Synthesis and evaluation of pseudosaccharin amine derivatives as potential elastase inhibitors

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Dedicated to my parents

"In chemistry, one's ideas, however beautiful, logical, elegant, imaginative that they may be in their own right, are simply without value unless they are actually applicable to the one physical environment we have- in short, they are only good if they work!"

R. B. Woodward

Publications

1) Rode, H. B.; Sprang, T.; Besch, A.; Loose, J.; Otto, H.-H. Pseudosaccharin amine derivatives: synthesis and elastase Inhibitory activity. *Die Pharmazie* **2005**, *60(10)*, 723-731.

2) Rode, H.; Koerbe, S.; Besch, A.; Methling, K.; Loose, J.; Otto, H.-H. Synthesis and in vitro evaluation of pseudosaccharin amine derivatives as potential elastase inhibitors. *Bioorg. Med. Chem.* **2005**, in press.

Presentation

1) Rode, H.; Besch, A.; Otto, H.-H. Studies on pseudosaccharin derivatives. A poster presented at the annual meeting of the Medicinal Chemistry "Frontiers in Medicinal Chemistry" Erlangen, 15-17 March **2004**.

aq.	Aqueous
BTAC	Benzyl triethyl ammonium chloride
BOC	*tert*-Butoxycarbonyl
δ	Chemical shift in ppm
CC	Column chromatography
J	Coupling constant in Hz
dec.	Decomposition
DSC	Differential scanning calorimetry
$CDCl_3$	Deuterochloroform
°C	Degree Celsius
DBU	1,8-Diazabicyclo[5.4.0]undec-7-en
DCM	Dichloromethane
$[D_6]DMSO$	Deuterodimethylsulfoxide
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
EtOH	Ethanol
AcOEt	Ethyl acetate
g	Gram
HDTMAB	Hexadecyltrimethylammonium bromide
HPLC	High performance liquid chromatography
HLE	Human Leukocyte Elastase
K_i	Inhibitor constant
LG	Leaving group
IBCF	Isobutyl chloroformate
IR	Infrared
M.p.	Melting point
MeOH	Methanol
μM	Micromolar
mg	Milligram
mM	Millimolar
pNA	4-Nitroanilide
NMM	N-Methyl morpholine
NMR	Nuclear magnetic resonance
PPE	Porcine Pancreatic Elastase
KI	Potassium iodide
R_f	Ratio of front
T_R	Retention time

RP-HPLC	Reverse phase high performance liquid chromatography
rt	Room temperature
THF	Tetrahydrofuran
TMS	Tetramethylsilane
TLC	Thin layer chromatography
TEA	Triethylamine
UV	Ultraviolet light

Amino acids

Ala	Alanine
Asp	Aspartic acid
Glu	Glutamic acid
Phe	Phenylalanine
Gly	Glycine
His	Histidine
Ile	Isoleucine
Leu	Leucine
Met	Methionine
α-Me-Ala	α-Methyl alanine
Pro	Proline
Tyr	Tyrosine
Ser	Serine
Val	Valine

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1. Introduction and theoretical background

Life depends on a well-orchestrated series of chemical reactions. Many of these reactions, however, proceed too slowly to sustain life on their own. Hence, nature has designed catalysts, which we now refer to as *enzymes*, to greatly accelerate the rates of these chemical reactions [1]. As the biocatalysts that regulate the rates at which all physiologic processes take place, enzymes occupy central roles in health and disease. While in health all physiologic processes occur in an ordered, regulated manner and homeostasis is maintained, homeostasis can be profoundly disturbed in pathologic states [2]. One of the most exiting fields of modern Enzymology is the application of enzyme inhibitors as drugs in human and veterinary medicine. Many of the drugs that are commonly used today function by inhibiting specific enzymes that are associated with the disease process [1]. Elastases are possibly the most destructive enzymes in the body, having the ability to degrade virtually all connective tissue components. Uncontrolled proteolytic degradation by elastase has been implicated in a number of pathological conditions [3].

1.1. Human Leukocyte Elastase (HLE)

HLE (EC 3.4.21.37) is often called as neutrophil elastase. It is one of the several hydrolytic enzymes contained in azurophil granules of human neutrophils and also found in thrombocytes, macrophages, in the spleen, aorta and skin, in snake venoms and in some microorganisms. Elastases are endopeptidases (serine proteases) which by definition are able to solubilize elastin by proteolytic cleavage [4, 5]. Besides elastin, collagen type III, proteoglycans, azocasein, haemoglobin, fibrinogen and histones are degraded, among other proteins. HLE is responsible (at least in part) for inflammatory and arthritis condition [4]. HLE consists of a single basic polypeptide chain of 218 amino acid residues, joined together by four disulfide bonds. It contains two asparagine linked carbohydrate side chains, and is in fact synthesized as a series of isoenzymes each containing different amounts of carbohydrate. The backbone architect of HLE and PPE is conserved, and the structures of the active sites near the cleavage site are very similar [6, 7]. The isoforms do obviously not differ in catalytic activity and are immunologically identical [8, 9]. Comparison of the sequence to other serine proteinases indicates only moderate homology with porcine pancreatic elastase (43.0%) or neutrophil cathepsin G (37.2%) [6]. The N-terminal amino acid sequence is strongly homologous with that of the porcine pancreatic elastase [8].

1.2. Porcine Pancreatic Elastase (PPE)

PPE (EC 3.4.21.36) is first described by BALO and BANGA in 1950 [10]. It consists of a single polypeptide chain of 240 amino acids [6]. The primary structure of PPE is homologous to that of trypsin and chymotrypsin. After processing to proelastase, it is stored in the zymogen granules and later activated to elastase in the duodenum [10]. Elastase contains no prosthetic groups or metal ions and is not subject to any allosteric activatory or inhibitory control. Its enzymatic activity results solely from the specific three-dimensional conformation which its single polypeptide chain adopts. Therefore, activity is lost by denaturation and conformational changes [11]. Elastase is a serine proteinase with broad substrate specificity. It preferentially cleaves peptide bonds at the carbonyl end of amino acid residues with small non-aromatic side chains, such as glycine, valine, leucine, isoleucine, and alanine. The wide specificity of elastase for non-aromatic uncharged side chains explains its unique ability to digest native elastin, a protein rich in aliphatic side chains. Elastase is also able to digest other proteins such as fibrin, haemoglobin, and casein, but not native collagen and keratin [11, 12].

1.3. Leukocyte Elastase-related diseases

1.3.1. ARDS and Lung injury

ARDS (Adult Respiratory Distress Syndrome) is a life-threatening noncardiogenic pulmonary oedema, which often occurs in association with sepsis, severe trauma, acute pancreatitis, and disseminated intravascular coagulation (DIC) as a consequential disease. Clinical characterization shows remarkable extravascular lung water with increased pulmonary microvascular permeability and impaired gas exchange. The pathological finding of ARDS shows severe lung tissue damage with a massive neutrophil infiltration into the lungs. In previous clinical studies, high levels of HLE activity and oxidised or cleaved forms of α1-PI were detected in the bronchoalveolar lavage fluids of ARDS patients. Thus, an effective new HLE inhibitor may be expected as a key to solving these problems [13, 14, 15].

1.3.2. Cystic fibrosis (CF)

CF is the most common lethal genetic disease among the Caucasian population. The characteristic pulmonary manifestations of CF are recurrent bacterial infections, chronic inflammation progressing to bronchiectasis and irreversible bronchiectatic cysts developments, resulting in death from respiratory failure in > 90% of patients. These clinical features of the disease undoubtedly result from CFTR (Cystic fibrosis

transmembrane conducting regulator) genetical dysfunction. The pathological findings of CF show a massive influx of activated neutrophils caused by recurrent infections, with mucous hypersecretion and the obstruction of small airways. The excessive pulmonary neutrophil-dominated inflammation is still the main factor leading to progressive lung damage in CF. Activated polymorphonuclear cells release high levels of proteolytic granule proteins, resulting in a proteinase-antiproteinase imbalance. The proteinases, including Leukocyte elastase, alters epithelial cell receptors, impede mucociliary transport by increasing mucus secretion and by slowing ciliary beat frequency, cleave immunoglobulins and complements and are directly toxic to the lung parenchyma [13, 14].

1.3.3. Pulmonary emphysema

Pulmonary emphysema is a slowly progressive disease characterised by irreversible enlargement of the respiratory airspaces of the lungs, with destruction of the alveolar walls. Since LAURRELL and ERIKSSON first reported the strong relationship between pulmonary emphysema at an early age and the inherited deficiency in α1-PI, HLE has been thought to be the major factor involved in the development of the disease in the hypothesis of elastase-antielastase imbalance theory. The two main types of human emphysema, centrilobular and panacinar emphysema are well known among many different recognised patterns of human pulmonary emphysema. Centrilobular emphysema, the most common form, is related to smoking, while the frequent panacinar emphysema is relatively independent of cigarette smoking and develops in childhood. Widespread panacinar emphysema is associated with severe α1-PI deficiency. Cigarette smoking experimentally activates neutrophils to release elastase and is regarded as the most predominant risk factor in the onset and progression of emphysema. It is well known fact that smokers have 5-10 folds more macrophages in their lungs than non-smokers, indicating that macrophages may play a pathogenic role in the mechanism of emphysema [13, 14, 15].

1.3.4. Smoking-related chronic bronchitis

Many previous studies have reported smoking-related chronic bronchitis among the various kinds of chronic bronchitis. Sputum samples of smoking-related chronic bronchitis patients are reported to be rich in proteolytic enzymes. The proteinase/inhibitor balance shifted towards the proteinase side in the sputum sol phase of smoking-related chronic bronchitis. As in pulmonary emphysema, the question of why some, but not all, smokers can acquire smoking-related chronic bronchitis has remained open to debate. The precise pathogenic mechanism of

chronic bronchitis has still not been fully solved. However, synthetic, HLE inhibitors may be useful candidates for investigating and treating smoking-related chronic bronchitis [13, 15].

1.3.5. Ischaemic-reperfusion injury

Neutrophils play an important role in the development of various ischaemic reperfusion-induced tissue injuries, such as pulmonary, hepatic and GI tissue. In case of cardiac surgery, the levels of TNF-α, IL-8 and IL-6 are elevated after reperfusion of cardiac operations using cardiac bypass along with increased HLE [13, 14, 15].

1.3.6. Rheumatoid arthritis (RA)

RA is characterized by the infiltration of mononuclear cells and polymorphonuclear neutrophils into the synovial tissue, with the implication of pathological degradation of cartilage and the formation of pannus tissue, following by joint destruction. Neutrophil elastase has a high affinity for cartilage tissue and degrades major cartilage tissue components, including proteoglycan and type II collagen. Polymorphonuclear neutrophils also induce elaborate reactive oxygen species such as superoxide anion, hydrogen peroxide and hypochlorous acid. HLE and reactive oxygen species cooperatively induce cartilage tissue damage. These facts make researcher anticipate that the HLE inhibitors may be highly effective method for RA therapy [13, 14, 15].

1.3.7. Gastric mucosal injury

Gastric mucosal injury generally has various causes, including drug abuse, stress and alcohol. Some of these causes have recently considered to be related to increased neutrophil accumulation and HLE. Gastric ulceration induced by non-steroidal anti-inflammatory drugs was reported to be concerned with a neutrophil dependent process [13, 14, 15].

1.4. Mechanism of peptide hydrolysis by HLE

The active-site of proteinase consists of two domains: an extended binding site where noncovalent binding interactions occur and a catalytic-site where the covalent bond forming and bond breaking reactions takes place. The key catalytic element of the serine proteinases is a serine hydroxyl group [14]. The mechanism of peptide bond hydrolysis by serine proteinase is depicted in figure 1. After complexation of the substrate with the extended binding site of enzyme (**III-1**); the hydroxyl group of

Ser-195 undergoes nucleophilic addition to the carbonyl carbon atom of the scissile peptide bond to form the tetrahedral intermediate **III-2** [13, 14, 15]. The nucleophilicity of the serine hydroxyl group is increased through hydrogen bond formation with the imidazole of His-57, which itself is involved in a hydrogen-bonding interaction with Asp-102. These three amino acid residues are known as the "catalytic triad" and are responsible for the proton transfer during peptide bond hydrolysis. Due to differences in the primary sequences of individual serine proteinases, the exact residue numbers for the catalytic residues may vary slightly. In accordance with the common practice, the chymotrypsin numbering (His-57, Asp-102, Ser-195) is used for all the serine proteinases.

While the precise movement of protons between the residues of the catalytic triad remains controversial, the proton on the serine hydroxyl group is ultimately transferred via His-57 to the nitrogen atom of the eventual amine product, thereby facilitating the collapse of the tetrahedral intermediate to the acyl-enzyme **III-3** and the departure of the C-terminal peptide fragment. The acyl enzyme is hydrolyzed by the catalytic addition of water to afford the N-terminal carboxylic acid fragment of the peptide, along with the free enzyme which is ready to repeat the cycle. In addition to the catalytic triad, another key feature of the catalytic site of the serine protease is the "Oxyanion hole" which is composed of the NH group of the Ser-195 and Gly-193. These two groups participate in a hydrogen-bonding interaction with the carbonyl oxygen atom; increasing its electrophilicity before addition of the Ser-195 hydroxyl group and stabilizing the oxyanion of the tetrahedral adduct [13, 14, 15].

Furthermore, the X-ray crystal structures of the non-covalent complexes of some natural inhibitors bound to a number of serine proteinases, including HLE, revealed that the carbonyl oxygen atom of the scissile amide was located in the oxyanion hole with its geometry distorted from trigonal towards tetrahedral. The pyramidalization of the carbonyl group was dramatically demonstrated when it was observed in the complex between bovine pancreatic trypsin inhibitor and anhydrotrypsin, in which the hydroxyl of Ser-195 has been removed, making a covalent bond formation impossible. This distortion from planarity serves to lower the activation energy required for formation of the tetrahedral intermediate. Indeed, polarization of the substrate carbonyl bond, resulting from strong electrostatic interactions within the oxyanion hole, has been postulated to be the main driving force behind acyl-enzyme formation. The tetrahedral intermediate **III-2** is the highest energy intermediate along the reaction coordinate for peptide bond hydrolysis. Factors within the active-site which stabilize this intermediate will lower the activation energy required for amide bond cleavage.
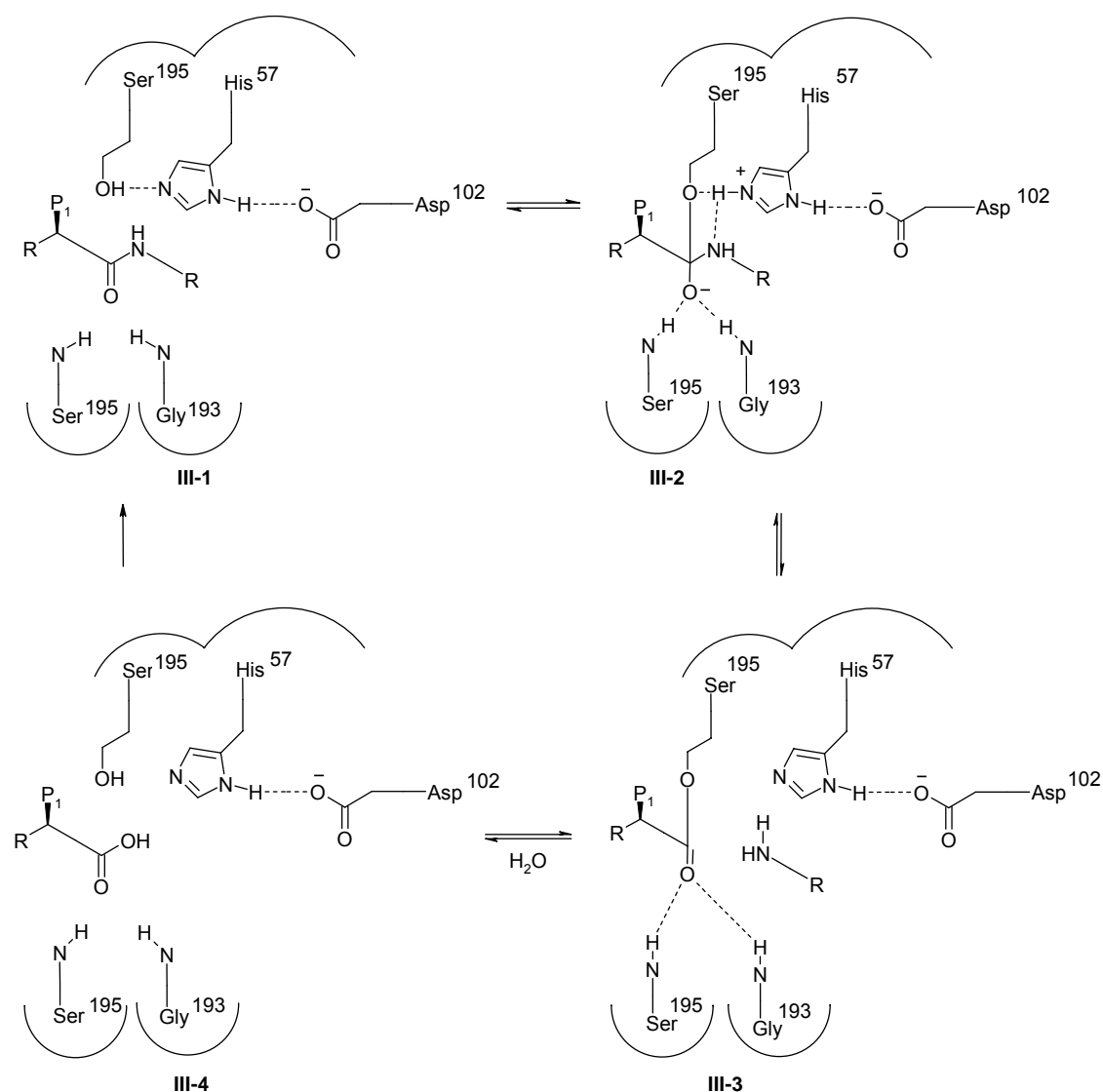
Fig. 1: Schematic representation for the mechanism of peptide bond hydrolysis by serine proteinases: **III-1**) initial enzyme-substrate complex; **III-2**) tetrahedral adduct with Ser-195; **III-3**) acyl-enzyme plus C-terminus amine product; **III-4**) regenerated catalytic triad plus N-terminus acid product [14].

The active-site of enzymes has evolved to be complementary to the transition-state for the particular chemical process they are catalyzing. Transition-state theory of the enzyme catalysis states that the transition state **(III-2)** is more tightly bound than the enzyme substrate complex **(III-1)**, acyl-enzyme **(III-3)**, or enzyme-product complex **(III-4)**. In an analogous fashion, inhibitors, which possess structural features that mimic the transition-state for the hydrolysis, will also be bound very tightly. This has been a widely employed tactic for inhibitor design. The intermediate **III-2** has generally been referred to as a transition-state, although a transition-state is a high energy structure which is undergoing bond formation or bond breakage. Thus, the

tetrahedral intermediate **III-2** is not the actual transition-state for catalysis, but rather a high energy "transition-state intermediate" between substrate and acyl-enzyme. In addition to the interaction within catalytic-site, the substrate also interacts with the enzyme through the amino acid residue extending to the either site of the scissile bond (figure 2) [16].
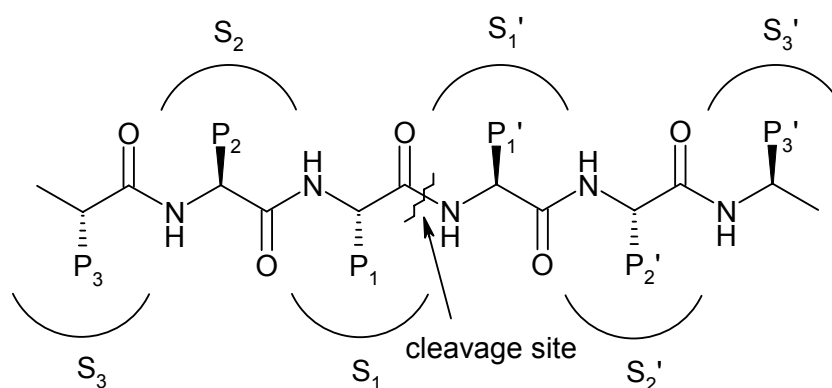
Fig. 2: Cartoon showing substrate/inhibitor residues (P) and protease binding sites (S). Prime and non-prime designations distinguish C-versus N-sides respectively of cleavage site [16].

Those residues extending towards the N-terminus end of the substrate are designed as P_1, P_2, P_3,.. …etc., while those towards the C-terminus end of the substrate are designed as P'_1, P'_2, P'_3, ….etc. The corresponding binding subsites on the enzyme are identified as S_1, S_1', and so on. The binding outside of the catalytic site occurs through non-covalent interactions such as hydrogen bonds and hydrophobic forces. The most important of these interactions is that between the S_1 subsite and P_1 residue. This interaction is the primary determinant of substrate specificity among the different serine proteinases, and it is generally possible to alter the enzyme selectivity of a substrate or inhibitor by modifying the group that binds in the S_1 subsite. While the ability to form a covalent attachment with Ser-195 forms the basis of most serine proteinase inhibitors, the cumulative binding forces obtainable in the extended binding pocket can be extremely strong hence sufficient to hold some inhibitors to the enzyme without covalent attachment to Ser-195. Indeed, many proteinaceous inhibitors of serine proteinases do not form covalent adducts but relies solely on non-covalent interactions [13, 14, 15]. The natural proteinase inhibitor, turkey ovomucoid inhibitor, has a dissociation constant only three fold greater against porcine pancreatic anhydroelastase than against PPE, indicating that interactions with the hydroxyl group of Ser-195 were of only secondary importance for binding [13, 14,]. In contrast to the proteinaceous inhibitors, low molecular-weight inhibitors generally

require the formation of a covalent adduct with Ser-195 to attain significant potency, since their small size limits the number of possible non-covalent interactions.

1.5. Elastase Inhibitors

The inhibitors can be divided into three broad classes depending on their mechanism of action: peptide-based inhibitors, heterocyclic inhibitors and acylating/alkylating agents.

1.5.1. Peptide based inhibitors

In peptide-based inhibitors, a short peptide interacts with the extended binding site of the enzyme as part of the enzyme inhibitory process, although the mechanistic basis of inhibition is usually the result of additional nonpeptidic structural features of the molecule. Certain C-terminal amides afforded moderately potent inhibitors of elastase. Compound **1** (figure 3) showed higher potency (K_i = 1.3 µM) against PPE than against HLE (K_i = 400 µM) [14]. Incorporation of D-amino acid residues into P_1 and P_2 position of the substrate resulted in a weak inhibition. **2** showed Ki of 120 µM against HLE [17]. A peptide ester substrate can be converted into an inhibitor by reversal of the position of the α-nitrogen and the α-carbon atom to form a carbamate or by replacement of the α-carbon with a nitrogen to yield the carbazate. Moderately active carbamate, **3** (K_i = 42.5 µM against PPE) [18] and carbazate, **4** (IC$_{50}$ = 0.28 µM) [19] are reported. Peptidyl aldehydes were believed to form a tetrahedral hemiacetal with the catalytic-site Ser-195 following initial binding of the unhydrated aldehyde and thus were proposed to be transition-state analogues of peptide substrates. A transition state inhibitor is one which mimics a transition-state or transition-state intermediate along the reaction coordinate, but is not processed further, thereby inhibiting the enzyme. This strategy capitalizes on the fact that the enzyme binds the transition-state tightest, and inhibitors whose structures are complimentary to that of the enzyme during the transition-state will be bound more tightly than those which resemble the substrate or the product. The design of transition-state analogues has been a dominant strategy for developing reversible inhibitors of HLE [14]. Modification of the peptide backbone of peptidic aldehydes had afforded highly potent HLE inhibitors.   N$^{\alpha}$-(1-adamantanesulphonyl)-N$^{\varepsilon}$-(4-carboxybenzoyl)-L-lysyl-L-alanyl-L-valinal was found to be a reversible and highly specific inhibitor of HLE (K_i = 0.06 µM) [20]. Aldehydes are relatively unstable and are liable to degrade by metabolic degradation. Replacement of the aldehydic proton by a different group would be expected to improve the stability. More importantly, if the replacement possessed a greater electron withdrawing capability, it would increase the

electrophilicity of the P_1 carbonyl group and thereby increase the potency of inhibitor [14].
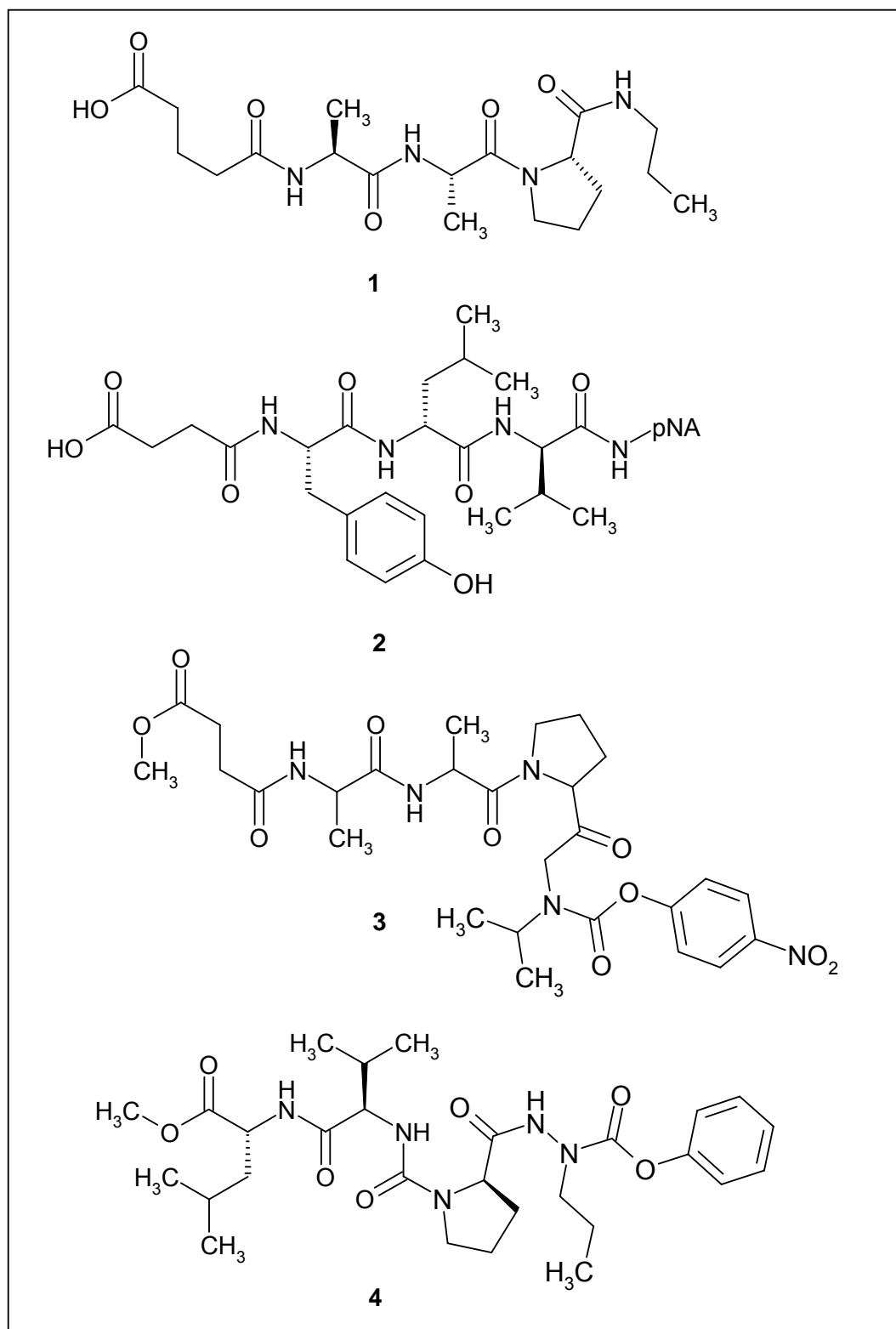
Fig. 3: Peptide based elastase inhibitors I
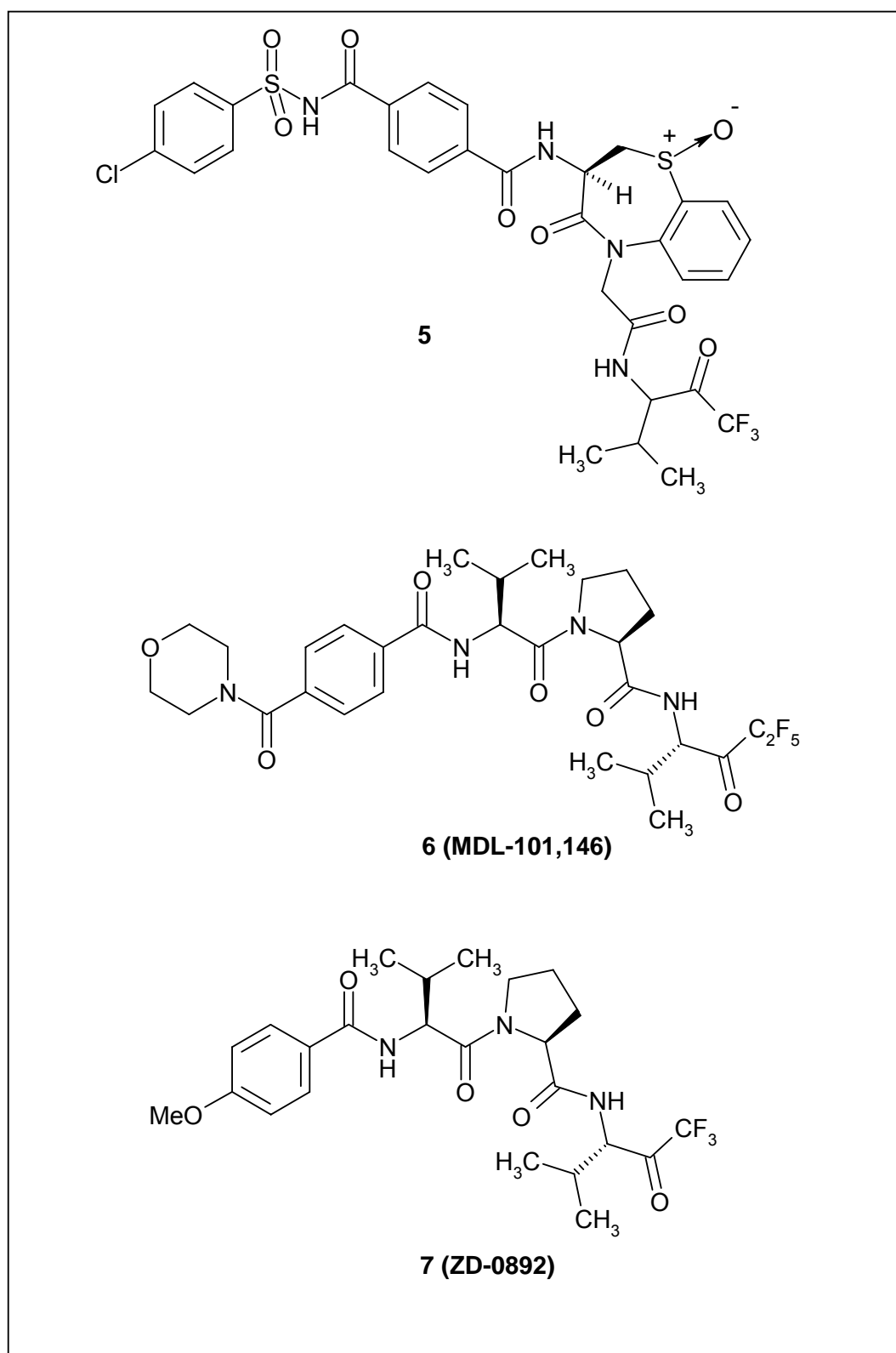
Fig. 4: Peptide based elastase inhibitors II
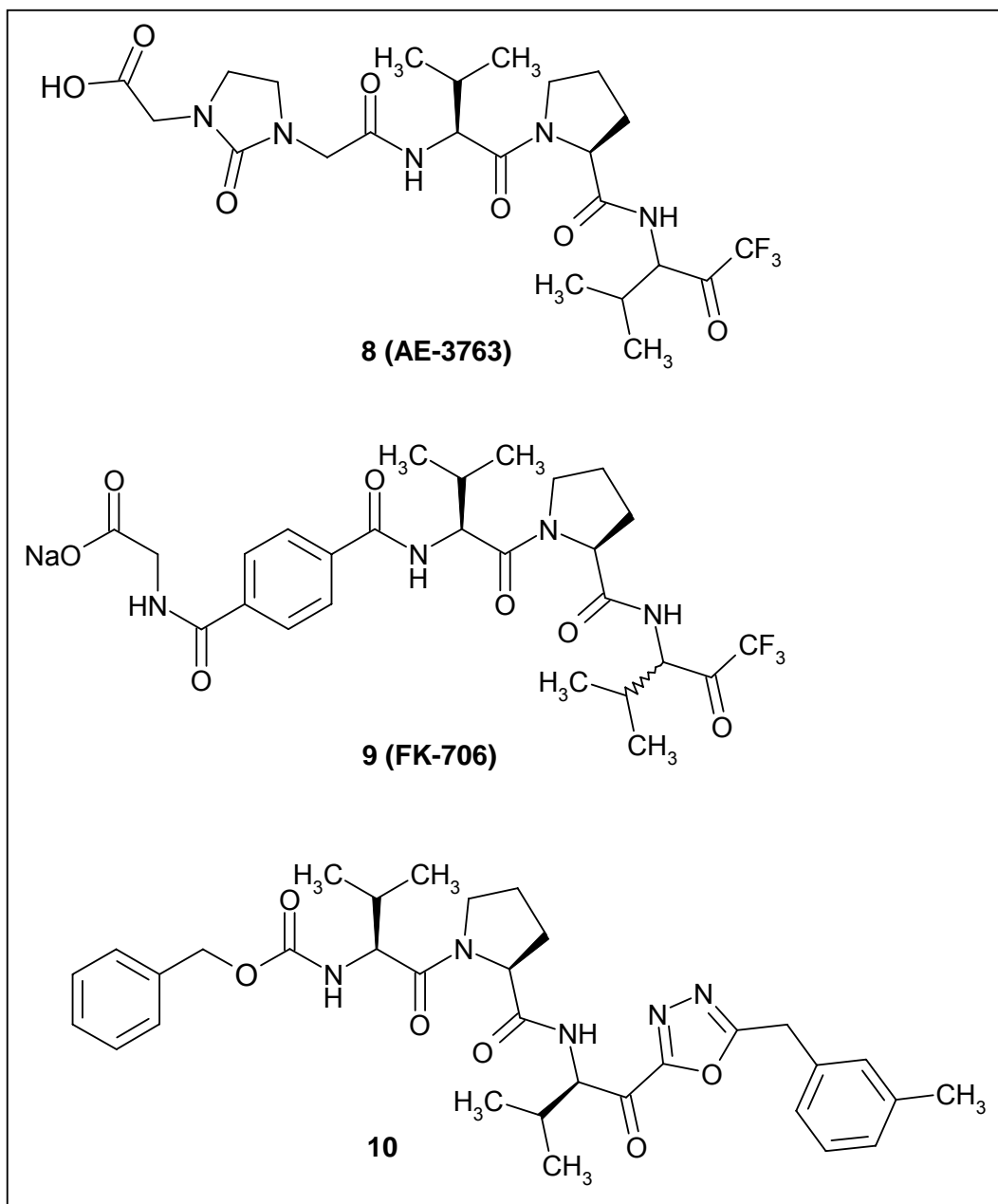
Fig. 5: Peptide based elastase inhibitors III

Investigating these mechanistic aspects has produced many highly potent trifluoromethyl and pentafluoroethyl ethyl peptidic inhibitors. Compound **5** (figure 4) was produced by Boehringer Ingelheim Pharmaceuticals and showed IC_{50} of 6.8 nM. This compound also showed in vivo activity upon administration [21]. **MDL - 101,146 (6)** is reported to posses oral activity with K_i of 25 nM [22]. Zeneca has patented peptidic trifluoromethyl ketones, which are both, orally active and obtained as a single diastereoisomer. **ZD-0892 (7)** is a potent inhibitor in vitro (K_i = 6.7 nM), and shows significant inhibition in an elastase-induced lung damage model [15].

Recently, **AE-3763 (8)** [13] (figure 5) (K_i = 3.2 nM) and **FK-706 (9)** [23] (K_i = 4.2 nM) were synthesized by Dainippon pharmaceuticals and Fujisawa Co Ltd respectively. **AE-3763** is in clinical development for the treatment of acute lung injury. Compound **FK-706** showed a competitive and slow-binding inhibition of HLE [23]. Researchers at Marion Merrell Dow research centre have developed pentafluoroethyl, heptafluoropropyl and nonafluorobutyl ketones, as the electrophilic entities. Most of the published data on these compounds are in the pentafluoroethyl ketone series [15]. EDWARD developed a series of heterocyclic ketones which activate the adjacent carbonyl group towards electrophilic attack by the hydroxyl group of Ser-195 of enzyme. He used benzoxazole as the heterocyclic component and elucidated the mechanism of the action of enzyme inhibition by probing the X-ray crystallographic studies on PPE-Inhibitor complex [3].

The extensive research at Cortech Inc. has led to the synthesis of one of the highest active elastase inhibitors. Compound **10** had a K_i value of 25 pM [13]. Boronic acid derivatives were developed as PPE and HLE inhibitors. Many of them have shown a high activity against elastase [24, 25].

Trifluoromethyl ketones, pentafluoromethyl ketones and aldehyde ketones have shown a reversible inhibition while the chloromethyl ketones are irreversible inhibitors of elastase. The two-step mechanism describes the enzyme inhibition by chloromethyl ketone types of compounds: formation of an initial reversible complex for which K_i can be calculated, followed by irreversible alkylation of His-57 [14, 26].

1.5.2. Heterocyclic Inhibitors

Heterocyclic inhibitors are those in which inhibition is the result of covalent attachment of a heterocyclic ring to one of the catalytic-site residues, usually with concomitant, temporary or permanent opening of the ring. In addition, this type of inhibitor does not utilize peptide recognition features for enzyme inactivation or selectivity. Small heterocyclic agents potentially offer advantages over the larger, peptide based inhibitors: increased proteinase stability, increased oral absorption and decreased structural complexity. However, heterocyclic inhibitors may suffer from increased hydrolytic instability and decreased enzyme selectivity. Enzyme-activated inhibitors and heterocyclic acylating agents are two classes of heterocyclic inhibitors [14].

1.5.2.1. Enzyme-activated inhibitors

Enzyme-activated inhibitors require initial covalent bond formation with a catalytic site residue to initiate a bond-breaking reaction within the heterocyclic ring which thereby

unmasks an even more reactive functionality. The newly generated reactive species then forms a second covalent bond with another catalytic residue. Later covalent bond is usually very stable and results in the irreversible inhibition [27]. Several substituted valero enol lactones are reported in 1992 as elastase inhibitors. Compound **11** (figure 6; K_a/K_i = 2.25 and 0.005 $\mu M^{-1} min^{-1}$ for HLE and PPE respectively) and **12** act as mechanism based inhibitors by forming an irreversible complex with elastase (alkylated enzyme) [28]. SPENCER developed ynenol lactones. Compound **13** (12000 ± 2000 $M^{-1} S^{-1}$) showed time and concentration dependent irreversible inactivation of HLE. Ynenol lactones that are both substituted α to the lactone carbonyl and unsubstituted at the acetylene terminus are rapid inactivators of HLE and inactivate PPE and trypsin more slowly [29]. Different 3-alkoxy-4-chloroisocoumarins, 3-alkoxy-4-chloro-7-nitroisocoumarins and 3-alkoxy-7-amino-4-chloroisocoumarins are developed as elastase inhibitors by POWERS [30]. Inactivation of HLE by 3-alkoxy-7-amino-4-chloroisocoumarin is irreversible, and less than 3% activity is regained upon extensive dialysis of the inactivated enzyme. The rate constant (K_{obsd} / [I]) was found to be 190000 for **14** by the progress curve method [30].

β-lactam based inhibitors are developed by the Merck group and have probably been the most intensely studied heterocyclic inhibitors of HLE, at least with respect to the breadth of the structure-activity investigations. Furthermore, the β-lactams are unique in that they are the only class of elastase inhibitors been used clinically for disease modification, albeit not yet for chronic diseases such as those associated with elastase [14]. The first reported β-lactam inhibitors were cephalosporins. Neutral cephalosporins (compounds not bearing the free carboxyl at position C-4) are reported by DOHERTY, and it was demonstrated that these derivatives can be potent time dependent inhibitors of HLE. Compound **15** showed IC$_{50}$ of 0.02 μgml^{-1} against HLE [31]. Dompe SpA, Synphar Laboratories and Japan Tobacco Inc. have developed different inhibitors based on β-lactams as acylating agents [15].

1.5.2.2. Heterocyclic acylating agents

Isatoic anhydrides act by promptly forming a covalent bond with the serine protease followed by a slow deacylation. The moderately active compound **16** [32] (figure 6) from this class is reported. Benzoxazinones based inhibitors show moderate activity against human neutrophil elastase, while *N*-arylbenzisothiazolinone 1,1-dioxides were found to form a ring opened acyl enzyme [33-35]. Compound **17** and **18** are potent inhibitors of HLE showing inhibition at IC$_{50}$ of 1 μgml^{-1} from these series [34, 35].
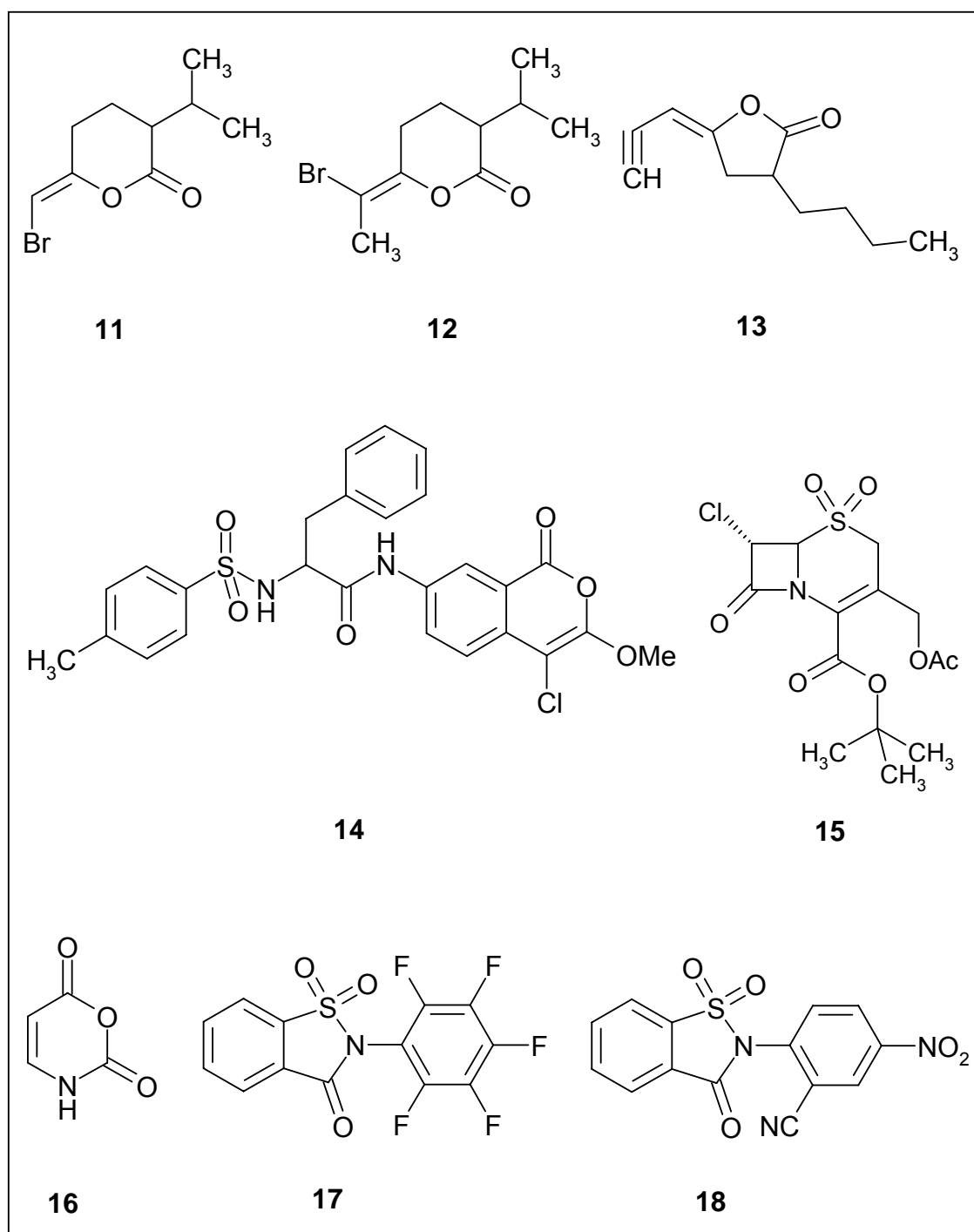
Fig. 6: Heterocycle based elastase inhibitors

1.5.3. Nonheterocyclic alkylating/acylating agents

Acylating or alkylating agents owe their inhibitory activity to the presence of a highly reactive functional group that forms a stable, and usually nonhydrolyzable, covalent bond with the Ser-195 [14, 36]. **Sivelestat (ONO-5046) (19)** (figure 7) is a competitive inhibitor (K_i = 0.2 µM) which suppresses lung damage induced in hamster by

elastase when administered intratracheally (ID 82 µg/Kg). Interestingly, when this drug was injected (100 mg/Kg/day/i.p) to arthritic rats, it protected cartilage from degradation and reduced the incidence and severity of collagen-induced arthritis. This compound has also been investigated for lung cancer and idiopathic interstitial pneumonia.

19 (ONO-5046, Sivelestat)   **20 (SR- 26831)**

21

22 (Midesteine, MR-889)
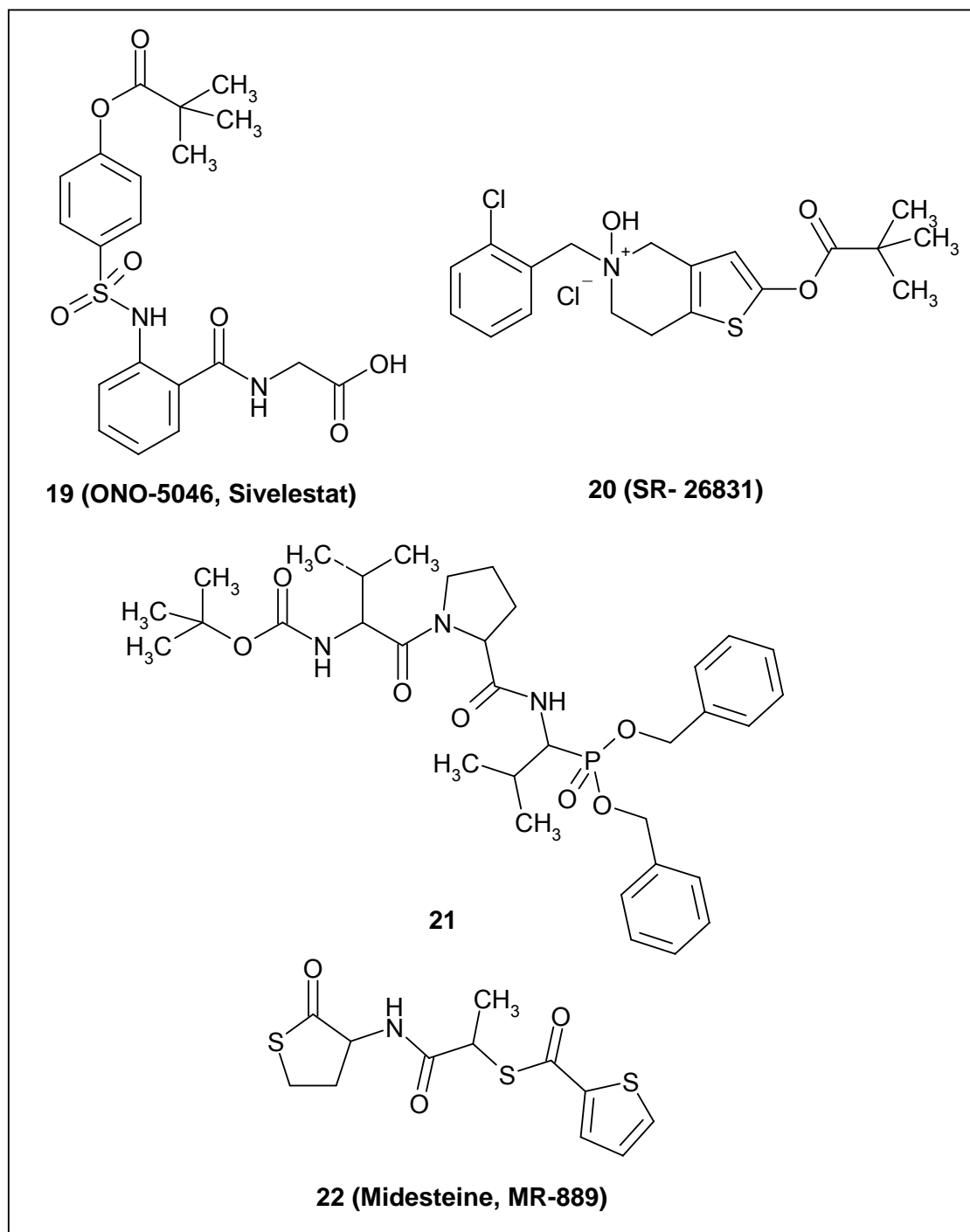
Fig. 7: Non-heterocyclic acylating or alkylating agents

Unfortunately, the bioavailability of **ONO-5046** is low which is mainly due to intestinal first pass metabolism. **Sivelestat** has been launched in Japan (2002) as an injectable formulation (**Elaspol**[®]) for the treatment of acute lung injury associated with systemic inflammatory response syndrome (SIRS) [16, 36, 37]. **SR-26831** (**20**), a tetrahydrothieno pyridine derivative is a potent non-competitive inhibitor of HLE and HPE (Human Pancreatic Elastase) showing activity in vitro with respective Ki values of 0.36 ± 0.08 and 3.5 ± 0.9 µM [38]. Peptidyl derivatives of diphenyl (*α*-aminoalkyl)phosphonates are specific and potent inhibitors of serine proteases. They are chemically stable, stable in plasma, do not react with acetylcholinesterase, and form very stable derivatives with the enzyme. Compound **21** ($K_{obsd}/[I]$ = 27000 $M^{-1}S^{-1}$, HLE) showed irreversible inhibition of HLE and PPE [39, 40]. **Midesteine** (**MR-889**) (**22**) is a reversible, slow binding, fully competitive serine protease inhibitor. It has some selectivity for neutrophil elastase (K_i = 1.4 µM), but also inhibits chymotrypsin. This compound is currently in preregistration (Italy, Medea Research) for the treatment of COPD. The active site of serine elastase is thought to attack the molecule at its thioester forming a thiophencarbonyl acyl enzyme complex, which resists further hydrolysis and effectively inhibits the enzyme. A crystal structure of the thiophencarbonyl adduct supports this hypothesis. A double-blind, randomised, placebo-controlled clinical trial in COPD patient showed that the compound was tolerated at dose of 500 mg b.i.d for 4 weeks [16, 41- 43].

2. Aim of the work

There is a vast interest in heterocyclic agents as elastase inhibitors. β-lactam based inhibitors have been developed by members of the research group of OTTO [44, 45]. Some saccharin derivatives are well reported elastase inhibitors [46-49]. The proposed mechanistic aspect of elastase inhibition reveals that the nucleophilic attack of Ser-195 of the enzyme on the carbonyl group of saccharin followed by the departure of the leaving group (indirect displacement) is essential (figure 8) [49].

Reactive intermediate

Reactivated HLE   +

LG is a leaving group
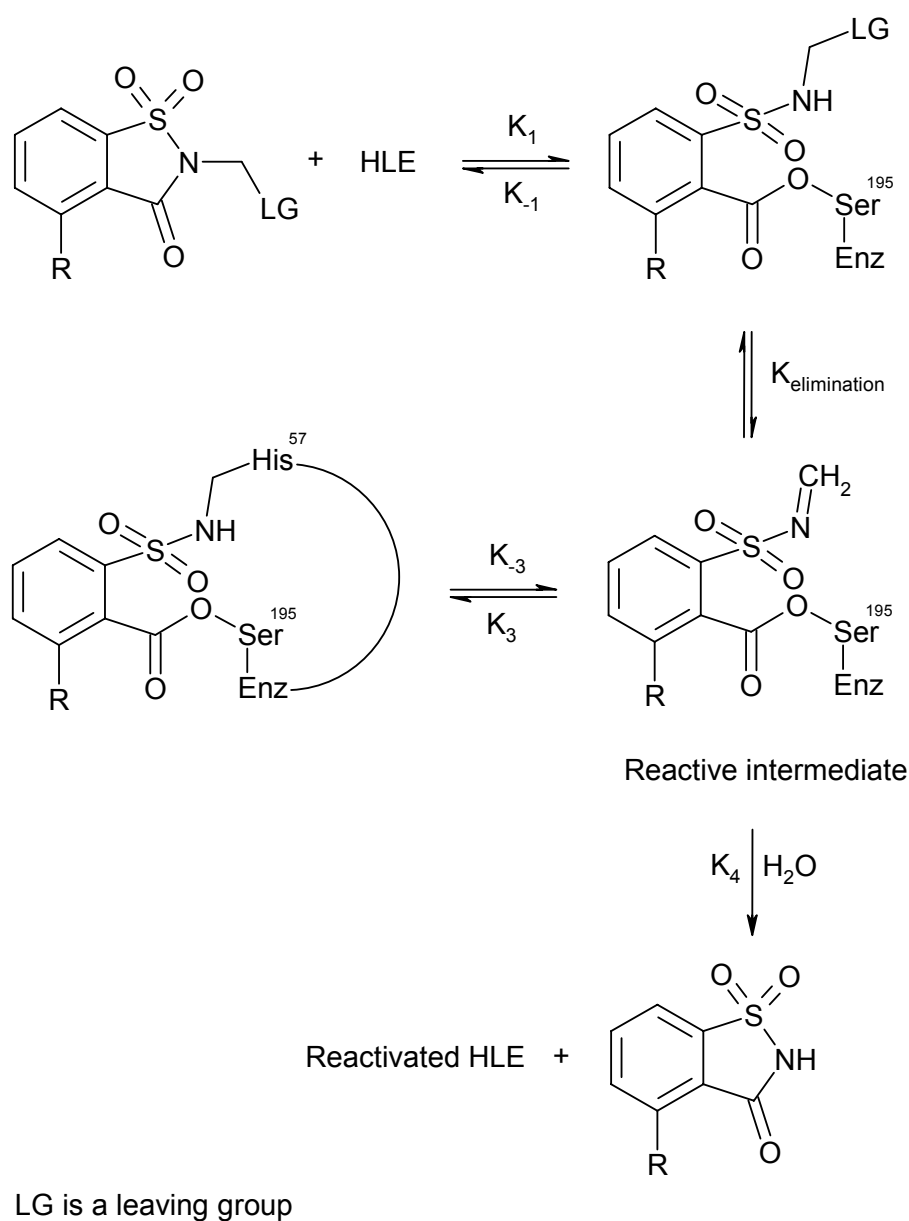
Fig. 8: Elastase inhibition by saccharin derivatives [49]

Here we planned to modify the saccharin derivatives in the following way.

1] Masking the carbonyl functionality of the saccharin nucleus

2] Reintroduction of a carbonyl functionality at the other part of the molecule along with a possible leaving group for the direct displacement (figure 9)

Hence, the structures **I** and **II** (figure 9) are planned for the synthesis and their screening against elastase activity. In these structures, X_1 and X_2 are different substituents. X_1 will be an alkyl or heterocyclic ring while X_2 will be different substituents. Peptide is planned for the synthesis in order to check the possibility of non covalent binding with the enzyme.

I   **II**

X_1 = Alkyl or heterocyclic substituents
X_2 = Different substituents
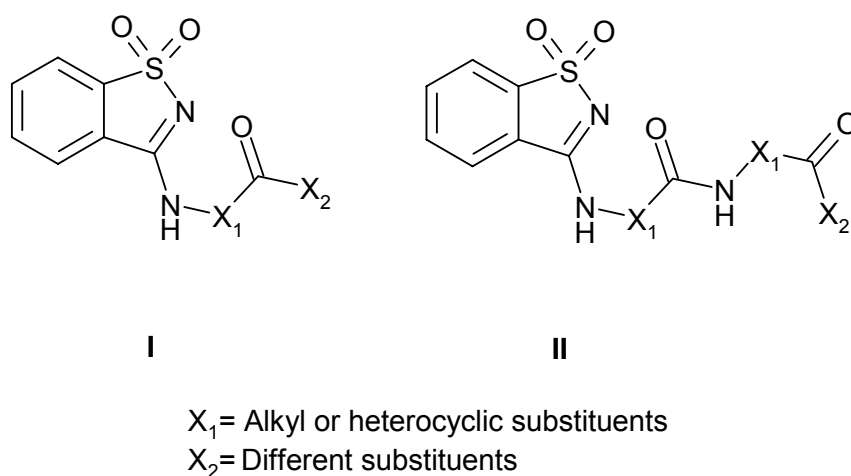
Fig. 9: Planned pseudosaccharinamine derivatives

3. Results and Discussion

3.1. Pseudosaccharinamines from (1,1-dioxobenzo[*d*]isothiazol-3-ylsulfanyl)acetonitrile

Pseudosaccharinamine was synthesised by SCHRADER in 1927 [50] from pseudosaccharin azide. He obtained *N*-phenyl-1,2-benzisothiazole-3-amine 1,1-dioxide by treatment of pseudosaccharin azide and aniline. The first use of 3-chloro-1,2-benzisothiazole 1,1-dioxide (pseudosaccharin chloride) was reported by MANNESSIER-MAMELI [51]. A Schiff's base was produced by the reaction of *N*-isocyanamine with saccharin and further addition of water leading to the formylation of the isonitrile group [52]. WHITEHEAD and TRAVERSO [53] prepared a number of 3-amino-1,2-benzisothiazole 1,1-dioxides for pharmacological evaluation as diuretic or hypoglycaemic agents. They obtained crystalline 3-amino-1,2-benzisothiazole 1,1-dioxides by refluxing saccharin with alkyl amines and arylamines at 130 °C for 8 h. Derivatives of lower boiling amines were synthesized by the amination of pseudosaccharin chloride.

Synthesis of thiosaccharin (**2**) [54] from saccharin (**1**) was carried out according to the literature (scheme 1). A relatively low yield of thiosaccharin was obtained from the reaction of **1** with P_2S_5 in refluxing dioxane, while the reaction without a solvent below 185 °C resulted in higher yields. In case of reaction in dioxane, water was added during work up and was present till 12 h, during that time the reconversion of thiosaccharin to saccharin might have reduced the yield of thiosaccharin. INOMATA [55] observed the reaction between the sodium salt of thiosaccharin and alkyl halides to give the 3-alkylthiopseudosaccharin, a crystalline thiol equivalent. The thiol was then liberated by treatment with piperidine. WHITEHEAD reported the formation of 3-hydrazino-1,2-benzisothiazole 1,1-dioxide from 3-methylmercapto-1,2-benzisothiazole 1,1-dioxide which was initially obtained from 3-mercapto-1,2-benzisothiazole 1,1-dioxide and aq. NaOH [56]. Amide proton abstraction by NaH followed by the addition of chloroacetonitrile in DMF resulted in *S*-alkylation forming (1,1-dioxo-1*H*-λ^6-benzo[*d*]isothiazol-3-ylsulfanyl)acetonitrile (**3**). Activation of the nitrile group of **3** by HCl in MeOH (over 1 week) did not result in an imido ester formation, indicating that under these conditions the nitrile group was not attacked by the nucleophile and the starting material was recovered. When the reaction of **3** with valine amide in the presence of CuCl was done at 80 °C in DMSO, the amidine was not produced indicating that the nitrile group is not prone to nucleophilic attack.
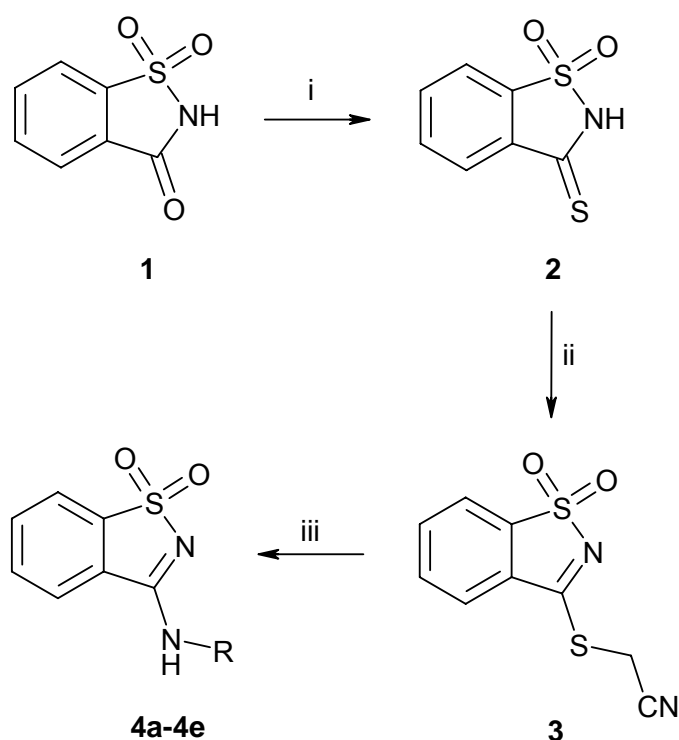
Scheme 1: Synthesis of pseudosaccharinamines from **3**
Reagents and conditions: (i) P_2S_5, heat or P_2S_5, dioxane, reflux, 6 h; (ii) NaH, DMF, 0° C; $ClCH_2CN$, rt, 10 h / reflux, 2 h; (iv) $DMSO/MeOH/CHCl_3/CH_2Cl_2$, amine/(amine HCl, TEA), rt

Table 1: Synthesis of pseudosaccharinamines from **3**

R	Reaction conditions	Yield (%)	Product
$CH[CH_2CH(CH_3)_2]CONH_2$	2 h, DMSO	13	**4a**
	24 h, MeOH	36	
	6 days, $CHCl_3$	68	
$CH(CH_2C_6H_5)CONH_2$	2 h, DMSO	21	**4b**
	5 h, MeOH	16	
	11 days, $CHCl_3$	60	
H	40 min, MeOH	48	**4c**
$CH(CH_2CH_2SCH_3)COOCH_3$	2 h, DCM	17	**4d**
$CH[CH_2CH(CH_3)_2]COOCH_3$	2 h, DCM	31	**4e**

In order to obtain the pseudosaccharinamines, different amines were treated with **3** (scheme 1). To optimize yields and conditions, reactions of different amino acid

derivatives and ammonia with **3** were carried out in different solvents, and under different conditions, to give the *N*-substituted derivatives **4a-4e**. Best results were obtained from reactions in CHCl$_3$ (table 1, scheme 1). In order to obtain pure **4**, CC was often required. Compounds **4** show tautomerism (figure 10), as the exocyclic nitrogen atom is part of a sulfonamidin function, and therefore, they do not form hydrochlorides. In solution the first tautomeric form (**I**) seems to be preferred. In the ^{1}H NMR spectra, a doublet is observed at δ 9.46 ppm (**4a**) for a proton on the nitrogen of the sulfonamidin functionality. Similarly in **4b**, **4d** and **4e**, the proton of the sulfonamidin functionality showed a doublet at δ 9.62, 9.68, and 9.69 ppm respectively.
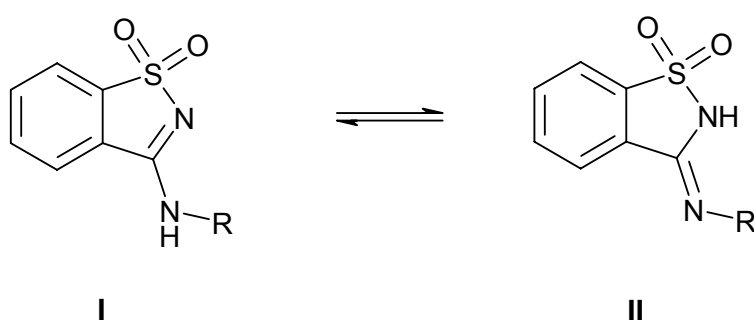
Fig. 10 Tautomerism in **4**

In the ^{1}H NMR spectrum of **4a**, a broad singlet was observed for the two protons of the amide while the α-H of leucine appeared as a multiplet at δ 4.63-4.66 ppm. β and γ protons of leucine appeared as a multiplet around δ 1.93-1.55 ppm. The two methyl groups appeared as two separate doublets at δ 0.93 and 0.90 ppm with a coupling constant of 5.81 Hz. Compounds **4a**, **4b**, **4d** and **4e** were analysed by HPLC using Chiralcel-OJ-R column. Their structures were further confirmed by ^{1}H NMR spectra.
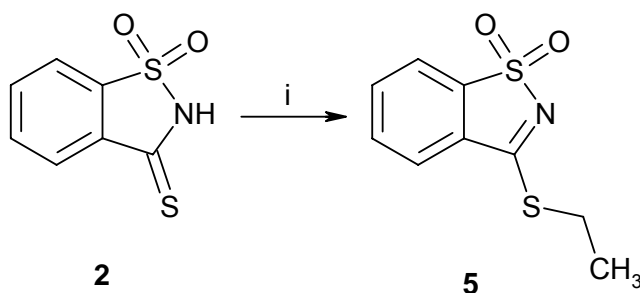
Scheme 2: Synthesis of *S*-ethyl pseudosaccharin
Reagents and conditions: (i) NaH, 0 °C, DMF, ethyl bromide, reflux, 2 h

The reaction between thiosaccharin (**2**) and ethyl bromide in the presence of NaH led to *S*-ethyl pseudosaccharin (**5**) (scheme 2) which was reported by MEADOW [54] from ethyl sulfate and thiosaccharin.

Additionally, we tried to obtain an isomer of **3**, thiosaccharin-2-acetonitrile (**9**), via the saccharin-2-acetonitrile (**7**), which was synthesized from saccharin sodium (**6**) and chloroacetonitrile in DMF (scheme 3). The reaction of **7** with P_2S_5 was not successful. Instead, the yellow compound (**8**) melting at 190-192 °C was isolated, indicating that the reaction had occurred at the carbonyl as well as at the nitrile group of **7**. Using two equivalents of P_2S_5 the yield of **8** was slightly increased. Hence, even this route was not appropriate for the synthesis of thiosaccharin-2-acetonitrile from **7**.
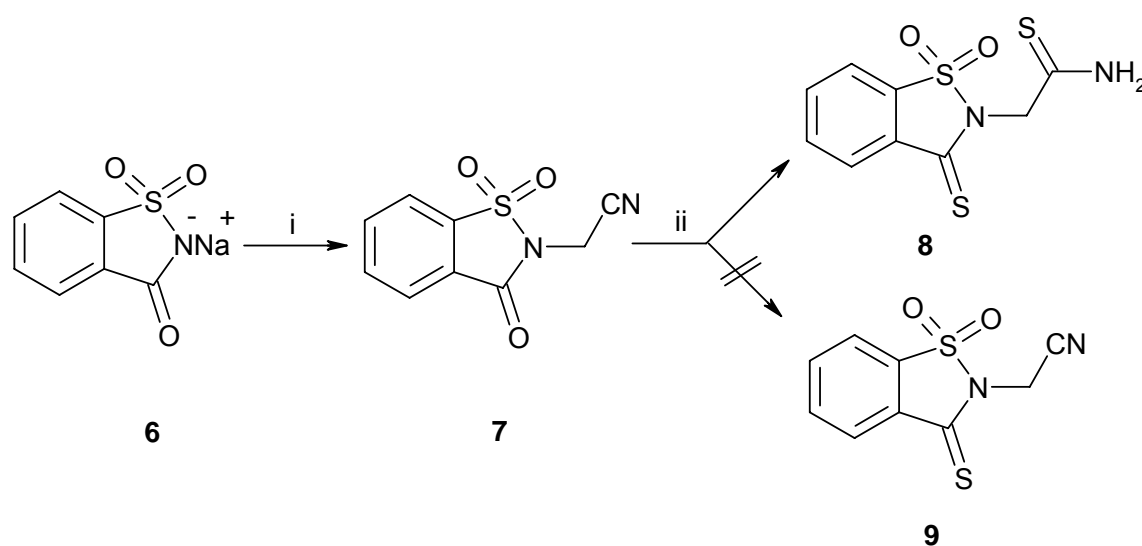
Scheme 3: Synthesis of **8**
Reagents and conditions: (i) ClCH$_2$CN, DMF, 100 °C, 4 h; (ii) P_2S_5, dioxane, reflux, 4 h

In the ^{13}C NMR spectrum of **8** (figure 11), a signal of the methylene carbon appeared at δ 49.79 ppm while aromatic carbons signals appeared at δ 121.21, 126.36, 129.83, 130.35, 135.15 and 135.25 ppm. Additional signals were observed at δ 195.81 ppm and δ 186.34 ppm and these peaks were absent in the DEPT spectrum, confirming the presence of two thio moieties in the structure. Further proof of the structure came from a mass spectrum of the compound where a molecular ion peak was found at 271.8 (272.37, calculated). In the ^{1}H NMR spectrum of **8** a singlet was observed at δ 4.95 ppm for the methylene protons while the aromatic protons were seen as two multiplets at around δ 8.36-7.96 ppm. The elemental anlysis of **8** is in aggrement with that of the calculated values. In the IR spectrum of **8**, C=N absorption was observed at 1637 cm^{-1} while the absorption of C=S was found at 1138 cm^{-1}.
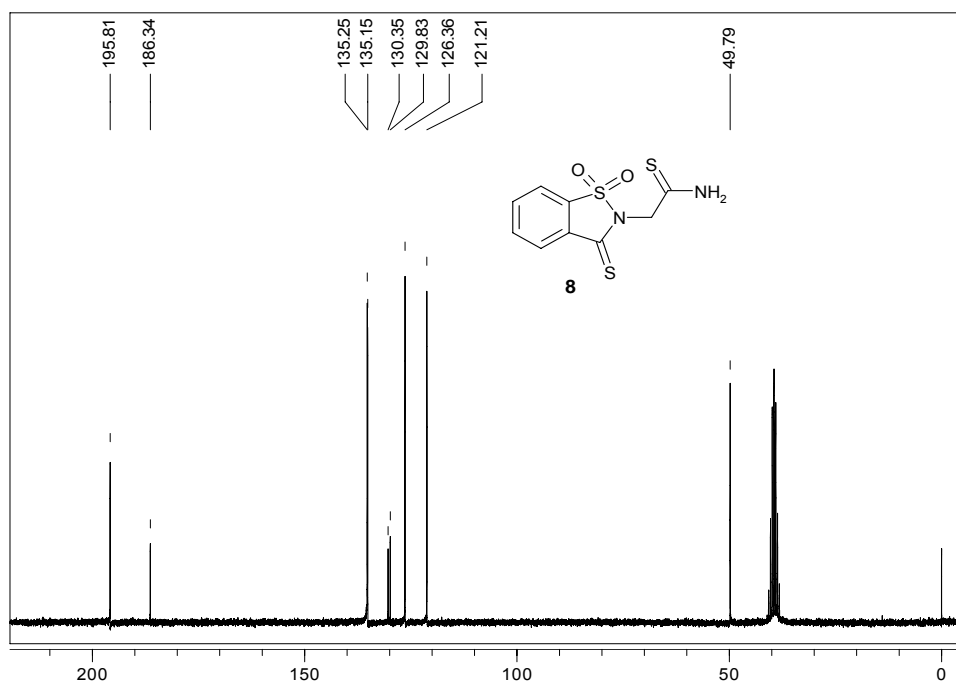
Fig. 11: ^{13}C NMR spectrum (50 MHz, 25 °C) of **8** in [D$_6$]DMSO

All pseudosaccharin derivatives **4** were characterized by their IR spectra. The absorption at $\approx$ 3400-3300 cm^{-1} was caused by NH, and absorption bands at 1140-1190 cm^{-1} and 1320-1390 cm^{-1} are due to the SO$_2$ group. A characteristic absorption between 1600 and 1640 cm^{-1} was attributed to the C=N moiety. The C=N band absorption for **4c** was shifted to a higher value, and was observed at 1679 cm^{-1}. The spectra of **4a** and **4b** showed two NH bands due to the primary amide and sulfonamidin group. The carbonyl absorptions were observed at 1675-1695 and 1740-1760 cm^{-1} due to amide and ester respectively.

3.2. Pseudosaccharinamine synthesis from thiosaccharinates

MANNESSIER-MAMELI reported a number of aryl-ammonium saccharinates while studying the difference in the behaviour of saccharin and thiosaccharin towards anilines [57-59]. Anisidines (*ortho* and *para*), phenetidines (*ortho* and *para*), naphthylamines (α and β), *para*-aminodimethylaniline and *para*-xylidine have been employed in the preparation of *ortho*-*N*-arylsulphonamides. The sulphonamides were also obtained from saccharin and aromatic amines by gentle boiling. At high temperature the sulphonamides yielded pseudosaccharinamines [60].
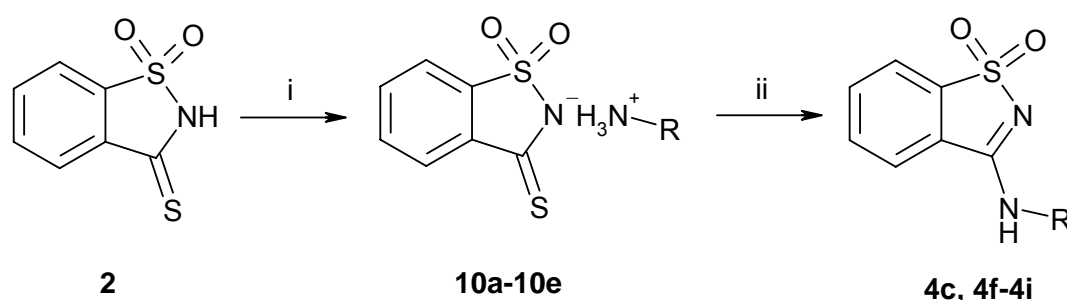
Scheme 4: Synthesis of **4** from **10**
Reagents and conditions: (i) Amine, MeOH, rt, 2 h; (ii) AcOH, reflux, 3-5 days

Table 2: Synthesis of **4** from **10**

Compound	R	Reaction time (days)	Yield (%)
4c	H	4	20
4f	C_6H_5	3	33
4g	$CH_2C_6H_5$	3	41
4h	C_6H_{11}	3	25
4i	$CH_2CH(CH_3)_2$	5	31

The synthesis of pseudosaccharinamines was carried out as shown in scheme 4, where the thiosaccharin was first converted into thiosaccharinates (**10a**-**10e**) by treating thiosaccharin (**2**) with different amines in methanol at rt for 2 h. Thus **10a**-**10e** were obtained as yellow crystalline solids. These salts upon acidification with aq. hydrochloric acid precipitated the thiosaccharin back. In the ^{1}H NMR spectra of thiosaccharinates, the amine protons were identified by a proton exchange with D_2O. The thiosaccharinates, **10**, were refluxed in glacial acetic acid for several hours (or

days) and compounds **4c, 4f-4i** were obtained. This reaction may be explained by equilibrium between **10**, ammonium acetate, **2** and the free amine, wherein the free amine attacks **2** forming **4**. The reaction was very slow and often required days and yields were low, ranging from 20 to 41% (scheme 4, table 2).

3.3. Pseudosaccharinamine synthesis from 3-ethoxybenzo[*d*]isothiazole 1,1-dioxide

In section 3.1, the synthesis of 3-amino pseudosaccharin from (1,1-dioxobenzo[*d*]isothiazole-3-ylsulfanyl)acetonitrile (**3**) is described. In this reaction a by-product, thiol, is also produced. The thiol has an unpleasant odour, and the reaction often required a CC in order to get the pure pseudosaccharinamines. Therefore, a strategy was designed to synthesise the pseudosaccharinamines from 3-ethoxybenzo[*d*]isothiazole 1,1-dioxide (**12**). By doing so two objects can be achieved, first, the unpleasant odour of the by-product will not be there as ethanol will be formed as a by-product, and, secondly, the by-product ethanol can be easily removed from the reaction mixture by just evaporating in vacuo and hence, possibly, CC will not be required.

The synthesis of 3-hydrazino-1,2-benzisothiazole 1,1-dioxide from 3-methoxy-1,2-benzisothiazole 1,1-dioxide is reported by WHITEHEAD et al [56] while the use of 3-(phthalimidoxy)-1,2-benzisothiazole 1,1-dioxide in the preparation of pseudosaccharin amine is reported by NOGUCHI et al [61] although in lower yield. In the literature an unexpected nucleophilic substitution was reported while studying the oxidation of sulfides to sulfoxides by H_2O_2 in the presence of a catalytic amount of 3-ethoxy-1,2-benzisothiazole 1,1-dioxide [62-63]. This unexpected nucleophilic participation and rearrangement of DBU was observed by PAGE [62, 63] while carrying out the oxidation of sulfides to sulfoxides by H_2O_2 (30% V/V) with DBU as a base and 3-ethoxy-1,2-benzisothiazole 1,1-dioxide as a catalyst. The unexpected formation of 3-[3'-(1"-azepin-2"-onyl)propylamino]-1,2-benzisothiazole 1,1-dioxide can be explained by a nucleophilic attack by one of the nitrogens of the DBU at the 3rd position of 3-ethoxy-1,2-benzisothiazole 1,1-dioxide, displacing the ethoxy anion, and further rearrangement in water to produce the unexpected product in 8% yield [62]. There is no direct intentional mention of the use of 3-ethoxy-1,2-benzisothiazole 1,1-dioxide for the synthesis of pseudosaccharinamine.

In order to obtain 3-ethoxybenzo[*d*]isothiazole 1,1-dioxide (**12**), a reaction of **6** with bromoethane was studied in DMF at 30-40 °C (scheme 5, table 3). The reaction led to the *N*-ethyl saccharin (**11**) and not to *O*-ethyl pseudosaccharin (**12**). Hence, at the given set of conditions, the *N*-alkylated product was isolated indicating that the nucleophilic attack was executed via the nitrogen of the saccharin anion on bromoethane. Thiosaccharin, saccharin, 3-aminobenz[*d*]isothiazole 1,1-dioxide all have in common that their anions are ambident (ambifunctional) [64, 65]. The ambident anion of saccharin is shown in figure 12.
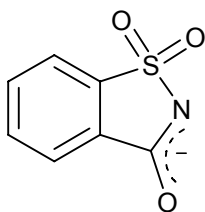
Fig. 12: Ambident anion of saccharin

The following generalization about the alkylation of anions possessing two different reactive positions (i.e. ambident anions) provides a simple rationale for the hitherto unsolved problem of carbon vs. oxygen alkylation, oxygen vs. nitrogen alkylation, etc. in anions derived from acetoacetic ester, phenols, nitroparaffins, α-pyridone, acid amides, thioamides etc: the greater the S_N^1 character of the transition state the greater is the preference for covalency formation with the atom of higher electronegativity and, conversely, the greater the S_N^2 contribution to the transition state the greater is the preference for bond formation to the atom of lower electronegativity [65]. Theoretical consideration of the reactions involving ambident anions is given by GOMPPER [64].

The saccharin anion (figure 12) may react via nitrogen or via oxygen in a nucleophilic reaction depending on such factors as temperature, the electrophile, the cation and the solvent [64, 65]. Reaction of sodium saccharin with alkyl halides in toluene with hexadecyltrimethylammonium bromide (HDTMAB) produces the *O*-alkyl product [66]. These *O*-alkyl products are also reported by MEADOE [67] from pseudosaccharin chloride and alcohols. The silver salt of saccharin promotes the *O*-alkylation [68] presumably through attack by silver on the halogen of the alkyl halide. Reaction of **6** with bromoethane in different reaction conditions resulted into *O*- and *N*-alkyl products (**11, 12**) (scheme 5, table 3).

When the reaction was carried out in DMF at 30 to 40 °C, only the *N*-substituted saccharin (**11**) was obtained, and the same result was obtained when the BTAC (benzyl triethylammonium chloride) was used and the reaction temperature was increased to 100 °C (entry 1 and 2, table 3). Both isomers, **11** and **12,** were produced in the ratio of 80:20 respectively when the reaction was carried out in the presence of HDTMAB (entry 3, table 3). When this reaction was allowed to continue till 4 days the overall yield was decreased and the ratio was found to be 75:25 for **11** and **12** respectively (entry 4, table 3). The reaction of the ambident anion (figure 12) with the alkyl chloride is enhanced in the presence of sodium iodide [69, 70]. The saccharin anion when reacted with bromoethane in the presence of KI in THF produced both isomers.
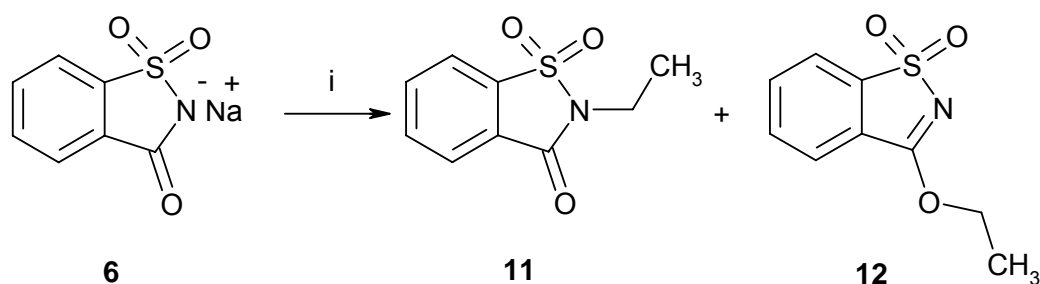
Scheme 5: Synthesis of *N*-ethyl saccharin (**11**) and *O*-ethyl pseudosaccharin (**12**) Reagents and conditions: (i) DMF/THF/Toluene, $BrCH_2CH_3$, catalyst (see table 3)

Table 3: Synthesis of **11** and **12** from **6** and bromoethane (in entry 5, **1** is used instead of **6**). The ratio of isomers is based on ^{1}H NMR measurements.

Entry No.	Catalyst	Reaction conditions	Yield (**11+12**, %)	**11:12**
1	-	DMF, 30-40 °C, 6 h	75	100:0
2	BTAC	DMF, 100 °C, 14 h	97	100:0
3	HDTMAB	DMF, 100 °C, 1 h	63	80:20
4	HDTMAB	DMF, 100 °C, 4 days	39	75:25
5	KI	THF, TEA, reflux, 2 days	17	60:40
6	HDTMAB	Toluene, reflux, 4h	06	0:100

When the reaction of **6** and bromoethane was carried out in toluene in the presence of HDTMAB, only the *O*-alkyl isomer was produced, but in low yield. The presence of both isomers (entry 3, 4, 5 of table 3) was clarified by ^{1}H NMR and HPLC analysis (figure 13, 14).

The two quartets at δ 4.66 and 3.86 ppm having a coupling constant of 7.20 Hz were seen in the ^{1}H NMR spectrum of the compound obtained from the reaction of **6** with bromoethane in the presence of HDTMAB (entry 4, table 3 and figure 13). One quartet appeared at higher chemical shift (δ 4.66 ppm) which is caused by the CH_2 group of **12**, as the CH_2 group is attached to the oxygen atom which is more electronegative than the nitrogen. The other quartet was seen at δ 3.86 ppm assignable to structure **11** where the CH_2 group is attached to the nitrogen atom. The similar situation was observed in case of the CH_3 groups (expanded region of the ^{1}H NMR spectrum in figure 13), where one triplet is observed at δ 1.54 ppm (*J* = 7.20 Hz) and the other at δ 1.45 ppm (*J* = 7.20 Hz) assignable to **12** and **11** respectively. The assignment of these structures was confirmed by comparing the spectra of the

two separate isomers obtained from two different reactions (entry 1 and 6, table 3). The presence of two isomers is also clearly visible by HPLC analysis (figure 14).
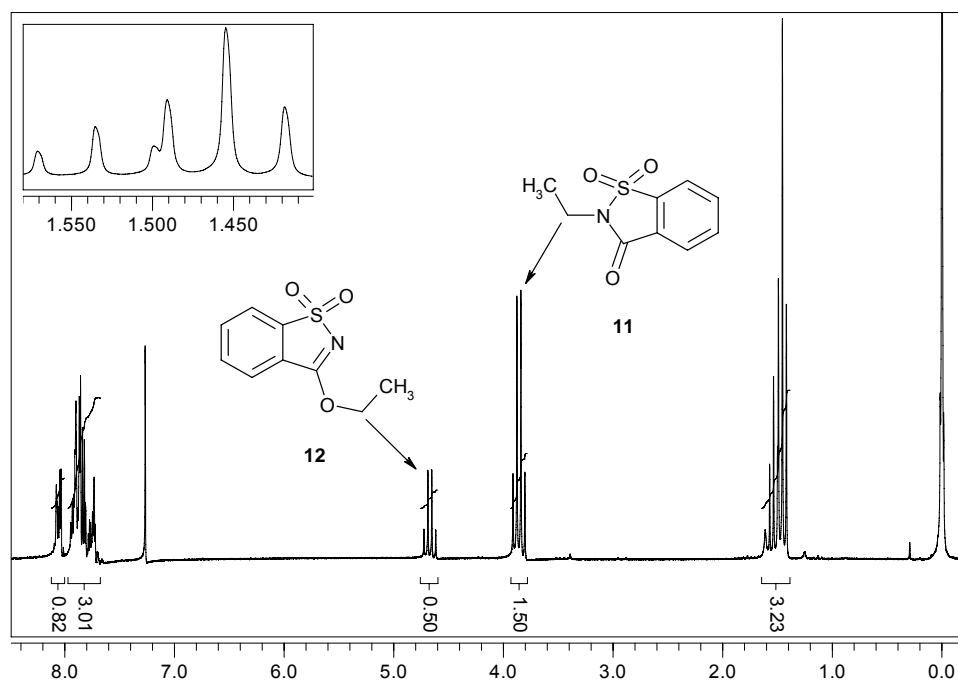
Fig. 13: ^{1}H NMR spectrum (200 MHz, 25 °C) of a mixture of *O*-alkyl (**12**) and *N*-alkyl (**11**) isomers (entry 4 in table 3) in CDCl$_3$
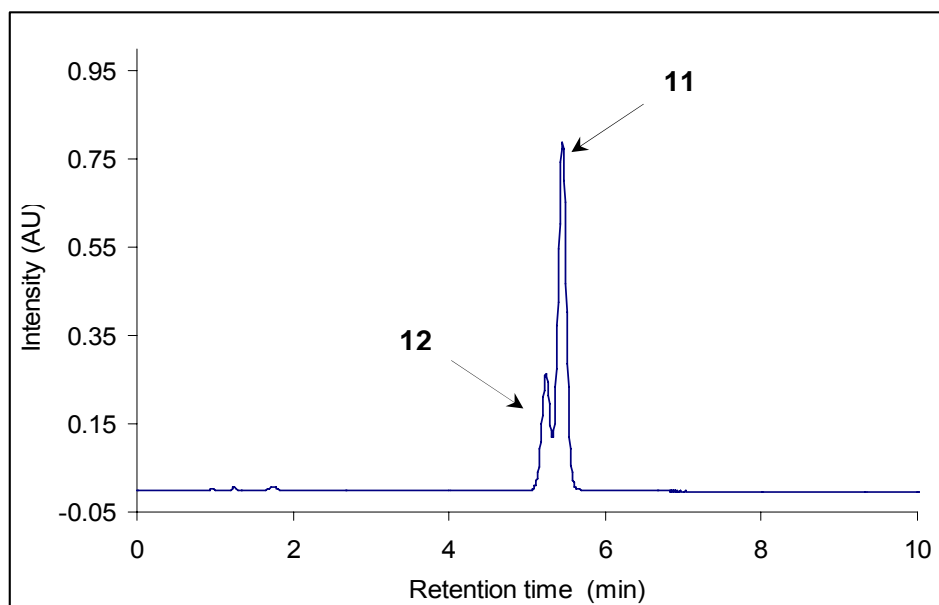
Fig. 14: Chromatogram of the product obtained as a mixture of two isomers (**11** and **12**, entry 4 in table 3) using a RP-18 column with MeCN/Water 1:1

In its IR spectra, **11** showed a strong absorption of carbonyl group at 1731 cm^{-1} and no absorption was observed for a C=N group; while in the IR spectrum of **12**, a strong absorption of C=N was observed at 1612 cm^{-1} which is a peculiar feature of the C=N of the five membered ring in pseudosaccharin derivatives. Compound **11** melted at 90-91 °C (93.5-94.5 °C) [70] while **12** showed a melting point at 211-214 °C (219 °C) [67].
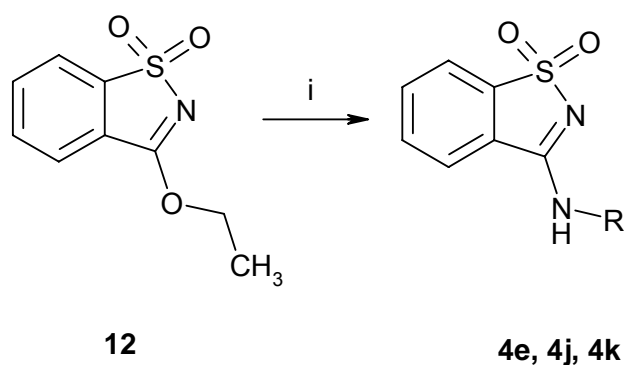
12   **4e, 4j, 4k**

Scheme 6: Synthesis of **4** from **12**
Reagents and conditions: (i) RNH$_2$, dioxane, reflux, one day

Table 4: Synthesis of **4e**, **4j**, **4k** from **12** along with chemical shifts (δ) of the α-H in ppm and coupling constants (*J*) in Hz

Compound	R	Yield (%)	[$\delta_{\alpha\text{-H}}$, *J*]
4e	CH[CH$_2$CH(CH$_3$)$_2$]COOCH$_3$	55	4.72-4.55 (m)
4j	CH[CH(CH$_3$)C$_2$H$_5$]COOCH$_3$	36	4.49 (t, 7.81)
4k	CH$_2$COOCH$_3$	52	4.32 (d, 5.70)

The desired pseudosaccharinamines were obtained by refluxing **12** with the amino acid methyl ester hydrochloride and TEA in dioxane for one day (scheme 6). The isolated product was pure and did not require CC.

The products **4e**, **4j**, **4k** were characterized by ^{1}H NMR, HPLC and elemental analysis. A doublet was observed at δ 9.69 ppm (*J* = 7.77 Hz) for the NH proton of the sulfonamidin functionality and a singlet at δ 3.71 ppm for the methoxy protons in the ^{1}H NMR of **4e**; while in **4j**, a doublet at δ 9.57 ppm (*J* = 7.81 Hz) for the NH proton and a singlet for the methoxy protons at δ 3.71 ppm were observed. A α proton of **4j** was well resolved and showed a triplet at δ 4.49 ppm with a coupling constant of 7.81 Hz. The spectrum of **4k** in [D$_6$]DMSO was well resolved and a

doublet was observed at δ 4.32 ppm for the α protons with a coupling constant of 5.70 Hz, while the NH proton showed a triplet at δ 9.93 ppm (J = 5.70 Hz).

Compound **4e** was obtained in 55% yield from **12** which is higher when compared with its synthesis from (1,1-dioxobenzo[*d*]isothiazole-3-ylsulfanyl)acetonitrile (**3**) (scheme 1, table 1), indicating a better possibility for the synthesis of pseudosaccharinamines from 3-ethoxybenzo[*d*]isothiazole 1,1-dioxide (**12**).

3.4. Pseudosaccharinamine derivatives from 3-chlorobenzo[*d*]isothiazole 1,1-dioxide and their further modifications

3.4.1. Pseudosaccharinamines

3-Chlorobenzo[*d*]isothiazole 1,1-dioxide (**13**) was obtained from **1** on treatment with $SOCl_2$ in the presence of a catalytic amount of DMF [71] (scheme 7).

1   **13**

4c   **4l**, R = 2-Cl   **4q**
                4m, R = 4-Cl
                4n, R = 3,4-$(OCH_3)_2$
                4o, R = 2-OCH_3
                4p, R = 3-CF_3
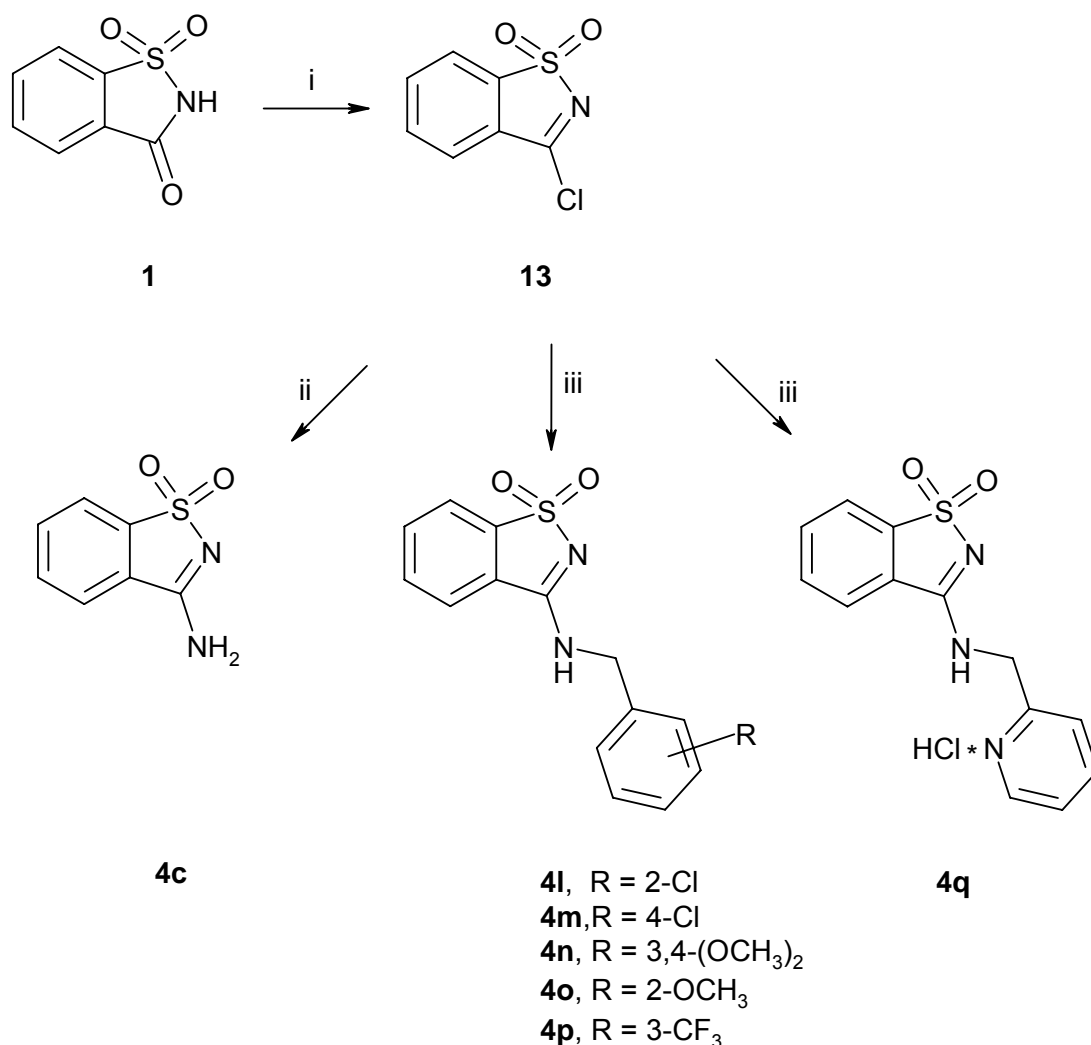
Scheme 7: Synthesis of pseudosaccharinamines from pseudosaccharin chloride Reagents and conditions: (i) $SOCl_2$, dioxane, cat. DMF, reflux, 2 days; (ii) Methanolic ammonia, rt, 2 h; (iii) Amine, dioxane, reflux, 4-5 h

An effective synthesis for **4** was carried out using **13**. Thus obtained compounds are relatively stable. The compounds **4l-4q** were obtained in moderate to good yields when amines were refluxed with **13** in dioxane (scheme 7). Compound **4q** was isolated as the hydrochloride. This compound dissolves in water. It's ^{1}H NMR

spectrum showed a very broad singlet at δ 6.60 ppm for HCl, a triplet at δ 10.59 ppm for NH of sulfonamidin and a doublet at δ 4.99 ppm for the methylene protons. **4q** underwent the D_2O exchange. The broad singlet disappeared when a drop of D_2O was added into the NMR sample. Furthermore, the NH proton disappeared and the doublet at δ 4.99 ppm collapsed into a singlet.

3.4.2. Amide and methyl ester derivatives

In order to obtain the target molecules, we synthesized different amino acid methyl esters. These amino acid methyl esters were obtained in high yields by reacting different amino acids with $SOCl_2$ in methanol, first at -10 °C and then refluxing the reaction mixture (scheme 8). All amino acid methyl esters were obtained as solids except the L-proline methyl ester.
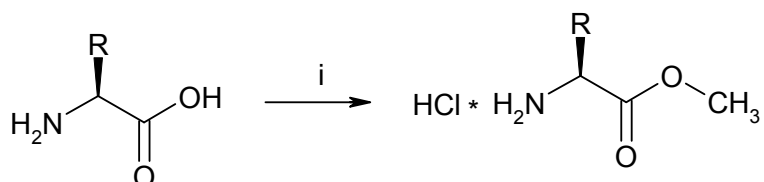
Scheme 8: Synthesis of amino acid methyl esters
Reagents and conditions: (i) MeOH, -10 °C, $SOCl_2$, reflux, 2 h. Note: The amino acids used were glycine, L-phenyl alanine, L-leucine, L-isoleucine, L-alanine, L-methionine, L-valine, α-methyl alanine, L-aspartic acid and L-proline.

The desired pseudosaccharinamine amides (**4a, 4b, 4r-4t**) and esters (**4d, 4e, 4j, 4k, 4u-4z**) were obtained as solids by reacting amides and amino acid methyl esters with **13** (scheme 9, table 5). TEA was used in order to liberate a free base wherever amine hydrochloride was used. **4s** was purified by CC with AcOEt/PE, 9/1. The similar compounds which were synthesized from different routes (section 3.1, 3.3) showed identical spectra.

All ester and amide derivatives were characterized by IR, NMR, HPLC and elemental analysis. In the ^{1}H NMR spectrum of **4y** in $CDCl_3$, two singlets were appeared for two methoxy group at δ 3.84 and δ 3.72 ppm, the β-CH_2 protons appeared as a doublet at δ 3.21 ppm (J = 4.10 Hz), the α-H proton appeared as a triplet of doublet at δ 5.08 ppm (J = 7.90, 4.10 Hz) while the sulfonamidin proton appeared as a doublet at δ 7.30 ppm (J = 7.90 Hz) and the aromatic protons appeared as two multiplets at δ 7.96-7.86, 7.81-7.65 ppm. The ^{1}H NMR spectra of these ester and amide derivatives (scheme 9, table 5) indicates that they are present in their sulfonamidin **I** form (figure 10).
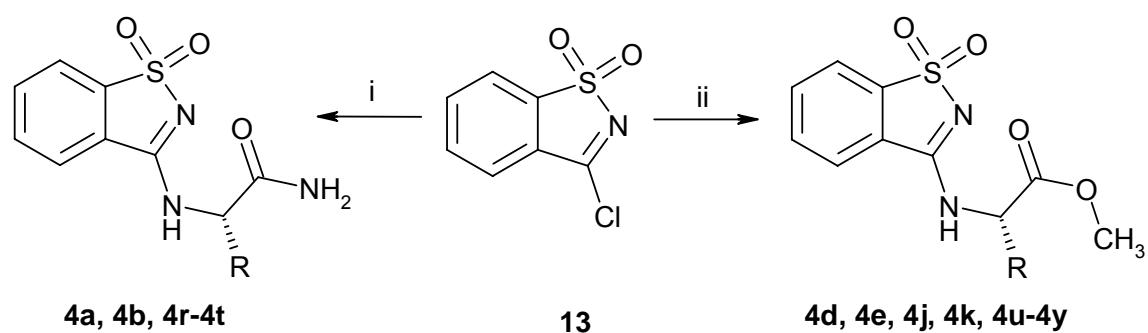
Scheme 9: Synthesis of the amide and ester derivatives
Reagents and conditions: (i) Amino acid amide hydrochloride, TEA, dioxane, reflux, 4-5 h; (ii) Amino acid methyl ester hydrochloride, TEA, dioxane, reflux, 1.5-5 h

Table 5: Ester and amide derivatives of pseudosaccharinamines with yields, melting points and specific rotations

Comp.	R	Yield (%)	mp (°C)	$[\alpha]_D^{20}$	
4a	$CH_2CH(CH_3)_2$	69	254-256	+18.33	(c = 2, DMSO)
4b	$CH_2C_6H_5$	58	214	-27.50	(c = 2, Dioxane)
4r	CH_3	55	236	+42.16	(c = 2, DMSO)
4s	$CH(CH_3)_2$	40	225	+12.00	(c = 2, DMSO)
4t	$CH(CH_3)C_2H_5$	72	247	-10.16	(c = 2, DMSO)
4d	$CH_2CH_2SCH_3$	31	166	-43.50	(c = 2, MeOH)
4e	$CH_2CH(CH_3)_2$	55	172	-48.00	(c = 2, MeOH)
4j	$CH(CH_3)C_2H_5$	43	168	-30.00	(c = 2, MeOH)
4k	H	34	214	-	
4u	$CH_2C_6H_5$	76	154	-51.66	(c = 2, MeOH)
4v	CH_3	41	232-234	-09.66	(c = 2, Acetone)
4w	$CH(CH_3)_2$	61	238	-38.00	(c = 2, MeOH)
4x	$(CH_3)_2$	39	235-238	-	
4y	CH_2COOCH_3	37	62	-24.16	(c = 2, MeOH)
4z	Proline analogue	61	185-187	-65.55	(c = 0.6, MeOH)

3.4.3. Ester hydrolysis

The ester derivatives were hydrolyzed by aq. NaOH in acetone (scheme 10).

4d, 4e, 4j, 4k, 4u-4y   **14a-14i**

4z   **14j**
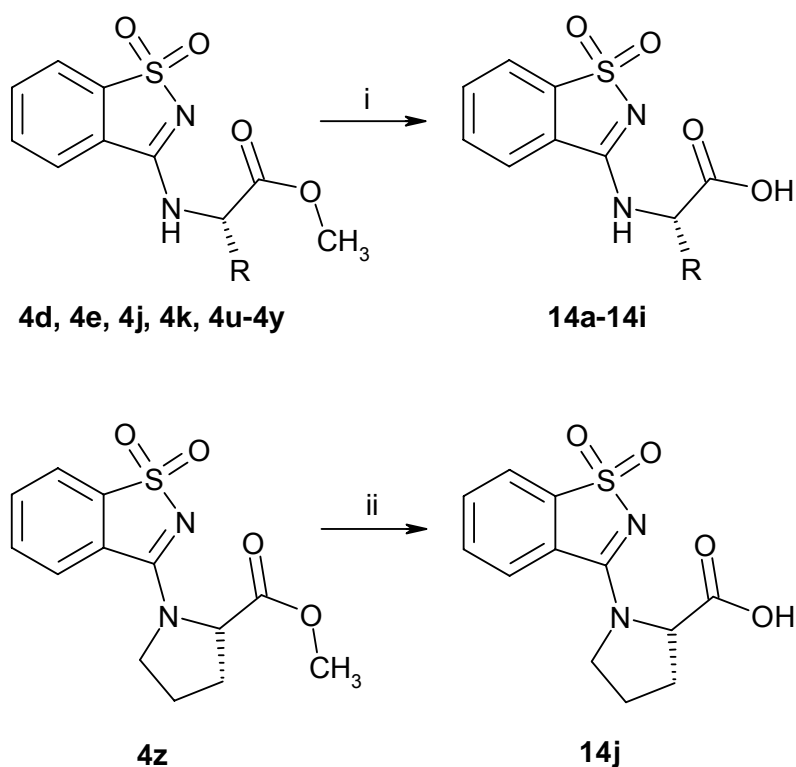
Scheme 10: Hydrolysis of the ester derivatives
Reagents and conditions: (i) aq. NaOH, Acetone, rt, 6 h; (ii) 5% HCl, reflux, 8 h

Table 6: Acid derivatives with chemical shifts (δ) in ppm of the NH and α-H protons with coupling constants (J) in Hz

Compound	R	[δ_{NH}, J]	[$\delta_{\alpha\text{-H}}$, J]
14a	$CH_2CH_2SCH_3$	9.58 (d, 7.30)	4.64 (q, 7.30)
14b	$CH_2CH(CH_3)_2$	9.56 (d, 7.77)	4.61-4.45 (m)
14c	$CH(CH_3)C_2H_5$	9.43 (d, 7.77)	4.42 (t, 7.77)
14d	H	9.84 (t, 6.00)	4.21 (d, 6.00)
14e	$CH_2C_6H_5$	9.70 (d, 7.95)	4.81-4.65 (m)
14f	CH_3	9.61 (d, 7.24)	4.62 (qn, 7.24)
14g	$CH(CH_3)_2$	9.41 (d, 7.95)	4.37 (t, 7.95)
14h	$(CH_3)_2$	9.18 (s)	-
14i	CH_2COOH	9.69 (d, 7.92)	4.83 (td, 7.92, 5.64)
14j	Proline analogue	-	4.65 (dd, 8.83, 3.89)

During the ester hydrolysis, a pale yellow colour was developed when the aq. NaOH was added to the solution in acetone. This colour change from colourless to pale yellow is possibly attributed to the anion of the sulfonamidin **II** (figure 10, figure 15) form of the ester. The anion can be mesomerically stabilized. Such change in tautomeric forms is observed by SCHRADER [50]. He observed that the pseudosaccharinhydrazide produced a yellow colour in alkaline medium which disappeared in acidic medium (figure 15).

Yellow   Colourless
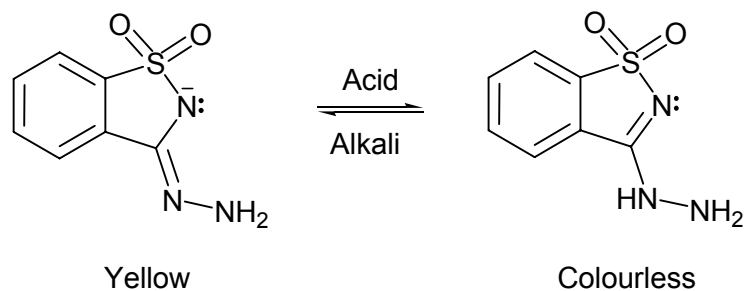
Fig. 15: Interconversion of the tautomeric forms [50]

Compounds **4d, 4e, 4j, 4k, 4u-4y** upon hydrolysis in acetone by aq. NaOH produced the acids **14a-14i**. Compound **4z** failed to give the hydrolyzed product (**14j**) under this alkaline condition. The product obtained from this reaction was characterized to be saccharin (**1**), indicating that the possible nucleophilic attack of OH⁻ on the C-N bond of **4z** was a prominent one.
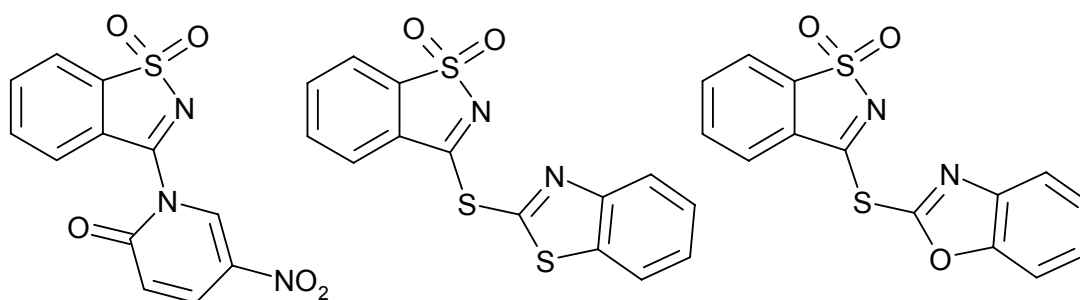
Fig. 16: Condensing reagents for the peptide synthesis [72, 73]

The nucleophilic attack on the C-N bond of the pseudosaccharinamine is a basis for the development of this class of agents as the condensing agent i.e. 3-(5-nitro-2-pyridon-1-yl)-1,2-benzisothiazole 1,1dioxide (figure 16) [72] is reported as a condensing reagent for the synthesis of peptides and esters; wherein the first step of the reaction involves the attack of COO⁻ on the C-N bond of the sulfonamidin.

Based on this strategy some reactive condensing agents are reported elsewhere (figure 16). Compound **14j** was then successfully synthesized by treating **4z** with 5% HCl at reflux for 8 h in 34% yield (scheme 10). It was observed that when the reaction time was increased up to one day, the desired **14j** was obtained along with saccharin, indicating that the C-N bond is prone to break under such conditions.

3.4.4. Different ester derivatives containing pseudosaccharinamines

Some other ester derivatives were synthesized from **14** (scheme 11). Compounds **15a-15d** were produced in moderate yields by refluxing **14c** in the respective alcohols with $SOCl_2$. The compounds **14c** and **14g** were first converted into their potassium salts. These salts were dried and then heated in chloroacetonitrile producing **15e** and **15h** respectively in low yields. **15f** and **15g** were synthesized by treating isoleucine benzyl ester tosylate and isoleucine *tert*-butyl ester hydrochloride with **13** and triethylamine in dioxane.

In the ^{1}H NMR spectrum of **15e**, two doublets were observed for the protons of the CH_2 group of the ester side chain (AB system). A classical interpretation of such an AB system is documented elsewhere [74]. One doublet was observed at δ 4.96 ppm with a coupling constant J_{AB} = 15.60 Hz, the other doublet at δ 4.76 ppm with the identical coupling constant. The external signal of both the doublets is attenuated and the internal signal is enhanced (*AB effect*, "roofing" symmetric to the centre of the AB system). A small difference in the chemical shifts of the coupled nuclei (H_A, H_B) (31.14 Hz) relative to the coupling constant (J_{AB} = 15.60 Hz) was observed in this AB system. The $\Delta v/J$ value was found to be 2.38. The signal of the α-H proton appeared as a doublet of doublets at δ 4.95 ppm (J = 8.00 Hz, J = 3.20 Hz) while the sulfonamidin proton was observed at δ 6.53 ppm (J = 8.00 Hz) as a doublet. β-H (CH) and γ-H (CH_2) protons were detected at δ 2.26-2.08 ppm and δ 1.69-1.25 ppm as multiplets respectively, while the two methyl groups were seen at δ 1.05-0.97 ppm as multiplets. The aromatic protons were seen at δ 7.94-7.88 ppm and δ 7.81-7.69 ppm as two multiplets. In its ^{13}C NMR spectrum a signal at δ 113.50 ppm was observed and was absent in the DEPT spectrum allowing us to assign it for the nitrile carbon. Signals at δ 49.14 and δ 25.69 ppm were assigned for the two methylene groups based on observations from the DEPT. No absorption was observed for the nitrile group in the IR spectrum of **15e**.

An AB system is also observed for the two protons of the CH_2 group of the ester side chain of **15f**. One proton of the ester side chain appeared as a doublet at δ 5.30 ppm

14c

15a R = CH$_2$CH$_3$
15b R = CH(CH$_3$)$_2$
15c R = CH$_2$CH$_2$CH$_2$CH$_3$
15d R = CH$_2$CH(CH$_3$)$_2$
15e R = CH$_2$CN

13

15f R = CH$_2$C$_6$H$_5$
15g R = C(CH$_3$)$_3$

14g

15h
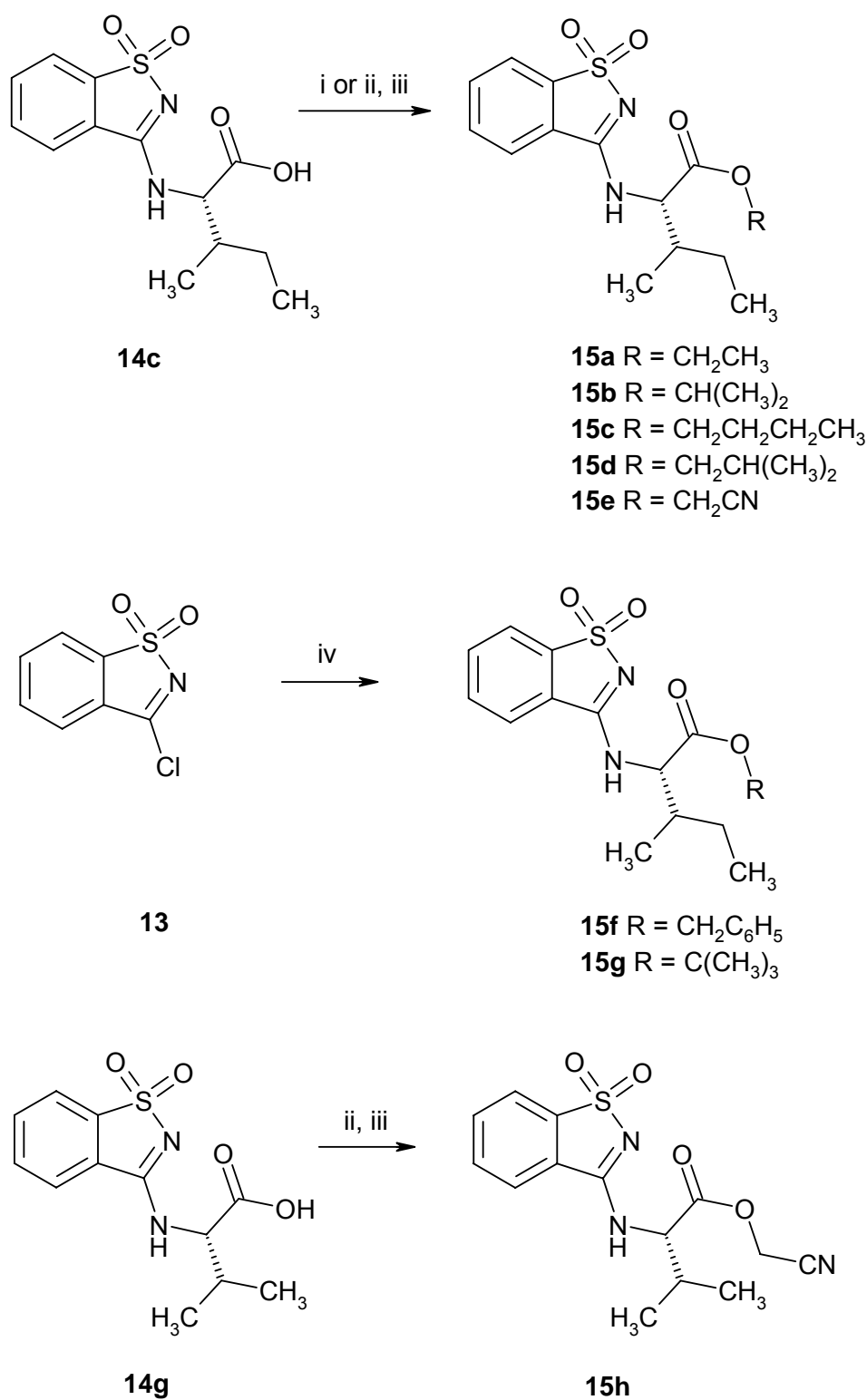
Scheme 11: Synthesis of ester derivatives (**15**)
Reagents and conditions: (i) SOCl$_2$, ROH, reflux, 4-18 h; (ii) aq. KOH, dry; (iii) ClCH$_2$CN, 80 °C, 30 min; (iv) Isoleucine benzyl ester tosylate/Isoleucine *tert*-butyl ester HCl, TEA, dioxane, reflux, 2 h

with a coupling constant of J_{AB} = 12.00 Hz, while the other as a doublet at δ 5.23 ppm with an identical coupling constant. A sulfonamidin proton of **15f** was seen at δ 6.85 ppm (J = 8.00 Hz) as a doublet and the α-H proton appeared as a doublet of doublets at δ 4.95 ppm (J = 8.00, 4.82 Hz).

An AB system was also observed in the ^{1}H NMR spectrum of **15h** for the protons on the CH$_2$ group of the ester side chain (figure 17). One proton of the CH$_2$ protons was at δ 4.95 ppm (J_{AB} = 15.60 Hz) as a doublet while the other showed a doublet at δ 4.76 ppm (J_{AB} = 15.60 Hz). The *AB effect* ("roofing") is clearly visible in the expanded region of figure 17. The difference in the chemical shift ($\Delta\nu$) was found to be 38.88 Hz while $\Delta\nu/J$ was found to be 2.49. The aromatic protons were present as two multiplets (δ 7.95-7.89, 7.82-7.67 ppm). A sulfonamidin proton was seen at δ 6.40 ppm (J = 8.28 Hz) as a doublet and the α-H proton appeared as a doublet of doublets at δ 4.92 ppm (J = 8.28 Hz, J = 4.90 Hz). The β-H proton was detected at δ 2.54-2.37 ppm as a multiplet while the two methyl groups were seen at δ 1.10 ppm and δ 1.08 ppm (J = 6.90 Hz) as two doublets.

In the ^{13}C NMR spectrum of **15h**, a signal at δ 49.23 ppm was assigned for the methylene carbon of the ester side chain, while the signal at δ 113.46 ppm was assigned to the nitrile based on a DEPT measurement. The nitrile group had not shown any absorption in the IR spectrum of **15h**.
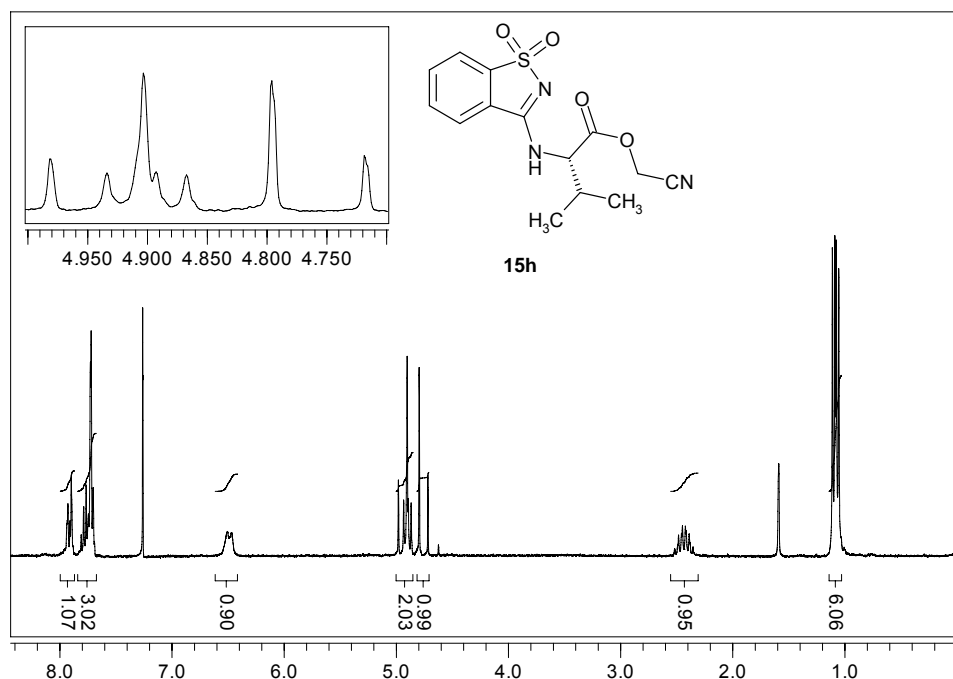
Fig.17: ^{1}H NMR spectrum (200 MHz, 25 °C) of **15h** in CDCl$_3$

3.4.5. Alcohol derivative: (2*S*,3*S*)-2-(1,1-dioxobenzo[*d*]isothiazol-3-ylamino)-3-methylpentan-1-ol

In order to synthesize the alcohol derivative, **14c** was converted into the anhydride in situ by the use of isobutyl chloroformate (IBCF) and *N*-methylmorpholine (NMM) at rt or at -15 °C in THF, and then we tried to reduce it by sodium borohydride (scheme 12). The reduction failed. The reduction was then carried out by using **4j** with lithium borohydride in 1,2-dimethoxyethane (DME) at -40 °C. This reaction proceeded smoothly and the desired alcohol **16** was obtained as a solid in 83% yield (scheme 12).

14c   **16**   **4j**
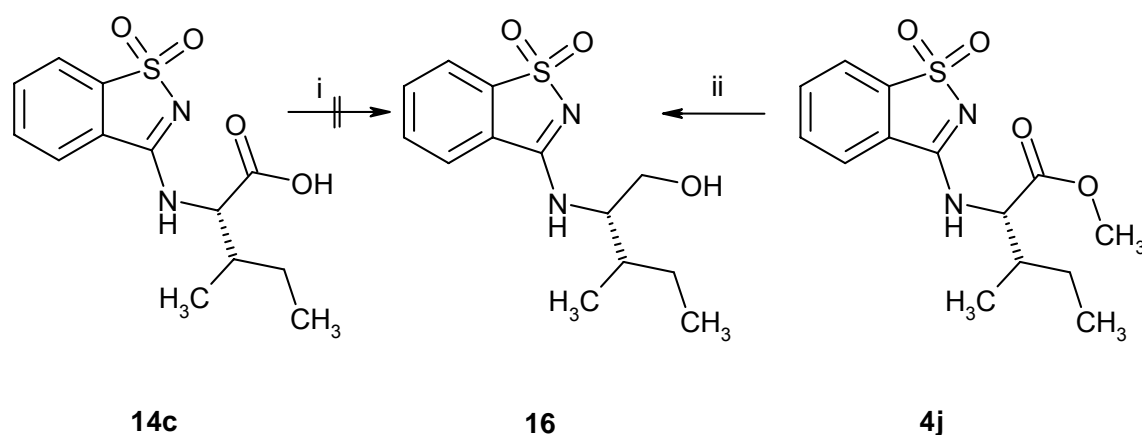
Scheme 12: Synthesis of **16**
Reagents and conditions: (i) IBCF, NMM, THF, rt / -15 °C, NaBH$_4$, MeOH, 0 °C , 1 h; (ii) DME, LiBH$_4$, -40 °C to rt, overnight

In the ^{1}H NMR spectrum of **4j**, a doublet at δ 9.57 ppm was observed for the sulfonamidin functionality, while the α-H proton showed a triplet at δ 4.49 ppm. The methoxy protons appeared as a singlet at δ 3.71 ppm and the β-H and γ-CH$_2$ protons appeared as multiplets. A doublet at δ 0.96 ppm for the γ-CH$_3$ and a triplet at δ 0.89 ppm for methyl protons were observed. **4j** showed a carbonyl carbon signal at 171.83 ppm in its ^{13}C NMR spectrum, and showed the carbonyl absorption at 1756 cm^{-1} in the IR spectrum.

In the ^{1}H NMR spectrum of **16**, a doublet for a sulfonamidin proton appeared at δ 6.72 ppm, the carbonyl carbon signal is absent in the ^{13}C NMR spectrum and also there was no carbonyl absorption seen in the IR spectrum. The hydroxyl group showed a signal at 3454 cm^{-1} in the IR spectrum.

3.4.6. Pseudosaccharin amine containing thiazole or thiophene ring analogues

The use of ethyl *syn*-(2-amino-thiazol-5-yl)-α-methoxyiminoacetate **(17a)** and ethyl *anti*-(2-amino-thiazol-5-yl)-α-methoxyiminoacetate as intermediates in the synthesis of derivatives of cephalosporins is reported [75-77]. **17a** is commercially available. The reaction between **13** and **17a** yielded the ester **18a** (60%, scheme 14), and by hydrolysis **19** was obtained in 90% yield (scheme 15). In the ^{1}H NMR spectra of **18a** and **19**, additional signals were detected indicating that isomerization had occurred during the reaction. This could be due to heat as the reaction mixture was refluxed in dioxane. In order to clarify the isomerization, we compared their ^{1}H NMR spectra with that of the starting material, ethyl *syn*-(2-amino-thiazol-5-yl)-α-methoxyiminoacetate (*Z* form) **(17a)**.

In [D$_6$]DMSO, the ^{1}H NMR spectrum of **17a** (for numbering see scheme 13) showed a broad singlet at δ 7.25 ppm for the NH$_2$ protons, a singlet at δ 6.90 ppm for 5'-H$_{thiazole}$, a quartet at δ 4.26 ppm for CH$_2$, a singlet at δ 3.87 ppm for OCH$_3$, and a triplet at δ 1.26 ppm for CH$_3$. In the ^{13}C NMR of the **17a** ([D$_6$]DMSO), signals at δ 168.59, 140.92, 108.38 ppm were caused by C$_{2'}$, C$_{4'}$ and C$_{5'}$ respectively. The signals at δ 146.73, 62.31 and 162.22 ppm were from C$_{6'}$, OCH$_3$ and C$_{7'}$ respectively. The methylene and methyl protons appeared at δ 61.26 ppm and δ13.80 ppm respectively. The data of ^{1}H and ^{13}C NMR were in agreement with those of the reported spectrum [75].
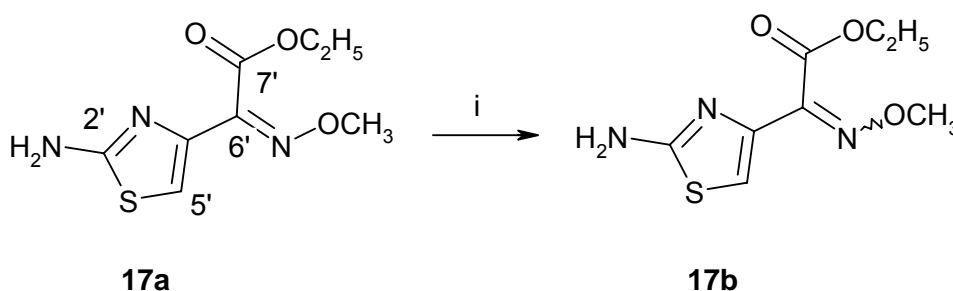
Scheme 13: Isomerization of **17a**
Reagents and conditions: UV$_{254\,nm}$, dioxane, 2 days

After irradiating **17a** with UV light for 2 days, we obtained **17b** where we observed additional signals in its ^{1}H NMR spectrum (scheme 13, figure 18).

Additional signals were: a singlet at δ 7.50 ppm for 5'-H$_{thiazole}$, a broad singlet at δ 7.14 ppm for NH$_2$, a quartet at δ 4.24 ppm (*J* = 7.14 Hz) for CH$_2$, a singlet at δ 3.98 ppm for OCH$_3$ and a triplet at δ 1.25 ppm (*J* = 7.14 Hz) for CH$_3$. These signals are

caused by the *anti* isomer (*E* form) indicating that the signals for the *E* form are shifted to higher δ values for 5'-H$_{thiazole}$, and the OCH$_3$ protons but to the lower δ values for CH$_2$, CH$_3$ and the NH$_2$ protons. The expanded region of ^{1}H NMR spectrum in figure 18 indicates the two mixed quartets. The ratio of isomers was found to be $\approx$ 2:8 (*E:Z*).
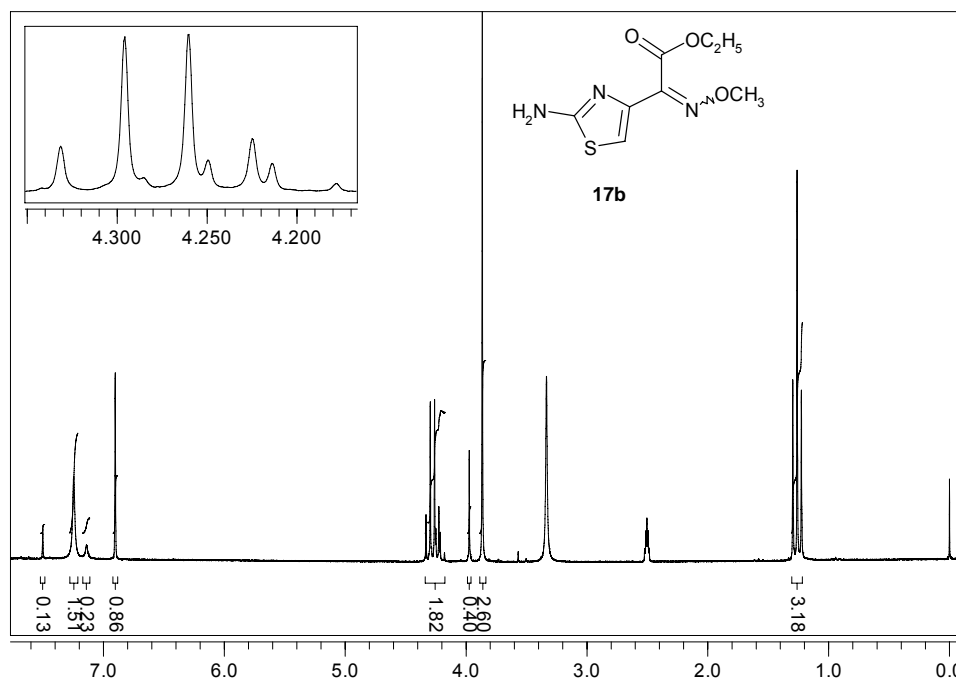
Fig. 18: ^{1}H NMR spectrum (200 MHz, 25 °C) of **17b** in [D$_6$]DMSO

Additional signals were also observed in the ^{13}C NMR spectrum of **17b**. The additional signals at δ 115.99, 62.91, 163.10 are assignable to C$_{5'}$, OCH$_3$, C$_{7'}$ respectively and they are shifted to higher δ values compared to **17a**, while the signals at δ 166.64, 137.46, 144.77, 61.24, 13.72 are assignable to C$_{2'}$, C$_{4'}$, C$_{6'}$, CH$_2$, CH$_3$ respectively and are shifted to lower δ values compared to **17a**. The ^{13}C NMR data of the additional signals of **17b** is in agreement with the reported data for the *E* isomer [75].

A similar situation was observed for **18a** (figure 19). Here we obtained two sets of signals representing the two different isomers. The dominant set showed the 5'-H$_{thiazole}$ singlet at δ 8.27 ppm, a quartet at δ 4.32 ppm for CH$_2$, a singlet for OCH$_3$ protons at δ 4.07 ppm and a triplet for the CH$_3$ group at δ 1.30 ppm, whereas the minor set showed signals that were shifted to lower δ values for 5'-H$_{thiazole}$, δ 7.83 ppm, and OCH$_3$, δ 3.96 ppm, but to higher δ values for CH$_2$, δ 4.37 ppm and for CH$_3$, δ 1.31 ppm. The NH proton was not seen in the spectrum. In the right and the left

expanded region of the ^1H NMR spectrum in figure 19 indicates the two mixed quartets and two mixed triplets respectively. Assuming the chemical shift dependency on E/Z isomerism being equivalent to that for the starting material, we deduced the ratio to be ≈ 7:3 (E:Z) for **18a**. The comparison of these signals is listed in table 7.
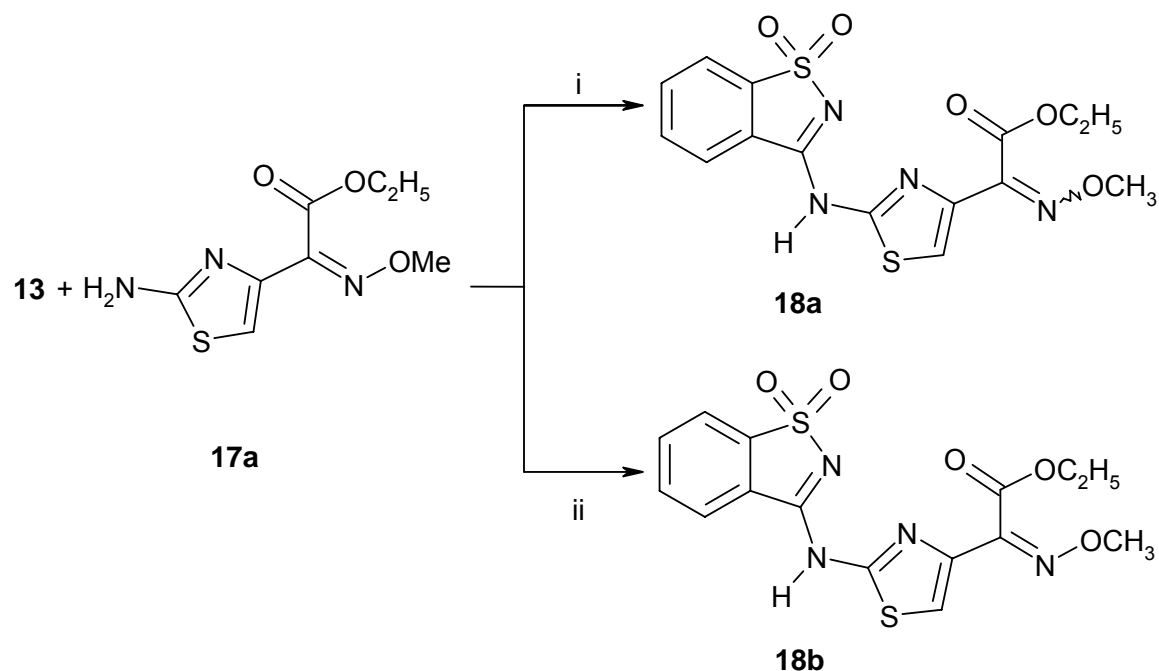
Scheme 14: Synthesis of **18a** and **18b**
Reagents and conditions: (i) Dioxane, reflux, 2 h; (ii) DCM, TEA, rt, 4 h

Table 7: Comparison of **18a** and **18b**

Group	δ_H (ppm)	
	18a	**18b**
5'-H	8.27, 7.83	7.83
OCH$_3$	4.07, 3.96	3.96
O$\underline{CH_2}$CH$_3$	4.32, 4.37	4.37
OCH$_2$$\underline{CH_3}$	1.30, 1.31	1.31

In the ^{13}C NMR spectrum of **18a**, we obtained the two sets of signals representing the two isomers. A major set showed signals at δ 162.58, 157.42, 156.74, 143.83, 141.10, 136.78, 134.05, 133.60, 126.86, 124.41, 124.18, 121.72, 63.42, 61.70, 13.80 ppm, while the minor set showed the signals at δ 161.77, 159.08, 156.52, 145.85,

141.05, 140.65, 134.13, 133.69, 126.70, 124.36, 121.77, 118.23, 62.77, 61.82 and 13.80 ppm.

Fig. 19: ^{1}H NMR spectrum (200 MHz, 25 °C) of **18a** in [D$_6$]DMSO

Furthermore, this assignment of **18a** was clarified by obtaining the single isomer **18b**. **18b** was obtained from the reaction between **13**, TEA and **17a** in dichloromethane at rt for 4 h (scheme 14). As expected, the spectra of **18b** showed the same signals which we had assigned to the minor set of the signals from structure **18a**.

Fig. 20: HPLC analysis of **18a** (blue) and **18b** (pink) using a Chiralcel OJ-R column with MeCN/Water 1:1

In HPLC measurements, only one peak with a retention time of 6.07 min was observed for **18b** while two peaks (retention times: 5.71 and 6.03 min) were observed for **18a** (figure 20). This information supports the *E*/*Z* mixture in **18a**. The HPLC using the calixarene as a stationary phase (CaltrexAlll 250X4 ml Kromasil Si 100/5 µm) in 35% MeCN: 65% 50 mM NaH_2PO_4, PH 3.5; also showed the separation of two isomers

A similar situation was observed in the ^{1}H NMR spectrum of **19**, where the ratio of isomers was found to be ≈ 8.5:1.5 (*E*:*Z*) based on ^{1}H NMR (scheme 15).

Scheme 15: Hydrolysis of **18a**
Reagents and conditions: (i) aq. NaOH, acetone, rt, 6 h

Furthermore **20**, **23** and **26** were reacted with **13** to produce **21**, **24**, **27** in 50, 83 and 87% yield in a short reaction time (30 min to 2 h) (scheme 16).

The hydrolysis of these ester derivatives (**21**, **24**, and **27**) was possible under basic conditions employing aq. NaOH in acetone. During hydrolysis, a deep orange-red colour was developed when the aq. NaOH was added indicating the possible conversion of sulfonamidin **I** to the anion of sulfonamidin **II** form as reported in section 3.4.3. Compound **22** was obtained in 87% yield, while **25** and **28** were obtained in 36 and 29%. These compounds were analysed by NMR, HPLC and elemental analysis. In the ^{1}H NMR spectrum of **24**, an AB system was observed for the thiophene protons. The 5'-H proton showed a doublet at δ 8.09 ppm and the 4'-H proton showed a doublet at δ 7.85 ppm. The sulfonamidin proton was observed at δ 11.07 ppm, while the methoxy protons were registered as a singlet at δ 3.89 ppm. The AB system was also seen in the hydrolyzed product **25**, where the 5'-H proton appeared at δ 8.01 ppm and the 4'-H proton appeared as a doublet at δ 7.92 ppm. The position of thiazole or thiophene protons in these heterocyclic derivatives is presented in the following table (table 8).

Scheme 16: Synthesis of heterocyclic ester derivatives and their hydrolysis
Reagents and conditions: (i) **13**, dioxane, reflux, 30 min-2 h; (ii) aq. NaOH, rt, 6 h-6 days

Table 8: Thiazole and thiophene protons of the heterocyclic derivatives. Values δ ppm

Compound	4'-H$_{Heterocyclic}$	5'-H$_{Heterocyclic}$
18b	-	7.83
21	-	7.30
22	-	7.25
24	7.85	8.09
25	7.92	8.01
27	7.00	7.30
28	7.31	7.34

In the ^{1}H NMR spectrum of **27**, the 5'-H proton appeared as a doublet at δ 7.30 ppm and the 4'-H proton showed a doublet at δ 7.00 ppm forming an AB system. Compound **28** also gave an AB system in the ^{1}H NMR spectrum from the thiophene protons. The 5'-H proton appeared at δ 7.34 ppm and the 4'-H proton appeared at δ 7.31 ppm.

3.4.7. Nitration of 3-(1,1-dioxobenzo[*d*]isothiazol-3-ylamino)thiophene-2-carboxylic acid methyl ester

Electrophilic substitution reactions in thiophene occur predominantly at the carbon atom and the substitution at the sulphur atom is relatively rare. Thiophene is the least reactive towards electrophiles among the five membered aromatic heterocycles. But thiophene is more reactive than benzene by the factor of 10^3-10^5. Electrophilic substitution reactions in thiophene proceed by forming an σ-complex (rate determining step). Thiophene undergoes electrophilic substitution reactions slowly and selectively at an α-position to sulphur rather than at β-position. The preferential electrophilic attack at an α-position in thiophene can be explained on the basis of stability of the transition state. The transition state resulting from the electrophilic attack at the α-position is stabilized over the transition state of β electrophilic attack (three resonating structures for an α attack and two resonating structure for β attack) [78]. LEUMANN [79] had obtained both isomers while studying the nitration of methyl 3-(acetylamino)thiophene-2-carboxylate. He obtained 51% of the α-nitro and 35% of the β-nitro isomer. While studying the nitration of 5-nitrothiophene-2-carbaldehyde, COGOLLI [80] had observed the *ipso* nitration. Nitration of the thiophene derivative **24** at -30 °C by nitrating mixture (conc. H_2SO_4 and conc. HNO_3) produced 18% of the nitro analogue **29** (scheme 17).

Scheme 17: Nitration of **24**
Reagents and conditions: (i) conc. H_2SO_4, conc. HNO_3, -30 ° C, 45 min, to rt

The structure of the product **29** can not be confirmed just by ^{1}H NMR as there is always a possibility of formation of the other isomer. In the ^{1}H NMR spectrum of the product, a sharp singlet appeared at δ 8.55 ppm assignable to a proton of the thiophene ring. Information obtained from ^{1}H NMR, ^{13}C NMR, DEPT, HMQC and HMBC reveals that a carbon at δ 125.58 ppm is assignable to a thiophene carbon bearing a proton, while carbons at δ 123.06, 133.65, 134.30, 121.74 ppm are assignable to CH of the aromatic part of the pseudosaccharin nucleus. A coupling is observed between an aromatic proton of the pseudosaccharin nucleus and a carbon at δ 157 ppm, allowing us to assign δ 157 ppm for the carbon of C=N. Also a coupling between the methoxy group signal at δ 3.93 ppm and that of the carbonyl group at δ 161 ppm was observed in HMBC. Unfortunately no coupling was observed between a proton at δ 8.55 ppm (thiophene bearing proton) and any of the carbons of thiophene ring. If we had isolated both isomers, we could have assigned the structure from the relative chemical shifts. But we obtained only one isomer. Although the possibility of formation of the other isomer can not be ruled out as in the ^{1}H NMR spectrum of the crude product, more products were indicated but these products could easily be neglected in crystallization and only one isomer was obtained as a major product. The Yields of other products were too low for isolation.

The structure of the nitro thiophene analogue was determined by X-ray crystallography and in fact the product was found to be the 5'-nitro analogue **29**. The crystal structure is presented in figure 21A and an ORTEP presentation of this molecule is shown in figure 21B.

Fig. 21 A: Crystal structure of **29**

Fig. 21 B: Molecular structure of **29**. Thermal ellipsoids are drawn at 50% probability level. First from the two chemically identical molecules present in the elemental cell is shown.

Formation of the 5'-nitro analogue can be explained on the basis of transition states (see the beginning of this section). The transition state involved in the formation of the 5'-nitro analogue probably is more stable compared to the transition state involved in the formation of the 4'-nitro analogue.

3.5. Peptide synthesis

Tripeptidic inhibitors should be more active than their corresponding dipeptidic analogues, which should be more active than their nonpeptidic analogues [14]. The most likely explanation for the large increase in activity of these peptidic analogues is the ability of the peptide moiety to form hydrogen bonds with the enzyme. The X-ray crystallographic study of the turkey ovomucoid inhibitor complexed with HLE was described by BODE et al. in 1986 [81].

That inhibitor was bound in the extended binding site in a manner similar to that observed for proteinaceous inhibitors bound to other serine proteinases. The P_3 to P_1 residues were found to bind to the enzyme in an *anti*-parallel, *β*-pleated sheet arrangement. In addition to the hydrogen bonds found in the oxyanion hole, three hydrogen bonds were observed between the inhibitor and the enzyme's extended binding pocket: the NH of the P_1 residue to the carbonyl of Ser-214; the carbonyl of P_3 to the NH of Val-216; and the NH of P_3 to the carbonyl of Val-216 (figure 22). The binding scheme depicted in figure 22 has also been observed in most of the X-ray crystal structures of complexes between PPE or HLE and peptidic inhibitors [14].

Fig. 22: Representation of the hydrogen bonding network between a serine proteinase and a peptide ligand [14]

To check the fact, that the dipeptides are more active than their non peptidic analogues, with our compound **15e**, we decided to synthesize some peptidic analogues of **15e**, hoping for better inhibition against HLE and PPE compared to **15e**. Furthermore, in order to produce more stable compounds, we decided to convert the ester linkage of **15e** into an amide function.

Different methods, acid chloride and fluoride method, active ester methods, carbodiimide methods, azide methods, methods based on phosphonium and uranium salts, are reported for the synthesis of peptides. An appropriate activation is required to perform the coupling reaction under mild conditions [82]. The mixed anhydride method of peptide bond formation involves aminolysis of an anhydride consisting of an N-protected amino acid and another acid.

Either carboxylic-carboxylic mixed anhydrides or carbonic-carboxylic mixed anhydrides are used, and both methods are well developed [82]. Isobutyl chloroformate is commonly used for the preparation of carbonic-carboxylic mixed anhydrides (figure 23).

R = isobutyl

Fig. 23: Mixed anhydride method using isobutyl chloroformate [82]

In the mixed anhydride method, the amide-forming reaction is complete within several minutes in an ice bath. The side-chain functional groups in the carboxyl component should be either in ester form or protected tentatively with a tertiary amine. In latter case, the reaction can be performed in water-containing solvents, but the products should be carefully separated because of its acidity. As a side reaction in this method, urethane formation occurs in the amine component (figure 23), but it can be neglected under usual experimental conditions [82].

During the peptide bond formation, generally the amino component or carboxyl component is protected by different protecting groups which can be easily removed under mild conditions. Among the amino protecting groups, carbamates, amides, imine derivatives, and enamine derivatives are of special value [80]. The tert-butyl carbamate (BOC) group is used extensively for amine protection. It is not hydrolyzed under basic conditions and is inert to many other nucleophilic reagents. The group

can be easily removed after the peptide synthesis. Hydrolysis of BOC group by 3 *M* HCl in ethyl acetate or methanol, CF_3COOH and PhSH at 20 °C, Me_3SiI in chloroform or acetonitrile, thermal cleavage (185 °C, 20-30 min), bromocatecholborane, Me_3SiI and PhOH in dichloromethane, 10% H_2SO_4 in dioxane is well documented [83].

In order to obtain more stable compounds than the esters, the ester group was converted into an amide moiety. Hence, synthesis of **30** was carried out from **14c** by the mixed anhydride method employing *N*-methylmorpholine (NMM) and isobutyl chloroformate (IBCF). The crude product **30** was purified by CC with EtOH/petroleum ether, 10%, with increasing polarity till 80%. **30** was then obtained as foam in 34% yield (scheme 18).

Scheme 18: Synthesis of **30**
Reagents and conditions: (i) THF, -25 °C, NMM, IBCF, -45 °C to rt, overnight

In order to synthesize the peptides, commercially available BOC-L-isoleucine (**31**) was used. Compound **32** was obtained in 38% from **31** and aminoacetonitrile HCl by a mixed anhydride method (scheme 19). The reactive anhydride of **31** was formed *in situ* at -25 °C with isobutyl chloroformate in the presence of *N*-methylmorpholine. The free base was obtained from aminoacetonitrile HCl in equimolar aq. NaOH and addition to the reactive anhydride was done at -45 °C. An attempt to use triethylamine to obtain the free base from aminoacetonitrile HCl failed. Hydrolysis of the BOC group was carried out in ethyl acetate saturated with HCl gas to produce **33** in 85% yields. Thus obtained **33** was highly hygroscopic (scheme 19). In the ^{1}H NMR of **33**, a doublet appeared at δ 4.15 ppm for the methylene protons of the amide side chain while the α, β and γ protons were appeared as multiplets.

Scheme 19: Synthesis of **33**
Reagents and conditions: (i) IBCF, NMM, THF, -25 °C, 30 min, -45 °C, aminoacetonitrile HCl in aq. NaOH, to rt, overnight; (ii) ethyl acetate saturated with HCl, 6 h, rt

The dipeptide derivatives **34** were synthesized by the mixed anhydride method (scheme 20, table 9). Peptides **34** were obtained in 5-51% yields. No urethane (side product) formation was observed under these mixed anhydride conditions. The obtained compounds **34** were solids. Compound **34f** was obtained as a mixture of rotamers as seen from the ^{1}H NMR of **34f**. In the IR spectra of **34**, the absorption of a nitrile group was absent; but its presence was confirmed by a signal around δ 113-118 ppm in the ^{13}C NMR spectra. The quaternary nature of the signal at δ 117.29 was confirmed by the DEPT of compound **34a**. The peptides **34** are obtained as polymorphic substances. Peptides (**34a**-**34f**) showed two polymorphic forms. The polymorphic forms of **34b** melted at 165 °C and 225 °C when measured by the Differential Scanning Calorimeter. All compounds were analyzed by NMR, HPLC and IR spectra. ^{1}H NMR spectra and ^{13}C NMR spectra of **34** were recorded in [D$_6$]DMSO. In the chiral HPLC measurements all the peptides showed only one retention time.

In the ^{1}H NMR spectrum of **34a**, a doublet was observed at δ 9.49 ppm with a coupling constant of 7.60 Hz for the NH proton. Only from this information, it was not possible to assign it to any specific proton of the NH functionality. But further a quartet is appeared at δ 4.70 ppm with the same coupling constant and hence, the doublet at δ 9.49 ppm now could be assigned to the NH proton of the sulfonamidin functionality and a quartet to the *α*-H of the methionine. A triplet was present at δ 8.78 ppm for the NH proton of the amido acetonitrile moiety, and a doublet was observed for a methylene proton of the amido acetonitrile moiety; this triplet and the doublet showed the same coupling constant (*J* = 5.50 Hz). The rest of the protons appeared as multiplets. Some of the ^{1}H NMR parameters of amide derivatives are listed in table 10.

All peptide derivatives were characterized by their IR spectra. The absorption at ≈ 3300 cm^{-1} was caused by NH, and the absorption bands at 1150-1164 cm^{-1} and 1340-1387 cm^{-1} are due to the SO$_2$ group. A characteristic absorption between 1602 and 1618 cm^{-1} was attributed to the C=N moiety of the five membered ring of the pseudosaccharin skeleton. The carbonyl absorptions were observed at 1650-1681 cm^{-1} due to the amide.

14j   **35**

Scheme 21: Synthesis of **35**
(i) IBCF, NMM, THF, -25 °C, 30 min, -45 °C, L-isoleucine methyl ester HCl, TEA, to rt, overnight

Furthermore, peptide **35** was synthesized from **14j** and L-isoleucine methyl ester (scheme 21) in 51% by mixed anhydride method. **35** was isolated as a colourless crystalline compound with a melting point of 174 °C. It was characterized by ^{1}H, ^{13}C NMR, HMQC and ^{1}H-^{1}H COSY experiments (figure 24).

14a	$R_1 = CH_2CH_2SCH_3$	
14b	$R_1 = CH_2CH(CH_3)_2$	
14c	$R_1 = CH(CH_3)C_2H_5$	
14f	$R_1 = CH(CH_3)_2$	
14g	$R_1 = CH_3$	

34a	$R_1 = CH_2CH_2SCH_3$
34b	$R_1 = CH_2CH(CH_3)_2$
34c	$R_1 = CH(CH_3)C_2H_5$
34d	$R_1 = CH(CH_3)_2$
34e	$R_1 = CH_3$

14j   **34f**

Scheme 20: Synthesis of dipeptide derivatives **34**
Reagents and conditions: (i) IBCF, NMM, THF, -25 °C, 30 min, -45 °C, **33**, TEA, to rt, overnight

Table 9: Peptide derivatives **34** with yields and specific rotations

Compound	R	$[\alpha]_D^{20}$		Yield (%)
34a	$CH_2CH_2SCH_3$	-57.50	(c = 2, MeOH)	13
34b	$CH_2CH(CH_3)_2$	-66.00	(c = 2, MeOH)	34
34c	$CH(CH_3)CH_2CH_3$	-106.50	(c = 2, MeOH)	51
34d	$CH(CH_3)_2$	-90.00	(c = 2, MeOH)	31
34e	CH_3	+6.66	(c = 2, DMSO)	17
34f	proline analogue	-97.00	(c = 2, MeOH)	05

Table 10: Selected ^{1}H NMR data of **30**, **34** and **35**. * denotes that the proton of sulfonamidin and NH of isoleucine is the same while $^\#$ denotes that the signal is mixed with other multiplets

Compound	30	34a	34b	34c
*N*H Ile	-*	8.40	8.44	8.52-8.35$^\#$
α-H Ile	4.56	4.27-4.04	4.30-4.10	4.47
NH	8.04	8.78	8.76	8.74
CH$_2$	4.19-4.07	4.15	4.15	4.14
NH sulfonamidin	8.45	9.49	9.47	9.41

Compound	34d	34e	34f	35
*N*H Ile	8.38	8.44-8.28$^\#$	8.70, 8.35	7.07
α-H Ile	4.19-4.07	4.27-4.06$^\#$	4.96-4.74	4.53
NH	8.75	8.76	8.80, 8.69	-
CH$_2$	4.13	4.27-4.06$^\#$	4.34-4.08$^\#$	-
NH sulfonamidin	9.37	9.50	-	-

Fig. 24: ^{1}H-^{1}H COSY spectrum of **35**

The COSY spectrum of **35** is shown in figure 24. In this spectrum, a coupling was observed for a doublet at δ 7.07 ppm to the doublet of doublets at δ 4.53 ppm. This doublet of doublets is unambiguously assignable to the α-H of isoleucine. A doublet of doublets at δ 4.92 ppm shows couplings to δ 2.10 ppm and 2.34 ppm. Hence, the doublet of doublets at δ 4.92 ppm is assigned to the α-H of proline and the multiplets at 2.10 and 2.34 ppm are assignable to the β-H of proline. A doublet of triplets at δ 4.24 ppm and multiplets at δ 4.05 ppm (δ 4.14-3.96 ppm) shows couplings to multiplets at δ 2.20 and δ 2.50 ppm. Hence, a doublet of triplets at δ 4.24 ppm and multiplets at δ 4.05 ppm are assignable to the δ-H of proline. The multiplets at δ 2.20 and δ 2.50 ppm can be assigned for the γ-H of the proline. The α-H of isoleucine shows a coupling with multiplets at δ 1.93 ppm. This multiplet at δ 1.93 then can be assigned for the β-H of isoleucine. A triplet at δ 0.90 ppm and a doublet at δ 0.94 ppm showed a coupling with multiplets at δ 1.93 ppm, 1.34 and 1.13 ppm.

3.6. Enzyme assay

An inhibitor of an enzyme is a compound that decreases the velocity of the reaction by binding to the enzyme [84]. The inhibitors are generally classified as reversible, tight binding or slow binding inhibitors [1].

3.6.1. Reversible inhibition

In this type of inhibition an enzyme is reversibly inhibited by the inhibitor. Reversible inhibitors that bind at the active site of the enzyme can be competitive, non-competitive and uncompetitive with respect to a substrate of the reaction. A competitive inhibitor 'compete' with the substrate for binding at the substrate binding site of enzyme. It is usually a close analogue of the substrate with which it competes [84]. Figure 25 provides a generalized scheme for the potential interactions between inhibitor, enzyme and substrate.

$$E + S \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} ES \xrightarrow{K_2} E + P$$

$$+$$
$$I$$

$$\Big\updownarrow K_i$$

$$EI$$

Fig. 25: Equilibrium scheme for enzyme turnover in the presence and absence of an inhibitor

In the scheme, K_1 is the rate of formation of the enzyme-substrate (ES) complex, K_{-1} is the equilibrium constant for the dissociation of the enzyme-substrate complex to the free enzyme and the free substrate, K_2 is the rate constant for the product formation from the ES and K_i is the dissociation constant for the enzyme-inhibitor (EI) complex. The dissociation constant is often referred as the inhibitor constant. The K_i is described by the following equation [85].

$$K_i = \frac{[E]\,[I]}{[EI]}$$

The backbone architect of HLE and PPE is conserved, and the structures of the active sites are very similar [6, 7]. Hence, the synthesized compounds were tested against PPE as well as against HLE. The inhibitory studies were done by carrying out the PPE and HLE assays. For the PPE assay, Suc-(L-Ala)$_3$-4-NA [10] was used as substrate and the rate of release of the product 4-nitroanilide was measured by a spectrophotometer at 410 nm over a period of 20 min in tris-buffer. In the HLE assay, N-methoxysuccinyl-Ala-Ala-Pro-Val-p-nitroanilide was used as substrate in HEPES buffer and the rate of release of 4-nitroanilide was measured at 410 nm over 20 min. A kinetic of **15h** was studied with PPE. A typical progress curve for PPE catalyzed reactions in the presence of different concentrations of **15h** and in the absence of **15h** is shown in figure 26. The substrate concentration used was 0.78 mM and the concentration of PPE was 28.9 nM.

Fig. 26: Absorption-time curve obtained at different concentrations of **15h** in PPE assay

The inhibition showed by **15h** is reversible as the straight lines, product formation increases linearly as time increases, were obtained in figure 26. In order to find out the subtype of the reversible inhibition, the obtained data was treated for a graphical transformation. A steady state model of enzyme kinetics was studied by HENRI and by MICHAELIS and MENTEN [1]. The steady state refers to a time period of the enzymatic reactions during which the rate of formation of the ES complex is exactly matched by its rate of decay to free enzyme and product. The HENRY-MICHAELIS-MENTEN equation (generally called as MICHAELIS-MENTEN equation) relates the initial velocity of an enzyme catalyzed reaction, υ, to the concentration S of the substrate, and two parameters, K_m and V_{max} (eq 1).

$$E + S \rightleftharpoons [ES] \longrightarrow E + P$$

$$\upsilon = \frac{V_{max}[S]}{K_m + [S]} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

V_{max} is the maximum reaction velocity and K_m is the MICHAELIS constant. K_m is defined as a substrate concentration that provides a reaction velocity that is half of the maximal velocity obtained under the saturating substrate conditions. The PPE assay was carried out in the presence of different concentrations of substrate (0.25 mM to 3.12 mM) and with two different inhibitor concentrations (for **15h**, ([I] = 0.42 mM and [I] = 0.84 mM). The rate of formation of the product was measured over a period of 20 min. The MICHAELIS-MENTEN curve was then obtained by plotting the substrate concentration vs the velocity of the reaction (figure 27). The curve indicates that the rate of formation of the product was decreased with increasing inhibitor concentrations.

Fig. 27: MICHAELIS-MENTEN curve in the presence of **15h**

Linearized plots are extremely useful in determining the mode of interaction between an enzyme and an inhibitor. The LINEWEAVER plot is an example of such plots. A LINEWEAVER-BURK plot was obtained by plotting 1/[S] vs 1/[V] (figure 28). The straight lines intersecting Y axis at one point and rise in K_m is a characteristic signature of a competitive inhibitor [85]. The lines intersect at their Y intercepts as a competitive inhibitor does not affect the apparent value of V_{max} [1]. In this plot, the slope is K_m/V_{max}, The Y intercept is $1/V_{max}$ and the intersection point on the abscissa is $-1/K_m$. The K_m

for PPE catalysed reaction of Suc-(L-Ala)$_3$-4-NA was found to be 5.56 mM (5.9 mM [86]) from the LINEWEAVER-BURK plot. The K_m was shifted at 6.70 mM and 8.06 mM in the presence of 0.42 mM and 0.84 mM of inhibitor **15h**, respectively. The MICHAELIS-MENTEN equation (eq 1) is modified in the presence of a competitive inhibitor and is stated in eq. 2.

$$\upsilon = \frac{V_{max}[S]}{K_m\left(1+\dfrac{[I]}{K_i}\right)+[S]} \quad\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$
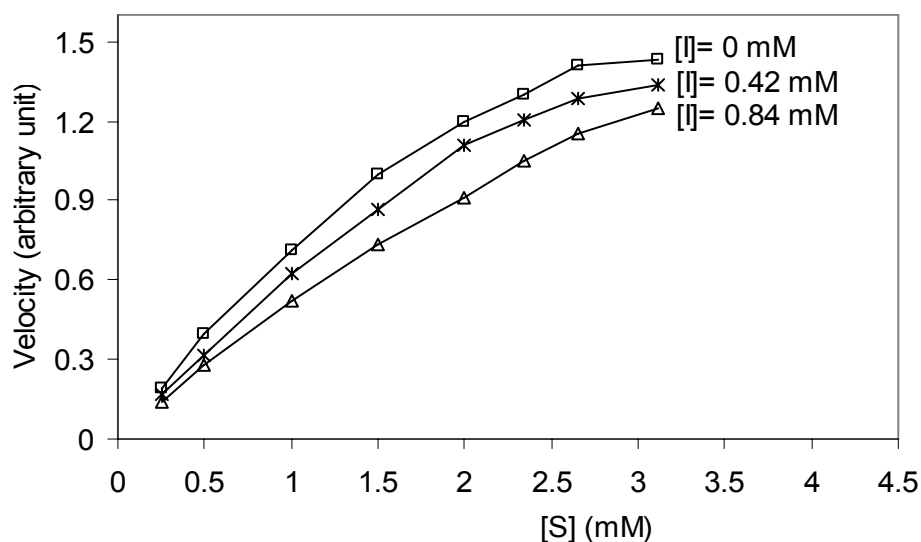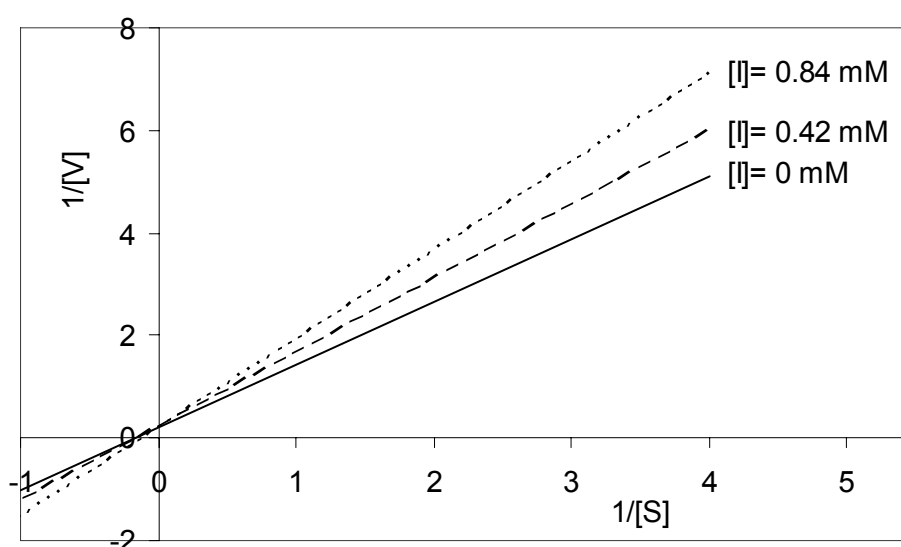
Fig. 28: LINEWEAVER-BURK double reciprocal plot in presence of **15h**

Determination of an inhibitor constant (K_i) is possible from the LINEWEAVER-BURK plot, but the method can be potentially misleading because it generally relies on a single inhibitor concentration for the determination of K_i [1]. The common method used nowadays for the determination of the inhibitor constant (K_i) is that introduced by DIXON [87].

3.6.2. Inhibitory activity of the compounds

The esters, acids, amides and alcohol derivatives were tested against PPE and HLE at 0.5 mM concentration. The peptides were also tested at 0.5 mM concentration against PPE and at 0.125 mM concentration against HLE. The results can be divided into three points.

1) The pseudosaccharin derivatives containing an amino acid analogue: The primary amide derivatives **4a**, **4b**, **4r-4t**, the acid derivatives **14a-14j**, the alcohol derivative

16, and the ester derivatives **4d, 4e, 4j, 4k, 4u-4z** were tested against PPE and against HLE. Only the ester derivatives **4j**, **4w** showed inhibition of both enzymes (table 11), while no inhibition was observed for acid, alcohol and primary amid derivatives. This is an important observation as **4j** is an isoleucine methyl ester analogue while **4w** is a valine methyl ester analogue which showed inhibition, while no other methyl ester analogues of other amino acids showed inhibition. This indicates that the isoleucine and valine play an important role in the inhibition. It is well documented that valine or alanine fit the S_1 pocket of HLE [14]. The presence of isoleucine at the P_1 position of the inhibitor is also reported [88]. Hence, possibly, the valine and isoleucine moiety of the pseudosaccharinamine derivatives occupy the S_1 pocket of the enzyme. It is also interesting to note that the alcohol derivative **16**, the primary amide derivatives **4a**, **4b**, **4r-4t**, and the acid derivatives **14a-14j** did not show any inhibition of HLE and PPE. When we look further into the structural details, we can find that **16** is an alcohol analogue of the isoleucine. Compounds **4a** and **4s** are primary amide derivatives containing isoleucine and valine, and **14c** and **14g** are acid derivatives containing the isoleucine and valine moieties. Interestingly, all these compounds are inactive. From the structure of these derivatives it becomes clear that the primary amides being weak electrophiles bound at an amino acid side chain, that the alcohol derivative devoid of an electrophilic centre at the amino acid side chain, and that the acid derivatives being weak electrophiles when looked at an amino acid side chain, possibly undergo a very slow reaction with a nucleophile. This is an important observation as the Ser-195 of the elastase executes a nucleophilic attack on the electrophilic centre. The ester derivatives are better electrophiles which is in agreement with our results. We found activity for the methyl ester derivatives of valine and isoleucine but not for the alcohol derivative, not for the primary amide derivatives, and not for the acid derivatives which all have valine or isoleucine in their structure. This indicates that not only the isoleucine or valine but also the electrophilic centre at the amino acid side chain is an important feature for an inhibitor. There might be a possibility of an attack of the enzyme on the sulphonamide of the pseudosaccharin nucleus. This attack is not completely ruled out. But the possibility of such a nucleophilic attack is low as all inactive derivatives, alcohol, primary amides and acids also contain a sulphonamide moiety.

Summarized, we suspect that the mechanism of the inhibition by the ester derivatives **4j** and **4w** involves the occupation of the S_1 pocket of the elastase by valine or isoleucine followed by a nucleophilic attack of Ser-195 on an electrophilic centre at the amino acid side chain.

For testing this explanation, the ester derivatives **15a**-**15h** containing isoleucine and valine were synthesized and tested. These ester derivatives showed inhibition of elastase except **15g** containing a *tert*-butyl ester of isoleucine. Perhaps, the *tert*-butyl moiety hinders the nucleophilic attack of the Ser-195. Therefore, more experiments are needed to clarify this result. Interestingly all other ester derivatives exhibited very weak inhibition of PPE while they showed high inhibition of HLE. This indicates that they are more specific towards HLE than towards PPE.

Compound **15b** produced a precipitate in the assay medium while testing it for PPE inhibition. The HLE inhibition by **15e** and **15h** at 0.5 mM concentration was very high and was not possible to measure. Therefore, the HLE inhibition of these two compounds was measured at 5 µM concentration. **15e** showed 69% inhibition while **15h** showed 50% inhibition of HLE activity at 5 µM concentrations. The higher inhibition of **15e** and **15h** is possibly contributed to the presence of a nitrile moiety in the structure. Compound **15e** was found to be specific towards HLE than towards PPE. The inhibition shown by the ester derivatives was found to be reversible (figure 26).

2) The pseudosaccharin derivatives containing a thiazole or thiophene ring: compounds **18a, 18b, 19, 21, 22, 24, 25** and **27** were tested at 0.5 mM concentration against both enzymes. Compound **28** was tested at 0.5 mM concentration against PPE and at 0.05 mM concentration against HLE while compound **29** was tested at 0.1 mM concentration against PPE and at 0.2 mM concentration against HLE. Compound **29** produced turbidity in the assay medium when tested at 0.5 mM concentration. Only compounds **22**, **28** and **29** showed an inhibition (table 11). **22** is an acid analogue of thiazole moiety while **28** is an acid analogue of thiophene moiety and **29** is an ester analogue of nitrothiophene derivative. More studies are indeed needed to understand the mechanism of action by these heterocycles.

3) The pseudosaccharin derivatives containing peptides: Compound **30** is a secondary amide analogue and showed 12% inhibition of both elastases (table 11). The peptidic derivatives **34** were found to be active against both elastases. The nitrile containing peptides (**34**) were tested at 125 µM concentrations against the HLE. Peptide **35** did not show any inhibition at concentration of 0.5 mM against HLE and PPE. The only difference between **34f** and **35** is the presence of a methyl ester moiety in **35** and the presence of an amido acetonitrile moiety in **34**. It is interested to note that the peptides **34a-34f** showed better inhibition of PPE than the non peptidic analogues **4j, 4w** and **15a-15e**.

4j, 15a-15g, 30　　　　**4w, 15h**　　　　　　**34a-34f**
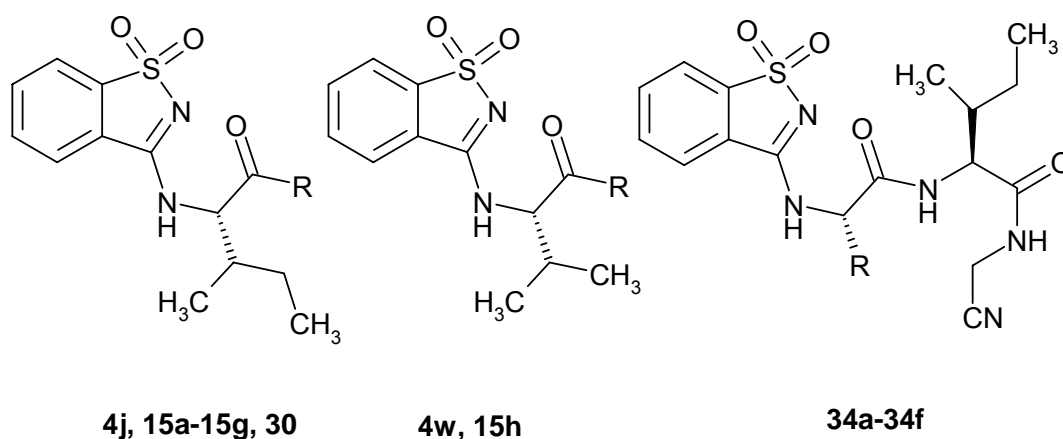
Table 11: Percent inhibition by different compounds at a concentration of 0.5 mM against PPE and HLE unless otherwise stated, 'a' denotes that the measurements were carried out with 2% cremophore; NI indicates that no inhibition was found.

Compound	R	PPE inhibition (%)	HLE inhibition (%)
4j	OCH_3	4	70
4w	OCH_3	4	65
15a	OCH_2CH_3	9	87
15b	$OCH(CH_3)_2$	-	83
15c	$OCH_2(CH_2)_2CH_3$	9	62
15d	$OCH_2CH(CH_3)_2$	8	75
15e	**OCH_2CN**	7	**69 at 5 µM**
15f	$OCH_2C_6H_5$	12	89
15g	$OC(CH_3)_3$	NI[a]	NI[a]
15h	**OCH_2CN**	47	**50 at 5 µM**
22	-	54	68
28	-	38	26 at 0.05 mM
29	-	32 at 0.1 mM[a]	15 at 0.2 mM
30	$NHCH_2CN$	12	12
34a	$CH_2CH_2SCH_3$	53	15 at 125 µM
34b	$CH_2CH(CH_3)_2$	35	04 at 125 µM
34c	$CH(CH_3)CH_2CH_3$	39	16 at 125 µM
34d	$CH(CH_3)_2$	29	04 at 125 µM
34e	CH_3	55	20 at 125 µM
34f	Proline analogue	23	13 at 125 µM
35	-	NI	NI

The possible reason for the higher activity of the peptides against PPE is that, in peptides one more amino acid residue is present which can occupy one more pocket of the active site of the enzyme producing better inhibition (figure 2).The nitrile containing peptides have been reported as cysteine protease inhibitors [89]. More recently, cyanoamides have been reported by FALGUEYRT [90] as inhibitors of the cysteine protease, human cathepsin K and L, and he proposed that the SH group of the cysteine attacks the nitrile.
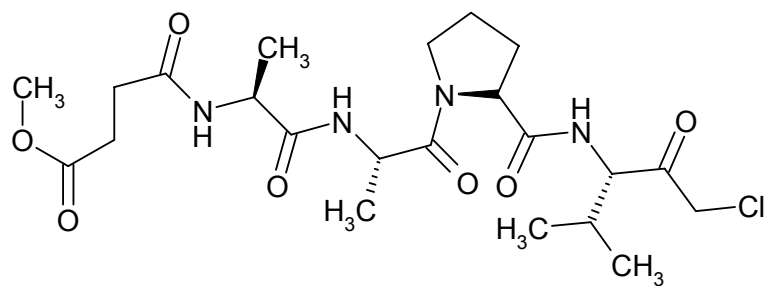
CMK (Chloromethyl ketone)
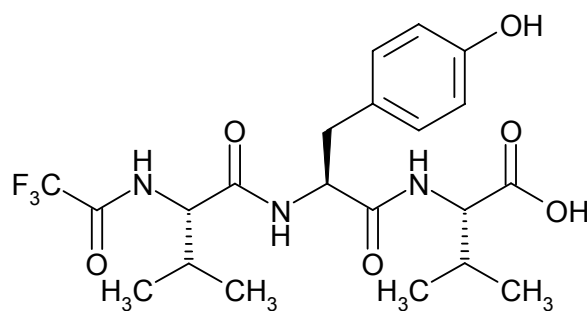
TFA (Trifluoroacetyl-val-tyr-val)

Fig. 29: CMK and TFA

CMK [91] (MeOSuc-Ala-Ala-Pro-Val-chloromethyl ketone) was used as a standard inhibitor for the HLE enzyme kinetics while TFA (Trifluoroacetyl-Val-Tyr-Val) [92] was used as a standard PPE inhibitor for the enzyme kinetics of PPE (figure 29). TFA showed a K_i of 0.56 µM against PPE [92]. CMK is reported as an irreversible inhibitor of HLE with a K_i value of 10 µM [91]. TFA showed 52% inhibition of the PPE at the concentration of 5 µM while the HLE inhibition by CMK was found to be 60% at the concentration of 2.5 µM.

An experiment was carried out to see whether compound **15e** is stable during the time of measurement of inhibition of both elastases. 0.5 mM of **15e** was incubated in tris-buffer and HEPES buffer in two separate test tubes. The aliquots were removed after 20 min and 4 h and were analysed by HPLC. The analysis showed that **15e** is stable in the both buffers. In another experiment, Suc-(Ala)$_3$-*p*-nitroanilide, the substrate for PPE, was added into the tris-buffer along with a 0.5 mM solution of **15e** and aliquots were removed after 20 min and 4 h and analyzed by HPLC. No degradation of **15e** was observed. 0.5 mM of **15e** was also incubated with methoxysuccinyl-Ala-Ala-Pro-Val-*p*-nitroanilide, the substrate for HLE, in HEPS buffer. The aliquots were removed after 20 min and 4 h and analyzed by HPLC. No degradation of **15e** was observed.

The reversible inhibition of **15e** was also found from an experiment in which **15e** and HLE were incubated in HEPES buffer for 4 h. After 4 h, the substrate was added and checked for the release of the product. It was observed that there was some recovery of the activity of HLE after 4 h.

Ester derivatives showing more than 50% inhibition against HLE and peptide derivatives **34c** and **34e** were further studied for determination of their K_i (table 12). K_i was determined from a DIXON plot. The initial velocity was measured as a function of the inhibitor concentration at two fixed concentrations of the substrate. The data were then plotted as 1/V as a function of the inhibitor concentration [I] for each substrate concentration, and the value of -K_i was determined from the x-axis value at which the lines intersect (figure 30, 31).

The different reference compounds are presented in table 12 along with their reported inhibitory constants (K_i). The only elastase inhibitor available in the market is ONO-5046 (Sivelestat). This compound has been launched in Japan (2002) as an injectable formulation (**Elaspol**®) for the treatment of acute lung injury associated with systemic inflammatory response syndrome (SIRS) [16]. However, although Sivelestat is commercially available in Japan, this clinical application is allowed under strictly ventilation-assisted circumstances. Furthermore, the clinical efficacy of sivelestat for acute lung injury has recently been controversial [93]. Erythromycin is a well studied antibiotic for the bactericidal effect. There is, however, an increasing body of evidence demonstrating that macrolide antibiotics may have activities not relates to their antibiotic properties. Hence, GORRINI et al had studied the inhibition of erythromycin and flurythromycin on HLE [94]. The author found that erythromycin at 50 µM concentration inhibits 15% HLE activity over 30 min. However, inhibition was temporary and full activity was regained within 1 h [94].

4j, 15a-15f   4w, 15h   34c, 34e
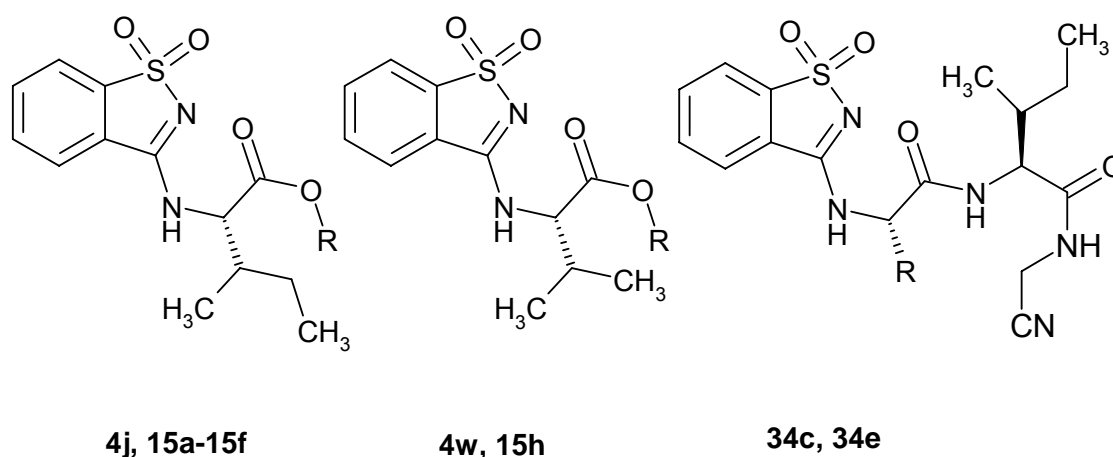
Table 12: Inhibitor constants (K_i) against HLE

Compound	R	K_i (µM)
4j	CH_3	45
4w	CH_3	60
15a	CH_2CH_3	36
15b	$CH(CH_3)_2$	31
15c	$CH_2(CH_2)_2CH_3$	36
15d	$CH_2CH(CH_3)_2$	57
15e	**CH_2CN**	**0.8**
15f	$CH_2C_6H_5$	38
15h	**CH_2CN**	**1.3**
34c	$CH(CH_3)CH_2CH_3$	70
34e	CH_3	47
ONO-5046	-	0.2 [37]
Midesteine (**MR-889**)	-	1.4 [16]
Erythromycin	-	2.98 [94]

The K_i values of the ester derivatives **4j, 4w, 15a-15d,** and **15f** are comparable. The K_i values of compound **15e** and **15h** are 0.8 µM and 1.3 µM respectively against HLE (figure 30, 31). The K_i value of ONO-5046, Midesteine and erythromycin is reported to be 0.2 µM, 1.4 µM and 2.98 µM respectively [37,16,94] and all three compounds showed the reversible inhibition [37,16,94]. The peptide **34c** and **34e** showed less inhibition of the HLE when compared with the nonpeptidic esters as seen from the table 12.
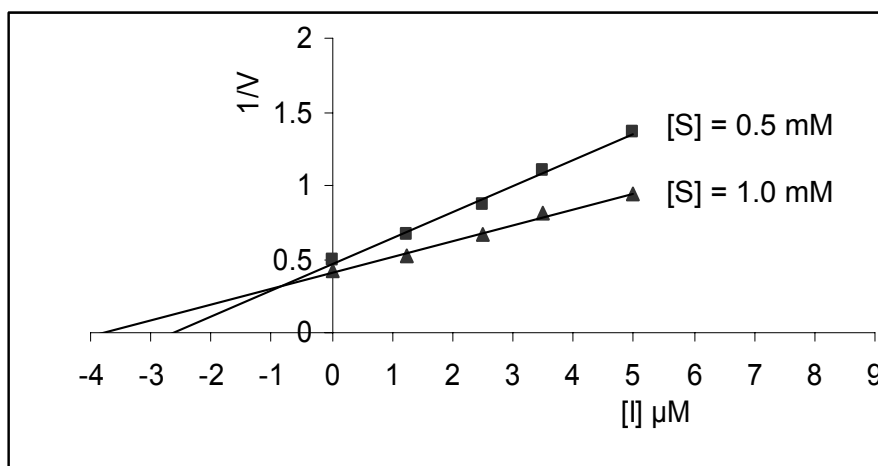
Fig. 30: Dixon plot showing the inhibition of HLE by **15e**
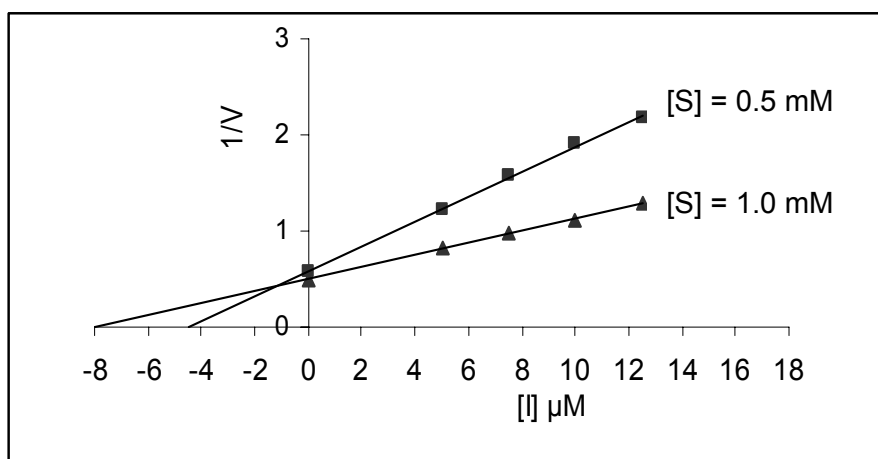
Fig.31: Dixon plot showing the inhibition of HLE by **15h**

When the structures of these two compounds are compared with the structures of the other ester derivatives, one can notice that there is a presence of an acetonitrile ester moiety which possibly is contributing for the higher activity of **15e** and **15h**. Further experiments are needed to conclude the mechanism of inhibition of HLE by **15e** and **15h**. **15e** can be a good candidate for the further chemical modification due to its high potency against HLE and at the same time its selectivity for HLE over PPE.

3.7. Molecular mechanics and docking studies

3.7.1. Molecular Mechanics

 Molecular geometry is the necessary starting point for most modelling treatment. Although modern NMR techniques can give some indication about interatomic distances for liquid samples, geometrical information, for large or medium-size molecules, is up to now mainly derived from crystallographic data. However, such information only concern crystals, where the geometry may be somewhat affected by pacing effects and is not automatically the same as for the reacting species in the biological media. In the X-ray data, if it gives the geometry of the more stable form, it says nothing about the energies and the possible existence of other low-energy structures. Suitable computational approaches are obviously needed for such applications. The methods of quantum mechanics are of course quite suited to predicting the geometric, electronic and energetic feature of known or unknown molecules [95]. Quantum mechanical method deals with the electron in a system, so that even if some of the electrons are ignored, a large number of the particles must be still considered, and the calculations are time-consuming. On the other hand, force field methods (also known as molecular mechanics) ignore the electronic motions and calculate the energy of a system as a function of the nuclear positions only. Molecular mechanics thus invariably is used to perform calculations on systems containing a significant number of atoms [96]. Molecular mechanics deals with the potential energy of a molecule. The potential energy of a molecule is obtained by adding all potentials resulted from bond stretching, angle bending, bond rotation, non-bonded interactions (electrostatic and van der Waals).

3.7.2. Docking

The action of drug molecules and the function of protein targets are governed by molecular recognition. Binding events between ligands and their receptors in biological systems form the basis of physiological activity and pharmacological effects of chemical compounds [97, 98]. Docking and virtual screening are computational tools to investigate the binding between macromolecular targets and potential ligands. They constitute an essential part of structure-based drug design, the area of medicinal chemistry that harnesses structural information for the purpose of drug discovery. The selective binding of a small-molecule as a ligand to a specific protein is determined by structural and energetic factors. For ligands of pharmaceutical interest, protein-ligand binding usually occurs through noncovalent interactions. The physical basis of noncovalent interactions is generally well

established through the theories of electromagnetic forces or, on a more fundamental level, of quantum mechanics [97, 98].

The primary forces acting between a protein and a ligand are of electrostatic nature. It is the interaction between explicit charges, dipoles, induced dipoles, and higher electric multipoles that lead to phenomena that are commonly referred to as salt bridges, hydrogen bonds, or van der WAALS interactions. In a simplified classification, it is only the charge-charge interactions. This interaction between two charges is of long range and considerable strength. In vacuum or uniform media it can be described by COULOMB's law. In aqueous solution of biomolecules, however, its application is complicated because of the presence of a large number of water molecules [97].

An interaction deserving special attention is that of hydrogen bonds. A hydrogen bond is defined as the interaction of an electronegative atom, the hydrogen-bond acceptor, with a hydrogen atom covalently bonded to an electronegative atom, the hydrogen bond donor. The major component of a hydrogen bond is the electrostatic interaction of the donor-hydrogen dipole with the negative partial charge of the acceptor. The special characteristics originate from the fact that the hydrogen atom is very small and can bear a considerable positive charge, such that the acceptor can contact the hydrogen atom at a shorter distance than expected from the van der WAALS radii. Hydrogen bonds are directed interactions showing a high angular dependency. This directionality arises from the anisotropic charge distribution around the acceptor atom (lone pairs) and the fact that the electron shells of donor and acceptor atom start to overlap at these short distances unless the ideal geometry is maintained. Hydrogen bonds are attributed an important role with respect to the specificity of the protein-ligand interactions. This is based on their directionality and the fact that they require a well-defined complementarity in the complex, a mutual arrangement of hydrogen-bond donors and acceptors [97]. Hydrogen bonds are often scored by simply counting the number of donor-acceptor pairs that fall in a given distance and angle range favourable for hydrogen bonding, weighted by penalty function for derivations from preset ideal values. A penalty function has been avoided in many studies and instead the use of a separate regression coefficient for strong, medium and weak hydrogen bonds was reported [98].

Weakly polar interactions in proteins and protein-ligand complexes are frequently phenomenologically analysed and classified in terms of the interacting partners. This especially includes interactions with π-systems, such as the NH-π, OH-π, or CH-π interactions, aromatic-aromatic interactions (parallel π-π stacking versus edge-to-face

interactions), and the cation-π interactions. All of these can mostly be rationalized in terms of electrostatic interactions, interactions between monopoles, dipoles, and quadrupoles. A more distinct character can be attributed to metal complexation, which can play a significant role in individual cases of protein-ligand interactions, as for example in metalloenzymes [97].

Hydrophobic or lipophilic interactions are often mentioned as additional contribution to protein-ligand interactions. These terms are used to describe the preferential association of nonpolar groups in aqueous solutions [97]. Hydrophobic interactions are the major driving force for complex formation. These interactions are usually estimated by the size of the contact surface at the receptor-ligand interface. Various approximations have been used, such as grid-based methods and volume-based methods [98].

Another important factor which must be considered in protein-ligand complex is entropy. Entropy terms account for the restriction of conformational degrees of freedom of the ligand and upon complex formation. A crude but useful estimate of this entropy contribution is the number of freely rotatable bonds of a ligand. This measure has the advantage of being a function of the ligand only. More elaborate estimate try to take into account the nature of each ligand half on either side of a flexible bond and the interaction they form with the receptor, since it is argued that purely hydrophobic contacts allow more residual motion in the ligand fragment [98].

Thermodynamically, the strength of the interaction between a protein and a ligand is described by the binding affinity or GIBBS free energy of binding. Assuming a simple equilibrium reaction of the form [97],
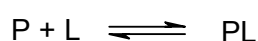
$$P + L \; \rightleftharpoons \; PL$$

between a protein P and a ligand L to give the complex PL, the dissociation constant K_d, or binding constant K_i, is generally used to describe the stability of complex formation:

$$K_d = [P]\,[L]/\,[PL]$$

From the experimentally measured equilibrium constant the binding affinity can be calculated as

$$\triangle G^0 = RT \ln K_d$$

Where R is the gas constant (8.314 J / molK) and T is the temperature. Experimentally determined binding constant K_i (K_d) are typically in the range of 10^{-2} to 10^{-12} M, corresponding to -10 to -70 kJ/mol. There are different programmes available to study the protein-ligand docking based on different methods. For e.g., systematic search (translational + rotational) method can be carried out with EUDOC program, Monte Carlo minimization method by ICM, Molecular dynamics (MD) by MDD, Genetic algorithm by GOLD and as well as others.

Molecular mechanics and docking studies were performed on the selected compounds. Energy minimized structures were obtained using the MMF94 force field method with the help of the programme "PC Model". Thus obtained structures were then used in order to generate the partial atomic charges by the *ab initio* method (Method: HARTREE FOCK, Basis set: 6-31G (d). These compounds were then docked into the active site of HLE. The structure of HLE was obtained from Brookhaven Protein Database (Structure No. 1H1B) and presented in the figure 32, while the catalytic triad of HLE is shown in figure 33. The docking process was carried out by "Discovery 3" minimization programme on 400 MHz MIPSR1200 (IP30) processor attached with the "Silicon Graphics Octane 2" work station. The docking study was performed with the help of "cvff" force field of the software "Insight II (2000)" from "Accelrys" (San Diego, CA). The figure 34 indicates the ten low energy conformers of **4w** obtained by docking this compound into the active site of the enzyme.
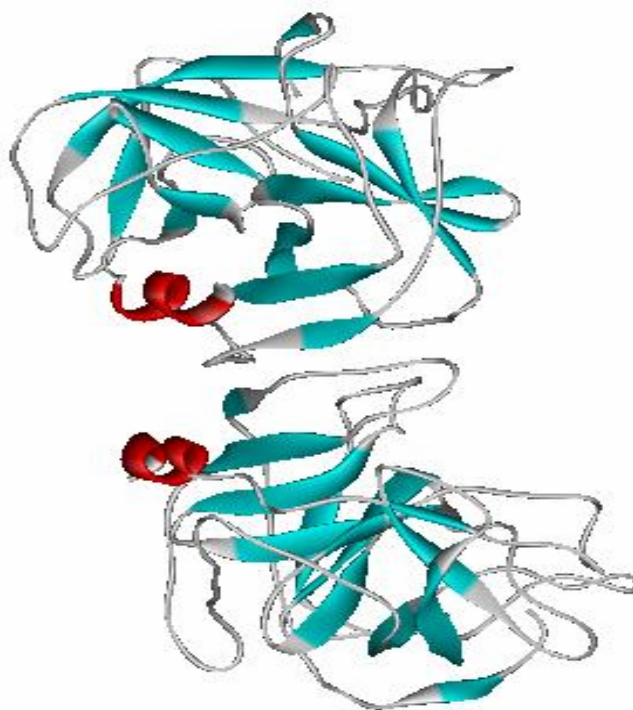
Fig.32: HLE shown in the flat ribbon display mode
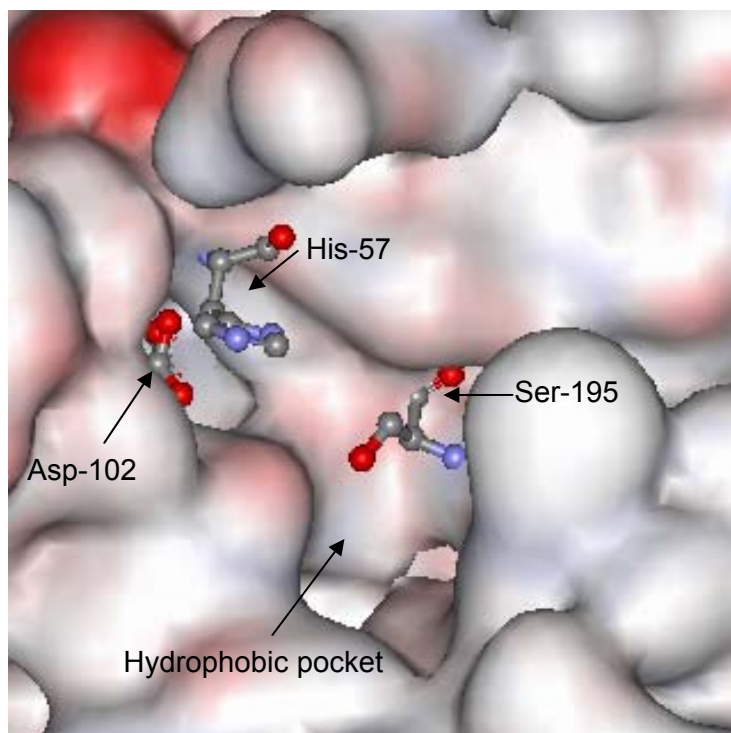
Fig.33: "Catalytic triad" of HLE
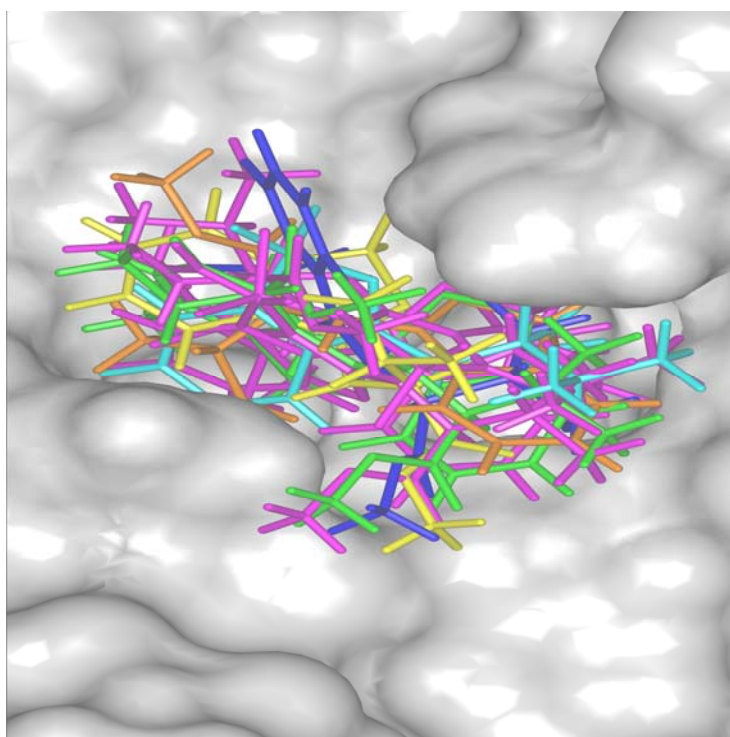
Fig. 34: Compound **4w** docked into the active site of HLE

Interesting results were obtained from the docking study. Five to ten low energy conformers were found for each compound. The complete list of all these conformers is presented in appendix. The lowest binding energy of the conformers is presented in table 13 along with their K_i values against HLE. The acetonitrile side chain of **15e** and **15h** was found to penetrate deeply into the hydrophobic pocket of the HLE (figure 35 and 36).
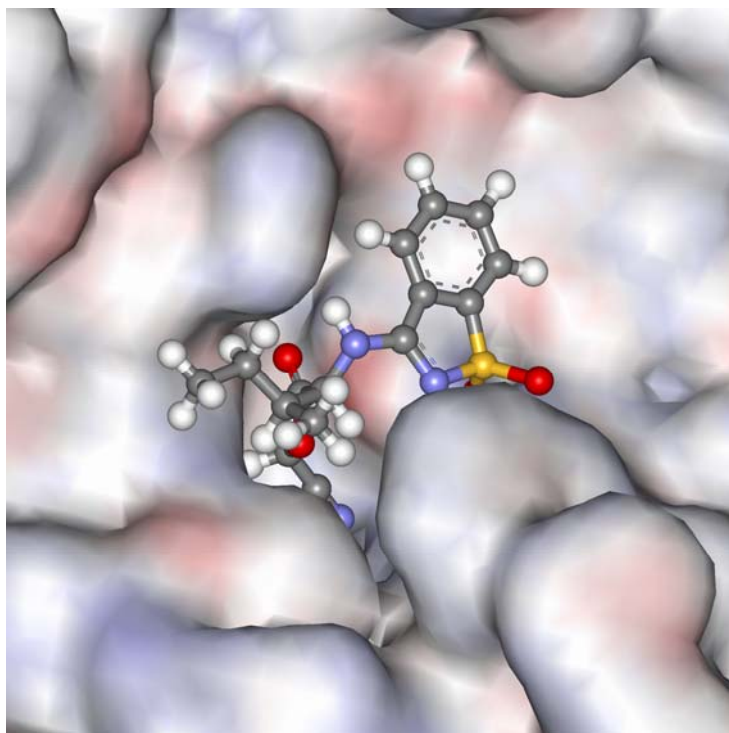
Fig. 35: Compound **15e** docked in the active site of HLE

The lowest binding energy of **15e** and **15h** was found to be -87.0 and -88.1 Kcal/mol respectively. These two conformers are the lowest binding energy conformers in the whole series. The lowest binding energy found for **15g** was -56.8 Kcal/mol. The important observation was that **15g** was unable to penetrate deep into the active site of HLE as shown in figure 37. The bulky *tert*-butyl group possibly prevents (force it out) **15g** entering into the hydrophobic pocket of the active site. This compound was also unable to inhibit the enzyme (table 13).

The lowest binding energy of **15f** and **15c** was found to be -80.6 and -78.8 Kcal/mol respectively. Compound **15d**'s lowest binding energy was found to be -47.5 Kcal/mol and this is the highest energy conformer in the whole series.
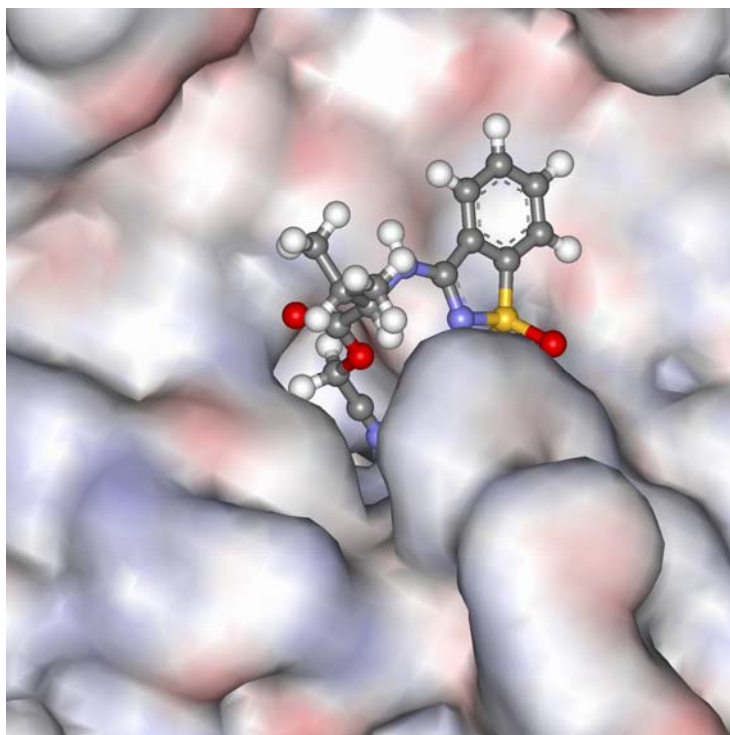
Fig. 36: Compound **15h** docked in the active site of HLE
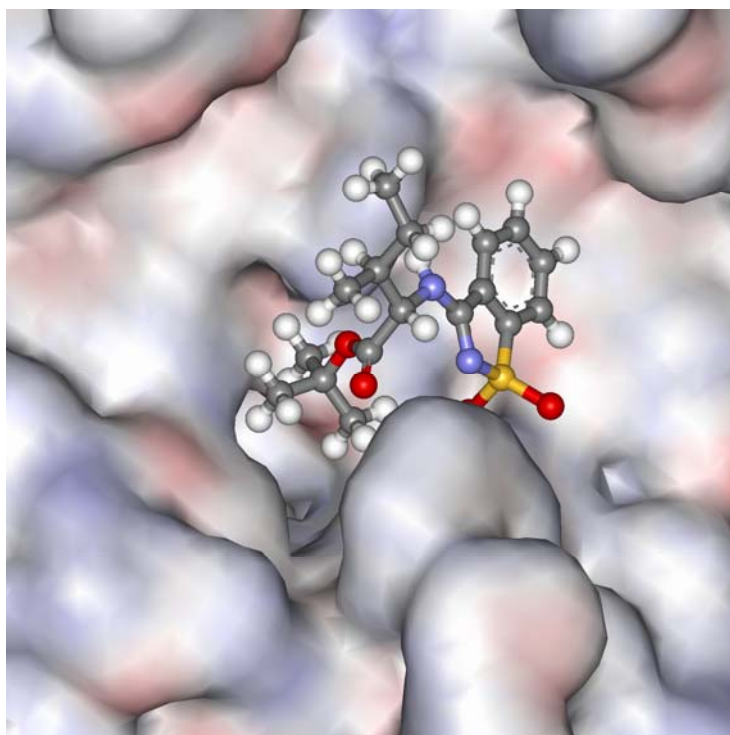
Fig. 37: Compound **15g** docked in the active site of HLE
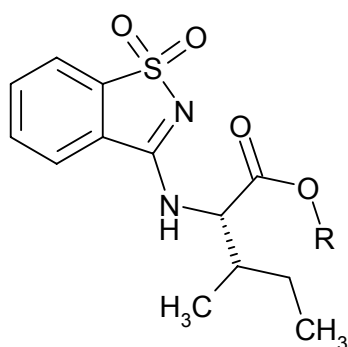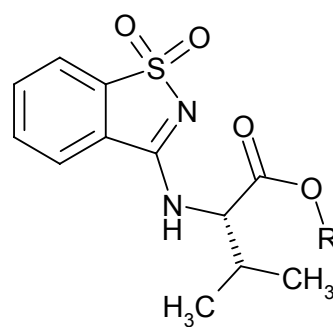
4j, 15a-15g   **4w, 15h**

Table 13: The lowest binding energy of the conformers (when docked) along with the K_i values against HLE. 'NA' indicates no activity.

Compound	R	K_i (µM)	Binding energy (Kcal/mol)
4j	CH_3	45	-54.2
4w	CH_3	60	-66.3
15a	CH_2CH_3	36	-64.4
15b	$CH(CH_3)_2$	31	-67.5
15c	$CH_2(CH_2)_2CH_3$	36	-78.8
15d	$CH_2CH(CH_3)_2$	57	-47.5
15e	CH_2CN	0.8	-87.0
15f	$CH_2C_6H_5$	38	-80.6
15g	$C(CH_3)_3$	NA	-56.8
15h	CH_2CN	1.3	-88.1
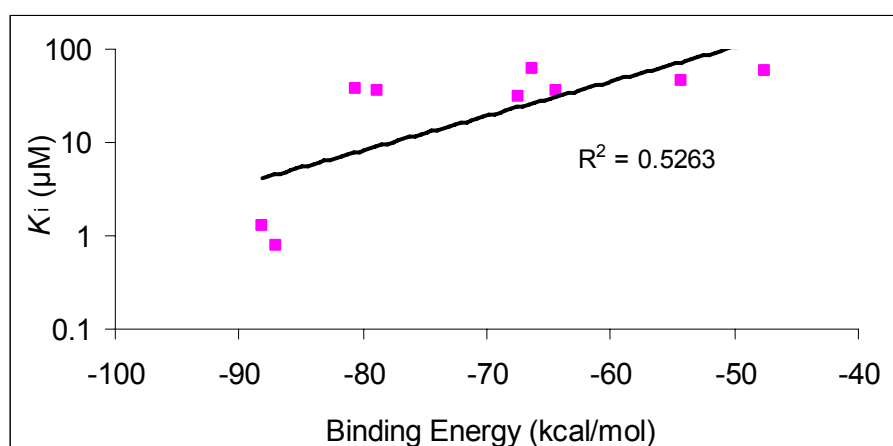
Fig. 38: Correlation of the binding energy and K_i

The graph of binding energy (Kcal/mole) vs ln K_i is shown in figure 38. A rough correlation was observed between the total binding energy and the ln K_i values, as predicted by following equation.

$$\triangle G^0 = RT \ln K_i$$

The regression coefficient was found to be 0.53. The docking study also predicted the conformers of compound **15e** and **15h** with lowest binding energy in the whole series.

4. Conclusions

The synthesis of pseudosaccharin amine derivatives was carried out from (1,1-dioxobenzo[*d*]isothiazol-3-ylsulfanyl)acetonitrile (**3**) and amines. The reaction was found to be slow. Thiosaccharinates (**10**) upon refluxing in absolute acetic acid also produced the pseudosaccharinamines but in low yields. Low yields of pseudosaccharinamines were then overcome by using the 3-ethoxybenzo[*d*]isothiazole 1,1-dioxide (**12**) and amines. This reaction produced pseudosaccharinamines in satisfactory yields. Different methyl ester and amide derivatives of pseudosaccharin amines were then produced in satisfactory to high yields from a classical route involving the reaction of 3-chlorobenzo[*d*]isothiazole 1,1-dioxide (**13**) with amino acid methyl esters or amino acid amides, respectively. Different esters, alcohol, and acid derivatives were synthesized and characterized. The peptides containing an aminoacetonitrile moiety were successfully synthesized by the mixed anhydride method. The synthesized esters, amides, alcohol, acid derivatives and peptides were screened for elastase inhibitory activity. The conclusions obtained from the inhibitory test can be divided into three points.

1) The pseudosaccharin derivatives containing an amino acid analogue: The primary amides, acids and alcohol derivative did not show any inhibition against both elastases (PPE and HLE) at 0.5 mM concentration. Only the ester analogues of isoleucine and valine showed activity against both the enzyme. These ester derivatives showed very weak inhibition of PPE at 0.5 mM but showed better inhibition of HLE. Cyanomethyl (2*S*,3*S*)-2-(1,1-dioxobenzo[*d*]isothiazol-3-ylamino)-3-methylpentanoate (**15e**) and cyanomethyl (2*S*)-2-(1,1-dioxobenzo[*d*]isothiazol-3-ylamino)-3-methylbutanoate (**15h**) were found to be the most active inhibitors of HLE with K_i values of 0.8 µM and 1.3 µM respectively.
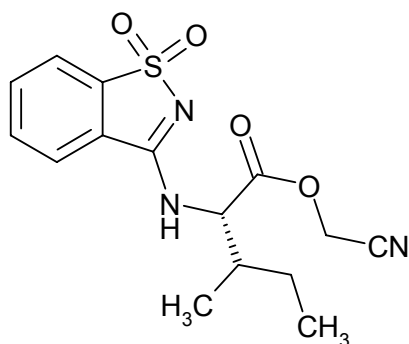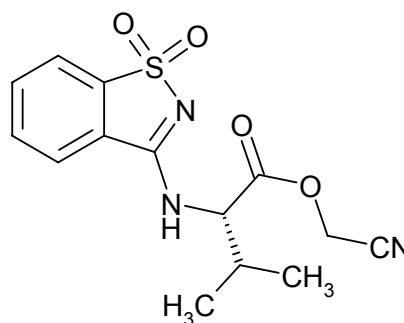
15e   **15h**

Compound **15h** showed some specificity towards HLE while **15e** showed better specificity towards HLE compared to PPE. Both compounds were found to be competitive reversible inhibitors of HLE. **15e** is stable during the biochemical studies, and might be a good candidate for further chemical modifications due to its high potency against HLE and at the same time its selectivity for HLE over PPE.

2) The pseudosaccharin derivatives containing a thiazole or thiophene ring: The acid derivative [2-(1,1-dioxobenzo[*d*]isothiazol-3-ylamino)thiazol-4-yl]acetic acid (**22**), 2-(1,1-dioxobenzo[*d*]isothiazol-3-ylamino)-thiophene-3-carboxylic acid (**28**) and 3-(1,1-dioxobenzo[*d*]isothiazol-3-ylamino)-5-nitro-thiophene-2-carboxylic acid methyl ester **(29)** showed inhibition of both elastases.

3) The pseudosaccharin derivatives containing peptides: All those peptides containing an aminoacetonitrile moiety (**34a-34f**) showed inhibition of PPE as well as of HLE. These peptides showed better inhibition of PPE than the non peptidic analogues (**4j, 4w, 15a-15f**). (2*S*)-2-{[1-(1,1-Dioxobenzo[*d*]isothiazol-3-yl-)pyrrolidine-2-carbonyl]amino}-(2*S*,3*S*)-3-methylpentanoic acid methyl ester (**35**) was found to be inactive against both enzymes at 0.5 mM concentration.

The docking experiments were performed on the selected nonpeptidic compounds. The acetonitrile moiety of **15e** and **15h** was found to penetrate deeply into the hydrophobic pocket of the active site of HLE.

5. Experimental

5.1. General Information

All the moisture free operations were performed by oven dried glassware under the atmosphere of nitrogen.

Column chromatography (CC) was performed by using Merck silica gel 60, Nr. 7734.

Melting points (M.p.) were determined by MEL-TEMP (Mel-Temp laboratories Inc, USA) melting point apparatus and are uncorrected. The melting of polymorphic substances was measured on PERKIN ELMER Differential Scanning Calorimeter at "Inorganic Chemistry Department of University of Halle (Saale).

Analytical TLC was performed on Merck TLC plates (Aluminium plates coated with silica gel 60 F_{254}, Nr.5554). Visualization of spots was carried out by using ultraviolet illumination (λ = 254 nm). Analytical data are reported as "ratio of front"-values (R_f -value).

Specific rotations were measured by using a Polartronic D apparatus (Schmidt Haensch GmbH).

Infrared spectra were obtained by using an IR spectrophotometer, Perkin-Elmer 1600 series FTIR. Absorption is reported in wave number ($\tilde{v}$).

Proton (^{1}H) nuclear magnetic resonance (NMR) spectra were measured with a Bruker DPX 200 (200 MHz) spectrometer, and carbon (^{13}C) NMR spectra were measured with a Bruker DPX 200 (50 MHz), both at 25 °C with tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported as parts per million (ppm, δ units). Coupling constants are reported in units of Hertz (Hz). The spectra were analysed by MESTREC NMR software. The following abbreviations were used -s: Singlet, bs: broad Singlet, d: Doublet, dd: Doublet of Doublets, dt: Doublet of Triplets, dq: Doublet of Quartets, t: Triplet, td: Triplet of Doublets, q: Quartet, qn: Quintet, spt: Septet, hpt: Heptet, oct: Octet, m: Multiplet.

Analytical purity was assessed at 30 °C by RP-HPLC using LaChrom apparatus series 7000, Merck Hitachi (Pump: L-7100, Diode-Array-Detector L-7450,

Auto sampler L-7200, thermostat column L-7350, Solvent degasser L-7612, Interface D-7000). Column used was LiChrospher 250-4, RP-18, 5 µm. The chiral compounds were also analysed by using a chiral column, LiChroCART 250-4, Chiralcel OJ-R, 5 µm. The measurement was carried out at λ_{max} 220 nm unless otherwise stated.

Mass spectra were taken on an AMD MS40, Varian MAT CH 7, MAT 731 (Elektronenstoßionisation EI, 70 eV).

Elemental analyses were performed on a Perkin-Elmer Elemental Analyzer 2400 CHN. The results are presented in%.

Tetrahydrofuran (THF) was dried with sodium and distilled in the presence of benzophenone prior to use. The other solvents were dried according to the following method.

Technical grade dioxane, concentrated HCl and water were refluxed for 10 h with a slow steam of nitrogen bubbling through the solution. The cold solution was treated with an excess potassium hydroxide pellets with shaking until some remained undissolved, and a strong alkaline aqueous layer ran off. The dioxane kept over potassium hydroxide pellets for 24 h. Thus obtained partially dry dioxane was then heated with sodium for   6-10 h till sodium remains bright. The dioxane was then distilled and kept in dark.

The commercial grade dichloromethane was purified by washing with portions of concentrated sulphuric acid, water, sodium carbonate solution, and water again. It was dried initially with calcium chloride and then distilled from calcium hydride.

Commercial grade THF was initially dried with calcium sulphate and distilled over calcium hydride. All other solvents were used without further purification.

Syn-ethyl (2-amino-4-thiazolyl)-α-methoxyiminoacetate (Cat 28,015-1), ethyl (2-amino-4- thiazolyl)acetate (Cat 22, 055-8), 3-aminothiophene-2-carboxylic acid methyl ester (Cat 23, 290-4) and 2-aminothiophene-3-carboxylic acid methyl ester (Cat 56, 308-0) were purchased from Aldrich. PPE (EC 3.4.21.36, $\approx$ 200 U/mg) and HLE (EC 3.4.21.37, $\approx$ 34 U/mg) were purchased from Serva. Suc-(Ala)$_3$-pNA, and *N*-methoxysuccinyl-(Ala)$_2$-Pro-Val-pNA were obtained from Bachem. TFA and CMK were purchased from Bachem.

5.2. Synthesis of the compounds

5.2.1. General method for the synthesis of amino acid methyl ester hydrochloride

0.1 mole of amino acid was added in 60 ml of methanol and stirred at -10 °C (ice-salt) for 10 min. In that added 8.03 ml (0.11 mol) of thionyl chloride drop wise over half an hour while maintaining the temperature below -5 °C. Reaction mixture was heated to reflux for 2 h. Methanol was removed in vacuo to obtain a thick liquid. Methanol (2 x 100 ml) was then added and again removed in vacuo to produce crystalline solid or liquid compound.

5.2.1.1. *L-Methionine methyl ester hydrochloride*

From 14.92 g of L-methionine. Yield: 18.20 g (92%); M.p. 142 °C (144-149 °C) [99]; $[\alpha]_D^{20}$ = -28.83 (c = 2, H_2O), [+22.1 (c = 1, H_2O)] [99].

5.2.1.2. *L-Leucine methyl ester hydrochloride*

From 13.10 g of L-leucine. Yield: 16.80 g (93%); M.p. 146 °C (148 °C) [100]; $[\alpha]_D^{20}$ = +13.66 (c = 2, H_2O), [+14.00 (c = 5, H_2O)] [100].

5.2.1.3. *L-Isoleucine methyl ester hydrochloride*

From 13.10 g of L-isoleucine. Yield: 14.52 g (80%); M.p. 92 °C (80 °C) [101]; $[\alpha]_D^{20}$ = +25.83 (c = 2, H_2O), [+ 36.3 (c = 2, MeOH)] [101].

5.2.1.4. *Glycine methyl ester hydrochloride*

From 7.50 g of glycine. Yield: 11.10 g (88%); M.p. 170-173 °C (178-180 °C) [101].

5.2.1.5. *L-Phenyl alanine methyl ester hydrochloride*

From 16.50 g of L-phenyl alanine. Yield: 11.10 g (84%); M.p. 157-159 °C (159-160 °C) [101]; $[\alpha]_D^{20}$ = -11.25 (c = 0.8, H_2O), [+16.1 (c = 0.5, MeOH)] [101].

5.2.1.6. *L-Alanine methyl ester hydrochloride*

From 8.90 g of L-alanine. Yield: 11.00 g (79%); M.p. 104-107 °C (104-108) [101]; $[\alpha]_D^{20}$ = +9.00 (c = 2, H_2O), [+6.5 (c = 2, MeOH)] [101].

5.2.1.7. L-*Valine methyl ester hydrochloride*

From 11.70 g of L-valine. Yield: 14.00 g (84%); M.p. 169-172 °C (164-167 °C) [101];
$[\alpha]_D^{20}$ = +16.5 (c = 2, H$_2$O), [+25.9 (c = 2, MeOH)] [101].

5.2.1.8. α-*Methyl alanine methyl ester hydrochloride*

From 10.30 g of α- methyl alanine. Yield: 14.50 g (95%); M.p. 165-167 °C;
IR: ν = 3426 (NH), 2956(CH), 1745 (CO) cm^{-1}; ^{1}H-NMR (CDCl$_3$): δ = 8.98 (bs, 1H,
NH), 3.85 (s, 3H, OMe), 2.30 (bs, 1H, HCl), 1.76 (s, 6H, 2Me); C$_5$H$_{12}$ClNO$_2$ (153.61),
calculated: C 39.05, H 7.81, N 9.11; found: C 37.57, H 8.34, N 9.37.

5.2.1.9. L-*Aspartic acid dimethyl ester hydrochloride*

From 13.30 g of L-aspartic acid. Yield: 15.00 g (83%); M.p. 110-113 °C (111-114 °C)
[100]; $[\alpha]_D^{20}$ = +11.0 (c = 2, H$_2$O), [+15.0 (c = 2, MeOH)] [101].

4.2.1.10. L-*Proline methyl ester hydrochloride*

From 11.50 g of L-proline. Yield: 10.00 g (61%); colourless liquid; $[\alpha]_D^{20}$ = -30.5
(c = 2, H$_2$O), [-28.9 (c = 0.5, H$_2$O)] [102].

5.2.2. *Benz[d]isothiazol-3(2H)-thione 1,1-dioxide* (2)

a) A mixture from benz[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (**1**) (5.0 g, 27 mmol) and
P$_2$S$_5$ (6.67 g, 30 mmol) was heated in a small round-bottom flask in an oil bath. The
bath temperature was allowed to rise slowly from 50 to 155 °C over 45 min. Heating
was continued for 15 min while the temperature rose to 170° C. The mixture was
extracted with toluene (1 l). The extract was concentrated and left for 12 h at room
temperature. The crystals were separated and dried. Yield: 4.1 g (75%).

b) Benz[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (**1**) (2.0 g, 11 mmol) dissolved in dioxane,
and P$_2$S$_5$ (4.85 g, 22 mmol) were refluxed for 6 h. After cooling to room temperature,
the precipitate was separated and the filtrate was concentrated to ca. 50%, poured
into ice cold water and left for 12 h. Work-up as a). Yield: 0.8 g (37%); M.p.
178-180 °C (Toluene, 178-178.5°C) [54]; R$_f$: 0.6 (MeOH/CH$_2$Cl$_2$, 1:3); IR: $\tilde{v}$ = 3338
(NH), 1368, 1150 (SO$_2$), 1120 (C=S); ^{1}H NMR (CDCl$_3$): δ = 8.25-8.17, 7.96-7.77 (2m,
4H, ar); HPLC: k' = -0.214, t$_0$ = 1.77 (RP-18, MeCN/H$_2$O, 1:1); C$_7$H$_5$NO$_2$S$_2$ (199.25),
calculated: C 42.20, H 2.53, N 7.03; found: C 42.28, H 2.46, N 6.92.

5.2.3. *(1,1-Dioxobenzo[d]isothiazol-3-ylsulfanyl)acetonitrile* (3)

At 0 °C and under N_2, NaH (1.56 g, 65.0 mmol) was slowly added with stirring to an ice-cold solution of benz[*d*]isothiazol-3(2*H*)-thione 1,1-dioxide (**2**) (6.0 g, 50.18 mmol) in DMF (100 ml). Stirring was continued for 30 minutes followed by the addition of chloroacetonitrile (5.1 ml, 67.29 mmol). Stirring was continued and progress of reaction was monitored by TLC. After 10 h, the mixture was poured into water and the precipitate was separated. Yield: 6.0 g (50%); M.p. 222-223 °C (MeCN/Acetone, 234.5-235.5) [55]; R_f: 0.5 (AcOEt/PE 6:4); IR: $\tilde{v}$ = 2991 (CH), 2256 (CN), 1616 (C=N), 1322, 1171 (SO_2), 1170 (C=S); ^{1}H NMR ([D_6]DMSO): δ = 8.18-8.12, 8.01-7.84 (2m, 4H, ar), 4.52 (s, 2H, CH_2); HPLC: k' = 1.41, t_0 = 1.77 (RP-18, MeCN/H_2O, 1:1); $C_9H_6N_2O_2S_2$ (238.29), calculated: C 45.37, H 2.54, N 11.76; found: C 44.99, H 2.27, N 11.63.

5.2.4. *(S)-2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)-4-methylpentanamide* (4a)

a) A mixture from (1,1-dioxobenzo[*d*]isothiazol-3-ylsulfanyl)acetonitrile (**3**) (0.3 g, 1.2 mmol) dissolved in DMSO (6 ml), and L-Leu-NH_2 (0.16 g, 1.2 mmol) in DMSO (3 ml) was stirred at room temperature for 2 h. It was then poured into water and extracted with AcOEt. The organic layer was washed with brine, dried with sodium sulfate, and concentrated in vacuo. Purification by CC (MeOH/CH_2Cl_2 3:7). Yield: 0.05 g (13%). b) L-Leu-NH_2 (0.22 g, 1.68 mmol) and (1,1-dioxobenzo[*d*]isothiazol-3-ylsulfanyl)acetonitrile (**3**) (0.40 g, 1.67 mmol) in MeOH (10 ml) were stirred at room temperature for 24 h. The solvent was evaporated in vacuo and the residue was purified by CC (AcOEt/PE 8:2). Yield: 0.18 g (36%). c) L-Leu-NH_2 (0.16 g, 1.2 mmol) and (1,1-dioxobenzo[*d*]isothiazol-3-ylsulfanyl)acetonitrile (**3**) (0.30 g, 1.2 mmol) in $CHCl_3$ were stirred at room temperature for 6 days, the solvent was evaporated in vacuo, and the residue was crystallized. Yield: 0.25 g (68%); M.p. 254-256 °C (MeOH); R_f: 0.66 (MeOH/CH_2Cl_2 1.5:8.5); $[\alpha]_D^{20}$ = +18.33 (c = 2, DMSO); IR: $\tilde{v}$ = 3450, 3325 (NH), 3067, 2958, 2929 (CH), 1686 (CO), 1628 (C=N), 1370, 1165 (SO_2); ^{1}H NMR ([D_6]DMSO): δ = 9.46 (d, J = 8.28, 1H, NH), 8.44-8.32, 8.03-7.92, 7.89-7.81 (3m, 4H, ar), 7.78 (bs, 1H, NH) 7.20 (bs, NH), 4.63-4.66 (m, 1H, α-H, Leu), 1.93-1.55 (m, 3H, [2H, β-H, Leu + 1H, γ-H, Leu]), 0.93 (3H, d, J = 5.90 Hz, δ-H, Leu) 0.90 (3H, d, J = 5.90 Hz, δ-H, Leu); HPLC: k' = 0.53, t_0 = 1.99 (RP-18, MeCN/H_2O, 1:1); HPLC: k' = 1.47, t_0 = 2.36 (Chiralcel OJ-R, MeCN/H_2O 2:8); $C_{13}H_{17}N_3O_3S$ (295.36), calculated: C 52.87, H 5.80, N 14.23; found: C 52.56, H 5.62, N 14.06.

5.2.5 *(S)-2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)-3-phenylpropanamide* (4b)

a) From L-Phe-NH$_2$ (0.21 g, 1.2 mmol) and (1,1-dioxobenzo[*d*]isothiazol-3-ylsulfanyl)acetonitrile (**3**) (0.3 g, 1.2 mmol) in DMSO (9 ml) as described for **4a**. Yield: 0.09 g (21%). b) From L-Phe-NH$_2$ (0.25 g, 1.5 mmol), and (1,1-dioxobenzo[*d*]isothiazol-3-ylsulfanyl)acetonitrile (**3**) (0.37 g, 1.5 mmol) in MeOH (10 ml) for 5 h as described for **4a**. Yield: 0.08 g (16%). c) From L-Phe-NH$_2$ (0.21 g, 1.2 mmol), and (1,1-dioxobenzo[*d*]isothiazol-3-ylsulfanyl)acetonitrile (**3**) (0.3 g, 1.2 mmol) in CHCl$_3$ (25 ml) for 11 days. Yield: 0.25 g (60%); M.p. 214 °C (AcOEt/PE); R$_f$: 0.66 (MeOH/CH$_2$Cl$_2$ 1.5:8.5); $[\alpha]_D^{20}$ = -27.50 (c = 2, Dioxane); IR: $\tilde{\nu}$ = 3444, 3311 (NH), 3100, 2934 (CH), 1691 (CO), 1618 (C=N), 1360, 1164 (SO$_2$); ^{1}H NMR ([D$_6$]DMSO): δ = 9.62 (d, *J* = 8.20 Hz, 1H, NH), 8.38-8.28, 7.98-7.25 (2m, 4H, ar), 7.42-7.10 [m, 5H, ar (Phe)], 4.80-4.65 (m, 1H, α-H, Phe), 3.30-3.00 (m, 2H, β-H, Phe); HPLC: k' = 0.62, t$_0$ = 1.99 (RP-18, MeCN/H$_2$O, 1:1); HPLC: k' = 3.33, t$_0$ = 2.36 (Chiralcel OJ-R, MeCN/H$_2$O 2:8); C$_{16}$H$_{15}$N$_3$O$_3$S (329.38), calculated: C 58.35, H 4.59, N 12.76; found: C 57.63, H 3.99, N 12.11.

5.2.6. *Benzo[d]isothiazol-3-ylamine 1,1-dioxide* (4c)

(1,1-Dioxobenzo[*d*]isothiazol-3-ylsulfanyl)acetonitrile (**3**) (0.3 g, 1.2 mmol) in methanolic ammonia (15 ml) was stirred for 0.5 h. The solution was evaporated in vacuo. Yield: 0.11 g (48%).; M.p. 307 °C (308 °C) [103]; R$_f$: 0.55 (MeOH/CH$_2$Cl$_2$ 1.5:8.5); IR: $\tilde{\nu}$ = 3340, 3282 (NH), 1679 (C=N), 1289, 1164 (SO$_2$); ^{1}H NMR ([D$_6$]DMSO): δ = 8.96 (bs, 2H, NH$_2$), 8.20-8.11, 8.02-7.93, 7.88-7.78 (3m, 4H, ar); HPLC: k' = 0.29, t$_0$ = 1.77 (RP-18, MeCN/H$_2$O, 1:1); C$_7$H$_6$N$_2$O$_2$S (182.20), calculated: C 46.15, H 3.32, N 15.37; found: C45.95, H 3.40, N 15.58.

5.2.7. *Methyl (S)-2 (1,1-dioxobenzo[d]isothiazol-3-ylamino)-4-methylsulfanyl-butanoate* (4d)

A mixture of L-Met-OMe∗HCl (0.42 g, 2.09 mmol) and Et$_3$N (0.32 ml, 2.30 mmol) in CH$_2$Cl$_2$ (5-10 ml) was stirred for 10 minutes. (1,1-dioxobenzo[*d*]isothiazol-3-ylsulfanyl)acetonitrile (**3**) (0.5 g, 2.09 mmol) in CH$_2$Cl$_2$ (5 ml) was added and stirring was continued for 2 h. After evaporation of the solvent, the residue was purified by CC (AcOEt/PE 8:2). Yield: 0.12 g (17%); M.p. 166 °C (MeOH/H$_2$O); R$_f$ = 0.56 (AcOEt/PE 7:3); $[\alpha]_D^{20}$ = -43.50 (c = 2, MeOH); IR: $\tilde{\nu}$ = 3329 (NH), 3101, 2953 (CH), 1755 (CO), 1613 (C=N), 1372, 1158 (SO$_2$); ^{1}H NMR ([D$_6$]DMSO): δ = 9.68 (d, *J* = 7.60 Hz, 1H, NH), 8.35-8.26, 8.04-7.97, 7.92-7.82 (3m, 4H, ar), 4.76 (q, *J* = 7.40 Hz,

1H, α-H, Met), 3.73 (s, 3H, OMe), 2.63 (dt, J = 6.90, 3.40 Hz, 2H, γ-H Met), 2.18 (q, J = 7.30 Hz, 2H, β-H, Met), 2.07 (s, 3H, SMe); HPLC: k' = 1.66, t_0 = 1.77 (RP-18, MeCN/H_2O, 1:1); HPLC: k' = 2.39, t_0 = 2.29 (Chiralcel OJ-R, MeCN/H_2O, 3:7); $C_{13}H_{16}N_2O_4S_2$ (328.41), calculated: C 47.55, H 4.91, N 8.53; found: C47.42, H 4.09, N 8.35.

5.2.8. *Methyl (S)-2 (1,1-dioxobenzo[d]isothiazol-3-ylamino)-4-methylpentanoate (4e)*

The compound was obtained from L-Leu-OMe∗HCl (0.17 g, 0.83 mmol), 0.20 gm of (1,1-dioxobenzo[*d*]isothiazol-3-ylsulfanyl)acetonitrile (**3**) (0.83 mmol) and 0.14 ml of Et$_3$N (1.00 mmol) as described for **4d**. Yield: 0.08 g (31%); M.p. 172 °C (MeOH/H_2O); R_f = 0.68 (AcOEt/PE 7:3); $[\alpha]_D^{20}$ = -48.00 (c = 2, MeOH); IR: $\tilde{v}$ = 3300 (NH), 2958, 2871 (CH), 1753 (CO), 1615 (C=N), 1369, 1155 (SO$_2$); ^{1}H NMR ([D$_6$]DMSO): δ = 9.69 (d, J = 7.77 Hz, 1H, NH), 8.40-8.27, 8.06-7.95, 7.93-7.81 (3m, 4H, ar), 4.72-4.55 (m, 1H, α-H, Leu), 3.71 (s, 3H, OMe), 1.90 (dd, J = 8.20, 2.00 Hz, 1H, β-H, Leu), 1.82-1.65 (m, 2H, [1H, β-H, Leu + 1H, γ-H, Leu]), 0.95 (d, J = 6.20 Hz, 3H, δ-H, Leu), 0.91 (d, J = 6.20 Hz, 3H, δ-H, Leu); HPLC: k' = 2.74, t_0 = 1.77 (RP-18, MeCN/H_2O, 1:1); HPLC: k' = 3.26, t_0 = 2.29 (Chiralcel OJ-R, MeCN/H_2O, 3:7); $C_{14}H_{18}N_2O_4S$ (310.37), calculated: C 54.18, H 5.85, N 9.03; found: C 53.78, H 4.74, N 8.98.

5.2.9. *3-(Ethylsulfanyl)benzo[d]isothiazole 1,1-dioxide* (5)

The compound was obtained from benz[*d*]isothiazol-3(2*H*)-thione 1,1-dioxide (**2**) (1.0 g, 5.02 mmol), ethyl bromide (0.53 ml, 7.03 mmol) and NaH (0.3 g, 7.5 mmol) in DMF by heating at 100 °C for 2 h. The work up was done as described for **3**. Yield: 0.3 g (26%); M.p. 183 °C (EtOH, 182.5-183 °C) [54]; R_f = 0.92 (AcOEt); IR: $\tilde{v}$ = 3085, 2987 (CH), 1616 (C=N), 1321, 1161 (SO$_2$); ^{1}H NMR (CDCl$_3$): δ = 7.90-7.62 (2m, 4H, ar), 3.38 (q, J = 7.60 Hz, 2H, CH$_2$), 1.51 (t, J = 7.60 Hz, 3H, SMe); HPLC: k' = 2.84, t_0 = 1.77 (RP-18, MeCN/H_2O 1:1); $C_9H_9NO_2S_2$ (227.31), calculated: C 47.56, H 3.99, N 6.16; found: C 47.22, H 3.67, N 5.98.

5.2.10. *(1,3-Dihydro-3-oxobenzo[d]isothiazol-2-yl)-acetonitrile 1,1-dioxide* (7)

Chloroacetonitrile (17 ml, 260 mmol) was added to a solution of saccharin sodium (**6**) (20.0 g, 98 mmol) in DMF (50 ml) and the mixture was heated at 100 °C for 4 h. The reaction mixture was allowed to cool at room temperature and then was poured into cold water. The precipitate was separated. Yield: 17.0 g (79%); M.p. 138-140 °C (AcOEt, 137-139 °C) [104]; R_f = 0.45 (AcOEt/PE 4:6); IR: $\tilde{v}$ = 3088, 2996 (CH),

2257(CN), 1746 (CO), 1338, 1170 (SO_2); ^{1}H NMR ([D_6]DMSO): δ = 8.43-8.37, 8.22-8.00 (2m, 4H, ar), 5.10 (s, 2H, CH_2); HPLC: k' = 1.43, t_0 = 1.76 (RP-18, MeCN/H_2O, 1:1); $C_9H_6N_2O_3S$ (222.22), calculated: C 48.64, H 2.72, N 12.61; found: C48.57, H 2.59, N 12.62.

5.2.11. *1,3-Dihydro-3-thioxobenzo[d]isothiazole-2-thioacetamide 1,1-dioxide* (8)

The mixture from P_2S_5 (2 g, 9 mmol), and a solution of (1,2-dihydro-3-oxobenzo[*d*]isothiazol-2-yl)acetonitrile 1,1-dioxide (**7**) (1.0 g, 4.50 mmol) in dioxane (20 ml) was refluxed for 4 h. The reaction mixture was cooled and filtered. The filtrate was concentrated to dryness and extracted with AcOEt. The extract was concentrated in vacuo, and the residue was purified by CC (AcOEt/PE 0.5:9.5 with increasing polarity). Yield: 0.3 g (24%); yellow solid; M.p. 190-192 °C; R_f = 0.6 (AcOEt/PE 4:6); IR: $\tilde{v}$ = 3372, 3299 (NH), 3148 (CH), 1637 (C=N), 1328, 1186 (SO_2), 1138 (C=S); ^{1}H NMR ([D_6]DMSO): δ = 9.93 (s, 1H, NH), 9.95 (s, 1H, NH), 8.36-8.22, 8.14-7.96 (2m, 4H, ar), 4.95 (s, 2H, CH_2); ^{13}C NMR ([D_6]DMSO): δ = 196.81 (C=S), 186.34 (C=S), 135.25, 135.15, 130.35, 129.83, 126.36, 121.21 (ar C), 49.79 (CH_2): DEPT: 135.86, 135.76, 126.96, 121.82 (ar CH), 50.39 (CH_2); HPLC: k' = 1.75, t_0 = 1.77 (RP-18, MeCN/H_2O, 1:1); EIMS m/z (rel.int.): 271.8 ([M^+], 7.54%), 238.8 (1.64), 221.8 (3.81), 165.8 (28.28), 147.3 (16.99), 121.0 (26.93), 102.8 (3.78), 69.8 (30.64), 66.7 (12.07), 28.0 (100); calculated M^+ = 272.37; $C_9H_8N_2O_2S_3$ (272.37), calculated: C 39.70, H 2.94, N 10.29; found: C 39.63, H 2.32, N 9.54.

5.2.12. General Procedure for the Synthesis of Thiosaccharinates (10)

Benz[*d*]isothiazol-3(2*H*)-thione 1,1-dioxide (**2**) (0.7 g, 3.51 mmol) and the equivalent amount of an amine in MeOH (20 ml) were stirred at room temperature for 2 h. The solvent was removed in vacuo, and the residue was crystallized as noted.

5.2.12.1. *Ammonium 1,1-dioxobenz[d]isothiazol-3-thion-2-id* (10a)

With 1 ml saturated solution of ammonia in MeOH. Yield: 0.58 g (76%); M.p. 209-211 °C (AcOEt/MeOH); R_f = 0.36 (MeOH/CH_2Cl_2 2:8); IR: $\tilde{v}$ = 3426 (NH), 1339, 1153 (SO_2), 1110 (C=S); ^{1}H NMR (D_2O): δ = 8.13-7.92, 7.26-7.57 (2m, 4H, ar); HPLC: k' = 0.92, t_0 = 2.00 (RP-18, MeCN/0.02M KH_2PO_4, 2:8, pH 2.72); $C_7H_8N_2O_2S_2$ (216.28), calculated: C 38.87, H 3.73, N 12.95; found: C 38.70, H 3.59, N 12.59.

5.2.12.2. *Anilinium 1,1-dioxobenz[d]isothiazol-3-thion-2-id* (10b)

From aniline (0.37 ml, 3.51 mmol). Yield: 0.80 g (78%); M.p. 305-308 °C (AcOEt, 290-300 °C) [56]; R_f = 0.28 (MeOH/CH$_2$Cl$_2$ 2:8); IR: $\tilde{v}$ = 3433 (NH), 1336, 1142, (SO$_2$), 1116 (C=S); ^{1}H NMR (D$_2$O): δ = 8.12-8.02, 7.90-7.73, 7.50-7.36, 7.32-7.15 (4m, 9H, ar); HPLC: k' = 0.89, t_0 = 2.00 (RP-18, MeCN/0.02M KH$_2$PO$_4$, 2:8, pH 2.72); C$_{13}$H$_{12}$N$_2$O$_2$S$_2$ (292.38), calculated: C 53.40, H 4.14, N 9.58; found: C 53.57, H 3.88, N 9.43.

5.2.12.3. *Benzylammonium 1,1-dioxobenz[d]isothiazol-3-thion-2-id* (10c)

From benzyl amine (0.38 g, 3.51 mmol). Yield: 0.88 g (82%); M.p. 178 °C (AcOEt); R_f = 0.48 (MeOH/CH$_2$Cl$_2$ 1:9); IR: $\tilde{v}$ = 3432 (NH), 3046 (CH), 1339, 1157, (SO$_2$), 1119 (C=S); ^{1}H NMR (D$_2$O): δ = 8.12-8.00, 7.90-7.72 (2m, 4 ar H), 7.51-7.40 [m, 5H, ar (Phenyl)], 4.21-4.13 (m, 2H, CH$_2$); HPLC: k' = 0.89, t_0 = 2.00 (RP-18, MeCN/0.02M KH$_2$PO$_4$, 2:8, pH 2.72); C$_{14}$H$_{14}$N$_2$O$_2$S$_2$ (306.41), calculated: C 54.88, H 4.61, N 9.14; found: C 55.38, H 3.65, N 9.01.

5.2.12.4. *Cyclohexylammonium 1,1-dioxobenz[d]isothiazol-3-thion-2-id* (10d)

From cyclohexylamine (0.35 g, 3.51 mmol). Yield: 0.78 g (74%); M.p. 172 °C (AcOEt); R_f = 0.60 (MeOH/CH$_2$Cl$_2$ 1:9); IR: $\tilde{v}$ = 3432 (NH), 3010, 2938 (CH), 1339, 1145, (SO$_2$), 1118 C=S); ^{1}H NMR (D$_2$O): δ = 8.13-8.02, 7.90-7.73 (2m, 4H, ar), 3.23-3.03, 2.07-1.90, 1.84-1.69, 1.68-1.54, 1.46-1.06 (5m, 11H$_{cyclohexyl}$, 5CH$_2$); HPLC: k '= 0.90, t_0 = 2.00 (RP-18, MeCN/0.02M KH$_2$PO$_4$, 2:8, pH 2.72); C$_{13}$H$_{18}$N$_2$O$_2$S$_2$ (298.43), calculated: C 52.32, H 6.08, N 9.39; found: C 52.53, H 5.59, N 9.41.

5.2.12.5. *Isobutylammonium 1,1-dioxobenz[d]isothiazol-3-thion-2-id* (10e)

From isobutylamine (0.26 g, 3.51 mmol). Yield: 0.77 g (79%); M.p. 212 °C (AcOEt); R_f = 0.60 (MeOH/CH$_2$Cl$_2$ 1:9); IR: $\tilde{v}$ = 3432 (NH), 3055, 2964 (CH), 1352, 1142, (SO$_2$), 1117 (C=S); ^{1}H NMR (D$_2$O): δ = 8.13-8.05, 7.90-7.77 (2m, 4H, ar), 2.85 (d, *J* = 6.97 Hz, 2H, CH$_2$), 1.94 (hpt, *J* = 6.97 Hz, CH), 0.94 (d, *J* = 6.97 Hz, 2 Me); HPLC: k' = 0.78, t_0 = 2.00 (RP-18, MeCN/0.02M KH$_2$PO$_4$, 2:8, pH 2.72); C$_{11}$H$_{16}$N$_2$O$_2$S$_2$ (272.39), calculated: C 48.50, H 5.92, N 10.28; found: C 48.81, H 5.34, N 10.27.

5.2.13. General Procedure for the Synthesis of 4 from 10

The thiosaccharinates, **10**, were refluxed in 20 ml of glacial acetic acid for 3-5 days. After cooling to rt, the precipitate was separated, washed with water and dried.

5.2.13.1. *Benzo[d]isothiazol-3-ylamine 1,1-dioxide* (4c)

From ammonium 1,1-dioxobenz[*d*]isothiazol-3-thion-2-id (**10a**) (0.3 g, 1.38 mmol), 4 days. Yield: 0.05 g (20%). For the analytical part see 5.2.6.

5.2.13.2. *N-(1,1-Dioxobenzo[d]isothiazol-3-yl)aniline* (4f)

From anilinium 1,1-dioxobenz[*d*]isothiazol-3-thion-2-id (**10b**) (0.4 g, 1.36 mmol), 3 days. Yield: 0.12 g (33%); M.p. 315 °C (315-317 °C) [57]; R_f = 0.75 (MeOH/CH$_2$Cl$_2$ 1.5:8.5); IR: $\tilde{\nu}$ = 3326 (NH), 3099, 3058 (CH), 1618 (C=N), 1353, 1155 (SO$_2$); ^{1}H NMR ([D$_6$]DMSO): δ = 8.54-8.46 (m, 1H, ar), 8.12-8.04 (m, 1H, ar), 7.95-7.82 (m, 4H, ar), 7.55-7.44 (m, 2H, ar), 7.32-7.23 (m, 1H, ar); HPLC: k' = 1.76, t_0 = 1.77 (RP-18, MeCN/H$_2$O, 1:1); C$_{13}$H$_{10}$N$_2$O$_2$S (258.30), calculated: C 60.45, H 3.90, N 10.85; found: C 60.64, H 3.59, N 10.75.

5.2.13.3. *N-(1,1-Dioxobenzo[d]isothiazol-3-yl)benzylamine* (4g)

From benzylammonium 1,1-dioxobenz[*d*]isothiazol-3-thion-2-id (**10c**) (0.3 g, 0.98 mmol), 3 days. Yield: 0.11 g (41%); M.p. 202 °C (209 °C) [53]; R_f = 0.4 (CH$_2$Cl$_2$); IR: $\tilde{\nu}$ = 3312 (NH), 3091, 3052 (CH), 1623 (C=N), 1350, 1151 (SO$_2$); ^{1}H NMR ([D$_6$]DMSO): δ = 9.94 (bs, 1H, NH), 8.27-8.19, 8.02-7.94, 7.88-7.79 (3m, 4H, ar), 7.43-7.29 [m, 5H, ar (Phe)], 4.70 (s, 2H, CH$_2$); HPLC: k' = 1.89, t_0 = 1.77 (RP-18, MeCN/H$_2$O, 1:1); C$_{14}$H$_{12}$N$_2$O$_2$S (272.33), calculated: C 61.75, H 4.44, N 10.29; found: C 61.59, H 3.17, N 9.90.

5.2.13.4. *N-(1,1-Dioxobenzo[d]isothiazol-3-yl)cyclohexylamine* (4h)

From cyclohexylammonium 1,1-dioxobenz[*d*]isothiazol-3-thion-2-id (**10d**) (0.4 g, 1.34 mmol), 3 days. Yield: 0.09 g (25%); M.p. 252 °C (248 °C) [53]; R_f = 0.56 (CH$_2$Cl$_2$); IR: $\tilde{\nu}$ = 3299 (NH), 3107, 3047, 2938 (CH), 1618 (C=N), 1350, 1154 (SO$_2$); ^{1}H NMR ([D$_6$]DMSO): δ = 9.15 (d, J = 7.63 Hz, 1H, NH), 8.23-8.20, 8.00-7.91, 7.86-7.77 (3m, 4 ar), 3.93-3.73, 2.04-1.91, 1.83-1.57, 1.48-1.11 (4 m, 11H$_{cyclohexyl}$); HPLC: k' = 2.55, t_0 = 1.85 (RP-18, MeCN/H$_2$O, 1:1); C$_{13}$H$_{16}$N$_2$O$_2$S (264.35), calculated: C 59.07, H 6.10, N 10.60; found: C 58.15, H 5.68, N 10.50.

5.2.13.5. *N-(1,1-Dioxobenzo[d]isothiazol-3-yl)isobutylamine* (4i)

From isobutylammonium 1,1-dioxobenz[*d*]isothiazol-3-thion-2-id (**10e**) (0.3 g, 1.09 mmol), 5 days. Yield: 0.08 g (31%); M.p. 216-217 °C; R_f = 0.40 (CH$_2$Cl$_2$); IR: $\tilde{\nu}$ = 3314 (NH), 3045, 2960 (CH), 1621 (C=N), 1351, 1152 (SO$_2$); ^{1}H NMR ([D$_6$]DMSO): δ = 9.42 (bs, 1H, NH), 8.30-8.18, 8.02-7.92, 7.88-7.78 (3m, 4H, ar), 3.31 (t, J = 7.02

Hz, 2H, CH$_2$CH), 2.09-1.92 (m, 1H, CH$_2$CH), 0.95 (d, J = 6.62 Hz, 6H, 2Me); HPLC: k' = 0.88, t_0 = 1.77 (RP-18, MeCN/H$_2$O, 1:1); C$_{11}$H$_{14}$N$_2$O$_2$S (238.31), calculated: C 55.44, H 5.92, N 11.76; found: C 55.15, H 4.78, N 11.68.

5.2.14. Synthesis of 11, (11+12 i.e. as a mixture of isomers) and 12

5.2.14.1. *2-Ethyl-1,1-dioxo-benz[d]isothiazol-3-one* (11)

Method A) Bromoethane (2.18 ml, 29.24 mmol) was added into the solution of saccharin sodium (**6**) (4.00 g, 19.49 mmol) in 40 ml DMF. The reaction mixture was heated at 30-40 °C for 6 h and then allowed to cool at room temperature. Water was added into the reaction mixture and the crystalline compound was separated, washed with plenty of water and dried. Yield: 3.10 g (75%).

Method B) Bromoethane (0.72 ml, 5.36 mmol) was added into a solution of saccharin sodium (**6**) (1.00 g, 4.87 mmol) in 5 ml of DMF. Benzyl triethyl ammonium chloride (0.10 g) was added into the reaction mixture and reaction mixture was heated at 100 °C for 14 h. The reaction mixture was then cooled at room temperature and poured into water. Solid filtered and washed with plenty of water and dried. Yield: 1.00 g (97%); M.p. 90-91 °C (93.5-94.5 °C) [70]; R$_f$ = 0.64 (AcOEt/PE 2:8); IR: $\tilde{\nu}$ = 3440 (NH), 3092, 3068, 3033, 2980 (CH), 1731 (C=O), 1364, 1162 (SO$_2$); ^{1}H NMR (CDCl$_3$): δ = 8.11-8.01, 7.95-7.88, 7.87-7.77 (3m, 4H, ar), 3.86 (q, J = 7.20 Hz, 2H, CH$_2$), 1.45 (t, J = 7.20 Hz, 3H, CH$_3$); HPLC: k' = 2.02, t_0 = 1.77 (RP-18, MeCN/H$_2$O, 1:1); C$_9$H$_9$NO$_3$S (211.24), calculated: C 51.17, H 4.29, N 6.63; found: C 50.64, H 4.49, N 6.48.

5.2.14.2. (11+12 *i.e. mixture of isomers*)

Method A) From saccharin sodium (**6**) (2.00 g, 9.74 mmol), bromoethane (1.44 ml, 10.72 mmol), hexadecyltrimethylammonium bromide (0.050 g) in DMF at 100 °C for 1 h. Work up as described for **11**. Yield: 1.30 g (63%); R$_f$ = 0.64 (AcOEt/PE 2:8) (spots of two isomers are overlying when checked by TLC and are not possible to distinguish) ; IR: $\tilde{\nu}$ = 3441 (NH), 3092, 3068, 3034, 2981 (CH), 1612 (C=N), 1731 (C=O), 1364, 1162 (SO$_2$); ^{1}H NMR (CDCl$_3$): δ = 8.09-8.01, 7.95-7.81, 7.79-7.70 (3m, 4H, ar), 4.66, 3.86 (2q, J = 7.20 Hz, 2H, CH$_2$), 1.54, 1.45 (2t, J = 7.20 Hz, 3H, CH$_3$), **11:12** $\approx$ 4:1 (based on ^{1}H NMR); HPLC: k' = 1.96, 2.02, t_0 = 1.77 (RP-18, MeCN/H$_2$O, 1:1), C$_9$H$_9$NO$_3$S (211.24), calculated: C 51.17, H 4.29, N 6.63; found: C 50.64, H 4.49, N 6.48.

Method B) same reaction as that of method "A" as described in 5.2.14.2 except the reaction time was increased up to 4 days. Yield: 0.80 g (39%); For analytical data

see above method "A"; **11:12** $\approx$ 3:1(based on ^{1}H NMR); HPLC: k' = 1.96, 2.02, t_0 = 1.77 (RP-18, MeCN/H_2O, 1:1); $C_9H_9NO_3S$ (211.24), calculated: C 51.17, H 4.29, N 6.63; found: C 50.64, H 4.49, N 6.48.

Method C) Benz[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (**1**) (1.00 g, 5.45 mmol) was suspended in 20 ml of dry THF. Triethylamine was then added drop wise into the suspension and stirred at room temperature for 10 min. 0.40 g of ethylbromide was added drop wise followed by addition of 0.02 g of KI and the reaction mixture was refluxed for 2 days. Reaction mixture was cooled to room temperature and the solid filtered off. Filtrate was removed in vacuo. Thus obtained solid was triturated in 10 ml of water, filtered and washed two times with plenty of water. Solid dried. Yield: 0.20 g (17%); For analytical data see above method "A". **11:12** $\approx$ 3:2 (based on ^{1}H NMR); HPLC: k' = 1.96, 2.02, t_0 = 1.77 (RP-18, MeCN/H_2O, 1:1); $C_9H_9NO_3S$ (211.24), calculated: C 51.17, H 4.29, N 6.63; found: C 50.64, H 4.49, N 6.48.

5.2.14.3. *3-Ethoxybenzo[d]isothiazole 1,1-dioxide* (12)

Sodium saccharin (**6**) (2.00 g, 9.75mmol), bromoethane (0.72 ml, 10.72 mmol), hexadecyltrimethylammonium bromide (0.20 g) was added into dry toluene and refluxed for 4 h. Toluene was removed in vacuo and the resulted solid was triturated in water and filtered. The solid was then dried and crystallized from ethanol. Yield: 0.12 g (6%); M.p. 211-214 °C (219 °C) [67]; R_f = 0.64 (AcOEt/PE 2:8); IR: $\tilde{\nu}$ = 3446 (NH), 3089, 2989 (CH), 1612 (C=N), 1350, 1162 (SO_2); ^{1}H NMR (CDCl$_3$): δ = 8.09-8.01, 7.95-7.81, 7.79-7.70 (3m, 4H, ar), 4.66 (q, *J* = 7.20 Hz, 2H, CH_2), 1.54 (t, *J* = 7.20 Hz, 3H, CH_3); HPLC: k' = 1.94, t_0 = 1.77 (RP-18, MeCN/H_2O, 1:1); $C_9H_9NO_3S$ (211.24), calculated: C 51.17, H 4.29, N 6.63; found: C 51.63, H 4.03, N 6.75.

5.2.15. *General procedure for the synthesis of 4e, 4j and 4k from 12*

Amine (7.10 mmol) and 1.2 equivalence of triethyl amine (1.19 ml, 8.52 mmol) was added to dioxane and stirred for 10 minutes at room temperature. 3-Ethoxy-benzo[*d*]isothiazole 1,1-dioxide (**12**) (1.50 g, 7.10 mmol) was then added into the reaction mixture and the reaction mixture was refluxed for one day. The reaction mixture was cooled at room temperature and the solid filtered off. The filtrate was evaporated in vacuo and thus obtained solid was washed with water (2 x 100 ml). The solid was then crystallized from MeOH:H_2O.

5.2.15.1 *Methyl (S)-2 (1,1-dioxobenzo[d]isothiazol-3-ylamino)-4-methyl-pentanoate* (4e)

From 1.29 g of L-Leu∗OMe-HCl and 3-ethoxybenzo[*d*]isothiazole 1,1-dioxide (**12**). Yield: 1.20 g (55%); for analytical data see 5.2.8.

5.2.15.2. *Methyl (2S,3S)-2-(1,1-dioxobenzo[d]isothiazol-3-ylamino)-3-methyl-pentanoate* (4j)

From 1.29 g of L-Ile-OMe∗HCl and 3-ethoxybenzo[*d*]isothiazole 1,1-dioxide (**12**). Yield: 0.80 g (36%); M.p.168 °C (MeOH/H$_2$O); R$_f$ = 0.68 (AcOEt/PE 7:3); $[\alpha]_D^{20}$ = -30.00 (c = 2, MeOH); IR: $\tilde{\nu}$ = 3309 (NH), 3097, 3050, 2969, 2879 (CH), 1756 (CO), 1612 (C=N), 1365, 1157 (SO$_2$); ^{1}H NMR ([D$_6$]DMSO): δ = 9.57 (d, *J* = 7.81 Hz, 1H, NH), 8.52-8.42, 8.04-7.94, 7.91-7.81 (3m, 4H, ar), 4.49 (t, *J* = 7.81 Hz, 1H, α-H, Ile), 3.71 (s, 3H, OMe), 2.15-1.97 (m, 1H, β-H, Ile), 1.63-1.11 (m, 2H, γ-H, Ile), 0.96 (d, *J* = 6.91 Hz, 3H, γ-H, Ile), 0.89 (t, *J* = 7.30 Hz, 3H, δ-H, Ile); 13C NMR CDCl$_3$: δ = 171.83, 159.83, 142.56, 133.17, 132.56, 127.85, 121.86, 121.03, 61.48, 59.45, 35.68, 25.87, 15.32, 11.28; HPLC: k '= 2.75, t$_0$ = 1.77 (RP-18, MeCN/H$_2$O, 1:1); HPLC: k' = 3.60, t$_0$ = 2.29 (Chiralcel OJ-R, MeCN/H$_2$O, 3:7); C$_{14}$H$_{18}$N$_2$O$_4$S (310.37), calculated: C 54.18, H 5.85, N 9.03; found: C 53.90, H 5.12, N 9.09.

5.2.15.3. *Methyl (1,1-dioxobenzo[d]isothiazol-3-ylamino)acetate* (4k)

From 0.89 g of glycine-OMe∗HCl and 3-ethoxybenzo[*d*]isothiazole 1,1-dioxide (**12**). Yield: 0.95 g (52%); M.p. 214 °C (MeOH/H$_2$O); R$_f$ = 0.45 (AcOEt/PE 7:3); IR: $\tilde{\nu}$ = 3343 (NH), 3051, 2959 (CH), 1759 (CO), 1625 (C=N), 1375, 1158 (SO$_2$); ^{1}H NMR ([D$_6$]DMSO): δ = 9.93 (t, *J* = 5.70 Hz, 1H, NH), 8.25-8.16, 8.04-7.96, 7.90-7.82 (3m, 4H, ar), 4.32 (d, *J* = 5.70 Hz, 2H, Gly), 3.72 (s, 3H, OMe); HPLC: k' = 1.42, t$_0$ = 1.81 (RP-18, MeCN/H$_2$O, 3:7); C$_{10}$H$_{10}$N$_2$O$_4$S (254.27), calculated: C 47.24, H 3.96, N 11.02; found: C 47.11, H 4.39, N 11.45.

5.2.16. *3-Chlorobenzo[d]isothiazole 1,1-dioxide* (13)

Benz[*d*]isothiazol-3(2*H*)-one 1,1-dioxide (**1**) (50.0 g, 272.3 mmol) was added in 200 ml of dioxane followed by addition of the catalytic amount of DMF (2 ml). 50 ml of SOCl$_2$ was cautiously added as the reaction is exothermic. The reaction mixture was refluxed for one day. Next day, 1 ml of DMF and 25 ml of thionyl chloride were added and the reaction mixture was refluxed for one more day. Reaction mixture was evaporated on rota vapour (**caution!** The operation must be carried out in a well ventilated area due to the strong and toxic odour of thionyl chloride). The obtained

solid was then crystallized two times from toluene. Care must be taken in handling of **13** as it is a strong vesicant and can produce painful chemical burns and a very persistent dermatitis [105]. Yield: 42.0 g (76%); M.p. 143-147 °C (Toluene) (Toluene, 140-145 °C) [71]; R_f = 0.63 (AcOEt/PE 4:6); IR: $\tilde{v}$ = 1605 (C=N), 1346, 1175 (SO$_2$); ^{1}H NMR (CDCl$_3$): δ = 8.15-7.85 (m, 4H, ar); HPLC: k' = 2.06, t_0 = 2.00 (RP-18, MeCN/H$_2$O, 1:1). C$_7$H$_4$ClNO$_2$S (201.63), calculated: C 41.70, H 2.00, N 6.95; found: C 41.52, H 1.74, N 6.98.

5.2.17. General method for the synthesis of 4c, 4l-4q from 13

3-Chlorobenzo[*d*]isothiazole 1,1-dioxide (**13**) and the amine were added to dioxane and refluxed for 4-5 h. The reaction mixture was evaporated in vacuo and the residue was washed with cold water (except **4q**) and crystallized.

5.2.17.1. *Benzo[d]isothiazol-3-ylamine 1,1-dioxide* (4c)

From 3-chlorobenzo[*d*]isothiazole 1,1-dioxide (**13**) (1.0 g, 4.96 mmol), and 20 ml of methanolic ammonia, 2 h, rt. Yield: 0.84 g (93%). For analytical data see 5.2.6.

5.2.17.2. *2-Chloro-N-(1,1-dioxobenzo[d]isothiazol-3-yl)benzylamine* (4l)

From 3-chlorobenzo[*d*]isothiazole 1,1-dioxide (**13**) (2.0 g, 9.90 mmol), and 2-chlorobenzylamine (1.4 g, 9.90 mmol) in 30 ml dioxane. Yield: 1.68 g (55%); M.p. 244 °C (Acetone); R_f = 0.577 (MeOH/CH$_2$Cl$_2$ 1.5:8.5); IR: $\tilde{v}$ = 3300 (NH), 3062, 2926 (CH), 1622 (C=N), 1345, 1158 (SO$_2$); ^{1}H NMR [D$_6$]DMSO: δ = 9.88 (s, 1H, NH), 8.32-8.21, 8.06-7.95, 7.91-7.80 (3m, 4H, ar), 7.58-7.45, 7.43-7.33 (2m, 4H, ar), 4.77 (s, 2H, CH$_2$); HPLC: k' = 2.35, t_0 = 1.85 (RP-18, MeCN/H$_2$O, 1:1); C$_{14}$H$_{11}$ClN$_2$O$_2$S (306.77), calculated: C 54.81, H 3.61, N 9.13; found: C 54.34, H 3.56, N 9.08.

5.2.17.3. *4-Chloro-N-(1,1-dioxobenzo[d]isothiazol-3-yl)benzylamine* (4m)

From 3-chlorobenzo[*d*]isothiazole 1,1-dioxide (**13**) (2.0 g, 9.90 mmol), and 4-chlorobenzylamine (1.4 g, 9.90 mmol) in 30 ml dioxane. Yield: 2.45 g (81%); M.p. 256 °C (Acetone); R_f = 0.70 (MeOH/CH$_2$Cl$_2$ 1.5:8.5); IR: $\tilde{v}$ = 3302 (NH), 3064, 2917 (CH), 1623 (C=N), 1349, 1153 (SO$_2$); ^{1}H NMR [D$_6$]DMSO: δ = 9.93 (bs, 1H, NH), 8.26-8.16, 8.02-7.94, 7.89-7.80 (3m, 4H, ar), 7.50-7.38 (m, 4H, ar), 4.69 (s, 2H, CH$_2$); HPLC: k' = 2.73, t_0 = 1.77 (RP-18, MeCN/H$_2$O, 1:1); C$_{14}$H$_{11}$ClN$_2$O$_2$S (306.77), calculated: C 54.81, H 3.61, N 9.13; found: C 54.16, H 3.18, N 8.95.

5.2.17.4. *3,4-Dimethoxy-N-(1,1-dioxobenzo[d]isothiazol-3-yl)benzylamine* (4n)

From 3-chlorobenzo[*d*]isothiazole 1,1-dioxide (**13**) (2.0 g, 9.90 mmol), and 3,4-dimethoxybenzylamine (1.66 g, 9.90 mmol) in 30 ml dioxane. Yield: 1.23 g (37%); M.p. 301-303 °C; R_f = 0.62 (MeOH/CH_2Cl_2 1.5:8.5); IR: $\tilde{\nu}$ = 3341 (NH), 3103, 2959, 2939 (CH), 1622 (C=N), 1359, 1155 (SO_2); ^{1}H NMR [D_6]DMSO: δ = 9.86 (t, *J* = 5.70 Hz, 1H, NH), 8.27-8.18, 8.04-7.95, 7.88-7.77 (3m, 4H, ar), 7.07 (d, *J* = 1.17 Hz, 1H, ar), 6.98-6.88 (m, 2H, ar), 4.62 (d, *J* = 5.70 Hz, 2H, CH_2), 3.75, 3.74 (2s, 2 OMe); HPLC: k' = 1.15, t_0 = 1.77 (RP-18, MeCN/H_2O, 1:1); $C_{16}H_{16}N_2O_4S$ (332.38), calculated: C 57.82, H 4.85, N 8.43; found: C 57.13, H 4.52, N 8.03.

5.2.17.5. *2-Methoxy-N-(1,1-dioxobenzo[d]isothiazol-3-yl)benzylamine* (4o)

From 3-chlorobenzo[*d*]isothiazole 1,1-dioxide (**13**) (2.0 g, 9.90 mmol), and 2-methoxybenzylamine (1.36 g, 9.90 mmol) in 30 ml dioxane. Yield: 2.0 g (67%); M.p. 219-221 °C (Acetone); R_f = 0.70 (MeOH/CH_2Cl_2 1.5:8.5); IR: $\tilde{\nu}$ = 3328 (NH), 3108, 2927 (CH), 1622 (C=N), 1366, 1155 (SO_2); ^{1}H NMR [D_6]DMSO: δ = 9.77 (t, *J* = 5.41 Hz, 1H, NH), 8.33-8.24, 8.05-7.94, 7.89-7.77 (3m, 4H, ar), 7.38-2.26, 7.09-6.90 (2m, 4H, ar), 4.66 (d, *J* = 5.41 Hz, CH_2), 3.85 (s, 3H, OMe); HPLC: k' = 2.01, t_0 = 1.77 (RP-18, MeCN/H_2O, 1:1); $C_{15}H_{14}N_2O_3S$ (302.35), calculated: C59.59, H 4.67, N 9.27; found: C 59.15, H 4.77, N 9.23.

5.2.17.6. *3-(Trifluoromethyl)-N-(1,1-dioxobenzo[d]isothiazol-3-yl)benzylamine* (4p)

From 3-chlorobenzo[*d*]isothiazole 1,1-dioxide (**13**) (2.0 g, 9.90 mmol), and 3-(trifluoromethyl)benzylamine (1.74 g, 9.90 mmol) in 30 ml dioxane. Yield: 2.86 g (85%); R_f = 0.78 (MeOH/CH_2Cl_2 1:9); M.p. 254-256 °C (Acetone); IR: $\tilde{\nu}$ = 3343 (NH), 3103, 2944 (CH), 1621 (C=N), 1348, 1160 (SO_2); ^{1}H NMR [D_6]DMSO: δ = 10.00 (bs, 1H, NH), 8.28-8.18, 8.05-7.97 (2m, 2H, ar), 7.91-7.78 (m, 3H, ar), 7.78-7.58 (m, 3H, ar), 4.83 (s, 2H, CH_2); HPLC: k' = 3.41 t_0 = 1.77 (RP-18, MeCN/H_2O, 1:1); $C_{15}H_{11}F_3N_2O_2S$ (340.33), calculated: C 52.94, H 3.26, N 8.23; found: C 52.84, H 3.15, N 8.19.

5.2.17.7. *N-(1,1-Dioxobenzo[d]isothiazol-3-yl)-2-pyridylmethyl amine hydrochloride* (4q)

From 3-chlorobenzo[*d*]isothiazole 1,1-dioxide (**13**) (2.0 g, 9.90 mmol), and 2-(amino methyl)pyridine (1.07 g, 9.90 mmol) in 30 ml dioxane. Yield: 1.53 g (50%); M.p. 260 °C (dec., Acetone); R_f = 0.55 (MeOH/CH_2Cl_2 1.5:8.5); IR: $\tilde{\nu}$ = 3440 (NH), 3097, 3050,

2995 (CH), 1626 (C=N), 1354, 1163 (SO$_2$); ^{1}H NMR [D$_6$]DMSO: δ = 10.59 (t, J = 5.70 Hz, 1H, NH), 8.84-8.75, 8.46-8.26, 8.03-7.94 (3m, 4H, ar), 7.92-7.72 (m, 4H, ar), 6.60 (bs, HCl), 4.99 (d, J = 5.70 Hz, 2H, CH$_2$); HPLC: k' = 0.73 t$_0$ = 1.77 (RP-18, MeCN/H$_2$O, 1:1); C$_{13}$H$_{12}$ClN$_3$O$_2$S (309.78), calculated: C 50.41, H 3.90, N 13.56; found: C 49.91, H 3.57, N 13.41.

5.2.18. General method for the synthesis of amide derivatives and methyl ester derivatives (4d, 4e, 4j, 4k, 4u-4z) from 13

Amino acid methyl ester HCl or amino acid amide hydrochloride and triethyl amine in dioxane were stirred for 10 min at rt. 3-Chlorobenzo[d]isothiazole 1,1-dioxide (**13**) was added and reaction mixture was refluxed for 4-5 h, unless otherwise stated. Reaction mixture was cooled down to 0 °C and then filtered. The filtrate was removed in vacuo and the resulting solid then washed with water and crystallized. (Triethyl amine was not used when the free amine was used in the reaction).

5.2.18.1.   *(S)-2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)-4-methylpentanamide* (4a)

From 3-chlorobenzo[d]isothiazole 1,1-dioxide (**13**) (3.00 g, 14.88 mmol), and L-Leu-NH$_2$ (1.92 g, 14.88 mmol) in 60 ml dioxane, see 4.2.18. Yield: 3.02 g (69%). For the analytical data see 5.2.4.

5.2.18.2.   *(S)-2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)-3-phenylpropanamide* (4b)

From 3-chlorobenzo[d]isothiazole 1,1-dioxide (**13**) (1.0 g, 4.96 mmol), and L-Phe-NH$_2$ (0.81 g, 4.96 mmol) in 30 ml dioxane, see 4.2.18. Yield: 0.95 g (58%). For the analytical data see 5.2.5.

5.2.18.3. *(S)-2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)propanamide* (4r)

From 3-chlorobenzo[d]isothiazole 1,1-dioxide (**13**) (1.0 g, 4.96 mmol), and L-Ala-NH$_2$∗HCl (0.62 g, 4.96 mmol), and Et$_3$N (1.38 ml, 9.92 mmol) in 30 ml dioxane. Yield: 0.60 g (55%); M.p. 236 °C (Acetone); R$_f$ = 0.50 (MeOH/CH$_2$Cl$_2$ 1.5:8.5); $[\alpha]_D^{20}$ = +42.16 (c = 2, DMSO); IR: $\tilde{\nu}$ = 3432, 3337 (NH), 3096, 3056, 2941 (CH), 1684 (CO), 1620 (C=N), 1361, 1160 (SO$_2$); ^{1}H NMR [D$_6$]DMSO: δ = 9.49 (bs, 1H, NH), 8.40-8.30, 8.03-7.92, 7.90-7.78 (3m, 4H, ar), 7.68 (bs, 1H, NH$_2$), 7.20 (bs, 1H, NH$_2$), 4.53 (qn, J = 6.10 Hz, 1H, α-H, Ala), 1.46 (d, J = 7.16 Hz, 3H, β-H, Ala); HPLC: k' = 0.83, t$_0$ = 2.00 (RP-18, MeCN/0.02M KH$_2$PO$_4$, 2:8, pH 2.72); HPLC: k' = 0.20, t$_0$ = 2.36

(Chiralcel OJ-R, MeCN/H$_2$O, 2:8); C$_{10}$H$_{11}$N$_3$O$_3$S (253.28), calculated: C 47.42, H 4.38, N 16.59; found: C 48.08, H 3.83, N 16.13.

5.2.18.4. *(S)-2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)-3-methylbutanamide (4s)*

From 3-chlorobenzo[*d*]isothiazole 1,1-dioxide (**13**) (4.0 g, 19.85 mmol), L-Val-NH$_2$*HCl (3.1 g, 20.33 mmol), and Et$_3$N (6.9 ml, 39.7 mmol) in 60 ml dioxane, CC (AcOEt/PE 9:1). Yield: 2.24 g (40%); M.p. 225 °C; R$_f$ = 0.56 (MeOH/CH$_2$Cl$_2$ 1.5:8.5); $[\alpha]_D^{20}$ = +12.00 (c = 2, DMSO); IR: $\tilde{\nu}$ = 3523, 3399 (NH), 3093, 3058, 2967, 2937 (CH), 1675 (CO), 1619 (C=N), 1362, 1162 (SO$_2$); ^{1}H NMR [D$_6$]DMSO: δ = 9.36 (d, *J* = 8.18 Hz, 1H, NH), 8.60-8.45, 8.05-7.94, 7.90-7.74 (3m, 5H, [4H ar + 1H, NH$_2$]), 7.31 (bs, 1H, NH$_2$), 4.36 (t, *J* = 8.18 Hz, 1H, α-H, Val), 2.32-2.11 (m, 1H, β-H, Val), 0.99 (d, *J* = 6.43 Hz, 6H, γ-H, Val); HPLC: k' = 2.62, t$_0$ = 1.92 (RP-18, MeCN/0.01M KH$_2$PO$_4$, 2:8, pH 2.95); HPLC: k' = 0.72, t$_0$ = 2.37 (Chiralcel OJ-R, MeCN/H$_2$O, 2:8); C$_{12}$H$_{15}$N$_3$O$_3$S (281.34), calculated: C 51.23, H 5.37, N 14.94; found: C 50.54, H 5.22, N 14.96.

5.2.18.5. *(2S,3S)-2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)-3-methylpentanamide (4t)*

From 3-chlorobenzo[*d*]isothiazole 1,1-dioxide (**13**) (1.0 g, 4.96 mmol), L-Ile-NH$_2$*HCl (0.83 g, 4.96 mmol), and Et$_3$N (1.38 ml, 9.92 mmol) in 30 ml dioxane. Yield: 1.07 g (72%); M.p. 247 °C (Acetone); R$_f$ = 0.60 (MeOH/CH$_2$Cl$_2$ 1.5:8.5); $[\alpha]_D^{20}$ = -10.16 (c = 2, DMSO); IR: $\tilde{\nu}$ = 3418, 3361 (NH), 2978, 2934, 2877 (CH), 1681 (CO), 1610 (C=N), 1366, 1163 (SO$_2$); ^{1}H NMR [D$_6$]DMSO: δ = 9.39 (d, *J* = 8.62 Hz, 1H, NH), 8.58-8.44, 8.04-7.93, 7.90-7.77 (3m, 5H, [4H ar + 1H, NH$_2$]), 7.30 (bs, 1H, NH$_2$), 4.39 (t, *J* = 8.62 Hz, 1H, α-H, Ile), 2.09-1.90 (m, 1H, β-H, Ile), 1.66-1.42, 1.36-1.11 (2m, 2H, γ-H, Ile), 0.96 (d, *J* = 6.87 Hz, 3H, γ-H, Ile), 0.86 (t, *J* = 7.16 Hz, 3H, δ-H, Ile); HPLC: k' = 2.53, t$_0$ = 1.79 (RP-18, MeCN/H$_2$O, 3:7); HPLC: k' = 1.45, t$_0$ = 2.36 (Chiralcel OJ-R, MeCN/H$_2$O, 2:8); C$_{13}$H$_{17}$N$_3$O$_3$S (295.36), calculated: C 52.87, H 5.80, N 14.23; found: C 52.71, H 5.44, N 14.24.

5.2.18.6. *Methyl (S)-2 (1,1-dioxobenzo[d]isothiazol-3-ylamino)-4-methylsulfanyl-butanoate* (4d)

From 3-chlorobenzo[*d*]isothiazole 1,1-dioxide (**13**) (7.0 g, 34.72 mmol), L-Met-OMe∗HCl (6.93 g, 34.72 mmol), and Et₃N (11.2 ml, 80.50 mmol) in 150 ml dioxane. Yield: 3.5 g (31%). For analytical data see 5.2.7.

5.2.18.7. *Methyl (S)-2 (1,1-dioxobenzo[d]isothiazol-3-ylamino)-4-methylpentano-ate* (4e)

From 3-chlorobenzo[*d*]isothiazole 1,1-dioxide (**13**) (7.0 g, 34.72 mmol), L-Leu-OMe∗HCl (6.3 g, 34.72 mmol), and Et₃N (11.2 ml, 80.50 mmol) in 150 ml dioxane. Yield: 6.0 g (55%). For analytical data see 5.2.8.

5.2.18.8. *Methyl (2S,3S)-2-(1,1-dioxobenzo[d]isothiazol-3-ylamino)-3-methylpen-tanoate* (4j)

From 3-chlorobenzo[*d*]isothiazole 1,1-dioxide (**13**) (7.0 g, 34.72 mmol), L-Ile-OMe∗HCl (6.3 g, 34.72 mmol), and Et₃N (11.2 ml, 80.50 mmol) in 150 ml dioxane. Yield: 4.8 g (43%). For analytical data see 5.2.15.2.

5.2.18.9. *Methyl (1,1-dioxobenzo[d]isothiazol-3-ylamino)acetate* (4k)

From 3-chlorobenzo[*d*]isothiazole 1,1-dioxide (**13**) (10.0 g, 49.58 mmol), and Gly-OMe∗HCl (8.7 g, 69.42 mmol), and Et₃N (16.5 ml, 119.04 mmol) in 200 ml dioxane. Yield: 4.3 g (34%). For analytical data see 5.2.15.3.

5.2.18.10. *Methyl (S)-2(1,1-dioxobenzo[d]isothiazol-3-ylamino)-3-phenylpropa-noate* (4u)

From 3-chlorobenzo[*d*]isothiazole 1,1-dioxide (**13**) (7.0 g, 34.7 mmol), L-Phe-OMe∗HCl (7.49 g, 34.7 mmol), and Et₃N (11.2 ml, 80.50 mmol) in 150 ml dioxane. Yield: 9.1 g (76%); M.p.154 °C (MeOH/H_2O); R_f = 0.66 (AcOEt/PE 7:3); $[\alpha]_D^{20}$ = -51.66 (c = 2, MeOH); IR: $\tilde{v}$ = 3307 (NH), 3059, 3027, 2952 (CH), 1740 (CO), 1616 (C=N), 1372, 1157 (SO_2); ^{1}H NMR [D₆]DMSO: δ = 9.80 (d, J = 7.70 Hz, 1H, NH), 8.30-8.22, 8.00-7.92, 7.88-7.80 (3m, 4H, ar), 7.36-7.16 [m, 5H, ar (Phe)], 4.90-4.75 (q, J = 7.60 Hz, 1H, α-H, Phe), 3.69 (s, 3H, OMe), 3.26 (dd, J = 5.75, 4.20 Hz, 2H, β-H, Phe); HPLC: k' = 2.75, t_0 = 1.77 (RP-18, MeCN/H_2O, 1:1); HPLC: k' = 6.23, t_0 = 2.29 (Chiralcel OJ-R, MeCN/H_2O, 3:7); $C_{17}H_{16}N_2O_4S$ (344.39), calculated: C 59.29, H 4.68, N 8.13; found: C 59.11, H 4.41, N 7.77.

5.2.18.11. *Methyl (S)-2-(1,1-dioxobenzo[d]isothiazol-3-ylamino)propionate* (4v)

From 3-chlorobenzo[*d*]isothiazole 1,1-dioxide (**13**) (10.0 g, 49.58 mmol), L-Ala-OMe∗HCl (9.68 g, 69.42 mmol), and Et_3N (16.56 ml, 119.04 mmol) in 200 ml dioxane. Yield: 5.5 g (41%); M.p. 232-234 °C (MeOH/Acetone/H_2O); R_f = 0.43 (AcOEt/PE 7:3); $[\alpha]_D^{20}$ = -9.66 (c = 2, Acetone); IR: $\tilde{\nu}$ = 3329 (NH), 3107, 3050, 2994, 2958 (CH), 1752 (CO), 1615 (C=N), 1368, 1158 (SO_2); ^{1}H NMR [D_6]DMSO: δ = 9.73 (d, *J* = 7.25, 1H, NH), 8.34-8.25, 8.04-7.95, 7.91-7.82 (3m, 4H, ar), 4.67 (qn, *J* = 7.25 Hz, 1H, α-H , Ala), 3.70 (s, 3H, OMe), 1.52 (d, *J* = 7.25 Hz, 3H, β-H, Ala); HPLC: k' = 0.82, t_0 = 1.77 (RP-18, MeCN/H_2O, 1:1); HPLC: k' = 1.73, t_0 = 2.36 (Chiralcel OJ-R, MeCN/H_2O, 2:8); $C_{11}H_{12}N_2O_4S$ (268.29), calculated: C 49.25, H 4.51, N 10.44; found: C 50.09, H 5.35, N 10.49.

5.2.18.12. *Methyl (S)-2-(1,1-dioxobenzo[d]isothiazol-3-ylamino)-3-methylbutano-ate* (4w)

From 3-chlorobenzo[*d*]isothiazole 1,1-dioxide (**13**) (20.0 g, 99.20 mmol), L-Val-OMe∗HCl (18.2 g, 109.12 mmol), and Et_3N (34.6 ml, 248.01 mmol) in 400 ml dioxane, 2 h reflux. Yield: 18.7 g (61%); M.p. 238 °C (Acetone/H_2O); R_f = 0.65 (AcOEt/PE 7:3); $[\alpha]_D^{20}$ = -38.00 (c = 2, MeOH); IR: $\tilde{\nu}$ = 3332 (NH), 3102, 3036, 2966, 2937 (CH), 1748 (CO), 1608 (C=N), 1359, 1155 (SO_2); ^{1}H NMR [D_6]DMSO: δ = 9.55 (d, *J* = 6.20 Hz, 1H, NH), 8.55-8.43, 8.05-7.96, 7.94-7.82 (3m, 4H, ar), 4.44 (t, *J* = 7.00 Hz, 1H, α-H, Val), 3.73 (s, 3H, OMe), 2.34-2.07 (m, 1H, β-H, Val), 1.04 (d, *J* = 6.90 Hz, 3H, γ-H, Val), 0.99 (d, J = 6.90 Hz, 3H, γ-H, Val); HPLC: k' = 1.70, t_0 = 1.77 (RP-18, MeCN/H_2O, 1:1); HPLC: k' = 2.05, t_0 = 2.29 (Chiralcel OJ-R, MeCN/H_2O, 3:7); $C_{13}H_{16}N_2O_4S$ (296.35), calculated: C 52.69, H 5.44, N 9.45; found: C 52.80, H 4.56, N 9.47.

5.2.18.13. *Methyl 2-(1,1-dioxobenzo[d]isothiazol-3-ylamino)-2-methylpropionate* (4x*)*

From 3-chlorobenzo[*d*]isothiazole 1,1-dioxide (**13**) (5.0 g, 24.80 mmol), α-Me-Ala-OMe∗HCl (3.80 g, 24.8 mmol), and Et_3N (8.62 ml, 62.0 mmol) in 60 ml dioxane, reflux for 2 h. Yield: 2.75 g (39%); M.p. 235-238 °C (Acetone); R_f = 0.45 (AcOEt/PE 8.5:1.5); IR: $\tilde{\nu}$ = 3301 (NH), 3100, 3043, 2991, 2951 (CH), 1753 (CO), 1615 (C=N), 1356, 1157 (SO_2); ^{1}H NMR [D_6]DMSO: δ = 9.40 (bs, 1H, NH), 8.40-8.30, 8.00-7.95, 7.90-7.80 (3m, 4H, ar), 3.61 (s, 3H, OMe), 1.60 (s, 6H, α-H, α-Me-

Ala); HPLC: k' = 1.04, t_0 = 1.77 (RP-18, MeCN/H_2O, 1:1); $C_{12}H_{14}N_2O_4S$ (282.32), calculated: C 51.05, H 5.00, N 9.92; found: C 50.81, H 4.71, N 9.63.

5.2.18.14. *Dimethyl (S)-2-(1,1-dioxobenzo[d]isothiazol-3-ylamino)succinate* (4y)

From 3-chlorobenzo[*d*]isothiazole 1,1-dioxide (**13**) (5.0 g, 24.8 mmol), L-Asp(OMe)$_2$*HCl (4.9 g, 24.8 mmol), and Et$_3$N (6.9 ml, 50 mmol) in 100 ml dioxane, reflux for 2 h. Yield: 3.0 g (37%); M.p. 62 °C (Acetone/MeOH/H_2O); R_f = 0.51 (AcOEt/PE 7:3); $[\alpha]_D^{20}$ = -24.16 (c = 2, MeOH); IR: $\tilde{v}$ = 3333 (NH), 3098, 2955 (CH), 1741 (CO), 1618 (C=N), 1370, 1164 (SO$_2$); ^{1}H NMR (CDCl$_3$): δ = 7.96-7.86, 7.81-7.65 (2m, 4H, ar), 7.30 (d, J = 7.90 Hz, 1H, NH), 5.08 (td, J = 7.90, 4.10 Hz, 1H, α-H, Asp), 3.84 (s, 3H, OMe), 3.72 (s, 3H, OMe), 3.21 (d, J = 4.10 Hz, 2H, β-H, Asp); HPLC: k' = 0.95, t_0 = 1.77 (RP-18, MeCN/H_2O, 1:1); HPLC: k' = 2.17, t_0 = 2.36 (Chiralcel OJ-R, MeCN/H_2O, 2:8); $C_{13}H_{14}N_2O_6S$ (326.33), calculated: C 47.85, H 4.32, N 8.58; found: C 47.38, H 3.71, N 8.23.

5.2.18.15. *Methyl (S)-1-(1,1-dioxobenzo[d]isothiazol-3-yl)pyrrolidine-2-carboxylate* (4z)

From 3-chlorobenzo[*d*]isothiazole 1,1-dioxide (**13**) (10.0 g, 49.58 mmol), L-Pro-OMe*HCl (8.1 g, 49.58 mmol), and Et$_3$N (13.8 ml, 99.2 mmol) in 200 ml dioxane, reflux for 1.5 h. Yield: 8.5 g (61%); M.p. 185-187 °C (Acetone); R_f = 0.27 (AcOEt/PE 8.5:1.5); $[\alpha]_D^{20}$ = -65.55 (c = 0.6, MeOH); IR: $\tilde{v}$ = 3462 (OH), 3003, 2959 (CH), 1746 (CO), 1605 (C=N), 1350, 1157 (SO$_2$); ^{1}H NMR [D$_6$]DMSO: δ = 8.23-8.14, 8.07-7.98, 7.93-7.78 (3m, 4H, ar), 4.75 (dd, J = 9.00, 4.00 Hz, 1H, α-H, Pro), 4.30-4.12 (m, 2H, δ-H, Pro), 3.69 (s, 3H, OMe), 2.46-2.25 (m, 1H, β-H, Pro), 2.23-1.95 (m, 3H, [1H, β-H, Pro + 2H, γ-H, Pro], Pro); HPLC: k' = 3.41, t_0 = 1.92 (RP-18, MeCN/H_2O, 1:1); HPLC: k' = 1.19, t_0 = 2.29 (Chiralcel OJ-R, MeCN/H_2O, 3:7); $C_{13}H_{14}N_2O_4S$ (294.33), calculated: C 53.05, H 4.79, N 9.52; found: C 53.30, H 4.20, N 9.60.

5.2.19. General Procedure for the Synthesis of Acid Derivatives 14 from 4

With stirring and cooled with an ice-water bath, 1N aq. NaOH solution (except **14j**) was added drop wise to a solution of the ester derivative in acetone over 5 min. Then the mixture was left without stirring for 6 h, acetone was removed under reduced pressure, the remaining aqueous layer was cooled to 0 °C, and acidified with 1N HCl

to pH 1-2. The precipitate was separated, washed with cold H_2O, crystallized, and dried over NaOH pellets.

5.2.19.1. *(S)-2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)-4-methylsulfanylbutyric acid* (14a)

From methyl (*S*)-2(1,1-dioxobenzo[*d*]isothiazol-3-ylamino)-4-methylsulfanylbutanoate (**4d**) (3.5 g, 10.67 mmol) in 80 ml acetone, and 26.63 ml 1N NaOH. Yield: 2.48 g (67%); M.p. 162 °C (Acetone/H_2O); R_f = 0.50 (MeOH/CH_2Cl_2 2.5:7.5); $[\alpha]_D^{20}$ = -29.6 (c = 2, DMSO); IR: $\tilde{v}$ = 3304 (NH, OH), 3112, 2918 (CH), 1722 (CO), 1614 (C=N), 1363, 1154 (SO_2); ^{1}H NMR [D_6]DMSO: δ = 9.58 (d, *J* = 7.30 Hz, 1H, NH), 8.35-8.27, 8.02-7.95, 7.89-7.82 (3m, 4H, ar), 4.64 (q, *J* = 7.30 Hz, 1H, α-H, Met), 2.70-2.55 (m, 2H, γ-H, Met), 2.24-2.10 (m, 2H, β-H, Met), 2.07 (s, 3H, SMe); HPLC: k' = -0.32, t_0 = 1.77 (RP-18, MeCN/H_2O, 1:1); HPLC: k' = 0.38, t_0 = 2.34 (Chiralcel OJ-R, MeCN/0.01M KH_2PO_4, 2:8, pH 2.95); $C_{12}H_{14}N_2O_4S_2$ (314.38), calculated: C 45.85, H 4.49, N 8.91; found: C 45.18, H 4.13, N 8.15.

5.2.19.2. *(S)-2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)-4-methylpentanoic acid* (14b)

From methyl (*S*)-2 (1,1-dioxobenzo[*d*]isothiazol-3-ylamino)-4-methylpentanoate (**4e**) (3.0 g, 9.67 mmol) in 80 ml acetone, and 25 ml 1N NaOH. Yield: 1.5 g (52%); M.p. 229 °C (AcOEt/PE); R_f = 0.62 (MeOH/CH_2Cl_2 2.5:7.5); $[\alpha]_D^{20}$ = -14.00 (c = 1, Dioxane); IR: $\tilde{v}$ = 3300 (NH, OH), 3103, 2961 (CH), 1731 (CO), 1614 (C=N), 1365, 1154 (SO_2); ^{1}H NMR [D_6]DMSO: δ = 9.56 (d, *J* = 7.77 Hz, 1H, NH), 8.37-8.26, 8.02-7.94, 7.90-7.81 (3m, 4H, ar), 4.61-4.45 (m, 1H, α-H, Leu), 1.98-1.63 (m, 3H, [2H, β-H + 1H, γ-H] Leu), 0.94 (d, *J* = 5.80 Hz, 3H, δ-H, Leu), 0.91 (d, *J* = 5.80 Hz, 3H, δ-H, Leu); HPLC: k' = 2.44, t_0 = 1.85 (RP-18, MeCN/0.02M KH_2PO_4, 3:7, pH 2.75); HPLC: k' = 0.74, t_0 = 2.34 (Chiralcel OJ-R, MeCN/0.01M KH_2PO_4, 2:8, pH 2.95); $C_{13}H_{16}N_2O_4S$ (296.35), calculated: C 52.69, H 5.44, N 9.45; found: C 52.69, H 6.48, N 9.33.

5.2.19.3. *(2S,3S)-2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)-3-methylpentanoic acid* (14c)

From methyl (2*S*,3*S*)-2-(1,1-dioxobenzo[*d*]isothiazol-3-ylamino)-3-methylpentanoate (**4j**) (4.0 g, 12.90 mmol) in 100 ml acetone, and 33 ml 1N NaOH. Yield: 2.92 g (76%); M.p. 211 °C (AcOEt/PE); R_f = 0.62 (MeOH/CH_2Cl_2 2.5:7.5); $[\alpha]_D^{20}$ = +11.5 (c = 2,

Acetone); IR: $\tilde{\nu}$ = 3280 (NH, OH), 3109, 2965 (CH), 1719 (CO), 1619 (C=N), 1361, 1157 (SO$_2$); ^{1}H NMR [D$_6$]DMSO: δ = 9.43 (d, J = 7.77 Hz, 1H, NH), 8.53-8.44, 8.02-7.94, 7.90-7.80 (3m, 4H, ar), 4.42 (t, J = 7.77 Hz, 1H, α-H, Ile), 2.15-1.96 (m, 1H, β-H, Ile), 1.64-1.44, (m, 1H, γ-H, Ile), 1.41-1.16 (m, 1H, 3H, γ-H, Ile), 0.98 (d, J = 6.71 Hz, 3H, γ-H, Ile), 0.89 (t, J = 7.24 Hz, δ-H, Ile); HPLC: k' = 2.02, t$_0$ = 1.85 (RP-18, MeCN/0.02M KH$_2$PO$_4$, 3:7, pH 2.95); HPLC: k' = 0.72, t$_0$ = 2.34 (Chiralcel OJ-R, MeCN/0.01M KH$_2$PO$_4$, 2:8, pH 2.95); C$_{13}$H$_{16}$N$_2$O$_4$S (296.35), calculated: C 52.69, H 5.44, N 9.45; found: C 52.55, H 4.71, N 9.13.

5.2.19.4. *(1,1-Dioxobenzo[d]isothiazol-3-ylamino)acetic acid* (14d)

From methyl (1,1-dioxobenzo[*d*]isothiazol-3-ylamino)acetate (**4k**) (4.0 g, 15.74 mmol) in 100 ml acetone, and 39.38 ml 1N NaOH. Yield: 1.50 g (40%); M.p. 255-257 °C (MeOH/H$_2$O; 259-260 °C)[71]; R$_f$ = 0.20 (MeOH/CH$_2$Cl$_2$ 2.5:7.5); IR: $\tilde{\nu}$ = 3289 (NH, OH), 3109, 3061, 2937 (CH), 1705 (CO), 1620 (C=N), 1361, 1156 (SO$_2$); ^{1}H NMR [D$_6$]DMSO: δ = 13.03 (bs, 1H, CO$_2$H), 9.84 (t, J = 6.00 Hz, 1H, NH), 8.25-8.17, 8.03-7.94, 7.91-7.80 (3m, 4H, ar), 4.21 (d, J = 6.00 Hz, 2H, α-H, Gly); HPLC: k' = 0.39, t$_0$ = 1.92 (RP-18, MeCN/0.01M KH$_2$PO$_4$, 2:8, pH 2.88); C$_9$H$_8$N$_2$O$_4$S (240.24), calculated: C 45.00, H 3.36, N 11.66; found: C 44.35, H 2.92, N 11.42.

5.2.19.5. *(S)-2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)-3-phenylpropionic acid* (14e)

From methyl (*S*)-2(1,1-dioxobenzo[*d*]isothiazol-3-ylamino)-3-phenylpropanoate (**4u**) (9.1 g, 26.42 mmol) in 200 ml acetone, and 67 ml 1N NaOH. Yield: 5.61 g (64%); M.p. 234°C (AcOEt); R$_f$ = 0.53 (MeOH/CH$_2$Cl$_2$ 2.5:7.5); $[\alpha]_D^{20}$ = -32.33 (c = 2, Acetone); IR: $\tilde{\nu}$ = 3327 (NH, OH), 3080, 2945 (CH), 1733 (CO), 1617 (C=N), 1360, 1151 (SO$_2$); ^{1}H NMR [D$_6$]DMSO: δ = 13.25 (bs, 1H, CO$_2$H), 9.70 (d, J = 7.95 Hz, 1H, NH), 8.31-8.21, 8.00-7.90), 7.88-7.78 (3m, 4H, ar), 7.38-7.14 (m, 5H, Phe), 4.81-4.65 (m, 1H, α-H, Phe), 3.30 (dd, J = 13.80, 4.80 Hz, 1H, β-H, Phe), 3.18 (dd, J = 13.80, 10.40 Hz, 1H, β-H, Phe); HPLC: k' = 2.88, t$_0$ = 1.89 (RP-18, MeCN/0.02M KH$_2$PO$_4$, 3:7, pH 2.75); HPLC: k' = 0.93, t$_0$ = 2.34 (Chiralcel OJ-R, MeCN/0.01M KH$_2$PO$_4$, 2:8, pH 2.95); C$_{16}$H$_{14}$N$_2$O$_4$S (330.36), calculated: C 58.17, H 4.27, N 8.48; found: C 58.80, H 4.51, N 8.43.

5.2.19.6. *(S)-2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)propionic acid* (14f)

From methyl (*S*)-2-(1,1-dioxobenzo[*d*]isothiazol-3-ylamino)propionate (**4v**) (5.0 g, 18.65 mmol) in 200 ml acetone, and 47 ml 1N NaOH. Yield: 2.90 g (61%); M.p. 277 °C (dec.); R_f = 0.23 (MeOH/CH$_2$Cl$_2$ 2.5:7.5); $[\alpha]_D^{20}$ = -3.00 (c = 2, DMSO); IR: $\tilde{\nu}$ = 3321 (NH, OH), 3100 (CH), 1760 (CO), 1614 (C=N), 1359, 1161 (SO$_2$); ^{1}H NMR [D$_6$]DMSO: δ = 9.61 (d, *J* = 7.24, 1H, NH), 8.37-8.25, 8.02-7.94, 7.90-7.79 (3m, 4H, ar), 4.62 (qn, *J* = 7.24 Hz, 1H, α-H, Ala), 1.52 (d, *J* = 7.24 Hz, 3H, β-H, Ala); HPLC: k' = 0.84, t_0 = 1.92 (RP-18, MeCN/0.01M KH$_2$PO$_4$, 2:8, pH 2.88); HPLC: k' = 0.16, t_0 = 1.87 (Chiralcel OJ-R, MeCN/0.01M KH$_2$PO$_4$, 2:8, pH 2.88); C$_{10}$H$_{10}$N$_2$O$_4$S (254.27), calculated: C 47.24, H 3.96, N 11.02; found: C 47.19, H 2.58, N 10.78.

5.2.19.7. *(S)-2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)-3-methylbutyric acid* (14g)

From methyl (*S*)-2-(1,1-dioxobenzo[*d*]isothiazol-3-ylamino)-3-methyl-butanoate (**4w**) (18.0 g, 54.87 mmol) in 200 ml acetone, and 137 ml 1N NaOH, 2 d. Yield: 6.0 g (35%); M.p. 238 °C (AcOEt/PE); R_f = 0.62 (MeOH/CH$_2$Cl$_2$ 2.5:7.5); $[\alpha]_D^{20}$ = -16.16 (c = 2, DMSO); IR: $\tilde{\nu}$ = 3318 (NH, OH), 3095, 2970, 2934 (CH), 1745 (CO), 1611 (C=N), 1357, 1157 (SO$_2$); ^{1}H NMR [D$_6$]DMSO: δ = 9.41 (d, *J* = 7.95 Hz, 1H, NH), 8.54-8.46, 8.02-7.91, 7.90-7.81 (3m, 4H, ar), 4.37 (t, *J* = 7.95 Hz, 1H, α-H, Val), 2.38-2.20 (m, 1H, β-H, Val), 1.04 (d, *J* = 6.70 Hz, 3H, γ-H, Val), 1.01 (d, *J* = 6.70 Hz, 3H, γ-H, Val); HPLC: k' = 0.37, t_0 = 2.34 (RP-18, MeCN/H$_2$O, 1:1); HPLC: k' = 0.52, t_0 = 2.57 (Chiralcel OJ-R, MeCN/0.01M KH$_2$PO$_4$, 2:8, pH 2.85); C$_{12}$H$_{14}$N$_2$O$_4$S (282.32), calculated: C 51.05, H 5.00, N 9.92; found: C 49.56, H 4.40, N 9.65.

5.2.19.8. *2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)-2-methylpropionic acid* (14h)

From methyl-2-(1,1-dioxobenzo[*d*]isothiazol-3-ylamino)-2-methyl-propionate (**4x**) (1.70 g, 6.02 mmol) in 35 ml acetone, and 15.0 ml 1N NaOH for 18 h. Yield: 0.85 g (53%); M.p. 279 °C; R_f = 0.47 (MeOH/CH$_2$Cl$_2$ 2.5:7.5); IR: $\tilde{\nu}$ = 3281 (NH, OH), 3104, 3056, 2994, 2942 (CH), 1722 (CO), 1613 (C=N), 1356, 1156 (SO$_2$); ^{1}H NMR [D$_6$]DMSO: δ = 12.80 (bs, 1H, OH), 9.18 (s, 1H, NH), 8.40-8.32, 8.00-7.93, 7.87-7.80 (3m, 4H, ar), 1.60 (s, 6H, α-H, α-Me-Ala); HPLC: k' = 0.56, t_0 = 1.77 (RP-18, MeCN/0.02M KH$_2$PO$_4$, 3:7, pH 2.95); C$_{11}$H$_{12}$N$_2$O$_4$S (268.29), calculated: C 49.25, H 4.51, N 10.44; found: C 49.26, H 3.93, N 10.69.

5.2.19.9. *(S)-2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)succinic acid* (14i)

From dimethyl (*S*)-2-(1,1-dioxobenzo[*d*]isothiazol-3-ylamino)succinate (**4y**) (2.0 g, 6.12 mmol) in 200 ml acetone, and 30 ml 1N NaOH. Yield: 0.75 g (41%); M.p. 215 °C; R_f = 0.84 (MeOH/H_2O 1:1, Reverse Phase TLC); $[\alpha]_D^{20}$ = -31.00 (c = 2, DMSO); IR: $\tilde{v}$ = 3372 (NH, OH), 3093, 2954 (CH), 1710 (CO), 1620 (C=N), 1363, 1151 (SO_2); ^{1}H NMR [D_6]DMSO: δ = 9.69 (d, *J* = 7.92 Hz, 1H, NH), 8.28-8.19, 8.03-7.96, 7.90-7.18 (3m, 4H, ar), 4.83 (td, *J* = 7.92, 5.64, Hz, 1H, α-H, Asp), 3.05-2.78 (m, 2H, β-H, Asp); HPLC: k' = 0.23, t_0 = 1.85 (RP-18, MeCN/0.01M KH_2PO_4, 2:8, pH 2.88); HPLC: k' = 0.52, t_0 = 2.57 (Chiralcel OJ-R, MeCN/0.01M KH_2PO_4, 2:8, pH 2.85); $C_{11}H_{10}N_2O_6S$ (298.28), calculated: C 44.30, H 3.38, N 9.39; found: C 42.85, H 3.37, N 9.03.

5.2.19.10. *(S)-1-(1,1-Dioxobenzo[d]isothiazol-3-yl)pyrrolidine-2-carboxylic acid (14j)*

Methyl (*S*)-2-(1,1-dioxobenzo[*d*]isothiazol-3-ylamino)pyrrolidine-2-carboxylate (**4z**) (2.0 g, 6.79 mmol) in 40 ml 5% HCl was refluxed for 8 h. After filtration the filtrate was left for 2 h, and then the resulted solid crystals were separated, washed with a few ml of H_2O, and dried. Yield: 0.64 g (34%); M.p. 253 °C; R_f = 0.32 (MeOH/CH_2Cl_2 2.5:7.5); $[\alpha]_D^{20}$ = -85.00 (c = 2, DMSO); IR: $\tilde{v}$ = 3452 (NH, OH) 3006, 2988, 2962, 2889 (CH), 1741 (CO), 1606 (C=N), 1334, 1154 (SO_2); ^{1}H NMR [D_6]DMSO: δ = 12.96 (bs, 1H, OH), 8.22-8.13, 8.06-7.98, 7.91-7.72 (3m, 4H, ar), 4.65 (dd, *J* = 8.80, 3.89 Hz, α-H, Pro), 4.29-4.09 (m, 2H, δ-H, Pro), 2.38-2.23 (m, 1H, β-H, Pro), 2.20-1.95 (m, 3H, [1H, β-H Pro + 2H, γ-H, Pro]); HPLC: k' = 0.76, t_0 = 1.92 (RP-18, MeCN/0.01M KH_2PO_4, 2:8, pH 2.88); HPLC: k' = 0.22, t_0 = 1.87 (Chiralcel OJ-R, MeCN/0.01M KH_2PO_4, 2:8, pH 2.88); $C_{12}H_{12}N_2O_4S$ (280.30), calculated: C 51.42, H 4.32, N 9.99; found: C 51.35, H 4.43, N 9.90.

5.2.20. Synthesis of the different ester derivatives 15

5.2.20.1. *Ethyl (2S, 3S)-2-(1, 1-dioxobenzo[d]isothiazol-3-ylamino)-3-methylpentanoate* (15a)

(2*S*,3*S*)-2-(1,1-Dioxobenzo[*d*]isothiazol-3-ylamino)-3-methylpentanoic acid (**14c**) (0.50 g, 1.68 mmol) was added to 20 ml of ethanol followed by the addition of 0.14 ml of thionyl chloride (1.86 mmol). The reaction mixture was refluxed for 4 h. Solvent was removed in vacuo, 10 ml of ethanol was added to the residue and the solution was concentrated to half on rota vapour. The concentrated solution was left overnight

at room temperature which yielded crystals on next day. The crystals were washed with petrol ether, and were crystallized from ethyl acetate/petrol ether. Yield: 0.28 g (52%); M.p.138 °C (AcOEt/PE); R_f = 0.63 (AcOEt/PE 1:1); $[\alpha]_D^{20}$ = -28.33 (c = 2, MeOH); IR: $\tilde{\nu}$ = 3307 (NH), 3098, 3050, 2968, 2968, 2935 (CH), 1749 (CO), 1612 (C=N), 1374, 1156 (SO_2); ^{1}H NMR (CDCl$_3$): δ = 7.92-7.88, 7.78-7.63 (2m, 4H, ar), 6.70 (d, J = 7.84 Hz, 1H, NH), 4.87 (dd, J = 7.84, 4.50 Hz, 1H, α-H, Ile), 4.30 (dq, J = 7.10, 0.74 Hz, 2H, OCH$_2$), 2.28-2.08 (m, 1H, β-H, Ile), 1.66-1.49 (m, 2H, γ-H, Ile), 1.35 (t, J = 7.10 Hz, 3H, CH$_3$), 1.03 (d, J = 7.30 Hz, 3H, γ-H, Ile), 0.97 (d, J = 6.90 Hz, 3H, δ-H, Ile); ^{13}C NMR (CDCl$_3$): δ = 171.51, 159.28, 142.60, 133.43, 132.87, 127.36, 122.07, 120.76, 62.25, 59.78, 37.97, 25.91, 15.19, 14.25, 11.65; HPLC: k' = 2.65, t_0 = 1.85 (RP-18, MeCN/H$_2$O, 1:1); HPLC: k' = 4.43, t_0 = 1.78 (Chiralcel OJ-R, MeCN/H$_2$O, 3:7); C$_{15}$H$_{20}$N$_2$O$_4$S (324.40), calculated: C 55.54, H 6.21, N 8.64; found: C 55.43, H 5.80, N 8.41.

5.2.20.2. *Isopropyl (2S,3S)-2-(1,1-dioxobenzo[d]isothiazol-3-ylamino)-3-methyl-pentanoate* (15b)

(2*S*,3*S*)-2-(1,1-Dioxobenzo[*d*]isothiazol-3-ylamino)-3-methylpentanoic acid (**14c**) (0.50 g, 1.68 mmol) and SOCl$_2$ (0.20 ml, 2.66 mmol) were added to 20 ml of 2-propanol. The reaction mixture was refluxed for 11 h. Reaction mixture was cooled down to room temperature and the solvent was removed in vacuo. The resulted solid was washed with 10 ml of water and crystallized from ethyl acetate and petrol ether to produce white fibrous crystals. Yield: 0.43 g (75%); M.p.120 °C (AcOEt/PE); R_f = 0.66 (AcOEt/PE 1:1); $[\alpha]_D^{20}$ = -27.50 (c = 2, MeOH); IR: $\tilde{\nu}$ = 3298 (NH), 3098, 3053, 2978, 2935 (CH), 1744 (CO), 1612 (C=N), 1373, 1155 (SO_2); ^{1}H NMR (CDCl$_3$): δ = 7.90-7.86, 7.76-7.63 (2m, 4H, ar), 6.83 (d, J = 7.78 Hz, 1H, NH), 5.15 (spt, J = 6.30 Hz, 1H, OCH), 4.83 (dd, J = 7.78, 4.44 Hz, 1H, α-H, Ile), 2.26-2.10 (m, 1H, β-H, Ile), 1.69-1.45 (m, 2H, γ-H, Ile), 1.32 (d, J = 6.30 Hz, 6H, 2Me), 1.04-0.96 (m, 6H, [3H, γ-H, Ile + 3H, δ-H, Ile]); ^{13}C NMR (CDCl$_3$): δ = 171.05, 159.24, 142.49, 133.34, 132.84, 127.33, 121.97, 120.74, 70.42, 59.79, 37.90, 25.90, 21.79, 21.77. 15.10, 11.60; HPLC: k' = 3.64, t_0 = 1.85 (RP-18, MeCN/H$_2$O, 1:1); HPLC: k' = 6.02, t_0 = 1.78 (Chiralcel OJ-R, MeCN/H$_2$O, 3:7); C$_{16}$H$_{22}$N$_2$O$_4$S (338.43), calculated: C 56.79, H 6.55, N 8.28; found: C 56.50, H 6.54, N 8.34.

5.2.20.3. *n*-Butyl(2S,3S)-2-(1,1-dioxobenzo[d]isothiazol-3-ylamino)-3-methylpentanoate (15c)

(2*S*,3*S*)-2-(1,1-Dioxobenzo[*d*]isothiazol-3-ylamino)-3-methylpentanoic acid (**14c**) (0.50 g, 1.68 mmol) and $SOCl_2$ (0.20 ml, 2.66 mmol) in 20 ml of n-butanol. The reaction mixture was refluxed for 13 h as described in 5.2.20.2. Yield: 0.14 g (24%); M.p. 98 °C (AcOEt/PE); R_f = 0.72 (AcOEt/PE 1:1); $[\alpha]_D^{20}$ = -25.50 (c = 2, MeOH); IR: $\tilde{\nu}$ = 3305 (NH), 3098, 2962, 2934 (CH), 1752 (CO), 1611 (C=N), 1364, 1156 (SO_2); ^1H NMR ($CDCl_3$): δ = 7.90-7.83, 7.72-7.63 (2m, 4H, ar), 6.86 (d, *J* = 7.92 Hz, 1H, NH), 4.88 (dd, *J* = 7.92, 4.60 Hz, 1H, α-H, Ile), 4.24 (t, *J* = 6.60 Hz, 2H, OCH_2), 2.27-2.07 (m, 1H, β-H, Ile), 1.76-1.26 (m, 6H, [4H, CH_2 + 2H, γ-H, Ile]), 1.04-0.93 (m, 9H [3H, Me + 3H, γ-H, Ile + 3H, δ-H, Ile]); ^{13}C NMR ($CDCl_3$): δ = 171.71, 159.31, 142.48, 133.37, 132.87, 127.29, 121.97, 120.76, 66.07, 59.82, 37.88, 30.47, 25.84, 19.07, 15.19, 13.56, 11.57; HPLC: k' = 5.72, t_0 = 1.85 (RP-18, MeCN/H_2O, 1:1); HPLC: k' = 11.13, t_0 = 1.78 (Chiralcel OJ-R, MeCN/H_2O, 3:7); $C_{17}H_{24}N_2O_4S$ (352.46), calculated: C 57.93, H 6.86, N 7.95; found: C 57.19, H 6.53, N 7.93.

5.2.20.4. *Isobutyl (2S,3S)-2-(1,1-dioxobenzo[d]isothiazol-3-ylamino)-3-methylpentanoate* (15d)

(2*S*,3*S*)-2-(1,1-Dioxobenzo[*d*]isothiazol-3-ylamino)-3-methylpentanoic acid (**14c**) (0.50 g, 1.68 mmol) and $SOCl_2$ (0.20 ml, 2.66 mmol) in 20 ml of isobutanol for 18 h at reflux as described for 5.2.20.2. Yield: 0.42 g (70%); M.p. 118-120 °C; R_f = 0.75 (AcOEt/PE 1:1); $[\alpha]_D^{20}$ = -26.00 (c = 2, MeOH); IR: $\tilde{\nu}$ = 3310 (NH), 3098, 2965 (CH), 1752 (CO), 1612 (C=N), 1367, 1157 (SO_2); ^1H NMR ($CDCl_3$): δ = 7.89-7.80, 7.74-7.63 (2m, 4H, ar), 7.00 (d, *J* = 8.00 Hz, 1H, NH), 4.90 (dd, *J* = 8.00, 4.80 Hz, 1H, α-H, Ile), 4.11-3.93 (m, 2H, OCH_2), 2.23-1.92 (m, 2H, [1H, $CH_2\underline{CH}$ + 1H, β-H, Ile), 1.69-1.25 (m, 2H, γ-H, Ile), 1.05-0.97 (m, 12 H [6H, 2Me + 3H, γ-H, Ile + 3H, δ-H Ile]); ^{13}C NMR ($CDCl_3$): δ =171.35, 159.04, 142.68, 133.47, 132.75, 127.38, 122.20, 120.62, 72.35, 59.64, 38.07, 27.68, 25.92, 19.10, 15.22, 11.69; HPLC: k' = 3.98, t_0 = 1.85 (RP-18, MeCN/H_2O, 1:1); HPLC: k' = 10.32, t_0 = 1.78 (Chiralcel OJ-R, MeCN/H_2O, 3:7); $C_{17}H_{24}N_2O_4S$ (352.46), calculated: C 57.93, H 6.86, N 7.95; found: C 57.30, H 6.14, N 7.69.

5.2.20.5. *Cyanomethyl (2S,3S)-2-(1,1-dioxobenzo[d]isothiazol-3-ylamino)-3-methyl pentanoate* (15e)

(2S,3S)-2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)-3-methylpentanoic acid (**14c**) (10 g, 33.6 mmol) was added to 33.60 ml of 2N aq. KOH (67.20 mmol) and stirred for 10 min at room temperature. The solvent was removed on rota vapour. The obtained solid was heated in 40 ml of chloroacetonitrile at 80 °C for 30 min. The reaction mixture was cooled to room temperature and then added drop wise into water. The aqueous layer was extracted with 100 ml of ethyl acetate. The organic layer was evaporated in vacuo and the obtained solid was crystallized from ethyl acetate and petrol ether. Yield: 0.24 g (42%); M.p. 172 °C (AcOEt/PE); R_f = 0.47 (AcOEt/PE 7:3); $[\alpha]_D^{20}$ = -60.00 (c = 2, MeOH); IR: $\tilde{\nu}$ = 3329 (NH), 3097, 3038, 2970, 2932 (CH), 1769 (CO), 1610 (C=N), 1376, 1161 (SO$_2$); ^{1}H NMR (CDCl$_3$): δ = 7.94-7.88, 7.81-7.69 (2m, 4H, ar), 6.53 (d, J = 8.00 Hz, 1H, NH), 4.96 (d, J = 15.60 Hz, 1H, OCH$_2$), 4.95 (dd, J = 8.00, 3.20 Hz, 1H, α-H, Ile), 4.76 (d, J = 15.60 Hz, 1H, OCH$_2$), 2.26-2.08 (m, 1H, β-H, Ile), 1.69-1.25 (m, 2H, γ-H, Ile), 1.05-0.97 (m, 6H, [3H, γ-H, Ile + 3H, δ-H, Ile]); ^{13}C NMR (CDCl$_3$): δ = 169.90, 159.61, 142.64, 133.69, 132.88, 126.95, 122.24. 120.94, 113.50, 59.52, 49.14, 38.01, 25.69, 15.25, 11.44; HPLC: k' = 2.41, t$_0$ = 1.85 (RP-18, MeCN/H$_2$O, 1:1); HPLC: k' = 3.25, t$_0$ = 1.78 (Chiralcel OJ-R, MeCN/H$_2$O, 3:7); C$_{15}$H$_{17}$N$_3$O$_4$S (335.38), calculated: C 53.72, H 5.11, N 12.53; found: C 53.48, H 4.11, N 12.58.

5.2.20.6. *Benzyl (2S,3S)-2-(1,1-dioxobenzo[d]isothiazol-3-ylamino)-3-methylpen-tanoate* (15f)

3.90 g of isoleucine benzyl ester tosylate (9.92 mmol) and triethyl amine (2.76 ml, 19.84 mmol) were added to 40 ml of dioxane. The reaction mixture was stirred at room temperature for 10 min. 3-Chlorobenzo[d]isothiazole 1,1-dioxide (**13**) (2.00 g, 9.92 mmol) was then added to the reaction mixture and the reaction mixture was refluxed for 2 and half h. Dioxane was removed in vacuo and water was added in it followed by the extraction with ethyl acetate (3 x 100ml). Ethyl acetate layer dried with sodium sulfate, evaporated and crystallized. Yield: 1.90 g (50%); M.p. 125-127 °C (AcOEt/PE); R_f = 0.69 (AcOEt/PE 1:1); $[\alpha]_D^{20}$ = -35.00 (c = 2, MeOH); IR: $\tilde{\nu}$ = 3320 (NH), 3094, 3069, 3030, 2964, 2932 (CH), 1738 (CO), 1608 (C=N), 1366, 1156 (SO$_2$); ^{1}H NMR (CDCl$_3$): δ = 7.88-7.81, 7.74-7.57 (2m, 4H, ar), 7.44-7.35 (m, 5H, ar, [Phe]), 6.85 (d, J = 8.00 Hz, 1H, NH), 5.30 (d, J = 12.00 Hz, 1H, OCH$_2$), 5.23 (d, J = 12.00 Hz, 1H, OCH$_2$), 4.95 (dd, J = 8.00, 4.82 Hz, 1H, α-H, Ile), 2.25-2.08 (m, 1H,

β-H, Ile), 1.63-1.19 (m, 2H, *γ*-H, Ile), 0.99-0.92 (m, 6H, [3H, *γ*-H, Ile + 3H, *δ*-H, Ile]); ^{13}C NMR (CDCl$_3$): δ = 171.24, 159.19, 142.67, 134.69, 133.46, 132.78, 128.84, 128.77, 128.60, 128.52, 127.30, 126.02, 122.16, 120.65, 67.93, 59.74, 38.08, 25.75, 15.19, 11.60; HPLC: k' = 7.40, t$_0$ = 1.85 (Chiralcel OJ-R, MeCN/H$_2$O, 4:6), C$_{20}$H$_{22}$N$_2$O$_4$S (386.47), calculated: C 62.16, H 5.74, N 7.25; found: C 62.37, H 6.01, N 7.27.

5.2.20.7. *Tert-butyl (2S,3S)-2-(1,1-dioxobenzo[d]isothiazol-3-ylamino)-3-methyl-pentanoate* (15g)

2.20 g of L-isoleucine *tert*-butyl ester HCl (9.92 mmol) and 2.76 ml of triethyl amine (19.84 mmol) were added to 40 ml of dioxane and stirred at room temperature for 10 min. 3-Chlorobenzo[*d*]isothiazole 1,1-dioxide (**13**) (2.00 g, 9.92 mmol) was added and the reaction mixture was refluxed for 2 and half h. The solvent was removed in vacuo and the resulted viscous material was triturated with 20 ml of water and then extracted by ethyl acetate (100ml x 3). The ethyl acetate layer was dried with sodium sulfate and evaporated in vacuo to obtain a highly viscous material which solidified after treatment with n-hexane. Yield: 2.30 g (66%); M.p. 118 °C; R$_f$ = 0.72 (AcOEt/PE 1:1); $[\alpha]_D^{20}$ = -25.00 (c = 2, MeOH); IR: $\tilde{\nu}$ = 3314 (NH), 3094, 2973, 2933 (CH), 1747 (CO), 1612 (C=N), 1367, 1156 (SO$_2$); ^{1}H NMR (CDCl$_3$): δ = 7.87-7.78, 7.72-7.58 (2m, 4H, ar), 7.04 (d, *J* = 7.70 Hz, 1H, NH), 4.74 (dd, *J* = 7.70, 4.86 Hz, 1H, *α*-H, Ile), 2.23-2.03 (m, 1H, *β*-H, Ile), 1.71-1.21 (m, 11H, [9H, CH$_3$ + 2H, *γ*-H, Ile]), 1.04-0.96 (m, 6H, [3H, *γ*-H, Ile + 3H, *δ*-H, Ile); ^{13}C NMR (CDCl$_3$): δ = 170.62, 159.00, 142.54, 133.31, 132.79, 127.44, 122.01, 120.62, 83.80, 59.95, 37.91, 28.08, 26.12, 15.04, 11.63; HPLC: k' = 5.23, t$_0$ = 1.85 (RP-18, MeCN/H$_2$O, 1:1); HPLC: k' = 8.19 t$_0$ = 1.78 (Chiralcel OJ-R, MeCN/H$_2$O, 3:7); C$_{17}$H$_{24}$N$_2$O$_4$S (352.46), calculated: C 57.93, H 6.86, N 7.95; found: C 58.04, H 7.04, N 8.16.

5.2.20.8. *Cyanomethyl (2S)-2-(1,1-dioxobenzo[d]isothiazol-3-ylamino)-3-methyl-butanoate* (15h*)*

From (*S*)-2-(1,1-Dioxobenzo[*d*]isothiazol-3-ylamino)-3-methylbutyric acid (**14g**) (1.00 g, 3.54 mmol), 1N KOH (4.25 ml, 4.25 mmol), 5 ml of chloroacetonitrile, 80 °C, 30 min as described in 5.2.20.5. Yield: 0.50 g (44%); M.p. 142 °C (AcOEt/PE); R$_f$ = 0.63 (AcOEt/PE 7:3); $[\alpha]_D^{20}$ = -68.00 (c = 2, MeOH); IR: $\tilde{\nu}$ = 3290 (NH), 3096, 3038, 2972 (CH), 1760 (CO), 1612 (C=N), 1375, 1156 (SO$_2$); ^{1}H NMR (CDCl$_3$): δ = 7.95-7.89, 7.82-7.67 (2m, 4H, ar), 6.40 (d, *J* = 8.28 Hz, 1H, NH), 4.95 (d, *J* = 15.60 Hz, 1H,

OCH$_2$), 4.92 (dd, J = 8.28, 4.90 Hz, 1H, α-H, Val), 4.76 (d, J = 15.60 Hz, 1H, OCH$_2$), 2.54-2.37 (m, 1H, β-H, Val), 1.10 and 1.08 (2d, J = 6.90 Hz, 6H, γ-H, Val); ^{13}C NMR (CDCl$_3$): δ = 169.98, 159.79, 142.78, 133.77, 132.89, 126.93, 122.37, 120.79, 113.46, 60.36, 49.23, 31.64, 18.63, 18.30; HPLC: k' = 1.88, t$_0$ = 1.92 (RP-18, MeCN/H$_2$O, 1:1), λ = 225 nm; HPLC: k' = 5.85, t$_0$ = 2.17 (Chiralcel OJ-R, MeCN/H$_2$O, 2.5:7.5); C$_{14}$H$_{15}$N$_3$O$_4$S (321.36), calculated: C 52.33, H 4.70, N 13.08; found: C 51.97, H 4.08, N 12.33.

5.2.21. *(2S,3S)-2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)-3-methyl-pentan-1-ol (16)*

9.00 g (30 mmol) of methyl (2*S*,3*S*)-2-(1,1-dioxobenzo[*d*]isothiazol-3-ylamino)-3-methylpentanoate (**4j**) was dissolved in 200 ml of 1,2-dimethoxy ethane and cooled to -40 °C. 1.8 g (82.64 mmol) of lithium borohydride (LiBH$_4$) was added at once and reaction mixture was stirred at -40 °C for half an h. The reaction mixture was allowed to attain room temperature and stirred overnight. The reaction mixture was cooled at ice bath temperature and 1N HCl was added drop wise till the reaction mixture turns to be neutral. The 1,2-dimethoxy ethane was removed in vacuo and the resulted aqueous layer was extracted two times with dichloromethane. The dichloromethane layer was dried with sodium sulfate and the solvent was removed in vacuo. The obtained solid was then crystallized from ethyl acetate and petrol ether to produce a white product. Yield: 6.80 g (83%); M.p. 162-165 °C (AcOEt/PE); R$_f$ = 0.62 (AcOEt/PE 8:2); $[\alpha]_D^{20}$ = -81.00 (c = 2, MeOH); IR: $\tilde{\nu}$ = 3454, 3259 (NH, OH), 3098, 3051, 2963 (CH), 1622 (C=N), 1377, 1158 (SO$_2$); ^{1}H NMR (CDCl$_3$): δ = 7.90-7.81, 7.77-7.64, 7.60-7.50 (3m, 4H, ar), 6.72 (d, J = 8.60 Hz, 1H, NH), 4.17-4.01 (m, 1H, CH$_2$), 3.95-3.83 (m, 2H, [1H, CH$_2$ + α-H, Ile]), 3.50 (bs, 1H, OH), 1.99-1.78 (1H, β-H, Ile), 1.72-1.48 (m, 4H, γ-H, Ile), 1.35-1.30 (m, 1H, γ-H, Ile), 0.99 (d, J = 6.80 Hz, 3H, γ-H, Ile), 0.97 (t, J = 7.30 Hz, 3H, δ-H, Ile); ^{13}C NMR (CDCl$_3$): δ 159.83, 142.56, 133.17, 132.56, 127.85, 121.86, 121.03, 61.48, 59.46, 35.68, 25.87, 15.32, 11.28; HPLC: k' = 0.90, t$_0$ = 1.77 (RP-18, MeCN/H$_2$O, 1:1); HPLC: k' = 2.95, t$_0$ = 1.73 (Chiralcel OJ-R, MeCN/H$_2$O, 2:8); C$_{13}$H$_{18}$N$_2$O$_3$S (282.36), calculated: C 55.30, H 6.43, N 9.92; found: C 55.29, H 5.89, N 9.66.

5.2.22. *Ethyl (Z)-(2-aminothiazol-5-yl)-α-methoxyiminoacetate* (17a)

M.p. 161 °C [77]; IR: $\tilde{\nu}$ = 3441 (NH), 2984, 2938 (CH), 1729 (C=O), 1618 (C=N), 1541 (NH$_2$ of the heterocyclic conjugated system), 1032 (C=N-OCH$_3$); UV (λ_{max} = 225) [77];

^{1}H NMR [D$_6$]DMSO: δ = 7.25 (bs, 2H, NH$_2$), 6.90 (s, 1H, 5'-H$_{thiazole}$), 4.26 (q, J = 7.14 Hz, 2H, CH$_2$), 3.87 (s, 3H, OMe), 1.26 (t, J = 7.14 Hz, 3H, Me); ^{13}C NMR [D$_6$]DMSO: δ = 168.59 (C$_{2'}$), 162.22 (C$_{7'}$), 146.73 (C$_{6'}$), 140.92 (C$_{4'}$), 108.38 (C$_{5'}$), 62.31 (OCH$_3$), 61.26 (CH$_2$), 13.80 (CH$_3$); HPLC: k' = 1.07, t$_0$ = 1.81 (RP-18, MeCN/H$_2$O, 1:1).

5.2.23. *Ethyl (E,Z)-(2-aminothiazol-5-yl)-α-methoxyiminoacetate* (17b)

Ethyl (*Z*)-(2-aminothiazol-5-yl)-α-methoxyiminoacetate (**17a**) (1.0 g) in dioxane was irradiated with UV light (λ = 254 nm) for 2 days. The solvent removed in vacuo to obtain a solid. Yield: 1.0 g (100%); M.p.128-132 °C; R$_f$ = 0.48 (MeOH/CH$_2$Cl$_2$ 0.2:9.8) (spots of two isomers are overlying when checked by TLC and are not possible to distinguish); IR: $\tilde{v}$ = 3442 (NH), 3129, 2984, 2938 (CH), 1730 (CO), 1619 (C=N), 1542 (NH$_2$ of the heterocyclic conjugated system); ^{1}H NMR [D$_6$]DMSO: δ = 7.50, 6.90 (2s, 1H, 5'-H$_{thiazole}$), 7.25, 7.14 (2bs, 2H, NH$_2$), 4.26, 4.24 (2q, J = 7.14 Hz, 2H, CH$_2$), 3.98, 3.87 (2s, 3H, OMe); 1.26, 1.25 (2t, J = 7.14 Hz, 3H, Me); *E:Z* $\approx$ 2:8; ^{13}C NMR [D$_6$]DMSO: δ = 168.59, 166.64 (C$_{2'}$), 163.10, 162.22 (C$_{7'}$), 146.73, 144.77 (C$_{6'}$), 140.92, 137.46 (C$_{4'}$), 115.99, 108.38 (C$_{5'}$), 62. 91, 62.31 (OMe), 61.26, 61.24 (CH$_2$), 13.80, 13.72 (Me); HPLC: k' = 1.06 and 1.40, t$_0$ = 1.81 (RP-18, MeCN/H$_2$O, 1:1); C$_8$H$_{11}$N$_3$O$_3$S (229.26), calculated: C 41.91, H 4.84, N 18.33; found: C 42.08, H 3.80, N 17.93.

5.2.24. *Ethyl (E,Z)-[2-(1,1-dioxobenzo[d]isothiazol-3-ylamino)thiazol-4-yl]-α-methoxyiminoacetate* (18a)

3-Chlorobenzo[*d*]isothiazole 1,1-dioxide (**13**) (3.0 g, 14.88 mmol) and ethyl *syn*-(2-aminothiazol-4-yl)-α-methoxyiminoacetate (3.42 g, 14.88 mmol) were added to 100 ml dioxane. The reaction mixture was refluxed for 2 h. Dioxane was removed in vacuo and the resulted material was added in 10 ml of water and filtered. The solid was separated and crystallized from Acetone/H$_2$O. Yield: 3.5 g (60%); M.p. 210-212 °C (Acetone/H$_2$O); R$_f$ = 0.71 (MeOH/CH$_2$Cl$_2$ 2.5:7.5) (spots of two isomers are overlying when checked by TLC and are not possible to distinguish); IR: $\tilde{v}$ = 3477 (NH), 2983, 2941 (CH), 1727 (CO), 1614 (C=N), 1369, 1160 (SO$_2$); ^{1}H NMR [D$_6$]DMSO: δ = 8.64-8.54, 8.18-8.08, 7.95-7.88 (3m, 4H, ar), 8.27, 7.83 (2s, 1H, 5'-H$_{thiazole}$), 4.37, 4.32 (2q, J = 7.14 Hz, 2H, CH$_2$), 4.07, 3.96 (2s, 3H, OMe), 1.30, 1.31 (2t, J = 7.14 Hz, 3H, Me); *E:Z* $\approx$ 7:3; ^{13}C NMR [D$_6$]DMSO: δ = 162.58, 161.77, 159.08, 157.42, 156.74, 156.52, 145.85, 143.83, 141.10, 141.05, 140.65, 136.78, 134.13, 134.05, 133.69, 133.60, 126.86, 126.70, 124.41, 124.36, 124.18, 121.77, 121.72, 118.23, 63.42, 62.77, 61.82, 61.70, 13.81, 13.80; HPLC: k' = 8.64 and 10.64, t$_0$ =

2.03 (Chiralcel OJ-R, MeCN/0.01M KH_2PO_4, 2:8, pH 2.88); HPLC: K' = 2.23, 2.41 (Chiralcel OJ-R, ACN/H_2O 1:1); $C_{15}H_{14}N_4O_5S_2$ (394.43), calculated: C 45.68, H 3.58, N 14.20; found: C 45.19, H 3.97, N 13.71.

5.2.25. *Ethyl (Z)-[2-(1,1-dioxobenzo[d]isothiazol-3-ylamino)thiazol-4-yl]-α-methoxyiminoacetate* (18b)

3-Chlorobenzo[*d*]isothiazole 1,1-dioxide (**13**) (0.50 g, 2.48 mmol), ethyl *syn*-(2-aminothiazol-4-yl)-α-methoxyiminoacetate (0.57 g, 2.48 mmol) and 0.35 ml of triethyl amine (2.54 mmol) were added to 20 ml of dichloromethane. The reaction mixture was stirred at room temperature for 6 h. The dichloromethane was removed in vacuo and the resulted solid was crystallized from acetone/methanol to produce the pale yellow crystals. Yield: 0.40 g (34%); M.p. 275-278 °C (dec.); R_f = 0.71 (MeOH/CH_2Cl_2 2.5:7.5); IR: $\tilde{\nu}$ = 3447 (NH), 2982, 2937 (CH), 1737 (CO), 1614 (C=N), 1356, 1173 (SO_2); ^{1}H NMR [D_6]DMSO: δ = 8.69-8.53, 8.19-8.08, 7.97-7.87 (3m, 4H, ar), 7.83 (s, 1H, 5'-$H_{thiazole}$), 4.37 (q, *J* = 7.14 Hz, 2H, CH_2), 3.96 (s, 3H, OMe), 1.31 (t, *J* = 7.14 Hz, 3H, Me); ^{13}C NMR [D_6]DMSO: δ = 161.77, 159.08, 156.52, 145.85, 141.05, 140.65, 134.13, 133.69, 126.70, 124.36, 121.77, 118.23, 62.77, 61.82, 13.80; HPLC: K' = 2.43 (Chiralcel OJ-R, ACN/ H_2O 1:1); $C_{15}H_{14}N_4O_5S_2$ (394.43), calculated: C 45.68, H 3.58, N 14.20; found: C 44.99, H 3.19, N 13.46.

5.2.26. *(E,Z)-[2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)thiazol-4-yl]-α-methoxyiminoacetic acid* (19)

Ethyl(*E,Z*)-[2-(1,1-dioxobenzo[*d*]isothiazol-3-ylamino)thiazol-4-yl]-α-methoxyimino-acetate (**18a**) (0.30 g, 0.79 mmol) was added to 20 ml of acetone and 1.98 ml of 1N aq. NaOH (1.98 mmol) was added drop wise with stirring at ice-bath temperature over 5 min. The reaction mixture was left at room temperature for 6 h. Acetone was removed under reduced pressure and the remained organic layer was cooled to 0 °C and acidified with 1N HCl to pH 1-2. The precipitate was separated, washed with cold water, crystallized and dried over NaOH pellets. Yield: 0.25 g (90%); M.p. 333-336 °C (dec.); R_f = 0.39 (MeOH/CH_2Cl_2 2.5:7.5) (spots of two isomers are overlying when checked by TLC and are not possible to distinguish); IR: $\tilde{\nu}$ = 3453 (NH, OH), 2947 (CH), 1708 (CO), 1600 (C=N), 1368, 1158 (SO_2); ^{1}H NMR [D_6]DMSO: δ = 8.65-8.55, 8.16-8.07, 7.95-7.85 (3m, 4H, ar), 8.25, 7.77 (2s, 1H, 5'-$H_{thiazole}$), 4.07, 3.95 (2s, 3H, OMe); ^{13}C NMR [D_6]DMSO: δ = 164.00, 163.28, 157.59, 156.87, 156.58, 144.78, 141.20, 141.15, 137.15, 134.09, 133.76, 133.68, 127.13, 124.45, 123.99, 121.77, 117.74, 63.23, 62.85. *E:Z* $\approx$ 8.5:1.5; HPLC: k' = -0.02 and 0.12, t_0 = 1.92 (Chiralcel

OJ-R, MeCN/0.01M KH_2PO_4, 2:8, pH 2.88); $C_{13}H_{10}N_4O_5S_2$ (366.38), calculated: C 42.62, H 2.75, N 15.29 found: C 41.86, H 2.87, N 14.60.

5.2.27. *Ethyl [2-(1,1-dioxobenzo[d]isothiazol-3-ylamino)thiazol-4-yl]acetate* (21)

From 3-chlorobenzo[*d*]isothiazole 1,1-dioxide (**13**) (3.0 g, 14.88 mmol) and ethyl (2-aminothiazol-4-yl)acetate (2.77 g, 14.88 mmol) in 70 ml dioxane at refluxed for 2 h as described in 5.2.18. Yield: 2.6 g (50%); M.p. 214°C (H_2O); R_f = 0.26 (MeOH/CH_2Cl_2 2.5:7.5); IR: $\tilde{v}$ = 3330 (NH), 3100, 2970, 2932 (CH), 1727 (CO), 1613 (C=N), 1350, 1157 (SO_2); ^{1}H NMR [D_6]DMSO: δ = 8.27 (bs, 1H, NH), 8.10-8.00, 7.91-7.80 (2m, 4H, ar), 7.30 (s, 1H, 5'-$H_{thiazole}$), 4.13 (q, *J* = 7.14 Hz, 2H, CH_2), 3.83 (s, 2H, CH_2), 1.25 (t, *J* = 7.14 Hz, Me); HPLC: k' = 3.90, t_0 = 1.85 (RP-18, MeCN/0.02M KH_2PO_4, 3:7, pH 2.95); $C_{14}H_{13}N_3O_4S_2$ (351.41), calculated: C 47.85, H 3.75, N 11.96; found: C 47.66, H 2.98, N 11.59.

5.2.28. *[2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)thiazol-4-yl]acetic acid* (22)

From ethyl [2-(1,1-dioxobenzo[*d*]isothiazol-3-ylamino)thiazol-4-yl]acetate **(21)** (0.5 g, 1.42 mmol) in 20 ml acetone and 2.84 ml 1N NaOH (2.84 mmol), according to 5.2.19. Yield: 0.40 g (87%); M.p. 304-306 °C (dec., AcOEt/MeOH/PE); R_f = 0.23 (MeOH/CH_2Cl_2 2.5:7.5); IR: $\tilde{v}$ = 3440 (NH, OH), 3129, 2920 (CH), 1715 (CO), 1613 (C=N), 1387, 1162 (SO_2); ^{1}H NMR [D_6]DMSO: δ = 8.26-8.15, 8.00-7.98, 7.88-7.93 (3m, 4H, ar), 7.25 (s, 1H, 5'-$H_{thiazole}$), 3.79 (s, 2H, CH_2); HPLC: k' = 1.42, t_0 = 1.85 (RP-18, MeCN/0.02M KH_2PO_4, 3:7, pH 2.85); $C_{12}H_9N_3O_4S_2$ (323.35), calculated: C 44.57, H 2.81, N 13.00; found: C 44.30, H 2.63, N 12.76.

5.2.29. *3-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)-thiophene-2-carboxylic acid methyl ester* (24)

3-Chlorobenzo[*d*]isothiazole 1,1-dioxide (**13**) (3.00 g, 14.88 mmol) and 2.33 g of 3-amino-thiophene-2-carboxylic acid methyl ester (14.88 mmol) were refluxed in 70 ml of dioxane. After cooling to room temperature, the solid was filtered off and washed with little acetone. The solid was dried to give the analytically pure compound. Yield: 3.96 g (83%); M.p. 284-287 °C, R_f = 0.87 (AcOEt/PE 8:2); IR: $\tilde{v}$ = 3446 (NH), 3090, 2990, 2944 (CH), 1683 (ester with intramolecular hydrogen bonds), 1616 (C=N), 1320, 1161 (SO_2); ^{1}H NMR [D_6]DMSO: δ = 11.07 (bs, 1H, NH), 8.19-8.10, 8.04-7.90 (2m, 4H, ar), 8.09 (d, *J* = 5.40 Hz, 1H, 5'-$H_{thiophene}$), 7.85 (d, *J* = 5.40 Hz, 1H, 4'-$H_{thiophene}$), 3.89 (s, 3H, OMe); ^{13}C NMR [D_6]DMSO: δ = 162.84, 156.30, 140.94, 140.83, 134.18, 133.72, 133.08, 127.12, 124.15, 122.63, 121.81, 116.71, 52.46;

HPLC: k' = 4.57, t_0 = 1.85 (RP-18, ACN/ H_2O 1:1); $C_{13}H_{10}N_2O_4S_2$ (322.36), calculated: C 48.44, H 3.13, N 8.69; found: C 48.38, H 2.97, N 8.63.

5.2.30. *3-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)-thiophene-2-carboxylic acid (25)*

0.50 g of 3-(1,1-Dioxobenzo[*d*]isothiazol-3-ylamino)-thiophene-2-carboxylic acid methyl ester (**24**) (1.55 mmol) was suspended in a mixture of 20 ml of acetone and 20 ml of methanol. The reaction mixture was stirred at room temperature for 10 min. 3.1 ml of 1N aq. NaOH (3.10 mmol) was added drop wise and the mixture was stirred for 5 min. After standing for 15 h at rt, the solvent was removed in vacuo and the resulted aqueous layer was filtered, concentrated and acidified with 1N HCl to pH 1-2. The aqueous layer was then extracted with ethyl acetate (2 x 100 ml) and the organic layer was dried with sodium sulfate. The organic layer was concentrated in vacuo and left overnight to produce the crystalline compound. Yield: 0.17 g (36%); M.p. 255-257 °C; R_f = 0.42 (MeOH/CH_2Cl_2 2.5:7.5); IR: $\tilde{v}$ = 3451 (NH), 3097 (CH), 1733 (CO), 1315, 1165 (SO_2); ^{1}H NMR [D_6]DMSO: δ = 11.31 (bs, NH /or OH), 8.17-8.10, 8.08-7.88 (2m, 4H, ar), 8.01 (d, *J* = 5.40 Hz, 1H, 5'-$H_{thiophene}$), 7.92 (d, *J* = 5.40 Hz, 1H, 4'-$H_{thiophene}$); ^{13}C NMR [D_6]DMSO: δ = 164.59, 155.73, 140.98, 140.82, 134.16, 133.73, 132.45, 127.20, 123.44, 122.30, 121.84, 117.02 ; HPLC: k' = 2.26, t_0 = 1.85 (RP-18, MeCN/0.02M KH_2PO_4, 3:7, pH 2.85); $C_{12}H_8N_2O_4S_2$ (308.34), calculated: C 46.75, H 2.62, N 9.09; found: C 46.40, H 2.45, N 8.74.

5.2.31. *2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)-thiophene-3-carboxylic acid methyl ester (27)*

3-Chlorobenzo[*d*]isothiazole 1,1-dioxide (**13**) (2.56 g, 12.70 mmol) and 2.00 g of 2-amino-thiophene-3-carboxylic acid methyl ester (12.70 mmol) were refluxed in 70 ml of dioxane for 30 min. Dioxane removed in vacuo and the resulted solid added to 10 ml of water. The solid then filtered off and dried, producing yellow-green fluorescent, analytical pure compound. Yield: 3.60 g (87%); M.p. 283-285 °C (dec.); R_f: 0.87 (AcOEt/PE 8:2); IR: $\tilde{v}$ = 3429 (NH), 3109, 3086, 2953 (CH), 1685 (ester with intramolecular hydrogen bonds), 1608 (C=N), 1310, 1162 (SO_2); ^{1}H NMR (CDCl$_3$): δ = 12.08 (bs, 1H, NH), 8.03-7.93, 7.85-7.74 (2m, 4H, ar), 7.30 (d, *J* = 5.70 Hz, 1H, 5'-$H_{thiophene}$), 7.00 (d, *J* = 5.70 Hz, 1H, 4'-$H_{thiophene}$), 3.95 (s, 3H, OMe); HPLC: k' = 2.64, t_0 = 1.81 (RP-18, ACN/H_2O 1:1); $C_{13}H_{10}N_2O_4S_2$ (322.36), calculated: C 48.44, H 3.13, N 8.69; found: C 47.93, H 2.18, N 7.90.

5.2.32. *2-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)-thiophene-3-carboxylic acid* (28)

2-(1,1-Dioxobenzo[*d*]isothiazol-3-ylamino)-thiophene-3-carboxylic acid methyl ester (**27**) (1.00 g, 3.10 mmol) was added to 20 ml of acetone followed by the addition of 6.20 ml of 1N NaOH (6.20 mmol). The reaction mixture was stirred for 5 min and left aside for 6 days. The acetone was removed in vacuo and the resulted solid was washed with 10 ml of water and dried. Yield: 0.28 g (29%); M.p. 265-267 °C (dec.); R_f: 0.37 (MeOH/CH_2Cl_2 2.5:7.5); IR: $\tilde{v}$ = 3434 (NH), 3090 (CH), 1664 (CO), 1366, 1163 (SO_2); ^{1}H NMR [D_6]DMSO: δ = 12.28 (bs, NH/OH), 8.18-8.09, 7.97-7.88 (2m, 4H, ar), 7.34 (d, J = 5.70 Hz, 1H, 5'-$H_{thiophene}$), 7.31 (d, J = 5.70 Hz, 1H, 4'-$H_{thiophene}$); ^{13}C NMR [D_6]DMSO: δ = 166.09, 154.89, 146.94, 141.29, 134.10, 133.73, 126.78, 125.18, 122.40, 121.93, 120.14, 117.89 ; HPLC: k' = 10.39, t_0 = 1.77 (RP-18, MeCN/0.01M KH_2PO_4, 2:8, pH: 2.88); $C_{12}H_8N_2O_4S_2$ (308.34), calculated: C 46.75, H 2.62, N 9.09; found: C 46.09, H 2.84, N 8.64.

5.2.33. *3-(1,1-Dioxobenzo[d]isothiazol-3-ylamino)-5-nitro-thiophene-2-carboxylic acid methyl ester* (29)

To a cooled (-30 °C) and stirred solution of 3-(1,1-Dioxobenzo[*d*]isothiazol-3-ylamino)-thiophene-2-carboxylic acid methyl ester (**24**) (2.00 g, 6.20 mmol) in 95% H_2SO_4 (10 ml), 2 ml of concentrated HNO_3 were added. The mixture was stirred at -30 °C for 45 minutes and allowed to warm to room temperature. The viscous liquid was poured on ice (10 g) and the resulting aq. phase extracted with dichloromethane (3 x 100 ml). The organic phase was separated, dried with sodium sulphate and evaporated in vacuo. The solid was crystallized from dichloromethane:methanol (9:1). Yield: 0.40 g (18%); M.p. 285-287 °C (MeOH/CH_2Cl_2); R_f = 0.80 (AcOEt/PE 8:2); IR: $\tilde{v}$ = 2958 (CH), 1707 (C=O), 1610 (C=N), 1344, 1174 (SO_2); ^{1}H NMR [D_6]DMSO: δ = 11.20 (bs, 1H, NH), 8.55 (s, 1H, H4'$_{thiophene}$), 8.28-8.19, 8.18-8.10, 8.01-7.92 (3m, 4H, ar), 3.93 (s, 3H, OMe); ^{13}C NMR [D_6]DMSO: δ = 160.98, 157.37, 152.00, 141.04, 137.58, 134.42, 133.79, 126.72, 125.50, 123.50, 123.11, 121.89, 53.37; HPLC: k' = 4.60, t_0 = 1.77 (RP-18, ACN/ H_2O 1:1); $C_{13}H_9N_3O_6S_2$ (367.36), calculated: C 42.50, H 2.47, N 11.44; found: C 42.50, H 2.11, N 11.42.

5.2.34. *N-(Cyanomethyl) (2S,3S)-2-(1,1-dioxobenzo[d]isothiazol-3-ylamino)-3-methyl pentanamide* (30)

(2*S*,3*S*)-2-(1,1-Dioxobenzo[*d*]isothiazol-3-ylamino)-3-methylpentanoic acid (**14c**) (2.00 g, 6.75 mmol) was dissolved in 40 ml of THF and cooled to -25 °C. The

reaction mixture was stirred for 10 min and *N*-methylmorpholine (0.74 ml, 6.75 mmol) was added followed by addition of 0.88 ml of isobutyl chloroformate (6.75 mmol) under the atmosphere of nitrogen. The reaction mixture was stirred for half an h at - 25 °C. Then it was cooled to -45 °C and a solution of amino acetonitrile [initially prepared by mixing amino acetonitrile HCl (0.625 g, 6.75 mmol) and 1.68 ml of 2N NaOH (6.75 mmol)] was added. The reaction mixture was stirred for 1 h at - 45°C and then left overnight at rt. Next day, the reaction mixture was filtered and the filtrate was diluted with ethyl acetate. The organic layer was washed with 1 N HCl followed by saturated $NaHCO_3$, brine and at the last with water. Organic layer was dried with sodium sulphate and removed in vacuum to obtain a foamy solid. Column chromatography in EtOH:Pet ether (10%, with increasing polarity, till 80%) gave a white foam. Yield: 0.76 g (34%); M.p. 220 °C; R_f = 0.58 (MeOH /CH_2Cl_2 1:9); $[\alpha]_D^{20}$ = -70.50 (c = 2, MeOH); IR: $\tilde{v}$ = 3331 (NH), 3060, 2966 (CH), 1669 (CO), 1617 (C=N), 1354, 1163 (SO_2); ^{1}H NMR ($CDCl_3$): δ = 8.45 (d, *J* = 8.00 Hz, 1H, NH), 8.04 (t, *J* = 5.96 Hz, 1H, NH), 7.76-7.51 (m, 4H, ar), 4.56 (t, *J* = 8.00 Hz, 1H, α-H, Ile), 4.19-4.07 (m, 2H, CH_2), 2.27-2.08 (m, 1H, β-H, Ile), 1.41-1.17 (m, 2H, γ-H, Ile), 1.05-0.88 (m, 6H, [3H, γ-H Ile + 3H, δ-H Ile]); ^{13}C NMR ($CDCl_3$): δ = 172.89, 160.48, 141.50, 133.92, 133.25, 127.26, 121.77, 121.52, 115.60, 62.02, 36.68, 27.88, 25.69, 15.12, 10.97; HPLC: k' = 1.28, t_0 = 2.72 (RP-18, MeCN/H_2O, 1:1); HPLC: k' = 0.49, t_0 = 1.94 (Chiralcel OJ-R, MeCN/H_2O, 1:1); $C_{15}H_{18}N_4O_3S$ (334.40), calculated: C 53.88, H 5.43, N 16.75; found: C 54.02, H 5.23, N 15.70.

5.2.35. (1*S*,2*S*)-[1-(Cyanomethyl-carbamoyl)-2-methyl-butyl]-carbamic acid *tert*-butyl ester (32)

From BOC-Ile-OH (**31**) (17.00 g, 73.50 mmol), 9.61 ml (73.50 mmol) of IBCF, 8.08 ml (73.50 mmol) of NNM, 6.80 g (73.50 mmol) of aminoacetonitrile HCl, 200 ml of THF and 18.38 ml of 4N NaOH (73.50 mmol) as described in 5.2.34. Yield: 7.60 g (38%); M.p. 123-126 °C (125-133.5 °C [89]); $[\alpha]_D^{20}$ = -23.50 (c = 2, MeOH); IR: $\tilde{v}$ = 3330, 3300 (NH), 2965, 2937, 2880 (CH), 1666 (C=O); ^{1}H NMR ($CDCl_3$): δ = 7.55 (bs, 1H, NH), 5.32 (d, *J* = 8.70 Hz, 1H, NH), 4.16 (m, 2H, CH_2), 4.00 (t, *J* = 8.70 Hz, 1H, α-H, Ile), 2.90-1.76 (m, 1H, β-H, Ile), 1.63-1.37 (m, 1H, γ-H, Ile), 1.45 (s, 9H, *tert*-butyl), 1.26-1.04 (m, 1H, γ-H, Ile), 1.00-0.85 (m, 6H, [3H, γ-H, Ile + 3H, δ-H, Ile]); ^{13}C NMR ($CDCl_3$): δ = 172.56, 156.32, 115.86, 80.63, 59.08, 37.00, 28.34, 27.27, 24.87, 15.52, 11.12; $C_{13}H_{23}N_3O_3$ (269.35), calculated: C 57.97, H 8.61, N 15.60; found: C 57.71, H 7.54, N 15.36.

5.2.36. *(2S,3S)-2-Amino-N-(cyanomethyl)-3-methylpentamide hydrochloride* (33)

(1*S*,2*S*)[1-(Cyanomethyl-carbamoyl)-2-methyl-butyl]-carbamic acid *tert*-butyl ester **(32)** (6.00 g, 22.8 mmol) was added to 50 ml of ethyl acetate (saturated with HCl gas) and the reaction mixture stirred at room temperature. During the reaction a solid separated. Ethyl acetate was removed in vacuo and the resulted gummy material was triturated with diethyl ether to produce a white solid (highly hygroscopic). Yield: 3.90 g (85%); $[\alpha]_D^{20}$ = +25.00 (c = 2, MeOH); IR: $\tilde{v}$ = 2969 (CH), 1682 (C=O); ^{1}H NMR (CDCl$_3$): δ = 4.15 (d, *J* = 2.68 Hz, 2H, CH$_2$), 3.78-3.87 (m, 1H, α-H, Ile), 2.08-1.78 (m, 1H, β-H, Ile), 1.50-1.30 (m, 1H, γ-H, Ile), 1.12-1.01 (m, 1H, γ-H, Ile), 0.93-0.76 (m, 6H, [3H, γ-H Ile + 3H, δ-H Ile]); ^{13}C NMR (CDCl$_3$): δ = 169.86, 116.16, 57.69, 36.25, 27.64, 24.13, 14.02, 13.54; C$_8$H$_{16}$ClN$_3$O (205.73), calculated: C 47.71, H 7.78, N 20.43; found: C 46.61, H 7.33, N 18.28.

5.2.37. *2-[(2S)-2-(1,1-dioxobenzo[d]isothiazol-3-ylamino)-4-methylsulfanyl-butyryl amino]-(2S,3S)-3-methyl-pentanoic acid cyanomethyl-amide* (34a)

From (*S*)-2-(1,1-Dioxobenzo[*d*]isothiazol-3-ylamino)-4-methylsulfanyl- butyric acid **(14a)** (0.63 g, 2.00 mmol), 30 ml THF, *N*-methylmorpholine (0.22 ml, 2.00 mmol), isobutyl chloroformate (0.26 ml, 2.00 mmol), (2*S*,3*S*)-2-Amino-N-(cyanomethyl)-3-methylpentamide hydrochloride (**33**) (0.41 g, 2.00 mmol) in 10 ml THF, triethyl amine (0.31 ml, 2.20 mmol), as described in 5.2.34. Yield: 0.12 g (13%); M.p. polymorphic substance, 70, 176 °C; R$_f$ = 0.51 (MeOH/CH$_2$Cl$_2$ 1:9); $[\alpha]_D^{20}$ = - 57.50 (c = 2, MeOH); IR: $\tilde{v}$ = 3318 (NH), 3060, 2965, 2931 (CH), 1655 (CO), 1616 (C=N), 1164 (SO$_2$); ^{1}H NMR [D$_6$]DMSO: δ = 9.49 (d, *J* = 7.60 Hz, 1H, NH), 8.78 (t, *J* = 5.50 Hz, 1H, NH), 8.40 (d, *J* = 8.40 Hz, 1H, NH), 8.39-8.29, 8.05-7.94, 7.91-7.79 (3m, 4H, ar), 4.70 (q, *J* = 7.60 Hz, 1H, α-H, Met), 4.27-4.04 (m, 1H, α-H, Ile), 4.15 (d, *J* = 5.50 Hz, 2H, CH$_2$), 2.75-2.41 (m, 2H, γ-H, Met, under [D$_6$]DMSO), 2.13-1.99 (m, 5H, [3H, SMe, Met + 2H, β-H, Met]), 1.87-1.66 (m, 1H, β-H, Ile), 1.56-1.05 (m, 2H, γ-H, Ile), 0.91-0.69 (m, 6H, [3H, γ-H Ile + 3H, δ-H, Ile]); ^{13}C NMR [D$_6$]DMSO: δ = 171.56, 169.87, 159.39, 142.08, 133.48, 133.03, 127.35, 123.41, 121.12, 117.29, 56.83, 55.77, 38.19, 36.40, 31.26, 29.69, 26.79, 24.31, 15.12, 14.63, 10.73; HPLC: k' = 1.21, t$_0$ = 1.77 (RP-18, MeCN/H$_2$O, 1:1), λ = 224 nm; HPLC: k' = 3.54, t$_0$ = 2.17 (Chiralcel OJ-R, MeCN/H$_2$O, 2.5:7.5); C$_{20}$H$_{27}$N$_5$O$_4$S$_2$ (465.60), calculated: C 51.59, H 5.85, N 15.04; found: C 51.83, H 6.53, N 14.28.

5.2.38. *(2S)-2-(1,1-dioxobenzo[d]isothiazol-3-ylamino)-4-methyl-pentanoic acid [(1S,2S)-1-(cyanomethyl-carbamoyl)-2-methyl-butyl]-amide* (34b)

(*S*)-2-(1,1-Dioxobenzo[*d*]isothiazol-3-ylamino)-4-methylpentanoic acid (**14b**) (0.63 g, 2.13 mmol), 30 ml THF, *N*-methylmorpholine (0.23 ml, 2.13 mmol), isobutyl chloroformate (0.28 ml, 2.13 mmol), (2*S*,3*S*)-2-amino-N-(cyanomethyl)-3-methylpentamide hydrochloride (**33**) (0.44 g, 2.13 mmol) in 10 ml THF, triethyl amine (0.33 ml, 2.34 mmol), as described in 5.2.34. Yield: 0.32 g (34%); M.p. polymorphic substance, 165, 225 °C; R_f = 0.48 (MeOH/CH$_2$Cl$_2$ 1:9); $[\alpha]_D^{20}$ = -66.00 (c = 2, MeOH); IR: $\tilde{\nu}$ = 3306 (NH), 3065, 2963, 2935 (CH), 1654 (CO), 1616 (C=N), 1387, 1162 (SO$_2$); ^{1}H NMR [D$_6$]DMSO: δ = δ 9.47 (d, *J* = 7.90 Hz, 1H, NH), 8.76 (t, *J* = 5.50 Hz, 1H, NH), 8.44 (d, *J* = 8.76 Hz,1H, NH), 8.41-8.30, 8.04-7.93, 7.89-7.79 (3m, 4H, ar), 4.81-4.62 (m, 1H, α-H, Leu), 4.30-4.10 (m, 1H, α-H, Ile) 4.15 (d, *J* = 5.50 Hz, 2H, CH$_2$), 1.89-1.42 (m, 6H, [2H, β-H, Leu + 1H, γ-H, Leu + 1H, β-H, Ile +2H, γ-H, Ile]), 0.98-0.75 (m, 12H, [6H, δ-H, Leu + 3H, γ-H, Ile + 3H, δ-H, Ile]); ^{13}C NMR [D$_6$]DMSO: δ = 172.15, 171.33, 159.83, 142.75, 134.04, 133.62, 127.99, 123.96, 121.71, 117.88, 65.40, 57.41, 55.48, 36.97, 27.37, 24.93, 23.56, 21.76, 15.68, 14.57, 11.30; HPLC: k' = 1.90, t_0 = 1.77 (RP-18, MeCN/H$_2$O, 1:1), λ = 222 nm; HPLC: k' = 4.41, t_0 = 2.17 (Chiralcel OJ-R, MeCN/H$_2$O, 2.5:7.5); C$_{21}$H$_{29}$N$_5$O$_4$S (447.56), calculated: C 56.36, H 6.53, N 15.65; found: C 55.09, H 7.18, N 15.02.

5.2.39. *(2S,3S)-2-(1,1-dioxobenzo[d]isothiazol-3-ylamino)-3-methyl-pentanoic acid [(1S,2S)-1-(cyanomethyl-carbamoyl)-2-methyl-butyl]-amide* (34c)

(2*S*,3*S*)-2-(1,1-Dioxobenzo[*d*]isothiazol-3-ylamino)-3-methylpentanoic acid (**14c**) (0.63 g, 2.13 mmol), 30 ml THF, *N*-methylmorpholine (0.23 ml, 2.13 mmol), isobutyl chloroformate (0.28 ml, 2.13 mmol), (2*S*,3*S*)-2-amino-*N*-(cyanomethyl)-3-methylpentamide hydrochloride (**33**) (0.44 g, 2.13 mmol) in 10 ml THF, triethyl amine (0.33 ml, 2.34 mmol), as described in 5.2.34. Yield: 0.48 g (51%); M.p. polymorphic substance, 193, 284 °C; R_f = 0.55 (MeOH/CH$_2$Cl$_2$ 1:9); $[\alpha]_D^{20}$ = -106.50 (c = 2, MeOH); IR: $\tilde{\nu}$ = 3305 (NH), 3065, 2966, 2934 (CH), 1681 (CO), 1616 (C=N), 1367, 1164 (SO$_2$); ^{1}H NMR [D$_6$]DMSO: δ = 9.41 (d, *J* = 8.11 Hz, 1H, NH), 8.74 (t, *J* = 5.50 Hz, 1H, NH), 8.52-8.35 (m, 2H, [1H, ar + 1H, NH]), 8.06-7.92, 7.88-7.78 (2m, 3H, ar), 4.47 (t, *J* = 8.85 Hz, 1H, α-H, Ile$_1$), 4.14 (d, *J* = 5.50 Hz, 2H, CH$_2$), 4.25-4.06 (m, 1H, α-H, Ile$_2$), 2.16-1.91 (m, 1H β-H, Ile$_1$), 1.85-1.66 (m, 1H, β-H, Ile$_2$), 1.62-1.41 (m, 2H, γ-H, Ile$_1$), 1.27-1.00 (m, 2H, γ-H, Ile$_2$), 0.95-0.72 (m, 12H, [3H, γ-H, Ile$_1$ + 3H, δ-H, Ile$_1$ + 3H, γ-H, Ile$_2$ + 3H, δ-H, Ile$_2$]); ^{13}C NMR [D$_6$]DMSO: δ = 172.00, 170.37, 159.86,

142.81, 134.02, 133.58, 127.93, 124.11, 121.69, 117.83, 61.38, 57.36, 36.77, 36.03, 27.33, 25.49, 24.94, 15.65, 15.46, 11.22, 10.85; HPLC: k' = 1.49, t_0 = 1.77 (RP-18, MeCN/H_2O, 1:1), λ = 224 nm; HPLC: k' = 3.89, t_0 = 2.17 (Chiralcel OJ-R, MeCN/H_2O, 2.5:7.5); $C_{21}H_{29}N_5O_4S$ (447.56), $C_{21}H_{29}N_5O_4S$ (447.56), calculated: C 56.36, H 6.53, N 15.65; found: C 55.77, H 6.53, N 15.23.

5.2.40. *2-[(2S)-2-(1,1-dioxobenzo[d]isothiazol-3-ylamino)-3-methyl-butyryl-amino]-(2S,3S)-3-methyl-pentanoic acid cyanomethyl-amide* (34d)

(*S*)-2-(1,1-Dioxobenzo[*d*]isothiazol-3-ylamino)-3-methylbutyric acid (**14g**) (0.63 g, 2.23 mmol), 30 ml THF, *N*-methylmorpholine (0.25 ml, 2.23 mmol), isobutyl chloroformate (0.30 ml, 2.23 mmol), (2S,3S)-2-amino-*N*-(cyanomethyl)-3-methylpentamide hydrochloride (**33**) (0.46 g, 2.23 mmol) in 10 ml THF, triethyl amine (0.34 ml, 2.45 mmol), as described in 5.2.34. Yield: 0.30 g (31%); M.p. polymorphic substance, 189, 200 °C; R_f = 0.48 (MeOH/CH_2Cl_2 1:9); $[\alpha]_D^{20}$ = -90.00 (c = 2, MeOH); IR: $\tilde{\nu}$ = 3315 (NH), 3092, 2967, 2935 (CH), 1654 (CO), 1615 (C=N), 1374, 1163 (SO_2); ^{1}H NMR [D_6]DMSO: δ = 9.37 (d, *J* = 8.52 Hz, NH), 8.75 (t, *J* = 5.70 Hz, NH), 8.49-8.44, 7.98-7.94, 7.83-7.80 (3m, 4H ar), 8.38 (d, *J* = 8.70 Hz, 1H, NH), 4.41 (t, *J* = 8.52 Hz, 1H, α-H, Val), 4.13 (d, *J* = 5.70 Hz 2H, CH_2), 4.19-4.07 (m, 1H, α-H, Ile), 2.25-2.09 (m, 1H, β-H, Val), 1.80-1.64 (m, 1H, β-H, Ile), 1.21-1.10 (m, 2H, γ-H, Ile), 0.96-0.74 (m, 12 H, [6H, γ-H, Val + 3H, γ-H, Ile + 3H, δ-H, Ile]); ^{13}C NMR [D_6]DMSO: δ = 171.44, 169.63, 159.40, 142.20, 133.43, 133.00, 127.34, 121.09, 117.24, 62.28, 56.75, 36.19, 29.90, 26.73, 24.35, 20.66, 19.15, 18.83, 15.05, 13.98; HPLC: k' = 1.24, t_0 = 1.77 (RP-18, MeCN/H_2O, 1:1), λ = 222 nm; HPLC: k' = 2.43, t_0 = 2.17 (Chiralcel OJ-R, MeCN/H_2O, 2.5:7.5); $C_{20}H_{27}N_5O_4S$ (433.53), calculated: C 55.41, H 6.28, N 16.15; found: C 54.44, H 6.19, N 15.28.

5.2.41. *2-[(2S)-2-(1,1-dioxobenzo[d]isothiazol-3-ylamino)-propionylamino]-(2S,3S)-3-methyl-pentanoic acid cyanomethyl-amide* (34e)

(*S*)-2-(1,1-Dioxobenzo[*d*]isothiazol-3-ylamino)propionic acid (**14f**) (0.63 g, 2.48 mmol), 30 ml THF, *N*-methylmorpholine (0.27 ml, 2.48 mmol), isobutyl chloroformate (0.32 ml, 2.48 mmol), (2*S*,3*S*)-2-Amino-*N*-(cyanomethyl)-3-methylpentamide hydrochloride (**33**) (0.50 g, 2.48 mmol) in 10 ml THF, triethyl amine (0.38 ml, 2.73 mmol), as described in 5.2.34. Yield: 0.17 g (17%); M.p. polymorphic substance, 163, 263 °C; R_f = 0.44 (MeOH/CH_2Cl_2 1:9); $[\alpha]_D^{20}$ = +6.66 (c = 2, DMSO); IR: $\tilde{\nu}$ = 3301 (NH), 3111, 3063, 2964, 2936 (CH), 1651 (CO), 1618 (C=N), 1354, 1150 (SO_2); ^{1}H

NMR [D$_6$]DMSO: δ = 9.50 (d, J = 7.52 Hz, 1H, NH), 8.76 (t, J = 5.42 Hz, 1H, NH), 8.44-8.28 (m, 2H, [1H, ar + 1H, NH]), 8.05-7.93, 7.90-7.78 (2m, 3H, ar), 4.70 (qn, J = 7.27 Hz, 1H, α-H, Ala), 4.27-4.06 (m, 3H, [1H, α-H, Ile + 2H, CH$_2$]), 1.86-1.61 (m, 1H, β-H, Ile), 1.56-1.32 (m, 2H, γ-H, Ile), 1.40 (d, J = 7.14, 3H, β-H, Ala), 1.27-1.01 (m, 2H, γ-H, Ile) 0.89-0.74 (m, 6H, [3H, γ-H, Ile + 3H, δ-H, Ile]); ^{13}C NMR [D$_6$]DMSO: δ = 172.19, 171.52, 159.45, 142.72, 134.02, 133.59, 128.01, 123.95, 121.70, 117.91, 57.31, 52.35, 37.14, 27.39, 24.89, 18.15, 15.73, 11.36; HPLC: k' = 0.71, t$_0$ = 1.77 (RP-18, MeCN/H$_2$O, 1:1), λ = 225 nm; HPLC: k' = 1.31, t$_0$ = 2.17 (Chiralcel OJ-R, MeCN/H$_2$O, 2.5:7.5); C$_{18}$H$_{23}$N$_5$O$_4$S (405.48), calculated: C 53.32, H 5.72, N 17.27; found: C 53.41, H 5.57, N 16.79.

5.2.42. *(2S)-1-(1,1-dioxobenzo[d]isothiazol-3-yl)-pyrrolidine-2-carboxylic acid [(1S,2S)-1-(cyanomethyl-carbamoyl)-2-methyl-butyl]-amide* (34f)

(*S*)-2-(1,1-Dioxobenzo[*d*]isothiazol-3-ylamino)pyrrolidine-2-carboxylic acid (**14j**) (0.63 g, 2.25 mmol), 30 ml THF, *N*-methylmorpholine (0.25 ml, 2.25 mmol), isobutyl chloroformate (0.30 ml, 2.25 mmol), (2*S*,3*S*)-2-Amino-*N*-(cyanomethyl)-3-methylpentamide hydrochloride (**33**) (0.46 g, 2.25 mmol) in 10 ml THF, triethyl amine (0.34 ml, 2.47 mmol), as described in 5.2.34. Yield: 0.05 g (5%); M.p. polymorphic substance, 220, 287 °C; R$_f$ = 0.58 (MeOH/CH$_2$Cl$_2$ 1:9); $[\alpha]_D^{20}$ = -97.00 (c = 2, MeOH); IR: $\tilde{\nu}$ = 3322 (NH), 3071, 2965, 2934 (CH), 1681 (CO), 1602 (C=N), 1344, 1162 (SO$_2$); ^{1}H NMR [D$_6$]DMSO: δ = 8.80 (t, J = 5.70 Hz, 0.35H, NH), 8.70 (d, J = 8.72 Hz, part of NH), 8.69 (t, J = 5.63 Hz, part of NH), 8.35 (d, J = 8.51 Hz, part of NH), 8.26-8.13, 8.09-7.96, 7.92-7.76, 7.74-7.62 (4m, 4H, ar), 5.43-5.27 (m, 1H, α-H, Pro), 4.96-4.74 (m, 1H, α-H, Ile), 4.34-4.08 (m, 4H, [2H, CH$_2$ + 2H, δ-H, Pro]), 2.36-2.04 (m, 3H, [2H, β-H, Pro + 1H, γ-H, Pro]), 2.01-1.71 (m, 2H, [1H, γ-H, Pro + 1H, β-H, Ile]), 1.29-1.04 (m, 2H, γ-H, Ile), 0.99-0.73 (m, 6H, [3H, γ-H, Ile + 3H, δ-H, Ile]); ^{13}C NMR [D$_6$]DMSO: δ = 171.64, 171.40, 170.30, 170.03, 158.29, 157.86, 143.62, 143.47, 133.40, 133.20, 133.03, 132.55, 127.45, 127.20, 126.46, 124.84, 121.92, 121.83, 117.31, 117.23, 67.65, 64.41, 63.71, 61.30, 59.65, 56.66, 52.55, 49.98, 36.59, 36.20, 32.65, 30.58, 29.03, 26.83, 24.63, 24.30, 15.22, 13.99, 10.77, 10.57; HPLC: k' = 0.70, t$_0$ = 1.77 (RP-18, MeCN/H$_2$O, 1:1), λ = 224 nm; HPLC: k' = 1.44, t$_0$ = 2.17 (Chiralcel OJ-R, MeCN/H$_2$O, 2.5:7.5); C$_{20}$H$_{25}$N$_5$O$_4$S (431.52), calculated: C 55.67, H 5.84, N 16.23; found: C 55.46, H 6.40, N 13.94.

5.2.43. *(2S)-2{[1-(1,1-Dioxo-benzo[d]-isothiazol-3-yl-)-pyrrolidine-2-carbonyl]-amino} -(2S,3S)-3-methyl pentanoic acid methyl ester* (**35**)

(*S*)-2-(1,1-Dioxobenzo[*d*]isothiazol-3-ylamino)pyrrolidine-2-carboxylic acid (**14j**) (0.480 g, 1.71 mmol), 17 ml THF, *N*-methylmorpholine (0.189 ml, 1.71 mmol), isobutyl chloroformate (0.22 ml, 1.71 mmol), isoleucine methyl ester HCl (0.310 g, 1.71 mmol) in 10 ml THF, triethyl amine (0.24 ml, 1.71 mmol), as described in 5.2.34. Yield: 0.35 g (51%); M.p. 174 °C (AcOEt/MeOH/PE); R_f = 0.42 (AcOEt/PE 8:2); $[\alpha]_D^{20}$ = -67.00 (c = 2, MeOH); IR: $\tilde{\nu}$ = 3371 (NH), 2959, 2878 (CH), 1734, 1690 (CO), 1604 (C=N), 1374, 1163 (SO$_2$); ^{1}H NMR (CDCl$_3$): δ = 7.96-7.84, 7.78-7.57 (3m, 4H, ar), 7.07 (d, *J* = 8.50 Hz, 1H, NH), 4.92 (dd, *J* = 7.65, 2.50 Hz, 1H, α-H, Pro), 4.53 (dd, *J* = 8.50, 4.86 Hz, 1H, α-H, Ile), 4.24 (dt, *J* = 9.22, 3.80 Hz, 1H, δ-H, Pro), 4.14-3.96 (m, 1H, δ'-H, Pro), 3.75 (s, 3H, OMe), 2.58-2.42 (m, 1H, γ-H, Pro), 2.41-2.27 (m, 1H, β-H, Pro), 2.25-2.15 (m, 1H, γ'-H, Pro), 2.13-2.04 (m, 1H, β'-H, Pro), 2.02-1.80 (m, 1H, β-H, Ile), 1.55-1.36, 1.35-1.12 (2m, 2H, γ-H, Ile), {0.94 (d, *J* = 6.80 Hz) + 0.90 (t, *J* = 7.20 Hz), 6H, [3H, γ-H Ile + 3H, δ-H, Ile]} ; ^{13}C NMR (CDCl$_3$): δ = 171.96, 169.55, 159.09, 144.26, 133.08, 132.66, 127.76, 125.36, 122.54, 65.00, 57.01, 52.01, 50.13, 37.68, 27.93, 25.55, 25.11, 15.51, 11.45; HPLC: k' = 6.12, t_0 = 1.85 (RP-18, MeCN/0.02M KH$_2$PO$_4$, 3:7, pH 2.85); HPLC: k' = 0.83, t_0 = 2.15 (Chiralcel OJ-R, MeCN/H$_2$O, 3:7); C$_{19}$H$_{25}$N$_3$O$_5$S (407.49), calculated: C 56.00, H 6.18, N 10.31; found: C 55.66, H 5.27, N 9.94.

5.3. X-ray crystallography of 3-(1,1-dioxobenzo[*d*]isothiazol-3-ylamino)-
5-nitro-thiophene-2-carboxylic acid methyl ester

Experimental data were collected on the KM4 kappa-geometry diffractometer equipped with Sapphire 2 CCD detector (Oxford Diffraction). Enhanced Mo Kα X-ray radiation source with a graphite monochromator was used. Measurement was carried out in four omega-scan runs - scan width 0.75°, exposure time 75 seconds per frame - consisting of 612 frames in total.

Determination of the elemental cell and data collection were carried out at 293(2) K. All preliminary calculations were performed using CrysAlis RED and CrysAlis CCD software package (Oxford Diffraction, 2005). The structure was solved by direct methods. Refinement was made against all reflections by the full-matrix least squares procedure based on F^2. All of the non-hydrogen atoms were refined with anisotropic thermal parameters. Hydrogen atoms were treated as riding with isotropic U values fixed as 1.4 times U_{eq} of the respective pivot atom. The amino hydrogen atoms H2A and H5A were found from Fourier maps and their coordinates were refined without constraints. Due to low value of absorption no correction was applied. The calculations were carried out using the SHELX-97 program package, run under WinGX 1.70.00 (Windows shell program).

5.4. Elastase inhibition studies

5.4.1. Buffers

Tris buffer pH 8: 6.5 g of tris-(hydroxymethyl)-aminomethane was dissolved in 500 ml of water (0.1 M, stock solution). 25 ml of this stock solution was mixed with 13.4 ml of 0.1 M HCl and volume was made up to 100 ml and pH was adjusted to 8.0.

HEPES buffer pH 7.5: 5.95 g of 2-[4-(2-hydroxyethyl)-1-piperazinyl]-ethane sulfonic acid was dissolved in 250 ml of water (0.1 M, stock solution). 2.99 g of sodium chloride was added into the 100 ml of stock solution and pH was adjusted to 7.5 with the help of aqueous sodium hydroxide.

5.4.2. Determination of percent inhibition

All the biochemical studies were analysed by computer program "Aspect Plus"

5.4.2.1. Percent inhibition of PPE

To a thermostatted solution of 820 µL Tris buffer, pH 8 (25 ml 0.1 M Tris buffer + 13.4 ml 0.1 M HCl) was added DMSO (100 µL), Porcine Pancreatic elastase (30 µL solution in 1 mM acetic acid, for a final enzyme concentration of 28.9 nM) and, finally, 50 µL Suc-Ala-Ala-Ala-*p*-nitroanilide in DMSO for a final substrate concentration of 0.78 mM. The rate of substrate hydrolysis was determined by monitoring the absorption at 410 nm for 20 min. The experiment was repeated in the presence of different compounds. The following compounds were tested at the concentration of 0.5 mM for the inhibitory activity: ester derivatives (**4j**, **4w**, **15a**-**15h**, **18a, 18b, 21, 24, 27**), amide derivatives (**4a**, **4b**, **4r-4t**), acid derivatives (**14a-14j**, **19, 22, 25, 28**), alcohol derivative (**16**), peptide (**35**), amide derivative containing acetonitrile moiety (**30**), peptides (**34a-34f**). Compound **29** was tested at 0.1 mM concentration against PPE.

5.4.2.2. Percent inhibition of HLE

To a thermostatted solution of 800 µL 0.1 M HEPES buffer containing 0.5 M NaCl, pH 7.5, was added DMSO (125 µL), Human leukocyte elastase (50 µL solution in 0.05 M sodium acetate buffer containing 0.4 M NaCl, pH 5.5, for a final enzyme concentration of 17 nM) and, finally, 25 µL methoxysuccinyl-Ala-Ala-Pro-Val-*p*-nitroanilide in DMSO for a final substrate concentration of 0.5 mM. The rate of substrate hydrolysis was determined by monitoring the absorption at 410 nm for 20 minutes. The experiment was repeated in the presence of 0.5 mM concentration of compound. The following compounds were tested at the concentration of 0.5 mM for

the inhibitory activity: ester derivatives (**4j**, **4w**, **15a**-**15h, 18a, 18b, 21, 24, 27**), amide derivatives (**4a**, **4b**, **4r-4t**), acid derivatives (**14a-14j**, **19, 22, 25**), alcohol derivative (**16**), peptide (**35**), amide derivative containing acetonitrile moiety (**17**). The peptides (**34a-34f**) were tested at 0.125 mM concentration against the HLE. Compound **28** was tested at 0.05 mM concentration against HLE while compound **29** was tested at 0.2 mM concentration against HLE. The percent inhibition of HLE by **15e** and **15h** was measured at 5 µM concentration as the inhibition by these two derivatives was very high at 0.5 mM concentration.

5.4.3. Determination of K_i

Determination of K_i was carried out according to the literature method [106-108].

To a thermostatted solution of 750 µL 0.1 M HEPES buffer containing 0.5 M NaCl, pH 7.5, was added DMSO (100 µL), Human leukocyte elastase (100 µL solution in 0.05 M sodium acetate buffer containing 0.4 M NaCl, pH 5.5, for a final enzyme concentration of 0.034 µM) and, finally, 50 µL methoxysuccinyl-Ala-Ala-Pro-Val-*p*-nitroanilide in DMSO for a final substrate concentration of 1 mM. The rate of substrate hydrolysis was determined by monitoring the absorption at 410 nm for 5 min. The experiment was repeated in the presence of varying amounts of the inhibitor **15a-15d, 15f** for final concentration: 12.5 µM, 25 µM, 37.5 µM; **15e** for final concentration: 1.25 µM, 2.5 µM, 3.5 µM, 5 µM; **15h** for final concentration: 5 µM, 7.5 µM, 10 µM, 12.5 µM; **34c, 34e** for final concentration of 31.25 µM, 62.50 µM, 93.75 µM) and at a constant final concentration of DMSO (15%) and the rates of substrate hydrolysis were determined. The series of experiments were repeated at one additional substrate concentration (final substrate concentration: 0.5 mM). All rates were determined in triplicate. The inverse of the average velocities was plotted against the final inhibitor concentration and K_i determined from the intersection of two lines (each R^2 >0.98).

Determination of K_i of **4j** and **4w** was carried out at three different concentrations of substrates. Inhibition by **4j** and **4w** (final inhibitor concentration 0.125, 0.25, 0.5 mM) was measured at a constant final concentration of DMSO (10%) and the rates of substrate hydrolysis were determined. The series of experiments was repeated at two additional substrate concentrations (final substrate concentrations: 0.5 and 0.3 mM). All rates were determined in triplicate. The inverse of the average velocities was plotted against the final inhibitor concentration and K_i was determined from the average intersection of the three lines (each R^2 >0.98).

5.4.4. Stability of cyanomethyl (2*S*,3*S*)-2-(1,1-dioxobenzo[*d*]isothiazol-3-ylamino)-3-methylpentanoate during the enzyme assay

Cyanomethyl (2*S*,3*S*)-2-(1,1-dioxobenzo[*d*]isothiazol-3-ylamino)-3-methylpentanoate (**15e**) was incubated in buffer solution with and without substrate. The samples were then removed after 20 minute and again after 4 hrs and analysed by HPLC. The set of samples were made as:

1) 900 µl of Tris buffer + 100 µl of **15e** in DMSO for final concentration of 0.5 mM

2) 850 µl of Tris buffer + 50 µl of Suc-Ala-Ala-Ala-*p*-nitroanilide (substrate) in DMSO + 100 µl of **15e** in DMSO for final concentration of 0.5 mM

3) 900 µl of HEPES buffer + 100 µl of **15e** in DMSO for final concentration of 0.5 mM

4) 875 µl of HEPES buffer + 25 µl of methoxysuccinyl-Ala-Ala-Pro-Val-*p*-nitroanilide (substrate) in DMSO + 100 µl of **15e** in DMSO for final concentration of 0.5 mM

5.5. Docking Studies

All calculations were done on SGI Octane2 workstations running at 400 MHz with a MIPSR12000 processor.

The program Gaussian98 was used to calculate the partial atomic charges for each inhibitor with the Hartree Fock (HF) method and the 6-31G (d) basis set. The structure used in the *ab initio* calculations was one obtained with PC Model (8.5), using the MMF94 force field and the random search program GMMX.

Molecular modeling was done with the program *InsightII* (2000) from *Accelrys* (San Diego, CA), using the *Discovery3* module and the cvff force field. Automated docking was done using the program *Affinity*. The X-ray crystal structure for human leukocyte elastase was obtained from the Brookhaven Protein Database (1H1B). Hydrogens were added to the enzyme and the bond order was automatically corrected for with *Insight*. The bond between the enzyme serine195 hydroxy group and the irreversibly bound inhibitor was deleted. All atoms of the enzyme within a 7 angstrom radius of the inhibitor were defined as the flexible binding site. The inhibitor was then deleted. The enzyme was then soaked in water to a depth of 7 angstroms for the first layer and then soaked again with water to a depth of 5 angstroms for the second water layer. The outer layer water molecules were then restrained while the rest of the system was minimized with a steepest descent method for 1000 interactions. Both layers of water were deleted and the remaining structure was used for docking experiments.

Solvent grids were constructed for both inhibitor and enzyme using the default parameters in *Affinity*. In the initial experiments, the inhibitor was placed outside of the active site and the program docked it automatically in various alignments within the active site and minimized for a maximum of 1000 interactions. The inhibitor was allowed to move in all directions a maximum of 3 angstroms and rotated by a maximum of 180°. The energy test was done with an energy range set to 100. The maximum energy tolerance was 1000. A maximum of the 10 most stable structures were stored and the binding energies calculated with Discovery3 according to the following equations:

Binding energy = (Total Energy of Complex) - (Energy of Enzyme) - (Energy of Inhibitor)

For the inhibitor **15e**, a very low energy binding structure with the acetonitrile group buried deep in the hydrophobic pocket near the active site SER195 was found.

However, for all other inhibitors, the automatic docking feature of Affinity did not find comparable structures with the ester side chain buried in the pocket. Thus, for all other inhibitors, manual docking was performed; i.e., the ester side chain was manually fitted into the binding site pocket, whereby care was taken to avoid any obvious stearic clashes. Next, an *Affinity* run was started as describe above, with the 10 most stable structures being stored. However, not all substances gave 10 stable structures.

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7. Appendix

7.1. X-ray crystal data of 29

Table 1. Crystal data and structure refinement for 29.

```
Identification code            29

Empirical formula              C13 H9 N3 O6 S2
Formula weight                 367.35
Temperature                    293(2) K
Wavelength                     0.71073 A

Crystal system, space group    Triclinic, P -1
Unit cell dimensions           a=8.4462(7)A alpha=100.754(7) deg.
                               b=12.5495(11)A beta=96.956(7) deg.
                               c=15.2557(14)A gamma=105.287(7)deg.

Volume                         1507.5(2) A^3

Z, Calculated density          4, 1.619 Mg/m^3

Absorption coefficient         0.391 mm^-1

F(000)                         752

Crystal size                   0.44 x 0.29 x 0.12 mm
Theta range for data collection 2.62 to 30.00 deg.
Limiting indices               -11<=h<=11, -17<=k<=17, -21<=l<=20
Reflections collected / unique 14750 / 8613 [R(int) = 0.0333]

Completeness to theta = 30.00  97.9%

Absorption correction          None

Refinement method              Full-matrix least-squares on F^2

Data / restraints / parameters 8613 / 0 / 443

Goodness-of-fit on F^2         1.088

Final R indices [I>2sigma(I)]  R1 = 0.0600, wR2 = 0.1559

R indices (all data)           R1 = 0.0693, wR2 = 0.1655

Largest diff. peak and hole    0.711 and -0.318 e.A^-3
```

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (A^2 x 10^3) for 29.
U (eq) is defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U(eq)
S(1)	7876(1)	3884(1)	1456(1)	34(1)
S(2)	8281(1)	933(1)	4859(1)	42(1)
N(1)	7960(2)	3218(2)	2289(1)	38(1)
N(2)	9528(2)	3541(2)	3741(1)	36(1)
N(3)	5301(3)	-54(2)	3723(1)	42(1)
O(1)	6285(2)	4085(2)	1295(1)	52(1)
O(2)	8372(2)	3285(2)	690(1)	48(1)
O(3)	4174(2)	-2(2)	3170(1)	55(1)
O(4)	5245(3)	-832(2)	4109(2)	68(1)
O(5)	11821(2)	3807(2)	5281(1)	47(1)
O(6)	11375(2)	2341(2)	5978(1)	48(1)
C(1)	9457(3)	5142(2)	2013(2)	33(1)
C(2)	10033(3)	6138(2)	1734(2)	46(1)
C(3)	11305(4)	6994(2)	2336(2)	57(1)
C(4)	11979(4)	6846(2)	3168(2)	53(1)
C(5)	11361(3)	5843(2)	3440(2)	40(1)
C(6)	10071(2)	4986(2)	2854(1)	31(1)
C(7)	9133(2)	3855(2)	2965(1)	31(1)
C(8)	8755(3)	2523(2)	3968(1)	32(1)
C(9)	7199(3)	1743(2)	3533(2)	36(1)
C(10)	6826(3)	873(2)	3966(2)	37(1)
C(11)	9476(3)	2198(2)	4707(1)	35(1)
C(12)	11014(3)	2871(2)	5344(2)	38(1)
C(13)	12858(3)	2927(3)	6659(2)	53(1)
S(3)	11994(1)	10806(1)	8408(1)	40(1)
S(4)	5574(1)	5771(1)	6858(1)	40(1)
N(4)	10485(2)	9600(2)	8056(1)	39(1)
N(5)	9233(2)	8041(2)	8627(1)	33(1)
N(6)	5555(3)	7074(2)	5630(1)	46(1)
O(7)	13266(3)	10819(2)	7864(1)	56(1)
O(8)	11299(3)	11736(2)	8489(2)	60(1)
O(9)	6148(3)	7924(2)	5358(1)	63(1)
O(10)	4332(3)	6290(2)	5216(2)	75(1)
O(11)	7795(2)	6159(2)	9336(1)	49(1)
O(12)	6092(2)	4651(1)	8287(1)	44(1)
C(14)	12651(3)	10588(2)	9485(1)	35(1)
C(15)	13942(3)	11271(2)	10175(2)	47(1)
C(16)	14187(3)	10876(3)	10953(2)	54(1)
C(17)	13169(4)	9847(2)	11034(2)	52(1)
C(18)	11866(3)	9169(2)	10339(2)	39(1)
C(19)	11628(2)	9561(2)	9556(1)	30(1)
C(20)	10386(2)	9038(2)	8700(1)	30(1)
C(21)	7995(2)	7431(2)	7874(1)	30(1)
C(22)	7594(3)	7811(2)	7076(1)	34(1)
C(23)	6318(3)	6980(2)	6499(1)	36(1)
C(24)	7004(2)	6351(2)	7848(1)	32(1)
C(25)	7026(3)	5729(2)	8577(2)	35(1)
C(26)	5853(5)	3981(3)	8964(2)	61(1)

Table 3.   Bond lengths [A] and angles [deg] for 29.

S(1)-O(1)	1.4323(18)
S(1)-O(2)	1.4361(18)
S(1)-N(1)	1.6507(19)
S(1)-C(1)	1.763(2)
S(2)-C(10)	1.697(2)
S(2)-C(11)	1.716(2)
N(1)-C(7)	1.308(3)
N(2)-C(7)	1.347(3)
N(2)-C(8)	1.399(3)
N(2)-H(2A)	0.88(3)
N(3)-O(3)	1.217(3)
N(3)-O(4)	1.224(3)
N(3)-C(10)	1.443(3)
O(5)-C(12)	1.221(3)
O(6)-C(12)	1.322(3)
O(6)-C(13)	1.450(3)
C(1)-C(2)	1.381(3)
C(1)-C(6)	1.392(3)
C(2)-C(3)	1.391(4)
C(2)-H(2)	0.9300
C(3)-C(4)	1.392(5)
C(3)-H(3)	0.9300
C(4)-C(5)	1.389(4)
C(4)-H(4)	0.9300
C(5)-C(6)	1.389(3)
C(5)-H(5)	0.9300
C(6)-C(7)	1.484(3)
C(8)-C(11)	1.394(3)
C(8)-C(9)	1.412(3)
C(9)-C(10)	1.365(3)
C(9)-H(9)	0.9300
C(11)-C(12)	1.467(3)
C(13)-H(13A)	0.9600
C(13)-H(13B)	0.9600
C(13)-H(13C)	0.9600
S(3)-O(8)	1.431(2)
S(3)-O(7)	1.434(2)
S(3)-N(4)	1.649(2)
S(3)-C(14)	1.763(2)
S(4)-C(23)	1.700(2)
S(4)-C(24)	1.719(2)
N(4)-C(20)	1.310(3)
N(5)-C(20)	1.343(3)
N(5)-C(21)	1.400(2)
N(5)-H(5A)	0.84(3)
N(6)-O(9)	1.222(3)
N(6)-O(10)	1.228(3)
N(6)-C(23)	1.442(3)
O(11)-C(25)	1.205(3)
O(12)-C(25)	1.334(3)
O(12)-C(26)	1.449(3)
C(14)-C(19)	1.381(3)
C(14)-C(15)	1.388(3)
C(15)-C(16)	1.383(4)
C(15)-H(15)	0.9300
C(16)-C(17)	1.386(4)
C(16)-H(16)	0.9300
C(17)-C(18)	1.395(3)
C(17)-H(17)	0.9300

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C(18)-C(19)              1.386(3)
C(18)-H(18)              0.9300
C(19)-C(20)              1.490(3)
C(21)-C(24)              1.383(3)
C(21)-C(22)              1.423(3)
C(22)-C(23)              1.364(3)
C(22)-H(22)              0.9300
C(24)-C(25)              1.475(3)
C(26)-H(26A)             0.9600
C(26)-H(26B)             0.9600
C(26)-H(26C)             0.9600

O(1)-S(1)-O(2)           116.41(12)
O(1)-S(1)-N(1)           109.99(11)
O(2)-S(1)-N(1)           109.26(11)
O(1)-S(1)-C(1)           111.45(11)
O(2)-S(1)-C(1)           111.32(11)
N(1)-S(1)-C(1)            96.60(9)
C(10)-S(2)-C(11)          89.52(10)
C(7)-N(1)-S(1)           109.66(14)
C(7)-N(2)-C(8)           126.70(18)
C(7)-N(2)-H(2A)          119(2)
C(8)-N(2)-H(2A)          115(2)
O(3)-N(3)-O(4)           125.1(2)
O(3)-N(3)-C(10)          118.6(2)
O(4)-N(3)-C(10)          116.3(2)
C(12)-O(6)-C(13)         117.00(19)
C(2)-C(1)-C(6)           123.0(2)
C(2)-C(1)-S(1)           129.99(19)
C(6)-C(1)-S(1)           107.03(14)
C(1)-C(2)-C(3)           116.5(2)
C(1)-C(2)-H(2)           121.8
C(3)-C(2)-H(2)           121.8
C(2)-C(3)-C(4)           121.7(2)
C(2)-C(3)-H(3)           119.2
C(4)-C(3)-H(3)           119.2
C(5)-C(4)-C(3)           120.8(2)
C(5)-C(4)-H(4)           119.6
C(3)-C(4)-H(4)           119.6
C(4)-C(5)-C(6)           118.3(2)
C(4)-C(5)-H(5)           120.9
C(6)-C(5)-H(5)           120.9
C(5)-C(6)-C(1)           119.77(19)
C(5)-C(6)-C(7)           130.5(2)
C(1)-C(6)-C(7)           109.67(17)
N(1)-C(7)-N(2)           123.96(18)
N(1)-C(7)-C(6)           116.99(18)
N(2)-C(7)-C(6)           119.06(18)
C(11)-C(8)-N(2)          121.07(19)
C(11)-C(8)-C(9)          112.35(18)
N(2)-C(8)-C(9)           126.54(19)
C(10)-C(9)-C(8)          109.73(19)
C(10)-C(9)-H(9)          125.1
C(8)-C(9)-H(9)           125.1
C(9)-C(10)-N(3)          124.6(2)
C(9)-C(10)-S(2)          115.90(17)
N(3)-C(10)-S(2)          119.44(16)
C(8)-C(11)-C(12)         125.68(19)
C(8)-C(11)-S(2)          112.46(16)
C(12)-C(11)-S(2)         121.70(16)
O(5)-C(12)-O(6)          125.7(2)
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O(5)-C(12)-C(11)              122.7(2)
O(6)-C(12)-C(11)              111.63(19)
O(6)-C(13)-H(13A)             109.5
O(6)-C(13)-H(13B)             109.5
H(13A)-C(13)-H(13B)           109.5
O(6)-C(13)-H(13C)             109.5
H(13A)-C(13)-H(13C)           109.5
H(13B)-C(13)-H(13C)           109.5
O(8)-S(3)-O(7)                117.16(13)
O(8)-S(3)-N(4)                109.67(12)
O(7)-S(3)-N(4)                109.93(11)
O(8)-S(3)-C(14)               110.86(12)
O(7)-S(3)-C(14)               110.92(12)
N(4)-S(3)-C(14)                96.31(10)
C(23)-S(4)-C(24)               89.12(10)
C(20)-N(4)-S(3)               109.72(15)
C(20)-N(5)-C(21)              126.90(18)
C(20)-N(5)-H(5A)              118(2)
C(21)-N(5)-H(5A)              114(2)
O(9)-N(6)-O(10)               124.5(2)
O(9)-N(6)-C(23)               118.5(2)
O(10)-N(6)-C(23)              117.1(2)
C(25)-O(12)-C(26)             116.8(2)
C(19)-C(14)-C(15)             122.7(2)
C(19)-C(14)-S(3)              107.78(15)
C(15)-C(14)-S(3)              129.52(19)
C(16)-C(15)-C(14)             117.0(2)
C(16)-C(15)-H(15)             121.5
C(14)-C(15)-H(15)             121.5
C(15)-C(16)-C(17)             120.9(2)
C(15)-C(16)-H(16)             119.5
C(17)-C(16)-H(16)             119.5
C(16)-C(17)-C(18)             121.7(2)
C(16)-C(17)-H(17)             119.2
C(18)-C(17)-H(17)             119.2
C(19)-C(18)-C(17)             117.4(2)
C(19)-C(18)-H(18)             121.3
C(17)-C(18)-H(18)             121.3
C(14)-C(19)-C(18)             120.26(19)
C(14)-C(19)-C(20)             109.28(18)
C(18)-C(19)-C(20)             130.45(19)
N(4)-C(20)-N(5)               123.65(18)
N(4)-C(20)-C(19)             116.87(18)
N(5)-C(20)-C(19)             119.48(18)
C(24)-C(21)-N(5)             121.09(18)
C(24)-C(21)-C(22)            112.71(18)
N(5)-C(21)-C(22)             126.20(18)
C(23)-C(22)-C(21)            108.87(19)
C(23)-C(22)-H(22)            125.6
C(21)-C(22)-H(22)            125.6
C(22)-C(23)-N(6)             124.6(2)
C(22)-C(23)-S(4)             116.54(16)
N(6)-C(23)-S(4)              118.88(17)
C(21)-C(24)-C(25)            126.73(18)
C(21)-C(24)-S(4)             112.76(15)
C(25)-C(24)-S(4)             120.43(15)
O(11)-C(25)-O(12)            125.3(2)
O(11)-C(25)-C(24)            123.3(2)
O(12)-C(25)-C(24)            111.37(18)
O(12)-C(26)-H(26A)           109.5
O(12)-C(26)-H(26B)           109.5
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H(26A)-C(26)-H(26B)          109.5
O(12)-C(26)-H(26C)           109.5
H(26A)-C(26)-H(26C)          109.5
H(26B)-C(26)-H(26C)          109.5
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Symmetry transformations used to generate equivalent atoms

Table 4. Anisotropic displacement parameters (A^2 x 10^3) for 29.
The anisotropic displacement factor exponent takes the form:
-2 pi^2 [h^2 a*^2 U11 + ... + 2 h k a* b* U12]

	U11	U22	U33	U23	U13	U12
S(1)	33(1)	32(1)	35(1)	12(1)	-1(1)	6(1)
S(2)	44(1)	38(1)	41(1)	19(1)	-1(1)	1(1)
N(1)	39(1)	30(1)	37(1)	14(1)	-4(1)	-1(1)
N(2)	38(1)	30(1)	32(1)	11(1)	-1(1)	0(1)
N(3)	44(1)	37(1)	38(1)	9(1)	4(1)	-1(1)
O(1)	35(1)	66(1)	58(1)	27(1)	2(1)	15(1)
O(2)	60(1)	43(1)	41(1)	7(1)	4(1)	17(1)
O(3)	43(1)	60(1)	49(1)	14(1)	-7(1)	-3(1)
O(4)	67(1)	48(1)	74(1)	32(1)	-6(1)	-12(1)
O(5)	42(1)	40(1)	49(1)	18(1)	-5(1)	-3(1)
O(6)	43(1)	49(1)	44(1)	24(1)	-6(1)	-1(1)
C(1)	32(1)	28(1)	39(1)	11(1)	5(1)	8(1)
C(2)	52(1)	36(1)	56(1)	21(1)	10(1)	13(1)
C(3)	61(2)	29(1)	76(2)	16(1)	12(1)	2(1)
C(4)	49(1)	31(1)	65(2)	0(1)	7(1)	-2(1)
C(5)	36(1)	32(1)	42(1)	1(1)	0(1)	2(1)
C(6)	31(1)	25(1)	37(1)	7(1)	5(1)	7(1)
C(7)	31(1)	28(1)	34(1)	10(1)	4(1)	7(1)
C(8)	33(1)	31(1)	31(1)	11(1)	5(1)	6(1)
C(9)	36(1)	35(1)	35(1)	11(1)	2(1)	6(1)
C(10)	38(1)	32(1)	35(1)	9(1)	2(1)	1(1)
C(11)	35(1)	33(1)	35(1)	13(1)	3(1)	4(1)
C(12)	36(1)	40(1)	33(1)	12(1)	2(1)	4(1)
C(13)	49(1)	58(2)	44(1)	21(1)	-8(1)	3(1)
S(3)	44(1)	32(1)	40(1)	12(1)	7(1)	3(1)
S(4)	38(1)	38(1)	35(1)	7(1)	-3(1)	1(1)
N(4)	41(1)	36(1)	34(1)	14(1)	0(1)	3(1)
N(5)	32(1)	32(1)	31(1)	12(1)	-3(1)	3(1)
N(6)	50(1)	57(1)	31(1)	9(1)	-2(1)	20(1)
O(7)	56(1)	55(1)	51(1)	15(1)	20(1)	1(1)
O(8)	72(1)	40(1)	75(1)	24(1)	13(1)	19(1)
O(9)	86(2)	68(1)	42(1)	27(1)	6(1)	28(1)
O(10)	65(1)	89(2)	49(1)	11(1)	-23(1)	5(1)
O(11)	50(1)	50(1)	39(1)	17(1)	-3(1)	2(1)
O(12)	54(1)	33(1)	42(1)	14(1)	4(1)	5(1)
C(14)	34(1)	33(1)	35(1)	5(1)	5(1)	5(1)
C(15)	42(1)	39(1)	48(1)	-2(1)	3(1)	1(1)
C(16)	46(1)	58(2)	40(1)	-6(1)	-9(1)	4(1)
C(17)	58(2)	55(2)	33(1)	4(1)	-7(1)	13(1)
C(18)	44(1)	39(1)	34(1)	10(1)	2(1)	10(1)
C(19)	30(1)	29(1)	29(1)	4(1)	2(1)	7(1)
C(20)	29(1)	28(1)	31(1)	6(1)	1(1)	6(1)
C(21)	28(1)	30(1)	30(1)	7(1)	1(1)	7(1)
C(22)	35(1)	34(1)	32(1)	11(1)	2(1)	10(1)
C(23)	36(1)	40(1)	29(1)	9(1)	2(1)	11(1)
C(24)	31(1)	34(1)	29(1)	10(1)	2(1)	6(1)
C(25)	34(1)	35(1)	36(1)	13(1)	7(1)	6(1)
C(26)	81(2)	47(1)	55(2)	29(1)	12(1)	6(1)

Table 5. Torsion angles [deg] for 29.

_____

O(1)-S(1)-N(1)-C(7)	-117.46(18)
O(2)-S(1)-N(1)-C(7)	113.60(17)
C(1)-S(1)-N(1)-C(7)	-1.75(18)
O(1)-S(1)-C(1)-C(2)	-61.9(2)
O(2)-S(1)-C(1)-C(2)	69.9(2)
N(1)-S(1)-C(1)-C(2)	-176.5(2)
O(1)-S(1)-C(1)-C(6)	116.68(16)
O(2)-S(1)-C(1)-C(6)	-111.53(16)
N(1)-S(1)-C(1)-C(6)	2.15(16)
C(6)-C(1)-C(2)-C(3)	1.1(4)
S(1)-C(1)-C(2)-C(3)	179.5(2)
C(1)-C(2)-C(3)-C(4)	1.1(4)
C(2)-C(3)-C(4)-C(5)	-2.2(5)
C(3)-C(4)-C(5)-C(6)	1.0(4)
C(4)-C(5)-C(6)-C(1)	1.1(3)
C(4)-C(5)-C(6)-C(7)	-177.8(2)
C(2)-C(1)-C(6)-C(5)	-2.3(3)
S(1)-C(1)-C(6)-C(5)	179.00(17)
C(2)-C(1)-C(6)-C(7)	176.9(2)
S(1)-C(1)-C(6)-C(7)	-1.9(2)
S(1)-N(1)-C(7)-N(2)	-178.87(17)
S(1)-N(1)-C(7)-C(6)	0.9(2)
C(8)-N(2)-C(7)-N(1)	-1.7(4)
C(8)-N(2)-C(7)-C(6)	178.53(19)
C(5)-C(6)-C(7)-N(1)	179.8(2)
C(1)-C(6)-C(7)-N(1)	0.8(3)
C(5)-C(6)-C(7)-N(2)	-0.5(3)
C(1)-C(6)-C(7)-N(2)	-179.47(19)
C(7)-N(2)-C(8)-C(11)	167.2(2)
C(7)-N(2)-C(8)-C(9)	-15.3(4)
C(11)-C(8)-C(9)-C(10)	0.1(3)
N(2)-C(8)-C(9)-C(10)	-177.6(2)
C(8)-C(9)-C(10)-N(3)	177.6(2)
C(8)-C(9)-C(10)-S(2)	-1.4(3)
O(3)-N(3)-C(10)-C(9)	-9.3(4)
O(4)-N(3)-C(10)-C(9)	171.6(2)
O(3)-N(3)-C(10)-S(2)	169.59(19)
O(4)-N(3)-C(10)-S(2)	-9.5(3)
C(11)-S(2)-C(10)-C(9)	1.7(2)
C(11)-S(2)-C(10)-N(3)	-177.3(2)
N(2)-C(8)-C(11)-C(12)	3.4(3)
C(9)-C(8)-C(11)-C(12)	-174.5(2)
N(2)-C(8)-C(11)-S(2)	178.97(16)
C(9)-C(8)-C(11)-S(2)	1.1(2)
C(10)-S(2)-C(11)-C(8)	-1.53(18)
C(10)-S(2)-C(11)-C(12)	174.2(2)
C(13)-O(6)-C(12)-O(5)	0.1(4)
C(13)-O(6)-C(12)-C(11)	-179.2(2)
C(8)-C(11)-C(12)-O(5)	0.2(4)
S(2)-C(11)-C(12)-O(5)	-175.0(2)
C(8)-C(11)-C(12)-O(6)	179.5(2)
S(2)-C(11)-C(12)-O(6)	4.3(3)
O(8)-S(3)-N(4)-C(20)	-113.04(18)
O(7)-S(3)-N(4)-C(20)	116.77(18)
C(14)-S(3)-N(4)-C(20)	1.79(18)
O(8)-S(3)-C(14)-C(19)	112.61(17)
O(7)-S(3)-C(14)-C(19)	-115.43(16)
N(4)-S(3)-C(14)-C(19)	-1.26(17)

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O(8)-S(3)-C(14)-C(15)                    -67.5(3)
O(7)-S(3)-C(14)-C(15)                     64.5(3)
N(4)-S(3)-C(14)-C(15)                    178.6(2)
C(19)-C(14)-C(15)-C(16)                    0.6(4)
S(3)-C(14)-C(15)-C(16)                  -179.3(2)
C(14)-C(15)-C(16)-C(17)                   -0.6(4)
C(15)-C(16)-C(17)-C(18)                    0.2(4)
C(16)-C(17)-C(18)-C(19)                    0.3(4)
C(15)-C(14)-C(19)-C(18)                   -0.1(3)
S(3)-C(14)-C(19)-C(18)                   179.79(17)
C(15)-C(14)-C(19)-C(20)                 -179.5(2)
S(3)-C(14)-C(19)-C(20)                     0.4(2)
C(17)-C(18)-C(19)-C(14)                   -0.3(3)
C(17)-C(18)-C(19)-C(20)                  178.9(2)
S(3)-N(4)-C(20)-N(5)                     177.40(17)
S(3)-N(4)-C(20)-C(19)                     -1.9(2)
C(21)-N(5)-C(20)-N(4)                      1.5(3)
C(21)-N(5)-C(20)-C(19)                  -179.26(18)
C(14)-C(19)-C(20)-N(4)                     0.9(3)
C(18)-C(19)-C(20)-N(4)                  -178.4(2)
C(14)-C(19)-C(20)-N(5)                  -178.37(19)
C(18)-C(19)-C(20)-N(5)                     2.3(3)
C(20)-N(5)-C(21)-C(24)                   172.4(2)
C(20)-N(5)-C(21)-C(22)                    -7.9(3)
C(24)-C(21)-C(22)-C(23)                   -0.2(3)
N(5)-C(21)-C(22)-C(23)                  -179.8(2)
C(21)-C(22)-C(23)-N(6)                   178.6(2)
C(21)-C(22)-C(23)-S(4)                     0.0(2)
O(9)-N(6)-C(23)-C(22)                      4.9(4)
O(10)-N(6)-C(23)-C(22)                  -174.4(2)
O(9)-N(6)-C(23)-S(4)                    -176.59(19)
O(10)-N(6)-C(23)-S(4)                      4.1(3)
C(24)-S(4)-C(23)-C(22)                     0.10(18)
C(24)-S(4)-C(23)-N(6)                   -178.52(18)
N(5)-C(21)-C(24)-C(25)                     3.2(3)
C(22)-C(21)-C(24)-C(25)                 -176.52(19)
N(5)-C(21)-C(24)-S(4)                    179.92(15)
C(22)-C(21)-C(24)-S(4)                     0.2(2)
C(23)-S(4)-C(24)-C(21)                    -0.20(17)
C(23)-S(4)-C(24)-C(25)                   176.80(18)
C(26)-O(12)-C(25)-O(11)                    6.7(4)
C(26)-O(12)-C(25)-C(24)                 -173.1(2)
C(21)-C(24)-C(25)-O(11)                    9.4(4)
S(4)-C(24)-C(25)-O(11)                  -167.15(19)
C(21)-C(24)-C(25)-O(12)                 -170.9(2)
S(4)-C(24)-C(25)-O(12)                    12.6(3)
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Symmetry transformations used to generate equivalent atoms

7.2. Docking data

Table: Binding Energies (kcal/mol) for docked structures found by Affinity.
Bold numbers correspond to the structures with the most negative binding energies.

Compound	4j	4w	15a	15b	15c
Structure					
1	-25.365	**-66.2712**	**-64.376**	**-67.4495**	**-78.788**
2	-49.975	-49.8978	-39.503	-44.845	-40.275
3	-51.526	-62.9945	-47.461	-45.695	-35.992
4	-34.424	-64.4098	-48.506	-46.4229	-50.378
5	-35.74	-65.0355	-44.535	-39.1224	-44.145
6	-40.236	-59.7989		-32.6004	-49.897
7	-42.247	-60.735		-38.3577	-41.593
8	**-54.153**	-48.9841		-43.7601	
9	-50.436	-48.977			
10	-46.997	-53.3414			

Compound	15d	15e	15f	15g	15h
Structure					
1	-41.1653	-85.793	**-80.565**	-37.052	-73.489
2	-39.6647	**-87.044**	-64.303	-48.765	-70.884
3	-40.9966	-52.208	-30.319	-53.603	-58.741
4	-42.967	-50.818	-25.769	-48.008	-64.824
5	-43.0601	-54.999	-34.358	-45.503	-61.972
6	-40.4258	-54.409	-41.096	-52.367	-69.577
7	**-47.511**	-49.516	-41.389	**-56.803**	-79.795
8	-41.711	-36.072	-48.923	-44.807	**-88.05**
9	-44.6791		-60.459	-51.506	-46.547
10	-26.4972		-67.483	-29.145	-40.835

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Prof. Dr. Hans -Hartwig Otto

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Greifswald, December 2005   Rode Haridas Baburao

Hiermit erkläre ich, daß diese Arbeit bisher von mir weder an der Mathematisch-Naturwissenschaftlichen Fakultät der Ernst-Moritz-Arndt-Universität Greifswald noch einer anderen wissenschaftlichen Einrichtung zum Zweck der Promotion eingereicht wurde.

Ferner erkläre ich, daß ich diese Arbeit selbständig verfaßt und keine anderen als die darin angegeben Hilfsmittel benutzt habe.

RESUME

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