, with the naphthyl occupying the S_3−S_1 subsite. This finding has guided the initial structure−activity relationship (SAR) exploration using combinatorial chemistry.[36a−c] As will be detailed later, modifications at the 4′-position afforded prototype leads with dramatically improved in vitro potencies. Their X-ray crystal structures in complex with recombinant human (rh) renin revealed features very distinct from the binding mode of peptide-derived TSA inhibitors. The terminology of "group replacement assisted binding" (GRAB) peptidomimetics was introduced to account for the topographical and dynamic aspects of these renin−inhibitor interactions.[29] The attractiveness of this remarkable class of DRIs has subsequently fueled drug discovery programs at several other pharmaceutical companies.

A HTS conducted at Pfizer led to the discovery of the 5-phenyl substituted 6-ethyl-2,4-diaminopyrimidine **10** as a weak inhibitor of rh-renin (Figure 6).[38a] Variation of the benzylic moiety employing parallel chemistry led to **11** with 7-fold improved activity. The X-ray cocrystal structure of the renin−**11** complex revealed an active site conformation in which the flap (residues Thr_{72}−Ser_{81}) is closed. Notably, the 2,4-diaminopyrimidine was identified as a novel planar center scaffold forming H-bonds with the two catalytic aspartates, while also providing constrained, favorable trajectories for both the 6-ethyl group binding into the S_1 pocket and for the difluorobenzylaminophenyl moiety extending into the S_3 and partly into the S_4 subsites (vide infra). This series was further optimized by conformational rigidification of the benzyl portion and introduction of a P_3^{sp} side chain (**12**, Figure 6), with additional modifications of the bicyclic (S_3−S_1)-motif leading to inhibitors exhibiting high in vitro potency and oral bioavailability (**34**−**37**, Figure 19).[38b]

Fragment-based drug discovery (FBDD) has become an approach of increasing importance over the past years in pharmaceutical research.[24b,c] The group at Pfizer has reported on an in silico docking approach using GOLD, combined with

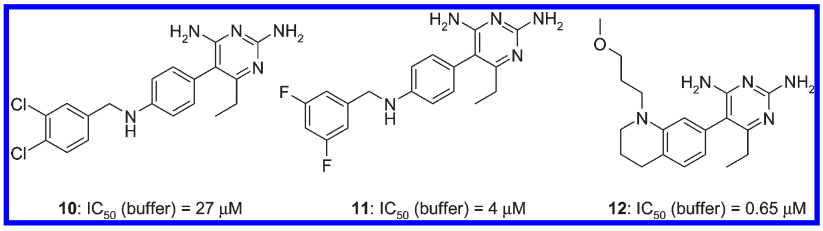


Figure 6. 2,4-Diaminopyrimidine-based DRIs originating from HTS.

a NMR auxiliary binding screen of a fragment library, to target specifically the renin S_2 pocket as part of the lead optimization efforts in the 6-ethyl-2,4-diaminopyrimidine series.[38c,d] The identified single fragment hit was shown by saturated transfer difference NMR via interligand NOEs in the presence of renin to be an active site coligand of the C4 aminomethyl analogue of compound **12** (Figure 6). Fragment-linking via a two-carbon spacer and cocrystallization of the resulting inhibitor confirmed the tethered fragment to be positioned in the S_2 pocket. Further elaboration using X-ray crystallographic data in combination with isothermal titration calorimetry (ITC) analyses resulted in significant potency improvement (cf. section "S_2 Specificity Pocket").[38c] This example highlights the potential of fragment-based approaches for mapping the renin active site. Future applications may uncover new promising lead templates and possibly so far unrecognized binding hot spots for renin.

Various in silico screening approaches have emerged recently and found application for renin hit discovery, as either alternative or complementary methods to the screening of physically available compound libraries. Researchers at Vitae Pharmaceuticals disclosed a novel class of DRIs, identified by a de novo structure-based design approach[39] using their computational tool Contour and starting from the X-ray structure of the peptidic inhibitor **4** bound to renin.[32] Interestingly, defined criteria for inhibitor design were to target exclusively the S_3-S_1 and S_3^{sp} pockets while leaving the S_2, S_4, and the prime-site pockets unoccupied. Furthermore, incorporation of a solubilizing basic amine that could interact with the catalytic aspartates was envisaged. Potent DRIs exhibiting reasonable pharmacokinetic (PK) properties and in vivo efficacy were identified as a result of design and synthesis iterations and further optimization of prototype leads.[39] The experimental X-ray structure of a renin−inhibitor complex was reportedly in close agreement with the predicted design model. It is noteworthy that de novo in silico design approaches targeting alternative, potentially pre-existing conformational ensembles of the renin active site[29] remain an extremely challenging task.

The tremendous progress in high sensitivity assay technologies and emerging powerful new enabling methodologies not only are providing new opportunities to enlarge the chemical space but also allow early validation of very low-affinity small molecule hits as active site binders. Furthermore, computational modeling approaches in combination with timely structural elucidation of an increasing number of renin−ligand complexes by X-ray crystallography have become even more indispensable for an efficient iterative drug discovery process. These approaches encompass hit finding and validation, hit selection, and progression toward enhanced inhibitory potency, all the way through to lead optimization and identification of preclinical candidates. This is most evident for the development of novel class DRIs in view of the multiple pre-existing conformational topographies of the renin active site.[29,36]

The impressive power of new screening technologies and advanced approaches in modern drug discovery is similarly reflected by the rapid development of nonpeptidic inhibitors of β-secretase (BACE-1, memapsin 2), a type-I membrane associated human aspartic proteases. BACE-1 is implicated in the proteolytic cleavage of β-amyloid precursor protein (APP) and subsequent formation of amyloid plaques and has attracted attention as a target for the treatment of Alzheimer's disease.[40] Since the first report of the potent octapeptide HE isostere inhibitor L-α-glutamyl-L-valyl-L-asparaginyl-(2*R*,4*S*,5*S*)-5-amino-4-hydroxy-2,7-dimethyloctanoyl-L-alanyl-L-α-glutamyl-L-phenylalanine (OM99-2) and its X-ray crystal structure in complex with the protease domain of β-secretase,[41] it took only a few years to bring forth a number of distinct classes of potent non-peptide inhibitors, incorporating either classical TSAs or alternative TS surrogates.[40] While tremendous advancements were made toward the design of BACE-1 inhibitors leading to significant reductions of $A\beta_{40-42}$ levels in animal models,[42] major hurdles such as brain penetration still need to be overcome with more promising development candidates.[40a]

The structural divergence of peptidomimetic inhibitors of renin and BACE-1 is striking and has limited a target enzyme family approach[43] beyond the application of some fundamental enzyme mechanism-related design principles. Nonpeptide BACE-1 inhibitors show high selectivity toward renin,[40] and vice versa, non-peptide DRIs have been reported to be inactive against BACE-1.[13,39] This can be rationalized by both the distinct substrate specificity and the active site architecture of the two enzymes. Relationships between renin and BACE-1 inhibitors with respect to enzyme−inhibitor interactions will be briefly cross-referenced in cases where this could be particularly instructive to emphasize the impact on specific inhibitor design.

(S_3-S_1)-Topographical Hot Spot

The first X-ray crystal structure reported for rh-renin revealed the S_3 and S_1 recognition sites to form a large and contiguous space without an apparent division between the two pockets.[31] A similar spacious and open topography of the S_3-S_1 site had been observed previously for several fungal aspartic proteases with and without bound peptide TSA inhibitors.[25,28,30,44,45] Furthermore, these enzyme−ligand complexes revealed the substrate-based inhibitors to bind in an extended β-strand conformation with the hydrophobic P_3 and P_1 residues being tightly constrained and positioned close to each other. Both P_3 and P_1 residues are shielded from solvent space by the flap β-hairpin in its "closed conformation". This S_3-S_1 binding topography was subsequently confirmed by the X-ray structures of peptide peptidomimetic DRIs, such as **4** (Figure 4).[30,32]

Different structure-based concepts have been investigated independently by several research groups based on the notion that the renin S_3 and S_1 pockets form a spacious hydrophobic

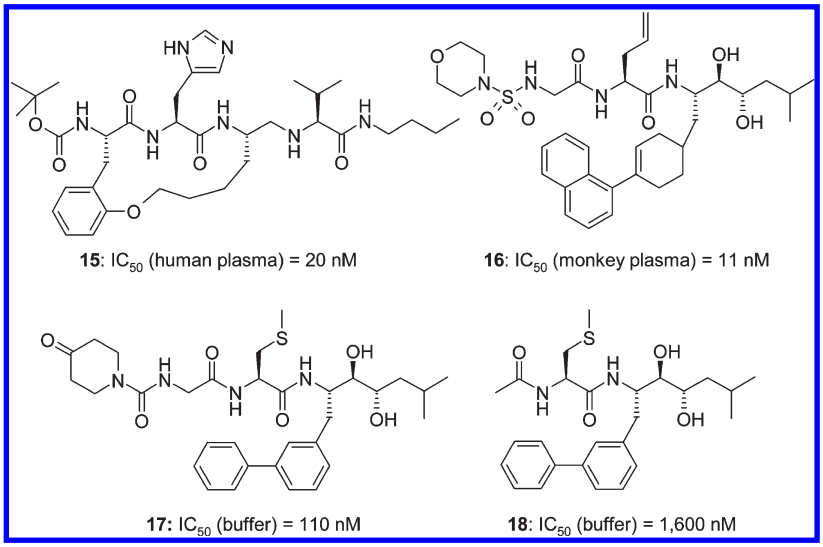


Figure 7. Peptide-based macrocyclic and (S_3−S_1)-topological DRIs.

cavity. The formation of large-surface van der Waals contacts to the hydrophobic amino acids lining the S_3−S_1 cavity was considered to be energetically favorable as a driving force for binding affinity. Design efforts therefore were aimed at maximizing the hydrophobic interactions to this key binding site by covalently linking the P_3 and P_1 side chains and ultimately at identifying novel structural classes of small-molecule DRIs with enhanced oral efficacy.[13,46−48] Conformational constraints have been introduced in peptide-based peptidomimetics by cross-linking alternate side chains to enhance potency and selectivity by locking the inhibitor in the bound conformation and in addition to potentially improve the PK properties of the more flexible acyclic congeners.[13,23a,23b,46] The macrocyclic inhibitor **15** bearing a ψ[CH_2NH]-reduced amide isostere (Figure 7) was 5 times more potent in vitro compared to the acyclic P_1 cyclohexylalanine-based analogue.[46] While macrocyclization between the P_3 and P_1 side chains of peptidomimetic DRIs has found only limited application, in contrast to substantial efforts made for the design of P_3−P_1-linked BACE-1 macrocyclic inhibitors,[40] the spatial proximity of other recognition sites has attracted more interest and provided potent macrocyclic TSA inhibitors of renin.[23a,b]

Dihydroxyethylene isostere peptidomimetics with topographically modified P_1(→P_3) side chains (**16**, Figure 7) have been explored by extending the P_1 cyclohexyl directly toward the S_3 binding pocket and by removing the linkage of P_3 to the peptide backbone. The P_3 glycine derivative **16** was found to be a potent inhibitor of monkey plasma renin.[47] Further truncation of the N-terminal backbone of these inhibitors was believed to be difficult to achieve without reducing the binding affinity.[47a] A similar design concept based on the X-ray structure of a tetrapeptide TSA in complex with rh-renin afforded inhibitors spanning the S_3−S_1 pocket, such as **17**, that were up to > 200-fold more potent than the respective P_1 phenyl analogues lacking S_3 interactions.[48] X-ray crystallography revealed the meta-biphenyl moiety of **17** to intrude into S_3−S_1 without completely filling this cavity, suggesting that additional lipophilic substituents could lead to more potent analogues. Removal of the P_4 residue produced analogues with a 14-fold drop in potency (cf. **18**, Figure 7).[48]

Novartis has reported the structure-based design of several unique structural series of non-peptide aminohydroxyethylene (AHE, Chart 1D) isosteres spanning the S_3−S_2' recognition sites and lacking the P_4−P_2 peptide backbone of previous inhibitors (**19**−**22**, Figure 8). These efforts have culminated in the discovery of **1**, a remarkably potent and highly selective first-in-class oral DRI.[11−13,49] The stepwise evolution of the design concept was primarily driven by the S_3−S_1 topography derived from the predicted active site conformation of **4** using a renin homology model. Weakly active AHE isostere fragments were grown directly into the large contiguous S_3−S_1 site, considered as a hydrophobic hot spot[50] for ligand binding, by extending at the P_1 position.[13] Most intriguingly, these novel classes of DRIs were discovered by X-ray crystallography to bind into a small and rigid cavity that extends from S_3 toward the enzyme core perpendicular to the binding cleft and is not utilized by known substrate-based inhibitors.[51] The interaction to this nonsubstrate S_3^{sp} subpocket is essential for strong renin inhibition by these topological TSA inhibitors.

The X-ray structure of **1** in complex to rh-renin is depicted in Figure 4.[12,51] The ligand is bound in an extended conformation spanning the S_3−S_2' recognition sites, leaving the S_4 and S_2 pockets unoccupied. Its P_1 isopropyl residue is more deeply positioned in the S_1 site compared to the P_1 cyclohexyl of the peptide-based inhibitor **4**, while the hydrophobic space-filling phenyl residue positions the methoxy group into the S_3 pocket. Remarkably, the ether oxygen is involved in a H-bond interaction with the hydroxyl of Thr_{12} mediated by S_3 bound water molecules. The extended flexible methoxypropoxy side chain penetrates deeply into the newly discovered S_3^{sp} cavity of renin (vide infra). The AHE isostere portion (Chart 1) forms reversible noncovalent tight binding interactions to both Asp_{35} and Asp_{215} via H-bonding and possibly electrostatic interactions. The structurally diverse (P_3−P_1)-pharmacophores of **19** and **21**−**22** also fully occupy the S_3−S_1 cavity, forming close van der Waals contacts to the large surface, as shown by the X-ray complex structures of related analogues.[51]

Inhibitor **1** and related AHE isosteres **19**−**22** represent landmark examples of a successful (S_3−S_1)-topography design concept supported by computer-aided molecular modeling and X-ray crystallography. Notably, the design of tetrapeptide-based aminohydroxyethylamine (AHEA, Chart 1H) isosteres spanning the S_1−S_3' sites and lacking the P_4−P_2 peptide portion was reported as early as 1989 and was similarly based on an endothiapepsin-derived renin homology model.[52,53] Inhibitor **23** (BW586C, Figure 9) bearing a tethered

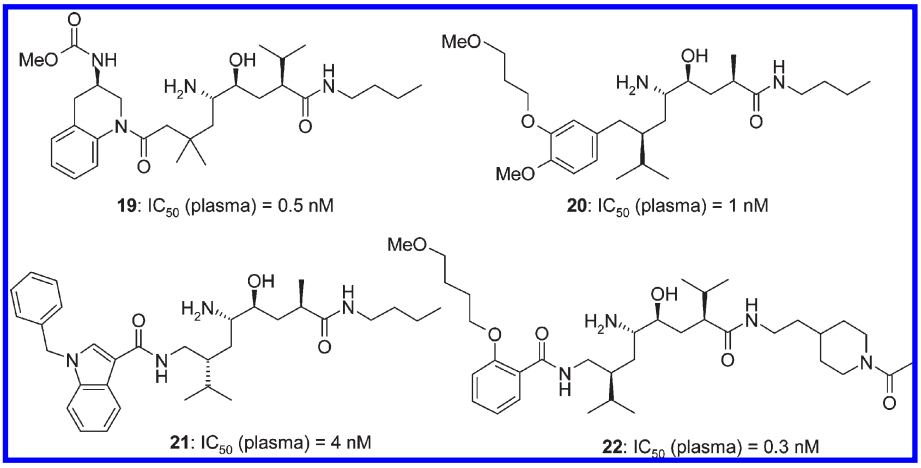


Figure 8. Nonpeptide (S_3-S_1)-topological peptidomimetic TSAs.

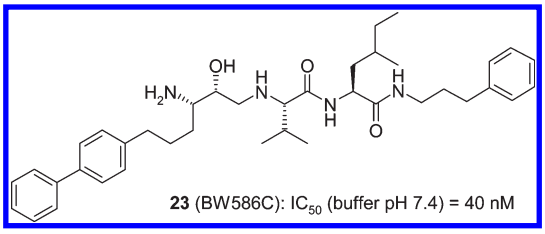


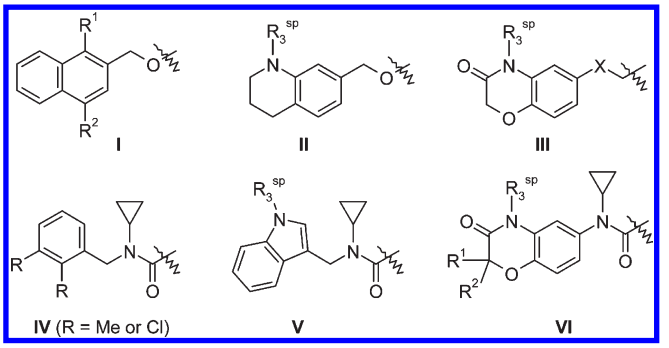
Figure 9. (S_3-S_1)-Topological tripeptide-based amino alcohol DRI.

P_1 biphenyl moiety was remarkably potent in vitro. SAR data for related amino alcohols with preferred (2*R*)(3*S*)-configuration indicated a significant potency increase when the P_1 residue was more lipophilic and extended.[52,53] An X-ray structure of **23** in complex with rh-renin, or any other aspartic protease, that could confirm the predicted occupancy of the S_3-S_1 cavity has not been reported.

Inhibitor **1** showed remarkable in vitro potency under more physiological conditions.[11,12] The methoxypropoxy side chain binding to S_3^{sp} turned out to be optimal for this inhibitor class, leading to high inhibitory affinity for plasma renin.[12,49] Significant increases in the IC_{50} of up to several orders of magnitude have been observed for analogues of **1** and other (S_3-S_1)-topological inhibitors[13] in the presence of plasma ("plasma shift"). Such plasma shifts have also been reported for peptide-derived[21a,54] and non-peptide DRIs from various structural classes.[36,39,55] The drop in potency toward plasma renin has been attributed to low solubility and high lipophilicity and/or high unspecific binding to plasma proteins reducing the free fraction in blood.[55] However, other yet to be uncovered factors may also contribute to this phenomenon.[21a,49,56] The relevance of in vitro plasma IC_{50} values as predictive descriptors for BP lowering potency of a given DRI in vivo has been under debate,[55] in particular in view of the emerging evidence for the importance of local renin inhibition in the kidney and other tissues.[57] Notably, an increase in plasma renin concentration (PRC) has been generally accepted as an important clinical surrogate marker for RAS blockade and inhibition of plasma renin activity (PRA) for the effectiveness of drug treatment using a DRI.[4]

Novel (S_3-S_1)-topological binding motifs have been identified from weakly active HTS hits (**8** and **10**, Figures 4 and 5),[36a,38a] and structure-based design has subsequently generated an array of privileged (P_3-P_1)-pharmacophores (**I**−**VI**, Chart 2). These structural moieties have in common that they

Chart 2. Topological (P_3-P_1)-Pharmacophores Used in Combination with Five- and Six-Membered Center Basic TS Surrogate Scaffolds of Non-Peptide DRIs (See Figures 5, 6, and 11)



have been linked to a basic center scaffold such as a piperidine or related mono- or bicyclic ring analogues (Figures 22 and 23)[36,55,58] and 2,4-diaminopyrimidines (**12**, Figure 6)[38] occupying the pivotal position between Asp_{32} and Asp_{215} by forming electrostatically enforced H-bonds (vide infra). These templates provide trajectories different from the P_1-tethering vector of AHE isosteres for extensions into the S_3-S_1 cavity. Optionally substituted naphthalene or tetrahydroquinoline as equipotent replacement (**I** and **II**, Chart 2), when bridged by a 3-methyleneoxy group to the 4-phenyl-piperidine scaffold, resulted in potent inhibitors classified as GRAB peptidomimetics[29] (cf. section "GRAB Peptidomimetic Class of DRIs"). The naphthalene occupies the S_3-S_1 site forming an edge-to-face interaction with the Phe_{117} residue.[38a,c] The position of the oxymethylene spacer and the proximal edge of the naphthyl partially overlap with the binding position of the P_1 isopropyl of **1**.

The nitrogen atom of the tetrahydroquinoline heterocycle provides an attachment point for side chains that can penetrate into the nonsubstrate S_3^{sp} cavity in a similar fashion as previously discovered for AHE isostere inhibitors **1** (Figure 2) and **19**−**22** (Figure 8). The 1,4-benzoxazin-3-one motif (**III**, Chart 2) was identified as a less hydrophobic tetrahydroquinoline replacement, being well accommodated by the S_3-S_1 site and providing an equally efficient trajectory for tethered P_3^{sp} residues.[58e] When attached to a 2-ketopiperazine center scaffold by a two-atom (thio)ether linkage, high affinity DRIs were obtained (vide infra). Subsequently, the 1,4-benzoxazinone pharmacophore and modifications thereof have attracted major interest by other groups.[15]

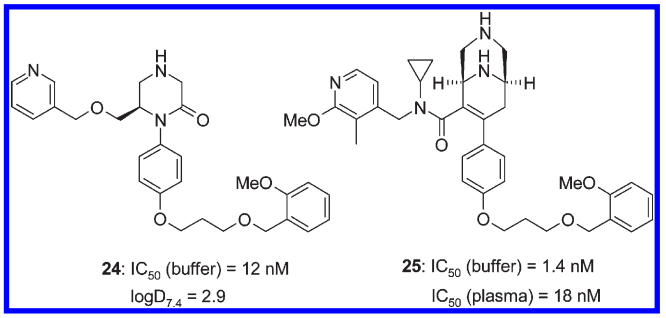


Figure 10. Renin inhibitor (P_3-P_1)-pharmacophores incorporating basic residues.

The 5-aryl-2,4-diaminopyrimidine (Figure 6)[38] constitutes a remarkable scaffold that functions as a basic TS surrogate by interacting with the catalytic dyad and in addition is part of a conformationally constrained (P_3-P_1)-pharmacophore. The X-ray structures of renin-bound **12** and related analogues revealed the C6 ethyl group to be positioned in the S_1 pocket closely overlapping with the P_1 isopropyl of **1**.[38] The 1,4-benzoxazinone of **34** (cf. Figure 19) extends into the S_3 pocket, with small alkyl groups at its (*S*)-configured C2 position fitting into a small indentation of the S_3 site, superimposing with the P_3 methoxy of **1**. The C2 phenyl of **35** is positioned within the hydrophobic but solvent exposed S_4 pocket, resulting in enhanced van der Waals contacts and hence increased binding affinities (**34** vs **35**).[38b] The C3 carbonyl favors a rigid conformation of the bicyclic moiety that directs the C2 phenyl toward S_4 and furthermore is involved in enzyme-water mediated H-bond interactions. Notably, a geminal disubstitution at C2 blocks this metabolical weak point prone to oxidative hydroxylation.

Researchers at Actelion were first to report the design of N-benzylated tertiary amides (**IV**, Chart 2) as a new pharmacophore tightly filling the hydrophobic S_3-S_1 pocket when attached to a 7-aryl substituted 3,9-diazabicyclo[3.3.1]nonene template (Figure 10) and related monocyclic core scaffolds.[55] These findings resulted from a structure-based design effort to identify GRAB peptidomimetics with superior pharmacokinetic properties. The nature of the bicyclic scaffold with its vinylic double bond at the 6,7-position required a replacement of the ether linkage of previous (P_3-P_1)-pharmacophores (for example, compare **25**, Figure 10, with **30** and **31**, Figure 16)[36,58] by a chemically stable functional spacer group. A parallel chemistry approach uncovered 2,3-disubstituted benzylamides with a *N*-cyclopropyl group to be optimal for in vitro potency, particularly in the presence of plasma.[55] The X-ray structure of **52** (ACT-077825/MK-8141, Figure 11 and Figure 23)[55] in complex with rh-renin revealed the *N*-cyclopropyl to be positioned in a small subpocket of the open-flap conformational topography of renin. Remarkably, the cyclopropyl binding position closely superimposes with that of the P_1 isopropyl residue of **1** in complex with the β-strand binding active site conformation (Figure 11). Also, the 2,3-disubstituted phenyl of **52** overlays with the P_3 phenyl group of **1** with close to perfect overlap of the 3-methyl and the 4-methoxy groups, respectively. Introduction of tethered H-bond acceptor groups to the C5 phenyl position of **52**, or related analogues, to target the S_3^{sp} pocket has also been investigated to optimize for potency.[15]

The tertiary benzylamide P_3-P_1 motif has been further investigated in combination with 3,4-disubstituted piperidines and related basic monocyclic center scaffolds by several other

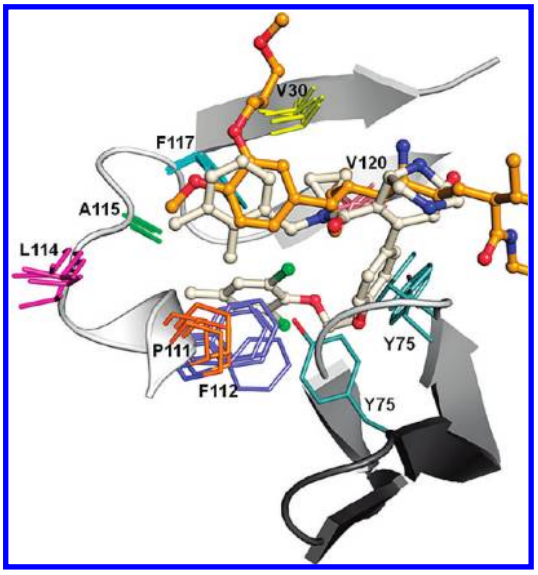


Figure 11. Conformational rigidity of the large contiguous S_3-S_1 binding site of renin, as illustrated by the overlay of several X-ray crystal structures of enzyme-bound inhibitors from distinct compound classes. Shown are the hydrophobic side chains lining the boundaries of S_3 (Thr_{12}, Gln_{13}, $\underline{Pro}_{111}$, $\underline{Phe}_{112}$, $\underline{Leu}_{114}$, $\underline{Ala}_{115}$, $\underline{Phe}_{117}$, Ser_{219}) and S_1 ($\underline{Val}_{30}$, Asp_{32}, $\underline{Tyr}_{75}$, Thr_{77}, $\underline{Phe}_{112}$, $\underline{Phe}_{117}$, $\underline{Val}_{120}$, Asp_{215}; the residues shown in the figure are underlined), as observed for **1** (orange), the GRAB peptidomimetic **52** (gray), the 2,4-diaminopyrimidine **34**, and the *N*-ureapiperidine **38**. Inhibitors **34** and **38** were omitted for clarity. A partial ribbon diagram is shown for the complex with **1** (gray) and in addition for the flap β-hairpin as observed for **52** (dark gray). Phe_{112} in the complex with **52** (flap pocket binder) is shifted significantly with respect to the other complexes. Large movements are observed only for the flap β-hairpin (e.g., 5.1 Å for the Cα of Tyr_{75} in the complex with **1** vs **52**).

groups.[15] These include analogues bearing extended side chains with terminal functional groups capable of forming a H-bond to Tyr_{14} of the S_3^{sp} subpocket (vide infra), such as N-tethered indoles (**V**, Chart 2).[15] Novartis has disclosed *N*-cyclopropyl-*N*-1,4-benzoxazinone carboxamides (**VI**) attached to a 3,5-disubstituted piperidine center scaffold leading to a new class of DRIs.[59]

The design of less hydrophobic (P_3-P_1)-pharmacophores has been explored by incorporation of basic amines or aromatic heterocycles into different chemotype DRIs.[36c,55a,56,58a,d] Analogues of **1** bearing a basic P_1 tertiary amine motif were reported to be potent toward plasma renin in vitro.[56] The group at Speedel has claimed P_3^{sp}-substituted pyridine analogues of **1** exhibiting high potency in vitro, albeit no detailed information on their potential was disclosed.[60] In the 2-ketopiperazine series of GRAB peptidomimetics, replacement of bicyclic P_3 motifs by simplified benzyl and pyridinyl ethers has been investigated using a Topliss approach.[58d] Notably, the 3-pyridinyl derivative **24** (Figure 10) was the only regioisomer shown to be active against rh-renin. The critical position of the pyridyl nitrogen atom was rationalized by H-bonding to Ser_{219} suggested by modeling. Inhibitor **24** had a more favorable profile with respect to solubility, cell permeability, and affinity to CYP3A4 in comparison to its phenyl congener.[58d] More hydrophilic 4-pyridinyl substituted P_3 tertiary amides were well tolerated when combined with the 3,9-diazabicyclo[3.3.1]nonene scaffold.[55a] Inhibitor **25** exhibited only a 10-fold plasma IC_{50} shift, while a 50-fold drop in plasma renin potency was observed for its 3-methoxy-2-methylphenyl analogue (buffer rh-renin IC_{50} = 0.62 nM). While increasing evidence has emerged for the

feasibility to design less hydrophobic S_3-S_1 binding motifs, it remains to be seen whether this strategy will lead to the design of novel DRIs with more promising characteristics.

The S_3-S_1 binding topography of renin recognized by structurally diverse classes of non-peptide peptidomimetics emulating the extended β-strand binding conformation of the active site is remarkably conserved and very similar to the topography recognized by substrate-based DRIs. The hydrophobic amino acids lining the S_3 and S1 pockets are located on four different sections of the protein chain (β-3, flap region, α h_{N2}, β-8). Their peptide backbone and side chains reveal only minor conformational differences in the X-ray crystal structures of various renin−inhibitor complexes (Figure 11). The carboxy-terminal boundary of the S_1 pocket is occupied by the aromatic ring of Tyr_{75}, which separates the S_1 from the S_2' pocket when the β-hairpin is in a closed conformation.

4-Phenylpiperidine GRAB peptidomimetics stabilize a renin active site conformation that is substantially different from the extended β-strand binding conformation observed for classical peptide-like inhibitors.[29,36,55,58] The distinct topography results from major movements of the β-hairpin, in particular of the backbone and side chains of Tyr_{75}, Leu_{73}, and Trp_{39}. This places the inhibitor C4 phenyl into the space vacated by the Tyr_{75} aromatic ring, thereby reconstituting the carboxy-terminal boundary of the S_1 pocket (vide infra). Most notably, these rearrangements have little impact on the conformation of the residues defining the boundaries of the S_3 pocket and major portions of S_1 (Figure 11). Moderate shifts, for example, of the Phe_{112} side chain deeper toward the bottom of the S_3 site (by ~1.5 Å) and of Val_{120} have been observed in some cases.[36] In the X-ray complexes with GRAB peptidomimetics, the renin flap region is in a more "open" conformation than observed for DRIs targeting the substrate-binding active site topography. The largest backbone displacement of ~6 Å away from the ligand position is noted for the Cα of Thr_{77} at the tip of the flap β-turn (Tyr_{75}-Ser_{76}-Thr_{77}-Gly_{78}). However, the Tyr_{75} phenyl side chain, rotated outward from its buried former position, is now directed toward the active site cleft, thereby closing the spatial gap to the enzyme-bound inhibitors (Figures 11 and 12).

The X-ray crystal structures of the topological peptidomimetics **28** and **29** (Figure 14) and a close analogue of **22** (Figure 8),[13,51] bearing a carbonyl group bridging the P_1 and P_3 residues, revealed a H-bond interaction to the Thr_{77} hydroxyl at the tip of the flap region in its closed conformation. In vitro SAR data suggested this Thr_{77} flap−inhibitor interaction to provide an important contribution to binding affinity.[13] In the case of peptide-derived inhibitors, the hydroxyl of Thr_{77} forms H-bonds to the nitrogen of the P_2/P_3 carboxamide and to the P_2 carbonyl.[30,32] In the renin-bound complex of several GRAB peptidomimetics, the reoriented Tyr_{75} phenylhydroxyl overlaid within a remarkably close ~1−2 Å distance with the hydroxyl side chain of Thr_{77} in the closed substrate-derived flap conformation observed for **4**, **28**, and **29** (Figure 12). This may suggest a potential role of the Tyr_{75} hydroxyl for the future design of novel GRAB peptidomimetic DRIs.

The hydrophobic and contiguous S_3-S_1 recognition pocket of renin is now well established as a key hot spot for high affinity ligand binding. All potent DRIs reported to date closely interact with this spacious topography of the target enzyme. The hydrophobic solvent exposed S_4 and the non-substrate S_3^{sp} cavity are directly accessible from S_3 and have been successfully exploited for novel inhibitor design. Also,

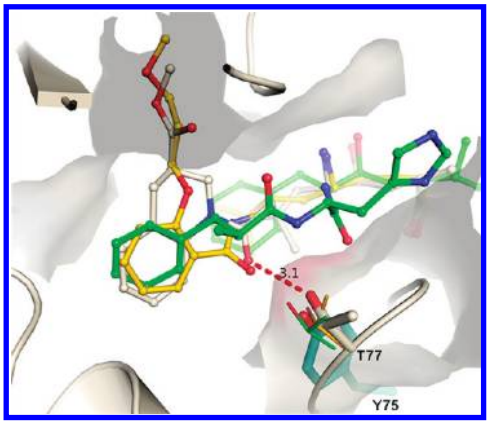


Figure 12. Hydrogen-bond interactions of the (S_3-S_1)-topological peptidomimetic **28** (gray) and a close analogue of **22** (yellow, PDB code 2v12),[51] as well as peptidic inhibitor **4** (green), with Thr_{77} of the flap β-turn. Portions of the enzyme chain in the complex with **28** are shown as a ribbon diagram in gray, and the Thr_{77} side chains in the different complexes are shown with the color coding of the respective inhibitors. Flap domains are in closed conformations. The hydroxyl of the flap Tyr_{75} (cyan) in complex with the GRAB peptidomimetic **32** (inhibitor not shown) is in a similar position as the Thr_{77} hydroxyl (0.9 Å distance) in complex with **28**. The H-bond between the carbonyl of **28** and the Thr_{77} hydroxyl is indicated.

both the S_3-S_1 subsite and the Trp_{39} "deep flap pocket" form a large open cleft in the renin conformational topography stabilized by GRAB peptidomimetics, as will be discussed in more detail in a later section devoted to this class of DRIs. This large accessible surface area may allow generation of unprecedented structural motifs binding to these, or possibly alternative, conformations, which could be combined with known center scaffolds interacting with the catalytic dyad. Conversely, it appears conceivable that the identification of new TS surrogates may offer distinct trajectories for novel (P_3-P_1)-pharmacophores with high ligand binding efficacy. Very likely, the S_3-S_1-topography of renin will remain a major target for future design of diverse non-peptide DRIs with possibly enhanced druglike characteristics.

Nonsubstrate S_3^{sp} Binding Pocket

Several investigators have independently discovered the presence of a distinct narrow cavity in native renin, extending from the S_3 site perpendicular to the active site cleft toward the center of the enzyme.[13,61−63] This pocket, now generally termed S_3^{sp}, is occupied by ordered water molecules and is not involved in substrate or peptide-based inhibitor binding. To our knowledge, Hanson et al.[61] were first to postulate this cavity as a potential auxiliary binding site based on computational energy surface analysis of a renin−peptide inhibitor complex and SAR data. The X-ray structure of **26** (SC-51106, Figure 13)[61] in complex with deglycosylated rh-renin demonstrated the β-(*S*)-methyl of the hydrocinnamoyl portion to occupy S_3^{sp} by replacing one enzyme-bound water. Lefker et al.[63] have reported the design of **27** (CP-305,202, Figure 13) and showed by X-ray that the flexible 3-pyridylpropoxy side chain was binding into the S_3^{sp} cavity.

Researchers at Ciba-Geigy/Novartis have described the design of topological AHE dipeptide isosteres bearing distinct hydrophobic (P_3-P_1)-pharmacophores.[12,13,49] The potent inhibitors **20** (Figure 8) and **28** and **29** (Figure 14) were discovered by X-ray crystallography to interact with the non-substrate S_3^{sp} pocket of renin. A key element of the evolving

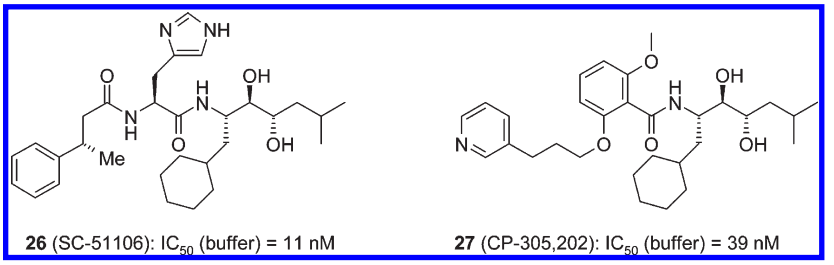


Figure 13. Early examples of DRIs binding to the S_3^{sp} subpocket of renin.

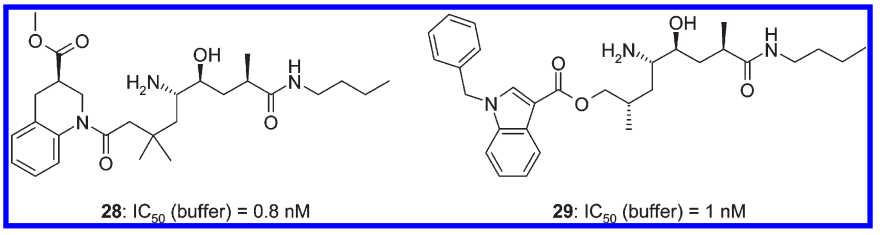


Figure 14. (S_3−S_1)-topological peptidomimetics binding to the nonsubstrate S_3^{sp} pocket.

design concept at Novartis has been to target H-bond interactions to Ser_{219} of renin.[13] This position is highly conserved as either serine or threonine in all aspartic proteases. Interactions with this residue had been recognized by in vitro SAR data and X-ray crystallography of multiple enzyme−inhibitor complexes to be important for the alignment of substrate-based peptidomimetics within the active site,[44] including renin and BACE-1 inhibitors.[40b] Hence, substitution of hydrophobic (P_3−P_1)-scaffolds of weakly active leads with suitable H-bond acceptors/donors was considered to mimic the conserved H-bond of the main chain P_3/P_2 carboxamide known to be essential for the tight binding of peptide-based inhibitors. The carboxylic ester **28** (Figure 15), a precursor analogue of **19**, was indeed shown by X-ray to interact with both the NH and OH of Ser_{219} (vide infra). Inhibitor **1**, but not the P_2' analogue **20**, forms a water-mediated H-bond between the proximal ether oxygen of the P_3^{sp} side chain and the Ser_{219} hydroxyl, which however is not critical for binding.[12,13,49]

The carboxylic ester of the tetrahydroquinoline analogue **28** is filling the S_3^{sp} cavity only partially with the methyl group being suboptimal in length and in addition forms a direct H-bond to Ser_{219} at the entrance of this site. In contrast, both the hydrophobic *N*-benzyl residue of the indole derivative **29** and the more polar flexible methoxypropoxy side chain of **20** penetrate deeply inside and fully occupy the S_3^{sp} pocket. Moreover, the terminal methoxy group of **20**, and likewise of **1**, is involved in H-bonding to the NH of Tyr_{14} at the bottom of the channel (Figure 15).[12,49,51] Most notably, these unprecedented binding interactions to the nonsubstrate S_3^{sp} site had not been predicted by molecular modeling. Further SAR efforts resulted in the identification of the preclinical candidates **19** and **22** (Figure 8) which exhibited high potency in vivo.[13]

The three-dimensional architecture of the renin S_3^{sp} subsite and its electrostatic surface properties are now well-defined by multiple enzyme−inhibitor X-ray crystal structures (Figure 15). This cavity constitutes a narrow channel extending from the P_3 pocket by about 10 Å in depth, flanked by residues located on five different segments of the enzyme. The hydrophilic nature of the channel is reflected by its capability to bind up to three water molecules in native renin and to accommodate inhibitors with a range of polar residues, including basic aliphatic amines.[36]

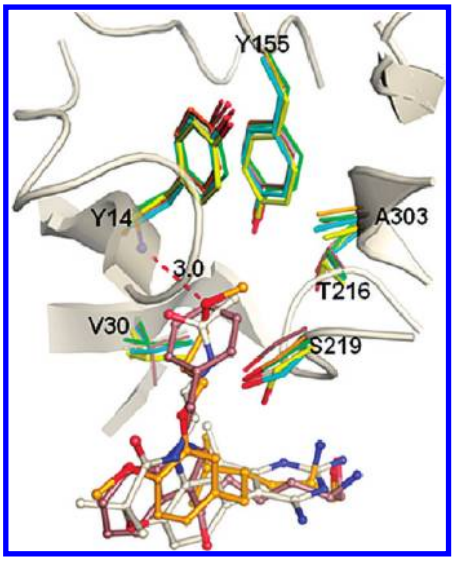


Figure 15. Illustration of the conformational rigidity of the renin S_3^{sp} pocket. Shown are the superimposed X-ray structures of rh-renin in complex with **1** (orange) (protein ribbon diagram in gray), *N*-benzylindole **29** (brown), and the *N*-ethylacetamide-substituted 2,4-diaminopyrimidine **34** (gray). Of the key side chains lining the boundaries of S_3^{sp} (Thr_{12}, Gln_{13}, $\underline{Tyr}_{14}$, $\underline{Val}_{30}$, $\underline{Tyr}_{155}$, $\underline{Thr}_{216}$, Gly_{217}, $\underline{Ser}_{219}$, and $\underline{Ala}_{303}$) those underlined are shown with the color codes used for the inhibitors. In addition, the corresponding side chains are shown for the renin complexes with inhibitor **4** (green), the *N*-hydroxypropyl analogue of 2-ketopiperazine **48** (PDB code 2fs4, cyan), and native renin (yellow).[31] The H-bond of the methoxypropoxy side chain of **1** to the backbone amide nitrogen of Tyr_{14} is highlighted.

Several lipophilic side chains lining the cavity boundaries explain the propensity of S_3^{sp} to accommodate equally well hydrophobic aliphatic and aryl ligands. Filling the S_3^{sp} pocket while displacing one or more bound water molecules and extruding them into bulk solvent is considered to be favorable because of entropic and enthalphic energy gains.[51]

Targeting binding interactions to the nonsubstrate S_3^{sp} pocket has significantly enlarged the arsenal for designing potent and selective DRIs and has been a key element in lead optimization of several new classes of non-peptide peptidomimetics including piperidines (**30** and **31**, Figure 16),[36] 2,4-diaminopyrimidines

(**34**−**37**, Figure 19),[38b] *N*-ureapiperidine-based reduced amide isosteres (**38**−**41**, Figure 20),[39] and 2-ketopiperazines (**46**−**50**, Figure 22).[58] Substituting distinct (P_3−P_1)-pharmacophores of various non-peptide peptidomimetics with optimized S_3^{sp}-binding residues has generated DRIs with a ≥50-fold increased potency,[13,19,39,58c] illustrating the importance of S_3^{sp}−ligand interactions for tight binding. Terminal alkyl ethers, acetamides, and alkyl carbamates were, in general, found to be optimal for in vitro potency toward rh-renin and more importantly often provided inhibitors with small in vitro plasma IC_{50} shifts (vide supra).[36,39,49] Conversely, combinations with hydrophobic P_3^{sp} motifs resulted in a significant loss of potency in the presence of plasma, even within inhibitor classes that generally demonstrated low lipophilicity and high solubility, as represented by **19**−**22** (Figure 8).[13,49] The specific structure−activity relationship for P_3^{sp} side chain modifications may be depending, to some extent, on the class of DRIs.[36,38b,39]

Introducing more hydrophilic P_3^{sp} side chains has also been explored as a strategy to modulate physicochemical and ADME properties of various classes of DRIs.[36,38,58] The group at Hoffmann-La Roche was the first to investigate this approach as part of the optimization of their highly lipophilic (log P > 5) GRAB peptidomimetic series (**30** and **31**, Figure 16).[36] The tetrahydroquinoline moiety of **30** (RO-65-2349) provides an ideal vector for hydrophilic extensions toward S_3^{sp}, as was suggested by the X-ray structure of **32** (Figure 18) bound to rh-renin.[36b,d] The less lipophilic and more soluble **31** (RO-66-1168), bearing a polar *N*-acetylaminoethyl residue, showed picomolar IC_{50} against rh-renin and most notably a 50-fold increase in potency against plasma renin. This compound demonstrated potent BP lowering efficacy in vivo but had poor PK properties in rat.[36d]

The group at Pfizer has subsequently used a similar concept to enhance the in vitro potency of related 2-ketopiperazine GRAB peptidomimetics and to address their strong affinities for CYP3A4.[58] Various linear side chain functionalities were investigated to probe the requirements for efficient S_3^{sp} binding interactions. These modifications resulted in subnanomolar inhibitors of rh-renin (cf. **46** vs **47**−**50**, Figure 22), albeit no IC_{50} values for plasma renin were reported. While a terminal hydroxyl, and particularly a carboxylic acid residue, reduced the affinity for CYP450 enzymes, these modifications were not well-tolerated in this series leading to modest in vitro potencies.[58e] The acidic carboxylate of these inhibitors does not interact with the S_3^{sp} cavity but rather is solvent exposed, as was shown by X-ray analysis (PDB code 2g26) of a 2-ketopiperazine analogue bearing a (1*H*-indol-1-yl)acetate moiety.[58f]

Extensive efforts were conducted at Pfizer to optimize the 2,4-diaminopyrimidine class of DRIs via modification of the P_3^{sp} side chain with the aim to modulate the physicochemical profile, CYP450 affinity, metabolic clearance, Caco-2 permeability, and in vivo PK.[38] Inhibitors **34**−**37** (Figure 19) illustrate key examples for an expanded exploration of S_3^{sp} binding motifs in combination with variations of the 1,4-benzoxazinone scaffold targeting the S_4 pocket. The persisting challenge to accomplish a proper balance between inhibitory potency and desirable ADME properties within this series will be further discussed in later sections.

The buried human renin S_3^{sp} cavity is characterized by high rigidity and hence a conserved van der Waals volume. Multiple X-ray crystal structures of apo-renin and renin-bound non-peptide inhibitors from different classes and bearing a diversity range of hydrophobic vs more polar S_3^{sp} binding motifs revealed only minor conformational movements for the amino acid side chains forming the boundaries of this pocket (Figure 15). Linear small side chains extending by about four to six atoms in length are preferred for optimal interactions with S_3^{sp}, in line with its well-defined elongated shape. Terminal H-bond acceptors form a favorable binding interaction with the NH of Tyr_{14}, by extruding the ordered water of apo-renin, as illustrated in Figure 15 for **1** and the 2,4-diaminopyrimidine **34**.[38b] The formation of a S_3^{sp} bound water-mediated H-bond to Thr_{216} has been observed for the *N*-hydroxypropyl analogue of 2-ketopiperazine **48** (Figure 22).[58e] An additional H-bonding opportunity involving Gly_{217} at the entrance of the S_3^{sp} site has been identified for a terminal methyl carbamate residue.[39] The lack of conformational flexibility of the S_3^{sp} cavity explains why sterically more demanding ligands are less tolerated, limiting the scope for structural modifications. Notably, the S_3^{sp} channel is present in other human aspartic proteases,[64] which in principle could have implications for the selectivity of DRIs (vide infra).

The discovery of the nonsubstrate S_3^{sp} cavity of renin as a targetable ligand binding site has contributed considerably to the recent advancements toward identifying several attractive new DRI chemotypes. The preferred P_3^{sp} pharmacophores reported to date, however, contain features such as a relatively high number of rotatable bonds and H-bond acceptors/donors that could limit oral absorption and in vivo metabolic stability or enhance the drug−drug interaction (DDI) potential. This is most evident when they are combined with unfavorable characteristics predominating in the basic pharmacophore of an inhibitor class. On the other hand, the design of potent non-peptide DRIs that are not dependent on interactions with S_3^{sp} has been successful. The most prominent example, the GRAB peptidomimetic **52** (Figure 23),[55a] had advanced to clinical trials. Future work will show whether novel class DRIs lacking a P_3^{sp} pharmacophore could offer advantages with respect to PK, in vivo efficacy, safety, and tolerability.

Basic Amine Transition-State Surrogates

The conceptual idea of modifying the dipeptide cleavage site of a peptide substrate sequence and of introducing a basic amino group at this position was reported as early as 1972 by Szelke et al. for the design of DRIs.[20,65] Replacing the scissile Leu-Val dipeptide of a minimal substrate sequence derived from angiotensinogen by a nonhydrolyzable reduced peptide bond isostere (ψ[CH_2NH], Chart 1C) generated the potent decapeptide L-prolyl-L-histidyl-L-prolyl-L-phenylalanyl-L-histidyl-L-leucyl-ψ(CH_2-NH)-L-valyl-L-isoleucyl-L-histidyl-L-lysine (H-142),[66] one of the first DRIs that underwent investigational clinical studies and that was shown to reduce BP and circulating AngI and AngII levels in humans.[67] The geometry of the reduced amide methylene was considered to be a mimic of the tetrahedral transition state for the proposed mechanism of peptide bond hydrolysis.[68] X-ray crystal structures of this and other peptidic inhibitors in complex with fungal aspartic proteases revealed the likely protonated nitrogen atom of the reduced amide to be in H-bond distance to only Asp_{215} of the catalytic dyad.[28,44,45]

Hydroxyl-containing dipeptide isosteres such as statine (Sta, Chart 1B)[69] of the pepstatin family of natural aspartic protease inhibitors[34] and the hydroxyethylene isostere (HE, Chart 1A)[20] have subsequently attracted the most attention and emerged as the key paradigm of aspartic protease inhibitor design. These TSAs, considered to resemble the

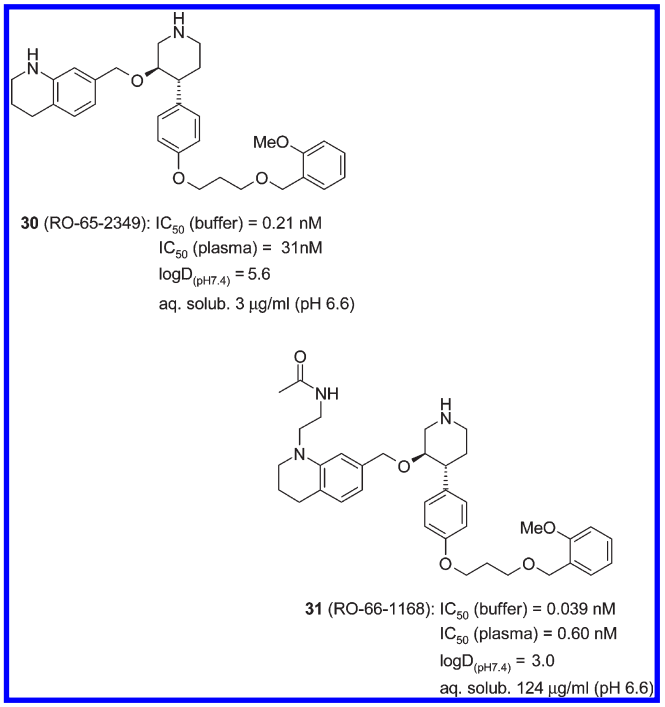


Figure 16. 3,4-Piperidine GRAB peptidomimetics: P_3^{sp} side chain approach.

geometry of the tetrahedral intermediate of enzyme-catalyzed amide bond hydrolysis, are engaged in a close H-bond network between the isostere hydroxyl and the active site Asp_{35} and Asp_{215} carboxylates. The binding position of the hydroxyl group is very similar to the site occupied by the extruded enzyme-bound "substrate" water molecule of native renin, suggesting at least in part a collected-substrate mechanism of inhibition.[25,27,29] A rich collection of highly effective acyclic hydroxyl-containing TSAs has evolved, and these have found wide application in the design of highly potent peptide-derived peptidomimetic DRIs (**2**−**7**, Figure 3).[20−23]

Relatively few attempts were made initially to design basic amine-based TSAs as a concept to overcome the limitations associated with peptide-derived peptidomimetic inhibitors. Several modified TSAs in which the hydroxyl is replaced by an isosteric NH_2 have been investigated anticipating favorable electrostatic interactions between a positively charged ammonium group and the Asp_{32}/Asp_{215} carboxylates. Incorporation of the aminoethylene (AE) motif (Chart 1E) into angiotensinogen-based peptide sequences has generated potent inhibitors of human renin and notably the first in vivo active inhibitors of rat renin.[70] The AE isostere approach was recently revisited for the design of soluble non-peptide isonicotinamide-derived BACE-1 inhibitors with good intracellular potency.[71] The cocrystal structure of an enzyme-bound analogue[71] has demonstrated the NH_2 to be in a binding position identical to that for the hydroxyl of an octapeptide BACE-1 inhibitor bearing a central hydroxyethylene dipeptide isostere and forming H-bonds to both catalytic aspartates.[41] Peptide-derived analogues containing aminostatine (AmSta, Chart 1F)[72,73] displayed potencies toward rh-renin similar to that of their Sta congeners and superior inhibition of rat renin.[72] Potent 1,2,4-triazolo[4,3-*a*]pyrazine-based inhibitors bearing a P_1 cyclohexyl-modified AmSta have been reported to reduce BP in Na-depleted marmosets when given parenterally.[73] For both Sta and AmSta, the 3(*S*)-configuration was preferred for binding, albeit the relative potency difference for the (*S*)- vs (*R*)-AmSta isostere was not very pronounced depending on the inhibitor framework. The lack of superior inhibitory affinity for AmSta vs Sta analogues was rationalized by unfavorable partial desolvation energy required for the ammonium group upon binding to the active site.[72c]

The hydroxyethylamine (HEA, Chart 1G) isostere has been envisaged to retain the favorable interactions of the tetrahedral carbon bearing the hydroxyl group and to mimic the dipeptide nitrogen atom during substrate hydrolysis. Initially, octapeptide HEA inhibitors of high potency against human renin have been reported.[74] Tripeptide-based (P_3−P_1)-spanning amino alcohols with a terminal NH_2 at the prime site showed only weak inhibitory affinities.[75] On the other hand, truncation of the nonprime site has generated moderately active P_1−P_3' tetrapeptides with primary or secondary amines at the P_1 position (AHEA, Chart 1H).[52,53] Interestingly, the reversed (*R*)-configuration is preferred for the AHEA hydroxyl. The cocrystal structure of a (*S*)-AHEA containing inhibitor bound to endothiapepsin has revealed the terminal amine to be in weak contact with Gly_{217} and the hydroxyl group to interact with both aspartates. By use of a human renin homology model, optimization of the occupancy of the S_1 pocket was achieved by extending the hydrophobic P_1 residue to afford the potent inhibitor **23** (Figure 9).[52,53]

Incorporation of the HEA dipeptide isostere has been very successful for development of several marketed antiviral HIV-1 protease inhibitors[76] and more recently for the design of BACE-1 inhibitors.[40] Furthermore, tertiary carbinamines have been designed as TS surrogates by simplification of the HEA motif to a primary amine, which in combination with a bioisosteric replacement of the P_1 carboxamide has culminated in a CNS penetrating BACE-1 inhibitor orally active in rhesus monkeys.[42]

The aminohydroxyethylene (AHE, Chart 1D) isostere of **1** and **19**−**22** constitutes an interesting variant of a basic amine central motif. Binding interactions to the catalytic dyad were found to be partially distinct from the H-bond pattern of classical hydroxyethylene (HE) isosteres. When incorporated into peptide-derived peptidomimetics, the HE hydroxyl is positioned symmetrically between Asp_{32} and Asp_{215} within the plane formed by the two carboxylates. In contrast, the X-ray structures of renin−bound **1** (Figures 4 and 17) and the tetrahydroquinoline analogue **28** revealed the NH_2 to be within binding distance of both the Gly_{217} carbonyl and the Asp_{215} carboxylate, possibly forming an electrostatic interaction.[51] The AHE isostere hydroxyl formed H-bonds to both aspartates, albeit its binding distance was closer to Asp_{32}. The hydroxyl of **20**, an analogue of **1** with modifications at P_1' and P_2', was engaged in a H-bond exclusively to Asp_{32}, while the NH_2 was within binding distance to the carbonyl of Gly_{217} but not to the aspartates.[49] These markedly altered H-bond patterns of the AHE moiety resulted from a positional shift of the ligands within the enzyme cleft and conformational adaptations of the isostere backbones. In addition, a slight movement of the Asp_{215} side chain was observed for **28**.[51] It is conceivable that novel amino alcohol motifs, possibly as part of a cyclic framework, will be designed in the future, leading to novel non-peptide DRIs.

The 4-phenylpiperidine scaffold was discovered by HTS as a novel druglike basic amine TS surrogate, first reported in 1999 by Hoffmann-La Roche.[36] X-ray crystallography of the potent prototype leads **32** and **33** (Figure 18), derived from the weakly active hit (*rac*)-**8** (Figure 5), demonstrated the

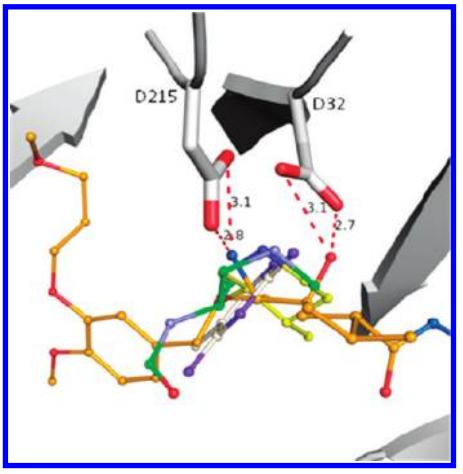


Figure 17. Overlay of different chemotype basic amine TS surrogate DRIs interacting with Asp_{32} and Asp_{215} of the catalytic center. Inhibitors shown are **1** (orange), the GRAB peptidomimetic **33** (yellow), the 2,4-diaminopyrimidine **34** (gray), and the $\psi[CH_2NH]$-reduced isostere **38** (green). For the rh-renin−**1** complex, residues Asp_{32}/Asp_{215} and a partial ribbon diagram are shown. To better distinguish the inhibitors, portions of their structures were omitted and nitrogen atoms were colored using different shades of blue.

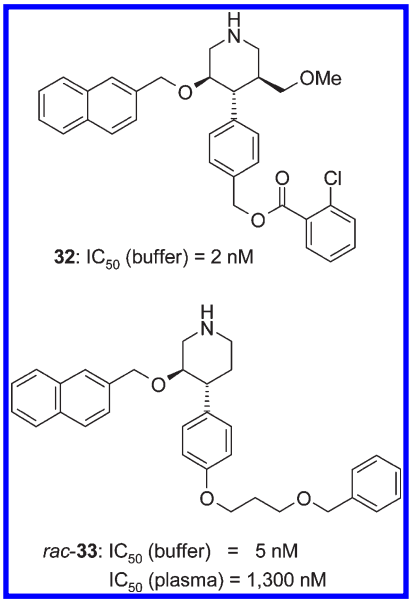


Figure 18. Early prototype 4-phenylpiperidine TS surrogate DRIs.

piperidine nitrogen atom to symmetrically occupy the pivotal position between the catalytic Asp_{32} and Asp_{215} of renin, similar to the hydroxyl group of classical dipeptide TS isosteres (Figure 17).[36a,b] The endocyclic nitrogen is most likely protonated, thus forming two enthalpically favorable electrostatically enforced H-bonds to both aspartates. In retrospect, it is remarkable that this intriguing new principle of efficiently blocking the catalytic site of an aspartic protease had not been uncovered earlier by de novo design considerations.[29] More strikingly, inhibitors **32** and **33** revealed a noncanonical ligand binding mode to a previously not observed conformational active site topography that is fundamentally different from the renin conformation targeted by substrate-derived peptidomimetics (vide infra). The C4 phenyl and substituents extending at its para-position are accommodated by a newly formed large hydrophobic binding site under the flap β-hairpin.[29,36] Furthermore, compared to the peptide-derived framework, the piperidine template provides a very distinct trajectory for topological extensions into the hydrophobic S_3-S_1 "superpocket", such as the naphthalene of **32** and **33** tethered at the C3 position.

From a conceptual perspective, piperidine and ring-size congeners have been postulated to constitute enzyme family directed privileged TS surrogates[36a,43] for the design of more druglike inhibitors of human and nonmammalian aspartic proteases. Indeed, novel non-peptide inhibitors based on mono- or bicyclic secondary amine center scaffolds have been described for the antimalarial target plasmepsin-II and -IV,[77] pepsin,[29,78] cathepsin D,[79] and the viral HIV-1 protease.[79] Intriguingly, the successful application of the piperidine core motif for the design of potent BACE-1 inhibitors has not been documented to the best of our knowledge.

Cyclic basic amine TS surrogates constitute highly versatile modules to attach structurally diverse peripheral pharmacophores that could reach into the enzyme specificity pockets flanking the core catalytic center or possibly target other yet uncovered ligand binding topographies of renin. Various piperidine-based inhibitors with distinct substitution patterns, including *trans*-2,5-, *cis*-3,5- and *trans*-3,5-disubstituted piperidines, and structurally diversified ring substitutions have been disclosed in the patent literature.[15] Related 3,4-*trans*-disubstituted pyrrolidines have also been explored by several groups and were claimed to be potent DRIs.[15] This is indicative of the intense interest in the design of such cyclic amine scaffolds and may suggest that the potential of diversely decorating such TS surrogates has not been fully exploited yet. Some evidence, based on the resemblance of common structural features, is emerging indicating that the design of such inhibitors with a binding pose allowing access to the prime site of renin is likely feasible.[15,36]

Predicting the binding interactions of unprecedented inhibitor chemotypes designed by de novo computational modeling relying on the enzyme structure−knowledge base and ligand structure commonalities remains challenging.[13,40b,79] Potent pyrrolidine-based HIV-1 protease inhibitors have been designed by decorating the 3,4-positions with side chains known from substrate-based inhibitors to optimally fill the S_2-S_2' recognition sites.[79] X-ray crystallography confirmed the pyrrolidine nitrogen of a prototype analogue to be positioned in binding distance to the catalytic dyad of the enzyme; however, the inhibitor adopted an unexpected binding pose which was distinct from the model on which the design was based. It was characterized by replacement of the structural flap water, a distinct H-bond pattern and altered occupation of the enzyme subsites, as well as a distortion of the geometry of the flap region.[36a,79] Hence, novel TS surrogates may potentially address previously unobserved energetically favored active site conformations, including deviations in the position and geometry of the catalytic aspartates.[40b,51,80]

The 2,4-diaminopyrimidine TS surrogate, discovered at Pfizer from an HTS hit (**10**, Figure 6), forms multiple H-bond interactions within the catalytic center of rh-renin, as shown by the X-ray crystal structures for several analogues of this inhibitor class (e.g., for **11**, **34**, and **36**; Figure 19).[19,38] The pyrimidine NH_2 at C2 is in binding distance to both catalytic Asp_{32} and Asp_{215}, and the N1 nitrogen interacts with the Asp_{32} carboxylate. Interactions of the pyrimidine NH_2 at C4 with Thr_{77} and Ser_{76} of the β-hairpin and of the N3 nitrogen with an enzyme-bound water contribute to the strong network of H-bonds and a significant gain in binding enthalpy as determined by ITC.[38c] The 2,4-diaminopyrimidine

framework, which is reminiscent of that of 2-aminopyridine and 2-aminoquinoline-based BACE-1 inhibitors,[81] was considered as a highly effective "recognition unit" for renin, and no efforts were made for further optimization of this particular subunit.[38a] Combining the TS surrogate scaffold at its 5-position with 2,2-disubstituted 1,4-benzoxazin-3-ones bearing a P_3^{sp} side chain resulted in analogues (**34**−**37**) exhibiting high potency in vitro. These inhibitors span the S_4/S_3 and S_1 sites of the extended β-strand binding active site conformation with closed flap conformation.[38] Various challenges to accomplish a proper balance between potency and desirable physicochemical and ADME properties were encountered for this class of DRIs (vide infra).[15,19,38b]

More than 30 years after the pioneering work by Szelke,[65] the incorporation of a ψ[CH_2NH]-reduced amide isostere has regained attraction as a viable concept for the design of new chemotype inhibitors of drug target aspartic proteases. First, this approach demonstrated success in generating open-chain and macrocyclic BACE-1 inhibitors with improved solubility, cell-permeability, and intracellular potency.[40b,82]

More recently, Vitae Pharmaceuticals has reported a new class of DRIs, exemplified by **38**−**40** (Figure 20), that emerged from an iterative structure-based de novo design (vide supra).[39] These inhibitors comprise a rigid 3-substituted *N*-ureapiperidine skeleton bridging the solubilizing basic amine TS surrogate and a substituted P_3 phenyl extended by a flexible P_3^{sp} side chain. Inhibitor **38** was confirmed by X-ray

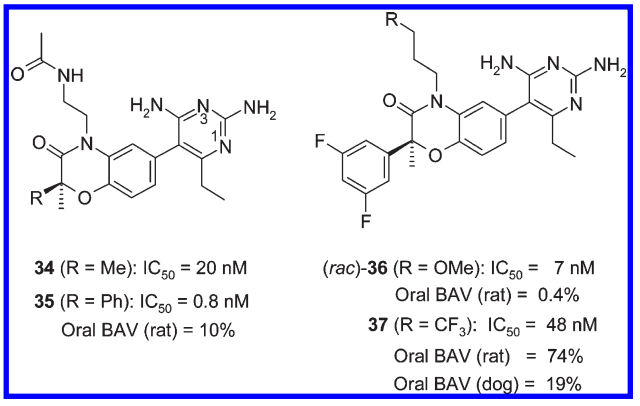


Figure 19. 2,4-Diaminopyrimidine-based peptidomimetics (IC_{50} in buffer).

to emulate the enzyme-bound β-strand conformation of peptide-derived peptidomimetics, as predicted by modeling. The piperidine scaffold serves to connect the hydrophobic P_1 cyclohexyl and the P_3 meta-Cl-phenyl in a nontopological fashion. The chlorine atom fills an additional hydrophobic subcavity formed by Pro_{118}, Phe_{119}, Ala_{122}, and Phe_{124}, resulting in a 15-fold potency increase.[39] Interestingly, the ψ[CH_2NH] nitrogen atom is positioned between both catalytic Asp_{32} and Asp_{215} carboxylates, in contrast to the binding mode observed for other reduced amide isostere inhibitors of aspartic proteases.[28,44,82] The tertiary hydroxyl of **38** was in H-bond distance (2.5 Å) to the γ-oxygen of Ser_{219} at the entrance of the S_3^{sp} cavity. This again illustrates the key role of Ser_{219} for the design of non-peptide DRIs by serving as a potential anchor position for the ligand framework within the enzyme cleft.[13,40b,44,51] Hence, the tertiary phenylcarbinol moiety of **38** constitutes a remarkably compact pharmacophore combining three key structural features important for strong inhibitor binding. Attachment of the P_3^{sp} side chain via an ether linkage and removal of the tertiary hydroxyl gave **39** with inferior potency but improved oral bioavailability in rat. Removal of the P_3^{sp} ethoxypropoxy chain resulted in a 70-fold IC_{50} increase, emphasizing the importance of binding to the S_3^{sp} cavity. The methylcarbamate analogue **40** showed a remarkably high potency toward plasma renin. Inhibitors **38** and **40** elicited a sustained BP reduction orally in a double-transgenic rat (dTGR) model.[39]

Further exploration of this chemotype has been a continued effort at Vitae, as judged from the patent literature.[83] Analogue **41** (Figure 20), bearing a regioisomeric modification of the basic amine TS surrogate and a more polar P_1 pyranyl ring, was claimed to be orally efficacious in a dTGR model.[83a] The *N*-carbonylpiperidine and related heterocyclic skeletons have been combined with various classical and novel TSAs.[15] Furthermore, the proximity of the P_3 phenyl and the P_1 cyclohexyl of renin-bound **38** and **40** has suggested growth of the phenyl template into S_1 in a topological fashion;[83b] however, no biological results have been reported to date.

Inhibitors **38**−**41** are reminiscent of the vicinal-diol TSA inhibitor **42** (CP-265,671) previously reported by Pfizer.[63] The tetrasubstituted cyclohexane provides a rigid vector for the phenethyl chain to reach into S_3 and for the ethyl to fill the S_2 pocket. The tertiary hydroxyl was incorporated to mimic the P_3/P_2 carbonyl of a peptidic inhibitor for Ser_{219}

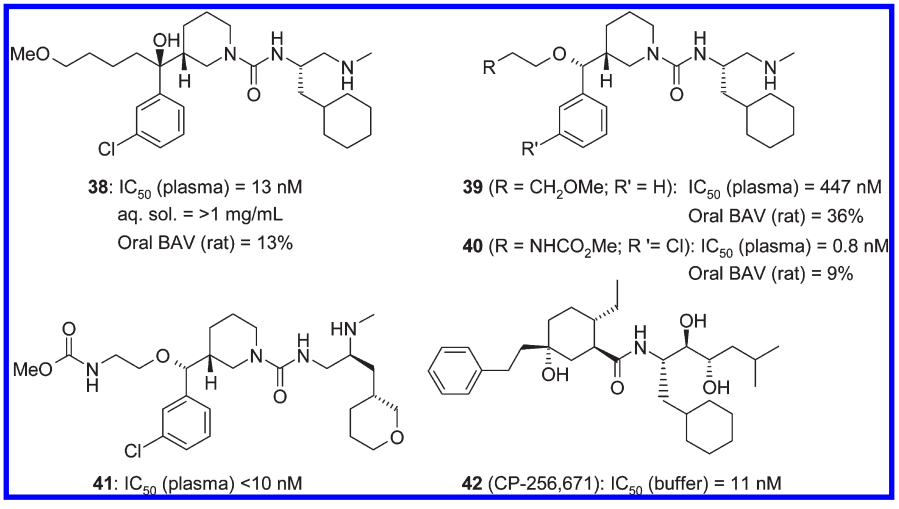


Figure 20. Non-peptide peptidomimetic ψ[CH_2NH]-reduced amide isosteres.

H-bonding.[44,63] Such compounds were abandoned because of low solubility, weak potency in plasma, and synthetic complexity but laid the foundation for the design of P_3^{sp}-tethered inhibitors like **27** (Figure 13).[63]

A diverse array of novel cyclic or acyclic basic "warheads" have been reported to potently inhibit BACE-1[40] and other aspartic proteases.[84] Some of these TS surrogates were utilized for the design of DRIs, such as *N*-acylamidines and *N*-acylguanidines;[15] however, no biological results were disclosed to date. Non-peptide inhibitors displaying a nonclassical binding mode without engaging in direct interactions to the catalytic dyad were identified for BACE-1[64,80] and plasmepsin-II (PM-II).[84] X-ray crystallography revealed a single water-mediated H-bonding between the two aspartates and either an amide NH, as for the BACE-1 ligand,[64] or the basic nitrogen of an *N*-alkylpiperidine as for the PM-II inhibitor.[84] Notably, the water molecules were in a position similar to that of the "catalytic" water of the native enzymes. Spiropiperidine-iminohydantoin BACE-1 inhibitors were found to interact with Asp_{32} and Asp_{228} via two bridging water molecules, forming a complex H-bonding network involving the N-benzylated piperidine and the exocyclic imino nitrogen atom.[80] Remarkably, BACE-1 adopted a different active site conformational topography by forming a new binding site under the flap β-hairpin.[80] While such atypical inhibitors of renin have not been reported, the possibility for a water-mediated mechanism to block the catalytic center may in principle exist also for renin.

GRAB Peptidomimetic Class of DRIs

The discovery of 3-substituted 4-arylpiperidines as a unique class of potent DRIs, bearing several key structural features unrelated to peptide-derived TSA inhibitors, constitutes a major breakthrough.[36] As already discussed, the finding that the center piperidine acts as a TS surrogate by interacting with the catalytic aspartates has created a new paradigm for the design of non-peptide inhibitors of renin and other aspartic proteases.[36a,43] Most intriguingly, these inhibitors bind to a renin active site topography that is very different from the enzyme conformation targeted by substrate-derived peptidomimetics.

Inhibitors **31** (Figure 16), **43** (RO-66-7219), and **44** (RO-66-1132, Figure 21) represent advanced potent and selective analogues of this new class of inhibitors,[36d] which were derived from the HTS hit *rac*-**8** (Figure 5) via further optimization of the prototype leads **32** and **33** (Figure 18).[36a−c] The bicyclic pharmacophores attached to the piperidine C3 span and partially fill the hydrophobic S_3-S_1 hot spot and can be further substituted by residues targeting the nonsubstrate S_3^{sp} cavity. The lipophilic piperidine C4 phenyl and its extension at the C4′ position occupy a large hydrophobic cleft located underneath the S_3-S_1 pocket at the core of the N-terminal domain, as unveiled by X-ray crystallography of **32** and **33** bound to rh-renin (Figure 24). This cavity results from major conformational movements of the flap adopting a more open conformation, which specifically involve concerted side chain rotations of the Tyr_{75}, Leu_{73}, and Trp_{39} residues. As a consequence, the H-bond between the phenolic OH of Tyr_{75} and the indole NH of Trp_{39} is disrupted, and the reorientation of the Trp_{39} indolylmethyl opens up a buried "deep flap pocket". Interestingly, this movement of Trp_{39} is not observed for the weak affinity inhibitors **8** and **9**, lacking the C4′ extension.[36a,b]

The piperidine C4 phenyl of **32** and **33** occupies, in a similar planar orientation, the space of the "gate-keeper" Tyr_{75} side chain[29] and thus reconstitutes the carboxy-terminal boundary of the S_1 pocket by forming an aromatic cluster of edge-to-face interactions with Tyr_{75} and Phe_{112}.[36a,b] The lipophilic C4′ flexible spacer spans the position previously occupied by the Trp_{39} indole, while the terminal phenyl moiety is accommodated by an extension of the deep flap cavity generated by additional subtle local rearrangements, particularly by Met_{107} (Figure 24). Initial lead optimization included attempts to rigidify the conformational flexibility of the polyether side chain by incorporation of cyclic constraints; however, the ortho-methoxy-benzyloxypropoxy residue (**30**, **31**, **43**, **44**) was found to be optimal for high affinity interactions to the flap binding topography.[36b] The assisting role of the inhibitor C4 phenyl in stabilizing the enzyme complex by replacing the Tyr_{75} aromatic ring has inspired to coin this class of DRIs as "group replacement assisted binding" or GRAB peptidomimetics.[29]

The explicitly hydrophobic nature of the early GRAB peptidomimetics is mainly attributed to the large lipophilic (P_3-P_1)-scaffold and the C4′ extended side chain, both being key contributors to high affinity interactions to renin. Various SAR strategies have been explored to improve the limitations of their physicochemical properties, recognized as the major culprit for significant plasma renin IC_{50} shifts, affinity to cytochrome P450 isozymes, and high metabolic clearance. Modifications of the core pharmacophore by replacing either the piperidine C4 phenyl or the side chain terminal phenyl by hydrophilic heteroaryl groups resulted mostly in significant in vitro potency loss.[36b,c] Attachment of solubilizing residues, which could either bind to the renin active site or alternatively point out into bulk solvent was found to be more promising.[36c,d] Inhibitor **31** with a hydrophilic extension into the nonsubstrate S_3^{sp} cavity is the most potent GRAB peptidomimetic toward plasma renin reported to date. The piperidine C5 position proved to be amenable for the introduction of polar substituents with up to two hydroxyl and/or alkoxy groups. The methoxymethyl of **32** was shown by X-ray to point toward the reoriented Tyr_{75} of the flap, while the C(4)−C(5)-*trans*-configured **43** and **44** potentially extend into the S_2' pocket. This has generated less hydrophobic analogues, albeit with limited impact on the in vitro potency against plasma renin (cf. **30**, Figure 16).[36c] The preclinical candidate **44** (Figure 21) demonstrated a superior PK profile and displayed a potent sustained BP reduction in dTGR and in monkeys.[36d,e,85b]

This first generation of *trans*-3,4-piperidine GRAB peptidomimetics has inspired other investigators to further exploit this chemotype with the aim to improve the in vitro and in vivo ADME profile. Pfizer has described initially 3-amino-4-arylpiperidines with preferred relative *cis*-configuration, by replacing the ether linkage at the piperidine C3 with a secondary amine spacer.[58a] The most potent analogue, *rac*-**45** (Figure 22), was shown by X-ray crystallography to bind to renin in a similar fashion compared to the 3,4-*trans*-disubstituted alkoxyarylpiperidines but with an additional H-bond formed between Gly_{217} and the exocyclic nitrogen atom. Further modifications of the center scaffold were subsequently described by eliminating one chirality center, leading to piperazine and 2-ketopiperazine analogues **46**−**50** with a "reversed" ether linkage.[58b−f] Ketopiperazine **46** showed moderate in vitro activity and short-lasting oral BP lowering effects in hypertensive double-transgenic mice.[58b,c] The 3,4-dihydro-2*H*-quinoline derivatives **47**[58c] and **48**[58d] bearing a P_3^{sp} *N*-acetylaminoethyl side chain revealed markedly enhanced in vitro potencies. Further variations of the (P_3-P_1)-bicyclic

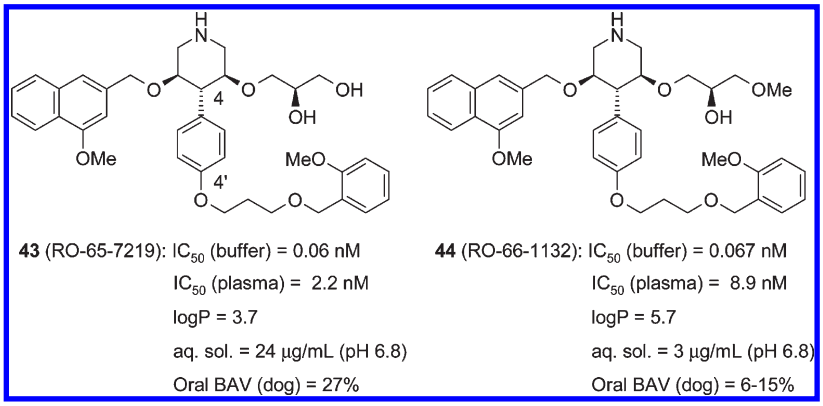


Figure 21. Advanced 3,4-piperidine-based GRAB peptidomimetics.

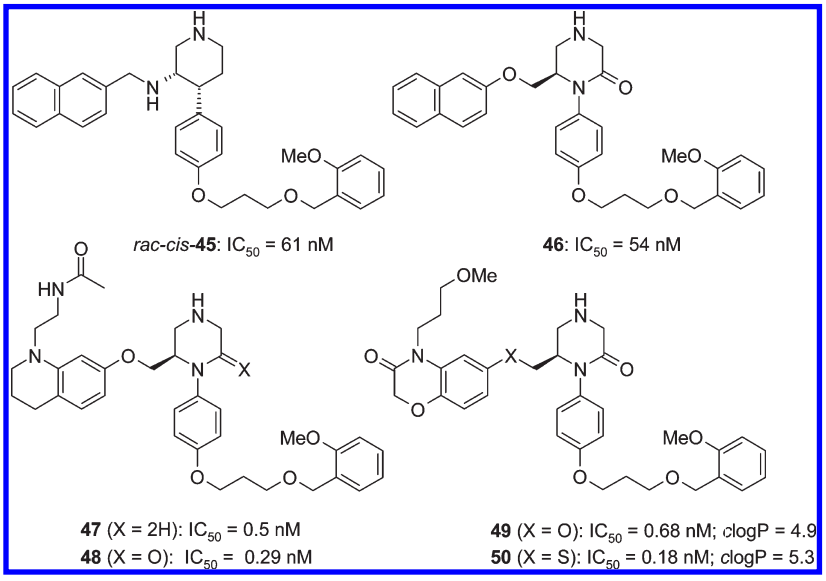


Figure 22. Piperidine-derived GRAB peptidomimetic inhibitors (IC_{50} in buffer).

moiety afforded the potent 1,4-benzoxazin-3-ones **49** and **50**.[58e] The lipophilicity of these inhibitors was reduced but remained noncompliant with Lipinski's rule,[86] suggesting a marked potency drop toward plasma renin to be very likely. Furthermore, most of the potent inhibitors of these series suffered from high affinities to CYP450 isozymes.[19,58] Modifications of the terminal ortho-methoxyphenyl and the propylenedioxy spacer were not envisaged, as this pharmacophore was considered to be optimal for binding into the flap pocket.[58e]

Actelion has reported a novel class of GRAB peptidomimetics based on a 3,9-diazabicyclo[3.3.1]nonene center scaffold (**51** and **52**, Figure 23), as well as modified bicyclic bridged templates.[55] Furthermore, related monocyclic tetrahydropyridine analogues were disclosed, representing the first achiral and in vitro potent DRIs known to date.[55c] Optimized inhibitors **51** and **52** were identified from a structure-based design approach involving multiparallel chemistry. Both analogues were potent toward rh-renin but showed a significant ~100-fold IC_{50} shift leading to moderate inhibitory affinities against plasma renin in vitro. This was explained by high plasma protein binding (> 99% for **52**) in human, dog, and monkey.[55a] The absolute configuration of the dibasic bicyclic core determines which of the two secondary ring nitrogen atoms interacts with the catalytic aspartates by forming H-bonds and expelling the enzyme-bound water, as shown by X-ray for renin-bound **51** and its equipotent (−)-(1*S*,5*R*)-enantiomer. The second nitrogen in turn is exposed toward bulk water and has served as anchor point for solubilizing groups, albeit none of the resulting inhibitors had a superior profile. This class of inhibitors comprise a benzylated *N*-cyclopropylcarboxamide as a novel P_3-P_1 motif (cf. Figure 11) providing high potencies toward plasma renin. Emphasis on rigidifying the conformational flexibility of the side chain in para of the 4-phenyl substituent led to the identification of the propyloxy (as in **51**) and 1,2-ethylenedioxy (as in **52**) spacers. A strained 2,6-substitution pattern with at least one ortho-chloro atom at the terminal phenyl, considered as a potential soft spot for metabolism, provided inhibitors with superior potencies. Inhibitors **51** and **52** were well absorbed in Wistar rats and proved to be orally efficacious in a dTGR model,[55a] as well as in salt-restricted, furosemide pretreated rhesus monkeys.[55b] The most preferred analogue, **52**, was progressed to phase IIa clinical trials.

The class of GRAB peptidomimetics is continuing to attract interest, as is implied by the large number of recent patent claims.[15] Design efforts are further exploring modifications of the flap binding motif in combination with new (P_3-P_1)-scaffolds and central piperidine replacements, including new substitution patterns like 2,4,5-trisubstituted piperidines. Design concepts that address the Trp_{39} flap pocket pharmacophore aim to reduce the number of rotatable bonds and to

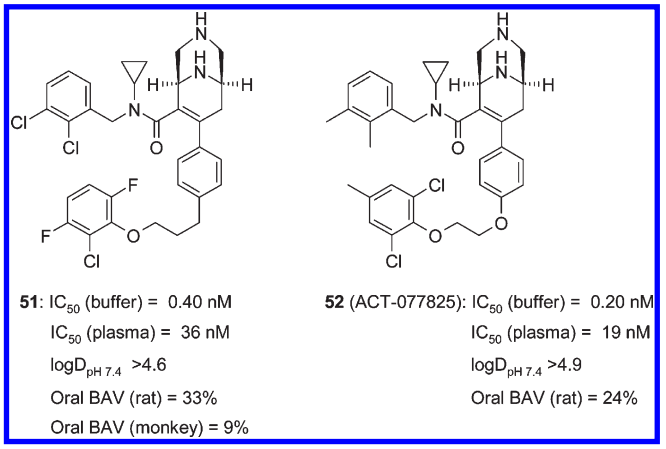


Figure 23. Second generation GRAB peptidomimetic DRIs.

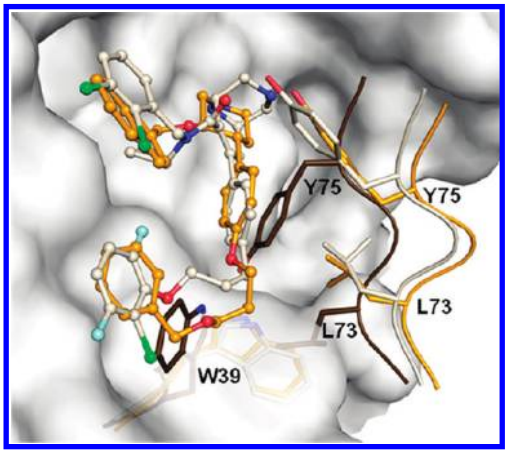


Figure 24. Superposition of X-ray structures of rh-renin complexed with GRAB peptidomimetics **33** (orange) and **51** (gray) binding to the open flap conformational topography. Partial peptide backbones and the side chains for Trp_{39}, Leu_{73}, and Tyr_{75} are shown for the two complexes using the same color code. The corresponding portions of the rh-renin−**1** complex are overlaid in brown. For the complex with **51** the portion of the active site behind the inhibitor is shown as a transparent surface.

replace the lipophilic C4′ portion by small polar side chains lacking the terminal aryl residue. Since more detailed biological data are currently not available, predictions of whether these approaches are likely to succeed in providing clinical candidates would be premature.

Notably, exploration of potent GRAB peptidomimetics, as reported to date, has been restricted to hydrophobic side chains of variable conformational flexibility that are exclusively attached to the para-position of the C4 phenyl binding into the Tyr_{75} pocket. Not surprisingly, analysis of X-ray crystal structures showed overall similar van der Waals contacts for such inhibitors to the open flap conformational topography and a conserved alignment of the side chains of Tyr_{75}, Leu_{73}, and Trp_{39} in the renin−inhibitor complexes (Figure 24).[36,55a] The 1,2-ethylenedioxy spacer of **52** adopted a more compact gauche conformation compared to the propyloxy spacer of **51**. Both terminal aryl groups are similarly positioned in the hydrophobic Trp_{37} flap pocket, albeit in the case of **52** the phenyl is not entirely filling out the cavity. In both cases, the ortho-chlorine occupied a hydrophobic indent, while the second ortho-halogen pointed straight toward the *N*-cyclopropyl in the S_1 site. The SAR of GRAB peptidomimetic inhibitors of plasmepsin II was shown to correlate with the degree to which the narrow well-defined flap pocket is filled by flexible ligand alkyl chains, in line with the principle of ideal volume occupancy for host−guest recognition.[77a]

The conformational mobility of the flap β-hairpin characterized by major spatial movements is part of its critical function to open and close the catalytic site, thereby enabling ligand binding and release. The disruption of flap domain H-bonds, such as between Tyr_{75} and Trp_{39}, is considered to be part of this dynamic process and hence to be at low energy cost involving low barrier conformational changes.[29,36a] It has been suggested that distinct pre-existing conformations of the renin active site are stabilized upon binding of complementary ligand structures rather than by an induced-fit adaptation binding mechanism.[29,87] Energetically favorable conformational ensembles with topographies distinct from that stabilized by known GRAB peptidomimetics may exist, which could be exploited for inhibitor design. Notably, 3,4-piperidine-based inhibitors that comprise a meta-extended C4 phenyl or more space-filling meta-biphenyl, benzothiophene, and N-substituted indole moieties at the C4 position of the TS surrogate have been claimed as novel DRIs.[15] Inspection by modeling would suggest that these residues could not be readily accommodated by the small Tyr_{75} pocket or may extend into the Trp_{37} deep flap pocket in a different manner as in the case of GRAB peptidomimetics bearing a para-phenyl substitution. Hence, distinct conformations of the flap region that could be stabilized by structurally distinct flap-binding pharmacophores are indeed likely to be accessible.

S_2 Specificity Pocket

Structural modification of the P_2 portion in peptide-derived peptidomimetics has played an important role for optimizing their potency and selectivity, as well as for designing analogues with improved pharmaceutical properties.[21−23] The large and bifurcated hydrophobic S_2 pocket of renin (Ser_{76}, Thr_{77}, Tyr_{220}, Phe_{242}, and His_{290}-Met_{292}), like that of related aspartic proteases, can accommodate a variety of both hydrophobic and polar pharmacophores and thus allows considerable variations in enzyme-ligand interactions. Indeed, a number of peptide-derived DRIs were shown by X-ray crystallography of their complexes to endothiapepsin to adopt a range of distinct P_2 side chain orientations as a consequence of the permissiveness of the S_2 pocket.[28,44,45] The fact that P_2 side chain conformations are not tightly constrained within this binding site has rendered accurate modeling predictions of ligand−enzyme interactions to S_2 rather difficult.[13,38c] The topography of both the S_2 and S_1' pockets forming a spacious open subsite has been exploited for the design of potent macrocyclic DRIs.[23a]

Most non-peptide classes of DRIs reported to date, including piperidine-based GRAB peptidomimetics (**43**, **44**, and **46**−**52**), as well as the (S_3−S_1)-topological peptidomimetics **1**, **19**−**22**, are not engaged in binding interactions to the S_2 specificity site. Topographic characteristics and promiscuity have suggested a limited attractiveness for explicitly targeting the S_2 pocket by rational de novo design of DRIs.[13,39] Such considerations, among other key aspects (vide supra), have formed the initial conceptual idea toward the design of (S_3−S_1)-topological peptidomimetic inhibitors that eventually led to the discovery of **1**.[12,13,49] Noteworthy, targeting optimized interactions to the S_2 pocket has been seminal in identifying potent inhibitors of BACE-1 with high selectivity against other human aspartic proteases.[40]

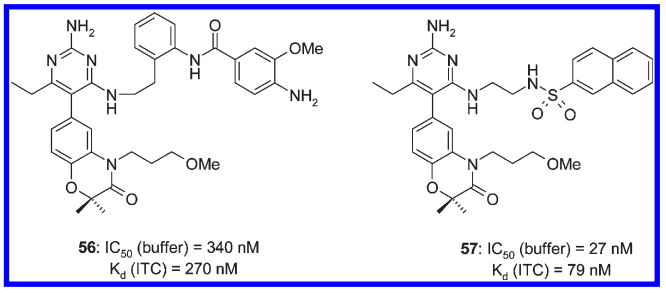


Figure 25. Non-peptide peptidomimetic DRIs binding to the S_2 pocket.

Analogues of 5-aryl-2,4-diaminopyrimidines (**34**−**37**, Figure 19) were shown by X-ray to be positioned with their 4-NH_2 group in close distance to Thr_{77} and Ser_{76} at the entry of the large S_2 pocket.[38] This suggested extending this position into the S_2 cavity as a strategy for lead optimization. A fragment-based NMR "second-site screening approach" using the 4-aminomethyl analogue of **12** (Figure 6) as the first-site ligand probe in combination with in silico GOLD docking to assess fragments as potential S_2 binders identified the (4-amino-3-methoxyphenyl)benzamide as single hit from a small library. Saturated transfer difference NMR via interligand NOEs in the presence of renin demonstrated this fragment to be an active site coligand.[38c,d] Modeling suggested tethering of the ortho-phenyl position of the fragment to the 4-NH_2 of **12** by an ethylene spacer. Cocrystallization of the resulting inhibitor **56** (Figure 25) with rh-renin confirmed the attached moiety to occupy a significant portion of the S_2 pocket.[38d] However, **56** was found to be 4-fold less potent compared to its direct 4-NH_2 analogue.[38b] ITC data suggested a substantial loss of binding enthalpy for **56**, which is largely compensated by a gain in binding entropy. This was attributed to flexing of the protein to accommodate the extension into S_2 and removal of ordered water from the pocket.[38c]

Guided by both X-ray and thermodynamics considerations, subsequent modifications of the spacer length and incorporation of functional groups predicted to interact more properly with the S_2 polar surface led to inhibitor **57** with improved enzyme inhibition and binding affinity (by ITC). Interestingly, the cocrystal structure of renin-bound **57** revealed two different orientations of the naphthalene extending into the S_2 pocket and alternative H-bonding patterns for the polar sulfonamide functionality at the bottom of this site. Furthermore, a portion of the renin active site determined by the peptide loop of residues Lys_{238}-Tyr_{244} adopted both a closed and a more open conformation, depending on the orientation of the ligand naphthalene.[38c]

ITC and X-ray data for **56** and **57** and related analogues suggested that binding to the large hydrophobic S_2 pocket is both enthalpy and entropy driven. A proper balance between nonpolar and polar interactions via correctly positioned functional groups that could compensate for displacement of ordered water molecules will likely be required for optimizing the interactions to the S_2 cavity.[38c] Noteworthy, it has been suggested that improving binding affinity is more difficult to achieve for inhibitors with entropically driven interactions, while inhibitors exhibiting favorable binding enthalpy may have a better potential for lead optimization.[88]

S_1'−S_2' Prime Site Specificity Pockets

Optimizing the interactions to the S_1' and S_2' recognition sites has played an important role for the initial development of peptide-based peptidomimetic DRIs. The introduction of polar or basic P_2' moieties has provided less lipophilic and more soluble analogues with improved PK properties.[23a] Dipeptide isostere TSAs with a truncated C-terminal portion have been designed to seek second generation DRIs with reduced molecular size (Figure 3),[20−23] and as a consequence, targeting the prime site became less attractive. In contrast, optimal interactions to both the S_1' and S_2' pockets were found to be critical for tight binding affinity of the (S_3−S_1)-topological class of TSAs such as **1** and **19**−**22**.[13] Truncated analogues with an optimized (P_3−P_1)-pharmacophore, but lacking the prime-site portion, were only moderately potent. The α,α-diMe β-alanine carboxamide of **1** constitutes a unique P_2' motif contributing significantly to the picomolar inhibition of plasma renin. Its terminal $CONH_2$ group forms a network of direct and water-mediated H-bonds to S_2' residues including Arg_{74} of the flap.[12,51]

More recent design approaches toward non-peptide DRIs have centered around the S_3−S_1 hydrophobic hot spot, the nonsubstrate S_3^{sp} cavity, and the flap β-hairpin binding sites. The prime-site portion of the active site has received much less attention and was even excluded conceptually for de novo inhibitor design.[39] Piperidine-based GRAB peptidomimetics bearing polar residues at the piperidine C5 position, like **43** and **44** (Figure 21) potentially extend into the S_2' pocket. Such modifications aimed to reduce the high lipophilicity of this class of DRIs.[36c,d]

The primarily hydrophobic S_1' site and the more polar S_2' pocket are flanked by Ser_{76} and Leu_{73}-Arg_{74}-Tyr_{75}, respectively, of the flexible flap β-hairpin in the inhibitor-bound closed conformation. The known dynamic flexibility of the flap region, and possibly other regions close to S_2', would suggest that distinct conformational ensembles of the renin prime site may well exist. Such alternative binding topographies could possibly be exploited for the design of new high-affinity pharmacophores. Interestingly, the renin S_2' subsite was described recently as a hot spot for binding of fragment-ligand probes using a computational approach based on the FTMAP algorithm for protein mapping.[89] Hence, the potential of the renin S_2' pocket of the renin active site may not have been fully recognized, albeit emerging evidence suggests that targeting this site has gained more interest recently.[15] The more hydrophilic nature of the S_2' pocket may offer attractive opportunities for modulating ADME properties or to address off-target selectivity.

Renin Selectivity Design

The gastric pepsins A and C, the lysosomal cathepsin D, as well as cathepsin E, are key off-targets within the human aspartic protease family for any clinical DRI.[90,91] High selectivity of a DRI against related human aspartic proteases including BACE-1 and -2 is generally considered as an essential criterion in view of a safe, life-long treatment for cardiovascular diseases such as hypertension. The significance of partial inhibition of these enzymes for clinical tolerability and safety, however, has not yet been fully elucidated. Successful design strategies to generate highly selective inhibitors of renin have relied mainly on structural differences of the substrate recognition sites within the enzyme family.[30,91] The emergence of renin active site topographies diverging from the β-strand binding catalytic cleft conformation and the discovery of diverse chemotype DRIs deserve further considerations with respect to enzyme selectivity design.

Piperidine-based TS surrogates have been demonstrated to serve as aspartic protease family specific warheads,[43,78,79]

which may imply a potential risk in achieving adequate selectivity within this protease family for DRIs comprising these and related structural features. Very limited in vitro structure−selectivity relationship data are currently available for newly disclosed potent non-peptide peptidomimetic DRIs.[12] For example, the 3,9-diazabicyclononene **51** (Figure 23) was reported to be a weak inhibitor of cathepsins D and E (IC_{50} = 2.7 and 8.1 μM, respectively).[55b] The 3,4-bis(aminomethyl)pyrrolidine has served as basic center scaffold to generate a submicromolar cathepsin D inhibitor and was predicted by modeling to bind into the S_2-S_2' specificity pockets.[79] On the other hand, the 2,4-diaminopyrimidine **12** (Figure 6) and related analogues with moderate affinity to renin were devoid of in vitro activity against cathepsins D and E and pepsin up to 100 μM.[38a] Also, prototype alkylamines **38** and **40** (Figure 20) of a novel class of S_3^{sp}-binding DRIs, showed greater than 1000-fold selectivity over pepsin, BACE-1, and cathepsins D and E.[39]

Competitive inhibition of porcine pepsin by a 3-alkoxy-4-arylpiperidine derivative structurally related to **8** (Figure 5) was shown to be pH-dependent with maximal inhibition at pH 5 (IC_{50} = 1.5 μM) and complete loss of in vitro activity at lower pH values.[78] Steady-state enzyme-kinetic studies demonstrated active site-directed inhibition of pepsin and significant conformational changes and perturbation of the ionization state of the catalytic aspartates upon binding of the ligand.[78] Reduced binding affinities under physiologically relevant conditions toward human aspartic proteases that function at low pH range could be a contributing factor leading to renin selectivity for basic TS surrogate classes of non-peptide DRIs.

The nonsubstrate S_3^{sp} binding cavity (vide supra) is not unique to renin within the human aspartic protease family. Inspection of the known X-ray crystal structures of human pepsin A (PDB code 1qrp), cathepsin D (PDB code 1lyb), and cathepsin E (PDB code 1tzs) revealed the presence of a conserved S_3^{sp} channel of similar size and topography compared to renin.[64] Interestingly, an aspartic acid (Asp_{303} in pepsin A; Asp_{323} in cathepsin D; Asp_{319} in cathepsin E) is located at the bottom of this channel in all cases, replacing the smaller hydrophobic Ala_{303} of renin. This suggests that the design of enzyme-specific ligand binding to the S_3^{sp} subsite is feasible and that such interactions could significantly contribute to the selectivity toward renin. However, the specific contributions of binding into the renin S_3^{sp} subsite to the selectivity of reported DRIs against other human aspartic proteases and to human vs nonprimate renin specificity appear less well-defined. Functional groups that could form H-bond or salt bridge interactions to Asp_{323}/Asp_{319} of the S_3^{sp} site of cathepsins D and E, respectively, may conceivably reduce renin selectivity. Conversely, the design of selective cathepsin D, E inhibitors by target-specific optimization of interactions to the nonsubstrate S_3^{sp} site could be explored. To our knowledge, aspects of structure−selectivity relationship related to the conserved S_3^{sp} channel of human aspartic proteases based on enzyme−inhibitor complex structures have not been studied in more detail.

Preclinical ADME Properties and Safety Profiling

A key driving force to identify new scaffolds potentially leading to orally efficacious DRIs was the desire to overcome the generally poor PK properties of most substrate-based peptidomimetics. The oral bioavailability of **1** was low to moderate in rat, dog, and marmoset (2.4%, 32%, and 16−30%), with the rat predicting best the human bioavailability.[11−14] Preclinical oral bioavailability data reported for representatives from different new classes of non-peptide DRIs in various species often ranged from low to moderate, albeit significantly higher oral bioavailability has been achieved in some cases.[38b,55c] The 3,9-diazabicyclo[3.3.1]nonene GRAB peptidomimetic **51** (Figure 23) showed bioavailability of 33%, 36%, and 9% in Wistar rat, dog, and rhesus monkey, respectively, with the elimination half-life being shortest in rat (5.5 h) and more sustained in dog and primates (10−11 h).[55b] The close analogue **52** displayed similar PK properties with sustained oral absorption in Wistar rats (24%, $t_{1/2}$ = 6.6 h, t_{max} = 10 h).[55a] The oral bioavailability of tetrahydropyridine analogues of **51** and **52**, disclosed by the same investigators, reached as high as 70% in rats.[55c] Oral bioavailability of the alkylamine inhibitor **38** (Figure 20) was modest in cynomolgus monkey (17%) and in rat (13%) because of incomplete GI absorption and high clearance but was significantly higher in dog (38%), with long terminal half-lives of ∼16 h in monkey and dog.[39a] All these reports hint at significant efforts invested in improving PK properties and highlight the difficulties in achieving this goal while maintaining other desired properties including potency. It remains to be seen whether any clinical candidate of such novel DRIs will exhibit acceptable ADME properties in humans.

The design of high-affinity DRIs exhibiting optimal ADME characteristics has become more demanding in line with the general paradigm shift in modern drug discovery that requires early evaluation and rigorous optimization of multiple additional parameters that predict, for example, the potential for toxicity.[92] High selectivity against antitargets including proteases, other enzyme classes as well as receptors, low potential for drug−drug interactions, and an acceptable in vitro/in vivo safety profile are considered to be key attributes of successful novel DRIs.

Many of the novel chemotype DRIs reported to date are characterized by relatively high molecular weight (MW > 500) and high lipophilicity ($\log P \geq 5$). A rather large contact surface area within the renin active site appears to be generally required to achieve maximal ligand binding efficacy for each inhibitor class, independent of the targeted enzyme conformation and recognition sites. Unfavorable physicochemical properties such as low aqueous solubility and lipophilicity may affect the in vitro potency against human plasma renin and hence could limit the in vivo efficacy of DRIs at reasonable dose levels.[36,38,49,55] Lipophilicity, molecular size, and H-bonding potential determine the ADME space predicting favorable PK properties, i.e., the potential to be delivered by the oral route.[93] Furthermore, high lipophilicity of compounds has recently been shown to be associated with increased promiscuity, i.e., a higher propensity to bind to multiple targets,[94] and with an increased likelihood of adverse or toxic events in vivo.[95]

A number of inhibitors from different new classes of non-peptide DRIs showed a significant in vitro affinity to cytochrome P450 isozymes, indicating a potential risk for high P450-mediated metabolic clearance and for in vivo drug−drug interactions (DDI). These include aminoarylpiperidines (e.g., **45**; Figure 22),[58a] 2-ketopiperazines (e.g., **47**; Figure 22),[58e] and 5-aryl-2,4-diaminopyrimidines (e.g., **36**; Figure 19).[38b] The in vitro metabolism of the *N*-alkyl-1,2,3,4-tetrahydroquinoline moiety, which has been incorporated as a common P_3-P_1 pharmacophore into several series of potent inhibitors by

human liver microsomes, has shown this moiety to be prone to metabolic aromatization.[58g] Compound **43** and the preclinical candidate **44** (Figure 21), both 3-arylmethoxy-4-arylpiperidine GRAB peptidomimetics, showed only moderate oral bioavailability (27% and 6−15%, respectively) in dogs due to extensive CYP3A4 mediated metabolism and/or P-glycoprotein-mediated efflux.[36e] The more soluble P_3^{sp}-substituted analogue **31** (Figure 16) was reported to have significantly inferior PK properties.[36d] The GRAB peptidomimetic **51** showed only weak CYP3A4 affinity ($IC_{50} > 10\ \mu M$). However, analogues in which one nitrogen of the center scaffold was replaced by nonpolar groups proved to be rather potent CYP3A4 inhibitors, and inhibition of CYP3A4 emerged as a selection criteria within this series.[55c] Inhibitor **51** revealed a potential for activation to reactive metabolites and for protein adduct formation in vitro in rat and human liver microsomes. Such effects were apparently less significant for the related clinical phase IIa inhibitor **52**.[55a]

Major hurdles have been encountered by Pfizer during lead optimization of the 2,4-diaminopyrimidine class of DRIs **34**−**37** (Figure 19) in an attempt to accomplish a favorable inhibitor profile for in vitro potency, CYP450 affinity, and metabolic stability, as well as PK properties.[19,38] Shortcomings of the most potent analogues identified were again attributed to their high lipophilicity. Various approaches for structural modifications were explored to render these inhibitors compliant with Lipinski's rule.[38,86] For example, inhibitor **35** bearing a polar *N*-(2-ethyl)acetamide P_3^{sp} side chain exhibited excellent in vitro potency toward rh-renin, good aqueous solubility, high human liver microsomal stability, as well as low affinity for CYP450 isozymes ($> 30\ \mu M$) rendering it superior to hydrophobic congeners. However, rat oral bioavailability of **35** was low (10%), likely due to low cell permeability and poor intestinal absorption. In contrast, inhibitors bearing an *N*-methoxypropyl P_3^{sp} residue and a more lipophilic S_4 residue, such as **36**, demonstrated strong CYP450 interactions, high microsomal clearance due to O-demethylation, and high liver first-pass elimination in rats (oral bioavailability of 0.4%). The 3-hydroxypropyl analogue of **36** showed no CYP450 inhibition ($> 30\ \mu M$), excellent stability toward liver microsomes, and good rat oral bioavailability (44%) but only moderate renin inhibition ($IC_{50} = 0.9\ \mu M$). Similarly, the CF_3-analogue **37** revealed high in vitro metabolic stability and improved oral bioavailability in rat (74%; $t_{1/2} = 3$ h) and dog (19%; $t_{1/2} = 10$ h) but again had only moderate potency and in addition suffered from strong CYP2D6 affinity.[38b] Remarkably, no in vivo pharmacology data have been reported for any analogues exhibiting high in vitro potency in the double-transgenic mouse (dTGM) model.[58c,96] The challenges of accomplishing a proper balance between desired target profile criteria, such as potency and ADME properties, have persisted throughout lead optimization. A preclinical development candidate was not identified from this class of DRIs at the time this research program was terminated.[15,19,38b]

Many of the novel DRIs comprise structural motifs, like the 4-arylpiperidine subunit of GRAB peptidomimetics, which are found in a number of other molecules known to bind to and modulate various types of drug targets or antitargets including G-protein-coupled receptors (GPCRs), transporters, and ion channels.[92] Cardiac ion channels play an increasingly important role. The hERG voltage-gated K^+ channel is widely recognized as a critical antitarget to be avoided from a drug safety perspective, as blocking this channel carries an increased risk of causing arrhythmias and torsades de pointes (TdP).[97] Several emerging non-peptide DRI chemotypes are characterized by a pharmacophore comprising a basic nitrogen atom, being crucial for the interaction with the catalytic aspartates of renin, in combination with one or more aromatic rings. These structural features may potentially match a well-accepted pharmacophore for hERG channel blockade.[97b,c] *N*-Ureapiperidine-based basic amine DRIs (**38**, Figure 20) were reported to be inactive against hERG (patch clamp $IC_{50} > 30\ \mu M$), while no in vitro affinity profile against GPCRs was disclosed.[39a] The in vitro cardiac safety profile of **1** indicated a very low risk of impacting cardiac repolarization and conduction time. The human hERG channel was very weakly inhibited (patch-clamp $IC_{25} = 671\ \mu M$), and no effects were observed on the action potential in the isolated rabbit heart up to $100\ \mu M$, suggesting no interaction of **1** with cardiac ion channels in tissues at clinically relevant concentrations.[98]

Early in vitro profiling for the potential to interfere with other ion channels such as the cardiac voltage-gated sodium ($Na_{v1.5}$) and calcium ($Ca_{v1.2}$) channels has received more attention recently.[99,100] Interfering with the physiological function of the $Na_{v1.5}$ channel may exert a malignant proarrhythmic potential in vivo.[99b] Assessment of the potential of novel chemotype DRIs to bind particularly to the $Na_v1.5$ channel needs to be considered in cases where specific structural alerts have been identified.[99a] Reports describing the binding affinities across antitarget panels of receptors and cardiac ion channels for key representatives of novel class DRIs have remained generally scarce to date.[38b] It will be interesting to learn more about the selectivity and safety profiles of advanced candidates from these series and about the medicinal chemistry path taken to optimize such compounds.

Significant advances have been made in the development of novel non-peptide DRIs with potent in vitro activity and oral efficacy in animal models (vide infra). While the structural diversity has increased remarkably during recent years, lead optimization to identify suitable drug candidates remains a challenge within each compound class. The criteria for candidate selection for cardiovascular indications such as hypertension have become increasingly demanding. Therefore, requirements needed for a long-term safe drug treatment are expected to further raise the hurdles for achieving the desirable preclinical target profile of any new DRI. As a result, the number of novel-class DRIs having reportedly entered clinical trials has remained remarkably low to date and none has progressed toward late stage development.[15]

Preclinical Pharmacology of Direct Renin Inhibition

Historically, the determination of in vivo potency and efficacy for antihypertensive agents has relied mainly on the use of rodents and dogs and to a lesser extent on other species. Numerous models of hypertension in the rat have been well characterized and are predictive of responses seen in hypertensive patients. However, the high specificity of human renin for human angiotensinogen was widely recognized as a significant limitation to the study of DRIs across species,[101] and the early in vivo assessment of human-specific DRIs has required the use of primates.[102] These animals are typically normotensive, and therefore, to detect effects on BP or changes in plasma renin activity (PRA) or total renin concentration, the use of a low sodium diet or diuretic pretreatment to activate the RAS is required.

The first DRI to demonstrate BP lowering effects following oral administration was *N*2-[(phenylmethoxy)carbonyl]-L-arginyl-L-arginyl-L-prolyl-L-phenylalanyl-L-histidyl-(3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoyl-L-isoleucyl-L-histidyl-*N*6-[(1,1-dimethylethoxy)carbonyl]-L-lysinemethyl ester (CGP29287).[103] This modified peptide produced a substantial hypotensive effect in furosemide-treated normotensive marmosets when given at a dose of 100 mg/kg. Several other less peptidic DRIs became available using chemical modifications intended to reduce the overall size and complexity and resulted in second generation inhibitors, including **2**, **4**, and **5** (Figure 3).[21b,23b] Early studies demonstrating BP lowering with **1** also relied on the use of marmosets.[104]

Although species other than non-human primates have been used less frequently, their value is nevertheless apparent from their acceptance as screening models for SAR studies or for basic mechanistic experiments. Early work by Pfizer utilized sodium-depleted guinea pigs or rats infused with hog renin.[35] Pfizer scientists also used the sodium-depleted guinea pig to assess the BP effects of the DRI **3** in combination with an ACEi or ARB, since enzyme affinities in this species are reportedly similar to those in man.[105] These RAS combinations, administered acutely to conscious, unrestrained guinea pigs, resulted in synergistic reductions in BP and were observed over a range of intravenous doses.

The development of transgenic rodent models began in the late 1980s and subsequently offered the potential to assess efficacy of DRIs in small animals. Techniques to measure BP and RAS biomarkers in the rat were already routinely utilized and therefore facilitated a more widespread use of such transgenic models. Early utilization of these transgenic techniques relied on the use of RAS gene constructs that interacted with the host RAS and therefore circumvented concerns of species specificity.[106] Separate lines of normotensive transgenic rats harboring either the human renin or angiotensinogen genes were first established. These strains were subsequently cross bred, yielding the double-transgenic rat (dTGR) characterized by severe hypertension and end organ damage.[107] To our knowledge, **5** was the first DRI shown to reduce arterial BP in this rat model.[107] Subsequent development and phenotyping of the double transgenic mouse (dTGM) essentially revealed pathology similar to that of the dTGR.[108]

These transgenic animals have become valuable tools for the evaluation of various DRI, including **1**. Renin inhibitor programs at several companies have identified novel, potent, and orally active compounds using these models. Piperidine-based GRAB peptidomimetic DRIs[36b−d] have been studied at Hoffmann-LaRoche in the dTGR model. For example, inhibitor **43** (Figure 21) elicited a slight reduction in BP and substantially reduced the progression of albuminuria.[85a] Furthermore, inhibitor **44** was shown to normalize BP and to prevent cardiac hypertrophy and albuminuria over a period of 4 weeks.[85b] The related analogue **30** (Figure 16) was evaluated by Pfizer and was shown to reduce BP, albuminuria, and left ventricular hypertrophy when administered to dTGM over a period of 4 weeks[96] The group at Pfizer has also reported novel aminoarylpiperidines and 2-ketopiperazines, including **45** and **46** (Figure 22), shown to reduce BP in the same dTGM model.[58a,c] Advanced analogues from the class of diazabicyclononene-based DRIs described by Actelion[55] and the alkylamine inhibitor series discovered by Vitae[39] were shown to elicit long-lasting BP reductions in the dTGR.

A desire to pursue more extensive profiling and ultimately differentiate **1** from other RAS blockers and other antihypertensive agents in general prompted testing in the spontaneously hypertensive rat (SHR), a well established hypertensive rat model. Although a reduction in BP was observed in the SHR, this effect was modest (~25 mmHg), required high doses (100 mg/kg per day), and subcutaneous delivery via osmotic minipump.[104] The moderate in vitro potency of **1** for rat renin (IC_{50} = 80 nM)[11] was consistent with its weak in vivo potency, thus limiting the use of the SHR model for further pharmacological evaluation, for example, for evaluating BP independent effects. Despite these limitations, it was possible to demonstrate a synergistic BP reduction when **1** was given in combination with either an ACEi or an ARB.[104] These findings in the rat corroborated those reported previously by Pfizer using the guinea pig.[105]

The availability of the dTGR overcomes limitations resulting from species cross-reactivity and now makes it feasible to explore potential additional benefits of renin inhibition. In contrast to the mild antihypertensive effect seen in SHR, subsequent studies with **1** in the dTGR demonstrated a potent and substantial BP response.[16] These studies also clearly revealed cardiac and renal benefits, including an improvement in cardiac hypertrophy and function, as well as a reduction in albuminuria and serum creatinine.[16] As already mentioned, similar effects were also observed with two different classes of DRIs, exemplified by **30**[96] and **44**.[85b] Experiments using a very low chronic dose of **1** (0.03 mg/kg per day) in dTGR showed only minimal effects on BP, yet a reduction in cardiac and renal damage.[109] These results implicate the RAS as an important pathophysiologic regulator of organ function and structure and moreover suggest that some of these benefits may be BP independent.

The transgenic (mRen-2)27 rat expressing the murine renin transgene Ren-2 also has become an important rodent model to study the pharmacology of DRIs. Diabetes can be induced readily in these hypertensive rats by the injection of streptozotocin and therefore provides a unique opportunity to assess the therapeutic potential of direct renin inhibition in an animal with the comorbid conditions of hypertension and diabetes. Compound **1** is a potent inhibitor of mouse renin (IC_{50} = 4.5 nM) and therefore makes possible the use of the (mRen-2)27 rat.[110] Chronic administration of **1** resulted in substantial antihypertensive and renal protective effects in this model[110] and was also shown to reduce albuminuria, glomerulosclerosis, and interstitial fibrosis[111] and to improve insulin sensitivity and skeletal muscle glucose transport.[112] Inhibitor **1** also decreased oxidative stress, induced beneficial remodeling of pancreatic structure,[113] and resulted in favorable effects on cardiac function and structure in diabetic, hypertensive mRen-2 rats.[114] These data provide evidence supporting organ protective benefits and extend the concept that direct renin inhibition, analogous to RAS inhibition, results in important tissue protective effects.[115]

Evidence to support differentiation is now being gathered as **1** is tested more extensively in animal models. Recent compelling data demonstrating an antiatherosclerotic effect in low density lipoprotein receptor null mice suggest that direct renin inhibition may provide optimal RAS blockade.[116] Inhibitor **1** (25 and 50 mg/kg per day) resulted in substantial reductions in lesion size in both the aortic root and arch. Although this study did not directly compare other RAS blockers, the effects reported with direct renin inhibition appeared to be greater than those described previously for

ACEi and ARBs. Another study using hypertensive apolipoprotein E null mice showed that **1** decreased BP, inhibited lesion progression, stabilized plaques, and reduced lipid core.[117] Improvements in atherosclerotic lesion size have also been demonstrated in Watanabe hypercholesterolemic rabbits.[118] Chronic administration of **1** (40 mg/kg per day; rabbit renin $IC_{50} = 11$ nM)[11] preserved endothelial function, as determined by increased cellular levels of nitric oxide, and reduced plaque area in this model. The overall benefit derived from RAS blockade in atherosclerosis is likely due to preventing the actions of AngII. However, a DRI may provide additional benefit by preventing the generation of AngI, AngII, and Ang breakdown products.[119]

The intracellular RAS is a new and emerging concept with potential therapeutic relevance[120,121] that extends our understanding beyond the classical concept of a systemic and tissue RAS. The importance of this system and its overall regulation are only now being defined. Stimulation of cardiac fibroblasts by high glucose activates the intracellular RAS resulting in elevated levels of intracellular AngII and in turn increased transforming growth factor-β and collagen I.[122] These biochemical changes were only partially attenuated by an ARB, whereas **1** completely prevented them. The same investigators also demonstrated that diabetes activates this system in cardiomyocytes and **1** was more effective at preventing increased oxidative stress and fibrosis than either ACE inhibition or AT1 receptor blockade.[123] Consequently, renin inhibition with **1** may offer additional tissue protective benefit in the setting of diabetes.

The prominent role of the RAS, specifically AngII, in the control of renal function has been known for many decades. The conventional view of the RAS had been that of a classical endocrine system. However, as specific pharmacologic tools became available to block the system at various points, evidence began to accrue suggesting the existence of an intrarenal RAS.[57] It soon became clear that the renal effects of AngII, such as renal vasoconstriction, are the result of both a systemic and tissue effect. AngII infused directly into the kidney, at doses that did not alter BP, resulted in renal vasoconstriction.[124] Blocking the effects of AngII therefore offered obvious therapeutic benefit. The early use of ACEi confirmed this idea as a substantial renal vasodilatation was noted.[125] Since it is widely recognized that ACE inhibition can alter the metabolism of several other vasoactive substances, including bradykinin, the ACEi-induced renal vasodilation may not be due only to the prevention of AngII formation. AngII antagonists available at that time were known to also possess agonist activity, rendering them of limited value in confirming the actions of AngII within the kidney.[126] Comparative studies in the marmoset using the ACEi enalaprilat and a modified peptide renin inhibitor indicated that the renal vasodilatation was primarily due to preventing the formation of AngII.[127] Renal vasodilation in response to direct renin inhibition was later confirmed using inhibitor 2.[128]

It was noted that the BP response to **5**, as well as to *N*-[(1*S*,2*R*,3*S*)-1-(cyclohexylmethyl)-3-cyclopropyl-2,3-dihydroxypropyl]-α-[[(2*S*)-2-[[[1,1-dimethyl-2-(4-morpholinyl)-2-oxoethyl]sulfonyl]methyl]-1-oxo-3-phenylpropyl]amino]-(α*S*)-1*H*-imidazole-4-propanamide (ciprokiren), in squirrel monkeys outlasted their effects on RAS biomarkers.[129] In another study, **5** was shown to induce greater renal vasodilation in squirrel monkeys than inhibitors **2** and **4**.[130] A study with radiolabeled **5** revealed a renal distribution and retention of the compound in the kidney long after its disappearance from the plasma compartment, suggesting a more pronounced inhibition of kidney renin.[131] Comparative experiments in guinea pigs demonstrated that for an equal effect on BP, **5** increased renal blood flow more than an ARB or an ACEi.[132] Subsequent studies in normal volunteers with **2** showed a greater increase in renal blood flow than with ACE inhibition, a response attributed to a local effect in renal tissue.[133,134] A marked renal vasodilatory response was also demonstrated with inhibitor **6** in normotensive men,[135] and could be explained by the propensity for this compound to penetrate tissues and consequently inhibit the renal RAS. The potential therapeutic benefit of intrarenal RAS inhibition, exclusive of a prominent effect within the plasma compartment, is suggested from comparative results obtained with the DRI **43**, cilazapril, and valsartan in dTGRs.[85a] Despite minimal effects on systemic BP, oral administration of **43** was equally efficacious in reducing albuminuria and in decreasing various biomarkers of renal inflammation. One possible explanation is that the kidney acts as a reservoir to sequester the DRI and thus provides for greater or more prolonged renin inhibition.

Further evidence supporting a unique renal specificity for DRIs has emerged from recent preclinical and clinical profiling studies with **1**. In healthy volunteers, **1** was shown to induce an increase in renal blood flow exceeding that observed with either an ACEi or an ARB.[136] In the rat, **1** distributed extensively to the kidney and was localized in the glomerulus.[110] Three weeks after stopping administration of **1**, drug levels in the kidney remained well above the IC_{50} for inhibition of renin, while plasma levels were below the limits of quantitation.[137] Interestingly, **1** was shown to accumulate in renin secretory granules and therefore to bind to renin and prorenin prior to release.[138] Taken together, these data provide a possible explanation for the observed long-lasting BP lowering effect of **1** in hypertensive patients and may support the idea of more complete blockade of the intrarenal RAS with DRIs.

It will be important ultimately to demonstrate that these potentially differentiating characteristics of **1** result in tangible therapeutic benefits. Preclinical data suggest that this is likely to occur. Inhibitor **1** was shown to lower BP, albuminuria, and serum creatinine in the hypertensive dTGR[16] and to reduce cystatin C and neutrophil gelatinase-associated lipocalin, two biomarkers of glomerular filtration and tubular damage, respectively.[139] In this same animal model, **1** reduced albuminuria at a dose that had only minimal effects on BP, suggesting a BP-independent renal protective effect.[109] Inhibitor **1** also reduced BP and albuminuria and suppressed renal transforming growth factor-β and collagen I expression in hypertensive, diabetic mRen-2 rats.[110] In a comparative study, **1** and the ACEi perindopril attenuated albuminuria and glomerulosclerosis to a similar extent in diabetic mRen-2 rats, even though BP was reduced to a greater extent by perindopril. Surprisingly, renal interstitial fibrosis was diminished more by **1**.[111] These results support the notion of an intrarenal action of **1** and a BP-independent effect. Increased generation of AngII occurs in renal podocytes in response to high glucose in the diabetic kidney.[140] Elevated AngII levels within the podocyte are causative for inducing podocyte injury, a principal feature of diabetic kidney disease. The DRI **1**, but not the ACEi captopril, decreased AngII production within the podocyte. This finding provides direct mechanistic evidence to functionally distinguish ACE inhibition from renin inhibition in the kidney. The clinical relevance of these findings awaits the outcome of several large ongoing clinical trials with **1**.

However, a small study in type 2 diabetic patients who are already on high dose losartan as background therapy (AVOID Study) has demonstrated an additional renal protective effect (reduction in albuminuria) with the addition of **1** to their daily drug regimen.[141]

Prorenin, Renin, and the (Pro)Renin Receptor

It is well recognized that the kidney is the primary source of renin and the only tissue capable of processing prorenin to renin. Notably, the kidney also releases prorenin.[142] In other tissues expressing the renin gene, prorenin is both the primary and final product.[143] Interestingly, prorenin represents 80−90% of all renin present in plasma, yet a physiologic or pathophysiologic role has not yet been demonstrated for this protein.[144] Plasma levels of prorenin are elevated in diabetic patients with underlying microvascular complications such as retinopathy and nephropathy,[145] thus implicating prorenin as a biomarker for vascular disease. However, convincing evidence to indicate a pathophysiologic role for plasma prorenin beyond that of a biomarker of disease has yet to be demonstrated.

Prorenin/renin binding proteins within tissues have been known for many years; however, their exact role is unclear.[146,147] Mesangial cells in culture have been shown to specifically bind renin and subsequently induce an increase in plasminogen activator inhibitor-1. These results suggest a potential biologic role for these receptors.[148] Mannose 6-phosphate/insulin-like growth factor II has been identified as another receptor for prorenin and renin[149] but appears to serve primarily as a clearance receptor.

Nguyen et al.[150] identified a novel receptor that binds prorenin and renin, induced intracellular signaling, and is designated as the (pro)renin receptor (P)RR. The (P)RR was localized to the glomerular mesangium and the subendothelium of coronary and renal arteries. Agonist binding resulted in a 4-fold increase in the catalytic activity of the enzyme−receptor complex. In addition to the proteolytic conversion of AngI to AngII, binding also resulted in initiation of an intracellular extracellularly regulated kinase 1/2 cascade that was independent of AngII. The identification of this receptor and the demonstration of an integrated response involving Ang-dependent and -independent mechanisms stimulated intense research into this novel RAS signaling cascade. Further investigation revealed that prorenin binding to this receptor resulted in increased expression of plasminogen activator inhibitor-1 in vascular smooth muscle cells, again an effect independent of AngII.[151] Renin binding was also shown to increase transforming growth factor-β and matrix proteins in mesangial cells, an effect that was not blocked by an ACEi, an ARB, or a DRI.[152]

Prorenin harbors a 43-amino-acid N-terminal propeptide that is believed to obstruct substrate access to the active site. Prorenin can be activated either by proteolytic cleavage of the propeptide or by a nonproteolytic mechanism. The latter can be induced by low temperature, low pH, or binding of prorenin to the (pro)renin receptor, all believed to trigger unfolding of the propeptide from the enzymatic cleft. The prosegment contains a "gate and handle" region that is critical for receptor binding during nonproteolytic activation.[153] Although renin and prorenin both have high affinity for the (P)RR, prorenin is considered the endogenous agonist.[154] A condition of mild obesity and impaired glucose tolerance is observed in transgenic rats and mice overexpressing the human renin gene, and these effects are independent of AngII.[155,156] Overexpression of the human (P)RR, an alternative approach for modulating this system, results in elevations in BP in the rat.[157] Recently, a decoy peptide, presumably binding to the active site of the (P)RR, denoted as the "handle region peptide" was shown to provide organ protective benefits in the kidney, eye, and heart when administered subcutaneously to rodents.[158] However, these results have not been consistently reproduced by other laboratories.[159] In addition, two recent studies have shown that prorenin does not induce any specific organ pathology or vasculopathy. Despite chronically elevated prorenin levels in transgenic mice overexpressing prorenin, no cardiac fibrosis or renal sclerotic changes were evident.[160] These investigators conclude that the mildly increased prorenin levels observed clinically in diabetic patients (3-fold) and those reported in their transgenic mice are insufficient to induce pathological responses. Similarly, glomerulosclerosis did not occur in transgenic rats despite substantial incremental increases in prorenin.[161] These results, taken together with the substantial body of clinical data from thousands of patients receiving chronic therapy with the DRI **1**, do not support a direct pathologic role for elevated prorenin or renin.

The recognition that the (P)RR binds both renin and prorenin and that the catalytic activity of bound renin increases 4-fold raises the intriguing question of whether or not a DRI could modify these effects. Initial studies indicate that **1** does not block the binding of prorenin or renin to the (P)RR.[159] Moreover, it has been shown in rat aortic vascular smooth muscle cells that the half-life of both prorenin and renin is increased by **1** binding to the receptor.[162] While several studies have begun to describe the biological effects that occur following agonist binding to the receptor, its pathophysiological relevance remains to be more fully characterized.

Clinical Pharmacology of the Direct Renin Inhibitor 1

A remarkable research effort has been put forth over the past decades directed at the discovery and development of DRIs. Initial clinical characterization of the effects of direct renin inhibition was accomplished with the use of various investigational drugs possessing suboptimal physicochemical and PK properties.[21−23,162] The opportunity to gain considerably more insight into the pharmacodynamic efficacy of DRIs became available with the clinical introduction of **1**.[163] Although the bioavailability of **1** was shown to be only 2.6% in humans, this represents a substantial improvement over that shown historically for DRIs as a class.[164,165] This enhancement represents more than a simple incremental improvement, especially when coupled with the remarkable potency of **1** for human renin, long duration of action, and the potential for distribution to the kidney. Steady-state C_{max} values are in the range 330−660 nM following a 300 mg dose of **1** and represent concentrations greater than 2 orders of magnitude above the IC_{50} for renin inhibition.[164,166] Importantly, **1** was also shown to have a long terminal half-life in human plasma.[167] From a functional standpoint and consistent with PK parameters, **1** was shown to induce dose-dependent and long-lasting inhibition of the RAS in humans.[168] Further confirmation of the anticipated benefit of oral renin inhibition came with the demonstration of a dose-dependent (75−300 mg of **1**) BP reduction in patients with mild to moderate hypertension.[169]

Early clinical results demonstrated that **1** has placebo-like tolerability and provides comparable or improved efficacy compared to angiotensin AT1 receptor blockade with

losartan[169] or irbesartan[170] and greater BP reductions than ramipril.[171] The antihypertensive response to **1** is sustained over 24 h (ambulatory BP) with high trough-to-peak ratios and thus represents a true once daily RAS blocker.[172] In a head-to-head comparative study, **1** (150 mg) was shown to reduce BP to a similar extent as 150 mg of irbesartan. Higher doses of **1** provided additional RAS blockade and greater BP reductions.[173] This study also demonstrated a potentially important biomarker divergence, showing that irbesartan and **1** both raised PRC; however, only **1** effectively inhibited PRA. In heart failure patients, an association between high PRA and increased cardiovascular risk has been shown.[174]

Combination therapy has become a cornerstone of the physician's approach to effective management of hypertension. Despite the vast armamentarium of antihypertensive drugs available today, high BP remains difficult to treat, especially in elderly individuals and in patients with existing comorbidities such as diabetes.[175] Inhibitor **1** has been shown to provide additive BP lowering efficacy when administered in combination with the diuretic hydrochlorothiazide, the ACEi ramipril, the ARBs irbesartan and valsartan,[176−178] and amlodipine.[179] These results indicate that **1** effectively reduced BP when administered alone or in combination with other antihypertensive agents. In summary, **1** offers an effective option for treating hypertensive patients.

With a wide variety of effective therapeutic options available, the decision of which agent to use can be a challenge for the physician. Efficacy and safety are clearly important factors. However, additional benefit that could be directly attributed to the use of a specific agent would be important in decision making. Unfortunately, with the introduction of any new agent, additional or unique benefit is usually theoretical rather than proven. It should be emphasized that the ultimate goal for controlling BP is to reduce the overall incidence of end organ failure. To this end, evidence is sought to demonstrate reduced cardiovascular morbidity and mortality.

Results from several small clinical studies have demonstrated a benefit beyond BP lowering. The intent is to use these findings to support larger, pivotal outcome studies that will ultimately demonstrate the long-term value of **1**. Collectively, the clinical program to evaluate the potential long-term benefit of **1** has been initiated and is known as the "Aspire Higher" clinical trial program.[5] It consists of a set of small scale studies focused on demonstrating cardiorenal protection in a short-term setting. These will then be followed by larger, long-term outcome studies aimed at directly demonstrating an organ protective benefit. One such acute study in healthy volunteers has recently demonstrated that **1** elicits a prominent renal vasodilation and that this response surpasses that seen with ACEi and ARBs.[136] This effect was long-lasting and associated with a natriuretic response. The now completed AVOID study demonstrated that **1**, when added to background therapy (losartan), further reduced the albumin/creatinine ratio in patients with type 2 diabetes and nephropathy.[141] This effect was independent of an effect on BP. These results further extend the preclinical observations and suggest a potentially important role for direct renin inhibition in renal (patho)physiology.

Two additional studies, ALLAY and ALOFT, provided early evidence to support a role for **1** in cardiac protection. The DRI **1** was shown to be as effective as losartan in attenuating left ventricular hypertrophy in hypertensive patients.[180] In patients with symptomatic heart failure (New York Heart Association class II to class IV), the addition of **1** to conventional therapy (ACEi or ARB, and a β-adrenoceptor antagonist) resulted in a further decrease in N-terminal pro-Brain natriuretic peptide, a proven biomarker that correlates with severity of cardiac severity.[181]

Future Directions

The disclosure of new classes of nonpeptide DRIs has continued over the past decade, suggesting that the opportunities offered by the newly uncovered topographical space and the increasing druglike ligand chemical space have not been fully exploited yet. The structural diversity of small-molecule DRIs is likely to further expand, and novel inhibitors are anticipated to be discovered in the future that may hold promise as candidates for clinical investigations. Future DRIs will need to demonstrate a favorable and differential clinical profile over existing therapies at attractive cost-benefit while facing increasingly demanding safety and tolerability standards by the regulatory authorities for cardiovascular drugs.

Several DRIs have reportedly progressed recently to the stage of early clinical investigations, indicating a revival of interest by several pharmaceutical companies in the development of DRIs.[15] The prospect of such more advanced DRIs reaching the patient for the treatment of cardiovascular diseases is difficult to predict, since only limited preclinical information is currently available. Preliminary data have been reported by Speedel on SPP635, SPP676, and SPP1148 (structures not disclosed) from phase I and on SPP635 from phase II clinical trials.[182] After a single dose in normal volunteers, SPP635 (100 mg) and SPP676 (30 mg) inhibited PRA to a similar extent as a 150 mg dose of **1**. SPP676 and SPP1148 were shown to be safe and well tolerated up to doses of 1800 and 600 mg, respectively. While SPP676 had a relatively short terminal half-life ($\sim$8 h), SPP1148 was reported to have a longer half-life of $\sim$30 h and to maximally inhibit PRA at the 50 mg single oral dose. SPP635, derived from the 3,4-piperidine GRAB peptidomimetics, showed an oral bioavailability of $\sim$30% and an elimination half-life of $\sim$24 h in phase I studies, in good agreement with the PK parameters determined by a microdosing approach. These data may suggest the potential for a favorable PK profile of SPP635 at clinically effective doses.[182] In a phase IIa study in patients with mild to moderate hypertension, SPP635 significantly reduced PRA and resulted in double-digit reductions in daytime ambulatory BP.[182] Novartis acquired Speedel and its portfolio of follow-up DRIs in 2008.

VTP-27999 (Vitae Pharmaceuticals, structure not disclosed) represents another novel DRI that has just entered phase I clinical trials.[183] VTP-27999 will be evaluated in a randomized double blind placebo controlled study in healthy volunteers to assess efficacy based on relevant biomarkers, as well as safety, tolerability, and PK.

The second generation GRAB peptidomimetic **52**[55a] has been reported by Actelion and Merck to be under codevelopment for the treatment of cardiovascular indications including renal and heart failure and hypertension.[184a] A randomized double-blind placebo and active comparator-controlled (enalapril 20 mg/day) study to determine the effectiveness and tolerability of **52** was completed. The drug candidate was given at 250 and 500 mg once daily over a 4-week treatment period to hypertensive patients. Results of this phase IIa trial have not been disclosed yet; however, further development of **52** has been recently discontinued.[184b]

As with any first-in-class drug, it is imperative that further characterization of **1** be undertaken. The intent is to more comprehensively define its clinical actions and to enhance our understanding of direct renin inhibition as a novel point of clinical intervention along the RAS cascade. With its market introduction, it now becomes possible for the first time to define in humans what had been for decades only a theoretical promise for direct renin inhibition. From a scientific perspective, **1** represents a novel and important pharmacologic probe that can be used to further expand our knowledge on the RAS in normal and diseased states. From a therapeutic point of view, it is important now to fully profile the effects of **1** in different patient populations and to clearly define the benefit derived from such an approach. The ultimate goal is to optimize therapy and to provide maximum benefit to the patient.

The accumulated evidence to date with **1** in cardiorenal disease indicates a potential for long-term benefit and provides a strong foundation on which to build our understanding of direct renin inhibition in cardiovascular disease. Outcome data from the planned and ongoing end point trials will provide definitive proof. One such study, ALTITUDE, aims to determine the effect of **1** added to conventional standard of care therapy (ACEi or ARB) in patients with type 2 diabetes and varying degrees of renal dysfunction.[185] The upcoming years will be an exciting time, as new data are forthcoming. This will hopefully provide evidence to support the hypothesis put forth more than 50 years ago that inhibition of AngI generation from angiotensinogen is the therapeutic approach "most likely to succeed" because renin is the initial and rate-limiting step of the RAS cascade.[3]

Methods

PDB codes, space groups, and resolution of the X-ray structures discussed in this article are as follows: glycosylated rh-renin (2ren, space group $I4$, 2.5 Å resolution);[31] **1** (2v0z, $P2_13$, 2.2 Å);[51] **4** (1rne, $P4_12_12$, 2.4 Å);[32] **29** (2v11, $P2_13$, 3.1 Å);[51] analogue of **22** (2v12, $P2_13$, 3.2 Å);[51] **28** (2v16, $P2_13$, 2.8 Å);[51] **34** (2g1r, $P2_13$, 2.4 Å);[38b] **38** (3gw5, $P2_12_12_1$, 2.0 Å);[39] *N*-hydroxypropyl analogue of **48** (2fs4, $P2_13$, 2.2 Å);[58e] **51** (3g70, $P2_12_12_1$, 2.0 Å);[55a] **52** (3g6z, $P2_12_12_1$, 2.0 Å).[55a] The X-ray structures (PDB codes, space groups, resolution) for **9** (1pr7, $P2_13$, 3.65 Å),[36a] **32** (1pr8, $P4_12_12$, 2.9 Å),[36a] and **33** (1uhq)[36b] had been made publicly available by the Protein Data Bank during a restricted time period. These data have been retracted in the meantime for unknown reasons. Shown X-ray structures were aligned onto the aliskiren complex using Coot (Emsley, P.; Cowtan, K. Coot: model-building tools for molecular graphics. *Acta Crystallogr.* **2004**, *D60*, 2126−2132). Figures were generated using PyMOL (DeLano, W. L. *The PyMOL Molecular Graphics System*; DeLano Scientific: San Carlos, CA, 2002; http://www.pymol.org).

Biographies

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Nikolaus Schiering received his Ph.D. from the University of Heidelberg (Germany) in 1991 for work conducted at the Max-Planck-Institute for Medical Research, Department of Biophysics, in the group of Emil F. Pai. From 1992 to 1995 he pursued postdoctoral studies at the Rosenstiel Center of Brandeis University, Waltham, MA, under the guidance of Gregory A. Petsko and Dagmar Ringe. He joined Pharmacia (Nerviano, Italy) in 1995 as a Research Scientist in the Department of Structural Chemistry. Since 2003, he has been working in the Structural Sciences Unit of the Expertise Platform Proteases at Novartis in Basel, Switzerland, as a Research Investigator.

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Jürgen Maibaum is Director and Leading Scientist within the Expertise Platform Proteases at NIBR in Basel, Switzerland. He graduated from the University of Münster, Germany, where he received his Ph.D. in Medicinal Chemistry in 1983 under the guidance of Professor Gottfried Blaschke. He was postdoctoral fellow between 1985 and 1987 at the University of Wisconsin, Madison, under the guidance of Professor Daniel H. Rich. He joined Ciba-Geigy (now Novartis) in 1989, where he has been engaged in drug discovery projects in the areas of cardiovascular disease, osteoporosis, metabolic disorders and obesity, and ophthalmology. In 2009, Jürgen was honored with the "Hero of Chemistry Award" by the American Chemical Society for his contributions to the discovery of aliskiren.

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