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Perspective

Protease Inhibitors: Current Status and Future Prospects

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1. Introduction

The four major classes of protease enzymes[1−4] (aspartic, serine, cysteine, and metallo) selectively catalyze the hydrolysis of polypeptide bonds. Their control over protein synthesis, turnover, and function enables them to regulate physiological processes such as digestion, fertilization, growth, differentiation, cell signaling/migration, immunological defense, wound healing, and apoptosis. Proteases of these classes are also crucial for disease propagation, and inhibitors of such proteases are emerging with promising therapeutic uses[3,5] in the treatment of diseases (Table 1) such as cancers,[6−8] parasitic, fungal, and viral infections (e.g. schistosomiasis,[9,10] malaria,[11,12] *C. albicans*,[13,14] HIV,[15−17] hepatitis,[18,19] herpes[20,21]), and inflammatory, immunological, respiratory,[22−25] cardiovascular,[26] and neurodegenerative disorders including Alzheimer's disease.[27] There are now many designed potent and selective protease inhibitors that slow or halt disease progression, inhibitors of the human immunodeficiency virus protease (HIV-1 protease) being notable for the speed with which they became available to humans.[17,28−31] To be effective as biological tools, protease inhibitors must be not only very potent but also highly selective in binding to a particular protease. As potential drugs, protease inhibitors must in addition have appropriate pharmacokinetic and pharmacodynamic properties.

Clues to how specific proteases selectively recognize small molecules most often come from peptide substrates for proteases. Although peptides display a diverse range of biological properties, their use as drugs is however usually compromised by their instability, low bioavailability, and poor pharmacological profiles. To be effective drugs, protease inhibitors need to have minimal peptide character, high stability to nonselective proteolytic degradation, good membrane permeability, long lifetimes in the bloodstream and in cells, low susceptibility to elimination, high selectivity for a protease, and good bioavailability (preferably by oral delivery). These properties usually require the compounds to have a low molecular weight (≤1000 Da).[32]

Protease inhibitors have been traditionally developed by natural product screening for lead compounds with subsequent optimization or by empirical substrate-based methods,[17] involving truncating polypeptide substrates to short peptides (<10 amino acids), replacing the cleavable amide bond by a noncleavable isostere, and optimizing inhibitor potency through trial and error structural modifications that progressively reduce the peptide nature of the molecule. This substrate-based drug design has been substantially improved in recent years with the availability of three-dimensional structural information for proteases, permitting receptor-based design. This involves using structural information about the active site of the receptor (or protease) and fitting into it selections of designed molecules with the aid of computers. Combinatorial chemistry also presents opportunities both to discover new molecular entities for assaying and to optimize lead structures for development of protease inhibitors.

Most proteases are sequence-specific, the size and hydrophobicity/hydrophilicity of enzyme sites defining possible binding amino acid side chains of polypeptide substrates. The standard nomenclature[33] used to designate substrate/inhibitor residues (e.g. P3, P2, P1, P1′,

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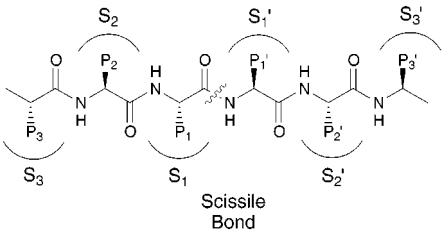


Figure 1. Standard nomenclature for substrate residues and their corresponding binding sites.[33]

P2′, P3′) that bind to corresponding enzyme subsites (S3, S2, S1, S1′, S2′, S3′) is shown in Figure 1.

Recently it has been convincingly demonstrated for a wide range of proteases that aspartic, serine, cysteine, and metallo proteases universally bind their inhibitors/substrates in extended or β-strand conformations; that is, the peptide backbone or equivalent is drawn out in a linear arrangement.[34,35] This common conformational requirement for recognition by proteases suggests new efforts to develop conformationally restricted inhibitors that adopt receptor-binding conformations and thus are entropically advantaged for binding to a protease. On the other hand, most of the many thousands of protease inhibitors that have been developed to date are relatively flexible molecules that have to use energy to rearrange into a protease-binding conformation. A possible trend in the development of more selective and potent protease inhibitors may be the use of more conformationally restricted molecules that are fixed in the protease-binding conformation. We now describe some of the better-studied small molecule inhibitors of aspartic, serine, cysteine, and metallo proteases, illustrate briefly how some of them bind to proteases, report their inhibitor potencies, and comment upon their pharmacological properties where available and their clinical prospects (Table 1).

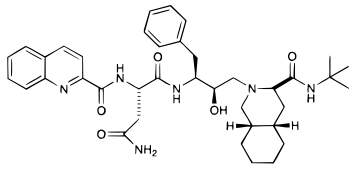
2. Aspartic Protease Inhibitors

Aspartic proteases[36] generally bind 6−10 amino acid regions of their polypeptide substrates which are typically processed with the aid of two catalytic aspartic acid residues in the active site. Thus there is usually considerable scope for building inhibitor specificity for a particular aspartic protease by taking advantage of the collective interactions between a putative inhibitor, on both sides of its scissile amide bond, and a substantial portion of the substrate-binding groove of the enzyme. Some aspartic proteases also have one or more flaps that close down on top of the inhibitor further adding to inhibitor−protease interactions and increasing the basis for selectivity. Figure 2 shows an example of the hydrogen-bonding interactions made between an aspartic protease (HIV-1 protease) and a selective cyclic peptidic inhibitor (K_i = 12 nM) as identified by an X-ray crystal structure.[37]

The general acid−base mechanism that is considered most likely for polypeptide hydrolysis catalyzed by aspartic proteases is depicted in Figure 3. The scissile amide bond undergoes nucleophilic attack by a water molecule which is itself partially activated by a deprotonated catalytic aspartic acid residue (Figure 3a). The protonated aspartic acid donates a proton to the amide bond nitrogen, generating a zwitterionic intermediate (Figure 3b) which collapses to the cleaved products (Figure 3c). The water molecule that binds between the enzyme (Ile50 and Ile150) and inhibitor is thought to position a peptide substrate, stretching the peptide bond out of planarity toward a tetrahedral transition state that is stabilized by a second water molecule.[38]

Aspartic protease−inhibitor crystal structures are currently available on the PDB database[39] for viral proteases (HIV-1, HIV-2, SIV, FIV), cathepsin D, renin, renin/chymosin, penicillopepsin, secreted aspartic protease, pepsin, mucoropepsin, retropepsin, saccharopepsin, rhizopuspepsin, and plasmepsin II.

2.1. HIV-1 Protease. The protease of the human immunodeficiency virus (HIV-1 protease) has proved to be an attractive drug target due to its essential role in the replicative cycle of HIV. Several low molecular weight inhibitors of HIV-1 protease (MW < 1000 Da) are now used in humans, including saquinavir (**1**), ritonavir (**2**), indinavir (**3**), nelfinavir (**4**), and amprenavir (**5**). These are among the first successful examples of receptor/structure-based designer drugs and were developed using structures of compounds bound in the active site of HIV-1 protease and with the knowledge of inhibitors of other aspartic proteases (e.g. renin).[29]



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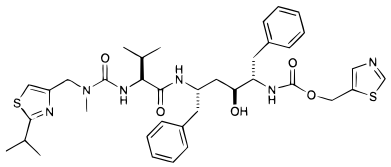

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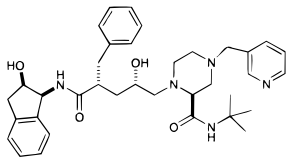

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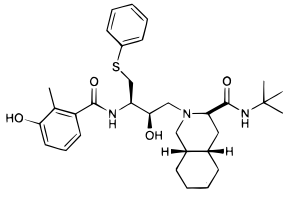

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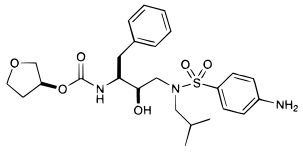

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All HIV-1 protease inhibitors developed so far target the active site substrate-binding groove of the homodimeric enzyme, a long cylindrical cavity that binds 6−7 amino acids via ionic, van der Waals, or H-bonding interactions.[29] Two catalytic aspartates in the middle of this cavity promote amide bond hydrolysis. Saquinavir (**1**) became the first protease inhibitor designed from a three-dimensional structure of a protease (structure-based design) to be approved for human use in 1996,[40,41]

Table 1. Examples of Proteases, Inhibitors, and Their Clinical Status

protease	function	disease	inhibitor and status
HIV-1 protease	HIV replication	AIDS	crixivan; available
renin	generation of angiotensin I	hypertension	preclinical
thrombin	blood coagulation	stroke, vascular clots, coronary infarction	argatroban; phase III (U.S.)
human neutrophil elastase	cleavage of elastin phagocytosis	inflammation, pulmonary disease	L-658,758; phase I
tryptases	phagocytosis	asthma	APC 366; phase II
C5/C3 convertases	complement activation	inflammation	FUT-187; phase II
cathepsin K	bone resorption	osteoporosis	APC 3328; preclinical
rhinovirus 3C protease	viral replication	common cold	AG7088; preclinical
ACE	generation of angiotensin II	hypertension	trandolapril, enalapril, captopril; available
MMPs	reconstruction of the cellular matrix	inflammation, cancer, muscular dystophy	marimistat; phase III
neutral endoprotease	release of ANP	hypertension	candoxatril; phase III
TACE	release of TNF-α	arthritis, multiple sclerosis	preclinical

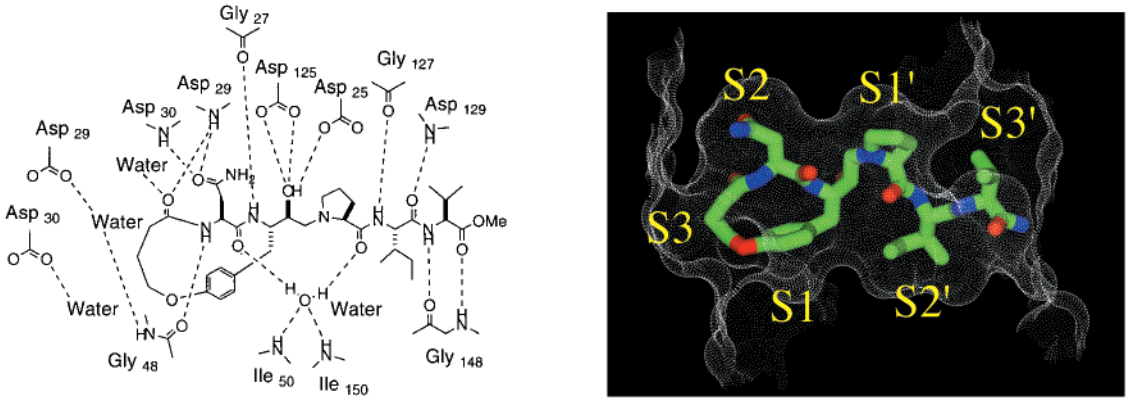


Figure 2. (a) Hydrogen-bonding interactions between a cyclic peptidomimetic inhibitor and HIV-1 protease.[37] The secondary hydroxyl of the hydroxyethylamine transition-state isostere makes hydrogen-bonding contacts with both catalytic aspartates of the enzyme. The water molecule that binds between the inhibitor and enzyme (Ile50 and Ile150) is thought to position a peptide substrate in the active site groove and to assist in stretching the peptide bond out of planarity, thus activating it for hydrolysis. (b) X-ray structure of the same cyclic peptidomimetic inhibitor bound to HIV-1 protease (PDB: 1cpi). The enzyme active site is delineated by a Connolly surface and can accommodate a heptapeptide. The inhibitor is totally encapsulated in the active site of the enzyme. This view looks down from the flap region of the enzyme, the secondary hydroxyl which interacts with the catalytic aspartates is pointing into the page, and the side chains can be seen to occupy six pockets (S3−S3′) in the active site.

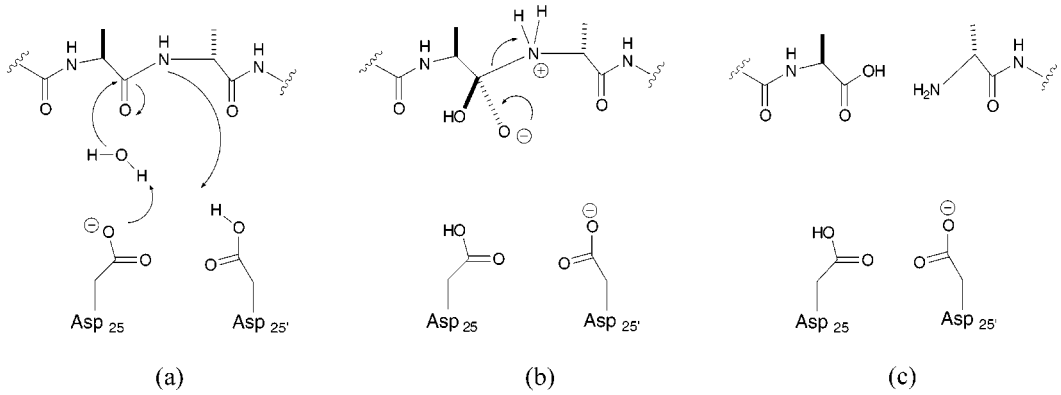


Figure 3. Catalytic mechanism for substrate hydrolysis by aspartic proteases. Nucleophilic attack of an activated water molecule on the scissile amide bond (a) and protonation of the amide nitrogen (b) give the zwitterionic intermediate (c) which collapses to the cleaved products.

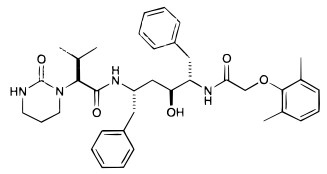
despite low oral bioavailability (1−4%) due to poor absorption and extensive first-pass degradation by cytochrome P450 (CYP 3A4).[42] It is active in cell culture against both HIV-1 and HIV-2 viruses (EC_{50} = 1−30 nM), and combinations with ritonavir (**2**), an inhibitor of cytochrome P450,[43] lead to greatly increased plasma concentrations. Ritonavir is itself a potent inhibitor of HIV-1 protease with high oral bioavailability (78% for 10 mg/kg po in rats, C_{max} = 2.6 μM, T_{max} = 2 h) and a plasma half-life of 1.2 h after a 5 mg/kg iv dose.[44] Indinavir or Crixivan (**3**) is another potent inhibitor of HIV-1 (K_i = 0.52 nM) and HIV-2 (K_i = 3.3 nM) proteases

which halts the spread of HIV infection in MT4 lymphoid cells at 25−50 nM and is orally bioavailable (90% in dogs). In humans, **3** is rapidly absorbed in a fasting state (T_{max} = 30−60 min), there is significant binding to plasma proteins (60%) (800 mg po every 8 h gives a C_{max} of 12 μM), and the main degradation pathway is via cytochrome P450.[45] The mesylate salt of nelfinavir (**4**, Agouron), approved for human use in 1997, is a lipophilic (log P = 4.1) protease inhibitor (K_i = 2 nM) with good oral bioavailability in rats (52%), dogs (31%), and monkeys (34%).[46] Amprenavir (**5**, K_i = 0.6 nM) is a water-soluble, orally bioavailable (>70%) inhibitor with plasma concentrations 20 times the EC_{90} value after 900−1200 mg doses, and its long $t_{1/2}$ of 7−10 h allows less frequent administration of drug thereby having the potential for less side effects with respect to other marketed HIV protease inhibitors described above.[32,47]

Viral resistance to 'monotherapy' with any of these drugs is a significant problem.[354] Serial passages of HIV-1 in vitro in the presence of increasing concentrations of a protease inhibitor cause rapid emergence of drug-resistant viral strains of HIV-1.[40] Resistance is due to amino acid substitutions in the protease, either in the inhibitor-binding active site or at remote residues which influence catalytic or binding efficiency. Many of the more common mutations (e.g. Gly48Val, Leu90Met (saquinavir); Val82Phe, Ile84Val, Leu90Met (ritonavir); Val82Phe, Ile84Val, Leu90Met (indinavir); Asp30Asn (nelfinavir); Ile50Val, Ile84Val (amprenavir)) are also found in the clinic. However cross-resistance is not always observed for inhibitor cocktails, so HIV patients who experience clinical failure with one protease inhibitor may still benefit from another protease inhibitor. Thus new HIV protease inhibitors with different resistance profiles are still being actively pursued.

A number of second-generation inhibitors have been developed. ABT-378 (**6**) was designed to inhibit mutant proteases produced in response to ritonavir. It is 10-fold more potent against ritonavir-resistant strains (e.g. Val82Phe, K_i = 1−4 pM; EC_{50} = 0.1 μM in MT-4 cells) and displays lower binding to serum proteins. Oral bioavailability is very poor (C_{max} < 0.1 μg/mL after 8 h) but is enhanced 77-fold (C_{max} = 5.5 μg/mL) when administered with ritonavir and is in early phase II clinical trials.[48] CGP-73547 (**7**) inhibits indinavir-resistant (IC_{50} = 0.03−0.1 μM) and saquinavir-resistant (IC_{50} = 0.004−0.1 μM) strains of HIV-1, is orally bioavailable (20% in rats, C_{max} = 90 min), and is in a phase I clinical trial.[49] One of the most promising preclinical candidates for HIV protease inhibition is palinavir (**8**). This compound is a very potent orally active inhibitor of HIV-1 (K_i = 31 pM) and HIV-2 (K_i = 134 pM) proteases with high antiviral activity (EC_{50}-(HIV-1) = 0.5−28 nM, EC_{50}(HIV-2) = 4−30 nM), low cytotoxicity (CC_{50} = 30-45 μM, TI > 1000), and reasonable pharmacokinetics (rats: $t_{1/2}$ = 0.7 h, C_{max}(5 mg/kg) = 1.6 μmol/L, F = 37%)[50] but still leads to resistant mutations predominantly Val32Ile and Ile84Ala.

2.2. Renin. The aspartic protease renin is involved in the rate-limiting first step of the renin−angiotensin (RAS) system, hydrolyzing the α_2-globulin angiotensinogen to release the 10-residue peptide angiotensin I. Because of its specificity, renin inhibitors are antihypertensive agents similar in action to ACE inhibitors and AII antagonists but free of some side effects associated with ACE inhibitor administration. For example zankiren (A-72517) (**9**), the first peptidic renin inhibitor with significant oral absorption (8% in monkey, 53% in dog, 24% in rat, 32% in ferret)[51] is a potent inhibitor (IC_{50} = 1.1 nM) of human plasma renin at pH = 7.4. A 10 mg/kg intraduodenal dose in sodium-depleted monkeys reduced mean arterial blood pressure by 32 mmHg (37%) for 2 h. The compound has also been administered to humans (C_{max} = 0.43 and 1.15 μL/mL after 125 and 259 mg doses, respectively) although bioavailability has not been reported.[52] Systolic blood pressure and diastolic blood pressure were reduced by 16 and 8 mmHg after a 125 mg dose. Zankiren (**9**) reduces the incidence of dry cough associated with ACE treatment.[53]



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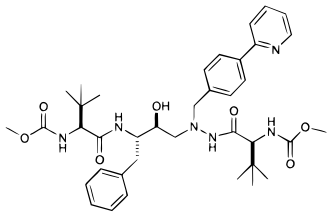

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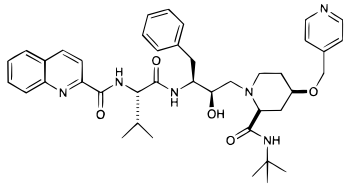

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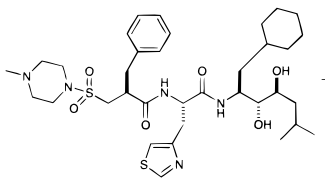
Renin inhibitors have mainly been developed by modifying substrate fragments from the angiotensinogen cleavage site,[54] but although several have entered phase II clinical trials,[55] their clinical progress has been hampered by their peptidic character which confers low stability and poor oral bioavailability in humans (CGP 38 560 (**10**),[56] < 1%, K_i = 0.7 nM; enalkiren (**11**),[54] 0.5%, K_i = 14 nM; remikiren (**12**),[57] 0.2%, K_i = 0.7 nM). Another hurdle to the development of renin inhibitors has been the high cost of production compared with current antihypertensives such as ACE inhibitors and AII receptor antagonists such as losartan (Dup-753). Renin inhibitors generally need to interact with five subsites (S4−S1′) of the enzyme to bind tightly and selectively compared with only three for ACE inhibitors. Consequently renin inhibitors tend to have a higher molecular weight, have more stereocenters, and are thus more expensive to manufacture.

Several renin inhibitors with low molecular weight, less peptidic character, and improved oral bioavailability have emerged recently. CP-108,671 (**13**) was designed from the cleavage site of angiotensinogen and the structure of the general aspartic protease inhibitor pepstatin.[58] It uses a cyclohexylnorstatine transition-



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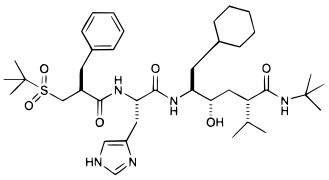

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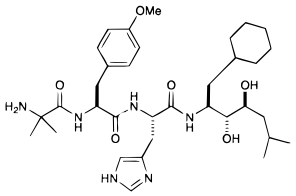

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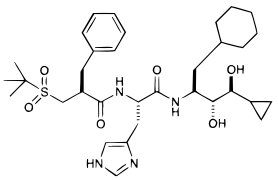

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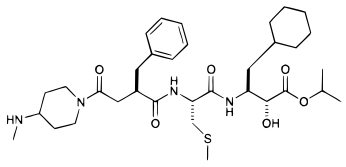
state analogue, a (*R*)-benzylsuccinate, at P3 for chymotrypsin stability and is a potent inhibitor of human plasma renin (IC_{50} = 4 nM). It is orally bioavailable (60% in dogs, 59% in marmosets, >27% in cynomolgus monkeys) with a half-life in dogs of 2.4 h after iv administration and lowers mean arterial blood pressure in sodium-depleted marmosets by 25 mmHg for at least 5 h after a 3 mg/kg oral dose. It is highly selective over most aspartic proteases (IC_{50}(porcine pepsin) = 2100 nM) but does weakly inhibit cathepsin D (IC_{50} = 550 nM). BILA 2157 BS (**14**) is another potent renin inhibitor (IC_{50} = 1.4 nM, pH = 6.0) with some selectivity (IC_{50}-(cathepsin D) = 550 nM) and oral activity (40% in cynomolgus monkeys after 3 mg/kg).[59] The P2−P3 amide bond has been replaced in this compound by a 2-substituted butanediamide moiety, the (2-amino-4-thiazolyl)methyl group at P2 and *N*-methyl-*N*-2-(2-pyridinyl)ethyl moiety at P4 are needed for oral activity, and there is only one stereogenic center and only one amino acid residue (glycinamide) left in the molecule.



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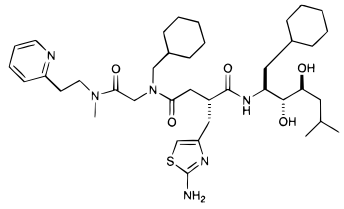

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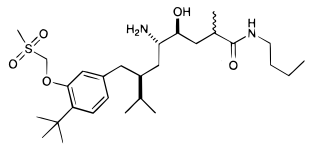
A combination of the X-ray crystal structure of CGP38560 (**9**) bound to renin[60] and previous deductions[61] that the S3 subsite can be accessed by extending the P1 residue of an inhibitor has led to inhibitor **15** (IC_{50} = 13 nM)[62] which lacks the P2−P4 amide backbone of CGP38560 yet retains good activity and specificity. Compound **16** (IC_{50} = 37 nM) is representative of a series of nonpeptidic piperidine-based inhibitors of human plasma renin.[63] It has a low molecular weight (517 Da), is membrane-permeable (log *D* = 1.9, pH 7.4) and is orally active in sodium-depleted marmosets with a maximal decrease in mean arterial blood pressure of 19 mmHg at 8.5 h after a 3 mg/kg dose. These compounds bind very differently to renin than most other inhibitors. An X-ray crystal structure of **17** shows that the protonated nitrogen of the piperidine ring is located between the two active site aspartic acid residues forming one hydrogen bond to each, thus fulfilling the function of the transition-state isosteres present in other inhibitors.[64] The naphthyl residue occupies the large hydrophobic S1/S3 subsite of renin, while the 4-phenyl ring of the inhibitor and the attached lipophilic tail disrupt a hydrogen bond between Tyr75 and Trp39 of the enzyme, lifting a portion of the flap region from Thr72 to Ser81. This binding induces the formation of a new hydrophobic pocket in the enzyme to accommodate the lipophilic tail and terminal phenyl group. Nonpeptidic, low molecular weight compounds such as **15** and **17** represent excellent progress toward the necessary features (oral bioavailability and economic production) for a renin-inhibiting drug but may need to be more selective.



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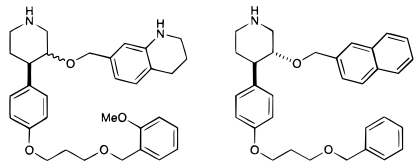
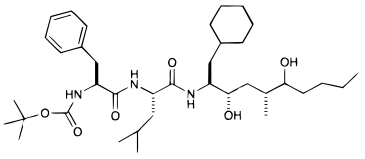


16 **17**

2.3. Plasmepsins. Plasmepsins I and II, found in the malarial parasite *Plasmodium falciparum*, are aspartic proteases that are believed to be essential for degradation of its major food source, human hemoglobin. Inhibition of these enzymes, which have 73% and 35% sequence homology with human cathepsin D, is therefore considered to be a viable therapeutic strategy for the treatment of malaria. Both plasmepsin I and II are believed to initially cleave the Phe33−Leu34 peptide bond of the α-chain of hemoglobin, followed by cleavage of the polypeptides into smaller fragments which are subsequently processed by the cysteine protease falcipain.[65] SC-5003 (**18**), the first peptidomimetic inhibitor reported to selectively inhibit plasmepsin I (IC_{50} = 500 nM; plasmepsin II, 22% at 10 μM), was active in vitro against the live parasite (IC_{50} = 2−5 μM) preventing hemoglobin degradation.[66] An X-ray crystal structure of plasmepsin II complexed to pepstatin A was used to develop peptidic inhibitors such as **19** (IC_{50} = 0.56 nM) which starves the live parasite in vitro (54% at 20 μM) as well as inhibits human cathepsin D (IC_{50} = 21 nM).[11]

Combinatorial synthesis is currently being used to generate inhibitor libraries for these enzymes. For example, the peptidic inhibitor **20** (IC_{50}(plasmepsin II) = 50 nM, IC_{50}(cathepsin D) = 320 nM) was developed from a 13000-compound library based on a statine transition-state isostere core. The library generated compounds with 31 different amino acids at the P2



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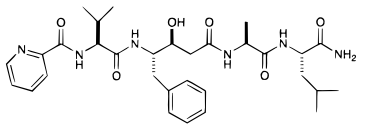

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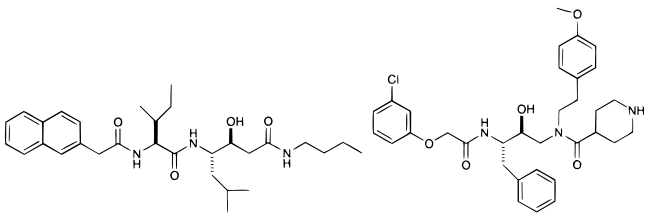
position and 20 different capping groups at P3, as well as explored conformational space at the P2′ position with a further eight groups.[67] Only two amide bonds remain in inhibitor **21** which was also developed using a combinatorial approach based on the hydroxyethylamine isosteric core. Inhibitor **21** (K_i = 4.3 nM) has a molecular weight of 594 Da with log *P* of 3.71, suggesting potential bioavailability, and a 10-fold selectivity over human cathepsin D (K_i = 63 nM).[68] While no data has been provided on the selectivity of **19**, **20**, or **21** against plasmepsin I as compared to plasmepsin II, given the similarity of the enzymes it is expected that these inhibitors can substantially inhibit plasmepsin I as well. All compounds developed to date are potent inhibitors of human cathepsin D, so better selectivity needs to be attained in orally active inhibitors of these enzymes. Interestingly, a combination of cysteine and aspartic protease inhibitors was recently found to be more effective than either compound alone in inhibiting *Plasmodium*-mediated hemoglobin degradation in both culture and a murine malaria model.[69] This synergy suggests that combination therapy may be a viable strategy for antimalarial treatment regimes of the future.



20 **21**

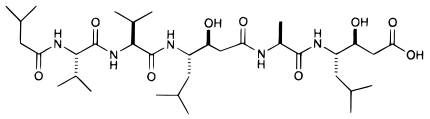
2.4. Cathepsin D. Human cathepsin D is an intracellular aspartic protease mainly found in lysosomes. It has a number of 'house-keeping' functions including degradation of cellular or phagocytosed proteins for reprocessing. The enzyme may be involved in a variety of disease states, including cancer and Alzheimer's disease. Clinical studies have shown that cathepsin D is overexpressed in breast cancer cells, and this seems to be associated with an increased risk of metastasis due to enhanced cell growth.[70] Cathepsin D or a similar aspartic protease is also thought to be involved in formation of β-amyloid peptide in Alzheimer's disease.[71,72] The availability of selective and potent inhibitors will help to further define the role of cathepsin D in disease and possibly lead to therapeutic agents.

Relatively few inhibitors of cathepsin D have been reported, partly because of its uncertain role as a viable target for therapeutic intervention. Human cathepsin D was cocrystallized with pepstatin A in 1993, and its structure[73] has promoted some inhibitor studies. One study suggests that entropy and solvation effects are key determinants of high affinity for pepstatin−cathepsin D binding.[74] Although a general inhibitor of aspartic proteases, pepstatin A (**22**) remains the most potent inhibitor known for human cathepsin D (K_i = 0.01 nM). The cyclic inhibitor **23** (K_i = 1.4 nM) is one of a series of inhibitors whose design is based on the X-ray structure and uses the fact that the enzyme-bound conformation of the P2 and P3′ residues of pepstatin are in close proximity to each other.[11] This allows cyclization of the inhibitor thereby increasing the proteolytic stability of the three amide bonds in the cycle. Compound **23** is only a weak inhibitor of plasmepsin II (IC_{50} = 1500 nM) due to an unfavorable interaction of the P2−P3′ linker with the flap of the enzyme.[11]

The benzophenone derivative **24** (IC_{50} = 210 nM) was developed from a lead obtained from compound library screening.[75] SAR studies showed that the hydroxyl, *n*-propyl, and rhodanine groups are important for good activity. No data was presented on specificity of **24** with respect to other aspartic proteases. Compound **25**, discovered from the statine-based combinatorial library developed for the inhibition of plasmepsin II, selectively inhibits cathepsin D (IC_{50} = 110 nM) over plasmepsin II (IC_{50} = 5800 nM).[67] Recently, another combinatorial approach based on the hydroxyethylamine transition-state isostere gave a series of potent and nonpeptidic inhibitors of cathepsin D; two examples are **26** (K_i = 9 nM)[76] and **27** (K_i = 0.7 nM).[77] The inhibitors were generated to prove the methodology but were not optimized for specificity against other aspartic proteases using combinatorial approaches.

2.5. Secreted Aspartic Protease. The *Candida* yeast strains *C. albicans*, *C. tropicalis*, and *C. parapsilosis* exist in small quantities in a healthy intestinal tract but become a health problem when the immune system is compromised. Such opportunistic infections arise in AIDS patients where *C. albicans* is a serious pathogen of the mucous membranes.[78] It is also the major cause of vaginitis[79] and has been implicated in liver toxicity and in development of multiple chemical allergies. *C. tropicalis* is the predominant cause of fungal infections in neutropenic cancer patients. These organisms have the ability to secrete into the host[80] several aspartic proteases (SAP, secreted aspartic protease) of broad specificity. These proteases are thought to be linked to the virulent effects of *Candida* strains in humans as protease-deficient mutants reduce virulence.[81,82]

The HIV-1 protease inhibitor indinavir (**3**) is a weak but specific inhibitor of SAP and greatly reduces the viability and growth of *C. albicans*.[78] These enzymes are therefore becoming attractive targets for therapeutic attack. Nine SAPs have been identified in the genome of *C. albicans* to date (SAP1−9).[83] From mutation experiments, SAP2 seems to be the dominant isoenzyme for the normal progression of systematic infection, while SAP1,3 are also important for overall virulence of *C. albicans*.[81] SAP4−6 appear to play a role in the process of induction of SAP2.[82] X-ray crystal structures have been determined for SAP2 complexed to pepstatin,



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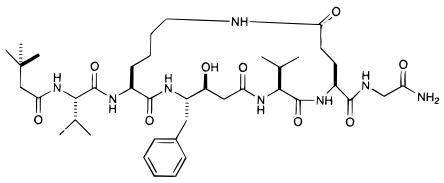

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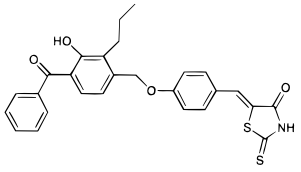

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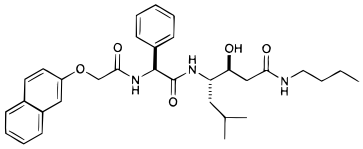

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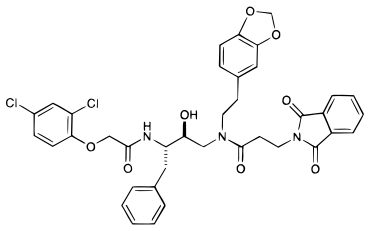

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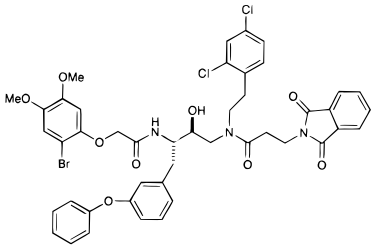

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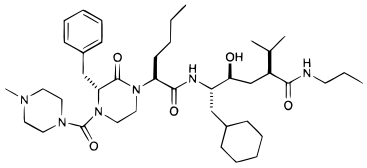
A-70450 (**28**),[84] a close homologue SAP2X bound to the same inhibitor,[14] and a SAP enzyme of *C. tropicalis*.[85]

Very little inhibitor design has been reported for SAP2. A-70450 (**28**) was originally designed to inhibit renin and later found to be a nonselective inhibitor of the SAP of *C. albicans* (IC_{50}(SAP) = 1.4 nM, IC_{50}(renin) = 7.1 nM, IC_{50}(cathepsin D) = 770 nM). This inhibitor incorporates the (*S*)-hydroxyethylene isostere with the hydroxyl group positioned in the crystal structure between the two catalytic aspartate residues. Interestingly, the terminal methylpiperazine ring of A-70450 is found to be in a boat conformation and occupies the S3 subsite of the enzyme together with the benzyl group of the ketopiperazine ring. The large S3 subsite is not found in other aspartic proteases, and this difference could be exploited to develop selective inhibitors for



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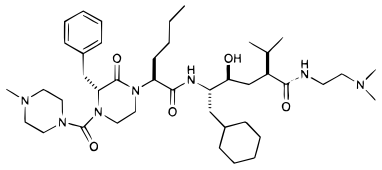

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SAP2. A limited structure−activity study, where P2′ substituents were varied, gave inhibitor **29** with much improved selectivity for SAP2 compared to renin or cathepsin D (IC_{50}(SAP) = 6.2 nM, IC_{50}(renin) > 100 nM, IC_{50}(cathepsin D) = 58000 nM.[13]

2.6. Summary. Most aspartic protease inhibitors that have been developed to date bind to their target enzyme through noncovalent interactions (i.e. hydrogen bonds, ionic or van der Waal's contacts). These compounds are therefore reversible inhibitors of proteases, and effective inhibition relies on the enzyme having very much higher affinity for the inhibitor than its natural substrate. High affinity for any particular aspartic protease has been achieved by trying to maximize the number of noncovalent interactions that the inhibitor makes with the enzyme. One approach that has proved very successful in this regard is the incorporation of a transition-state isostere into designed inhibitors. A transition-state isostere is defined as a functional group that can mimic the tetrahedral transition-state of amide bond hydrolysis (Figure 3b can be regarded as a very rough approximation of the transition state) but cannot itself be hydrolyzed by the enzyme. It has been hypothesized that stable structures which can resemble the transition state for an enzyme reaction will be bound more tightly than the substrate for the enzyme-catalyzed reaction. Studies on the general aspartic protease inhibitor pepstatin (which incorporates the statine transition-state isostere) suggest that this increased affinity, which can be as great as a 10^4-fold, is not only due to mimicry of the transition state of amide bond hydrolysis but also due to the displacement of the catalytic water molecule hydrogen bonded to the catalytic aspartates.[86] Some transition-state isosteres that have been used for the design of aspartic protease inhibitors are depicted in Figure 4, and examples of their use can be seen in all the inhibitors described above.

3. Serine Protease Inhibitors

Serine proteases[1,87−89] are classified by their substrate specificity, particularly by the type of residue found at P1, as either trypsin-like (positively charged residues Lys/Arg preferred at P1), elastase-like (small hydrophobic residues Ala/Val at P1), or chymotrypsin-like (large hydrophobic residues Phe/Tyr/Leu at P1). The active site of these enzymes consists of a catalytic triad of Ser195, His57, and Asp102 residues (chymotrypsin numbering system) and an oxyanion hole. The substrate binds in the active site forming a Michaelis complex, exposing

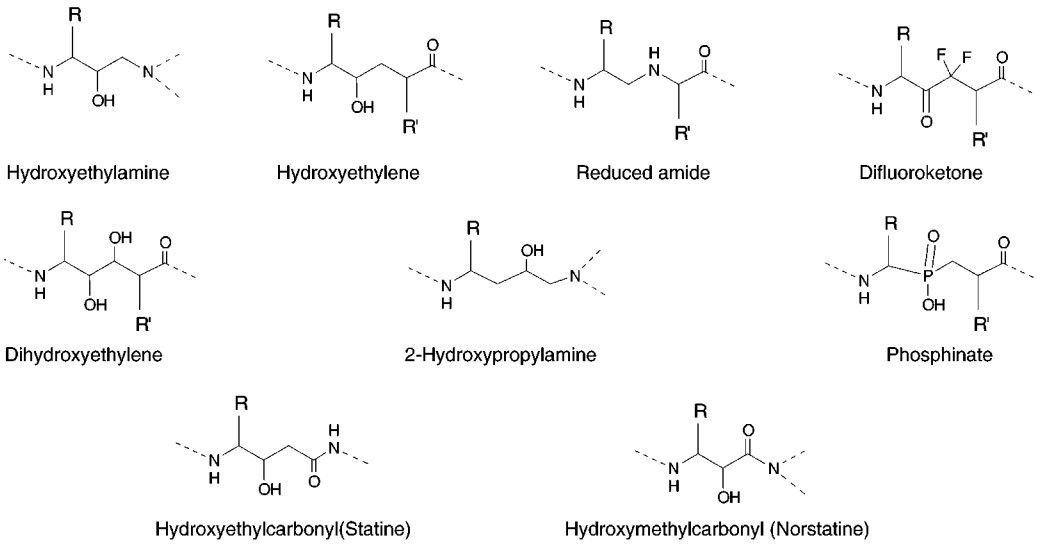


Figure 4. Structure of some of the most common transition-state isosteres that have been used to replace scissile amide bonds in the development of aspartic protease inhibitors.

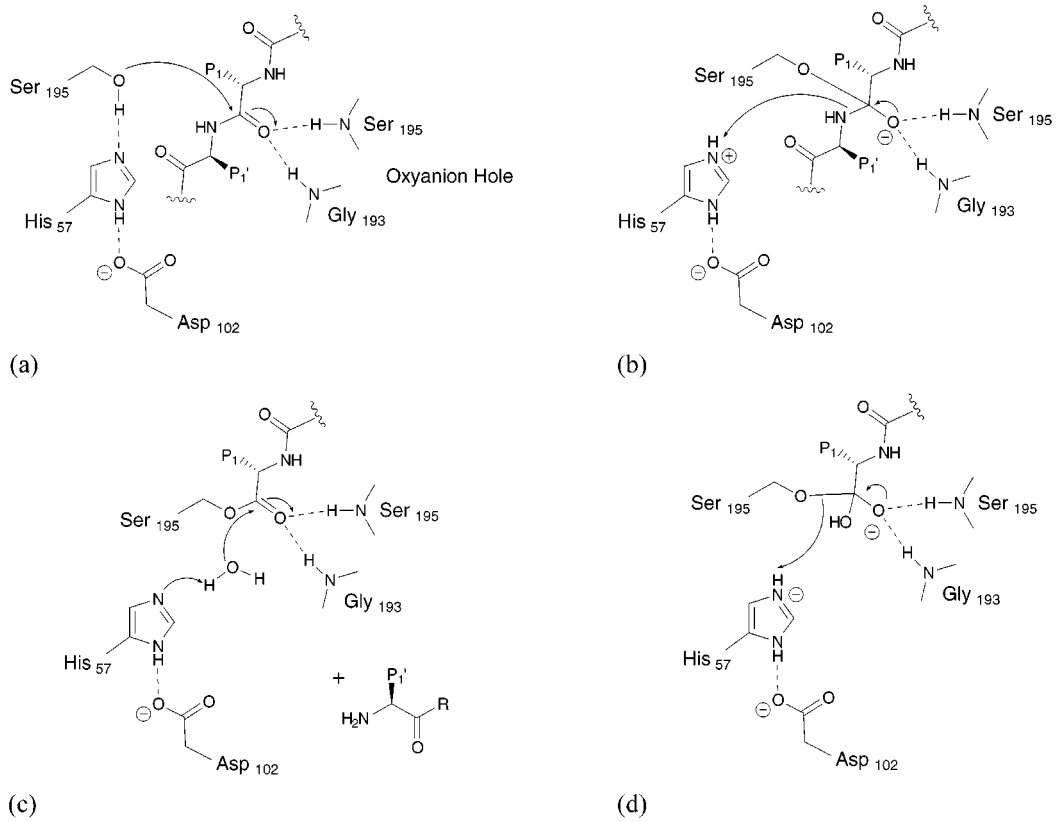


Figure 5. General catalytic mechanism for serine proteases. (a) Hydrogen-bonding interactions with histidine and aspartate residues of the catalytic triad activate the serine hydroxyl for nucleophilic attack of the scissile amide bond, forming the first hemiacetal tetrahedral intermediate. (b) Proton transfer from His57 to the amine of the tetrahedral intermediate facilitates expulsion of the C-terminal fragment of the substrate to give the covalent acyl complex. (c) Water attacks the complex to form the second tetrahedral intermediate (d) which collapses via acid-assisted catalysis by His57 to regenerate Ser195 and the N-terminal fragment of the cleaved substrate.

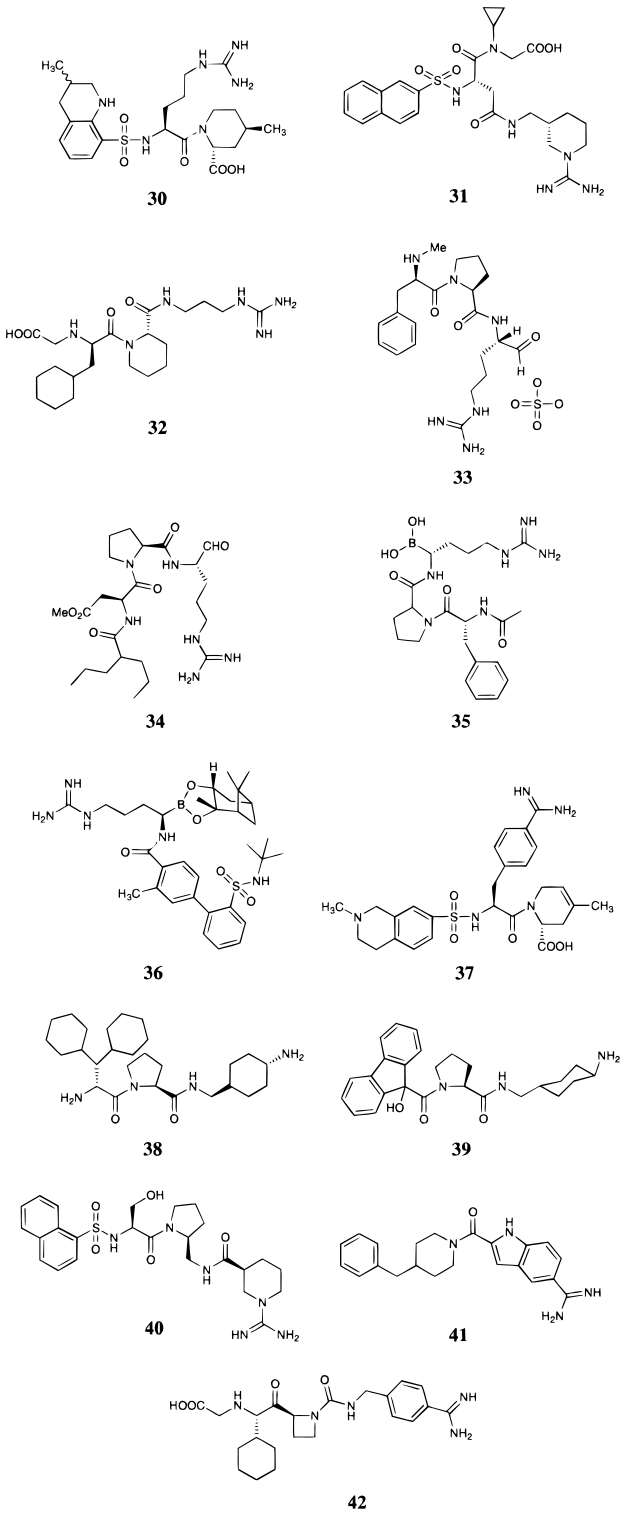
the carbonyl group of the scissile amide bond to nucleophilic attack by the active site serine hydroxyl, under base catalysis by the imidazole side chain of His57 (Figure 5a). The resulting tetrahedral intermediate is stabilized by hydrogen bonding to the backbone NH of Ser195 and Gly193, which form the oxyanion hole. Proton transfer from His57 to the amine of the tetrahedral intermediate facilitates expulsion of the amine fragment as leaving group (Figure 5b). The covalent acyl−enzyme complex is attacked by water, with formation of a new tetrahedral intermediate (Figure 5c) which subsequently breaks down via acid-assisted catalysis by His57 to form the carboxyl fragment of the cleaved substrate and regenerate Ser195 (Figure 5d).

Serine proteases for which protease−inhibitor X-ray crystal structural data is available on the PDB database[39] include trypsin, α-chymotrypsin, γ-chymotrypsin, human neutrophil elastase, α-lytic protease, thrombin,

subtilisin, human cytomegalovirus, proteinase A, achromobacter, human cathepsin G, glutamic acid-specific protease, carboxypeptidase D, blood coagulation factor VIIa, porcine factor 1XA, mesentericopeptidase, HCV protease, and thermitase.

3.1. Thrombin. Thrombin is a trypsin-like serine protease that plays a central role in hemostasis and induces platelet aggregation and secretion.[90,91] It is a member of the trypsin family of serine proteases and the final enzyme in the blood coagulation cascade. Thrombin hydrolyzes factors V, VIII, and XIII and fibrinogen, releasing fibrinopeptides A and B, which generate fibrin. The polymerization of fibrin produces the core of a blood clot. The substrate pocket S1 of thrombin is optimized to recognize an arginine side chain, while the S2 and S3 subsites are hydrophobic in contrast to trypsin. The S2 subsite is smaller than the S3, typically accommodating a proline residue or a small hydrophobic unit, while the S3 subsite can bind larger residues (e.g. D-Phe). Thousands of thrombin inhibitors have been discovered over the last 20 years, some of which are orally active and under clinical evaluation.[92] A natural product isolated from the medicinal leech called hirudin (66 amino acids) and its analogue hirulog (20 amino acids) are polypeptides that have been under clinical scrutiny as thrombin inhibitors.[90] Both peptides bind tightly to thrombin ($K_d = 2 \times 10^{-14}$ and 2.3×10^{-9} M, respectively), penetrate the thrombus, and neutralize thrombin bound to fibrin thereby reducing platelet deposition and thrombus growth. Hirulog ($t_{max} = 15-19$ min, $t_{1/2} = 0.18-0.55$ h, C_{max} = dose-dependent) combines a fragment of the C-terminus of hirudin with an N-terminal fragment D-Phe-Pro-Arg-Pro(Gly) that interacts with the catalytic site of thrombin. This D-Phe-Pro-Arg-Pro unit has been the basis for numerous small molecule active-site-directed competitive inhibitors of thrombin, including argatroban (MD-805, **30**), napsagatran (RO46-6240, **31**), inogatran (**32**), efegatran (LY 294468, **33**), CVS-1123 (**34**), DuP714 (**35**), UK156406 (**37**), and melagatran (**42**).

Argatroban (Novastan, MD-805) (**30**) is a potent arginine derivative ($K_i = 39$ nM) which inhibits platelet aggregation by clot-associated thrombin. It is a 64:36 mixture of 21(*R*) and 21(*S*) diastereomers, with the latter being twice as potent as the former in an in vitro coagulation assay but also less soluble in aqueous buffer.[93] Argatroban monohydrate is in phase III clinical trials in the United States and is approved for iv use in Japan for treating peripheral arterial occlusive disease.[94] It lacks oral bioavailability and has a short duration of action ($t_{1/2} = 40$ min) in humans. Another inhibitor in clinical trials is napsagatran (**31**), a potent and reversible inhibitor that is 6000 times more selective for thrombin ($K_i = 0.3$ nM) than trypsin ($K_i = 1.9$ μM) and significantly decreases the intracoronary thrombus at low dose.[95] This compound has similar antithrombotic effects to heparin, one of the most widely used anticoagulants.[96,97] Inogatran (**32**) is a selective and competitive inhibitor of thrombin ($K_i = 15$ nM),[98] occupying S1−S3 enzyme subsites and inhibiting platelet aggregation ($IC_{50} = 17$ nM) without interacting directly with the nucleophilic serine of thrombin.[99] It is a synthetic peptidomimetic with good pharmacokinetic properties for iv administration in clinical trials.[100]



Efegatran (LY 294468, **33**) is a tripeptidic argininal inhibitor of thrombin that is clinically well-tolerated and displays dose-dependent anticoagulant activity above 0.63 mg/kg/h.[90] It has been developed as a parenteral anticoagulant for the treatment of acute coronary syndromes. Efegatran has undergone phase II clinical trials in unstable angina and acute myocardial infarction patients.[101] Another argininal inhibitor which has entered clinical trials as an orally administered drug is CVS-1123 (**34**). It was recently reported to prevent arterial and venous thrombosis after iv administration in dogs and also prevented coronary artery thrombosis

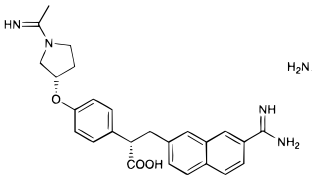
after oral administration.[102,103] Similarly, DuP714 (**35**) is a boroarginine tripeptide containing D-Phe-Pro-Arg,[104,105] which binds to thrombin with high affinity ($K_d = 4.1 \times 10^{-11}$ M) and inhibits thrombin-mediated platelet activation and fibrinogen cleavage (K_i(thrombin) = 40 pM) but is not selective (K_i(trypsin) = 45 pM). Clinical studies were not undertaken due to liver toxicity.[90] To reduce the peptidic nature of DuP714 (**35**), various biaryl-substituted akylboronate esters **36** (K_i(thrombin) = 0.21 nM, K_i(trypsin) = 0.70 nM) were synthesized as potent thrombin inhibitors.[106]

UK156406 (**37**) is another peptidomimetic currently in clinical trials (K_i = 0.46 nM); however, it is only moderately selective against trypsin and anticoagulant parameters returned to baseline within 8 h.[92,107] It has oral bioavailability in dogs of 45% and a relatively short plasma half-life of 48 min. Compound **38** (K_i = 56 pM) is 2100-fold more selective for thrombin than trypsin and 90% orally bioavailable in dogs; it suffers in vivo from high protein binding.[108] Compound L-372,460 (**39**) is selective for thrombin (K_i = 1.5 nM) over trypsin (K_i = 860 nM) and efficacious in a rat model of arterial thrombosis at an infusion rate of 10 μg/kg/min.[109] It is orally bioavailable in dogs (74%) and monkeys (39%) with a serum half-life of 4 h and is a clinical candidate for the treatment of thrombogenic disorders. BMS-189090 (**40**) is also selective for thrombin (K_i = 3.6 nM) over trypsin and other serine proteases and has an X-ray crystal structure similar to PPACK (D-Phe-Pro-Arg-CH_2Cl) bound to thrombin.[110]

LY178550 (**41**) has a basic amidine which forms a salt bridge with Asp189 within the active site of thrombin, while the 4-benzylpiperidine interacts with the S2 and S3 binding sites via hydrophobic interactions.[111] The indole NH of the inhibitor forms a hydrogen bond with the γ-oxygen of the active site Ser195. This compound has a high K_{ass} of 2×10^6 M^{-1}, which indicates strong interaction between inhibitor and enzyme. Unfortunately, this compound is not selective over trypsin but has good selectivity toward other serine proteases. Melagatran (**42**) is a potent orally active inhibitor of thrombin (K_i = 2 nM),[112] which prevents or delays formation of electrically induced occlusive thrombus in the canine coronary artery and prolongs clotting time to twice the control value in coagulation assays at low concentrations.[113,114] While thrombin inhibitors are excellent anticoagulants, their action on platelet function disposes individuals to excessive bleeding. Other compounds with antithrombotic activity but without this side effect are more desirable for clinical use and are actively being sought.

3.2. Factor Xa. Factor Xa is another trypsin-like serine protease present in the prothrombinase complex and is responsible for cleaving prothrombin to form thrombin.[115] At the site of injury, the prothrombinase complex is unaffected by thrombin inhibitors, therefore maintaining a prothrombotic state by producing thrombin continuously. A number of natural product inhibitors of factor Xa have been reported such as TAP (tick anticoagulant peptide), antistasin, and ecotin. Compared to thrombin inhibitors, there are relatively few reports of small molecule inhibitors of factor Xa.

DX-9065a (**43**) (K_i = 41 nM) is an inhibitor of factor Xa. It is an amidinonaphthalene derivative which is orally active and highly selective over other serine proteases (e.g. K_i(thrombin) > 2000 μM, K_i(chymotrypsin) > 2000 μM, K_i(trypsin) = 0.62 μM).[116,117] It does not affect platelet aggregation, has prolonged antifactor Xa activity and inhibition of thrombin generation, causes a reduction in tissue factor-induced mortality in mice (ED_{50} = 56 mg/kg), and inhibits stasis-induced thrombosis in rabbits (ED_{50}(iv) = 0.03 mg/kg, ED_{50}(sc) = 0.3 mg/kg, ED_{50}(po) = 50.5 mg/kg).[118] Computer models[119,120] and an X-ray crystal structure[121] of **43** bound to the enzyme have been reported. The naphthamidine group was predicted by modeling to be fixed in the S1 pocket via a salt bridge to Asp189, which was confirmed by the crystal structure. Hydrophobic interactions around the pocket also contribute to the high binding affinity, while the pyrrolidine ring binds to the other major interaction site at S4. DX-9065a is a promising candidate in the treatment and prevention of thrombotic disease.[122]



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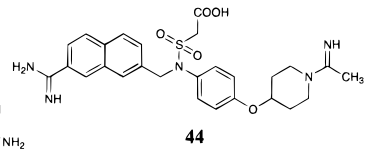

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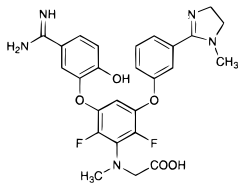

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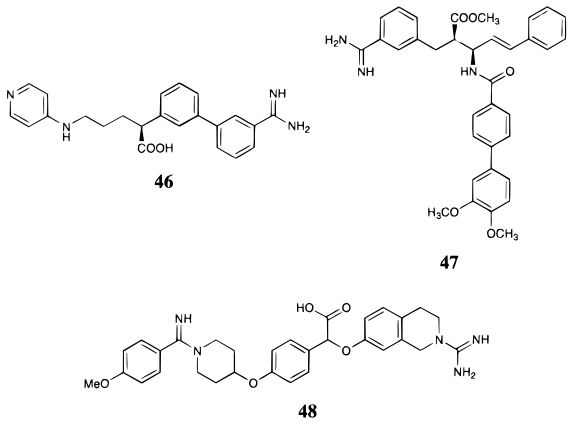
A similar naphthamidine derivative is YM-60828 (**44**), which is selective, potent, and bioavailable (K_i(factor Xa) = 1.3 nM, K_i(thrombin) > 100 μM) and inhibits the enzyme in the prothrombinase complex with an IC_{50} of 7.7 nM.[123] It is orally active, significantly reduces the incidence of occlusion, and improves carotid arterial potency at 30 mg/kg po in an electrically induced artery thrombosis model in rats.[124] This compound also exerted antithrombotic effects in a dose-dependent manner in a venous thrombosis and an arteriovenous shunt model in rats with ID_{50} of 0.081 and 0.010 mg/kg, respectively.[125]

ZK-807191 (**45**) is a potent (K_i = 0.1 nM) and selective inhibitor of factor Xa (3200-fold over trypsin) and is orally active (20% bioavailability) in dogs and monkeys. Substitution at the 4-position and a phenol group opposite the amidine function contribute to the selectivity and potency of this molecule.[92] Other selective submicromolar inhibitors of factor Xa reported recently are FX-2212 (**46**, IC_{50} = 0.27 μM),[126] β-alanine derivatives **47** (IC_{50} = 0.03 μM),[127] and amidinotetrahydroisoquinoline derivatives **48** (K_i = 0.026 μM).[128] To date no factor Xa inhibitors have been developed as therapeutic agents, but they could conceivably become valuable anticoagulants in the future since they demonstrate good antithrombotic activity without the bleeding complications associated with thrombin inhibitors.

3.3. Elastase. Elastase (EC 3.4.21.37) is a serine protease implicated in adult respiratory distress syn-

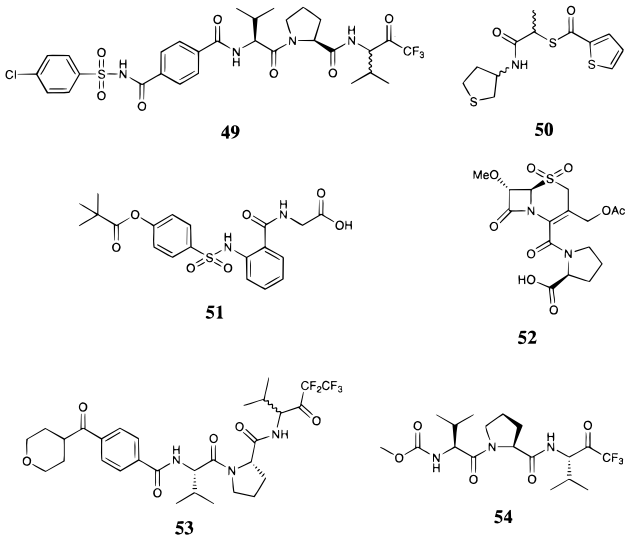


46 **47** **48**

drome (ARDS), rheumatoid arthritis, pulmonary emphysema, cystic fibrosis, and chronic bronchitis. Human neutrophil elastase is released from human polymorphonuclear leukocytes in response to inflammatory stimuli and is responsible for the degradation of connective tissue proteins such as collagen, elastin, laminin, fibronectin, and proteoglycan. Normally elastase activity is tightly regulated by endogenous inhibitors such as secretory leukocyte protease inhibitors and α1-protease inhibitors, but an imbalance between proteases and antiproteases can lead to degradation of healthy tissue and disease development.

Numerous inhibitors of elastase have been reported during the past decade.[129] Although no elastase inhibitors have been approved for commercial use, various electrophilic carbonyl derivatives (ICI-200800 (**49**), MR889 (**50**), MDL101,146 (**53**), ZD8321 (**54**)) and heterocyclic derivatives (L-658,758 (**52**)) have been investigated and are currently undergoing clinical trials for emphysema and other pulmonary diseases. Inhibitors such as ICI-200880 (**49**)[130] and ICI-200355[131,132] are acylsulfonamide trifluoromethyl ketone derivatives of a Val-Pro-Val template, (K_i = 0.5 and 0.6 nM, respectively). The Val-Pro-Val residues bind to the S1−S3 sites of the enzyme, while the electrophilic ketone interacts with the catalytic serine nucleophile in the active site to form a hemiketal adduct. Both compounds significantly reduce elastase-induced lung hemorrhage when administered intratracheally at 0.3 μmol/animal up to 36 h prior to elastase installation.[133] Although ICI-200880 has undergone human trials, it has low oral bioavailability and is limited to intratracheal administration. In a recent article, clinical trials for this compound were reported to have been discontinued.[134]

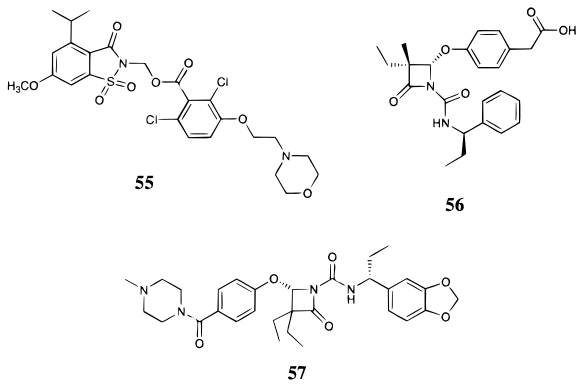
The cyclic thiol MR889 (**50**) is in clinical evaluation for the treatment of chronic obstructive pulmonary disease (COPD).[135] It is a reversible, slow-binding inhibitor (K_i = 1.38 μM) with good kinetic properties, and it may be given to COPD patients at 500 mg b.i.d. safely for a period of 4 weeks with no major side effects.[136,137] ONO-5046 (**51**) is a competitive inhibitor (K_i = 0.2 μM, IC_{50} = 0.044 μM) which also suppresses lung damage induced in hamsters by elastase administered intratracheally (ID_{50} = 82 μg/kg).[138] Interestingly, when this drug was injected (100 mg/kg/day) intraperitoneally to arthritic rats, it was found to protect cartilage from degradation and reduce the incidence and severity of collagen-induced arthritis.[139,140] This compound has also been investigated as a chemotherapeutic



49 **50** **51** **52** **53** **54**

agent for lung cancer[141] and idiopathic interstitial pneumonia.[142] With its low toxicity and good in vivo properties, it may soon enter human trials.[143]

The cephalosporin derivative L-658,758 (**52**), which is a potent and irreversible inhibitor of elastase (k_{obs}/[I] = 3800 M^{-1} s^{-1}, ED_{50} = 5 μg/animal, $t_{1/2}$ = 21 h),[144,145] has been investigated as a topical aerosol for the treatment of pulmonary diseases. While intratracheal administration of drugs may be the preferred method for the treatment of pulmonary diseases, other elastase-induced inflammatory and nonpulmonary disorders such as rheumatoid arthritis and atherosclerosis require orally bioavailable remedies. Various heterocyclic derivatives such as penicillins,[146,147] penems,[148] β-lactams,[149,150] isocoumarins,[151] benzisothiazolones,[152] WIN63759 (**55**), alkylazetidinones (**56**), and many others have been shown to be potent elastase inhibitors. In particular, elastase inhibitors such as WIN63759 (K_i = 0.013 nM) have good pharmacokinetic properties in dogs when administered orally and can be detected in the lung (C_{max} = 0.47 μg/mL).[153] Similarly, compound **56** (k_{obs}/[I] = 91000 M^{-1} s^{-1}) is an orally active heterocyclic derivative causing 98% inhibition 30 min after 30 mg/kg was administered to hamsters. Even after 5 h, there was still 51% inhibition (ED_{50} = 3.7 mg/kg, $t_{1/2}$ = 5 h).[154] Another heterocyclic inhibitor disclosed recently is DMP-777 (**57**), an orally active pyrrolidine *trans*-lactam which has entered phase II clinical trials for inflammatory-related disorders.[134,155]

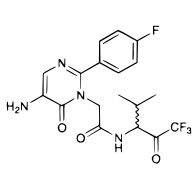


55 **56** **57**

Many inhibitors which have been developed have been based on the Val-Pro-Val template such as ICI-200880,

MDL101,146 (**53**), and ZD8321 (**54**). Although peptidic inhibitors have generally been demonstrated to have poor oral bioavailability characteristics, **53** is an orally active pentafluoroethyl ketone inhibitor (K_i = 20 nM, ED_{50} = 15 mg/kg) which has recently entered human trials.[156] This compound inhibited lung hemorrhage in rats and hamsters by 69% and 74% by oral administration of 50 and 25 mg/kg, respectively.[157] An enol actate prodrug of MDL101,146 showed improved oral bioavailability with an ED_{50} of 9 mg/kg.[158]

The trifluoromethyl ketone derivative ZD8321 (**54**) has also been shown to display oral activity (K_i = 13 nM). It is a potent inhibitor with excellent in vivo activity (ED_{50}(iv) = 0.51 mg/kg, ED_{50}(oral) = 2.0 mg/kg) including oral bioavailability of 75%, 84%, and 70% in hamsters, rats, and dogs, respectively.[159] Various trifluoromethyl ketone[160] analogues have been synthesized with different nonpeptidic structures, including pyrimidone **58**, pyridone **59**, β-carbolinone **60,** and pyridopyrimidine **61**. The pyridone analogue **59** was found to possess excellent intratracheal activity (K_i = 9.4 nM, ED_{50} = 2 nmol/animal),[161] while the β-carbolinone **60** displayed significant in vitro potency (K_i = 6.1 nM) but was not orally active.[162] Pyrimidone **58** (K_i = 101 nM, ED_{50}(oral) = 7.5 mg/kg)[163,164] and pyridopyrimidine **61** (K_i = 0.95 nM) have more promising oral bioavailability.[165] Another interesting nonpeptide derivative CE-1037 (**62**) is a potent inhibitor (K_i = 0.45 nM) which has undergone phase II clinical trials as a therapeutic for cystic fibrosis.[134]



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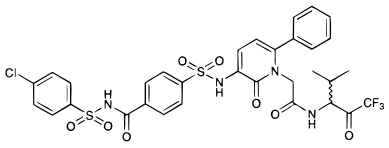

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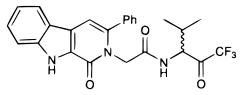

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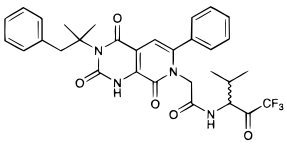

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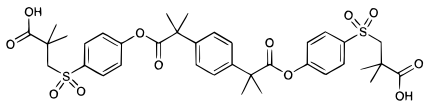

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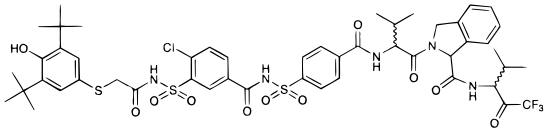

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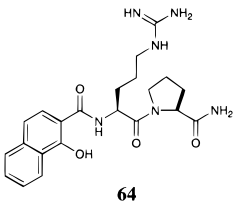
In a recent report, it was demonstrated that the destructive potential of elastase in the lung was enhanced by reactive oxygen radical species. A perhydroindole derivative with an antioxidant moiety attached (**63**, IC_{50} = 26 nM) was found to reduce hemorrhage by 50% when given 72 h prior to elastase challenge (15 nmol/animal it). This compound had excellent stability and retention in the lung as compared to 18 h for ICI-200880.[166] Novel pharmacophores such as *trans*-lactams, *trans*-lactones,[155] thienooxazinones,[167] thiadiazolidinone dioxides,[168] and hydrazinopeptides[169] have also been reported in the literature as potent elastase inhibitors worthy of further investigation.

3.4. Tryptase. Tryptase (EC 3.4.21.59) is a trypsin-like serine protease that is the major secretory product of human mast cells. This enzyme is released in response to mast cell activators along with histamine, heparin, and other mast cell proteases.[170,171] Tryptase has been implicated as an inflammatory mediator in different inflammatory and allergic conditions such as conjuctivitis, rhinitis, and especially asthma. The S1 pocket is large enough to accommodate a range of simple P1 nitrogen bases (e.g. Arg, Lys). Homology models[172] and the crystal structure[173] of the enzyme have indicated preference for simple aromatic and aliphatic nitrogen bases at P1.

Three types of synthetic tryptase inhibitors have been reviewed,[174] including peptidic inhibitors such as APC-366 (**64**, K_i = 0.84 μM), which is currently in phase II clinical trials for the treatment of asthma,[5] dibasic inhibitors (**65**), and Zn^{2+} binding inhibitors BABIM (**66**).[175] The arginine residue on APC-366 is proposed to bind in the S1 pocket, with flanking residues occupying prime and nonprime sides of the active site. It is a time-dependent irreversible inhibitor where presumably the active site serine nucleophile attacks the hydroxynaphthoyl ring leading to the acylated enzyme. Treatment of allergic sheep with APC-366 by inhalation abolished airway hyperresponsiveness caused by tryptase, while intradermal tryptase administration also induced a dose-dependent cutaneous response in allergic sheep.[176,177] In a clinical trial, 32% reduction of the early response asthma reaction was achieved and over 60% of the late response; thus it was concluded that the effects of APC-366 found in sheep were reflected in the clinical trial as well.[178]



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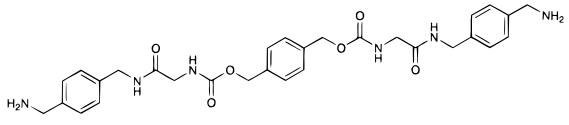

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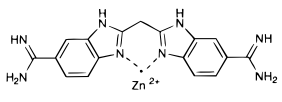

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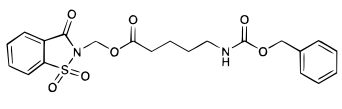

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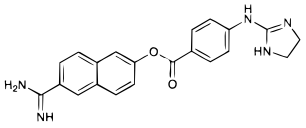
A glycine derivative (**65**) is very selective and potent for tryptase (K_i(tryptase) = 0.6 nM, K_i(trypsin) = 84 μM). These dibasic benzylamine inhibitors are C_2-symmetric, one end of the molecule fitting the S1 pocket or the acidic surface of the enzyme while the other end interacts with either the S1 pocket or the acidic region on a second monomer. This is made possible by the

pseudosymmetry of the active tryptase tetramer, which places the individual active sites within reasonable distance of one another.[174]

The discovery of zinc-mediated inhibitors was recently reported,[175] in which Zn^{2+} coordinates between the two catalytic residues (Ser195, His57) and the nitrogens of the inhibitor. Zn^{2+} itself inhibits some enzymes such as cytomegalovirus protease, cysteine protease, and HIV-1 protease by binding at their active sites. The presence of the ion enhances the activity of the inhibitors, as in the case of BABIM (**66**); however, this zinc-mediated inhibition is suppressed by metal-chelating agents such as EDTA. Therefore, K_i is 50 nM for **66** in the presence of zinc but only 2.5 μM in the additional presence of EDTA.

Most recently, 1,2-benzisothiazol-3-one 1,1-dioxide inhibitors were reported. These inhibitors show good selectivity for tryptase over elastase and trypsin. In a delayed-type hypersensitivity mouse model of skin inflammation, a 5% solution of compound **67** was found to reduce edema by 69% compared to control animals. This compound was also found to reduce myeloperoxidase content, an enzyme marker of polymorphonuclear leukoyte infiltration, by 96%.[179]

3.5. Complement Convertases. C3 and C5 convertases respectively process the third and fifth plasma proteins of the human complement system, which becomes activated during the immune response to infection or injury.[180,181] These proteases are essential for complement activation via both classical (antibody-dependent) and alternate (antibody-independent) pathways. Their inhibition down-regulates the complement cascade and the generation of potent pro inflammatory proteins C3a[182] and C5a,[183] which are overexpressed in chronic inflammatory conditions such as rheumatoid arthritis, pancreatitis, Crohn's disease, ARDS, sepsis, and Alzheimer's disease.



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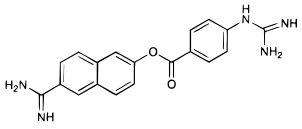

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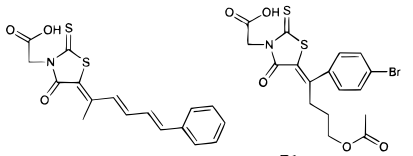
Sepimostat mesylate (FUT-187, **68**) is an orally active serine protease inhibitor with potent inhibitory activity against enzymes involved in the pathogenesis of pancreatitis as well as displaying anticomplement activity.[184] The autodigestive process starts with the activation of trypsin, which in turn activates other enzymes such as elastase and phospholipase A2. These enzymes are associated with the pathogenesis of pancreatitis, while complement has also been suggested to play a role in the development of pancreatitis. K_i values for sepimostat mesylate against trypsin, pancreatic kallikrein, plasma kallikrein, plasma thrombin, factor Xa, and C1r were 0.097, 0.029, 0.61, 0.57, 2.5, 20.4, and 6.4 μM, respectively. Sepimostat mesylate also inhibited complement-mediated hemolyses in classical and alternative pathways, IC_{50} = 0.17 and 3.5 μM, respectively.[185] On the central and peripheral nervous systems, no serious side effects were observed for sepimostat mesylate in a general pharmacology study in mice and rats. In a similar study, no adverse effects on the cardiovascular and respiratory systems were observed in dogs.[186]

Several reported studies have evaluated sepimostat mesylate in patients with postgastrectomy reflux esophagitis. Patients were given 100, 200, or 300 mg/day po for 4 weeks in a double-blind phase II study in order to determine the clinical effect and optimum dose of the compound for this condition.[187,188] Improvement of endoscopic findings were obtained at the higher doses. In another 4-week study, 78% of patients reported a reduction in chief subjective symptoms. Sepimostat mesylate was found to display a wide margin of safety, with no adverse reactions accompanying excellent therapeutic results.[189]

A closely related naphthamidine derivative, nafamostat mesylate (FUT-175, **69**),[190] is another synthetic protease inhibitor widely used for the treatment of acute pancreatitis and disseminated intravascular coagulation in Japan. FUT-175 caused 50% inhibition of the production of C3a and C5a generated by C3/C5 convertase activity at 4.0 μM.[191] Compound **69** can also exert beneficial effects on glomerulonephritis with hypocomplementemia by inhibiting complement activation.[192] This compound is even more potent than gabexate mesylate in its inhibitory effect on other proteases (IC_{50}(thrombin) = 1.9 μM, IC_{50}(plasmin) = 2.9 μM, IC_{50}(collagenase) = 0.42 mM, IC_{50}(cathepsin D) > 1 mM, IC_{50}(factor Xa) = 2.1 μM). However, it has been reported to cause hyperkalemia because of reduced urinary excretion of potassium.[193]

3.6. Hepatitis C−NS3 Protease. Chronic infection by the hepatitis C virus can lead to progressive liver injury, cirrhosis, and liver cancer. A serine protease known as NS3 protease, a flavivirus protease, is thought to be essential for viral replication and has become a target for anti-HCV drugs.[40] The X-ray crystal structure of the NS3 protease shows it to be monomeric with two domains: a trypsin-like fold and a structural zinc-binding site.[19,194,195] Its substrate specificity is however different from that of cellular serine proteases with a cysteine residue in the P1 position of peptide substrates.[196,197] NS3 protease is becoming the most studied target for the development of anti-HCV therapeutics. Numerous nonpeptidic compounds have been reported to inhibit the protease at micromolar concentrations, including thiazolidine derivatives RD4-6204 (**70**) and RD4-6205 (**71**), which exhibit moderate selectivity toward other serine proteases (**70**, IC_{50}(HCV) = 9.7 μg/mL, IC_{50}(chymotrypsin) = 10.8 μg/mL, IC_{50}(trypsin) = 12.6 μg/mL, IC_{50}(plasmin) > 50 μg/mL, IC_{50}(elastase) = 21.6 μg/mL; **71**, IC_{50}(HCV) = 6.4 μg/mL, IC_{50}(chymotrypsin) = 15.6 μg/mL, IC_{50}(trypsin) = 18.3 μg/mL, IC_{50}(plasmin) > 50 μg/mL, IC_{50}(elastase) > 50 μg/mL).[198] A chemical library of 2000 compounds was screened for HCV inhibition, and a number of compounds, **72**−**74** (IC_{50} = 3.2, 6.5, and 6.2 μM, respectively), were active against NS3 protease. These compounds were however not selective for NS3 over other serine proteases such as chymotrypsin.[199]

A number of peptidic inhibitors have been reported with submicromolar activity. These compounds were designed from the substrate sequences and so were expected to exhibit better selectivity than the nonpeptidic inhibitors discussed above. Hexapeptides based on the cleavage sites NS4A−NS4B (**75**) and NS5A−NS5B (**76**) were found to be effective inhibitors of the enzyme (K_i = 0.6 μM, IC_{50} = 28 μM, respectively).[200,201] A



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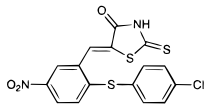

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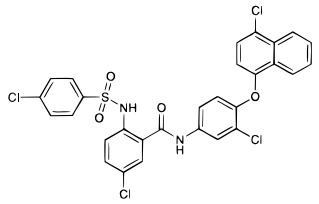

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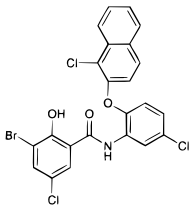

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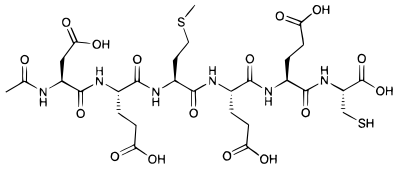
D-amino acid scan of peptide **75** gave rise to a potent inhibitor **77** ($IC_{50} = 4\ \mu M$).[201] This peptide was further optimized where the cysteine residue was replaced by norvaline and the carboxylic acid moiety was replaced by an activated carbonyl group, resulting in the submicromolar inhibitor **78** ($IC_{50} = 0.64\ \mu M$).[202] Similarly, replacing the P2 and P4 residues of **76** with unnatural amino acids gave **79** which was even more potent ($IC_{50} = 0.05\ \mu M$).[203] Like most peptidic inhibitors, these compounds will likely suffer from poor bioavailability.[17] However the major unsolved problem in this field is the lack of good cellular and in vivo models for HCV infection, and this is in part responsible for the fact that there are currently no HCV-NS3 protease inhibitors approved for the treatment of HCV infection. With the crystal structure of HCV-NS3 protease and related viral proteases now available, this field should grow rapidly in the next few years with new inhibitors being developed through a combination of structure-based, substrate-based, analogue-based drug design methods perhaps using lead molecules developed through combinatorial chemistry approaches.



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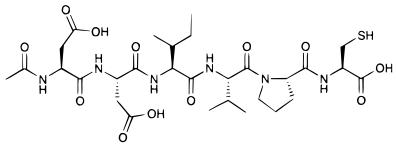

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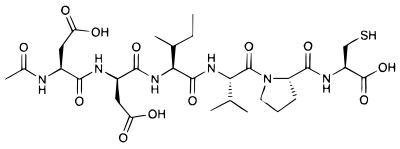

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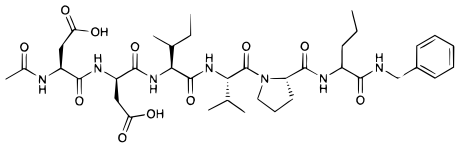

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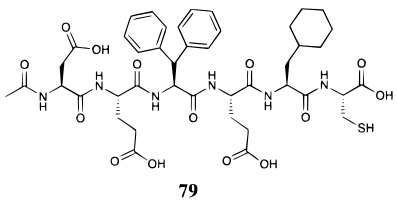

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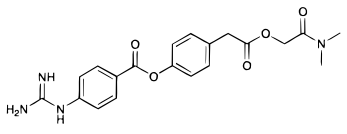
3.7. Broad-Spectrum Serine Protease Inhibitors. Aprotinin,[204,205] a naturally occurring broad-spectrum proteinaceous serine protease inhibitor isolated from bovine lung tissue, inhibits kallikrein and plasmin. The use of aprotinin to reduce blood loss during and after cardiac operations was reported in the literature more than 20 years ago. Aprotinin has several possible mechanisms of action, including inhibition of the inflammatory cascades activated by contact with the foreign surface of the bypass machine. This contact triggers the generation of plasmin and kallikrein. Aprotinin inhibits these serine proteases, affecting fibrinolysis, reducing the inflammatory actions of kallikrein and the subsequent involvement of complement and immune cell activation.[206] Clinical trials have proven that high-dose aprotinin is effective in reducing blood loss and transfusion requirements associated with primary cardiac procedures such as coronary artery bypass graft (CABG) or heart valve replacement surgery.[204]

Aprotinin was also found to have no adverse effects in pediatric cardiac surgery[207] and is well-tolerated with minor allergic reactions being the most frequently reported adverse effect in other uses.[208] However there is a possibility that aprotinin could create a prothrombic state leading to early graft occlusion and formation of microthrombi in renal and coronary vasculatures.[205] Aprotinin therapy has other major pharmacologic actions to reduce the inflammatory response to the period of extracorporeal circulation and operation, incidence and severity of pulmonary hypertension, and inflammatory actions on the heart and brain.[209] Aprotinin administration has important clinical benefits in patients undergoing reoperation, arterial switch procedures, heart, lung, and orthotopic liver transplantation.[210,211]

Gabexate mesylate (FOY-305, **80**) is a potent small molecule (MW 417 Da) serine protease inhibitor, which blocks clotting activity by thrombin but is inactivated rapidly by plasma esterases. It also inhibits trypsin, kallikrein, plasmin, C1 esterase, phospholipase A2, and prostaglandin synthesis. Gabexate has been used for several years in clinical therapy of acute pancreatitis and disseminated intravascular coagulation.[212] Recently, it has also been reported that gabexate modulates the immune response in the experimental model of lung injury by acute pancreatitis in rats.[213] Early administration of **80** significantly improved mortality



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in severe pancreatitis and achieved significantly earlier recovery of abdominal pain, hyperamylasmia and leu-

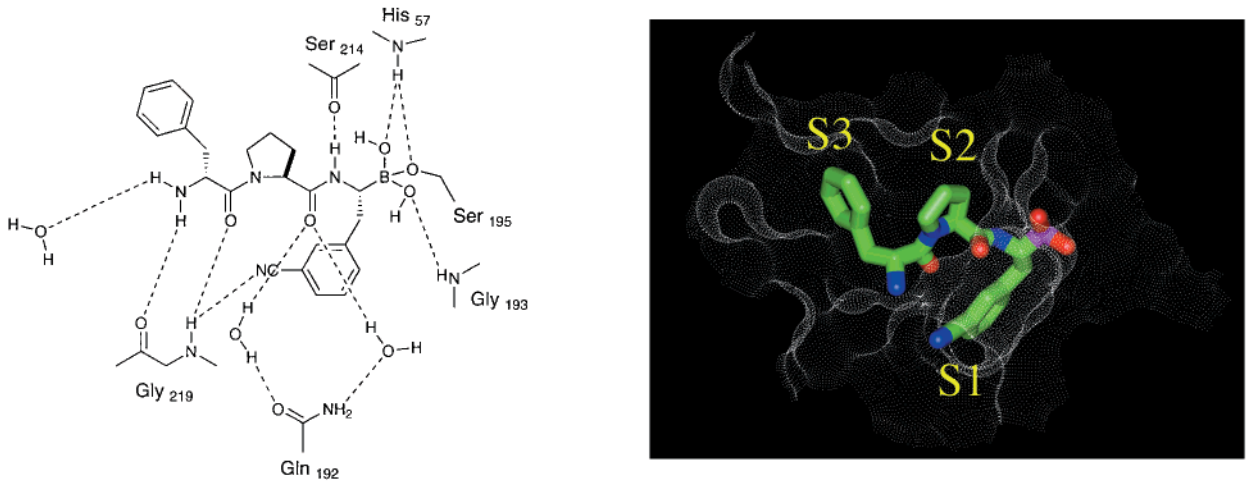


Figure 6. (a) Hydrogen-bonding interactions between H-D-Phe-Pro-boroPhe(*m*-CN)-OH and the serine protease trypsin. The inhibitor is covalently bound to the enzyme via the active site serine which forms a tetrahedral complex with the boronic acid group. The *m*-cyano function makes a hydrogen-bonding interaction with the backbone nitrogen of Gly219. This interaction gives enhanced binding affinity (10−100 times) to this inhibitor with respect to H-D-Phe-Pro-boroPhe-OH for thrombin, factor Xa, and trypsin.[216] (b) X-ray crystal structure of the inhibitor bound to trypsin. The active site of this enzyme (PDB: 1auj), as for most other serine proteases, consists of a shallow groove that is relatively exposed to solvent and can accommodate 3−5 amino acids.

kocytosis in mild to moderate pancreatitis.[193] In a pilot clinical trial where the effect of gabexate mesylate on blood loss in cardiac valve replacement surgery was assessed, it was concluded that this compound may be a promising alternative to aprotinin.[214] As gabexate mesylate is a synthetic serine protease inhibitor, it has advantages over aprotinin which has other biological characteristics leading to allergic or anaphylactoid adverse reactions. The effects of gabexate mesylate and nafamostat mesylate on the postischemic recovery of function and enzyme leakage were also investigated. Nafamostat mesylate and gabexate mesylate, at 5 and 100 μM, respectively, have myocardial protective effects, suggesting that serine proteases play an important role in the progress of myocardial damage during ischemia.[215]

3.8. Summary. Typically serine proteases have active site clefts that are relatively exposed to solvent, often permitting access to polypeptide loops of putative substrates or inhibitors (e.g. endogenous proteinaceous inhibitors such as serpins) which nevertheless present their protease-binding domains in an extended strand conformation.[34,35] Frequently serine protease inhibitors have only 3−5 amino acid residues or their equivalent and thus interact with only a small region of the enzyme. This leads to one of the major problems with designing inhibitors for serine proteases − obtaining selectivity. For example considerable effort had to be expended in developing thrombin inhibitors because of the similarity of the active site for other serine proteases, notably trypsin. Figure 6 highlights the hydrogen bonds made between one thrombin inhibitor and the enzyme trypsin as determined from an X-ray crystal structure,[216] and receptor-based design of protease inhibitors is becoming increasingly valuable for differentiating inhibitor selectivity.

The thousands of small molecule serine protease inhibitors that have been designed to date can be broadly categorized as either reversible or irreversible. Briefly, *irreversible* inhibitors usually are substrate-like and possess an electrophilic functional group capable of reacting with the active site serine or histidine residues to form a covalent bond between enzyme and inhibitor. Many such inhibitors have been described with terminal electrophilic groups such as alkyl fluorophosphates, chloromethyl ketones, and sulfonyl fluorides, the latter being used extensively as affinity labels in enzymology. Most *reversible* inhibitors that have been described make hydrogen bonds and ionic and van der Waal's interactions with the subsites of the enzyme as well as form reversible tetrahedral transition-state-like complexes with the catalytic serine hydroxyl of the enzyme. Reversible transition-state analogues usually possess an electrophilic functional group (isostere) located at the C-terminus of the P1 residue. A few examples are aldehydes such as **33**, boronic acids or their esters such as **35** and **36,** and activated ketones such as the trifluoromethyl ketone **49**.[87,129] As these compounds mimic the transition state of amide bond hydrolysis when bound to the active site of the enzyme, they generally display much greater binding affinity (10−1000-fold) than inhibitors that do not possess an electrophilic isostere.

For the development of therapeutically useful serine protease inhibitors with good pharmacological characteristics, it has generally been accepted that reversible inhibitors are preferred over irreversible ones. Irreversible inhibitors would be expected to covalently bind with many nucleophiles en route to the intended target most likely resulting in toxic side effects. In addition, an irreversible inhibitor would be required to have a high degree of selectivity for its target enzyme; otherwise it could inactivate other serine proteases with concomitant side effects. The greatest effort has therefore been directed to the development of reversible inhibitors and,

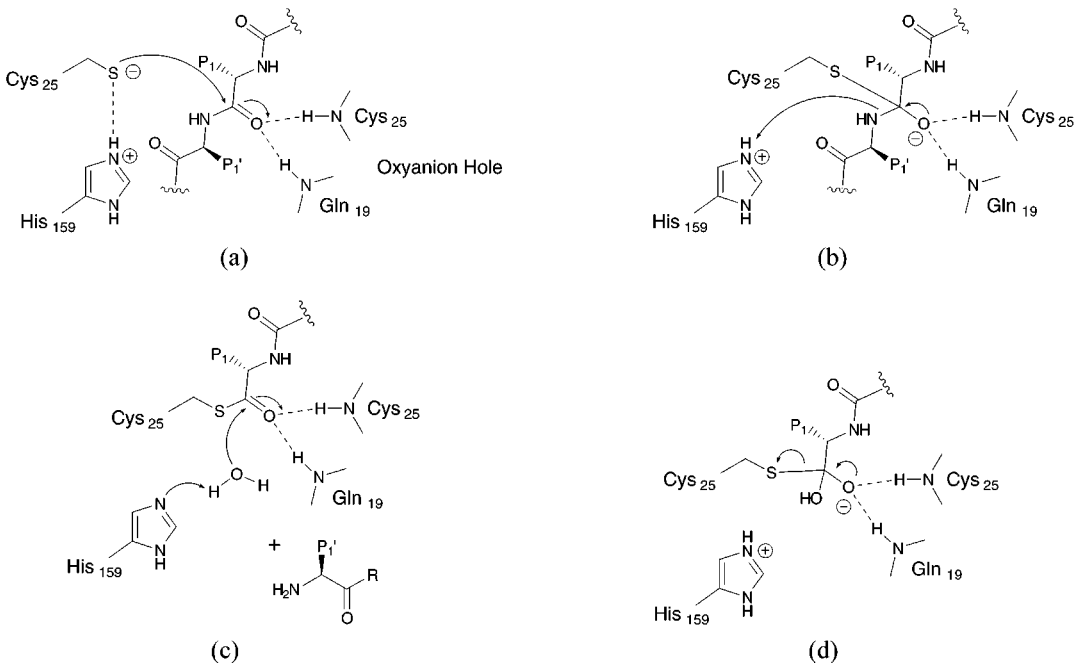


Figure 7. Proposed catalytic mechanism for cysteine proteases. (a) The thiol group of the active site cysteine and the imidazole ring of histidine are believed to exist as a thiolate/imidazolium ion pair. The thiolate anion is therefore highly nucleophilic and readily attacks the scissile amide bond. (b) The tetrahedral intermediate produced is stabilized by the oxyanion hole. This intermediate collapses, via acid-assisted catalysis, to the thioester intermediate (c) with release of the C-terminal substrate fragment. (d) Water hydrolysis gives the regenerated active site and the N-terminal substrate fragment.

in particular, those possessing an electrophilic isostere in order to achieve greater affinity for the intended target.

The presence of a reactive electrophilic site in many of these reversible inhibitors can however be expected to lead to many pharmacological problems when these compounds are administered in humans or mammmals. Aldehydes for example are easily oxidized to the corresponding carboxylic acids and are prone to racemization in the presence of acid or base if a chiral center is present at the α-carbon of the P1 residue. Inhibitors possessing this isostere would be expected to display poor bioavailability, and it will be interesting to see if any clinical candidates such as the thrombin inhibitor efegatran **(33)** are successful drugs.

After many years of intensive research aimed at the development of thrombin inhibitors with good selectivity and pharmacokinetic properties, it seems that the best strategy is to remove the electrophilic isostere altogether.[217] Nine out of the 13 inhibitors (**30**−**42**) shown for thrombin are not transition-state analogues and rely solely on ionic interactions at P1 with guanidine or amidine functional groups with hydrogen-bonding and hydrophobic contacts to other parts of the binding groove of the enzyme. Indeed, the *trans*-aminocyclohexylglycine inhibitor **39** was developed from a transition-state analogue that contained a *trans*-aminocyclohexylglycine ketoamide at the P1 position. The α-ketoamide isostere was removed due to concerns of stability, toxicity, and difficulty of synthesis. The activity of the compound decreased from K_i = 90 pM to K_i = 5 nM, but selectivity for thrombin over trypsin (∼2000-fold) was retained and the compound exhibited a better pharmacokinetic profile. This strategy has also been used for the deveopment of inhibitors of factor Xa and tryptase. These researchers have also shown that basic groups such as amines, amidines, and guanidines are not needed in the P1 position.[218,219] The introduction of a hydrophobic group such as 2,5-dichlorobenzyl was also found to lead to low nanomolar and subnanomolar inhibitors of thrombin with greater than a 1000-fold selectivity over trypsin. This latter result suggests that inhibitors which lack the electrophilic isostere can also be designed for chymotrypsin-like and elastase-like enzymes.

4. Cysteine Protease Inhibitors

Cysteine (thiol) proteases[4,220−222] exist in three structurally distinct classes which are either papain-like (e.g. cathepsins), ICE-like (caspases), or picorna-viral (similar to serine proteases with cysteine replacing serine). The papain group of cysteine proteases has been the most studied until recently. The active site of papain contains a catalytic triad of Cys25, His159, and Asn175. However, it is debatable whether the catalytic dyad of Cys and His is sufficient for full catalytic activity. Asn175 has been proposed to orientate His159, so that the imidazole group of His159 polarizes the thiol group of Cys25, allowing deprotonation even at neutral to weakly acidic pH, and the resulting thiolate/imidazolium ion pair is highly nucleophilic.

Cysteine proteases hydrolyze amide bonds in much the same manner as serine proteases. A noncovalent Michaelis complex is formed upon substrate binding. The thiolate anion then attacks the carbonyl carbon of the scissile amide bond (Figure 7a). A tetrahedral intermediate is produced which is stabilized by the oxyanion hole (Figure 7b). This is followed by the acylation of the enzyme and the liberation of the first product. Hydrolysis of the acyl−enzyme leads to the formation of the second tetrahedral intermediate (Figure 7c). Following the collapse of the second intermedi-

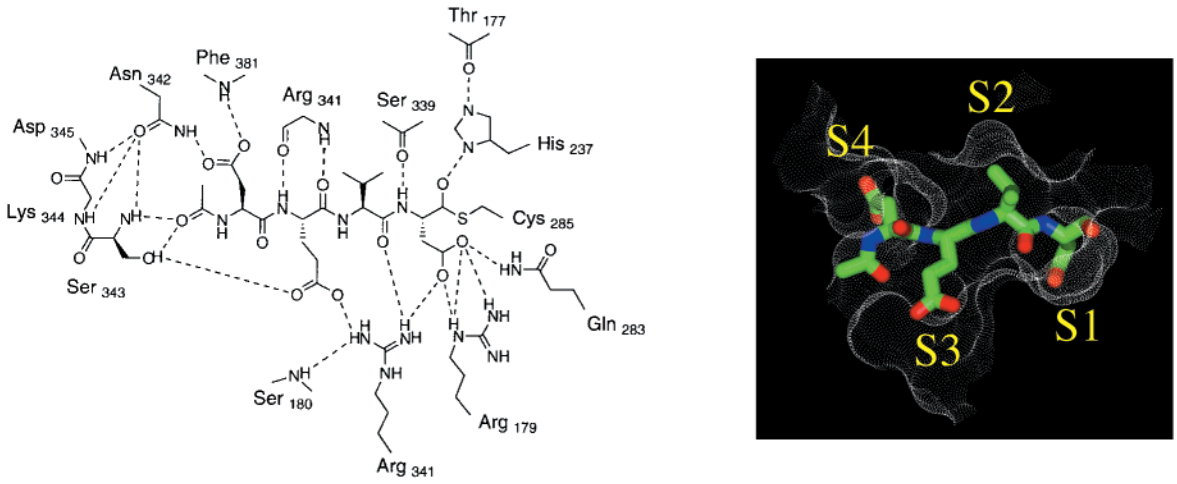


Figure 8. (a) Hydrogen-bonding interactions between Ac-Asp-Glu-Val-Asp-CHO and the cysteine protease caspase-3.[268] The enzyme forms a covalent hemithioketal complex with the aldehyde function of the inhibitor and forms an extensive hydrogen-bonding network to all other residues of the inhibitor including the amide backbone. The large number of hydrogen-bonding and ionic interactions with the P1 aspartate makes it easy to see why caspases in general have an absolute requirement for aspartic acid as the P1 residue in substrate hydrolysis. (b) X-ray structure of the inhibitor bound to caspase-3 showing the extended conformation of the inhibitor in the active site (PDB: 1pau).

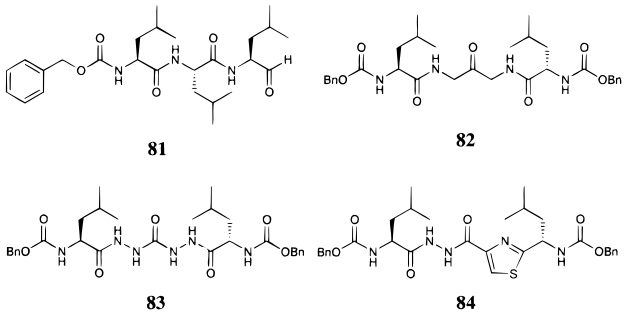
ate, the product acid is released and the free enzyme is regenerated (Figure 7d).

The biggest problem in designing inhibitors for cysteine proteases is the similarity in their substrate affinities and proteolytic mechanisms with serine proteases, particularly the common requirement of an electrophilic isostere that reacts selectively, and preferably reversibly, with the catalytic nucleophile (cysteine thiolate versus serine hydroxyl). Although the spatial configurations of the catalytic triads of serine and cysteine proteases are quite similar, it appears that the oxyanion hole and the negatively charged tetrahedral intermediate are the central features of the catalytic mechanisms of serine proteases, while cysteine proteases stabilize the later more neutral acyl intermediate. The development of potent, reversible, and selective transition-state analogues could potentially take advantage of this mechanistic difference.

Like serine proteases, cysteine proteases tend to have relatively shallow, solvent-exposed active sites that can accommodate short substrate/inhibitor segments of protein loops (e.g. from endogenous inhibitors such as cystatins) or strands. Most inhibitors developed to date tend to be 2−4 amino acids or their equivalent in length, interacting with the nonprime subsites of the enzymes and terminating with various electrophilic isosteres. Figure 8 shows the hydrogen-bonding pattern of a tetrapeptide−aldehyde inhibitor (Ac-Asp-Glu-Val-Asp-CHO) binding to a cysteine protease from the caspase family (caspase-3).[223]

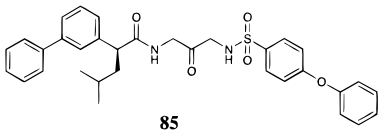
Protease−inhibitor X-ray crystal structures are also available from the PDB database[39] for cysteine proteases such as papain, cathepsin B, ICE, actinidin, glycyl endopeptidase, cruzain, and calpain.

4.1. Cathepsin K. A large number of cathepsins are intracellular cysteine proteases of the papain superfamily, usually but not exclusively found in lysosomes, and are active at acidic to neutral pH. Cathepsin K is selectively expressed in osteoclasts and has been proposed to play an important role in osteoclast-mediated bone resorption.[224,225] Cathepsin K inhibitors are promising therapeutics for the treatment of diseases characterized by excessive bone loss such as osteoporosis.[5] A leupeptin analogue Cbz-Leu-Leu-Leu-CHO (**81**) has been fundamental in the development of cathepsin K inhibitors. This peptidic aldehyde is a potent inhibitor of cathepsin K ($K_{i,app}$ = 1.4 nM), inhibits parathyroid hormone (PTH)-stimulated resorption, and, when administered at 30 mg/kg ip, significantly reduces bone loss and hind paw edema in an adjuvant arthritic rat model.[225]



Based on the observation that leupeptin and Cbz-Leu-Leu-Leu-CHO bind to the active site of papain in opposite directions, a 1,3-bis(acylamino)-2-propanone inhibitor **(82)** was generated and developed from the superposition of the two bound structures which span the whole active site. This compound is selective over the related cysteine proteases such as cathepsins B and L and papain ($K_{i,app}$(cathepsin K) = 22 nM, $K_{i,app}$(papain) > 10 μM, $K_{i,app}$(cathepsin L) = 0.34 μM, $K_{i,app}$(cathepsin B) = 1.3 μM).[226] Other developed inhibitors which span the whole active site are 1,5-diacylcarbohydrazide derivatives such as **83**.[227] These compounds are potent, selective, and kinetically irreversible inhibitors ($K_{i,app}$ = 0.7 nM, $k_{obs}/[I] = 3.1 \times 10^6$ M^{-1} s^{-1}), where X-ray

crystal structure analysis indicated the addition of the catalytic cysteine residue to the central urea carbonyl. A similar diacylhydrazine analogue **84**, containing a thiazole amide bond isostere, is also a potent and selective inhibitor ($K_{i,app}$(cathepsin K) = 10 nM, $K_{i,app}$(cathepsin L) = 0.7 μM, $K_{i,app}$(cathepsin B) = 5.2 μM, $K_{i,app}$(cathepsin S) > 1 μM).[227]



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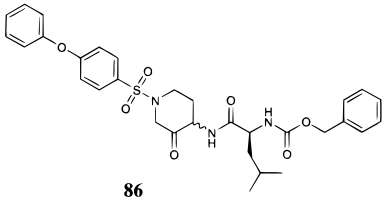

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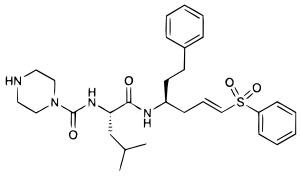

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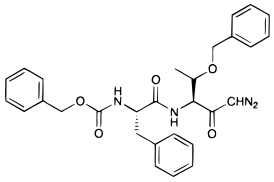
A potent peptidomimetic inhibitor **85** based on the 1,3-bis(acylamino)-2-propanone scaffold has been developed ($K_{i,app}$(cathepsin K) = 1.4 nM, $K_{i,app}$(cathepsin L) > 1 μM, $K_{i,app}$(cathepsin B) > 10 μM, $K_{i,app}$(cathepsin S) = 0.91 μM). The Cbz-Leu moieties in the molecule were replaced by 3-biphenylyl and 4-(phenoxyphenyl)-sufonyl groups resulting in 500-fold selectivity over human cathepsins B, L, and S.[228] Another subnanomolar peptidomimetic inhibitor **86** was developed by the incorporation of a sulfonamide moiety into a conformationally constrained 1,3-diamino ketone inhibitor. The design of this compound removed most of the structural liabilities associated with a peptidic amide ($K_{i,app}$(cathepsin K) = 0.5 nM, $K_{i,app}$(cathepsin L) > 1 μM, $K_{i,app}$(cathepsin B) > 1 μM).[229]

Based on the success of vinyl sulfones[230] as cysteine protease inhibitors, the irreversible inhibitor APC-3328 (**87**) was developed (k_{second} = 5.7 × 10^6 M^{-1} s^{-1}). The crystal structure of human cathepsin K complexed to APC-3328 revealed that the inhibitor closely mimics substrate binding from P1 to P3 and extends to the S1′ side of the binding cleft.[231] This compound is currently in preclinical development and may lead to clinical candidates for osteoporosis.

4.2. Cathepsin B. Cathepsin B[8] is a lysosomal cysteine protease, which plays various digestive and processing roles inside cells to maintain normal cellular metabolism. However, when overexpressed, it has been associated with several pathophysiological conditions such as tumor metastasis, inflammation, bone resorption, and myocardial infarction. Cathepsin B can facilitate tumor progession through direct degradation of the extracellular matrix and membrane components and indirectly through the cascade activation of other proteases such as MMPs, cathepsins D and L, and serine proteases such as plasmin.

Among the earliest reported inhibitors are peptidic diazomethyl ketones and aldehydes. Diazomethyl ketones inactivate cathepsin B by alkylation of the active site cysteine residue. For example Cbz-Phe-Ala-CHN_2 has been reported to inactivate cathepsin B (k_{inact} = 6.8 × 10^4 M^{-1} s^{-1}) and cathepsin L effectively. The closely related cathepsin L inhibitor Cbz-Phe-Phe-CHN_2 is less effective in inhibiting cathepsin B (k_{inact} = 2.1 × 10^4 M^{-1} s^{-1}), indicating that bulky hydrophobic residues are not well-tolerated in the S1 pocket of cathepsin B. However, an *O*-benzylthreonine replacement at P1 yielded a more effective inhibitor **88** (k_{inact} = 1.8 × 10^7 M^{-1} s^{-1}), indicating that the phenyl ring can occupy more remote space.[232]



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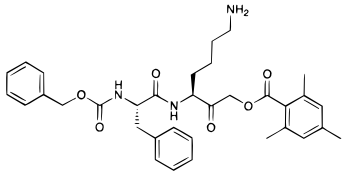

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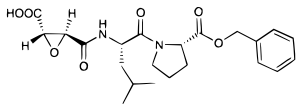

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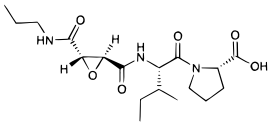

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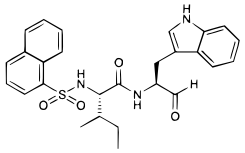
Peptidyl (acyloxy)methyl ketone derivatives have been reported to be potent and selective cathepsin B inhibitors.[233,234] Compound **89** was shown to be effective in vivo, with potent inhibition against the liver enzyme by three different routes of administration (ED_{50}(po) = 18 mg/kg, ED_{50}(ip) = 5.0 mg/kg, ED_{50}(sc) = 2.4 mg/kg).[235] Other peptidic inhibitors of great interest are epoxysuccinyl derivatives such as E-64 (**93**), isolated from cultures of *Aspergillus japonicus*, that alkylate the active site residue of cysteine proteases. E-64 is a broad-spectrum inhibitor which also effectively inhibits cathepsin L and calpain.[236,237] More selective derivatives have been developed such as **90** (k_{second} = 8.7 × 10^3 M^{-1} s^{-1})[238] and CA-074 (**91**).

CA-074 in particular was reported to rescue 67% of monkey hippocampal cornuammonis 1 (CA1) neurons from delayed neuronal death on day 5 after ischemia, when administered intravenously immediately after the ischemia insult.[239,240] The methyl ester prodrug of **91**, on the other hand, was reported to inhibit both stimulated and basal bone resorption in vitro. This compound also inhibited the resorptive activity of isolated rat osteoclast cultured on bone slices with a maximal effect at 50 μM.[241] These encouraging in vivo results suggest that selective and potent cathepsin B inhibitors may become important clinical therapeutics.

4.3. Cathepsin L. Cathepsin L, a lysosomal cysteine protease secreted by osteoclasts, participates in bone resorption by degrading extracellular matrix proteins such as collagen. Osteoporosis is characterized by low bone mass, most often caused by the increase of bone resorption over bone formation. Therefore, selective cathepsin L inhibitors may be good candidates as antiosteoporotic agents. Aldehyde (Cbz-Phe-Phe-CHO, IC_{50} = 0.74 nM)[242] and diazomethyl ketone (Cbz-Phe-Phe-CHN_2, IC_{50} = 50 nM) derivatives have been reported to be selective inhibitors of cathepsin L.[243,244] In a recent report, an analogue Cbz-Phe-Tyr-CHO proved

to be a selective inhibitor (IC_{50}(cathepsin L) = 0.85 nM, IC_{50}(cathepsin B) = 85.1 nM, IC_{50}(calpain II) = 184 nM, IC_{50}(α-chymotrypsin) > 1000 ng/mL), and ip administration (5−10 mg/kg/day) to ovariectomized mice, a model of osteoporosis, for 4 weeks suppressed bone weight loss in a dose-dependent manner. Another aldehyde derivative **92** was reported to be the first example of an orally active cathepsin L inhibitor. It is a potent, selective, and reversible inhibitor (IC_{50} = 1.9 nM). At an oral dose of 50 mg/kg, this compound inhibited the release of calcium ions and hydroxyproline from bone in vitro and prevented bone loss in ovariectomized mice.[245]



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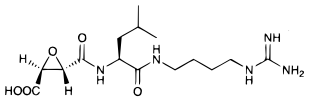

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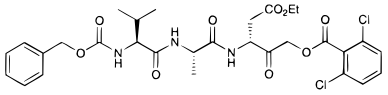
Cathepsin L has also been implicated in tumor metastasis, the process where tumor cells detach from the primary lesion and migrate through lymph or blood vessels to form new foci at distant sites. Lewis lung carcinoma H-59 cell line, which is highly invasive and preferentially metastatic to the liver, expresses high levels of cathepsin L but lower levels of cathepsin B. Treatment with E-64 (**93**) at 100 μg/mL inhibited formation of experimental liver metastases by up to 90%.[237] These results warrant further development of cathepsin L inhibitors as potential therapeutics for the treatment of osteoporosis and also as antimetastatic agents.

4.4. Caspases. Caspase is a new term given to ICE-like cysteine proteases with absolute specificity for aspartic acid at P1 in their substrates.[246−249] Interleukin-1 converting enzyme (ICE) (EC 3.4.22.36), now renamed caspase-1,[250] hydrolyzes the 31-kDa pro-IL-1 in monocytes to the active 17-kDa cytokine IL-1β. Among many functions, this cytokine is a key inflammatory mediator, and ICE inhibitors are promising therapeutic candidates for the treatment of inflammatory diseases.[251,252] The structure of ICE shows that it is quite different from the papain family. It is a heterodimer with an active site consisting of a catalytic dyad of Cys285 and His237. Numerous peptidyl inhibitors of ICE are known, and X-ray crystal structures have been reported for ICE complexed to two potent tetrapeptide−aldehyde inhibitors, Ac-Tyr-Val-Ala-Asp-CHO and Ac-Trp-Glu-His-Asp-CHO, which show an extended inhibitor conformation bound in the active site.[253] Ac-Tyr-Val-Ala-Asp-CHO (K_i = 760 pM),[254] a peptide based on the substrate sequence, forms the basis of subsequent inhibitor design, while the more potent Ac-Trp-Glu-His-Asp-CHO (K_i = 56 pM) was discovered to be the optimal tetrapeptide sequence for ICE using a positive scanning peptide substrate library.[255] An *N*-methyl scan of the tripeptide backbone of a broad-spectrum caspase inhibitor, Cbz-Val-Ala-Asp-CHO, was conducted to investigate the hydrogen-bonding pattern of ICE. The results indicated that the P1 and P3 amide hydrogens were required for high-affinity binding, so P2 and P3 residues were replaced for the development of peptidomimetics.[256]

The first demonstration of an effective ICE inhibitor in a chronic disease model was the prodrug VE-13045 (**94**). The aspartyl side chain ester in the compound is rapidly hydrolyzed in vivo, yielding a bioactive molecule based on the Cbz-Val-Ala-Asp template. Inhibition of ICE activity blocks secretion of both IL-1β and IL-1α, and compound **94** was shown to reduce inflammation and progression of arthritis when administered to mice with established disease. In a DBA/1J mouse model of type II collagen-induced arthritis, prophylactic treatment with VE-13045 (50 and 100 mg/kg/day) significantly delayed the onset of inflammation, with a 60% overall reduction in disease severity.[257] In another report, this compound was also observed to decrease pancreatitis severity when administered before the induction of pancreatitis by feeding a choline-deficient, methionine-supplemented diet.[258]



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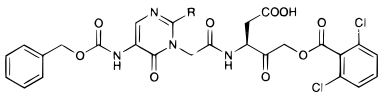
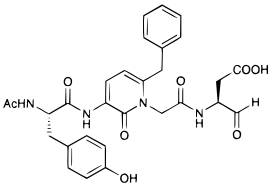


95 R = 2-thienyl



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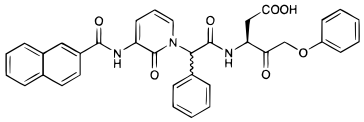
To increase bioavailability, peptidomimetic inhibitors of ICE were developed. The first examples were 5-aminopyrimidin-6-one derivatives such as **95** (k_{obs}/[I] = 272000 M^{-1} s^{-1}).[259] Based on the success of such derivatives, structure−activity relationships were explored for the S2 pocket to yield a potent peptidic aldehyde inhibitor with the 3-aminopyrid-2-one moiety (**96**, K_i = 54 nM).[260] Other pyridone-based peptidomimetics with different activated carbonyl moieties such as phenyl ketomethyl ether **97** and aminomethylene ketone **98** have also been reported to be extremely potent ICE inhibitors (K_i = 1.3 and 0.37 nM, respectively).[261,262]



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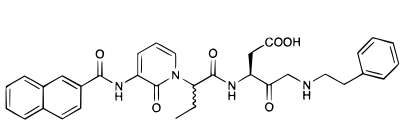

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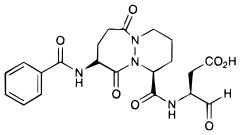

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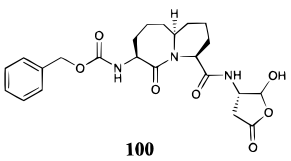
Bicyclic peptidomimetics such as pyridazinodiazepine derivatives have been shown to be potent inhibitors of

ICE. In particular, compound **99** is a potent inhibitor (K_i = 5 nM) and was selected as a candidate for oral administration to dogs. This compound was stable in ex vivo liver slice assays, and it was found to be 12−17% orally bioavailable in the standard iv/po dog model.[263] Most recently, conformationally constrained bicyclic caspase inhibitors **100** and **101** were reported.[264] Caspase-3 (apopain or CPP32), which is believed to be a key executioner in apoptosis, has been implicated in neurodegenerative diseases such as Alzheimer's disease.[252,265−267] The three-dimensional structure (Figure 8) of caspase-3 complexed with a substrate-based tetrapeptide−aldehyde inhibitor, Ac-Asp-Glu-Val-Asp-CHO (K_i = 0.35 nM),[268] revealed an extended conformation within the active site with alternating side chains projecting on the same face of the inhibitor.[223,269] The major difference between ICE and caspase-3 is the S4 pocket of the enzyme. An aspartyl residue in the P4 position is crucial for potent caspase-3 inhibition, the acidic residue interacting with the positively charged S4 pocket while a bulky hydrophobic residue is preferred in P4 for ICE. Compound **100** is a bicyclic derivative which is selective for ICE (IC_{50}(ICE) = 36 nM, IC_{50}(caspase-3) > 10 μM) due to the bulky Cbz group in P4, while compound **101** is a selective caspase-3 inhibitor (IC_{50}(caspase-3) = 18 nM, IC_{50}(ICE) = 10.4 μM) due to the aspartic acid moiety at P4.[264]



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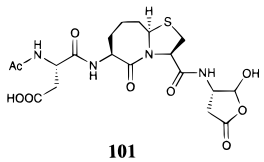
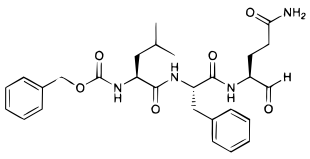


101

4.5. Rhinovirus 3C Protease. Human rhinoviruses (HRV) are small icosahedral RNA viruses which are responsible for the common cold. Since they occur in over a hundred serotypes, the development of a vaccine seems unlikely, whereas the inhibition of their component cysteine proteases 2A and 3C, which are essential for replication as they produce both structural and enzymatic viral proteins, represents a possible therapeutic strategy against the cold.[270−272] Rhinovirus 3C protease is a 20-kDa picorna-viral protease with high sequence and backbone structural homology with trypsin-like serine proteases, but with an active site cysteine instead of serine nucleophile that selectively catalyzes hydrolysis of glutamine−glycine peptide bonds.[273,274] Substrate processing studies have revealed that Leu-Phe-Gln-Gly-Pro is the consensus sequence required for recognition by the protease.

Numerous peptidic and nonpeptidic inhibitors have been reported as rhinovirus 3C protease inhibitors.[40] The peptidic aldehyde based on the substrate sequence **102** is a moderately active inhibitor (K_i = 3.6 μM, EC_{50} = 66 μM, TC_{50} = 398 μM), while the P1 isosteric replacement for glutamine improved the activity of another peptidic aldehyde **103** (K_i = 5 nM, EC_{50} = 1.3 μM, TC_{50} = 63 μM).[275] In a recent review, AG6084 (**104**, K_i = 6 nM), another peptidyl aldehyde inhibitor, was reported to inhibit the replication of different HRV serotypes and related piocornaviruses Coxsackie viruses A21 and B3, enterovirus 70, and echovirus 11. This compound was able to reduce the levels of infectious virus, even when administered late in the HRV life cycle. Such broad-spectrum antiviral activity may make it a potential clinical candidate.[40]



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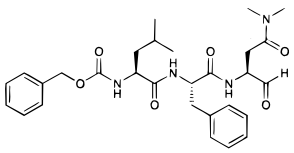

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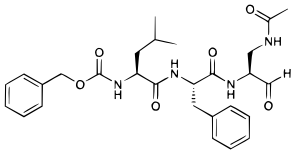

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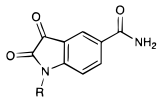
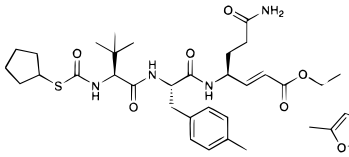


105 R = CH_2-2-benzo[*b*]thiopene,



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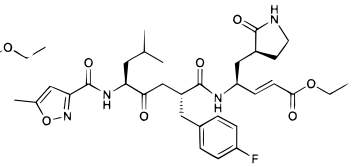

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On the other hand, nonpeptidic 1,5-disubstituted isatins **105** (K_i = 2 nM) were found to have excellent selectivity for HRV-14 3C protease. Unfortunately, these compounds were either relatively inactive or did not display efficacy below their toxic concentrations (**105**, EC_{50} > 5.6 μM, TC_{50} = 5.6 μM) when evaulated in cultured cells infected by HRV.[276]

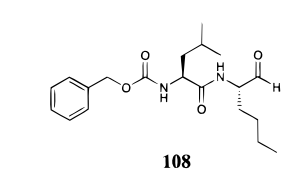
Other peptidic derivatives such as halomethyl ketones,[277] azapeptides,[278] and bromomethyl ketone hydrazides[279] have also been reported as good 3C protease inhibitors. Tripeptides which contain various Michael acceptor moieties have been demonstrated previously to be excellent cysteine protease inhibitors. Michael acceptor analogues[280,281] based on the substrate sequence of Cbz-Leu-Phe-Gln give reasonably potent inhibitors.[282] However when the Cbz moiety and the P2 and P3 residues were modified, the resulting inhibitor **106** displayed potent irreversible inhibition and potent antiviral activity (k_{obs}/[I] = 800000 M^{-1} s^{-1}, EC_{50} = 56 nM).[283]

Most recently, peptidomimetics containing a ketomethylene replacement and an ethyl propenoate Michael acceptor moiety have been synthesized and evaluated.[284] One of the most potent analogues, AG7088 (**107**), has demonstrated excellent selectivity and antirhinoviral activity (k_{obs}/[I] = 1090000 M^{-1} s^{-1}, EC_{50} = 5 nM).[285] This compound is the result of structure-assisted design where the P1 glutamine was replaced by a lactam moiety and the backbone amide between the P2 and P3 residues was replaced by the ketomethylene mimetic. This compound is reported to have entered clinical trials.[286,287]

4.6. Calpains. Calpains (EC 3.4.22.17) comprise a family of cytosolic cysteine proteases consisting of at least six distinct members. These neutral enzymes are calcium-activated and are ubiquitously distributed in various cells. Although the precise functions of calpains

are yet to be elucidated, they have been postulated to be involved in protein turnover, protein kinase C activation, and cytoskeleton and cell membrane organization and also to interact with various membrane receptors and calmodulin-binding proteins.[288] Calpains are believed to play key roles in the pathology of disorders such as stroke, Alzheimer's disease, muscular dystrophy, cataract, and arthritis.[289] Most of the reported inhibitors in the literature are active-site-directed peptidic compounds, and they have been used widely in studies to determine the role of calpains. However, their lack of selectivity poses major problems for the development therapeutics.[290,291]

Numerous peptidic aldehydes have been synthesized and tested as calpain inhibitors,[292] for example Cbz-Val-Phe-CHO and calpeptin (**108**). Calpain has been found to accommodate a range of amino acids at P1, while Leu and Val appeared to be most common at the P2 position. Cbz-Val-Phe-CHO is a potent inhibitor of calpain (K_i = 7 nM) that is reported to protect rat erythrocyte membrane-associated cyoskeletal proteins from degradation (IC_{50} = 1 μM).[293] Calpeptin is a cell-penetrating calpain inhibitor (ID_{50}(calpain I in platelets) = 0.04 μM) which completely abolished calpain activity in platelets after a 30-min incubation.[293,294] Among the many peptidic aldehydes synthesized, an interesting *N*-arylsulfonylproline analogue **109** was recently reported. This compound incorporated a substituted proline at P2 and was found to be potent and very selective toward calpains (IC_{50}(calpain) = 28 nM, IC_{50}(cathepsin B) > 10 μM).[295]



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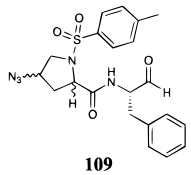

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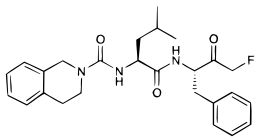

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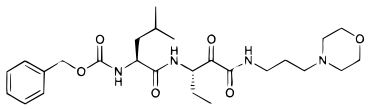

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Potent carbonyl derivatives such as fluoromethyl ketones, Cbz-Leu-Leu-Tyr-CH_2F and Cbz-Leu-Tyr-CH_2F, have been reported to inactivate calpain; however, these compounds also inhibit cathepsin L.[296,297] On the other hand, compound **110** is a potent and selective fluoromethyl ketone inhibitor, having a tetrahydroisoquinoline-containing urea motif as N-terminal capping group ($k_{obs}/[I]$ = 276000 M^{-1} s^{-1}) that is selective over cathespins B and L by 36- and 4-fold, respectively.[298] Another calpain inhibitor of interest is an α-ketoamide derivative **111**. With the activated carbonyl moiety located in the center of the molecule, this compound is able to span both sides of the scissile amide bond. Although this compound displayed only a moderate K_i of 41 μM, postocclusion administration at 3 mg/kg gave a dose-dependent neuroprotective effect (32% reduction in infarct volume) after focal brain ischemia in rats.[299,300]

4.7. Summary. To date there are few electrophilic isosteres appropriate for developing selective, *reversible* inhibitors of cysteine proteases.[221] This may partly explain why very few cysteine protease inhibitors have advanced to early clinical trials.[286] So far, C-terminal aldehydes and ketones, which react with the active site cysteine to form reversible thioacetal transition-state analogues, are the most used isosteres for developing reversible cysteine protease inhibitors. As discussed in the serine protease section, incorporation of aldehyde isosteres into designed inhibitors is expected to lead to poor pharmacokinetic properties and possible lack of selectivity over similar cysteine or serine proteases in the body. Certain activated ketones have been found to be much more selective for cysteine over serine proteases and have the added advantage of permitting access to the S1′−S3′ subsites of an enzyme leading to greater selectivity.[4] This isostere and prime side extension have been used to great effect for the development of inhibitors of cathepsin K with enhanced selectivity over similar proteases such as cathepsins B, L, and S. Peptidyl nitriles show some selectivity for cysteine over serine proteases, with the protein−nitrile adduct resembling the neutral acyl intermediate, but incorporation of this isostere generally leads to reversible inhibitors of low potency for any particular enzyme as compared to aldehydes or ketones.

In contrast to the paucity of reversible isosteres for cysteine protease inhibitor development, a great number of functional groups capable of *irreversibly* alkylating the active site cysteine have been incorporated into peptides including: fluoromethyl ketones, epoxides, diazomethyl ketones, acyloxymethyl ketones, Michael acceptors (α,*β*-unsaturated carbonyl compounds, vinyl sulfones), and ketomethylsulfonium salts to mention a few.[220] These functional groups are activated electrophiles that take advantage of the greater nucleophilic character of the active site cysteine and are generally quite selective for cysteine versus serine proteases. Unfortunately they are still highly reactive to numerous other nucleophiles in the body especially other thiols. These concerns have severely limited the development of such irreversible inhibitors as therapeutic agents. A recent report[230] however has shown that cysteine protease inhibitors containing a vinyl sulfone electrophile are unreactive to thiols such as glutathione and can be considered to be effectively inert in the absence of an enzyme's catalytic machinery. It remains to be seen whether vinyl sulfone electrophiles will provide irreversible inhibitors with the pharmacokinetic and pharmacological profiles required of therapeutic agents in the future.

5. Metalloprotease Inhibitors

All known metalloproteases use a zinc atom to effect amide bond hydrolysis. Figure 9 depicts the currently accepted catalytic mechanism for amide bond hydrolysis by matrix metalloproteases (MMPs). The Zn^{2+} ion is generally tetrahedrally coordinated to three donor groups from the enzyme and a water molecule. The water is also hydrogen-bonded to the carboxylate side chain of a glutamic acid and is therefore activated for nucleophilic attack (Figure 9a). Coordination by the carbonyl group of the scissile amide bond to zinc is presumably followed by nucleophilic attack of the zinc-bound water with simultaneous proton transfer to the

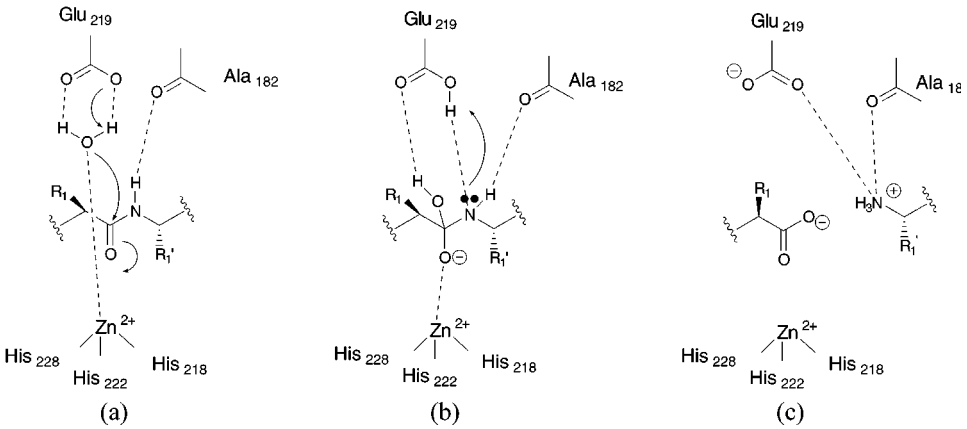


Figure 9. Catalytic mechanism for MMPs. (a) The catalytic water molecule is hydrogen-bonded to the zinc atom and the carboxylate side chain of a glutamic acid residue and is therefore activated for nucleophilic attack. (b) Coordination of the carbonyl carbon of the amide bond is followed by nucleophilic attack of the zinc-bound water to give the tetrahedral intermediate (c) which collapses via acid-mediated catalysis to products.

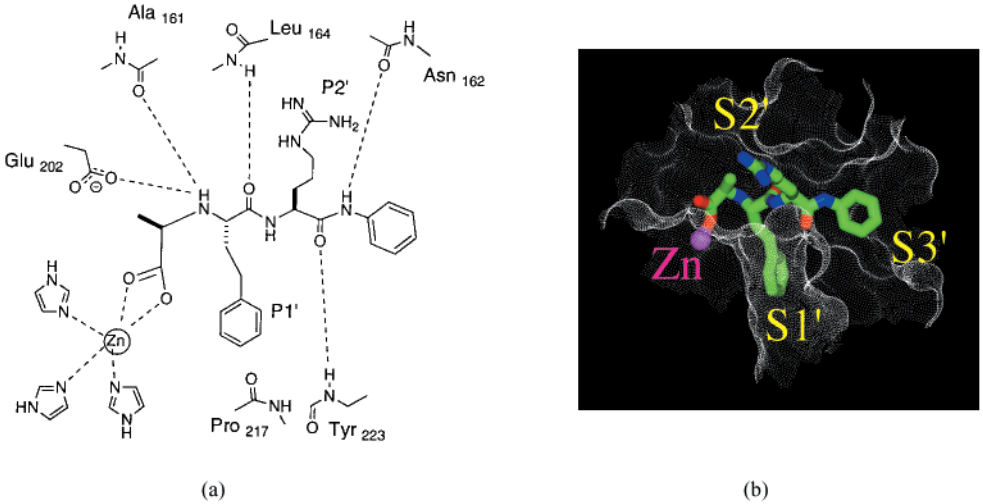


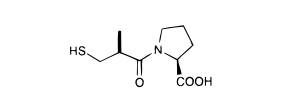
Figure 10. (a) Hydrogen-bonding interactions between an *N*-carboxyalkyl peptide inhibitor and stromelysin-1 (MMP-3). A carboxylate is the zinc-binding group in this case, with a homophenylalanine residue making hydrophobic contacts with the extraordinarily large S1′ subsite of this enzyme, as can be seen from the X-ray structure (b) (PDB: 1sln).[329] The primed side of the active site of most MMPs consists of a depression flanked by two protein segments: the bulge-forming segment and the wall-forming segment. Peptidic inhibitors or substrates can insert between the two segments and form extensive hydrogen-bonding interactions with the enzyme via their amide backbone.

carboxylate giving the zinc-complexed tetrahedral intermediate (Figure 9b). Transfer of a proton from the glutamic acid to the amide nitrogen is followed by the collapse of the tetrahedral intermediate with the generation of a salt bridge between glutamic acid and free amine of the cleaved substrate (Figure 9c). The *N*-carboxyalkyl inhibitor (Figure 10a) is a typical example of a metalloprotease inhibitor and is shown bound to stromelysin-1 (MMP-3) in Figure 10.

Metalloproteases for which inhibitor−protease crystal structures are available on the PDB database include thermolysin, matrilysin, neutrophil collagenase, interstitial collagenase, atrolysin C, stromelysin, carboxypeptidase A, and TNF-α convertase.

5.1. Angiotensin-Converting Enzyme. Angiotensin-converting enzyme (ACE) is a zinc metalloprotease responsible for the formation of the octapeptide angiotensin II by cleaving two amino acids off the C-terminal end of its precursor decapeptide angiotensin I. Angiotensin II stimulates G protein-coupled angiotensin II (type I) receptors causing potent vasoconstriction. Inhibition of ACE decreases levels of angiotensin II in the body leading to a decrease in blood pressure. ACE is one of the most studied drug design targets, and inhibitor development has been extensively described in numerous reviews.[301,302] Ten ACE inhibitors are approved by the FDA for therapeutic use including captopril (**112**) (IC_{50} = 23 nM), enalapril (**113**) and enalaprilat (the diacid metabolite of enalapril) (IC_{50} = 4.5 nM), lisinopril (IC_{50} = 4.7 nM), benazepril (IC_{50} = 1.7 nM), moexipril (**114**)[303] (IC_{50} = 2.6 nM), trandolapril (**115**)[304] (IC_{50} = 0.93 nM), fosinopril (**116**) (IC_{50} = 1 nM), ramipril (IC_{50} = 4 nM), and quinapril (IC_{50} = 8.3 nM). The IC_{50} values shown are for deesterified active metabolites. Zofenopril (**117**) (IC_{50} = 0.4 nM) is currently in phase III clinical trials and may be commercially available soon.[305]

These drugs are useful not only for the treatment of hypertension but also for hypertensive crises, endothelial dysfunction, myocardial infarction, congestive heart failure, and renal protection.[306] The inhibitors are small, low molecular weight compounds that generally occupy



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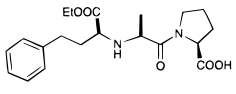

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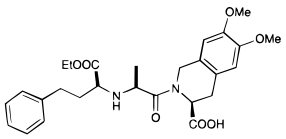

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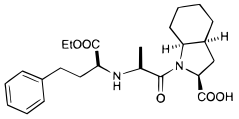

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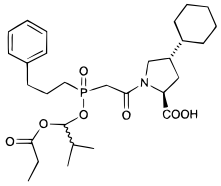

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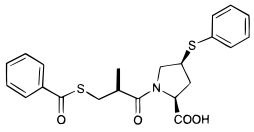

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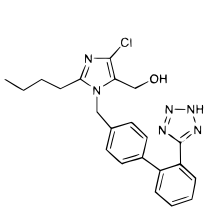
only the S1, S1′, and S2′ subsites of ACE, all employing a chelating group that binds to the active site zinc, resulting in high inhibitor potency and selectivity. Captopril (**112**) was the first compound of this class to be developed and the only one apart from zofenopril **(117)** which employs a thiol group as the zinc ligand. Fosinopril **(116)** is the only agent which employs a phosphinic acid, while all others can be considered to be derivatives of enalapril which use a carboxyl group to interact with the zinc atom of ACE.

The success of enalapril initiated the development of a series of homophenylalanine derivatives with a long duration of action (>24 h) of which moexipril (**114**) and trandolapril (**115**) are the most recent to be approved for human use (1996). With the exception of captopril (duration of action is 6−10 h) all ACE inhibitors can be dosed once per day. All compounds except for captopril and lisinopril need to be administered as ester prodrugs for good oral bioavailability, the ester group being rapidly cleaved by the liver to generate active drug. Enalaprilat, the diacid of enalapril, is the only compound available for iv administration in cases of extensive liver damage. Oral bioavailability in humans for all compounds except enalprilat is typically high, ranging from 50−80%.

ACE is a nonspecific enzyme which cleaves not only angiotensin I but also bradykinin, substance P, and other peptide hormones. Inhibition of ACE leads to side effects such as cough, skin rash, and angioneurotic edema. These side effects seem to be associated with a build-up of bradykinin in the body. In the past few years a number of angiotensin II receptor antagonists such as losartan (**118**),[307] irbesartan (**119**),[308] and candersartan cilexitil (**120**)[309] have emerged from clinical trials. These low molecular weight compounds are lipophilic, potent, and inexpensive to manufacture. Losartan (MW 422 Da, IC_{50} = 19 nM) and irbesartan have oral bioavailabilities in humans of 33% and 60−90%, respectively.[310] These drugs are as effective as ACE inhibitors for the treatment of hypertension but do not affect levels of bradykinin in the body and are devoid of side effects such as cough and angioneurotic edema.[311] They may have the potential to compete effectively with ACE inhibitors as drugs of choice for the treatment of hypertension in the marketplace.



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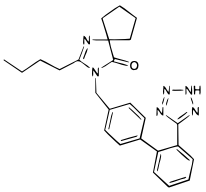

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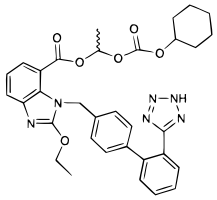

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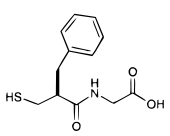
5.2. Neutral Endopeptidase. The neutral endopeptidase (NEP) like ACE is a zinc metalloprotease. It also has been shown to be the main protagonist in the breakdown of the atrial natriuretic peptide (ANP), which is secreted by the heart into the circulation to decrease blood pressure, raise the urinary excretion of water and sodium, and lower plasma renin and aldosterone levels. The enzyme also cleaves endogenous enkephalins in the brain.[312] The therapeutic potential of inhibitors for this enzyme may be in analgesia and for the treatment of hypertension.[313]

The design of selective inhibitors of NEP has essentially mirrored the development of ACE inhibitors, since both enzymes have similar active site topology. Thus small peptide mimics that can occupy the S1, S1′, and S2′ subsites of the enzyme display good potency. Thiorphan (**121**) was the first potent NEP inhibitor described (IC_{50} = 4 nM for both *R* and *S* diastereomers) and was found to substantially inhibit ACE ($IC_{50}(R)$ = 860 nM, $IC_{50}(S)$ = 140 nM). Specificity for NEP can be improved substantially by exploiting differences in the size of the S1 and S1′ subsites. For example, the homotyrosine moiety of SCH39370 (**122**) which interacts with the S1 subsite together with the β-hydroxy GABA residue at the C-terminal end of the molecule (location of the terminal carboxyl in NEP has been found to be less critical than for ACE) makes it highly specific for NEP (IC_{50} = 11 nM) over ACE (IC_{50} > 10000 nM). Orally bioavailable ester prodrugs of both compounds **121** and **122** have been synthesized together with hundreds of other inhibitors over the years. Since 1980 these compounds have been used as biological probes to establish the pharmacological role of NEP, but none have as yet progressed through clinical trials. The most likely drug for human use is possibly candoxatril (**123**),[314] (Pfizer) an ester prodrug of UK-69578 (candoxatrilat) (IC_{50} = 28 nM), which is currently in late-stage phase III clinical trials for the treatment of conjestive heart failure.



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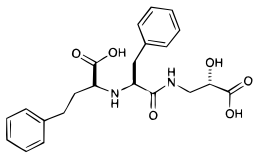

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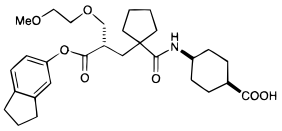

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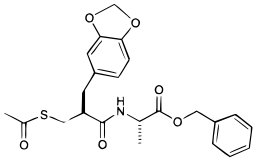
5.3. Dual Angiotensin-Converting Enzyme/Neutral Endopeptidase Inhibitors. The success of ACE

inhibitor therapy is dependent on several factors, including the severity of hypertension, the plasma renin activity, and the sodium status of the patient. ACE inhibitor therapy is only effective in 40−50% of people suffering from hypertension, usually those with high renin activity, and is enhanced by coadministration of diuretics. Such coadministration can lead to side effects such as depletion of potassium in the body. Selective NEP inhibitors show significant diuresis and naturesis in humans without potassium loss and have been shown to produce this effect in low-renin animal models.[315] These observations and the fact that the inhibition of ACE or NEP can lead to a reduction in blood pressure by reducing levels of angiotensin II or increasing levels of ANP have led to the development of broader-spectrum inhibitors that potently inhibit both enzymes and are thus more effective for the treatment of hypertension. Such dual inhibitors have been shown to possess synergistic effects which are more beneficial than monotherapy with ACE or NEP inhibitors alone.[316]

The structure−activity data needed for the design of dual inhibitors has largely come from the development of specific inhibitors of NEP. A large number of compounds in these studies were found to potently inhibit both ACE and NEP. The first rationally designed compound however was fasidotril (**124**),[317] obtained by the sequential modification of thiorphan (**121**). Based on stereochemical preferences, the methylenedioxy-substituted phenyl ring of **121** was proposed to bind to the S1 subsite of ACE and the S1′ subsite of NEP, thereby giving potent inhibition for both enzymes. The biologically active metabolite fasidotrilat (with free acid and thiol functions present) is a potent inhibitor of ACE (K_i = 9.8 nM) and NEP (K_i = 5.6 nM). Fasidotril (**124**) has high oral activity against both enzymes in vivo (ED_{50} = 0.2−0.5 mg/kg mouse) and was found to display a combination of biological effects due to the inhibition of either ACE or NEP. A recent study[318] has shown the potential usefulness of fasidotril and other NEP/ACE inhibitors in pathological disorders associated with sodium retention such as congestive heart failure (CHF). Fasidotril is currently in phase II clinical trials.



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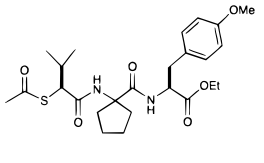

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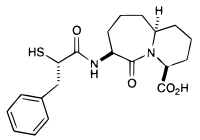

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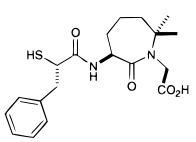

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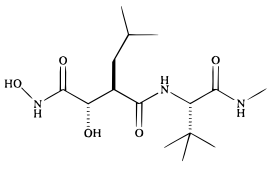
Other clinical candidates being assessed presently include CGS 30440 (**125**), sampatrilat,[319] omapatrilat (**126**) (IC_{50}(ACE) = 5 nM, IC_{50}(NEP) = 8 nM),[320−322] and the derivative **127** (IC_{50}(ACE) = 12 nM, IC_{50}(NEP) = 63 nM).[323] Omaprilat (**126**) is the most advanced inhibitor, being in phase III clinical trials, effectively inhibits both enzymes in vivo at 0.1 μmol/kg, and is long-acting. A single dose of 12 mg/kg in rats decreased mean arterial blood pressure by approximately 40 mmHg for 24 h and seems to be at least as effective as fosinopril (**116**) as an ACE inhibitor.

5.4. Matrix Metalloprotease Inhibitors. The MMPs are a family of structurally related zinc metalloproteases that degrade and remodel structural proteins in the extracellular matrix, such as membrane collagens, aggrecan, fibronectin, and laminin. They have been implicated in tissue remodeling at various stages of human development, wound healing, and disease. Although inhibited naturally by endogenous tissue inhibitors of metalloproteases (TIMPs), an imbalance caused by overexpression and activation of MMPs results in tissue degradation. It is thought that MMPs are important in the growth and spread of malignant tumors and development of chronic diseases such as multiple sclerosis, arthritis, fibrosis, and other inflammatory conditions.[320] For these reasons MMPs are considered to be attractive targets for inhibitor development and treatment of these disorders.

Currently, there are 17 known human MMP enzymes, all sharing significant sequence homology. The ones of current therapeutic interest are fibroblast collagenase (MMP-1), neutrophil collagenase (MMP-8), collagenase-3 (MMP-13), gelatinase A (MMP-2), gelatinase B (MMP-9), stromelysin-1 (MMP-3), stromelysin-2 (MMP-10), matrilysin (MMP-7), membrane-type-1-MMP (MT1-MMP), and aggrecanase.[324,325] Although inhibitor development has been the subject of much research since the early 1980s, three-dimensional crystal and solution structures of inhibitors bound to MMP-1,3,7,8 and MT1-MMP have come only recently (since 1994),[326] and this structural data has greatly accelerated research. There are numerous reviews on metalloprotease inhibitor development now available.[1,6,326−328]

At least 10 MMP inhibitors are undergoing evaluation in clinical trials for the treatment of cancer, arthritis, and multiple sclerosis.[327] Of these marimastat (**128**) is the most notably advanced. This peptidomimetic is the end result of years of substrate-based inhibitor design, based on the Gly-Ile and Gly-Leu collagenase (MMP-1) cleavage sites. It has characteristic features which are common to most of the hundreds of substrate-based inhibitors described so far. The most important feature of these inhibitors is the zinc-binding component, a terminal hydroxamate proving to be the most effective ligand for enhancing the potency of inhibitors for MMPs. SAR studies of these inhibitors have been used to determine a ranking with respect to the zinc-binding



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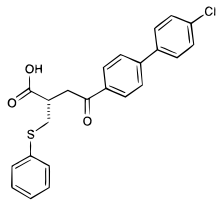

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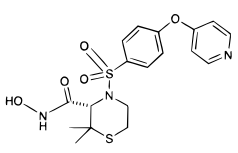

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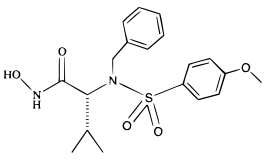

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Table 2. Inhibitor Potencies of MMP Inhibitors

compound	MMP-1	MMP-2	MMP-3	MMP-7	MMP-8	MMP-9	MMP-13
marimastat[a] (128)	5	6	200	20	2	3	
Bay-12-9566[b] (129)	5000	11	143			301	1470
AG 3340[b] (130)	8.2	0.083	0.27				0.038
CGS-27023A[b] (131)	33	20	43			8	
RS-130830[b] (132)	590	0.22	9.3	1200		0.58	0.52
Ro-3203555[b] (133)	3	154	527		4	59	3
134[b]	24		18	30		2.7	
135[b]	<1	3	<1				
136[b]	>30000		30				
137[b]		3000	18				
138[b]	2000	10	500				

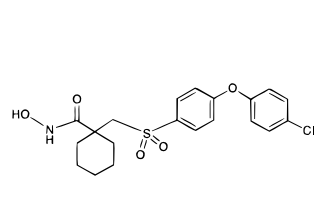
[a] IC_{50} values (nM). [b] K_i values (nM).

group for the inhibition of fibroblast collagenase (MMP-1): hydroxamate ≫ formylhydroxylamine > sulfydryl > phosphinate > aminocarboxylate > carboxylate.[6]

In general, like ACE and NEP, the greatest potency can be obtained by designing inhibitors to interact with at least the S1, S1′, and S2′ subsites of the enzymes. SAR studies have also shown that the S1′ subsite differs most among the MMPs and a certain degree of specificity can be achieved by varying the P1′ residue of the inhibitor. Introduction of larger P1′ substituents generally gives greater specificity for MMP-2,9,3[329] at the expense of MMP-1,7. Oral bioavailability has been the greatest stumbling block for these substrate-based inhibitors. Fortuitously, it was found that the combination of α-hydroxy and P2′ *tert*-butyl groups of marimastat gives the inhibitor high oral bioavailability (20−50%). Marimistat (**128**) is a broad-spectrum MMP inhibitor having a low nanomolar (IC_{50} ∼ 2−20 nM) activity against MMP-1,2,7,8,9 and IC_{50} of 200 nM for MMP-3 (Table 2). It is currently in phase III clinical trials and is being evaluated for the treatment of invasive cancers and metastasis.[328] After a 50 mg oral dose to healthy volunteers, a C_{max} of 192 μg/L, T_{max} of 1.9 h, and $t_{1/2}$ of 7−10 h were observed.

Due to the difficulty of developing orally bioavailable inhibitors via the substrate-based approach, synthetic and natural product libraries have also been screened in order to obtain leads for the design of nonpeptidic MMP inhibitors with better bioavailability. Bay-12-9566 (**129**)[330] and CGS-27023A (**131**)[331] are two orally bioavailable inhibitors developed by optimization of lead compounds found by this strategy. Bay-12-9566 (**129**) is selective for MMP-2 and is currently in phase III clinical trials, while CGS-27023A (**131**) is a broad-spectrum inhibitor undergoing phase I clinical evaluation.

In recent years receptor-based drug design strategies have begun to dominate inhibitor development for MMPs. X-ray crystal structures have been determined for the orally active compounds **130** (phase III),[332] **131** (phase I),[333] and **133** (phase I),[334] all developed via this strategy. By exploiting differences in the active site of the MMPs, the compounds are generally much more potent and specific for particular members of the MMP family, though lack of selectivity is still an important issue for most MMP inhibitors. Inhibitors **134**−**138** were reported in the first 6 months of 1999. A series of bissulfonamides was described by researchers at Procter and Gamble with a view to developing simple achiral MMP inhibitors. The best compound **134** was a good inhibitor of MMP-1,3,7,9 (IC_{50} = 2−30 nM). An X-ray structure of **134** bound to stromelysin showed that one of the *p*-methoxyphenyl groups binds in the S1′ subsite while the other binds in the S1/S2 pocket.[335] Workers at DuPont have reported further work on macrocyclic MMP inhibitors (e.g. **135**) which can be considered to be derivatives of marimastat. The macrocyclic linkage is directed away from the active site into solvent and was thought to provide a means of altering the physical properties of the compounds without affecting the binding affinity. Compound **135** is generally representative of the activity of these derivatives with low nanomolar activity for MMP-1,3,9.[336]



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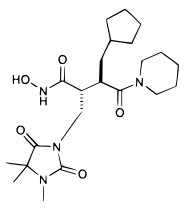

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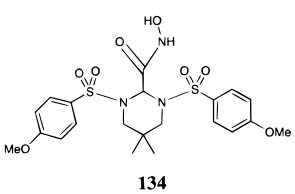

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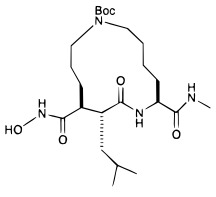

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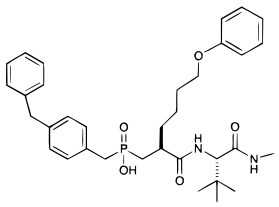

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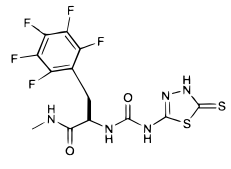

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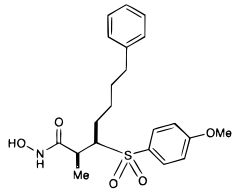

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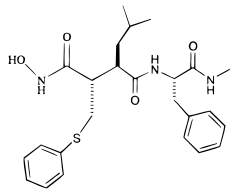
Researchers at Pfizer have reported a series of phosphinate-based inhibitors that occupy S2 and S1′−S3′ subsites of MMP-1,13. They found that an inhibitor

capable of interacting with the S2 subsite could impart greater selectivity for particular MMPs. Compound **136** is >1000-fold more selective for MMP-13 than MMP-1.[337] Pharmacia and Upjohn researchers have reported a series of thiadiazole urea inhibitors that are selective for stromelysin (MMP-3), the best being **137** which inhibits MMP-3 with a K_i = 18 nM and gelatinase (MMP-2) with a K_i = 3000 nM. The compound does not inhibit any of the collagenases. When **137** was cocrystallized with stromelysin it was found that, contrary to the expected binding mode, the inhibitor bound to the S1−S3 subsites with the exocylic sulfur interacting with the active site zinc. It was reasoned that the unprimed subsites of MMPs in general vary greatly between members of the family as compared to the primed subsites and could explain the specificity of **137**.[338] Rhone-Poulenc has recently disclosed a series of dual inhibitors of phosphodiesterase type-4 (PDE4) and MMPs. PDE4 inhibitors have been shown to be useful for the treatment of inflammatory diseases (arthritis, multiple sclerosis, atopic dermatitis, psoriasis; a number of inhibitors are currently in clinical development[339]) where MMP inhibitors are also effective. Compound **138** is the best of the series and inhibits PDE4 (K_i = 30 nM) as well as MMP-1 (K_i = 2 μM), MMP-2 (K_i = 10 nM), and MMP-3 (K_i = 500 nM).[340]

5.5. Tumor Necrosis Factor-α-Converting Enzyme. The zinc metalloenzyme tumor necrosis factor-α convertase (TACE) cleaves a membrane-bound protein (pro-TNF-α) releasing to the circulation a 17-kDa proinflammatory and immunomodulatory cytokine, tumor necrosis factor-α (TNF-α).[341] TNF-α plays an important signaling role in inflammatory diseases such as rheumatoid arthritis (RA), multiple sclerosis, and Crohn's disease[342] and is involved in the recruitment and retention of inflammatory cells and the production and regulation of inflammatory mediators such as prostaglandins and leukotrienes.[343] Recent studies demonstrated that TNF-α and its receptors are overexpressed in the synovium and cartilage−pannus junction of RA joints[344] and that monoclonal antibodies against TNF-α are antiinflammatory in patients with RA.[344,345] However the therapeutic effects of anti-TNF-α antibodies only last a short time due to their inappropriate pharmacokinetic profiles.

A number of strategies for inhibiting the formation of TNF-α are currently being investigated for the treatment of inflammatory disorders, and a recent review has been published on the subject.[346] One attractive strategy is the development of low molecular weight inhibitors of TACE. The enzyme was purified and cloned in 1997 and a crystal structure determined in 1998.[347] One study has also established that only the single proteinase TACE is required for the shedding of TNF-α from T-cells and myeloid cells.[348] So far TACE inhibitor development has evolved from MMP inhibitor design programs, two early studies showing that hydroxamate-based broad-spectrum MMP inhibitors (**139**) and (**140**) could also reduce the production of TNF-α by inhibiting TACE.[349,350] Since then a handful of other MMP inhibitors have also been shown to inhibit TACE,[346] reflecting structural similarities between the active sites of MMPs and TACE.[347]

Very few studies directed to specific inhibitors of



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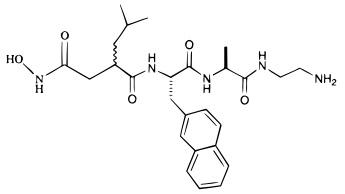

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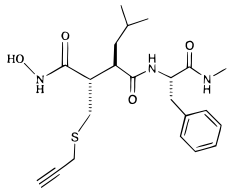

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TACE have been reported to date. Of note however is a series of compounds that are the subject of a patent application.[351] Compound **141** is claimed to be an inhibitor of TNF-α formation and soluble CD23, a protein that is associated with autoimmune disease and allergy. The compound presumably inhibits TACE and a similar metalloprotease responsible for the production of CD23. Compound **141** inhibits TNF formation by 83% at 1 μM in human monocytes and has also been shown to be specific for TACE with respect to MMP-1 (IC_{50} > 10 μM).

5.6. Summary. Low potency has generally been observed for inhibitors designed to interact only with the nonprime side subsites of metalloproteases. It has been suggested that weak binding at these nonprime side subsites is desirable for normal substrate processing; otherwise the carboxylic acid product could also effectively inhibit the enzyme. Inhibitor design for most metalloproteases therefore has focused on small inhibitors capable of interacting with the prime side pockets and incorporating a zinc-binding ligand. The general strategy of incorporating a zinc-binding ligand, such as a carboxylic acid, sulfhydryl, phosphinate, hydroxamate, or formylhydroxylamine, for the designed inhibitors has proven to be highly successful. The ACE inhibitors for example were the first protease inhibitors to reach the marketplace in the early 1980s. The design of inhibitors which can interact principally with the prime side subsites of metalloproteases has led to selectivity problems especially in the design of MMP inhibitors. It is now becoming apparent that increased selectivity between the members of the MMP family can be achieved by designing molecules that can make noncovalent interactions at the S2 and S1 subsites as well as at the usual S1′−S3′ subsites.

6. Future Prospects

Protease inhibitor discovery began with natural product screening and substrate-derived analogue-based drug design, then progressed with incorporation of mechanism-based drug design strategies, and more recently has advanced further using computer-assisted structure-based inhibitor design using three-dimensional structures of proteases determined by X-ray crystallography and NMR spectroscopy. Today the design of protease inhibitors involves a powerful combination of all of these traditional drug discovery

approaches, supplemented by de novo drug design, combinatorial chemistry, and phage display techniques and supported by rapid robotic assay methods to find or optimize inhibitor leads from vast chemical libraries. These techniques are currently being refined and expanded to accelerate the discovery process for new protease inhibitors. Coupled with advances in molecular and cellular biology, protein chemistry, microbiology, structural biology, and molecular pharmacology, there can be little doubt that the next decades will see identification of many new proteases as targets for inhibitor development.

For example, genomic and biochemical comparisons will increasingly be made between normal and diseased cells, between host and pathogen (e.g. human/virus, animal/parasite), and between healthy and unhealthy individuals, and from these studies new or overexpressed proteases will be identified. These proteases will need to be sequenced, synthesized in milligram quantities by expression, structurally characterized and their enzymology characterized. Small molecule inhibitors of these proteases will also need to be designed, synthesized, and tested for activity and especially selectivity against both disease-related and normal proteases, cells, animals, and humans. On the basis of what is illustrated in this article, it does not take much imagination to anticipate that protease inhibitors will increasingly become valuable molecular probes for improving our understanding of biological processes as well as commercially valuable drug candidates for treating diseases.

This compilation has assembled some important facts for several hundred of the most extensively reported inhibitors of over 30 proteolytic enzymes from the aspartic, serine, cysteine, and metallo classes of proteases. A key *conformational* requirement of all these proteases is their universal recognition of peptides, substrate analogues, and inhibitors in an extended backbone conformation.[34,35] This structural motif is unlike all other elements of secondary structure in providing maximum separation and exposure of all component amino acids for interaction with solvent or protease. By contrast, the side chains of helices, turns, and sheets shelter most of their peptide backbone amides which tend to adopt intramolecular hydrogen-bonded arrangements. It follows that folding into secondary and tertiary structures protects polypeptides from recognition and degradation by proteolytic enzymes, and there is ample evidence that protein denaturation does indeed accelerate degradation by proteases.

In the face of this common conformational requirement for recognition by a protease, it is surprising that the vast majority of protease inhibitors developed to date are quite flexible molecules. This flexibility is expected to raise the entropic barrier for protease binding, and it would seem advantageous to examine prospective inhibitors that are more restrained to a protease-binding conformation and thus need not reorganize prior to binding with a protease. Alternatively, the energetic barrier for inhibitor−protease interactions could be reduced by designing compounds that minimize their degree of solvation by water, thus reducing the penalty for their desolvation. A third approach is to design compounds that replace key water molecules in the active site of the enzyme, a strategy that has been notably employed with success for cyclic urea and other inhibitors of HIV-1 protease.[352,353]

A key *mechanistic* feature of many protease inhibitors is the presence of a transition-state isostere that simulates to some extent the transition state of amide bond hydrolysis. The incorporation of such amide bond surrogates has proven to be a very successful strategy for rapidly converting protease substrates into inhibitors, another benefit being increased affinity by $10-10^4$-fold of a peptide for a protease. Although quite a few transition-state isosteres are already known, it seems reasonable to think that further advances can be made in more closely approaching the transition state and thereby further increasing inhibitor affinity for a protease active site. As more is learned through structural insights about the detailed mechanisms of protease-mediated cleavage, it may also be possible to better design protease inhibitors to make specific interactions with active site residues near the catalytic site and so interfere more effectively with cooperative interactions that comprise catalytic mechanisms. This might involve inhibiting conformational changes needed by the protease for substrate processing, such as blocking the movements of active site residues, flaps, cofactor binding, and other distortions within the substrate-binding groove. Some proteases require more than one polypeptide subunit for creation of an active site, so compounds that interfere with association of subunits could be effective protease inhibitors. To date this approach has not been effective due to the need for extremely high-affinity molecules for competing with subunits.

Inhibitor *composition* has been an important feature of protease inhibitor design to date. As drugs, protease inhibitors need, on the one hand, to be designed to adopt an extended conformation and make hydrogen bonds to, and/or interact through hydrophobic effects with, specific proteases while at the same time avoiding degradation en route to the desired protease through interactions with numerous other proteases in the digestive tract, in plasma, and inside cells. This problem is compounded by the fact that natural peptide substrates for proteases, while providing clues to molecular interactions with proteases and thus to inhibitor design, are very susceptible to such degradation and, as well, suffer from other major delivery problems such as low membrane permeability, rapid elimination from plasma, high first-pass metabolism, and very low oral bioavailability. Thus for oral and systemic delivery to humans and animals, the most successful drugs based upon protease inhibition have tended to be small organic molecules (MW $<$ 300−500 Da) with few or no peptide bonds, significant lipophilicity (usually 100$\times$ water solubility), and high selectivity for both the specific protease and the clinical indication being targeted. Selectivity is still an outstanding issue to be resolved for most protease inhibitors, and it is very likely that this requirement will see more complexity added by way of additional appendages to lead compounds, raising the likely molecular weights of future generations of protease inhibitors to 600−1000 Da. Compromises between increased complexity, pharmacokinetic profiles, and drug affordability will challenge medicinal chemists to find new general methods for the simple creation of new small

molecules which are potent, selective, and bioavailable protease inhibitors or to find better methods for efficient delivery of peptidic or protein inhibitors to proteases. It is hoped that this review can help stimulate new efforts toward achieving such goals.

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Biographies

Donmienne Leung received her B.Sc. (Hons) degree from the School of Chemistry of the University of Melbourne in 1997. She is currently completing her Ph.D. degree in the Centre for Drug Design and Development. Her Ph.D. studies are directed at designing and developing competitive reversible protease inhibitors, particularly for serine and cysteine proteases involved in viral and parasitic infections, Alzheimer's disease, and apoptosis.

Giovanni (John) Abbenante received his B.Sc. (Hons) degree from the Department of Chemistry of the University of Adelaide and his Ph.D. degree on the synthesis of $GABA_b$ receptor antagonists from Flinders University in 1992. He undertook postdoctoral studies on the synthesis of ladderanes at Central Queensland University and of HIV-1 protease inhibitors at the University of Queensland. He is currently a Senior Research Officer in the Chemistry Group of the Centre for Drug Design and Development with interests in drug design and in the synthesis of protease inhibitors, macrocycles, receptor antagonists, and peptidomimetics.

David P. Fairlie received a B.Sc. (Hons) degree from the University of Adelaide and a Ph.D. from the University of New South Wales and did postdoctoral research at Stanford University and University of Toronto. He has held research/teaching appointments in six Australian universities and has led the Chemistry Group in the Centre for Drug Design and Development since 1991. He is Scientific Director of Promics Pty Ltd. Research interests are in chemical synthesis (organic, inorganic), molecular recognition (DNA-, RNA-, protein-, metal-binding compounds), peptide and protein mimetics, enzyme inhibitors and receptor antagonists for viral infections, inflammatory disorders, cancers, and neurodegenerative diseases, and in mechanisms of chemical reactions, biological processes, disease development, and drug action.

References

(1) Babine, R. E.; Bender, S. L. Molecular recognition of protein−ligand complexes: Applications to drug design. *Chem. Rev.* **1997**, *97*, 1359−1472.
(2) Ripka, A. S.; Rich, D. H. Peptidomimetic design. *Curr. Opin. Chem. Biol.* **1998**, *2*, 441−52.
(3) Craik, M. S.; Debouck, C. Proteases as Therapeutic Targets. In *Perspectives in Drug Discovery and Design*; McKerrow, J. H., James, M. N. G., Eds.; ESCOM: Leiden, 1995; Vol. 2, pp 1−125.
(4) Shaw, E. Cysteinyl proteinases and their selective inactivation. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1990**, *63*, 271−347.
(5) Seife, C. Blunting nature's Swiss army knife [news]. *Science* **1997**, *277*, 1602−3.
(6) Beckett, R. P.; Davidson, A. H.; Drummond, A. H.; Whittaker, M. Recent advances in matrix metalloproteinase inhibitor research. *Drug Discov. Today* **1996**, *1*, 16−26.
(7) Johnson, L. L.; Dyer, R.; Hupe, D. J. Matrix metalloproteinases. *Curr. Opin. Chem. Biol.* **1998**, *2*, 466−71.
(8) Yan, S.; Sameni, M.; Sloane, B. F. Cathepsin B and human tumor progression. *Biol. Chem.* **1998**, *379*, 113−23.
(9) Becker, M. M.; Harrop, S. A.; Dalton, J. P.; Kalinna, B. H.; McManus, D. P.; Brindley, P. J. Cloning and characterization of the *Schistosoma japonicum* aspartic proteinase involved in hemoglobin degradation. *J. Biol. Chem.* **1995**, *270*, 24496−501.
(10) Brindley, P. J.; Kalinna, B. H.; Dalton, J. P.; Day, S. R.; Wong, J. W.; Smythe, M. L.; McManus, D. P. Proteolytic degradation of host hemoglobin by schistosomes. *Mol. Biochem. Parasitol.* **1997**, *89*, 1−9.
(11) Silva, A. M.; Lee, A. Y.; Gulnik, S. V.; Maier, P.; Collins, J.; Bhat, T. N.; Collins, P. J.; Cachau, R. E.; Luker, K. E.; Gluzman, I. Y.; Francis, S. E.; Oksman, A.; Goldberg, D. E.; Erickson, J. W. Structure and inhibition of plasmepsin II, a hemoglobin-degrading enzyme from Plasmodium falciparum. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 10034−9.
(12) Li, Z.; Chen, X.; Davidson, E.; Zwang, O.; Mendis, C.; Ring, C. S.; Roush, W. R.; Fegley, G.; Li, R.; Rosenthal, P. J.; et al. Antimalarial drug development using models of enzyme structure. *Chem. Biol.* **1994**, *1*, 31−7.
(13) Abad-Zapatero, C.; Goldman, R.; Muchmore, S. W.; Hutchins, C.; Stewart, K.; Navaza, J.; Payne, C. D.; Ray, T. L. Structure of a secreted aspartic protease from C. albicans complexed with a potent inhibitor: implications for the design of antifungal agents. *Protein Sci.* **1996**, *5*, 640−52.
(14) Abad-Zapatero, C.; Goldman, R.; Muchmore, S. W.; Hutchins, C.; Oie, T.; Stewart, K.; Cutfield, S. M.; Cutfield, J. F.; Foundling, S. I.; Ray, T. L. Structure of secreted aspartic proteinases from Candida − Implications for the design of antifungal agents. *Adv. Exp. Med. Biol.* **1998**, *436*, 297−313.
(15) Wlodawer, A.; Erickson, J. W. Structure-based inhibitors of HIV-1 protease. *Annu. Rev. Biochem.* **1993**, *62*, 543−85.
(16) Darke, P. L.; Huff, J. R. HIV protease as an inhibitor target for the treatment of AIDS. *Adv. Pharmacol. (San Diego)* **1994**, *5*, 399−454.
(17) West, M. L.; Fairlie, D. P. Targeting HIV-1 protease: a test of drug-design methodologies. *Trends Pharmacol. Sci.* **1995**, *16*, 67−75.
(18) Kim, J. L.; Morgenstern, K. A.; Lin, C.; Fox, T.; Dwyer, M. D.; Landro, J. A.; Chambers, S. P.; Markland, W.; Lepre, C. A.; O'Malley, E. T.; Harbeson, S. L.; Rice, C. M.; Murcko, M. A.; Caron, P. R.; Thomson, J. A. Crystal structure of the hepatitis C virus NS3 protease domain complexed with a synthetic NS4A cofactor peptide [published erratum appears in *Cell* **1997**, *89*, 159]. *Cell* **1996**, *87*, 343−55.
(19) Love, R. A.; Parge, H. E.; Wickersham, J. A.; Hostomsky, Z.; Habuka, N.; Moomaw, E. W.; Adachi, T.; Hostomska, Z. The crystal structure of hepatitis C virus NS3 proteinase reveals a trypsin-like fold and a structural zinc binding site. *Cell* **1996**, *87*, 331−42.
(20) Gibson, W.; Hall, M. R. Assemblin, an essential herpesvirus proteinase. *Drug Des. Discov.* **1997**, *15*, 39−47.
(21) Shieh, H. S.; Kurumbail, R. G.; Stevens, A. M.; Stegeman, R. A.; Sturman, E. J.; Pak, J. Y.; Wittwer, A. J.; Palmier, M. O.; Wiegand, R. C.; Holwerda, B. C.; Stallings, W. C. Three-dimensional structure of human cytomegalovirus protease [published erratum appears in *Nature* **1996,** *384*, 288]. *Nature* **1996**, *383*, 279−82.
(22) Bernstein, P. R.; Edwards, P. D.; Williams, J. C. Inhibitors of human leukocyte elastase. *Prog. Med. Chem.* **1994**, *31*, 59−120.
(23) Hugli, T. E. Protease inhibitors: novel therapeutic application and development. *Trends Biotechnol.* **1996**, *14*, 409−12.
(24) Fath, M. A.; Wu, X.; Hileman, R. E.; Linhardt, R. J.; Kashem, M. A.; Nelson, R. M.; Wright, C. D.; Abraham, W. M. Interaction of secretory leukocyte protease inhibitor with heparin inhibits proteases involved in asthma. *J. Biol. Chem.* **1998**, *273*, 13563−9.
(25) Tanaka, R. D.; Clark, J. M.; Warne, R. L.; Abraham, W. M.; Moore, W. R. Mast cell tryptase: a new target for therapeutic intervention in asthma. *Int. Arch. Allergy Immunol.* **1995**, *107*, 408−9.
(26) Stubbs, M. T.; Bode, W. A player of many parts: the spotlight falls on thrombin's structure. *Thromb. Res.* **1993**, *69*, 1−58.
(27) Vassar, R.; Bennett, B. D.; Babu-Kahn, S.; Kahn, S.; Mendiaz, E. A.; et al. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science* **1999**, *286*, 735−41.
(28) Vacca, J. P.; Dorsey, B. D.; Schleif, W. A.; Levin, R. B.; McDaniel, S. L.; Darke, P. L.; Zugay, J.; Quintaro, J. C.; Blahy, O. M.; Roth, E.; et al. L-735,524: an orally bio-available human immunodeficiency virus type 1 protease inhibitor. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4096−100.
(29) Kempf, D. J.; Sham, H. L. HIV protease inhibitors. *Curr. Pharm. Des.* **1996**, *2*, 225−46.
(30) Molla, A.; Granneman, G. R.; Sun, E.; Kempf, D. J. Recent developments in HIV protease inhibitor therapy. *Antiviral Res.* **1998**, *39*, 1−23.
(31) March, D. R.; Fairlie, D. P. *Designing new antiviral drugs for AIDS: HIV-1 Protease and its inhibitors*; R. G. Landes Co.: Austin, TX, 1996.
(32) Kim, E. E.; Baker, C. T.; Dwyer, M. D.; Murcko, M. A.; Rao, B. G.; Tung, R. D.; Navia, M. A. Crystal structure of HIV-1 protease in complex with VX-478, a potent and orally bioavailable inhibitor of the enzyme. *J. Am. Chem. Soc.* **1995**, *117*, 1181−2.
(33) Schechter, I.; Berger, A. On the size of the active site in proteases. I. Papain. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 157−62.

(34) Fairlie, D. P.; Tyndall, J. D. A.; Reid, R. C.; Wong, A. K.; Abbenante, G.; Scanlon, M. J.; March, D. R.; Bergman, D. A.; Chai, C. L. L.; Burkett, B. A. Conformational selection of inhibitors and substrates by proteolytic enzymes. Implications for drug design and polypeptide processing. *J. Med. Chem.* **1999**, accepted for publication.
(35) Tyndall, J. D. A.; Fairlie, D. P. Conformational Homogeneity in Molecular Recognition by Proteolytic enzymes. *J. Mol. Recognit.* **1999**, *12*, 1−8.
(36) James, M. N. G.; Sielecki, A. R. Aspartic proteases and their catalytic pathway inhibitors. In *Biological macromolecules and assemblies: Active sites of enzymes*; Jurnak, F. A., McPherson, A., Eds.; John Wiley & Sons: New York, 1987; Vol. 3, p 413.
(37) Martin, J. L.; Begun, J.; Schindeler, A.; Wickramasinghe, W. A.; Alewood, D.; Alewood, P. F.; Bergman, D. A.; Brinkworth, R. I.; Abbenante, G.; March, D. R.; Reid, R. C.; Fairlie, D. P. Molecular recognition of macrocyclic peptidomimetic inhibitors by HIV-1 protease. *Biochemistry* **1999**, *38*, 7978−88.
(38) Chatfield, D. C.; Brooks, B. R. HIV-1 Protease cleavage mechanism eluciated with molecular-dynamics simulation. *J. Am. Chem. Soc.* **1995**, *117*, 5561−72.
(39) PDB: www.rcsb.org/pdb.
(40) Patick, A. K.; Potts, K. E. Protease inhibitors as antiviral agents. *Clin. Microbiol. Rev.* **1998**, *11*, 614−27.
(41) Pakyz, A.; Israel, D. Overview of protease inhibitors. *J. Am. Pharm. Assoc. (Washington)* **1997**, *NS37*, 543−51.
(42) Wacher, V. J.; Silverman, J. A.; Zhang, Y.; Benet, L. Z. Role of P-glycoprotein and cytochrome P450 3A in limiting oral absorption of peptides and peptidomimetics. *J. Pharm. Sci.* **1998**, *87*, 1322−30.
(43) Kempf, D. J.; Marsh, K. C.; Kumar, G.; Rodrigues, A. D.; Denissen, J. F.; McDonald, E.; Kukulka, M. J.; Hsu, A.; Granneman, G. R.; Baroldi, P. A.; Sun, E.; Pizzuti, D.; Plattner, J. J.; Norbeck, D. W.; Leonard, J. M. Pharmacokinetic enhancement of inhibitors of the human immunodeficiency virus protease by coadministration with ritonavir. *Antimicrob. Agents Chemother.* **1997**, *41*, 654−60.
(44) Lea, A. P.; Faulds, D. Ritonavir. *Drugs* **1996**, *52*, 541−6 (discussion 547−8).
(45) Lacy, M. K.; Abriola, K. P. Indinavir: a pharmacologic and clinical review of a new HIV protease inhibitor. *Conn. Med.* **1996**, *60*, 723−7.
(46) Shetty, B. V.; Kosa, M. B.; Khalil, D. A.; Webber, S. Preclinical pharmacokinetics and distribution to tissue of AG1343, an inhibitor of human immunodeficiency virus type 1 protease. *Antimicrob. Agents Chemother.* **1996**, *40*, 110−4.
(47) Adkins, J. C.; Faulds, D. Amprenavir. *Drugs* **1998**, *55*, 837−42 (discussion 843−4).
(48) Sham, H. L.; Kempf, D. J.; Molla, A.; Marsh, K. C.; Kumar, G. N.; Chen, C. M.; Kati, W.; Stewart, K.; Lal, R.; Hsu, A.; Betebenner, D.; Korneyeva, M.; Vasavanonda, S.; McDonald, E.; Saldivar, A.; Wideburg, N.; Chen, X.; Niu, P.; Park, C.; Jayanti, V.; Grabowski, B.; Granneman, G. R.; Sun, E.; Japour, A. J.; Norbeck, D. W.; et al. ABT-378, a highly potent inhibitor of the human immunodeficiency virus protease. *Antimicrob. Agents Chemother.* **1998**, *42*, 3218−24.
(49) Bold, G.; Fassler, A.; Capraro, H. G.; Cozens, R.; Klimkait, T.; Lazdins, J.; Mestan, J.; Poncioni, B.; Rosel, J.; Stover, D.; Tintelnot-Blomley, M.; Acemoglu, F.; Beck, W.; Boss, E.; Eschbach, M.; Hurlimann, T.; Masso, E.; Roussel, S.; Ucci-Stoll, K.; Wyss, D.; Lang, M. New aza-dipeptide analogues as potent and orally absorbed HIV-1 protease inhibitors: candidates for clinical development. *J. Med. Chem.* **1998**, *41*, 3387−401.
(50) Lamarre, D.; Croteau, G.; Wardrop, E.; Bourgon, L.; Thibeault, D.; Clouette, C.; Vaillancourt, M.; Cohen, E.; Pargellis, C.; Yoakim, C.; Anderson, P. C. Antiviral properties of palinavir, a potent inhibitor of the human immunodeficiency virus type 1 protease. *Antimicrob. Agents Chemother.* **1997**, *41*, 965−71.
(51) Kleinert, H. D.; Stein, H. H.; Boyd, S.; Fung, A. K.; Baker, W. R.; Verburg, K. M.; Polakowski, J. S.; Kovar, P.; Barlow, J.; Cohen, J.; et al. Discovery of a well-absorbed, efficacious renin inhibitor, A-74273. *Hypertension* **1992**, *20*, 768−75.
(52) Menard, J.; Boger, R. S.; Moyse, D. M.; Guyene, T. T.; Glassman, H. N.; Kleinert, H. D. Dose-dependent effects of the renin inhibitor zankiren HCl after a single oral dose in mildly sodium-depleted normotensive subjects. *Circulation* **1995**, *91*, 330−8.
(53) Sunman, W.; Sever, P. S. Nonangiotensin effects of angiotensin-converting enzyme inhibitors [editorial]. *Clin. Sci. (Colch)* **1993**, *85*, 661−70.
(54) Rosenberg, S. H. Renin Inhibitors. In *Progress in Medicinal Chemistry*; Ellis, G. P., Luscombe, D. K., Eds.; Elsevier Science: New York, 1995; Vol. 32, pp 37−115.
(55) Kleinert, H. D. Renin inhibition. *Cardiovasc. Drugs Ther.* **1995**, *9*, 645−55.
(56) de Gasparo, M.; Cumin, F.; Nussberger, J.; Guyenne, T. T.; Wood, J. M.; Menard, J. Pharmacological investigations of a new renin inhibitor in normal sodium-unrestricted volunteers. *Br. J. Clin. Pharmacol.* **1989**, *27*, 587−96.
(57) Kleinbloesem, C. H.; Weber, C.; Fahrner, E.; Dellenbach, M.; Welker, H.; Schroter, V.; Belz, G. G. Hemodynamics, biochemical effects, and pharmacokinetics of the renin inhibitor remikiren in healthy human subjects. *Clin. Pharmacol. Ther.* **1993**, *53*, 585−92.
(58) Hoover, D. J.; Lefker, B. A.; Rosati, R. L.; Wester, R. T.; Kleinman, E. F.; Bindra, J. S.; Holt, W. F.; Murphy, W. R.; Mangiapane, M. L.; Hockel, G. M.; et al. Discovery of inhibitors of human renin with high oral bioavailability. *Adv. Exp. Med. Biol.* **1995**, *362*, 167−80.
(59) Simoneau, B.; Lavallee, P.; Anderson, P. C.; Bailey, M.; Bantle, G.; Berthiaume, S.; Chabot, C.; Fazal, G.; Halmos, T.; Ogilvie, W. W.; Poupart, M. A.; Thavonekham, B.; Xin, Z.; Thibeault, D.; Bolger, G.; Panzenbeck, M.; Winquist, R.; Jung, G. L. Discovery of nonpeptidic P2−P3 butanediamide renin inhibitors with high oral efficacy. *Bioorg. Med. Chem.* **1999**, *7*, 489−508.
(60) Buhlmayer, P.; Caselli, A.; Fuhrer, W.; Goschke, R.; Rasetti, V.; Rueger, H.; Stanton, J. L.; Criscione, L.; Wood, J. M. Synthesis and biological activity of some transition-state inhibitors of human renin. *J. Med. Chem.* **1988**, *31*, 1839−46.
(61) Plummer, M. S.; Shahripour, A.; Kaltenbronn, J. S.; Lunney, E. A.; Steinbaugh, B. A.; Hamby, J. M.; Hamilton, H. W.; Sawyer, T. K.; Humblet, C.; Doherty, A. M.; et al. Design and synthesis of renin inhibitors: incorporation of transition-state isostere side chains that span from the S1 to the S3 binding pockets and examination of P3-modified renin inhibitors. *J. Med. Chem.* **1995**, *38*, 2893−905.
(62) Goschke, R.; Cohen, N. C.; Wood, J. M.; Maibaum, J. Novel 2,7-dialkyl substituted 5(S)-amino-4(S)hydroxy-8-phenyl-octanecarboxamides as in vitro potent peptidomimetic inhibitors of human renin. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2735−40.
(63) Guller, R.; Binggeli, A.; Breu, V.; Bur, D.; Fischli, W.; Hirth, G.; Jenny, C.; Kansy, M.; Montavon, F.; Muller, M.; Oefner, C.; Stadler, H.; Vieira, E.; Wilhelm, M.; Wostl, W.; Marki, H. P. Piperidine-renin inhibitors compounds with improved physicochemical properties. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1403−8.
(64) Vieira, E.; Binggeli, A.; Breu, V.; Bur, D.; Fischli, W.; Guller, R.; Hirth, G.; Marki, H. P.; Muller, M.; Oefner, C.; Scalone, M.; Stadler, H.; Wilhelm, M.; Wostl, W. Substituted piperidines—highly potent renin inhibitors due to induced fit adaptation of the active site. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1397−402.
(65) Francis, S. E.; Sullivan, D. J., Jr.; Goldberg, D. E. Hemoglobin metabolism in the malaria parasite Plasmodium falciparum. *Annu. Rev. Microbiol.* **1997**, *51*, 97−123.
(66) Francis, S. E.; Gluzman, I. Y.; Oksman, A.; Knickerbocker, A.; Mueller, R.; Bryant, M. L.; Sherman, D. R.; Russell, D. G.; Goldberg, D. E. Molecular characterization and inhibition of a Plasmodium falciparum aspartic hemoglobinase. *EMBO J.* **1994**, *13*, 306−17.
(67) Carroll, C. D.; Patel, H.; Johnson, T. O.; Guo, T.; Orlowski, M.; He, Z. M.; Cavallaro, C. L.; Guo, J.; Oksman, A.; Gluzman, I. Y.; Connelly, J.; Chelsky, D.; Goldberg, D. E.; Dolle, R. E. Identification of potent inhibitors of Plasmodium falciparum plasmepsin II from an encoded statine combinatorial library. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2315−20.
(68) Haque, T. S.; Skillman, A. G.; Lee, C. E.; Habashita, H.; Gluzman, I. Y.; Ewing, T. J.; Goldberg, D. E.; Kuntz, I. D.; Ellman, J. A. Potent, low-molecular-weight non-peptide inhibitors of malarial aspartyl protease plasmepsin II. *J. Med. Chem.* **1999**, *42*, 1428−40.
(69) Semenov, A.; Olson, J. E.; Rosenthal, P. J. Antimalarial synergy of cysteine and aspartic protease inhibitors. *Antimicrob. Agents Chemother.* **1998**, *42*, 2254−8.
(70) Rochefort, H.; Liaudet-Coopman, E. Cathepsin D in cancer metastasis: a protease and a ligand. *APMIS* **1999**, *107*, 86−95.
(71) Papassotiropoulos, A.; Bagli, M.; Feder, O.; Jessen, F.; Maier, W.; Rao, M. L.; Ludwig, M.; Schwab, S. G.; Heun, R. Genetic polymorphism of cathepsin D is strongly associated with the risk for developing sporadic Alzheimer's disease. *Neurosci. Lett.* **1999**, *262*, 171−4.
(72) Wolfe, M. S.; Xia, W.; Moore, C. L.; Leatherwood, D. D.; Ostaszewski, B.; Rahmati, T.; Donkor, I. O.; Selkoe, D. J. Peptidomimetic probes and molecular modeling suggest that Alzheimer's gamma-secretase is an intramembrane-cleaving aspartyl protease. *Biochemistry* **1999**, *38*, 4720−7.
(73) Baldwin, E. T.; Bhat, T. N.; Gulnik, S.; Hosur, M. V.; Sowder, R. C. d.; Cachau, R. E.; Collins, J.; Silva, A. M.; Erickson, J. W. Crystal structures of native and inhibited forms of human cathepsin D: implications for lysosomal targeting and drug design. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 6796−800.
(74) Majer, P.; Collins, J. R.; Gulnik, S. V.; Erikson, J. W. Substrate-based subsite specificity mapping of human cathepsin D using statine-based inhibitors. *Protein Sci.* **1997**, *6*, 1458−66.

(75) Whitesitt, C. A.; Simon, R. L.; Reel, J. K.; Sigmund, S. K.; Phillips, M. L.; Shadle, J. K.; Heinz, L. J.; Koppel, G. A.; Hunden, D. C.; Lifer, S. L.; Berry, D.; Ray, J.; Little, S. P.; Liu, X. D.; Marshall, W. S.; Panetta, J. A. Synthesis and structure−activity relationships of benzophenones as inhibitors of cathepsin D. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2157−62.

(76) Kick, E. K.; Roe, D. C.; Skillman, A. G.; Liu, G.; Ewing, T. J.; Sun, Y.; Kuntz, I. D.; Ellman, J. A. Structure-based design and combinatorial chemistry yield low nanomolar inhibitors of cathepsin D. *Chem. Biol.* **1997**, *4*, 297−307.

(77) Lee, C. E.; Kick, E. K.; Ellman, J. A. General solid-phase synthesis approach to prepare mechanism-based aspartyl protease inhibitor libraries. Identification of potent cathepsin D inhibitors. *J. Am. Chem. Soc.* **1998**, *120*, 9735−47.

(78) Gruber, A.; Speth, C.; Lukasser-Vogl, E.; Zangerle, R.; Borg-von Zepelin, M.; Dierich, M. P.; Wurzner, R. Human immunodeficiency virus type 1 protease inhibitor attenuates *Candida albicans* virulence properties in vitro. *Immunopharmacology* **1999**, *41*, 227−34.

(79) De Bernardis, F.; Arancia, S.; Morelli, L.; Hube, B.; Sanglard, D.; Schafer, W.; Cassone, A. Evidence that members of the secretory aspartyl proteinase gene family, in particular SAP2, are virulence factors for Candida vaginitis. *J. Infect. Dis.* **1999**, *179*, 201−8.

(80) Naglik, J. R.; Newport, G.; White, T. C.; Fernandes-Naglik, L. L.; Greenspan, J. S.; Greenspan, D.; Challacombe, S. J.; Agabian, N. In vivo analysis of secreted aspartyl proteinase expression in human oral candidiasis. *Infect. Immun.* **1999**, *67*, 2482−90.

(81) Hube, B.; Sanglard, D.; Odds, F. C.; Hess, D.; Monod, M.; Schafer, W.; Brown, A. J. P.; Gow, N. A. R. Disruption of each of the secreted aspartyl proteinase genes SAP1, SAP2, and SAP3 of *Candida albicans* attenuates virulence. *Infect. Immun.* **1997**, *65*, 3529−38.

(82) Sanglard, D.; Hube, B.; Monod, M.; Odds, F. C.; Gow, N. A. A triple deletion of the secreted aspartyl proteinase genes SAP4, SAP5, and SAP6 of *Candida albicans* causes attenuated virulence. *Infect. Immun.* **1997**, *65*, 3539−46.

(83) Monod, M.; Hube, B.; Hess, D.; Sanglard, D. Differential regulation of SAP8 and SAP9, which encode two new members of the secreted aspartic proteinase family in *Candida albicans*. *Microbiology* **1998**, *144*, 2731−7.

(84) Cutfield, S. M.; Dodson, E. J.; Anderson, B. F.; Moody, P. C.; Marshall, C. J.; Sullivan, P. A.; Cutfield, J. F. The crystal structure of a major secreted aspartic proteinase from *Candida albicans* in complexes with two inhibitors. *Structure* **1995**, *3*, 1261−71.

(85) Symersky, J.; Monod, M.; Foundling, S. I. High-resolution structure of the extracellular aspartic proteinase from Candida tropicalis yeast. *Biochemistry* **1997**, *36*, 12700−10.

(86) Rich, D. H. Pepstatin-derived inhibitors of aspartic proteinases. A close look at an apparent transition-state analogue inhibitor. *J. Med. Chem.* **1985**, *28*, 263−73.

(87) Powers, J. C.; Harper, J. W. Inhibitors of serine proteases. *Proteinase Inhib.* **1986**, 55−152.

(88) Rawlings, N. D.; Barrett, A. J. Families of serine peptidases. *Methods Enzymol.* **1994**, *244*, 19−61.

(89) Barrett, A. J.; Rawlings, N. D. Families and clans of serine peptidases. *Arch. Biochem. Biophys.* **1995**, *318*, 247−50.

(90) Verstraete, M. Modulating platelet function with selective thrombin inhibitors. *Haemostasis* **1996**, *26*, 70−7.

(91) Sanderson, P. E. J.; NaylorOlsen, A. M. Thrombin inhibitor design. *Curr. Med. Chem.* **1998**, *5*, 289−304.

(92) Vacca, J. P. Thrombosis and coagulation. *Annu. Rep. Med. Chem.* **1998**, *33*, 81−90.

(93) Rawson, T. E.; VanGorp, K. A.; Yang, J.; Kogan, T. P. Separation of 21-(R)- and 21-(S)-argatroban: solubility and activity of the individual diastereoisomers [letter]. *J. Pharm. Sci.* **1993**, *82*, 672−3.

(94) Schwartz, R. P.; Becker, J.-C. P.; Brooks, R. L.; Hursting, M. J.; Joffrion, J. L.; Knappenberger, G. D.; Kogan, T. P.; Kogan, P. W.; McKinney, A. A. The preclinical and clinical pharmacology of Novastan (Argatroban): A small-molecule, direct thrombin inhibitor. *Clin. Appl. Thromb. Hemost.* **1997**, *3*, 1−15.

(95) Hilpert, K.; Ackermann, J.; Banner, D. W.; Gast, A.; Gubernator, K.; Hadvary, P.; Labler, L.; Muller, K.; Schmid, G.; Tschopp, T. B.; et al. Design and synthesis of potent and highly selective thrombin inhibitors. *J. Med. Chem.* **1994**, *37*, 3889−901.

(96) Bounameaux, H.; Ehringer, H.; Hulting, J.; Rasche, H.; Rapold, H. J.; Zultak, M. An exploratory trial of two dosages of a novel synthetic thrombin inhibitor (napsagatran, Ro 46-6240) compared with unfractionated heparin for treatment of proximal deep-vein thrombosis - - results of the European multicenter ADVENT trial. *Thromb. Haemost.* **1997**, *78*, 997−1002.

(97) Roux, S.; Tschopp, T.; Baumgartner, H. R. Effects of napsagatran (Ro 46-6240), a new synthetic thrombin inhibitor and of heparin in a canine model of coronary artery thrombosis: comparison with an ex vivo annular perfusion chamber model. *J. Pharmacol. Exp. Ther.* **1996**, *277*, 71−8.

(98) Teger-Nilsson, A. C.; Bylund, R.; Gustafsson, D.; Gyzander, E.; Eriksson, U. In vitro effects of inogatran, a selective low molecular weight thrombin inhibitor. *Thromb. Res.* **1997**, *85*, 133−45.

(99) Preville, P.; He, J. X.; Tarazi, M.; Siddiqui, M. A.; Cody, W. L.; Doherty, A. M. An efficient preparation of the potent and selective pseudopeptide thrombin inhibitor, inogatran. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1563−6.

(100) Eriksson, U. G.; Renberg, L.; Bredberg, U.; Teger-Nilsson, A. C.; Regardh, C. G. Animal pharmacokinetics of inogatran, a low-molecular-weight thrombin inhibitor with potential use as an antithrombotic drug. *Biopharm. Drug Dispos.* **1998**, *19*, 55−64.

(101) Jackson, C. V.; Satterwhite, J.; Roberts, E. Preclinical and clinical pharmacology of efegatran (LY294468): A novel antithrombin for the treatment of acute coronary syndromes. *Clin. Appl. Thromb. Hemost.* **1996**, *2*, 258−67.

(102) Rebello, S. S.; Miller, B. V.; Basler, G. C.; Lucchesi, B. R. CVS-1123, a direct thrombin inhibitor, prevents occlusive arterial and venous thrombosis in a canine model of vascular injury. *J. Cardiovasc. Pharmacol.* **1997**, *29*, 240−9.

(103) Cousins, G. R.; Friedrichs, G. S.; Sudo, Y.; Rebello, S. S.; Rote, W. E.; Vlasuk, G. P.; Nolan, T. G.; Mendoza, C.; Lucchesi, B. R. Orally effective CVS-1123 prevents coronary artery thrombosis in the conscious dog. *Circulation* **1996**, *94*, 1705−12.

(104) Kettner, C.; Mersinger, L.; Knabb, R. The selective inhibition of thrombin by peptides of boroarginine. *J. Biol. Chem.* **1990**, *265*, 18289−97.

(105) Knabb, R. M.; Kettner, C. A.; Timmermans, P. B.; Reilly, T. M. In vivo characterization of a new synthetic thrombin inhibitor. *Thromb. Haemost.* **1992**, *67*, 56−9.

(106) Quan, M. L.; Wityak, J.; Dominguez, C.; Duncia, J. V.; Kettner, C. A.; Ellis, C. D.; Liauw, A. Y.; Park, J. M.; Santella, J. B.; Knabb, R. M.; Thoolen, M. J.; Weber, P. C.; Wexler, R. R. Biaryl substituted alkylboronate esters as thrombin inhibitors. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1595−600.

(107) Allen, M.; Abel, S. M.; Barber, C. G.; Cussans, N. J.; Danilewicz, J. C.; Elliss, D.; Hawkeswood, E.; Herron, M.; Holland, S.; Fox, D. N. A.; James, K.; Kobylecki, R. J.; Overington, J. P.; Pandit, J.; Parmer, H.; Powling, M.; Rance, D. J.; Taylor, W.; Shepperson, N. B. 215th ACS National Meeting, Dallas, TX, Mar 29−Apr 2, 1998; Abstr. MEDI 200.

(108) Tucker, T. J.; Lumma, W. C.; Lewis, S. D.; Gardell, S. J.; Lucas, B. J.; Baskin, E. P.; Woltmann, R.; Lynch, J. J.; Lyle, E. A.; Appleby, S. D.; Chen, I. W.; Dancheck, K. B.; Vacca, J. P. Potent noncovalent thrombin inhibitors that utilize the unique amino acid D-dicyclohexylalanine in the P3 position. Implications on oral bioavailability and antithrombotic efficacy. *J. Med. Chem.* **1997**, *40*, 1565−9.

(109) Brady, S. F.; Stauffer, K. J.; Lumma, W. C.; Smith, G. M.; Ramjit, H. G.; Lewis, S. D.; Lucas, B. J.; Gardell, S. J.; Lyle, E. A.; Appleby, S. D.; Cook, J. J.; Holahan, M. A.; Stranieri, M. T.; Lynch, J. J.; Lin, J. H.; Chen, I. W.; Vastag, K.; NaylorOlsen, A. M.; Vacca, J. P. Discovery and development of the novel potent orally active thrombin inhibitor N-(9-hydroxy-9-fluorenecarboxy)prolyl *trans*-4-aminocyclohexylmethyl amide (L-372,460): Coapplication of structure-based design and rapid multiple analogue synthesis on solid support. *J. Med. Chem.* **1998**, *41*, 401−6.

(110) Malley, M. F.; Tabernero, L.; Chang, C. Y.; Ohringer, S. L.; Roberts, D. G.; Das, J.; Sack, J. S. Crystallographic determination of the structures of human alpha-thrombin complexed with BMS-186282 and BMS-189090. *Protein Sci.* **1996**, *5*, 221−8.

(111) Chirgadze, N. Y.; Sall, D. J.; Klimkowski, V. J.; Clawson, D. K.; Briggs, S. L.; Hermann, R.; Smith, G. F.; Gifford-Moore, D. S.; Wery, J. P. The crystal structure of human alpha-thrombin complexed with LY178550, a nonpeptidyl, active site-directed inhibitor. *Protein Sci.* **1997**, *6*, 1412−7.

(112) Gustafsson, D.; Antonsson, T.; Bylund, R.; Eriksson, U.; Gyzander, E.; Nilsson, I.; Elg, M.; Mattsson, C.; Deinum, J.; Pehrsson, S.; Karlsson, O.; Nilsson, A.; Sorensen, H. Effects of melagatran, a new low-molecular-weight thrombin inhibitor, on thrombin and fibrinolytic enzymes. *Thromb. Haemost.* **1998**, *79*, 110−8.

(113) Mehta, J. L.; Chen, L. Y.; Nichols, W. W.; Mattsson, C.; Gustafsson, D.; Saldeen, T. G. P. Melagatran, an oral active-site inhibitor of thrombin, prevents or delays formation of electrically induced occlusive thrombus in the canine coronary artery. *J. Cardiovasc. Pharmacol.* **1998**, *31*, 345−51.

(114) Lila, C.; Gloanec, P.; Cadet, L.; Herve, Y.; Fournier, J.; Leborgne, F.; Verbeuren, T. J.; DeNanteuil, G. Large scale preparation of protected 4-aminomethylbenzamidine. Application to the synthesis of the thrombin inhibitor, melagatran. *Synth. Commun.* **1998**, *28*, 4419−29.

(115) Kaiser, B. Factor Xa versus factor IIa inhibitors. *Clin. Appl. Thromb. Hemost.* **1997**, *3*, 16−24.

(116) Hara, T.; Yokoyama, A.; Ishihara, H.; Yokoyama, Y.; Nagahara, T.; Iwamoto, M. Dx-9065a, a New Synthetic, Potent Anticoagulant and Selective Inhibitor For Factor-Xa. *Thromb. Haemost.* **1994**, *71*, 314−9.

(117) Hara, T.; Yokoyama, A.; Tanabe, K.; Ishihara, H.; Iwamoto, M. DX-9065a, an orally active, specific inhibitor of factor Xa, inhibits thrombosis without affecting bleeding time in rats. *Thromb. Haemost.* **1995**, *74*, 635−9.

(118) Herbert, J. M.; Bernat, A.; Dol, F.; Herault, J. P.; Crepon, B.; Lormeau, J. C. DX 9065A, a novel, synthetic, selective and orally active inhibitor of factor xa: In vitro and in vivo studies. *J. Pharm. Exp. Ther.* **1996**, *276*, 1030−8.

(119) Katakura, S.; Nagahara, T.; Hara, T.; Iwamoto, M. A novel factor Xa inhibitor: structure−activity relationships and selectivity between factor Xa and thrombin. *Biochem. Biophys. Res. Commun.* **1993**, *197*, 965−72.

(120) Katakura, S.; Nagahara, T.; Hara, T.; Kunitada, S.; Iwamoto, M. Molecular model of an interaction between factor Xa and DX-9065a, a novel factor Xa inhibitor: Contribution of the acetimidoylpyrrolidine moiety of the inhibitor to potency and selectivity for serine proteases. *Eur. J. Med. Chem.* **1995**, *30*, 387−94.

(121) Brandstetter, H.; Kuhne, A.; Bode, W.; Huber, R.; vonderSaal, W.; Wirthensohn, K.; Engh, R. A. X-ray structure of active site-inhibited clotting factor Xa − Implications for drug design and substrate recognition. *J. Biol. Chem.* **1996**, *271*, 29988−92.

(122) Morishima, Y.; Tanabe, K.; Terada, Y.; Hara, T.; Kunitada, S. Antithrombotic and hemorrhagic effects of DX-9065a, a direct and selective factor Xa inhibitor: Comparison with a direct thrombin inhibitor and antithrombin III-dependent anticoagulants. *Thromb. Haemost.* **1997**, *78*, 1366−71.

(123) Taniuchi, Y.; Sakai, Y.; Hisamichi, N.; Kayama, M.; Mano, Y.; Sato, K.; Hirayama, F.; Koshio, H.; Matsumoto, Y.; Kawasaki, T. Biochemical and pharmacological characterization of YM-60828, a newly synthesized and orally active inhibitor of human factor Xa. *Thromb. Haemost.* **1998**, *79*, 543−8.

(124) Kawasaki, T.; Sato, K.; Sakai, Y.; Hirayama, F.; Koshio, H.; Taniuchi, Y.; Matsumoto, Y. Comparative studies of an orally active factor Xa inhibitor, YM-60828, with other antithrombotic agents in a rot model of arterial thrombosis. *Thromb. Haemost.* **1998**, *79*, 410−6.

(125) Sato, K.; Kawasaki, T.; Hisamichi, N.; Taniuchi, Y.; Hirayama, F.; Koshio, H.; Matsumoto, Y. Antithrombotic effects of YM-60828, a newly synthesized factor Xa inhibitor, in rat thrombosis models and its effects on bleeding time. *Br. J. Pharmacol.* **1998**, *123*, 92−6.

(126) Kamata, K.; Kawamoto, H.; Honma, T.; Iwama, T.; Kim, S. H. Structural basis for chemical inhibition of human blood coagulation factor Xa. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 6630−5.

(127) Klein, S. I.; Czekaj, M.; Gardner, C. J.; Guertin, K. R.; Cheney, D. L.; Spada, A. P.; Bolton, S. A.; Brown, K.; Colussi, D.; Heran, C. L.; Morgan, S. R.; Leadley, R. J.; Dunwiddie, C. T.; Perrone, M. H.; Chu, V. Identification and initial structure−activity relationships of a novel class of nonpeptide inhibitors of blood coagulation factor Xa. *J. Med. Chem.* **1998**, *41*, 437−50.

(128) Kucznierz, R.; Grams, F.; Leinert, H.; Marzenell, K.; Engh, R. A.; von der Saal, W. Tetrahydro-isoquinoline-based factor Xa inhibitors. *J. Med. Chem.* **1998**, *41*, 4983−94.

(129) Edwards, P. D.; Bernstein, P. R. Synthetic inhibitors of elastase. *Med. Res. Rev.* **1994**, *14*, 127−94.

(130) Williams, J. C.; Stein, R. L.; Giles, R. E.; Krell, R. D. Biochemistry and pharmacology of ICI 200,880, a synthetic peptide inhibitor of human neutrophil elastase. *Ann. N. Y. Acad. Sci.* **1991**, *624*, 230−43.

(131) Sommerhoff, C. P.; Krell, R. D.; Williams, J. L.; Gomes, B. C.; Strimpler, A. M.; Nadel, J. A. Inhibition of human neutrophil elastase by ICI 200,355. *Eur. J. Pharmacol.* **1991**, *193*, 153−8.

(132) O'Riordan, T. G.; Otero, R.; Mao, Y.; Lauredo, I.; Abraham, W. M. Elastase contributes to antigen-induced mucociliary dysfunction in ovine airways. *Am. J. Respir. Crit. Care Med.* **1997**, *155*, 1522−8.

(133) Williams, J. C.; Falcone, R. C.; Knee, C.; Stein, R. L.; Strimpler, A. M.; Reaves, B.; Giles, R. E.; Krell, R. D. Biologic characterization of ICI 200,880 and ICI 200,355, novel inhibitors of human neutrophil elastase. *Am. Rev. Respir. Dis.* **1991**, *144*, 875−83.

(134) Anderson, G. P.; Shinagawa, K. Neutrophil elastase inhibitors as treatments for emphysema and chronic bronchitis. *Curr. Opin. Anti-inflam. Immun. Invest. Drugs* **1999**, *1*, 29−38.

(135) Luisetti, M.; Sturani, C.; Sella, D.; Madonini, E.; Galavotti, V.; Bruno, G.; Peona, V.; Kucich, U.; Dagnino, G.; Rosenbloom, J.; Starcher, B.; Grassi, C. MR889, a neutrophil elastase inhibitor, in patients with chronic obstructive pulmonary disease: a double-blind, randomized, placebo-controlled clinical trial. *Eur. Respir. J.* **1996**, *9*, 1482−6.

(136) Luisetti, M.; Piccioni, P. D.; Donnini, M.; Peona, V.; Pozzi, E.; Grassi, C. Studies of MR 889, a new synthetic proteinase inhibitor. *Biochem. Biophys. Res. Commun.* **1989**, *165*, 568−73.

(137) Baici, A.; Pelloso, R.; Horler, D. The kinetic mechanism of inhibition of human leukocyte elastase by MR889, a new cyclic thiolic compound. *Biochem. Pharmacol.* **1990**, *39*, 919−24.

(138) Kawabata, K.; Suzuki, M.; Sugitani, M.; Imaki, K.; Toda, M.; Miyamoto, T. ONO-5046, a novel inhibitor of human neutrophil elastase. *Biochem. Biophys. Res. Commun.* **1991**, *177*, 814−20.

(139) Iwamura, H.; Moore, A. R.; Willoughby, D. A. Interaction between neutrophil-derived elastase and reactive oxygen species in cartilage degradation. *Biochim. Biophys. Acta* **1993**, *1156*, 295−301.

(140) Kakimoto, K.; Matsukawa, A.; Yoshinaga, M.; Nakamura, H. Suppressive effect of a neutrophil elastase inhibitor on the development of collagen-induced arthritis. *Cell. Immunol.* **1995**, *165*, 26−32.

(141) Inada, M.; Yamashita, J.; Ogawa, M. Neutrophil elastase inhibitor (ONO-5046-Na) inhibits the growth of human lung cancer cell lines transplanted into severe combined immunodeficiency (scid) mice. *Res. Commun. Mol. Pathol. Pharmacol.* **1997**, *97*, 229−32.

(142) Kobayashi, J.; Kitamura, S. How can we monitor and treat patients with acute exacerbations of idiopathic interstitial pneumonia. *Nihon Kyobu Shikkan Gakkai Zasshi* **1996**, *34*, 195−9.

(143) Watanabe, F.; Sato, M.; Kato, A.; Murakami, T.; Higashi, Y.; Yata, N. First-pass metabolism of ONO-5046 (N-[2-[4-(2,2-dimethylpropionyloxy) phenylsulfonylamino]benzoyl]aminoacetic acid), a novel elastase inhibitor, in rats. *Biol. Pharm. Bull.* **1997**, *20*, 392−6.

(144) Finke, P. E.; Shah, S. K.; Ashe, B. M.; Ball, R. G.; Blacklock, T. J.; Bonney, R. J.; Brause, K. A.; Chandler, G. O.; Cotton, M.; Davies, P.; et al. Inhibition of human leukocyte elastase. 4. Selection of a substituted cephalosporin (L-658,758) as a topical aerosol. *J. Med. Chem.* **1992**, *35*, 3731−44.

(145) Pacholok, S. G.; Davies, P.; Dorn, C.; Finke, P.; Hanlon, W. A.; Mumford, R. A.; Humes, J. L. Formation of polymorphonuclear leukocyte elastase: alpha 1 proteinase inhibitor complex and A alpha (1−21) fibrinopeptide in human blood stimulated with the calcium ionophore A23187. A model to characterize inhibitors of polymorphonuclear leukocyte elastase. *Biochem. Pharmacol.* **1995**, *49*, 1513−20.

(146) Thompson, K. R.; Finke, P. E.; Shah, S. K.; Ashe, B. M.; Dahlgren, M. E.; Maycock, A. L.; Doherty, J. B. Inhibition of human leukocyte elastase. 6. Inhibiton by 6-substituted penicillin esters. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2283−8.

(147) Thompson, K. R.; Finke, P. E.; Shah, S. K.; Ashe, B. M.; Dahlgren, M. E.; Dellea, P. S.; Fletcher, D. S.; Hand, K. M.; Maycock, A. L.; Doherty, J. B. Inhibition of human leukocyte elastase. 7. Inhibiton by 6-substituted penicillin amides. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2289−94.

(148) Finke, P. E.; Dahlgren, M. E.; Weston, H.; Maycock, A. L.; Doherty, J. B. Inhibition of human leukocyte elastase. 5. Inhibiton by 6-alkyl substituted penem benzyl esters. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2277−82.

(149) Maillard, J. L.; Favreau, C.; Reboud-Ravaux, M.; Kobaiter, R.; Joyeau, R.; Wakselman, M. Biological evaluation of the inhibition of neutrophil elastase by a synthetic beta-lactam derivative. *Eur. J. Cell Biol.* **1990**, *52*, 213−8.

(150) Knight, W. B.; Green, B. G.; Chabin, R. M.; Gale, P.; Maycock, A. L.; Weston, H.; Kuo, D. W.; Westler, W. M.; Dorn, C. P.; Finke, P. E.; et al. Specificity, stability, and potency of monocyclic beta-lactam inhibitors of human leucocyte elastase. *Biochemistry* **1992**, *31*, 8160−70.

(151) Kerrigan, J. E.; Oleksyszyn, J.; Kam, C. M.; Selzler, J.; Powers, J. C. Mechanism-based isocoumarin inhibitors for human leukocyte elastase. Effect of the 7-amino substituent and 3-alkoxy group in 3-alkoxy-7-amino-4-chloroisocoumarins on inhibitory potency. *J. Med. Chem.* **1995**, *38*, 544−52.

(152) Hlasta, D. J.; Ackerman, J. H.; Court, J. J.; Farrell, R. P.; Johnson, J. A.; Kofron, J. L.; Robinson, D. T.; Talomie, T. G.; Dunlap, R. P.; Franke, C. A. A novel class of cyclic beta-dicarbonyl leaving groups and their use in the design of benzisothiazolone human leukocyte elastase inhibitors. *J. Med. Chem.* **1995**, *38*, 4687−92.

(153) Hlasta, D. J.; Subramanyam, C.; Bell, M. R.; Carabateas, P. M.; Court, J. J.; Desai, R. C.; Drozd, M. L.; Eickhoff, W. M.; Ferguson, E. W.; Gordon, R. J.; et al. Orally bioavailable benzisothiazolone inhibitors of human leukocyte elastase. *J. Med. Chem.* **1995**, *38*, 739−44.

(154) Finke, P. E.; Shah, S. K.; Fletcher, D. S.; Ashe, B. M.; Brause, K. A.; Chandler, G. O.; Dellea, P. S.; Hand, K. M.; Maycock, A. L.; Osinga, D. G.; et al. Orally active beta-lactam inhibitors of human leukocyte elastase. 3. Stereospecific synthesis and structure−activity relationships for 3,3-dialkylazetidin-2-ones. *J. Med. Chem.* **1995**, *38*, 2449−62.

(155) Macdonald, S. J.; Belton, D. J.; Buckley, D. M.; Spooner, J. E.; Anson, M. S.; Harrison, L. A.; Mills, K.; Upton, R. J.; Dowle, M. D.; Smith, R. A.; Molloy, C. R.; Risley, C. Syntheses of *trans*-5-oxo-hexahydro-pyrrolo[3,2-b]pyrroles and *trans*-5-oxo-hexahydro-furo[3,2-b]pyrroles (pyrrolidine *trans*-lactams and *trans*-lactones): new pharmacophores for elastase inhibition. *J. Med. Chem.* **1998**, *41*, 3919−22.

(156) Cregge, R. J.; Durham, S. L.; Farr, R. A.; Gallion, S. L.; Hare, C. M.; Hoffman, R. V.; Janusz, M. J.; Kim, H. O.; Koehl, J. R.; Mehdi, S.; Metz, W. A.; Peet, N. P.; Pelton, J. T.; Schreuder, H.

A.; Sunder, S.; Tardif, C. Inhibition of human neutrophil elastase. 4. Design, synthesis, X-ray crystallographic analysis, and structure−activity relationships for a series of P2-modified, orally active peptidyl pentafluoroethyl ketones. *J. Med. Chem.* **1998**, *41*, 2461−80.

(157) Angelastro, M. R.; Baugh, L. E.; Bey, P.; Burkhart, J. P.; Chen, T. M.; Durham, S. L.; Hare, C. M.; Huber, E. W.; Janusz, M. J.; Koehl, J. R.; et al. Inhibition of human neutrophil elastase with peptidyl electrophilic ketones. 2. Orally active PG-Val-Pro-Val pentafluoroethyl ketones. *J. Med. Chem.* **1994**, *37*, 4538−53.

(158) Burkhart, J. P.; Koehl, J. R.; Mehdi, S.; Durham, S. L.; Janusz, M. J.; Huber, E. W.; Angelastro, M. R.; Sunder, S.; Metz, W. A.; Shum, P. W.; et al. Inhibition of human neutrophil elastase. 3. An orally active enol acetate prodrug. *J. Med. Chem.* **1995**, *38*, 223−33.

(159) Veale, C. A.; Bernstein, P. R.; Bohnert, C. M.; Brown, F. J.; Bryant, C.; Damewood, J. R., Jr.; Earley, R.; Feeney, S. W.; Edwards, P. D.; Gomes, B.; Hulsizer, J. M.; Kosmider, B. J.; Krell, R. D.; Moore, G.; Salcedo, T. W.; Shaw, A.; Silberstein, D. S.; Steelman, G. B.; Stein, M.; Strimpler, A.; Thomas, R. M.; Vacek, E. P.; Williams, J. C.; Wolanin, D. J.; Woolson, S. Orally active trifluoromethyl ketone inhibitors of human leukocyte elastase. *J. Med. Chem.* **1997**, *40*, 3173−81.

(160) Edwards, P. D.; Andisik, D. W.; Bryant, C. A.; Ewing, B.; Gomes, B.; Lewis, J. J.; Rakiewicz, D.; Steelman, G.; Strimpler, A.; Trainor, D. A.; Tuthill, P. A.; Mauger, R. C.; Veale, C. A.; Wildonger, R. A.; Williams, J. C.; Wolanin, D. J.; Zottola, M. Discovery and biological activity of orally active peptidyl trifluoromethyl ketone inhibitors of human neutrophil elastase. *J. Med. Chem.* **1997**, *40*, 1876−85.

(161) Bernstein, P. R.; Gomes, B. C.; Kosmider, B. J.; Vacek, E. P.; Williams, J. C. Nonpeptidic inhibitors of human leukocyte elastase. 6. Design of a potent, intratracheally active, pyridone-based trifluoromethyl ketone. *J. Med. Chem.* **1995**, *38*, 212−5.

(162) Veale, C. A.; Damewood, J. R., Jr.; Steelman, G. B.; Bryant, C.; Gomes, B.; Williams, J. Nonpeptidic inhibitors of human leukocyte elastase. 4. Design, synthesis, and in vitro and in vivo activity of a series of beta-carbolinone-containing trifluoromethyl ketones. *J. Med. Chem.* **1995**, *38*, 86−97.

(163) Veale, C. A.; Bernstein, P. R.; Bryant, C.; Ceccarelli, C.; Damewood, J. R., Jr.; Earley, R.; Feeney, S. W.; Gomes, B.; Kosmider, B. J.; Steelman, G. B.; et al. Nonpeptidic inhibitors of human leukocyte elastase. 5. Design, synthesis, and X-ray crystallography of a series of orally active 5-aminopyrimidin-6-one-containing trifluoromethyl ketones. *J. Med. Chem.* **1995**, *38*, 98−108.

(164) Brown, F. J.; Andisik, D. W.; Bernstein, P. R.; Bryant, C. B.; Ceccarelli, C.; Damewood, J. R., Jr.; Edwards, P. D.; Earley, R. A.; Feeney, S.; Green, R. C.; et al. Design of orally active, nonpeptidic inhibitors of human leukocyte elastase. *J. Med. Chem.* **1994**, *37*, 1259−61.

(165) Edwards, P. D.; Andisik, D. W.; Strimpler, A. M.; Gomes, B.; Tuthill, P. A. Nonpeptidic inhibitors of human neutrophil elastase. 7. Design, synthesis, and in vitro activity of a series of pyridopyrimidine trifluoromethyl ketones. *J. Med. Chem.* **1996**, *39*, 1112−24.

(166) Portevin, B.; Lonchampt, M.; Canet, E.; De Nanteuil, G. Dual inhibition of human leukocyte elastase and lipid peroxidation: in vitro and in vivo activities of azabicyclo[2.2.2]octane and perhydroindole derivatives. *J. Med. Chem.* **1997**, *40*, 1906−18.

(167) Gutschow, M.; Neumann, U. Novel thieno[2,3-d][1,3]oxazin-4-ones as inhibitors of human leukocyte elastase. *J. Med. Chem.* **1998**, *41*, 1729−40.

(168) Kuang, R.; Venkataraman, R.; Ruan, S.; Groutas, W. C. Use of the 1,2,5-thiadiazolidin-3-one 1,1-dioxide and isothiazolidin-3-one 1,1-dioxide scaffolds in the design of potent inhibitors of serine proteinases. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 539−44.

(169) Guy, L.; Vidal, J.; Collet, A.; Amour, A.; Reboud-Ravaux, M. Design and synthesis of hydrazinopeptides and their evaluation as human leukocyte elastase inhibitors. *J. Med. Chem.* **1998**, *41*, 4833−43.

(170) Schwartz, L. B. Tryptase: a mast cell serine protease. *Methods Enzymol.* **1994**, *244*, 88−100.

(171) Walls, A. F. The roles of neutral proteases in asthma and rhinitis. In *Asthma and rhinitis*; Busse, W. W., Holgate, S. T., Eds.; Blackwell: Oxford, 1995; pp 801−825.

(172) Matsumoto, R.; Sali, A.; Ghildyal, N.; Karplus, M.; Stevens, R. L. Packaging of proteases and proteoglycans in the granules of mast cells and other hematopoietic cells. A cluster of histidines on mouse mast cell protease 7 regulates its binding to heparin serglycin proteoglycans. *J. Biol. Chem.* **1995**, *270*, 19524−31.

(173) Stubbs, M. T.; Morenweiser, R.; Sturzebecher, J.; Bauer, M.; Bode, W.; Huber, R.; Piechottka, G. P.; Matschiner, G.; Sommerhoff, C. P.; Fritz, H.; Auerswald, E. A. The three-dimensional structure of recombinant leech-derived tryptase inhibitor in complex with trypsin. Implications for the structure of human mast cell tryptase and its inhibition. *J. Biol. Chem.* **1997**, *272*, 19931−7.

(174) Rice, K. D.; Tanaka, R. D.; Katz, B. A.; Numerof, R. P.; Moore, W. R. Inhibitors of tryptase for the treatment of mast cell-mediated diseases. *Curr. Pharm. Des.* **1998**, *4*, 381−96.

(175) Katz, B. A.; Clark, J. M.; FinerMoore, J. S.; Jenkins, T. E.; Johnson, C. R.; Ross, M. J.; Luong, C.; Moore, W. R.; Stroud, R. M. Design of potent selective zinc-mediated serine protease inhibitors. *Nature* **1998**, *391*, 608−12.

(176) Clark, J. M.; Abraham, W. M.; Fishman, C. E.; Forteza, R.; Ahmed, A.; Cortes, A.; Warne, R. L.; Moore, W. R.; Tanaka, R. D. Tryptase inhibitors block allergen-induced airway and inflammatory responses in allergic sheep. *Am. J. Respir. Crit. Care Med.* **1995**, *152*, 2076−83.

(177) Molinari, J. F.; Scuri, M.; Moore, W. R.; Clark, J.; Tanaka, R.; Abraham, W. M. Inhaled tryptase causes bronchoconstriction in sheep via histamine release. *Am. J. Respir. Crit. Care Med.* **1996**, *154*, 649−53.

(178) Van Noorden, J. F. IBC's 2nd International Conference on Protease Inhibitors: Novel Therapeutic Applications and Development, Washington DC, 24−26 February. *Acta Histochem.* **1997**, 249−55.

(179) Combrink, K. D.; Gulgeze, H. B.; Meanwell, N. A.; Pearce, B. C.; Zulan, P.; Bisacchi, G. S.; Roberts, D. G.; Stanley, P.; Seiler, S. M. 1,2-Benzisothiazol-3-one 1,1-dioxide inhibitors of human mast cell tryptase. *J. Med. Chem.* **1998**, *41*, 4854−60.

(180) Whaley, K. *Complement in health and disease*; MTP Press Ltd.: Lancaster, 1987.

(181) Sim, E. *The natural immune system: Humoral factors*; Oxford University Press: Oxford, 1993.

(182) Ember, J. A.; Hugli, T. E. Complement factors and their receptors. *Immunopharmacology* **1997**, *38*, 3−15.

(183) Gerard, C.; Gerard, N. P. C5A anaphylatoxin and its seven transmembrane-segment receptor. *Annu. Rev. Immunol.* **1994**, *12*, 775−808.

(184) Oda, M.; Ino, Y.; Nakamura, K.; Kuramoto, S.; Shimamura, K.; Iwaki, M.; Fujii, S. Pharmacological studies on 6-amidino-2-naphthyl[4-(4,5-dihydro-1H-imidazol-2-yl)amino] benzoate dimethane sulfonate (FUT-187). I: Inhibitory activities on various kinds of enzymes in vitro and anticomplement activity in vivo. *Jpn. J. Pharmacol.* **1990**, *52*, 23−34.

(185) Nakamura, K.; Johmura, A.; Oda, M.; Ino, Y.; Uchiyama, H.; Ohtani, H.; Miyazaki, H.; Kurumi, M.; Akizawa, Y.; Oka, T. Inhibitory effects of sepimostat mesilate (FUT-187) on the activities of trypsin-like serine proteases in vitro. *Yakugaku Zasshi* **1995**, *115*, 201−12.

(186) Sepimostat Mesilate. *Drugs Future* **1997**, *22*, 502−7.

(187) Abe, O.; Yoshino, K.; Kimura, K.; Suzuki, H.; Aoki, T.; Hiki, Y.; Mitomi, T.; Makuuchi, H. Early phase II study of FUT-187 (sepimostat mesilate) on reflux esophagitis−mainly on postgastrectomy reflux esophagitis. *Gan To Kagaku Ryoho* **1994**, *21*, 845−52.

(188) Shiozaki, H.; Inoue, M.; Tamura, S.; Iwanaga, T.; Imaoka, M.; Furukawa, H.; Hiratsuka, M.; Kikkawa, N.; Kobayashi, K.; Okamura, J.; Takada, N.; Ogawa, Y.; Yamada, T.; Takami, M.; Takada, T.; Okuda, H.; Yano, T.; Satomi, T.; Kawasaki, T.; Oshima, S.; Yamasaki, K.; Imamoto, H.; Noguchi, S.; Fujimoto, N.; Mori, T.; et al. Effect of FUT-187, oral serine protease inhibitor, on inflammation in the gastric remnant. *Gan To Kagaku Ryoho* **1996**, *23*, 1971−9.

(189) Makuuchi, H.; Kanno, K.; Mitomi, T. Clinical usefulness of FUT-187 (sepimostat mesilate) on reflux esophagitis and gastritis after gastrectomy. *Gan To Kagaku Ryoho* **1995**, *22*, 777−83.

(190) Ikari, N.; Sakai, Y.; Hitomi, Y.; Fujii, S. New synthetic inhibitor to the alternative complement pathway. *Immunology* **1983**, *49*, 685−91.

(191) Inagi, R.; Miyata, T.; Maeda, K.; Sugiyama, S.; Miyama, A.; Nakashima, I. FUT-175 as a potent inhibitor of C5/C3 convertase activity for production of C5a and C3a. *Immunol. Lett.* **1991**, *27*, 49−52.

(192) Fujita, Y.; Inoue, I.; Inagi, R.; Miyata, T.; Shinzato, T.; Sugiyama, S.; Miyama, A.; Maeda, K. Inhibitory effect of FUT-175 on complement activation and its application for glomerulonephritis with hypocomplementemia. *Nippon Jinzo Gakkai Shi* **1993**, *35*, 393−7.

(193) Kitagawa, M.; Naruse, S.; Ishiguro, H.; Hayakawa, T. Pharmaceutical development for treating pancreatic diseases. *Pancreas* **1998**, *16*, 427−31.

(194) Love, R. A.; Parge, H. E.; Wickersham, J. A.; Hostomsky, Z.; Habuka, N.; Moomaw, E. W.; Adachi, T.; Margosiak, S.; Dagostino, E.; Hostomska, Z. The conformation of hepatitis C virus NS3 proteinase with and without NS4A: a structural basis for the activation of the enzyme by its cofactor. *Clin. Diagn. Virol.* **1998**, *10*, 151−6.

(195) Yan, Y.; Li, Y.; Munshi, S.; Sardana, V.; Cole, J. L.; Sardana, M.; Steinkuehler, C.; Tomei, L.; De Francesco, R.; Kuo, L. C.; Chen, Z. Complex of NS3 protease and NS4A peptide of BK strain hepatitis C virus: a 2.2 A resolution structure in a hexagonal crystal form. *Protein Sci.* **1998**, *7*, 837−47.

(196) Koch, J. O.; Bartenschlager, R. Determinants of substrate specificity in the NS3 serine proteinase of the hepatitis C virus. *Virology* **1997**, *237*, 78−88.
(197) Zhang, R.; Durkin, J.; Windsor, W. T.; McNemar, C.; Ramanathan, L.; Le, H. V. Probing the substrate specificity of hepatitis C virus NS3 serine protease by using synthetic peptides. *J. Virol.* **1997**, *71*, 6208−13.
(198) Sudo, K.; Matsumoto, Y.; Matsushima, M.; Fujiwara, M.; Konno, K.; Shimotohno, K.; Shigeta, S.; Yokota, T. Novel hepatitis C virus protease inhibitors: thiazolidine derivatives. *Biochem. Biophys. Res. Commun.* **1997**, *238*, 643−7.
(199) Kakiuchi, N.; Komoda, Y.; Komoda, K.; Takeshita, N.; Okada, S.; Tani, T.; Shimotohno, K. Non-peptide inhibitors of HCV serine proteinase. *FEBS Lett.* **1998**, *421*, 217−20.
(200) Steinkuhler, C.; Biasiol, G.; Brunetti, M.; Urbani, A.; Koch, U.; Cortese, R.; Pessi, A.; De Francesco, R. Product inhibition of the hepatitis C virus NS3 protease. *Biochemistry* **1998**, *37*, 8899−905.
(201) Llinas-Brunet, M.; Bailey, M.; Fazal, G.; Goulet, S.; Halmos, T.; Laplante, S.; Maurice, R.; Poirier, M.; Poupart, M. A.; Thibeault, D.; Wernic, D.; Lamarre, D. Peptide-based inhibitors of the hepatitis C virus serine protease. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1713−8.
(202) Llinas-Brunet, M.; Bailey, M.; Deziel, R.; Fazal, G.; Gorys, V.; Goulet, S.; Halmos, T.; Maurice, R.; Poirier, M.; Poupart, M. A.; Rancourt, J.; Thibeault, D.; Wernic, D.; Lamarre, D. Studies on the C-terminal of hexapeptide inhibitors of the hepatitis C virus serine protease. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2719−24.
(203) Ingallinella, P.; Altamura, S.; Bianchi, E.; Taliani, M.; Ingenito, R.; Cortese, R.; De Francesco, R.; Steinkuhler, C.; Pessi, A. Potent peptide inhibitors of human hepatitis C virus NS3 protease are obtained by optimizing the cleavage products. *Biochemistry* **1998**, *37*, 8906−14.
(204) Davis, R.; Whittington, R. Aprotinin. A review of its pharmacology and therapeutic efficacy in reducing blood loss associated with cardiac surgery. *Drugs* **1995**, *49*, 954−83.
(205) Robert, S.; Wagner, B. K. J.; Boulanger, M.; Richer, M. Aprotinin. *Ann. Pharmacother.* **1996**, *30*, 372−80.
(206) Smith, C. R. Management of bleeding complications in redo cardiac operations. *Ann. Thorac. Surg.* **1998**, *65* (Suppl. S), S2−S8.
(207) Pouard, P. Review of efficacy parameters. *Ann. Thorac. Surg.* **1998**, *65* (Suppl. S), S40−S43.
(208) Dobkowski, W. B.; Murkin, J. M. A risk-benefit assessment of aprotinin in cardiac surgical procedures. *Drug Saf.* **1998**, *18*, 21−41.
(209) Royston, D. Aprotinin versus lysine analogues: The debate continues. *Ann. Thorac. Surg.* **1998**, *65* (Suppl. S), S9−S19.
(210) Rich, J. B. The efficacy and safety of aprotinin use in cardiac surgery. *Ann. Thorac. Surg.* **1998**, *66*, S6−11 (discussion S25−8).
(211) Llamas, P.; Cabrera, R.; Gomez-Arnau, J.; Fernandez, M. N. Hemostasis and blood requirements in orthotopic liver transplantation with and without high-dose aprotinin. *Haematologica* **1998**, *83*, 338−46.
(212) Pezzilli, R.; Barakat, B.; MorselliLabate, A. M. Evaluation of the immunomodulatory effect of gabexate mesilate in patients with severe acute pancreatitis. *Curr. Ther. Res. Clin. Exp.* **1998**, *59*, 710−6.
(213) Okumura, Y.; Inoue, H.; Fujiyama, Y.; Bamba, T. Effects of serine protease inhibitors on accumulation of polymorphonuclear leukocytes in the lung induced by acute pancreatitis in rats. *J. Gastroenterol.* **1995**, *30*, 379−86.
(214) Parodi, F.; Cordero, G.; DeGasperis, C.; Minola, P.; Dellora, C.; Innocenti, P. Gabexate mesilate, a new synthetic serine protease inhibitor: A pilot clinical trial in valvular heart surgery. *J. Cardiothorac. Vasc. Anesth.* **1996**, *10*, 235−7.
(215) Shibata, T.; Yamamoto, F.; Suchiro, S.; Kinoshita, H. Effects of protease inhibitors on postischemic recovery of the heart. *Cardiovasc. Drugs Ther.* **1997**, *11*, 547−56.
(216) Lee, S. L.; Alexander, R. S.; Smallwood, A.; Trievel, R.; Mersinger, L.; Weber, R. C.; Kettner, C. New inhibitors of thrombin and other trypsin-like proteases: hydrogen bonding of an aromatic cyano group with a backbone amide of the P1 binding site replaces binding of a basic side chain. *Biochemistry* **1997**, *36*, 13180−6.
(217) Sanderson, P. E. Small, noncovalent serine protease inhibitors. *Med. Res. Rev.* **1999**, *19*, 179−97.
(218) Tucker, T. J.; Brady, S. F.; Lumma, W. C.; Lewis, S. D.; Gardell, S. J.; Naylor-Olsen, A. M.; Yan, Y.; Sisko, J. T.; Stauffer, K. J.; Lucas, B. J.; Lynch, J. J.; Cook, J. J.; Stranieri, M. T.; Holahan, M. A.; Lyle, E. A.; Baskin, E. P.; Chen, I. W.; Dancheck, K. B.; Krueger, J. A.; Cooper, C. M.; Vacca, J. P. Design and synthesis of a series of potent and orally bioavailable noncovalent thrombin inhibitors that utilize nonbasic groups in the P1 position. *J. Med. Chem.* **1998**, *41*, 3210−9.
(219) Lyle, T. A.; Chen, Z.; Appleby, S. D.; Freidinger, R. M.; Gardell, S. J.; Lewis, S. D.; Li, Y.; Lyle, E. A.; Lynch, J. J. J.; Mulichak, A. M.; Ng, A. S.; Naylor-Olsen, A. M.; Sanders, W. M. Synthesis, evaluation, and crystallographic analysis of L-371,912: A potent and selective active-site thrombin inhibitor. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 67−72.
(220) Otto, H.-H.; Schirmeister, T. Cysteine proteases and their inhibitors. *Chem. Rev.* **1997**, *97*, 133−71.
(221) Rasnick, D. Small synthetic inhibitors of cysteine proteases. *Perspect. Drug Discov. Des.* **1996**, *6*, 47−63.
(222) Rawlings, N. D.; Barrett, A. J. Families of cysteine peptidases. *Methods Enzymol.* **1994**, *244*, 461−86.
(223) Rotonda, J.; Nicholson, D. W.; Fazil, K. M.; Gallant, M.; Gareau, Y.; Labelle, M.; Peterson, E. P.; Rasper, D. M.; Ruel, R.; Vaillancourt, J. P.; Thornberry, N. A.; Becker, J. W. The three-dimensional structure of apopain/CPP32, a key mediator of apoptosis. *Nat. Struct. Biol.* **1996**, *3*, 619−25.
(224) Drake, F. H.; Dodds, R. A.; James, I. E.; Connor, J. R.; Debouck, C.; Richardson, S.; LeeRykaczewski, E.; Coleman, L.; Rieman, D.; Barthlow, R.; Hastings, G.; Gowen, M. Cathepsin K, but not cathepsins B, L, or S, is abundantly expressed in human osteoclasts. *J. Biol. Chem.* **1996**, *271*, 12511−6.
(225) Votta, B. J.; Levy, M. A.; Badger, A.; Bradbeer, J.; Dodds, R. A.; James, I. E.; Thompson, S.; Bossard, M. J.; Carr, T.; Connor, J. R.; Tomaszek, T. A.; Szewczuk, L.; Drake, F. H.; Veber, D. F.; Gowen, M. Peptide aldehyde inhibitors of cathepsin K inhibit bone resorption both in vitro and in vivo. *J. Bone Miner. Res.* **1997**, *12*, 1396−406.
(226) Yamashita, D. S.; Smith, W. W.; Zhao, B. G.; Janson, C. A.; Tomaszek, T. A.; Bossard, M. J.; Levy, M. A.; Oh, H. J.; Carr, T. J.; Thompson, S. K.; Ijames, C. F.; Carr, S. A.; McQueney, M.; Dalessio, K. J.; Amegadzie, B. Y.; Hanning, C. R.; AbdelMeguid, S.; DesJarlais, R. L.; Gleason, J. G.; Veber, D. F. Structure and design of potent and selective cathepsin K inhibitors. *J. Am. Chem. Soc.* **1997**, *119*, 11351−2.
(227) Thompson, S. K.; Halbert, S. M.; Bossard, M. J.; Tomaszek, T. A.; Levy, M. A.; Zhao, B. G.; Smith, W. W.; AbdelMeguid, S. S.; Janson, C. A.; Dalessio, K. J.; McQueney, M. S.; Amegadzie, B. Y.; Hanning, C. R.; DesJarlais, R. L.; Briand, J.; Sarkar, S. K.; Huddleston, M. J.; Ijames, C. F.; Carr, S. A.; Garnes, K. T.; Shu, A.; Heys, J. R.; Bradbeer, J.; Zembryki, D.; LeeRykaczewski, L.; James, I. E.; Lark, M. W.; Drake, F. H.; Gowen, M.; Gleason, J. G.; Veber, D. F. Design of potent and selective human cathepsin K inhibitors that span the active site. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 14249−54.
(228) DesJarlais, R. L.; Yamashita, D. S.; Oh, H. J.; Uzinskas, I. N.; Erhard, K. F.; Allen, A. C.; Haltiwanger, R. C.; Zhao, B. G.; Smith, W. W.; AbdelMeguid, S. S.; Dalessio, K.; Janson, C. A.; McQueney, M. S.; Tomaszek, T. A.; Levy, M. A.; Veber, D. F. Use of X-ray cocrystal structures and molecular modeling to design potent and selective non-peptide inhibitors of cathepsin K. *J. Am. Chem. Soc.* **1998**, *120*, 9114−5.
(229) Marquis, R. W.; Yamashita, D. S.; Ru, Y.; LoCastro, S. M.; Oh, H. J.; Erhard, K. F.; DesJarlais, R. L.; Head, M. S.; Smith, W. W.; Zhao, B. G.; Janson, C. A.; AbdelMeguid, S. S.; Tomaszek, T. A.; Levy, M. A.; Veber, D. F. Conformationally constrained 1,3-diamino ketones: A series of potent inhibitors of the cysteine protease cathepsin K. *J. Med. Chem.* **1998**, *41*, 3563−7.
(230) Palmer, J. T.; Rasnick, D.; Klaus, J. L.; Bromme, D. Vinyl sulfones as mechanism-based cysteine protease inhibitors. *J. Med. Chem.* **1995**, *38*, 3193−6.
(231) Linnevers, C. J.; McGrath, M. E.; Armstrong, R.; Mistry, F. R.; Barnes, M. G.; Klaus, J. L.; Palmer, J. T.; Katz, B. A.; Bromme, D. Expression of human cathepsin K in Pichia pastoris and preliminary crystallographic studies of an inhibitor complex. *Protein Sci.* **1997**, *6*, 919−21.
(232) Shaw, E.; Wikstrom, P.; Ruscica, J. An exploration of the primary specificity site of cathepsin B. *Arch. Biochem. Biophys.* **1983**, *222*, 424−9.
(233) Krantz, A.; Copp, L. J.; Coles, P. J.; Smith, R. A.; Heard, S. B. Peptidyl (acyloxy)methyl ketones and the quiescent affinity label concept: the departing group as a variable structural element in the design of inactivators of cysteine proteinases. *Biochemistry* **1991**, *30*, 4678−87.
(234) Krantz, A. Peptidyl (acyloxy)methanes as quiescent affinity labels for cysteine proteinases. *Methods Enzymol.* **1994**, *244*, 656−71.
(235) Wagner, B. M.; Smith, R. A.; Coles, P. J.; Copp, L. J.; Ernest, M. J.; Krantz, A. In vivo inhibition of cathepsin B by peptidyl (acyloxy)methyl ketones. *J. Med. Chem.* **1994**, *37*, 1833−40.
(236) Feng, M. H.; Chan, S. L.; Xiang, Y.; Huber, C. P.; Lim, C. The binding mode of an E-64 analogue to the active site of cathepsin B. *Protein Eng.* **1996**, *9*, 977−86.
(237) Navab, R.; Mort, J. S.; Brodt, P. Inhibition of carcinoma cell invasion and liver metastases formation by the cysteine proteinase inhibitor E-64. *Clin. Exp. Metast.* **1997**, *15*, 121−9.

(238) Gour-Salin, B. J.; Lachance, P.; Plouffe, C.; Storer, A. C.; Menard, R. Epoxysuccinyl dipeptides as selective inhibitors of cathepsin B. *J. Med. Chem.* **1993**, *36*, 720−5.
(239) Tsuchiya, K.; Kohda, Y.; Yoshida, M.; Zhao, L.; Ueno, T.; Yamashita, J.; Yoshioka, T.; Kominami, E.; Yamashima, T. Postictal blockade of ischemic hippocampal neuronal death in primates using selective cathepsin inhibitors. *Exp. Neurol.* **1999**, *155*, 187−94.
(240) Yamashima, T.; Kohda, Y.; Tsuchiya, K.; Ueno, T.; Yamashita, J.; Yoshioka, T.; Kominami, E. Inhibition of ischaemic hippocampal neuronal death in primates with cathepsin B inhibitor CA-074: a novel strategy for neuroprotection based on 'calpain-cathepsin hypothesis'. *Eur. J. Neurosci.* **1998**, *10*, 1723−33.
(241) Hill, P. A.; Buttle, D. J.; Jones, S. J.; Boyde, A.; Murata, M.; Reynolds, J. J.; Meikle, M. C. Inhibition of bone resorption by selective inactivators of cysteine proteinases. *J. Cell. Biochem.* **1994**, *56*, 118−30.
(242) Woo, J. T.; Sigeizumi, S.; Yamaguchi, K.; Sugimoto, K.; Kobori, T.; Tsuji, T.; Kondo, K. Peptidyl aldehyde derivatives as potent and selective inhibitors of cathepsin L. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1501−4.
(243) Kirschke, H.; Shaw, E. Rapid interaction of cathepsin L by Z-Phe-PheCHN12 and Z-Phe-AlaCHN2. *Biochem. Biophys. Res. Commun.* **1981**, *101*, 454−8.
(244) Rifkin, B. R.; Vernillo, A. T.; Kleckner, A. P.; Auszmann, J. M.; Rosenberg, L. R.; Zimmerman, M. Cathepsin B and L activities in isolated osteoclasts. *Biochem. Biophys. Res. Commun.* **1991**, *179*, 63−9.
(245) Yasuma, T.; Oi, S.; Choh, N.; Nomura, T.; Furuyama, N.; Nishimura, A.; Fujisawa, Y.; Sohda, T. Synthesis of peptide aldehyde derivatives as selective inhibitors of human cathepsin L and their inhibitory effect on bone resorption. *J. Med. Chem.* **1998**, *41*, 4301−8.
(246) Thornberry, N. A.; Rano, T. A.; Peterson, E. P.; Rasper, D. M.; Timkey, T.; Garcia-Calvo, M.; Houtzager, V. M.; Nordstrom, P. A.; Roy, S.; Vaillancourt, J. P.; Chapman, K. T.; Nicholson, D. W. A combinatorial approach defines specificities of members of the caspase family and granzyme B. Functional relationships established for key mediators of apoptosis. *J. Biol. Chem.* **1997**, *272*, 17907−11.
(247) Talanian, R. V.; Quinlan, C.; Trautz, S.; Hackett, M. C.; Mankovich, J. A.; Banach, D.; Ghayur, T.; Brady, K. D.; Wong, W. W. Substrate specificities of caspase family proteases. *J. Biol. Chem.* **1997**, *272*, 9677−82.
(248) Margolin, N.; Raybuck, S. A.; Wilson, K. P.; Chen, W.; Fox, T.; Gu, Y.; Livingston, D. J. Substrate and inhibitor specificity of interleukin-1 beta-converting enzyme and related caspases. *J. Biol. Chem.* **1997**, *272*, 7223−8.
(249) Thornberry, N. A. Caspases: key mediators of apoptosis. *Chem. Biol.* **1998**, *5*, R97−103.
(250) Alnemri, E. S.; Livingston, D. J.; Nicholson, D. W.; Salvesen, G.; Thornberry, N. A.; Wong, W. W.; Yuan, J. Human ICE/CED-3 protease nomenclature [letter]. *Cell* **1996**, *87*, 171.
(251) Cerretti, D. P.; Kozlosky, C. J.; Mosley, B.; Nelson, N.; Van Ness, K.; Greenstreet, T. A.; March, C. J.; Kronheim, S. R.; Druck, T.; Cannizzaro, L. A.; et al. Molecular cloning of the interleukin-1 beta converting enzyme. *Science* **1992**, *256*, 97−100.
(252) Talanian, R. V.; Allen, H. J. Roles of caspases in inflammation and apoptosis: Prospects as drug discovery targets. *Annu. Rep. Med. Chem.* **1998**, *33*, 273−82.
(253) Thornberry, N. A.; Bull, H. G.; Calaycay, J. R.; Chapman, K. T.; Howard, A. D.; Kostura, M. J.; Miller, D. K.; Molineaux, S. M.; Weidner, J. R.; Aunins, J.; et al. A novel heterodimeric cysteine protease is required for interleukin-1 beta processing in monocytes. *Nature* **1992**, *356*, 768−74.
(254) Chapman, K. T. Synthesis of a potent, reversible inhibitor of interleukin-1β converting enzyme. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 613−8.
(255) Rano, T. A.; Timkey, T.; Peterson, E. P.; Rotonda, J.; Nicholson, D. W.; Becker, J. W.; Chapman, K. T.; Thornberry, N. A. A combinatorial approach for determining protease specificities: application to interleukin-1beta converting enzyme (ICE). *Chem. Biol.* **1997**, *4*, 149−55.
(256) Dolle, R. E.; Singh, J.; Rinker, J.; Hoyer, D.; Prasad, C. V.; Graybill, T. L.; Salvino, J. M.; Helaszek, C. T.; Miller, R. E.; Ator, M. A. Aspartyl alpha-((1-phenyl-3-(trifluoromethyl)-pyrazol-5-yl)oxy)methyl ketones as interleukin-1 beta converting enzyme inhibitors. Significance of the P1 and P3 amido nitrogens for enzyme-peptide inhibitor binding. *J. Med. Chem.* **1994**, *37*, 3863−6.
(257) Ku, G.; Faust, T.; Lauffer, L. L.; Livingston, D. J.; Harding, M. W. Interleukin-1 beta converting enzyme inhibition blocks progression of type II collagen-induced arthritis in mice. *Cytokine* **1996**, *8*, 377−86.
(258) Norman, J.; Yang, J.; Fink, G.; Carter, G.; Ku, G.; Denham, W.; Livingston, D. Severity and mortality of experimental pancreatitis are dependent on interleukin-1 converting enzyme (ICE). *J. Interferon Cytokine Res.* **1997**, *17*, 113−8.
(259) Dolle, R. E.; Prouty, C. P.; Prasad, C. V.; Cook, E.; Saha, A.; Ross, T. M.; Salvino, J. M.; Helaszek, C. T.; Ator, M. A. First examples of peptidomimetic inhibitors of interleukin-1 beta converting enzyme. *J. Med. Chem.* **1996**, *39*, 2438−40.
(260) Golec, J. M.; Mullican, M. D.; Murcko, M. A.; Wilson, K. P.; Kay, D. P.; Jones, S. D.; Murdoch, R.; Bemis, G. W.; Raybuck, S. A.; Luong, Y. P.; Livingston, D. J. Structure-based design of nonpeptidic pyridone aldehydes as inhibitors of interleukin-1β converting enzyme. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2181−6.
(261) Semple, G.; Ashworth, D. M.; Baker, G. R.; Batt, A. R.; Baxter, A. J.; Benzies, D. W.; Elliot, L. H.; Evans, D. M.; Franklin, R. J.; Hudson, P.; Jenkins, P. D.; Pitt, G. R.; Rooker, D. P.; Sheppard, A.; Szelke, M.; Yamamoto, S.; Isomura, Y. Pyridone-based peptidomimetic inhibitors of interleukin-1β-converting enzyme (ICE). *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1337−42.
(262) Semple, G.; Ashworth, D. M.; Batt, A. R.; Baxter, A. J.; Benzies, D. W.; Elliot, L. H.; Evans, D. M.; Franklin, R. J.; Hudson, P.; Jenkins, P. D.; Pitt, G. R.; Rooker, D. P.; Yamamoto, S.; Isomura, Y. Peptidomimetic aminomethylene ketone inhibitors of interleukin-1 beta-converting enzyme (ICE). *Bioorg. Med. Chem. Lett.* **1998**, *8*, 959−64.
(263) Dolle, R. E.; Prasad, C. V.; Prouty, C. P.; Salvino, J. M.; Awad, M. M.; Schmidt, S. J.; Hoyer, D.; Ross, T. M.; Graybill, T. L.; Speier, G. J.; Uhl, J.; Miller, B. E.; Helaszek, C. T.; Ator, M. A. Pyridazinodiazepines as a high-affinity, P2−P3 peptidomimetic class of interleukin-1 beta-converting enzyme inhibitor. *J. Med. Chem.* **1997**, *40*, 1941−6.
(264) Karanewsky, D. S.; Bai, X.; Linton, S. D.; Krebs, J. F.; Wu, J.; Pham, B.; Tomaselli, K. J. Conformationally constrained inhibitors of caspase-1 (interleukin-1 beta converting enzyme) and of the human CED-3 homologue caspase-3 (CPP32, apopain). *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2757−62.
(265) Chinnaiyan, A. M.; Dixit, V. M. The cell-death machine. *Curr. Biol.* **1996**, *6*, 555−62.
(266) Cohen, G. M. Caspases: the executioners of apoptosis. *Biochem. J.* **1997**, *326*, 1−16.
(267) Garcia-Calvo, M.; Peterson, E. P.; Leiting, B.; Ruel, R.; Nicholson, D. W.; Thornberry, N. A. Inhibition of human caspases by peptide-based and macromolecular inhibitors. *J. Biol. Chem.* **1998**, *273*, 32608−13.
(268) Nicholson, D. W.; Ali, A.; Thornberry, N. A.; Vaillancourt, J. P.; Ding, C. K.; Gallant, M.; Gareau, Y.; Griffin, P. R.; Labelle, M.; Lazebnik, Y. A.; et al. Identification and inhibition of the ICE/CED-3 protease necessary for mammalian apoptosis. *Nature* **1995**, *376*, 37−43.
(269) Mittl, P. R.; Di Marco, S.; Krebs, J. F.; Bai, X.; Karanewsky, D. S.; Priestle, J. P.; Tomaselli, K. J.; Grutter, M. G. Structure of recombinant human CPP32 in complex with the tetrapeptide acetyl-Asp-Val-Ala-Asp fluoromethyl ketone. *J. Biol. Chem.* **1997**, *272*, 6539−47.
(270) Couch, R. B. Rhinoviruses. In *Virology*; Fields, B. N., Knipe, D. M., Eds.; Raven Press: New York, 1990; pp 607−629.
(271) Rueckert, P. R. Piconaviridae and their replication. In *Virology*; Fields, B. N., Knipe, D. M., Eds.; Raven Press: New York, 1990; pp 507−548.
(272) Diana, G. D.; Pevear, D. C. *Antipicornavirus drugs: current status*; International Medical Press Ltd.: London, 1997; pp 401−408.
(273) Matthews, D. A.; Smith, W. W.; Ferre, R. A.; Condon, B.; Budahazi, G.; Sisson, W.; Villafranca, J. E.; Janson, C. A.; McElroy, H. E.; Gribskov, C. L.; et al. Structure of human rhinovirus 3C protease reveals a trypsin-like polypeptide fold, RNA-binding site, and means for cleaving precursor polyprotein. *Cell* **1994**, *77*, 761−71.
(274) Matthews, D.; Smith, W. W.; Ferre, R. A.; Condon, B.; Budahazi, G.; Sisson, W.; Villafranca, J. E.; Janson, C.; McElroy, H.; Gribskov, C.; Worland, S. Crystal-Structure Of Human Rhinovirus Type-14 3c-Protease. *J. Cell. Biochem.* **1994** (Suppl. 18D), 170.
(275) Webber, S. E.; Okano, K.; Little, T. L.; Reich, S. H.; Xin, Y.; Fuhrman, S. A.; Matthews, D. A.; Love, R. A.; Hendrickson, T. F.; Patick, A. K.; Meador, J. W.; Ferre, R. A.; Brown, E. L.; Ford, C. E.; Binford, S. L.; Worland, S. T. Tripeptide aldehyde inhibitors of human rhinovirus 3C protease: Design, synthesis, biological evaluation, and cocrystal structure solution of P-1 glutamine isosteric replacements. *J. Med. Chem.* **1998**, *41*, 2786−805.
(276) Webber, S. E.; Tikhe, J.; Worland, S. T.; Fuhrman, S. A.; Hendrickson, T. F.; Matthews, D. A.; Love, R. A.; Patick, A. K.; Meador, J. W.; Ferre, R. A.; Brown, E. L.; DeLisle, D. M.; Ford, C. E.; Binford, S. L. Design, synthesis, and evaluation of nonpeptidic inhibitors of human rhinovirus 3C protease. *J. Med. Chem.* **1996**, *39*, 5072−82.
(277) Molla, A.; Hellen, C. U.; Wimmer, E. Inhibition of proteolytic activity of poliovirus and rhinovirus 2A proteinases by elastase-specific inhibitors. *J. Virol.* **1993**, *67*, 4688−95.

(278) Venkatraman, S.; Kong, J.; Nimkar, S.; Wang, Q. M.; Aube, J.; Hanzlik, R. P. Design, synthesis, and evaluation of azapeptides as substrates and inhibitors for human rhinovirus 3C protease. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 577−80.
(279) Kati, W. M.; Sham, H. L.; McCall, J. O.; Montgomery, D. A.; Wang, G. T.; Rosenbrook, W.; Miesbauer, L.; Buko, A.; Norbeck, D. W. Inhibition of 3C protease from human rhinovirus strain 1B by peptidyl bromomethyl ketonehydrazides. *Arch. Biochem. Biophys.* **1999**, *362*, 363−75.
(280) Kong, J. S.; Venkatraman, S.; Furness, K.; Nimkar, S.; Shepherd, T. A.; Wang, Q. M.; Aube, J.; Hanzlik, R. P. Synthesis and evaluation of peptidyl Michael acceptors that inactivate human rhinovirus 3C protease and inhibit virus replication. *J. Med. Chem.* **1998**, *41*, 2579−87.
(281) Dragovich, P. S.; Zhou, R.; Skalitzky, D. J.; Fuhrman, S. A.; Patick, A. K.; Ford, C. E.; Meador, J. W., 3rd; Worland, S. T. Solid-phase synthesis of irreversible human rhinovirus 3C protease inhibitors. Part 1: Optimization of tripeptides incorporating N-terminal amides. *Bioorg. Med. Chem.* **1999**, *7*, 589−98.
(282) Dragovich, P. S.; Webber, S. E.; Babine, R. E.; Fuhrman, S. A.; Patick, A. K.; Matthews, D. A.; Lee, C. A.; Reich, S. H.; Prins, T. J.; Marakovits, J. T.; Littlefield, E. S.; Zhou, R.; Tikhe, J.; Ford, C. E.; Wallace, M. B.; Meador, J. W.; Ferre, R. A.; Brown, E. L.; Binford, S. L.; Harr, J. E. V.; DeLisle, D. M.; Worland, S. T. Structure-based design, synthesis, and biological evaluation of irreversible human rhinovirus 3C protease inhibitors. 1. Michael acceptor structure−activity studies. *J. Med. Chem.* **1998**, *41*, 2806−18.
(283) Dragovich, P. S.; Webber, S. E.; Babine, R. E.; Fuhrman, S. A.; Patick, A. K.; Matthews, D. A.; Reich, S. H.; Marakovits, J. T.; Prins, T. J.; Zhou, R.; Tikhe, J.; Littlefield, E. S.; Bleckman, T. M.; Wallace, M. B.; Little, T. L.; Ford, C. E.; Meador, J. W.; Ferre, R. A.; Brown, E. L.; Binford, S. L.; DeLisle, D. M.; Worland, S. T. Structure-based design, synthesis, and biological evaluation of irreversible human rhinovirus 3C protease inhibitors. 2. Peptide structure−activity studies. *J. Med. Chem.* **1998**, *41*, 2819−34.
(284) Dragovich, P. S.; Prins, T. J.; Zhou, R.; Fuhrman, S. A.; Patick, A. K.; Matthews, D. A.; Ford, C. E.; Meador, J. W., 3rd; Ferre, R. A.; Worland, S. T. Structure-based design, synthesis, and biological evaluation of irreversible human rhinovirus 3C protease inhibitors. 3. Structure−activity studies of ketomethylene-containing peptidomimetics. *J. Med. Chem.* **1999**, *42*, 1203−12.
(285) Dragovich, P. S.; Prins, T. J.; Zhou, R.; Webber, S. E.; Marakovits, J. T.; Fuhrman, S. A.; Patick, A. K.; Matthews, D. A.; Lee, C. A.; Ford, C. E.; Burke, B. J.; Rejto, P. A.; Hendrickson, T. F.; Tuntland, T.; Brown, E. L.; Meador, J. W., 3rd; Ferre, R. A.; Harr, J. E.; Kosa, M. B.; Worland, S. T. Structure-based design, synthesis, and biological evaluation of irreversible human rhinovirus 3C protease inhibitors. 4. Incorporation of P1 lactam moieties as L-glutamine replacements. *J. Med. Chem.* **1999**, *42*, 1213−24.
(286) Matthews, D. A.; Dragovich, P. S.; Webber, S. E.; Fuhrman, S. A.; Patick, A. K.; Zalman, L. S.; Hendrickson, T. F.; Love, R. A.; Prins, T. J.; Marakovits, J. T.; Zhou, R.; Tikhe, J.; Ford, C. E.; Meador, J. W.; Ferre, R. A.; Brown, E. L.; Binford, S. L.; Brothers, M. A.; DeLisle, D. M.; Worland, S. T. Structure-assisted design of mechanism-based irreversible inhibitors of human rhinovirus 3C protease with potent antiviral activity against multiple rhinovirus serotypes. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 11000−7.
(287) Patick, A. K.; Binford, S. L.; Brothers, M. A.; Jackson, R. L.; Ford, C. E.; Diem, M. D.; Maldonado, F.; Dragovich, P. S.; Zhou, R.; Prins, T. J.; Fuhrman, S. A.; Meador, J. W.; Zalman, L. S.; Matthews, D. A.; Worland, S. T. In vitro antiviral activity of AG7088, a potent inhibitor of human rhinovirus 3C protease [in process citation]. *Antimicrob. Agents Chemother.* **1999**, *43*, 2444−50.
(288) Johnson, P. Calpains (intracellular calcium-activated cysteine proteinases): structure−activity relationships and involvement in normal and abnormal cellular metabolism. *Int. J. Biochem.* **1990**, *22*, 811−22.
(289) Saido, T. C.; Sorimachi, H.; Suzuki, K. Calpain: new perspectives in molecular diversity and physiological-pathological involvement. *FASEB J.* **1994**, *8*, 814−22.
(290) Wang, K. K. Developing selective inhibitors of calpain. *Trends Pharmacol. Sci.* **1990**, *11*, 139−42.
(291) Wang, K. K.; Yuen, P. W. Calpain inhibition: an overview of its therapeutic potential. *Trends Pharmacol. Sci.* **1994**, *15*, 412−9.
(292) Iqbal, M.; Messina, P. A.; Freed, B.; Das, M.; Chatterjee, S.; Tripathy, R.; Tao, M.; Josef, K. A.; Dembofsky, B.; Dunn, D.; Griffith, E.; Siman, R.; Senadhi, S.; Biazzo, W.; Bozyczko-Coyne, D.; Meyer, S. L.; Ator, M. A.; Bihovsky, R. Subsite requirements for peptide aldehyde inhibitors of human calpain I. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 539−44.
(293) Mehdi, S.; Angelastro, M. R.; Wiseman, J. S.; Bey, P. Inhibition of the proteolysis of rat erythrocyte membrane proteins by a synthetic inhibitor of calpain [published erratum appears in *Biochem. Biophys. Res. Commun.* **1989**, *159*, 371]. *Biochem. Biophys. Res. Commun.* **1988**, *157*, 1117−23.
(294) Ariyoshi, H.; Shiba, E.; Kambayashi, J.; Sakon, M.; Tsujinaka, T.; Uemura, Y.; Mori, T. Characteristics of various synthetic peptide calpain inhibitors and their application for the analysis of platelet reaction. *Biochem. Int.* **1991**, *23*, 1019−33.
(295) Tripathy, R.; Gu, Z. Q.; Dunn, D.; Senadhi, S. E.; Ator, M. A.; Chatterjee, S. P2-proline-derived inhibitors of calpain I. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2647−52.
(296) Angliker, H.; Anagli, J.; Shaw, E. Inactivation of calpain by peptidyl fluoromethyl ketones. *J. Med. Chem.* **1992**, *35*, 216−20.
(297) Chatterjee, S.; Ator, M. A.; Bozyczko-Coyne, D.; Josef, K.; Wells, G.; Tripathy, R.; Iqbal, M.; Bihovsky, R.; Senadhi, S. E.; Mallya, S.; O'Kane, T. M.; McKenna, B. A.; Siman, R.; Mallamo, J. P. Synthesis and biological activity of a series of potent fluoromethyl ketone inhibitors of recombinant human calpain I. *J. Med. Chem.* **1997**, *40*, 3820−8.
(298) Chatterjee, S.; Josef, K.; Wells, G.; Iqbal, M.; Bihovsky, R.; Mallamo, J. P.; Ator, M. A.; Bozyczko-Coyne, D.; Mallya, S.; Senadhi, S.; Siman, R. Potent fluoromethyl ketone inhibitors of recombinant human calpain I. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1237−40.
(299) Bartus, R. T.; Hayward, N. J.; Elliott, P. J.; Sawyer, S. D.; Baker, K. L.; Dean, R. L.; Akiyama, A.; Straub, J. A.; Harbeson, S. L.; Li, Z.; et al. Calpain inhibitor AK295 protects neurons from focal brain ischemia. Effects of postocclusion intra-arterial administration. *Stroke* **1994**, *25*, 2265−70.
(300) Li, Z.; Ortega-Vilain, A. C.; Patil, G. S.; Chu, D. L.; Foreman, J. E.; Eveleth, D. D.; Powers, J. C. Novel peptidyl alpha-keto amide inhibitors of calpains and other cysteine proteases. *J. Med. Chem.* **1996**, *39*, 4089−98.
(301) Adang-Aep; Hermkens, P. H. H.; Linders, J. T. M.; Ottenheijm, H. C. J.; Vanstaveren, C. J. Case-histories of peptidomimetics − Progression from peptides to drugs. *Recl. Trav. Chim. Pays Bas J. R. Netherlands Chem. Soc.* **1994**, *113*, 63−78.
(302) Gante, J. Peptidomimetics − Tailored Enzyme−Inhibitors. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1699−720.
(303) Brogden, R. N.; Wiseman, L. R. Moexipril. A review of its use in the management of essential hypertension. *Drugs* **1998**, *55*, 845−60.
(304) Peters, D. C.; Noble, S.; Plosker, G. L. Trandolapril. An update of its pharmacology and therapeutic use in cardiovascular disorders. *Drugs* **1998**, *56*, 871−93.
(305) Giusti, A.; Bertolotti, M.; Llabres, S.; Carre, A.; Casini, A. Comparison of the efficacy of zofenopril or enalapril in patients with mild to moderate hypertension. *Am. J. Hypertens.* **1999**, *12*, 26A−27A.
(306) White, M. C. Pharmacologic, pharmacokinetic, and therapeutic differences among ACE inhibitors. *Pharmacotherapy* **1998**, *18*, 588−99.
(307) Pruitt, J. R.; Olson, R. E. Angiotensin II receptor antagonists: Design, SAR and development of Losartan. In *Medicinal Chemistry of the Renin-Angiotensin System*; Timmermans, P. B. M. W. M., Wexler, R. R., Eds.; Elsevier Science: New York, 1994; pp 121−155.
(308) Gillis, J. C.; Markham, A. Irbesartan. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic use in the management of hypertension. *Drugs* **1997**, *54*, 885−902.
(309) McClellan, K. J.; Goa, K. L. Candesartan cilexetil. A review of its use in essential hypertension. *Drugs* **1998**, *56*, 847−69.
(310) Vachharajani, N. N.; Shyu, W. C.; Chando, T. J.; Everett, D. W.; Greene, D. S.; Barbhaiya, R. H. Oral bioavailability and disposition characteristics of irbesartan, an angiotensin antagonist, in healthy volunteers. *J. Clin. Pharmacol.* **1998**, *38*, 702−7.
(311) Pylypchuk, G. B. ACE inhibitor- versus angiotensin II blocker-induced cough and angioedema. *Ann. Pharmacother.* **1998**, *32*, 1060−6.
(312) Roques, B. P.; Noble, F.; Dauge, V.; Fournie-Zaluski, M. C.; Beaumont, A. Neutral endopeptidase 24.11: structure, inhibition, and experimental and clinical pharmacology. *Pharmacol. Rev.* **1993**, *45*, 87−146.
(313) Schwartz, J. C.; Gros, C.; Lecomte, J. M.; Bralet, J. Enkephalinase (EC 3.4.24.11) inhibitors: protection of endogenous ANF against inactivation and potential therapeutic applications. *Life Sci.* **1990**, *47*, 1279−97.
(314) Richards, A. M.; Wittert, G. A.; Crozier, I. G.; Espiner, E. A.; Yandle, T. G.; Ikram, H.; Frampton, C. Chronic inhibition of endopeptidase 24.11 in essential hypertension: evidence for enhanced atrial natriuretic peptide and angiotensin II. *J. Hypertens.* **1993**, *11*, 407−16.

(315) Flynn, G. A.; French, G. F.; Dage, R. C. Dual inhibitors of angiotensin converting enzyme and neutral endopeptidase: Design and therapeutic rationale. In *Hypertension: Pathology, Diagnosis, and Management*; Laragh, J. H., Brenner, B. M., Eds.; Raven Press: New York, 1995; pp 3099−114.
(316) French, J. F.; Anderson, B. A.; Downs, T. R.; Dage, R. C. Dual inhibition of angiotensin-converting enzyme and neutral endopeptidase in rats with hypertension. *J. Cardiovasc. Pharmacol.* **1995**, *26*, 107−13.
(317) Gros, C.; Noel, N.; Souque, A.; Schwartz, J. C.; Danvy, D.; Plaquevent, J. C.; Duhamel, L.; Duhamel, P.; Lecomte, J. M.; Bralet, J. Mixed inhibitors of angiotensin-converting enzyme (EC 3.4.15.1) and enkephalinase (EC 3.4.24.11): rational design, properties, and potential cardiovascular applications of glycopril and alatriopril. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 4210−4.
(318) Marie, C.; Mossiat, C.; Lecomte, J. M.; Bralet, J. Acute natriuretic effect of fasidotrilat, a mixed inhibitor of neutral endopeptidase and angiotensin I-converting enzyme, in rats with heart failure. *Pharmacology* **1998**, *56*, 291−6.
(319) Wallis, E. J.; Ramsay, L. E.; Hettiarachchi, J. Combined inhibition of neutral endopeptidase and angiotensin-converting enzyme by sampatrilat in essential hypertension. *Clin. Pharmacol. Ther.* **1998**, *64*, 439−49.
(320) Robl, J. A.; Sun, C. Q.; Stevenson, J.; Ryono, D. E.; Simpkins, L. M.; Cimarusti, M. P.; Dejneka, T.; Slusarchyk, W. A.; Chao, S.; Stratton, L.; Misra, R. N.; Bednarz, M. S.; Asaad, M. M.; Cheung, H. S.; Abboa-Offei, B. E.; Smith, P. L.; Mathers, P. D.; Fox, M.; Schaeffer, T. R.; Seymour, A. A.; Trippodo, N. C. Dual metalloprotease inhibitors: mercaptoacetyl-based fused heterocyclic dipeptide mimetics as inhibitors of angiotensin-converting enzyme and neutral endopeptidase. *J. Med. Chem.* **1997**, *40*, 1570−7.
(321) Trippodo, N. C.; Robl, J. A.; Asaad, M. M.; Fox, M.; Panchal, B. C.; Schaeffer, T. R. Effects of omapatrilat in low, normal, and high renin experimental hypertension. *Am. J. Hypertens.* **1998**, *11*, 363−72.
(322) Burnett, J. C., Jr. Vasopeptidase inhibition: a new concept in blood pressure management. *J. Hypertens. Suppl.* **1999**, *17*, S37−43.
(323) Robl, J. A.; Sulsky, R.; Sieber-McMaster, E.; Ryono, D. E.; Cimarusti, M. P.; Simpkins, L. M.; Karanewsky, D. S.; Chao, S.; Asaad, M. M.; Seymour, A. A.; Fox, M.; Smith, P. L.; Trippodo, N. C. Vasopeptidase inhibitors: incorporation of geminal and spirocyclic substituted azepinones in mercaptoacyl dipeptides. *J. Med. Chem.* **1999**, *42*, 305−11.
(324) Arner, E. C.; Pratta, M. A.; Trzaskos, J. M.; Decicco, C. P.; Tortorella, M. D. Generation and characterization of aggrecanase. A soluble, cartilage-derived aggrecan-degrading activity. *J. Biol. Chem.* **1999**, *274*, 6594−601.
(325) Arner, E. C.; Pratta, M. A.; Decicco, C. P.; Xue, C. B.; Newton, R. C.; Trzaskos, J. M.; Magolda, R. L.; Tortorella, M. D. Aggrecanase. A target for the design of inhibitors of cartilage degradation [in process citation]. *Ann. N. Y. Acad. Sci.* **1999**, *878*, 92−107.
(326) Bode, W.; Fernandez-Catalan, C.; Tschesche, H.; Grams, F.; Nagase, H.; Maskos, K. Structural properties of matrix metalloproteinases. *Cell. Mol. Life Sci.* **1999**, *55*, 639−52.
(327) Whittaker, M.; Brown, P. Recent advances in matrix metalloproteinase inhibitor research and development. *Curr. Opin. Drug Discov. Dev.* **1998**, *1*, 157−64.
(328) Summers, J. B.; Davidsen, S. K. Matrix metalloproteinase inhibitors and cancer. In *Annual Reports in Medicinal Chemistry*; Bristol, J. A., Ed.; Academic Press: Michigan, 1998; Vol. 33, pp 131−40.
(329) Becker, J. W.; Marcy, A. I.; Rokosz, L. L.; Axel, M. G.; Burbaum, J. J.; Fitzgerald, P. M.; Cameron, P. M.; Esser, C. K.; Hagmann, W. K.; Hermes, J. D.; et al. Stromelysin-1: three-dimensional structure of the inhibited catalytic domain and of the C-truncated proenzyme. *Protein Sci.* **1995**, *4*, 1966−76.
(330) Nelson, N. J. Inhibitors of angiogenesis enter phase III testing [news]. *J. Natl. Cancer Inst.* **1998**, *90*, 960−3.
(331) MacPherson, L. J.; Bayburt, E. K.; Capparelli, M. P.; Carroll, B. J.; Goldstein, R.; Justice, M. R.; Zhu, L.; Hu, S.; Melton, R. A.; Fryer, L.; Goldberg, R. L.; Doughty, J. R.; Spirito, S.; Blancuzzi, V.; Wilson, D.; O'Byrne, E. M.; Ganu, V.; Parker, D. T. Discovery of CGS 27023A, a nonpeptidic, potent, and orally active stromelysin inhibitor that blocks cartilage degradation in rabbits. *J. Med. Chem.* **1997**, *40*, 2525−32.
(332) Santos, O.; McDermott, C. D.; Daniels, R. G.; Appelt, K. Rodent pharmacokinetic and antitumor efficacy studies with a series of synthetic inhibitors of matrix metalloproteinases. *Clin. Exp. Metast.* **1997**, *15*, 499−508.
(333) Brewster, M.; Lewis, E. J.; Wilson, K. L.; Greenham, A. K.; Bottomley, K. M. Ro 32-3555, an orally active collagenase selective inhibitor, prevents structural damage in the STR/ORT mouse model of osteoarthritis. *Arthritis Rheum.* **1998**, *41*, 1639−44.
(334) Lewis, E. J.; Bishop, J.; Bottomley, K. M.; Bradshaw, D.; Brewster, M.; Broadhurst, M. J.; Brown, P. A.; Budd, J. M.; Elliott, L.; Greenham, A. K.; Johnson, W. H.; Nixon, J. S.; Rose, F.; Sutton, B.; Wilson, K. Ro 32-3555, an orally active collagenase inhibitor, prevents cartilage breakdown in vitro and in vivo. *Br. J. Pharmacol.* **1997**, *121*, 540−6.
(335) Pikul, S.; McDow Dunham, K. L.; Almstead, N. G.; De, B.; Natchus, M. G.; Anastasio, M. V.; McPhail, S. J.; Snider, C. E.; Taiwo, Y. O.; Rydel, T.; Dunaway, C. M.; Gu, F.; Mieling, G. E. Discovery of potent, achiral matrix metalloproteinase inhibitors. *J. Med. Chem.* **1998**, *41*, 3568−71.
(336) Cherney, R. J.; Wang, L.; Meyer, D. T.; Xue, C. B.; Arner, E. C.; Copeland, R. A.; Covington, M. B.; Hardman, K. D.; Wasserman, Z. R.; Jaffee, B. D.; Decicco, C. P. Macrocyclic hydroxamate inhibitors of matrix metalloproteinases and TNF-alpha production [in process citation]. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1279−84.
(337) Reiter, L. A.; Rizzi, J. P.; Pandit, J.; Lasut, M. J.; McGahee, S. M.; Parikh, V. D.; Blake, J. F.; Danley, D. E.; Laird, E. R.; Lopez-Anaya, A.; Lopresti-Morrow, L. L.; Mansour, M. N.; Martinelli, G. J.; Mitchell, P. G.; Owens, B. S.; Pauly, T. A.; Reeves, L. M.; Schulte, G. K.; Yocum, S. A. Inhibition of MMP-1 and MMP-13 with phosphinic acids that exploit binding in the S2 pocket. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 127−32.
(338) Jacobsen, E. J.; Mitchell, M. A.; Hendges, S. K.; Belonga, K. L.; Skaletzky, L. L.; Stelzer, L. S.; Lindberg, T. J.; Fritzen, E. L.; Schostarez, H. J.; O'Sullivan, T. J.; Maggiora, L. L.; Stuchly, C. W.; Laborde, A. L.; Kubicek, M. F.; Poorman, R. A.; Beck, J. M.; Miller, H. R.; Petzold, G. L.; Scott, P. S.; Truesdell, S. E.; Wallace, T. L.; Wilks, J. W.; Fisher, C.; Goodman, L. V.; Kaytes, P. S.; et al. Synthesis of a series of stromelysin-selective thiadiazole urea matrix metalloproteinase inhibitors. *J. Med. Chem.* **1999**, *42*, 1525−36.
(339) Burnouf, C.; Pruniaux, M. P.; Szilagyi, C. M. Phosphodiesterase 4 inhibitors. In *Annual Reports in Medicinal Chemistry*; Bristol, J. A., Ed.; Academic Press: Michigan, 1998; Vol. 33, pp 91−109.
(340) Groneberg, R. D.; Burns, C. J.; Morrissette, M. M.; Ullrich, J. W.; Morris, R. L.; Darnbrough, S.; Djuric, S. W.; Condon, S. M.; McGeehan, G. M.; Labaudiniere, R.; Neuenschwander, K.; Scotese, A. C.; Kline, J. A. Dual inhibition of phosphodiesterase 4 and matrix metalloproteinases by an (arylsulfonyl)hydroxamic acid template. *J. Med. Chem.* **1999**, *42*, 541−4.
(341) Black, R. A.; Rauch, C. T.; Kozlosky, C. J.; Peschon, J. J.; Slack, J. L.; Wolfson, M. F.; Castner, B. J.; Stocking, K. L.; Reddy, P.; Srinivasan, S.; Nelson, N.; Boiani, N.; Schooley, K. A.; Gerhart, M.; Davis, R.; Fitzner, J. N.; Johnson, R. S.; Paxton, R. J.; March, C. J.; Cerretti, D. P. A metalloproteinase disintegrin that releases tumour-necrosis factor-alpha from cells. *Nature* **1997**, *385*, 729−33.
(342) Vassalli, P. The pathophysiology of tumor necrosis factors. *Annu. Rev. Immunol.* **1992**, *10*, 411−52.
(343) Firestein, G. S. Cytokine networks in rheumatoid arthritis: implications for therapy. *Agents Actions Suppl.* **1995**, *47*, 37−51.
(344) Maini, R. N.; Elliott, M. J.; Brennan, F. M.; Feldmann, M. Beneficial effects of tumour necrosis factor-alpha (TNF-alpha) blockade in rheumatoid arthritis (RA) [published erratum appears in *Clin. Exp. Immunol.* **1995,** *102*, 443]. *Clin. Exp. Immunol.* **1995**, *101*, 207−12.
(345) Elliott, M. J.; Maini, R. N.; Feldmann, M.; Long-Fox, A.; Charles, P.; Bijl, H.; Woody, J. N. Repeated therapy with monoclonal antibody to tumour necrosis factor alpha (cA2) in patients with rheumatoid arthritis. *Lancet* **1994**, *344*, 1125−7.
(346) Newton, R. C.; Decicco, C. P. Therapeutic potential and strategies for inhibiting tumor necrosis factor-alpha. *J. Med. Chem.* **1999**, *42*, 2295−314.
(347) Maskos, K.; Fernandez-Catalan, C.; Huber, R.; Bourenkov, G. P.; Bartunik, H.; Ellestad, G. A.; Reddy, P.; Wolfson, M. F.; Rauch, C. T.; Castner, B. J.; Davis, R.; Clarke, H. R.; Petersen, M.; Fitzner, J. N.; Cerretti, D. P.; March, C. J.; Paxton, R. J.; Black, R. A.; Bode, W. Crystal structure of the catalytic domain of human tumor necrosis factor-alpha-converting enzyme. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 3408−12.
(348) Cerretti, D. P. Characterization of the tumour necrosis factor alpha-converting enzyme, TACE/ADAM17. *Biochem. Soc. Trans.* **1999**, *27*, 219−23.
(349) Mohler, K. M.; Sleath, P. R.; Fitzner, J. N.; Cerretti, D. P.; Alderson, M.; Kerwar, S. S.; Torrance, D. S.; Otten-Evans, C.; Greenstreet, T.; Weerawarna, K.; et al. Protection against a lethal dose of endotoxin by an inhibitor of tumour necrosis factor processing. *Nature* **1994**, *370*, 218−20.
(350) McGeehan, G. M.; Becherer, J. D.; Bast, R. C., Jr.; Boyer, C. M.; Champion, B.; Connolly, K. M.; Conway, J. G.; Furdon, P.; Karp, S.; Kidao, S.; et al. Regulation of tumour necrosis factor-alpha processing by a metalloproteinase inhibitor. *Nature* **1994**, *370*, 558−61.

(351) Smithkline Beecham. Hydroxamic acid based inhibitors of the formation of CD23 and TNF. WO-09743250, 1997.
(352) Yamazaki, T.; Hinck, A. P.; Wang, Y. X.; Nicholson, L. K.; Torchia, D. A.; Wingfield, P.; Stahl, S. J.; Kaufman, J. D.; Chang, C. H.; Domaille, P. J.; Lam, P. Y. Three-dimensional solution structure of the HIV-1 protease complexed with DMP323, a novel cyclic urea-type inhibitor, determined by nuclear magnetic resonance spectroscopy. *Protein Sci.* **1996**, *5*, 495−506.
(353) Lam, P. Y.; Jadhav, P. K.; Eyermann, C. J.; Hodge, C. N.; Ru, Y.; Bacheler, L. T.; Meek, J. L.; Otto, M. J.; Rayner, M. M.; Wong, Y. N.; et al. Rational design of potent, bioavailable, nonpeptide cyclic ureas as HIV protease inhibitors. *Science* **1994**, *263*, 380−4.
(354) Erickson, J. W. The not-so-great escape. *Nature Struct. Biol.* **1995**, *2*, 523−9.

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