A NEW CLASS OF CARBAMOYLATING AGENTS BASED ON THE CYCLOSULFAMIDE SCAFFOLD

A Thesis by

Qingliang Yang

M. S., Sichuan University, 2001

B. S., Sichuan University, 1998

Submitted to the Department of Chemistry
and the faculty of the Graduate School of
Wichita State University
in partial fulfillment of
the requirements for the degree of
Master of Science

December 2006

A NEW CLASS OF CARBAMOYLATING AGENTS BASED ON THE CYCLOSULFAMIDE SCAFFOLD

I have examined the final copy of this thesis for form and content, and recommended that it be accepted in partial fulfillment of the requirement for the degree of Master of Science with a major in Chemistry.

_____
William C. Groutas, Committee Chair

We have read this thesis
and recommend its acceptance:

_____
William T. Stevenson, Committee Member

_____
Erach R. Talaty, Committee Member

_____
Kandatege Wimalasena, Committee Member

_____
Lop-Hing Ho, Committee Member

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Groutas for his kindness, guidance and support throughout my studies and research at Wichita State University, and for the opportunity given to me to broaden my knowledge and interest in organic chemistry and medicinal chemistry. I am very grateful for such a wonderful teacher in my life.

I would also like to thank Dr. Ho, Dr. Stevenson, Dr. Talaty, and Dr. Wimalasena for serving on my thesis committee.

Many thanks must be extended to Tzutshin Wong, Zhong Lai, Bingfan Du, and Dengfeng Dou for their help and valuable discussion about synthesis, and to Dr. Kevin R. Alliston, Xiangdong Gan, Swathi Mohan, and Yi Li for doing the biochemical studies.

Finally I want to thank my family which has provided the support that makes everything possible and worthwhile.

ABSTRACT

The neutrophil-derived serine proteases human leukocyte elastase (HLE) and proteinase 3 (PR3) have been implicated in various inflammatory diseases such as pulmonary emphysema, chronic bronchitis, asthma, cystic fibrosis, rheumatoid arthritis, psoriasis, and others. Poor regulation of the activity of these enzymes by their endogenous protein inhibitors is believed to result in the degradation of the major components of extracelluar matrix. Agents that function as potent and selective inhibitors of these enzymes are of potential therapeutic value. The research described herein was an attempt to design and synthesize a class of non-covalent inhibitors of HLE and PR3 based on the cyclosulfamide scaffold. However during the course of these studies a new class of covalent inhibitors of HLE was discovered.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Boc	*tert*-Butyloxycarbonyl
COPD	Chronic Obstructive Pulmonary Disease
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
HATU	*O*-(7-Azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate
HCV	*Hepatitis C virus*
HLE	Human Leukocyte Elastase
MF	Molecular Formula
MW	Molecular Weight
α_1-PI	α_1-Protease Inhibitor
PR3	Proteinase 3
SLPI	Secretory Leukoprotease Inhibitor
TEA	Triethylamine
TFA	Trifluoroacetic Acid
THF	Tetrahydrofuran

CHAPTER 1

INTRODUCTION

1.1 Overview of Proteases

Proteases are enzymes which selectively catalyze the hydrolysis of amide bonds in peptides and proteins. Proteases are involved in numerous important physiological processes including protein turnover, digestion, blood coagulation, wound healing, fertilization, cell differentiation and growth, cell signaling, and apoptosis. Uncontrolled proteolysis can lead to many diseases including emphysema, stroke, viral infections, cancer, Alzheimer's disease, inflammation, and arthritis. [1]

1.1.1 Classification of Proteases

Based on their catalytic mechanisms, proteases are classified as serine, cysteine, aspartic and metallo proteases. All the enzymes in each class have the same mechanism of action, but differ in their substrate specificity. For the serine and cysteine proteases, either the hydroxyl group of a serine residue or the thiolate ion of a cysteine residue serves as a nucleophile that attacks the carbonyl carbon of a peptide bond. Metalloproteases and aspartic proteases use a water molecule as a nucleophile.

1.1.2 Berger and Schechter Nomenclature[2]

The Berger and Schechter nomenclature has become generally accepted and used for the interaction of a substrate with a protease (Figure 1.1). This system is based on a schematic interaction of amino acid residues of the substrate with specific binding subsites located at the active site of a protease. By convention, the subsites on the protease are designated as S (for subsites) and the amino acid residues of substrate are designated as P (for peptide). The numbering of each residue is given from the scissile bond. P_1, P_2, P_3…P_n refer to amino acid

residues of the substrate (or inhibitor) in the amino terminus direction from the scissile bond and P_1', P_2', P_3'…P_n' refer to amino acid residues in the carboxyl terminus direction from the scissile bond. The corresponding subsites of the enzyme are referred to S_1, S_2, S_3…S_n and S_1', S_2', S_3'…S_n' respectively. P_1 is the primary specificity amino acid residue and S_1 is the primary specificity subsite.

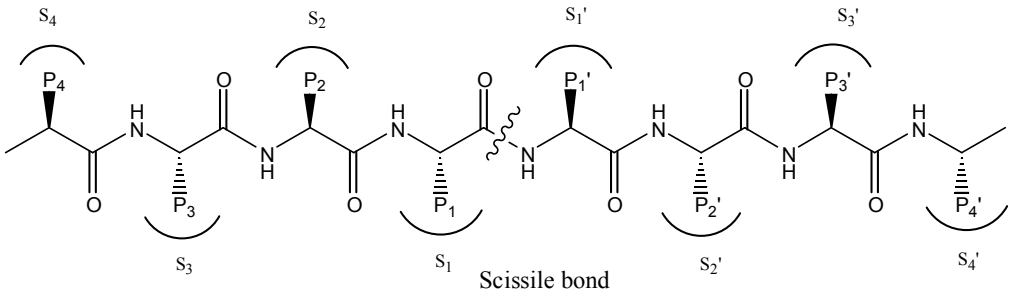


Figure 1.1 Berger and Schechter nomenclature for proteases.

1.2 Serine Proteases

Serine proteases have been one of the most extensively studied family of enzymes. Serine proteases are classified by their substrate specificity, specifically by the type of residue found at P_1, as either trypsin-like (positively charged residues Lys/Arg preferred at P_1), elastase-like (small hydrophobic residues Ala/Val at P_1), or chymotrypsin-like (large hydrophobic residues Phe/Tyr/Leu at P_1). [3]

1.2.1 Catalytic Mechanism of Serine Proteases

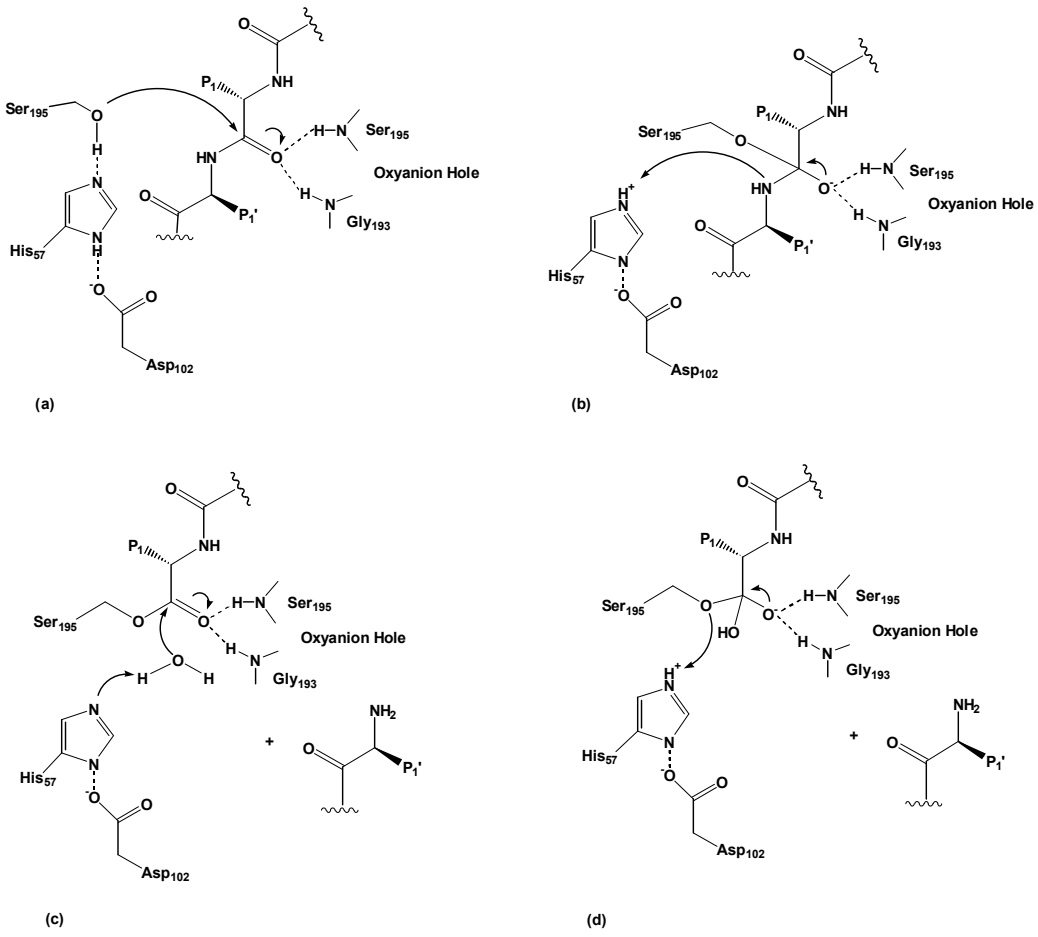


Figure 1.2 General catalytic mechanism of serine proteases.

The proposed catalytic mechanism of serine proteases is shown in Figure 1.2. The active site of these enzymes consists of a catalytic triad of Ser_{195}, His_{57}, and Asp_{102} residues and an oxyanion hole. After the substrate binds to the active site, the hydroxyl group of Ser_{195}, under general base catalysis by the imidazole side chain of His_{57}, attacks the carbonyl carbon of the scissile amide bond (Figure 1.2a). The resulting tetrahedral intermediate is stabilized by hydrogen bonding to the backbone NH of Ser_{195} and Gly_{193}, which form the oxyanion hole. Proton transfers from His_{57} to the amine of the tetrahedral intermediate, which facilitates the

intermediate to collapse and releases the amino fragment as a leaving group (Figure 1.2b). Then the resulting acyl-enzyme complex is attacked by water, assisted by base catalysis by His_{57}, and forming a new tetrahedral intermediate (Figure 1.2c). The breakdown of this intermediate releases the carboxyl fragment of the substrate and regenerates Ser_{195} (Figure 1.2d).

Serine proteases for which protease-inhibitor X-ray crystal structural data is available in the PDB database[4] include trypsin, α-chymotrypsin, human neutrophil elastase, α-lytic protease, thrombin, subtilisin, human cytomegalovirus proteinase A, human cathepsin G, glutamic acid-specific protease, blood coagulation factor VIIa, HCV protease, and many others.[1]

1.2.2 Substrate Specificity of HLE and PR3

Our major targets, HLE and PR3, both belong to the chymotrypsin superfamily of serine proteases and play an important role in a range of biological processes. As is typical of the chymotrypsin family of serine proteases, these two enzymes consist of two domains of β-barrel structures and display a high degree of sequence similarity: the amino acid sequence of PR3 has a 54% identity to that of HLE. [5]

1.2.2.1 Human Leukocyte Elastase (HLE)

HLE (EC 3.4.21.37) consists of 218 amino acids with a large number of arginine residues on its outer surface. HLE is a strongly basic glycoprotein. HLE has two asparagine N-linked carbohydrate side chains and four intramolecular disulfide bonds linking eight half-cysteine residues.[6] The difference in carbohydrate content results in four isoforms of HLE with molecular weight for 24 to 30kDa. The catalytic properties of these isoforms are identical.

The S_1 pocket of HLE is hemispheric in shape and hydrophobic in nature. The pocket entrance lies between the flat sides of the peptide backbone of residues 214-216, Phe_{228} and the disulfide bridge Cys_{191}-Cys_{220}. Studies of interactions between HLE and substrate or inhibitors

indicate that the S_1 subsite usually does not accommodate large or polar amino acid residues. HLE prefers a valine residue at its S_1 subsite, and other small hydrophobic residues, such as leucine, norvaline and isoleucine. Proline has been shown to be the optimal P_2 residue. The S_1' subsites of HLE are hydrophobic in nature. [7]

1.2.2.2 Proteinase 3 (PR3)

PR3 (EC 3.4.21.76) has a molecular weight of about 26.8 kDa with 224 amino acid residues per molecule. The composition of PR3 is very similar to that of HLE. The high sequence similarity between HLE and PR3 results in close similarity between the active sites of the two enzymes. However, there are also significant differences between their binding subsites: according to the X-ray crystal structure of PR3[8] and studies of binding interactions of PR3 with synthetic substrates, [9] the S_4 to S_3' subsites in PR3 are more polar than the corresponding sites in HLE.

The S_1 pocket of PR3 is smaller than that of HLE, due to differences of several residues in the vicinity of the S_1 pocket. Val_{190} and Ala_{213} located at the bottom of the S_1 pocket of HLE are replaced by Ile and Asp in PR3. PR3 prefers small aliphatic residues, such as Ala, Val, Nva and Abu. Amino acids with two or three-carbon straight alkyl side chains are found to give the best monomeric substrates, [9] while HLE can tolerate larger side chains such as Ile, Leu and Nle.[10] This primary specificity difference is further supported by the fact that the physiologic inhibitors of HLE like SLPI (secretory leukoprotease inhibitor) with Leu as P_1 residue show no inhibition toward PR3. Elafin, on the other hand, with Ala as P_1 residue is a good inhibitor of PR3.

Substrate kinetics and inhibition studies indicate that the introduction of proline at P_2 position has a significant effect on binding affinity between PR3 and its substrates or inhibitors (same effect as with other serine proteases).[11] It orients substrate binding to the enzyme in a

productive mode. Close examination of PR3 shows that S_2 is a deep and polar pocket in comparison with that of HLE, mainly due to the substitution of residue Leu_{99} in S_2 pocket by Lys_{99}.

Asp_{61} makes both the S_1' and S_3' pockets smaller and more polar. The S_2' pocket of PR3 is more polar than that of HLE due to the difference of several residues in the S_2' pockets: the replacement of Leu_{143} and Ile_{151} by Arg_{143} and Pro_{151}. [8]

A summary of the specificity of HLE and PR3 discussed above is given in Table1.1.

Table 1.1 Subsite specificity of HLE and PR3

Substrate or inhibitor	HLE [a]	PR3[a]
P_2'	hydrophobic (Leu-143, Ile-151)	polar group (Arg-143, Pro-151)
P_1'	hydrophobic (His-57, SS42-58, Phe-41)	small, polar group (His-57, Phe-41)
P_1	medium size hydrophobic: Leu, Val (Val-190, Phe-192, Ala-213, Phe-228, SS192-220)	small hydrophobic: Ala, Val, Nva, Abu (Ile-190, Phe-192, Asp-213, Phe-228)
P_2	Pro (Leu-99, Phe-215, His-57)	Pro or polar group (Lys-99, Phe-215, His-57)
P_3	Ala (Val-216, Phe-192)	Ala (solvent exposed)

[a]Amino acid residues in parentheses are the residues involved in substrate recognition.

1.3 Role in Human Disease

Both HLE and PR3 are neutrophil-derived serine proteases which play a major role in the extracellular proteolytic activity associated with inflammatory states. They are involved in assisting the neutrophil in its migration to the site of inflammation, as well as in tissue remodeling and wound healing.

Under normal conditions, neutrophils are the first line of host defense against bacterial and fungal infections.[12] These cells are first produced in the bone marrow, and then enter the

bloodstream, where they form a circulating and migrating pool. When appropriately stimulated, these cells migrate from blood to tissue, where they ingest invading pathogens through a process called phagocytosis (eating by cells). These phagocyctic cells function via the combined action of reactive oxygen species generated by the respiratory burst oxidase, and the intracellular release of antibacterial proteins and proteolytic enzymes.

These proteolytic enzymes are stored in the azurophilic and specific granules of neutrophils, and include elastase, cathepsins D and G, proteinase 3, plasminogen activator and collagenase.

Many inflammatory diseases such as pulmonary emphysema, bronchitis, rheumatoid arthritis, psoriasis, and others are associated with a massive influx of neutrophils.[13] During inflammation, a large number of neutrophils migrate to inflammatory sites and subsequently release oxidative species and hydrolytic enzymes into both the intracellular and extracellular medium, which results in cell leakage or cell death.[14] Human elastase, cathepsin G and proteinase 3 represent examples of hydrolytic enzymes. These three enzymes are not only capable of digesting denatured proteins and polypeptides, but also degrading constituents of the extracellular matrix. They degrade various proteins, such as elastin, collagen, fibronectin and base membrane components.[15] The uncontrolled activity of these hydrolytic enzymes ultimately leads to the onset of disease.

The protease-antiprotease imbalance is a widely used hypothesis for explaining the role of enzymes in inflammatory diseases, such as chronic obstructive pulmonary disease (COPD). It is believed that the imbalance between proteases and their physiological protein inhibitors leads to the onset of disease. The imbalance could be caused by either increased burden of proteases or a deficiency (genetic or acquired) of functional antiproteases or both. For example, studies of

pulmonary disease suggest that the pathogenesis of the disease is caused by a deficiency of α_1-PI (α_1-protease inhibitor or α_1-antitrypsin) that can arise either because of a genetic defeat or, more commonly, because of the inactivation of α_1-PI by the combined effects of oxidants in cigarette smoke and endogenous oxidants.[16] Evidence also indicates a correlation between the severity of emphysema and proteolytic enzyme burden. Severity is also inversely correlated with proteolytic enzyme inhibitory capacity.[17]

According to this hypothesis, agents that can redress the protease-antiprotease imbalance could be useful therapeutic agents for inflammatory diseases. Strategies that have been used to restore the protease/antiprotease imbalance include (a) using endogenous protein inhibitors such as α_1-PI and SLPI as supplementation therapy; and (b) using low molecular weight synthetic inhibitors. The use of synthetic inhibitors offers several advantages: greater oral absorption, less proteolytic inactivation, greater enzyme selectivity and decreased risk of immunological response. Therefore, the development of low molecular weight inhibitors is a promising avenue of investigation that may lead to the emergence of useful therapeutic agents for chronic obstructive pulmonary disease and other inflammatory diseases.

1.4 Synthetic Inhibitors of Serine Proteases

According to the protease-antiprotease imbalance, agents that can redress the protease-antiprotease imbalance could be useful therapeutic agents for inflammatory disease. Intensive efforts have been focused on the discovery of low molecular weight synthetic inhibitors.[1, 18-21] From a kinetics point of view, synthetic inhibitors can be classified as reversible and irreversible inhibitors. But the most informative approach for classifying serine protease inhibitors is through their mechanism of action, which eventually determines their kinetic behavior during inhibition. The mechanism of action used by serine protease inhibitors includes non-covalent inhibition,

transition state analog inhibition, mechanism-based and acylating inhibition and affinity labeling inhibition. Affinity label inhibitors utilize highly reactive functional groups to inactivate serine proteases. The toxic nature of this kind of agents, arising from their high reactivity and lack of selectivity, has excluded them as candidates for drug development, thus will not be discussed here. All other inhibitory mechanisms are discussed below.

1.4.1 Transition State Analog Inhibitors

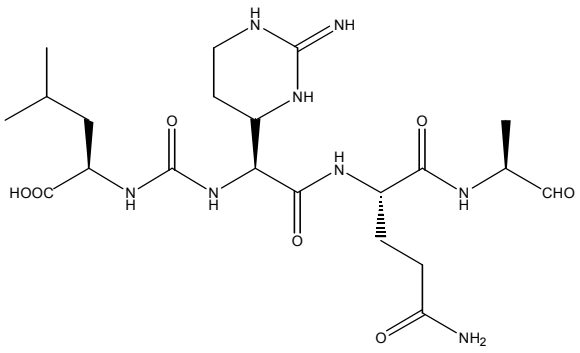
A transition state inhibitor is an inhibitor that mimics a transition state or transition state intermediate along the reaction coordinate. The basis of designing transition state inhibitor is that an enzyme binds the transition state more tightly than the ground state. Inhibitors whose structures mimic the transition state will also be bound tightly. The complex is stable and is not processed further, thereby inhibiting the enzyme.

Transition state analog inhibitors incorporate in their structure a serine trap which allows them to bind reversibly with an enzyme to form an EI complex, which, in the case of serine proteases, goes further to form a reversible covalent bond with the catalytic Ser_{195} (E-I). The dissociation constant K_I is an index of stability of the EI complex and also an index of inhibitory potency.

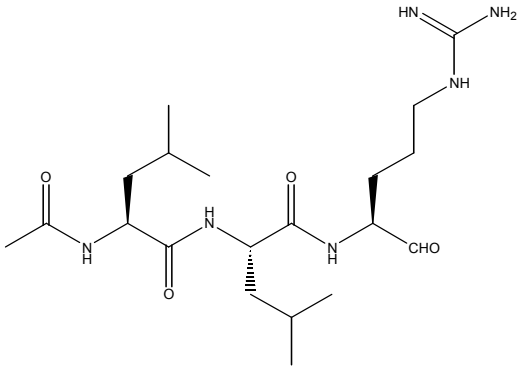
$$E + I \underset{K_I}{\rightleftharpoons} E \cdot I \rightleftharpoons E\text{-}I$$

Peptidyl aldehydes were first described as "transition state analog" inhibitors of serine proteases. They form reversible covalent adducts with serine proteases, in which the active site serine oxygen reacts with the aldehyde to form a hemiacetal. The hemiacetal hydroxyl group interacts with the oxyanion hole. Only the P_n-S_n binding interactions between enzyme and inhibitors exist in this adduct, while the P_n'-S_n' interactions are excluded because of the nature of

the aldehyde structure. The natural products elastatinal and leupeptin are representative examples of this class.



Elastatinal, inhibitor of elastase, K_I =240 nM



Leupeptin, inhibitor of trypsin, K_I = 400 nM

Peptidyl ketones can provide both potential P_n-S_n and P_n'-S_n' interactions with serine proteases. The corresponding ketal adduct formed by a regular ketone and serine protease is much less stable than the one resulting from an aldehyde. Increasing the stability of ketone-enzyme adducts, which parallels the potency of the inhibitors, has been achieved by using electron-withdrawing groups to increase the electrophilicity of the ketone group in peptidyl ketone inhibitors. Activated ketones, such as α-trifluoromethyl ketones, α-difluoromethylene ketones, α-ketoesters, α-ketoamides, α-ketoheterocycles, have been successfully used in the design of transition state serine protease inhibitors. [22-24] Peptidyl boronic acids have also been developed as transition state analog inhibitors of serine proteases. [25]

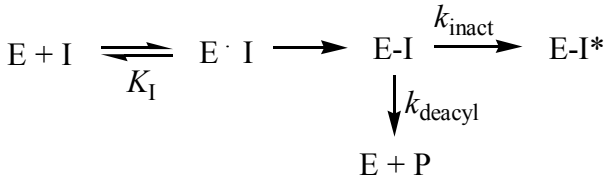
1.4.2 Mechanism-based Inhibitors and Acylating Agents

Mechanism-based inhibitors are a class of inhibitors which utilize the target enzyme mechanism to achieve inhibition. According to this broad definition, as suggested by Pratt (1992) [26] and Krantz (1992), [27] many heterocyclic inhibitors can be classified as mechanism-based

inhibitors of serine proteases. They act as substrates, and in the process of enzymatic catalysis, they react with their target serine proteases to form stable enzyme-inhibitor complexes, leading to the termination of the catalytic cycle.

However, a more narrow definition of mechanism-based inhibition has also been used. According to Silverman (1988),[28] a mechanism-based inhibitor is defined as an unreactive compound, whose structure resembles that of either the substrate or product of the target enzyme, and which undergoes a catalytic transformation by the enzyme to a reactive species that, prior to release from the active site, inactivates the enzyme. This definition has grouped many heterocyclic inhibitors that can form "double-hit" complexes with proteases as mechanism-based inhibitors, while those only form stable acyl-enzyme complexes with proteases as "acylating agents".

Mechanism-based inhibitors are typically irreversible inhibitors. The inactivation process involves the active site of an enzyme. The inhibitor forms a reversible complex (EI) which subsequently progresses to a reactive stable acyl-enzyme (E-I), which contains a reactive species. The E-I intermediate undergoes either deacylation to lead to product and free enzyme, or undergoes further reaction with a secondary active site residue of the enzyme to lead to permanent inactivation of the enzyme.



Mechanism-based inhibitors are time-dependent inhibitors. The apparent second order rate constant k_{inat}/K_I is an index of inhibitory potency. The ratio k_{deacyl}/k_{inact}, called the partition

ratio (defined as the number of molecules of inhibitor necessary to inactivate a single molecule of enzyme), is an index of the inhibition efficiency of a mechanism-based inhibitor.

Most heterocyclic inhibitors inhibit their target serine proteases by first acylating the active site serine residue, then unmasking a latent inhibitory functionality to irreversibly inactivate the enzyme. Since the second functionality is more reactive and only generated at the active site of the enzyme, mechanism-based inhibitors may show a high degree of selectivity.

Mechanism-based inhibitors, with their "drug-like" heterocyclic structures and potential higher selectivity, should have been the favored choice for drug development. Unfortunately, most of the reported mechanism-based inhibitors of serine proteases lack the required chemical stability. [19]

Several classes of acylating agents have been reported as serine protease inhibitors, including azapeptides, acyl imidazole and esters. [21] They acylate their target enzymes and give stable acyl enzyme complexes, where the newly formed ester bond is not positioned for nucleophilic attack by the deacylation water. Again, the stability problem is the reason hindering their clinical application. [19]

1.4.3 Non-covalent Inhibitors

Inhibitors of this kind can form a non-covalent complex with a serine protease, and reversible inhibition is often observed. Interactions between these inhibitors and their target enzymes are mainly through ionic, hydrogen bonding, and hydrophobic interactions and surface complementarities.

$$E + I \underset{K_I}{\rightleftharpoons} E \cdot I$$

A great number of natural protein inhibitors of serine proteases have been identified. Most of these protein inhibitors belong to this class. Many protein inhibitors are highly effective against a variety of serine proteases. However, most of them are non-human proteins, which would induce an immunogenic response, thus are not suitable for clinical development.

Many x-ray crystal structures of protease-inhibitor complexes show that the inhibitor is bound to the active site in a substrate-like fashion.[29] These studies have yielded a wealth of information related to the substrate recognition and catalytic mechanism of serine proteases, which is critical for the design of small molecule inhibitors of serine proteases through a structure-based approach.

Non-covalent inhibition of serine proteases with small molecules has emerged as one of the favored choices for drug discovery, because of the higher possibility to afford safe and orally bioavailable clinical drugs.[1] However, highly potent and non-covalent inhibition of HLE is expected to be difficult to achieve with small molecules, because of the limited number of weak bond interactions and the lack of ionic interactions available for inhibitor design. By far, most reported potent inhibitors of HLE are either transition state analog inhibitors or mechanism-based inhibitors.

CHAPTER 2

INHIBITOR DESIGN RATIONALE AND RESEARCH GOALS

2.1 Design Rationale

We have previously described the structure-based design of a highly functionalized heterocyclic scaffold (1,2,5-thiadiazolidin-3-one 1,1 dioxide, I) [30-31] and have demonstrated that 1) derivatives based on (I) function as potent covalent inhibitors of serine proteases; 2) these inhibitors show near absolute selectivity between neutral, basic and acidic serine proteases, namely, inhibitors with small hydrophobic side chains at the P_1 position are found to be effective inhibitors of elastase, those with aromatic side chains inhibit cathepsin G, and those with basic side chains inhibit trypsin; 3) scaffold (I) makes possible the exploitation of favorable binding interactions with both the S and S' subsites and potentially, subtle structural differences in the S and S' subsites of closely related proteases, like HLE and PR3.

Based on our findings with (I), we reasoned that functionalized surrogate scaffolds capable of mimicking (I) in terms of the spatial orientation of attached recognition elements, may lead to new classes of serine protease inhibitors. Indeed, studies with functionalized 4-imidazolidinone derivatives have provided evidence in support of this hypothesis.[32] Furthermore, we hypothesized that the replacement of the carbonyl group in (I) with a methylene group would yield a functionalized cyclic sulfamide structure (II) that could function as a non-covalent reversible inhibitor of a serine protease (Figure 2.1). The interactions between the inhibitor and the enzyme are mainly through hydrogen bonding, hydrophobic interactions and surface

complementarities.

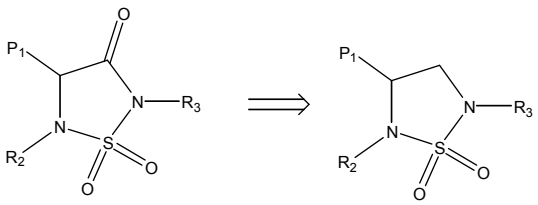


Figure 2.1 Design rationale of inhibitor (II).

2.2 Research Goals

Based on the forgoing discussion, the research goals were the following:

a) Design and synthesis of non-covalent reversible inhibitors of serine protease based on a cyclic sulfamide scaffold; and

b) In vitro biochemical evaluation (potency and selectivity) of the inhibitors.

CHAPTER 3

EXPERIMENTAL

The ^{1}H and ^{13}C NMR spectra were recorded on a Varian XL-300 or XL-400 NMR spectrometer. A Hewlett-Packard diode array UV/VIS spectrophotometer was used in vitro evaluation of the inhibitors. Melting points were determined on a Mel-Temp apparatus and are uncorrected.

Reagents were purchased from chemical suppliers such as Aldrich, Sigma, Lancaster, Fisher, Acros Organics, TCI and Bachem. The flash columns for purifying compounds were packed with silica gel (60Å, 32-63μm) obtained from Sorbent Technologies. Thin layer chromatography was performed using Analtech silica gel plates. The TLC plates were visualized using iodine vapor and/or UV light.

Synthesis of compound 1. A solution of *N*-chlorosulfonyl isocyanate (13.0 mL, 0.15 mol) in methylene chloride (200 mL) was cooled in an ice bath and a solution of t-butyl alcohol (11.12 g, 0.15 mol) in methylene chloride (100 mL) was added dropwise with stirring. After the stirring continued for 10 minutes, the reaction mixture was transferred to a separatory funnel and added dropwise to a solution of (L) leucine methyl ester hydrochloride (27.25 g, 0.15 mol) and triethylamine (30.4 g, 0.30 mol) in methylene chloride (300 mL) in an ice bath. The resulting mixture was then stirred at room temperature overnight. The reaction mixture was washed with 5% HCl (2×75 mL) and brine (75 mL). The organic solution was separated and dried over anhydrous Na_2SO_4, then concentrated to yield a white solid (45.84 g, 94%): mp 89-91 °C; ^{1}H NMR ($CDCl_3$) δ 0.93 (t, 6H), 1.44 (s, 9H), 1.59 (m, 2H), 1.81 (m, 1H), 3.74 (s, 3H), 4.19 (m, 1H), 5.56 (d, 1H), 7.18 (bs, 1H).

Synthesis of compound 2. To a solution of compound **1** (4.86 g, 15 mmol) in dry THF (20 mL) was added dropwise 2 M lithium borohydride THF solution (7.5 mL, 15 mmol) over a dry ice-acetone bath. After the reaction was stirred for 3 hours, absolute ethanol (50 mL) was added dropwise. The reaction was then stirred overnight at room temperature, then cooled to 0 °C, and neutralized by 5% HCl. The resulting mixture was concentrated and then diluted with H_2O (10 mL), extracted with ethyl acetate (3×25 mL). The organic phase was dried over anhydrous Na_2SO_4, and concentrated. The crude product was purified using flash chromatography (hexane/EtOAc, 75:25) to afford a white solid (1.93 g, 43%): mp 114-116 °C; ^{1}H NMR ($CDCl_3$) δ 0.92 (d, 6H), 1.36 (m, 1H), 1.42 (m, 1H), 1.44 (s, 9H), 1.71 (m, 1H), 3.47 (m, 2H), 3.71 (m, 1H), 5.50 (d, 1H).

Synthesis of compound 3. To a solution of compound **2** (2.96 g, 10 mmol) in dry THF (25 mL) was added methanesulfonyl chloride (1.15 g, 10 mmol) in an ice bath. Triethylamine (1.35 g, 13.3 mmol) was then added dropwise. After the reaction was stirred for 4 hours at room temperature, the solvent was evaporated and the residue was taken up in ethyl acetate (50 mL) and washed with 5% HCl (2×10 mL) and brine (10 mL). The organic phase was dried over anhydrous Na_2SO_4, then concentrated to yield an oil (3.32 g, 89%): ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 6H), 1.42 (m, 2H), 1.44 (s, 9H), 1.71 (m, 1H), 3.03 (s, 3H), 3.71 (m, 1H), 4.19 (dd, 1H), 4.30 (dd, 1H), 5.40 (dd, 1H).

Synthesis of compound 4. To a solution of compound **3** (3.74 g, 10 mmol) in dry acetonitrile (45 mL) was added dropwise a solution of DBU (1.83 g, 12 mmol) in dry acetonitrile (25 mL). The mixture is stirred for 5 hours and then the solvent was evaporated. The residue was taken up in ethyl acetate (50 mL) and washed with 5% HCl (2×15 mL), 5% $NaHCO_3$ (2×15 mL) and brine (15 mL). The organic phase was dried over anhydrous Na_2SO_4, and then concentrated.

The crude product was purified using flash chromatography (hexane/EtOAc, 85:15) to afford a white solid (1.50 g, 54%): mp 97-99 °C; ^{1}H NMR ($CDCl_3$) δ 0.93 (d, 6H), 1.46 (m, 1H), 1.56 (s, 9H), 1.59 (m, 1H), 1.74 (m, 1H), 3.41 (t, 2H), 3.81 (m, 1H), 4.00 (dd, 1H), 4.27 (d, 1H).

Representative procedure for the synthesis of compounds 5a-g. To a solution of 3-methoxyphenylacetic acid (0.25 g, 1.5 mmol) in dry methylene chloride (8 mL) was added HATU (0.68 g, 1.8 mmol), followed by TEA (0.30 g, 3 mmol) and compound **4** (0.42 g, 1.5 mmol) in dry methylene chloride (5 mL). The reaction was stirred at room temperature for 4 hours and then the solvent was evaporated. The residue was taken up in ethyl acetate (50 mL) and washed with 5% HCl (2×8 mL), 5% $NaHCO_3$ (2×8 mL) and brine (2×8 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated. The crude product was purified using flash chromatography (hexane/EtOAc, 95:5) to give compound **5a** as an oil (0.32 g, 50%):^{1}H NMR ($CDCl_3$) δ 0.92 (t, 6H), 1.57 (m, 2H), 1.58 (s, 9H), 1.63 (m, 1H), 3.78 (m, 2H), 3.79 (s, 3H), 4.03 (q, 2H), 4.54 (m, 1H), 6.80-7.22 (m, 4H).

Synthesis of compound 5b. Compound **5b** was prepared from compound **4** as described for **5a** in 44% yield as a white solid: mp 98-100 °C ; ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 6H), 1.57 (m, 2H), 1.58 (s, 9H), 1.63 (m, 1H), 3.78 (m, 2H), 3.79 (s, 3H), 4.01 (q, 2H), 4.54 (m, 1H), 6.82-7.23 (m, 4H).

Synthesis of compound 5c. Compound **5c** was prepared from compound **4** as described for **5a** in 22% yield as an oil: ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 6H), 1.57 (m, 2H), 1.58 (s, 9H), 1.63 (m, 1H), 3.78 (m, 2H), 4.02 (q, 2H), 4.54 (m, 1H), 6.90-7.30 (m, 4H).

Synthesis of compound 5d. Compound **5d** was prepared from compound **4** as described for **5a** in 10% yield as an oil: ^{1}H NMR ($CDCl_3$) δ 0.96 (dd, 6H), 1.58 (s, 9H), 1.62 (m, 2H), 1.96 (m, 1H), 3.63 (dd, 2H), 4.00 (dd, 2H), 4.99 (m, 1H), 7.45-7.79 (m, 5H).

Synthesis of compound 5e. Compound **5e** was prepared from compound **4** as described for **5a** in 20% yield as an oil: ^{1}H NMR ($CDCl_3$) δ 0.94 (dd, 6H), 1.57 (m, 2H), 1.58 (s, 9H), 1.61 (m, 1H), 2.98 (m, 3H), 3.10 (m, 1H), 3.73 (m, 2H), 4.53 (m, 1H), 7.20 (m, 5H).

Synthesis of compound 5f. Compound **5f** was prepared from compound **4** as described for **5a** in 13% yield as an oil: ^{1}H NMR ($CDCl_3$) δ 0.96 (dd, 6H), 1.58 (s, 9H), 1.60 (m, 2H), 1.96 (m, 1H), 3.63 (dd, 2H), 4.00 (dd, 2H), 4.99 (m, 1H), 6.99-7.58 (m, 9H).

Synthesis of compound 5g. Compound **5g** was prepared from compound **4** as described for **5a** in 11% yield as an oil: ^{1}H NMR ($CDCl_3$) δ 0.92 (dd, 6H), 1.59 (m, 1H), 1.60 (s, 9H), 1.70 (m, 2H), 3.82 (m, 2H), 4.56 (q, 2H), 4.58 (m, 1H), 7.40-7.84 (m, 7H).

Representative procedure for the synthesis of compounds 6a-g. Compound **5a** (0.58 g, 1.36 mmol) was treated with TFA/methylene chloride (1:1 v/v, 15 mL) and stirred for 3 hours at room temperature. The solvent and excess TFA was removed and the residue was taken up in ethyl acetate (35 mL) and washed with saturated $NaHCO_3$ (2×10 mL) and brine (10 mL). The organic phase was dried over anhydrous Na_2SO_4 and then concentrated to yield compound **6a** as an oil (0.38 g, 87%): ^{1}H NMR ($CDCl_3$) δ 0.92 (dd, 6H), 1.55 (m, 2H), 1.72 (m, 1H), 3.23 (dd, 1H), 3.64 (dd, 1H), 3.79 (s, 3H), 4.03 (q, 2H), 4.54 (m, 1H), 6.78-7.22 (m, 4H).

Synthesis of compound 6b. Compound **6b** was prepared from compound **5b** as described for **6a** in 100% yield as a white solid: mp 135-137 °C ; ^{1}H NMR ($CDCl_3$) δ 0.92 (dd, 6H), 1.57 (m, 2H), 1.72 (m, 1H), 3.23 (dd, 1H), 3.64 (dd, 1H), 3.79 (s, 3H), 4.01 (q, 2H), 4.54 (m, 1H), 6.83-7.21 (m, 4H).

Synthesis of compound 6c. Compound **6c** was prepared from compound **5c** as described for **6a** in 91% yield as a white solid: mp 141-144 °C; ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 6H), 1.57 (m, 2H), 1.72 (m, 1H), 3.23 (d, 1H), 3.64 (dd, 1H), 4.01 (q, 2H), 4.54 (m, 1H), 6.82-7.32 (m, 4H).

Synthesis of compound 6d. Compound **6d** was prepared from compound **5d** as described for **6a** in 100% yield as an oil: ^{1}H NMR ($CDCl_3$) δ 0.96 (d, 6H), 1.60 (m, 2H), 1.84 (m, 1H), 3.21 (dd, 1H), 3.64 (dd, 2H), 4.20 (bs, 1H), 4.83 (m, 1H), 7.42-7.80 (m, 5H).

Synthesis of compound 6e. Compound **6e** was prepared from compound **5e** as described for **6a** in 98% yield as an oil: ^{1}H NMR ($CDCl_3$) δ 0.94 (dd, 6H), 1.57 (m, 2H), 1.68 (m, 1H), 2.98 (m, 4H), 3.24 (dd, 1H), 3.64 (dd, 1H), 4.54 (m, 1H), 7.26 (m, 5H).

Synthesis of compound 6f. Compound **6f** was prepared from compound **5f** as described for **6a** in 89% yield as an oil: ^{1}H NMR ($CDCl_3$) δ 0.96 (dd, 6H), 1.61 (m, 2H), 1.82 (m, 1H), 3.20 (m, 1H), 3.62 (m, 1H), 4.71 (m, 1H), 6.90-7.60 (m, 9H).

Synthesis of compound 6g. Compound **6g** was prepared from compound **5g** as described for **6a** in 84% yield as an oil: ^{1}H NMR ($CDCl_3$) δ 0.90 (dd, 6H), 1.50 (m, 2H), 1.70 (m, 1H), 3.22 (d, 1H), 3.64 (dd, 1H), 3.80 (bs, 1H), 4.51 (m, 2H), 4.52 (m, 1H), 7.40-7.84 (m, 7H).

Representative procedure for the synthesis of compounds 7a-g. To a solution of compound **6a** (0.37 g, 1.13 mmol) in dry methylene chloride (10 mL) was added TEA (0.12 g, 1.13 mmol) and 2-phenethyl isocyanate (0.24 g, 1.58 mmol) successively. The reaction mixture was gently refluxed for one hour. The solvent was then evaporated and the residue was taken up in ethyl acetate (50 mL) and washed with 5% HCl (2×10 mL) and brine (10 mL). The organic phase was dried over anhydrous Na_2SO_4, and then concentrated. The crude product was purified using flash chromatography (hexanes/EtOAc, 85:15) to afford **7a** (oil, 0.41 g, 77%): ^{1}H NMR ($CDCl_3$) δ 0.92 (dd, 6H), 1.59 (m, 3H), 2.86 (t, 2H), 3.57 (m, 2H), 3.80 (s, 3H), 3.86 (m, 2H), 4.00 (q, 2H), 4.50 (m, 1H), 6.05 (t, 1H), 6.81-7.38 (m, 9H).

Synthesis of compound 7b. Compound **7b** was prepared from compound **6b** as described for **7a** in 95% yield as an oil: ^{1}H NMR ($CDCl_3$) δ 0.92 (dd, 6H), 1.59 (m, 3H), 2.86 (t,

2H), 3.57 (m, 2H), 3.80 (s, 3H), 3.86 (m, 2H), 4.00 (q, 2H), 4.50 (m, 1H), 6.05 (t, 1H), 6.86-7.38 (m, 9H).

Synthesis of compound 7c. Compound **7c** was prepared from compound **6c** as described for **7a** in 72% yield as an oil: ^{1}H NMR ($CDCl_3$) δ 0.96 (dd, 6H), 1.59 (m, 3H), 2.86 (t, 2H), 3.59 (m, 2H), 3.86 (m, 2H), 4.02 (q, 2H), 4.50 (m, 1H), 6.05 (t, 1H), 6.95-7.38 (m, 9H).

Synthesis of compound 7d. Compound **7d** was prepared from compound **6d** as described for **7a** in 78% yield as a white solid: mp 77-78 °C; ^{1}H NMR ($CDCl_3$) δ 0.96 (dd, 6H), 1.64 (m, 2H), 1.86 (m, 1H), 2.78 (t, 2H), 3.45 (m, 2H), 3.62 (dd, 1H), 4.23 (dd, 1H), 4.84 (m, 1H), 5.95 (t, 1H), 7.12-7.80 (m, 10H).

Synthesis of compound 7e. Compound **7e** was prepared from compound **6e** as described for **7a** in 87% yield as a white solid: mp 88-90 °C; ^{1}H NMR ($CDCl_3$) δ 0.96 (d, 6H), 1.59 (m, 3H), 2.84 (t, 2H), 3.01 (m, 4H), 3.53 (m, 2H), 3.83 (m, 2H), 4.44 (m, 1H), 5.98 (t, 1H), 7.20 (m, 10H).

Synthesis of compound 7f. Compound **7f** was prepared from compound **6f** as described for **7a** in 70% yield as an oil: ^{1}H NMR ($CDCl_3$) δ 0.96 (dd, 6H), 1.60 (m, 2H), 1.81 (m, 1H), 2.80 (t, 2H), 3.45 (m, 2H), 3.62 (dd, 1H), 4.21 (dd, 1H), 4.80 (m, 1H), 5.95 (t, 1H), 7.01-7.50 (m, 14H).

Synthesis of compound 7g. Compound **7g** was prepared from compound **6g** as described for **7a** in 69% yield as an oil: ^{1}H NMR ($CDCl_3$) δ 0.90 (dd, 6H), 1.60 (m, 3H), 2.90 (t, 2H), 3.60 (m, 2H), 3.94 (d, 2H), 4.51 (m, 2H), 4.52 (m, 1H), 6.08 (t, 1H), 7.22-7.84 (m, 12H).

Synthesis of compound 8. To a solution of compound **6b** (0.55 g, 1.68 mmol) in dry methylene chloride (10 mL) was added TEA (0.17 g, 1.68 mmol) and 2-phenethyl isothiocyanate (0.38 g, 2.35 mmol) successively. The reaction mixture was gently refluxed for one hour. The

solvent was then evaporated and the residue was taken up in ethyl acetate (50 mL) and washed with 5% HCl (2×10 mL) and brine (10 mL). The organic phase was dried over anhydrous Na_2SO_4, and then concentrated. The crude product was purified using flash chromatography (hexane/EtOAc, 95:5) to afford **8** (oil, 0.03 g, 4%): ^{1}H NMR ($CDCl_3$) δ 0.92 (dd, 6H), 1.60 (m, 3H), 2.96 (t, 2H), 3.79 (s, 3H), 3.90 (m, 2H), 3.95 (t, 2H), 4.10 (m, 1H), 4.24 (d, 1H), 4.44 (m, 1H), 6.82-7.58 (m, 9H).

The structures of compounds **1-8** are listed in Figure 3.1 and their spectral properties are shown in Table 3.1.

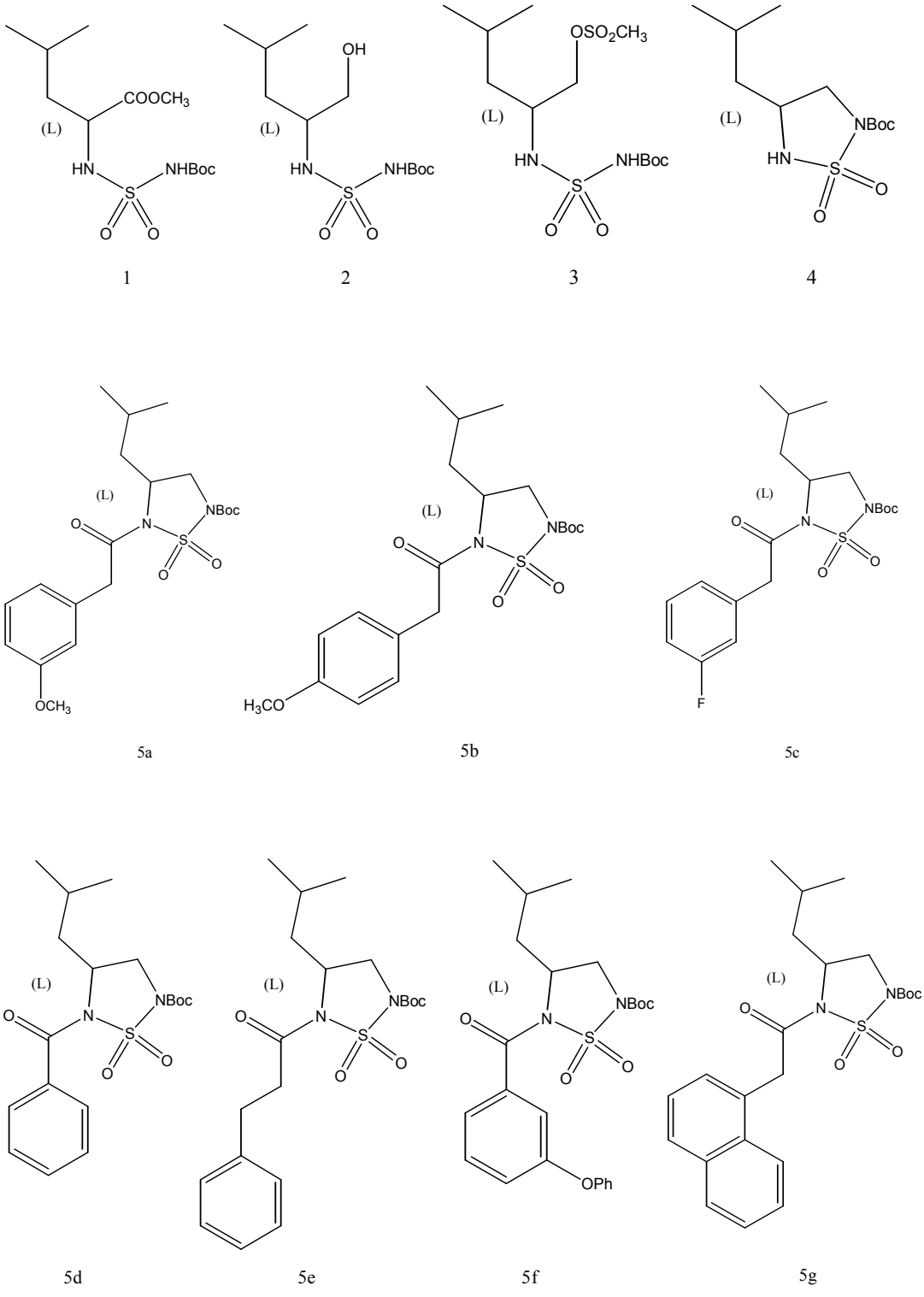
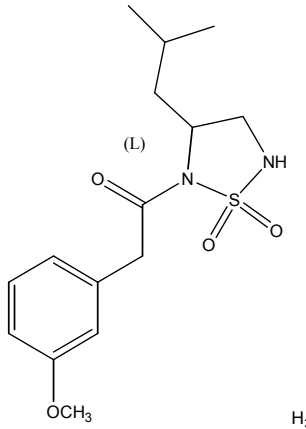
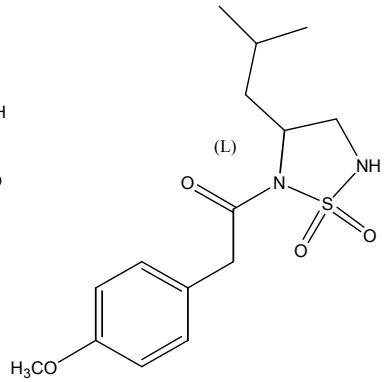


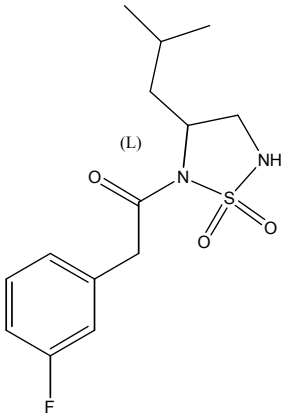
Figure 3.1 Structures of compounds 1-8.



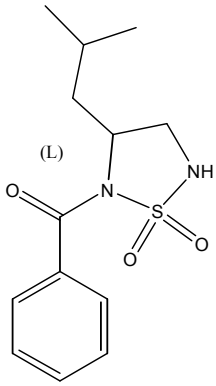
6a



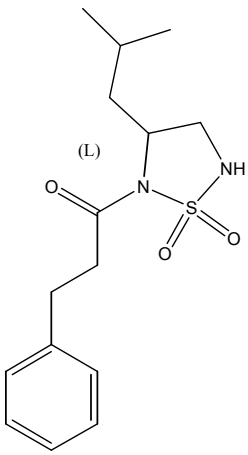
6b



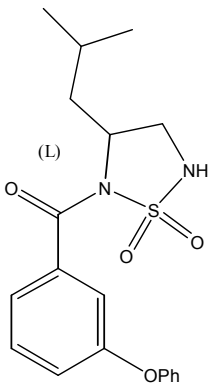
6c



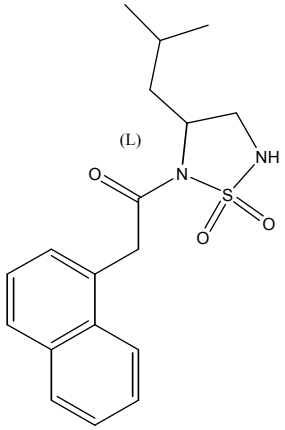
6d



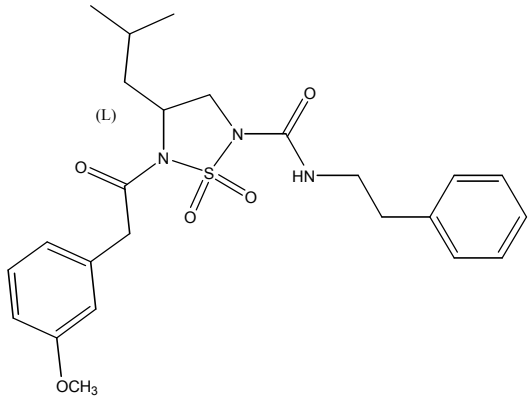
6e



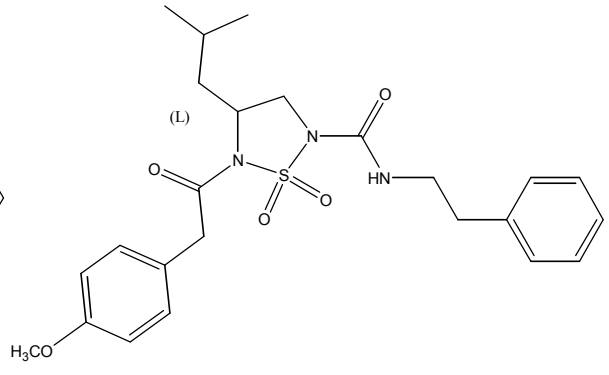
6f



6g

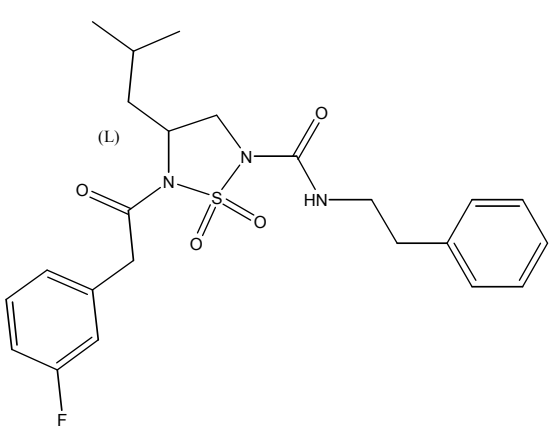


7a

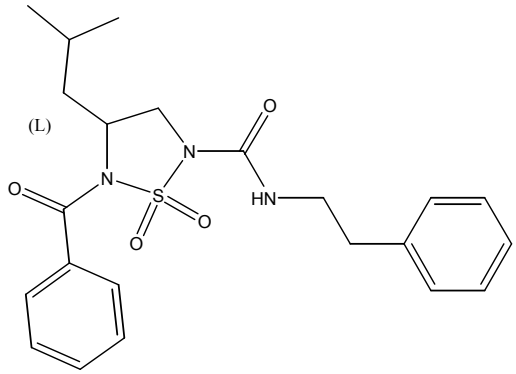


7b

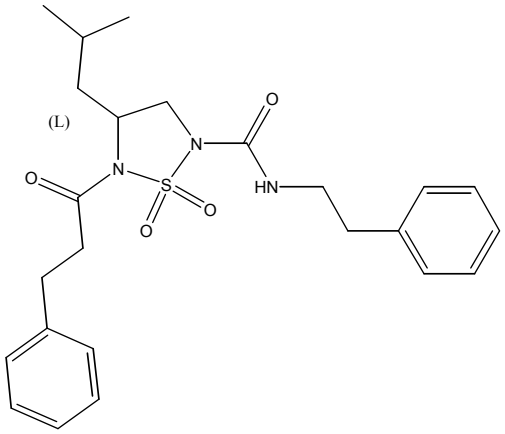
Figure 3.1 Structures of compounds 1-8 (continued).



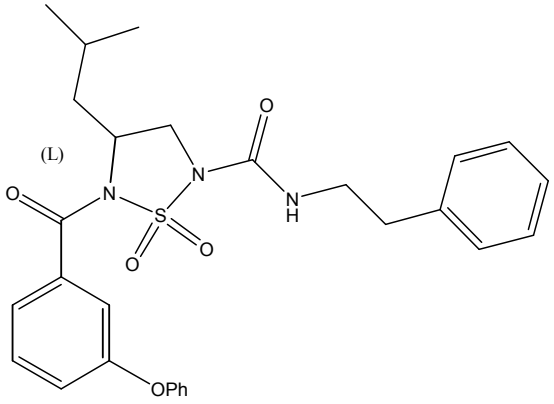
7c



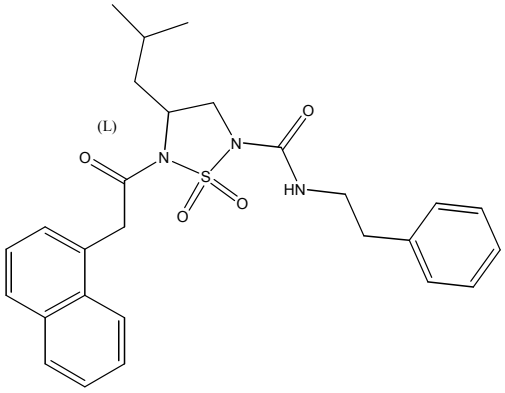
7d



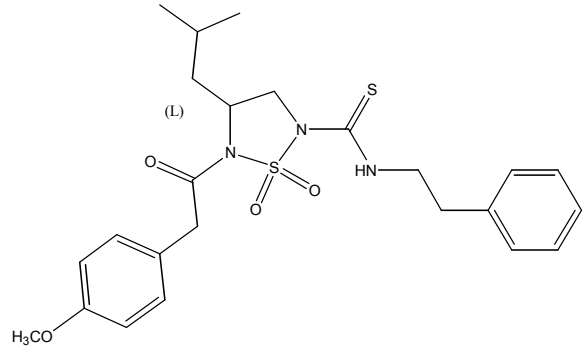
7e



7f



7g



8

Figure 3.1 Structures of compounds 1-8 (continued).

Table 3.1 Physical and spectral data of compounds 1-8

Compound	MF	MW	^{1}H NMR Data (δ)
1	$C_{12}H_{24}N_2O_6S$	324.39	($CDCl_3$) 0.93 (t, 6H), 1.44 (s, 9H), 1.59 (m, 2H), 1.81 (m, 1H), 3.74 (s, 3H), 4.19 (m, 1H), 5.56 (d, 1H), 7.18 (bs, 1H)
2	$C_{11}H_{24}N_2O_5S$	296.38	($CDCl_3$) 0.92 (d, 6H), 1.36 (m, 1H), 1.42 (m, 1H), 1.44 (s, 9H), 1.71 (m, 1H), 3.47 (m, 2H), 3.71 (m, 1H), 5.50 (d, 1H)
3	$C_{12}H_{26}N_2O_7S_2$	374.48	($CDCl_3$) 0.92 (t, 6H), 1.42 (m, 2H), 1.44 (s, 9H), 1.71 (m, 1H), 3.03 (s, 3H), 3.71 (m, 1H), 4.19 (dd, 1H), 4.30 (dd, 1H), 5.40 (dd, 1H)
4	$C_{11}H_{22}N_2O_4S$	278.37	($CDCl_3$) 0.93 (d, 6H), 1.46 (m, 1H), 1.56 (s, 9H), 1.59 (m, 1H), 1.74 (m, 1H), 3.41 (t, 2H), 3.81 (m, 1H), 4.00 (dd, 1H), 4.27 (d, 1H)
5a	$C_{20}H_{30}N_2O_6S$	426.53	($CDCl_3$) 0.92 (t, 6H), 1.57 (m, 2H), 1.58 (s, 9H), 1.63 (m, 1H), 3.78 (m, 2H), 3.79 (s, 3H), 4.03 (q, 2H), 4.54 (m, 1H), 6.80-7.22 (m, 4H)
5b	$C_{20}H_{30}N_2O_6S$	426.53	($CDCl_3$) 0.92 (t, 6H), 1.57 (m, 2H), 1.58 (s, 9H), 1.63 (m, 1H), 3.78 (m, 2H), 3.79 (s, 3H), 4.01 (q, 2H), 4.54 (m, 1H), 6.82-7.23 (m, 4H)
5c	$C_{19}H_{27}FN_2O_5S$	414.49	($CDCl_3$) 0.92 (t, 6H), 1.57 (m, 2H), 1.58 (s, 9H), 1.63 (m, 1H), 3.78 (m, 2H), 4.02 (q, 2H), 4.54 (m, 1H), 6.90-7.30 (m, 4H)
5d	$C_{18}H_{26}N_2O_5S$	382.48	($CDCl_3$) 0.96 (dd, 6H), 1.58 (s, 9H), 1.62 (m, 2H), 1.96 (m, 1H), 3.63 (dd, 2H), 4.00 (dd, 2H), 4.99 (m, 1H), 7.45-7.79 (m, 5H)
5e	$C_{20}H_{30}N_2O_5S$	410.53	($CDCl_3$) 0.94 (dd, 6H), 1.57 (m, 2H), 1.58 (s, 9H), 1.61 (m, 1H), 2.98 (m, 3H), 3.10 (m, 1H), 3.73 (m, 2H), 4.53 (m, 1H), 7.20 (m, 5H)
5f	$C_{24}H_{30}N_2O_6S$	474.57	($CDCl_3$) 0.96 (dd, 6H), 1.58 (s, 9H), 1.60 (m, 2H), 1.96 (m, 1H), 3.63 (dd, 2H), 4.00 (dd, 2H), 4.99 (m, 1H), 6.99-7.58 (m, 9H)
5g	$C_{23}H_{30}N_2O_5S$	446.56	($CDCl_3$) 0.92 (dd, 6H), 1.59 (m, 1H), 1.60 (s, 9H), 1.70 (m, 2H), 3.82 (m, 2H), 4.56 (q, 2H), 4.58 (m, 1H), 7.40-7.84 (m, 7H)
6a	$C_{15}H_{22}N_2O_4S$	326.41	($CDCl_3$) 0.92 (dd, 6H), 1.55 (m, 2H), 1.72 (m, 1H), 3.23 (dd, 1H), 3.64 (dd, 1H), 3.79 (s, 3H), 4.03 (q, 2H), 4.54 (m, 1H), 6.78-7.22 (m, 4H)
6b	$C_{15}H_{22}N_2O_4S$	326.41	($CDCl_3$) 0.92 (dd, 6H), 1.57 (m, 2H), 1.72 (m, 1H), 3.23 (dd, 1H), 3.64 (dd, 1H), 3.79 (s, 3H), 4.01 (q, 2H), 4.54 (m, 1H), 6.83-7.21 (m, 4H)
6c	$C_{14}H_{19}FN_2O_3S$	314.38	($CDCl_3$) 0.92 (t, 6H), 1.57 (m, 2H), 1.72 (m, 1H), 3.23 (d, 1H), 3.64 (dd, 1H), 4.01 (q, 2H), 4.54 (m, 1H), 6.82-7.32 (m, 4H)
6d	$C_{13}H_{18}N_2O_3S$	282.36	($CDCl_3$) 0.96 (d, 6H), 1.60 (m, 2H), 1.84 (m, 1H), 3.21 (dd, 1H), 3.64 (dd, 2H), 4.20 (bs, 1H), 4.83 (m, 1H), 7.42-7.80 (m, 5H)
6e	$C_{15}H_{22}N_2O_3S$	310.41	($CDCl_3$) 0.94 (dd, 6H), 1.57 (m, 2H), 1.68 (m, 1H), 2.98 (m, 4H), 3.24 (dd, 1H), 3.64 (dd, 1H), 4.54 (m, 1H), 7.26 (m, 5H)
6f	$C_{19}H_{22}N_2O_4S$	374.46	($CDCl_3$) 0.96 (dd, 6H), 1.61 (m, 2H), 1.82 (m, 1H), 3.20 (m, 1H), 3.62 (m, 1H), 4.71 (m, 1H), 6.90-7.60 (m, 9H)
6g	$C_{18}H_{22}N_2O_3S$	346.44	($CDCl_3$) 0.90 (dd, 6H), 1.50 (m, 2H), 1.70 (m, 1H), 3.22 (d, 1H), 3.64 (dd, 1H), 3.80 (bs, 1H), 4.51 (m, 2H), 4.52 (m, 1H), 7.40-7.84 (m, 7H)
7a	$C_{24}H_{31}N_3O_5S$	473.59	($CDCl_3$) 0.92 (dd, 6H), 1.59 (m, 3H), 2.86 (t, 2H), 3.57 (m, 2H), 3.80 (s, 3H), 3.86 (m, 2H), 4.00 (q, 2H), 4.50 (m, 1H), 6.05 (t, 1H), 6.81-7.38 (m, 9H)
7b	$C_{24}H_{31}N_3O_5S$	473.59	($CDCl_3$) 0.92 (dd, 6H), 1.59 (m, 3H), 2.86 (t, 2H), 3.57 (m, 2H), 3.80 (s, 3H), 3.86 (m, 2H), 4.00 (q, 2H), 4.50 (m, 1H), 6.05 (t, 1H), 6.86-7.38 (m, 9H)
7c	$C_{23}H_{28}FN_3O_4S$	461.55	($CDCl_3$) 0.96 (dd, 6H), 1.59 (m, 3H), 2.86 (t, 2H), 3.59 (m, 2H), 3.86 (m, 2H), 4.02 (q, 2H), 4.50 (m, 1H), 6.05 (t, 1H), 6.95-7.38 (m, 9H)

Table 3.1 (continued)

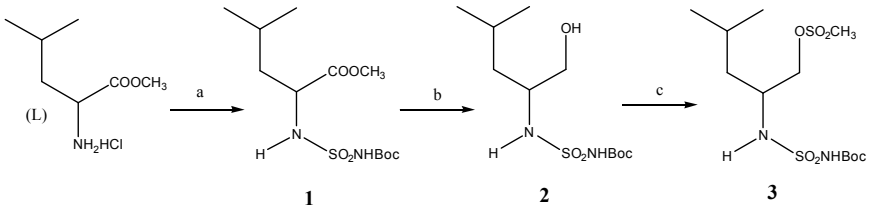
Compound	MF	MW	^{1}H NMR Data (δ)
7d	$C_{22}H_{27}N_3O_4S$	429.53	($CDCl_3$) 0.96 (dd, 6H), 1.64 (m, 2H), 1.86 (m, 1H), 2.78 (t, 2H), 3.45 (m, 2H), 3.62 (dd, 1H), 4.23 (dd, 1H), 4.84 (m, 1H), 5.95 (t, 1H), 7.12-7.80 (m, 10H)
7e	$C_{24}H_{31}N_3O_4S$	457.59	($CDCl_3$) 0.96 (d, 6H), 1.59 (m, 3H), 2.84 (t, 2H), 3.01 (m, 4H), 3.53 (m, 2H), 3.83 (m, 2H), 4.44 (m, 1H), 5.98 (t, 1H), 7.20 (m, 10H)
7f	$C_{28}H_{31}N_3O_5S$	521.63	($CDCl_3$) 0.96 (dd, 6H), 1.60 (m, 2H), 1.81 (m, 1H), 2.80 (t, 2H), 3.45 (m, 2H), 3.62 (dd, 1H), 4.21 (dd, 1H), 4.80 (m, 1H), 5.95 (t, 1H), 7.01-7.50 (m, 14H)
7g	$C_{27}H_{31}N_3O_4S$	493.62	($CDCl_3$) 0.90 (dd, 6H), 1.60 (m, 3H), 2.90 (t, 2H), 3.60 (m, 2H), 3.94 (d, 2H), 4.51 (m, 2H), 4.52 (m, 1H), 6.08 (t, 1H), 7.22-7.84 (m, 12H)
8	$C_{24}H_{31}N_3O_4S_2$	489.65	($CDCl_3$) 0.92 (dd, 6H), 1.60 (m, 3H), 2.96 (t, 2H), 3.79 (s, 3H), 3.90 (m, 2H), 3.95 (t, 2H), 4.10 (m, 1H), 4.24 (d, 1H), 4.44 (m, 1H), 6.82-7.58 (m, 9H)

CHAPTER 4

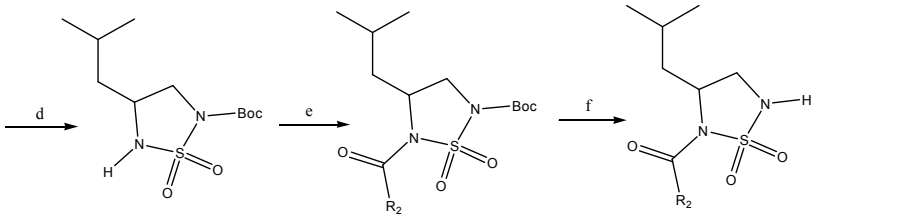
RESULTS AND DISCUSSION

4.1 Synthesis of Inhibitors

The synthetic route shown in Figure 4.1 was used in the synthesis of inhibitors 7a-g. Since HLE shows a strong preference for medium size hydrophobic side chains (Leu, Val) at the P_1 position (L) leucine methyl ester was used as starting material. It was allowed to undergo reaction with the *N*-chlorosulfonyl isocyanate and *t*-butyl alcohol adduct in the presence of triethylamine to give a substituted amino acid ester **1**, which was then reduced to alcohol **2** using lithium borohydride. The resulting alcohol was activated with methanesulfonyl chloride to give compound **3** and then cyclized to yield heterocyclic compound **4** using DBU as a base. It was then coupled with an appropriate acid to give the corresponding derivatives **5a-g**, through a standard coupling procedure. Removal of the Boc group with trifluoroacetic acid followed by reaction with 2-phenethyl isocyanate, yielded the final compounds **7a-g**. Compound **8** was also synthesized by reacting **6b** with 2-phenethyl isothiocyanate (Figure 4.2).



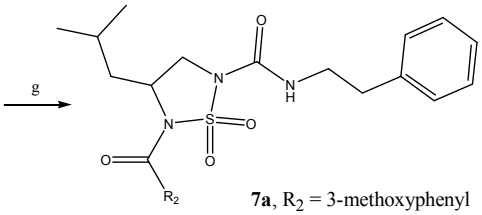
1 **2** **3**



4

5a, R_2 = 3-methoxyphenyl
5b, R_2 = 4-methoxyphenyl
5c, R_2 = 3-fluorobenzyl
5d, R_2 = benzyl
5e, R_2 = phenethyl
5f, R_2 = 3-phenoxyphenyl
5g, R_2 = 1-naphthylmethyl

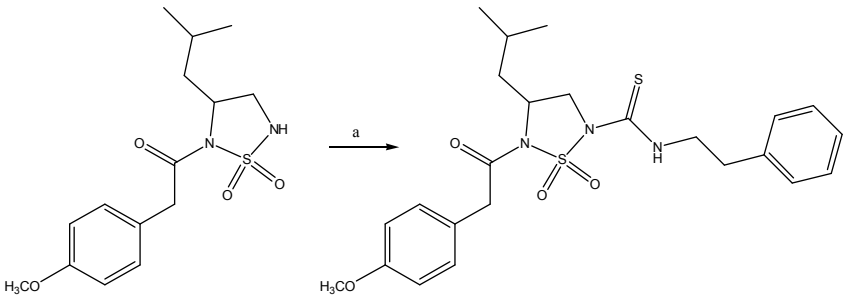
6a, R_2 = 3-methoxyphenyl
6b, R_2 = 4-methoxyphenyl
6c, R_2 = 3-fluorobenzyl
6d, R_2 = benzyl
6e, R_2 = phenethyl
6f, R_2 = 3-phenoxyphenyl
6g, R_2 = 1-naphthylmethyl



7a, R_2 = 3-methoxyphenyl
7b, R_2 = 4-methoxyphenyl
7c, R_2 = 3-fluorobenzyl
7d, R_2 = benzyl
7e, R_2 = phenethyl
7f, R_2 = 3-phenoxyphenyl
7g, R_2 = 1-naphthylmethyl

[a]$ClSO_2N{=}C{=}O$/t-BuOH/TEA/CH_2Cl_2; [b]$LiBH_4$/THF; [c]CH_3SO_2Cl/TEA/THF; [d]DBU/CH_3CN; [e]R_2COOH/HATU/TEA; [f]TFA/CH_2Cl_2; [g]$PhCH_2CH_2N{=}C{=}O$/TEA/CH_2Cl_2.

Figure 4.1 Synthesis of inhibitors 7a-g.



[a]$PhCH_2CH_2N=C=S/TEA/CH_2Cl_2$.

Figure 4.2 Synthesis of inhibitor 8.

4.2 Biochemical Studies

Compounds **7a-g** were initially screened against HLE. Thus, incubation of compound **7e** with HLE led to rapid and time-dependent loss of enzyme activity (Figure 4.3). The enzyme was found to be inhibited irreversibly, and there was no regain in enzymatic activity after a 24 hour incubation period.

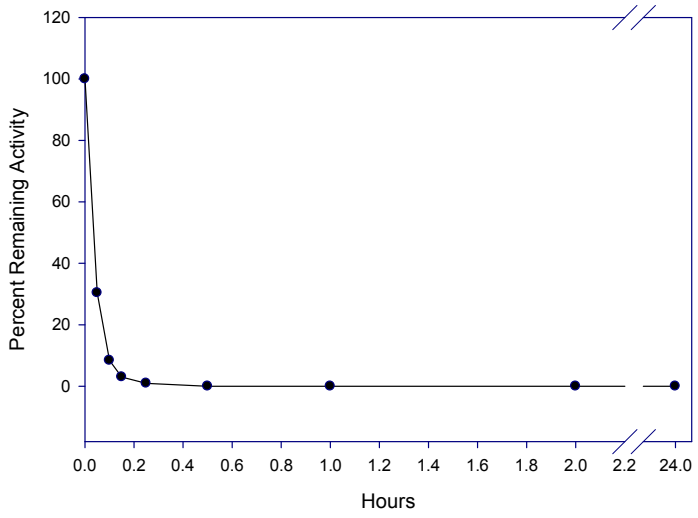


Figure 4.3 Time-dependent inhibition of HLE by compound 7e.

The inhibitory activity of compounds **7a-g** toward HLE was then determined using the progress curve method.[30] The k_{inact}/K_I values, which reflect the potency of each inhibitor, are listed in Table 4.1.

Table 4.1 Inhibitory activity of compounds 7a-g toward HLE

compound	k_{inact}/K_I $M^{-1}s^{-1}$ [a]
7a	350
7b	350
7c	750
7d	890
7e	1260
7f	980
7g	1050

[a] Determined using the progress curve method.[30]

It is evident from the results shown in Table 4.1 that compounds **7a-g** are fairly efficient covalent inhibitors of HLE. A tentative mechanism for the inactivation of HLE by this series of compounds is shown in Figure 4.4, where binding of the inhibitor to the active site of the enzyme apparently places the phenethyl group in the S_1 pocket. This is followed by nucleophilic attack by the active site serine$_{195}$, leading to the formation of a carbamoylated enzyme. The greater stability of the latter toward aqueous hydrolysis, as compared to an acyl enzyme, accounts for the irreversible nature of the inactivation. The nature of the R_2 group does not appear to play a major role in the efficiency of the inactivation process, accounting for the small variations in the k_{inact}/K_I values. The fact that compound **6e** is devoid of any inhibitory activity toward HLE is supportive of the proposed mechanism. Further studies are needed to establish unequivocally the mechanism of action of compounds **7a-g**.

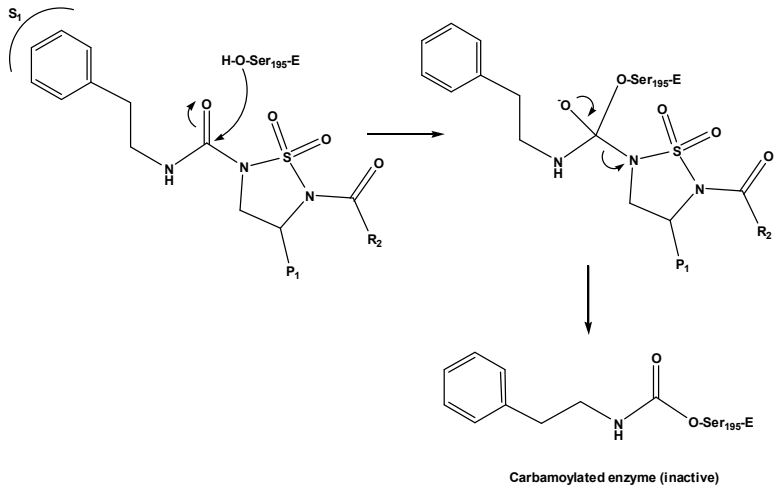


Figure 4.4 Postulated mechanism of inhibition of HLE by compounds 7a-g.

CHAPTER 5

CONCLUSION

The main focus of the studies described herein has been the discovery of *non-covalent* inhibitors of serine proteases that incorporate in their structure the cyclosulfamide scaffold. During the course of these studies, the serendipitous discovery of a new class of carbamoylating agents of serine proteases based on the cyclosulfamide scaffold was made. These agents are potentially valuable molecular probes of this class of enzymes.

REFERENCES

LIST OF REFERENCES

1 Abbenante, G.; Fairlie D. P. Protease Inhibitors in the Clinic. *Medicinal Chemistry* 2005, 1, 71-104.

2 Schechter, I.; Berger, A. On the Size of the Active Site in Proteases. *Biochem. Biophys. Res. Comm.* 1967, 255, 3931-3934.

3 Barrett, A. J.; Rawlings, N. D. Families and Clans of Serine Peptidases. *Arch. Biochem. Biophys.* 1995, 318, 247-250.

4 PDB: www.rcsb.org/pdb.

5 Campanelli, D.; Melchior, M.; Fu, Y.; Nakata, M.; Shuman, H.; Nathan, C.; Gabay, J. E. Cloning of cDNA for Proteinase 3: A Serine Protease, Antibiotic, and Autoantigen from Human Neutrophils. *J. Exp. Med.* 1990, 172, 1709-1715.

6 Navia, M. A.; Springer, J. P.; Lin, T.; Williams, H. R.; Firestone, R. A.; Pisano, J. M.; Doherty, J. B.; Finke, P. E.; and Hoogsteen, K. Crystallographic Study of a β-Lactam Inhibitor Complex with Elastase at 1.84 Å Resolution. *Nature* 1987, 327(7), 79-82.

7 Bode, W.; Meyer, E.; Powers, J. C. Human Leukocyte and Porcine Pancreatic Elastase: X-ray Crystal Structures, Mechanism, Substrate Specificity, and Mechanism-based Inhibitors. *Biochemistry* 1989, 28(5), 1951-1963.

8 Fujinaga, M., Chernaia, M. M.; Halenbeck, R.; Koths K.; James, M. N. G. The Crystal Structure of PR3, a Neutrophil Serine Proteinase Antigen of Wegener's Granulomatosis Antibodies. *J. Mol. Biol.* 1996, 261(2), 267-278.

9 Brubaker, M. J.; Groutas, W. C.; Hoidal, J. R. and Rao, N. V. Human Neutrophil Proteinase 3: Mapping of the Substrate Binding Site Using Peptidyl Thiobenzyl Esters. *Biochem. Biophys. Res. Comm.* 1992, 188(3), 1318-1324.

10 Harper, J. W.; Cook, R. R.; Roberts, C. J.; McLaughlin, B. J.; Powers J. C. Active Site Mapping of the Serine Proteases Human Leukocyte Elastase, Cathepsin G, Porcine Pancreatic Elastase, Rat Mast Cell Proteases I and II, Bovine Chymotrypsin Aα, and *Staphlococcus aureus* Protease V-8 Using Tripeptide Thiobenzyl Ester Substrates. *Biochemistry* 1984, 23, 2995-3002.

11 Rao, N. V.; Wehner, N. G.; Marshall, B. C.; Sturrock, A.; Huecksteadt, T.; Rao, G. V.; Gray, B. H.; Hoidal J. R. Proteinase 3: a Polymorphonuclear Leukocyte Serine Proteinase. *Ann. N. Y. Acad. Sci.* 1991, 624, 60-68.

12 Edward S. W. *Biochemistry and Physiology of the Neutrophil,* Cambridge University Press, New York, 1994.

13 Travis, J.; Pike, R.; Imamura, T.; Potempa, J. The Role of Proteolytic Enzyme within the Development of Pulmonary Emphysema and Periodontal Disease. *Am. J. Respir. Crit. Care Med.* 1994, 150, 143-146.

14 Gabay, J. E.; Almeida R. P. Antibiotic Peptides and Serine Protease Homology in Human Polymorphonuclear Leukocyte: Defensins and Azurocidin. *Curr. Op. Immun.* 1993, 5, 97-102.

15 Weiss, S. J. Tissue Destruction by Neutrophils. *N. Engl. J. Med.* 1989, 320, 365-376.

16 Doring, G. Role of Neutrophil Elastase in Chronic Inflammation. *Am. J. Respir. Crit. Care Med.* 1994, 150, 114-117.

17 Fujita, J.; Nelsonn, N. L. Evaluation of Elastase and Antielastase Balance in Patients with Chronic Bronchitis and Pulmonary Emphsema. *Am. J. Respir. Dis.* 1990, 142, 57-62.

18 Leung, D.; Abbenante, G.; Fairle, D. P. Protease Inhibitors: Current Status and Future Prospects. *J. Med. Chem.* 2000, 43(3), 305-341.

19 Powers, J. C.; Asgian, J. L.; Ekici, O. D.; James, K. E. Irreversible Inhibitors of Serine, Cysteine, and Threonine Proteases. *Chem. Rev.* 2002, 102, 4639-4750.

20 Ilies, M. A.; Scozzafava, A.; Supuran, C. Therapeutic Applications of Serine Protease Inhibitors. *Expert Opin. Ther. Patents.* 2002, 12(8), 1181-1214.

21 Zhong, J.; Groutas, W. C. Recent Development in the Design of Mechanism-based and Alternate Substrate Inhibitors of Serine Proteases. *Curr. Top. Med. Chem.* 2004, 4, 1203-1216.

22 Veale, C. A.; Bernstein, P. R.; Bohnert, C. M.; Brown, F. J.; Bryant, C.; Damewood, J. R.; 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, 3173-3181.

23 Peet, N. P.; Burkhart, J. P.; Angelastro, M. R.; Giroux, E. L.; Mehdi, S.; Bey, P; Kolb, M.; Neises, B.; Schirlin, D. Synthesis of Peptidyl Fluoromethyl Ketones and Peptidyl α- Keto Esters as Inhibitors of Porcine Pancreatic Elastase, Human Neutrophil Elastase, and Rat and Human Neutrophil Cathepsin G. *J. Med. Chem.* 1990, 33, 394-407.

24 Edwards, P. D.; Wolanin, D. J.; Andisik, D. W.; Davis, M. W. Peptidyl α-Ketoheterocyclic Inhibitors of Human Leukocyte Elastase. 2. Effect of Varying the Heterocyclic Ring on in Vitro Potency. *J. Med. Chem.* 1995, 38, 76-85.

25 Kettner, C. A.; Shennvi, A. B. Inhibitors of Serine Proteinases Leukocyte Elastase Pancreatic Elastase Cathepsin G and Chymotrypsin by Peptide Boronic Acids. *J. Biol. Chem.* 1984, 259 (24), 15106-15114.

26 Pratt, R. F. On the Definition and Classification of Mechanism-Based Enzyme Inhibitors. *Bioorg. Med. Chem. Lett.* 1992, 2, 1323-1326.

27 Krantz, A. A Classification of Enzyme Inhibition. *Bioorg. Med. Chem. Lett.* 1992, 2, 1327-1334.

28 Silverman, R. B. *Mechanism-based Enzyme Inactivation: Chemistry and Enzymology,* Vols 1 and 2, CRC Press, Boca Raton, FL, 1988.

29 Bode, W.; Huber, R. Natural Protein Proteinase Inhibitors and Their Interaction with Proteinases**.** *Eur. J. Biochem.* 1992, 204, 433-451.

30 Groutas, W. C.; Kuang, R.; Venkataraman, R. ; Epp, J. B. ; Ruan, S. ; Prakash, O. Structure-based Design of a General Class of Mechanism-based Inhibitors of the Serine Proteinases Employing a Novel Amino Acid Derived Heterocyclic Scaffold. *Biochemistry* 1997, 36, 4739-4750.

31 Kuang, R.; Epp, J. B.; Ruan, S.; Yu, H.; Huang P.; He, S.; Tu J.; Schechter, N. M.; Turbov, J.; Froelich, C. J.; Groutas, W. C. A General Inhibitor Scaffold for Serine Proteases with a (Chymo)trypsin-like Fold: Solution-phase Construction and Evaluation of the First Series of Libraries of Mechanism-based Inhibitors. *J. Am. Chem. Soc.* 1999, 121, 8128-8129.

32 Wei, L.; Gan, X.; Zhong, J.; Allistion, K. R.; Groutas, W. C. Noncovalent Inhibitors of Human Leukocyte Elastase Based on the 4-Imidazolidinone Scaffold. *Bioorg. Med. Chem.* 2003, 11, 5149-5153.