The Relationship Between Inhibitors of Eukaryotic and Prokaryotic Serine Proteases

Monika I. Konaklieva[1,*] and Balbina J. Plotkin[2]

[1]*American University, Washington DC 20016, USA*

[2]*Midwestern University, Chicago IL, 60515, USA*

Abstract: The ability to inhibit serine proteases is a major focus in the pharmaceutical industry. Serine proteases of medical importance range in phylogenetic diversity from the metallo-proteases, which play a role in pulmonary hypertension, and destruction of the lung parenchyma in emphysema, to those proteases (beta-lactamases), which play a role in the resistance of bacteria to beta-lactam antibiotics. In both the mammalian and microbial systems, the development of serine protease inhibitors has been a focal strategy spurring investigations in the area of serine protease dependent prodrugs that incorporate a bactericidal moiety as well as other classes of metalloprotease inhibitors.

Keywords: Serine proteases, β-lactamases, human leukocyte elastase, serine protease inhibitors, β-lactams.

INTRODUCTION

Serine proteases are a broad family of proteins that can quite literally control the life and death of cells regardless of their phylogenetic placement in the taxonomic order. Cell cycling, programmed cell death, environmental survival are but a few of the life cycle events in prokaryotes and eukaryotes that are regulated by the actions of this diverse group of proteases. Likewise, their activity is regulated by seemingly equally diverse cohort of inhibitors. Serine protease inhibitors are found throughout the natural environment, from soil (β-lactams) to mammalian serpins (serine protease inhibitors), which inhibit the activity of both circulating and tissue proteases by acylating the serine hydroxyl at the active site [1]. The work of Irving *et al*. [2] suggests phylogenetic relationships and conserved residues in the mechanism of conformational change, which is triggered in the serpin while maintaining the covalent linkage between the protease and serpin. Irreversible serine acylating protease inhibitors generally fall into three basic categories, these being the β-lactams, peptide-based inhibitors and the non-amide inhibitors [3]. The focus of this review is to compare the interaction of amide-based inhibitors with serine proteases from diverse phylogenetic origins and discuss the underlying structural constraints that are essential for activity, regardless of inhibitor category.

The ability to survive in a variety of harsh environments, whether it be the cytoplasm of mammalian host cell or the soil is dependent on the presence of a mechanism to handle inhibitory substances, including serine proteases, thus, explaining the apparently ubiquitous nature of these compounds. Genomic sequencing has allowed the examination of genetic heritage of serine proteases and individual protein domains. Eukaryotic signaling domain homologues have been described in archea and bacteria with apparent ancient ancestry [4]. Phylogenetic distribution of signaling domains is well reviewed by Ponting, *et al*. [5]. Conversely, prokaryotic homologs have been identified for eukaryotic enzymes, such as trypsin-like serine proteases and matrix metalloproteases. Eukaryotic signaling domains are believed to have arisen early with the apparent ancient ancestry the result of horizontal gene transfer [6]. Von Willebrand factor A (vWFA), a multidomain glycoprotein, which is present in mammals and mediates platelet adhesion to damaged endothelium, has signaling domains that are recognized in a variety of other proteins including collagens and integrins. Prokaryotes also carry vWFA domains, which demonstrate conservation of the serine as well as aspartic acid residues that are known from the mammalian vWFA to interact with divalent cations. It has been postulated that prokaryotic metal binding domains function in a fashion similar to that in mammalian cells i.e., they protect bacteria such as *E. coli* from the harmful effects of heavy metals [7]. In chronic infections such as tuberculosis, caused by *Mycobacterium tuberculosis*, one can speculate that where β-lactamase production is occurring within granulomas, the added burden of exogenous serine protease could overwhelm mammalian serpins present contributing to the breakdown of the granuloma and systemic spread of the organism. There can be a further speculation on whether the presence of chronic infections by β-lactamase producing bacteria may contribute to the formation of athromas, and degradation of tissues in fibrinolytic diseases such as emphysema. The emergence of microbial resistance to existing β-lactamase inhibitors has spurred investigations in the area of β-lactamase dependent prodrugs that incorporate a bactericidal moiety. In addition, certain β-lactams have been identified as inhibitors of serine enzymes produced by viruses, fungi and mammals. Development of β-lactam inhibitors of enzymes, which have active-site serine, such as human leukocyte elastase, human cytomegalovirus protein, prostate specific antigen, thrombin, herpesvirus, co-enzyme A independent transacylase, γ-aminobutyric acid (GABA) aminotransferase, and human cytosolic phospholipase A_2, have attracted interest in all areas of medicinal chemistry. Efforts have been

*Address correspondence to this author at the Department of Chemistry, American University, 4400 Massachusetts Avenue, NW, Washington, DC, 20016-8014, USA; Tel: (202) 885-1761; Fax: (202) 885-1752; E-mail: mkonak@american.edu

1389-5575/04 $45.00+.00

directed toward identification of the structure-activity relationship of the newly developed or modified β-lactams, as well as other carbamate-containing inhibitors such as the mammalian peptide serine inhibitors, e.g. serpins.

The current paradigm is that protease inhibitor efficacy is dependent on achieving *in situ* concentrations in the nanomolar range, and a high level of substrate specificity. However, there appear to be proteases from evolutionarily diverse backgrounds, such as β-lactamase and elastase which are inhibited by clavulanate, a β-lactamase inhibitor. When the focus of comparison is shifted from a phylogenetic approach to that of structure-function, examination of inhibitor structure finds that there is little diversity between certain groupings of serine protease inhibitor compounds. The carbamate-based classes of inhibitors, such as the β-lactams and peptide-based inhibitors, demonstrate this fundamental similarity between serine protease inhibitor structures.

NON-PEPTIDE INHIBITORS. THE β-LACTAM PARADIGM

The cross-clan ability of inhibitors to function is best demonstrated by β-lactam ring containing antimicrobials. There is little similarity between the target of β-lactams, the transpeptidases (penicillin binding proteins, PBPs), serine β-lactamases, and the rest of the serine enzymes, either structurally or with regard to amino acid (AA) sequence [8a, 9, 10]. Serine enzymes catalyze different types of reactions and thus can be classified as proteases, lipases, etc. Serine proteases have an active-site serine, which is involved in a hydrolysis of polypeptide bonds. The contemporary

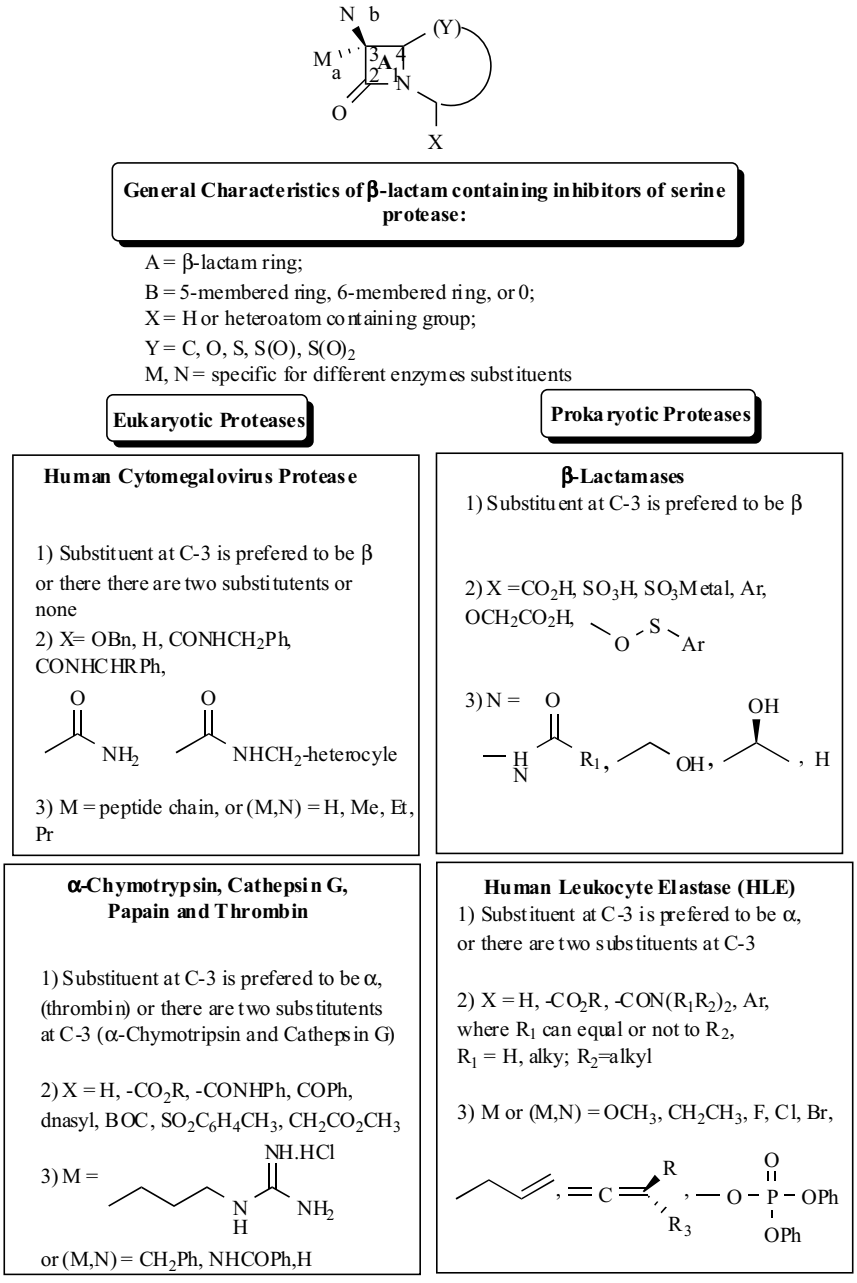

Fig. (1).

classification of the serine enzymes is, in general, based on amino acid sequence data now available for more than 600 proteases, or on phylogenetic relationships [11]. The data show that there may be as many as 60 evolutionary lines of proteases with separate origins. Classification by families and clans has been suggested as a complement to classification by catalytic type [9]. Enzymes that have high sequence similarity tend to group into the same lineage. Based on this classification schema, the generation of enzymes with new functions depends on two separate processes-modifying the catalytic machinery and optimizing the contacts with substrates.

The serine superfamily of bacterial enzymes and eukarytoic serine proteases may have evolved a similar strategy for their biocatalysis, namely utilizing serine as a nucleophile, for a similar purpose, i.e., survival in a harsh environment. This might be an example of evolutionary forces that led to similar catalytic strategies, accomplished by different pathways. In general, serine proteases have active site clefts that are relatively exposed to solvent, often permitting access to polypeptide loops of substrates or inhibitors (such as endogenous proteinaceous inhibitors such as serpins) which nevertheless present their protease-binding domains in an extended strand conformation [12]. Frequently, synthetic serine protease inhibitors have only 3-5 amino acid residues or their equivalent and thus interact with only a small region of the enzyme. This leads to one of the major problems of designing inhibitors of serine proteases, obtaining selectivity. For example, considerably big numbers of thrombin inhibitors needed to be developed to achieve selectivity, due to the similarity of the active site for other serine proteases, notably trypsin. β-Lactams not only exhibit utility as inhibitors of bacterial prokaryotic β-lactamases, but have activity as inhibitors of eukaryotic mammalian active-site serine enzymes such as human leukocyte elastase, porcine pancreatic elastase, prostate specific antigen, co-enzyme A-independent transacylase, thrombin, α-chymotrypsin, cathepsin-G, γ-aminobutyric

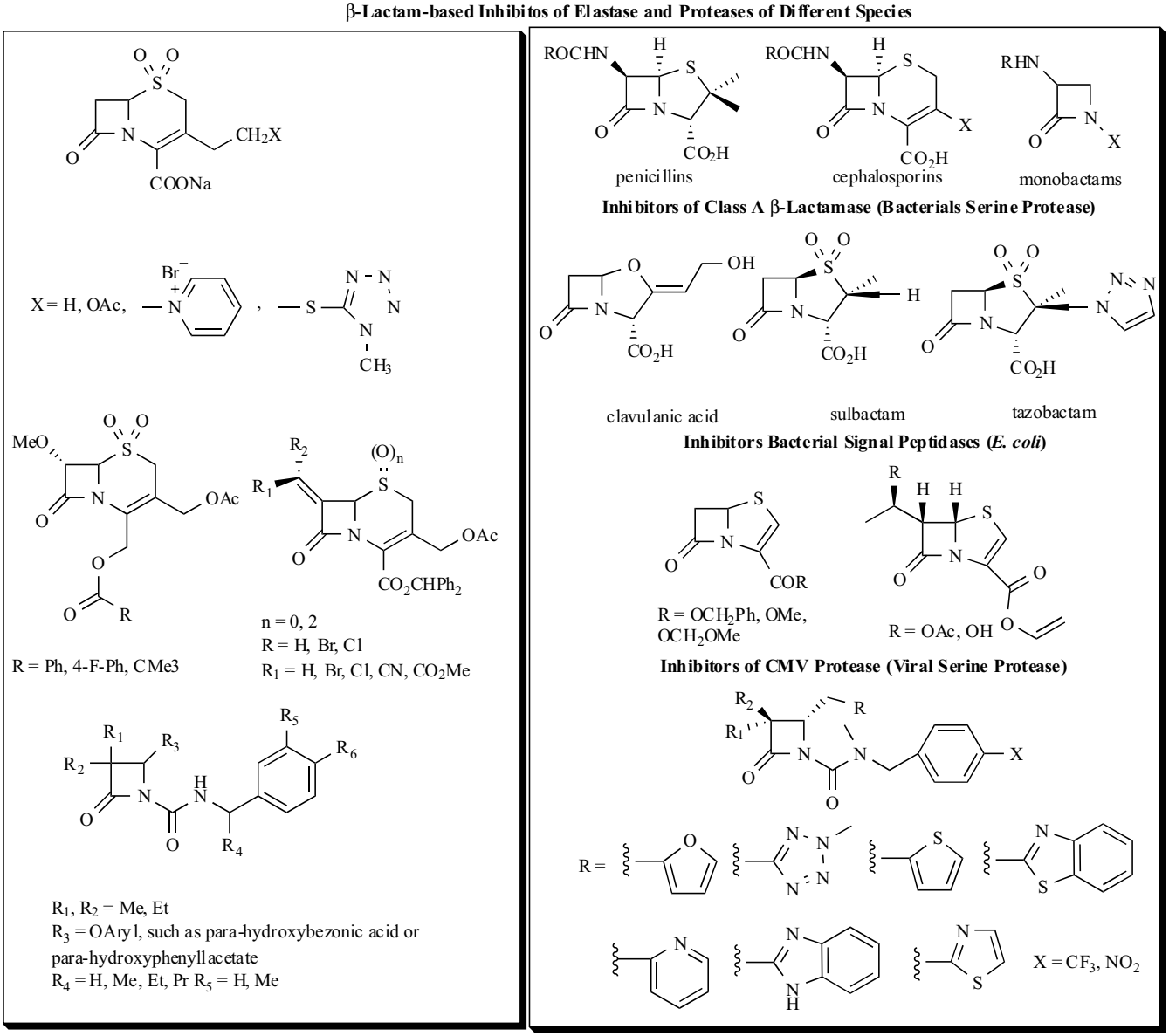

Fig. (2).

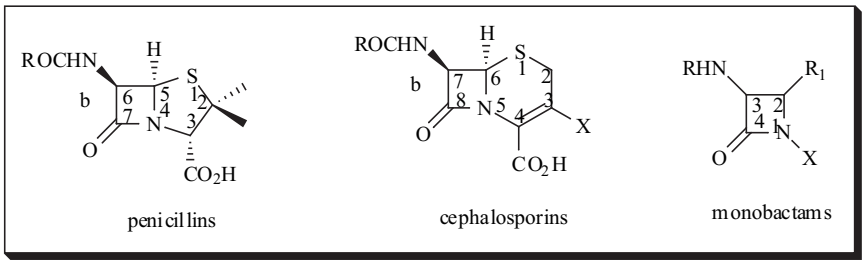

Fig. (3).

acid (GABA) transferase, human cytosolic phospholipase A_2, and in cholesterol absorption in general. In addition, activity has been demonstrated against viral (cytomegalovirus) and fungal serine protease (Fig. (**1**)). As demonstrated by the β-lactams, serine protease inhibitors, regardless of taxonomic kingdom, can share the same catalytic mechanism, i.e. their active site contains a serine residue, which acts as a nucleophile. Thus, the active-site serine undergoes acylation, followed by deacylation in the enzymes, they can act as a platform in the development of novel serine protease inhibitors.

β-Lactams are found in the natural environment as by-products of fungal fermentation. Bacterial resistance to β-lactams generally operates by three different mechanisms: decreased access of antimicrobials to the targets in bacterial cell wall (efflux pumps), altered PBPs (affinity of binding decreased) and β-lactamase production. The latter is by far the most efficient of the resistance mechanisms [13]. Of the

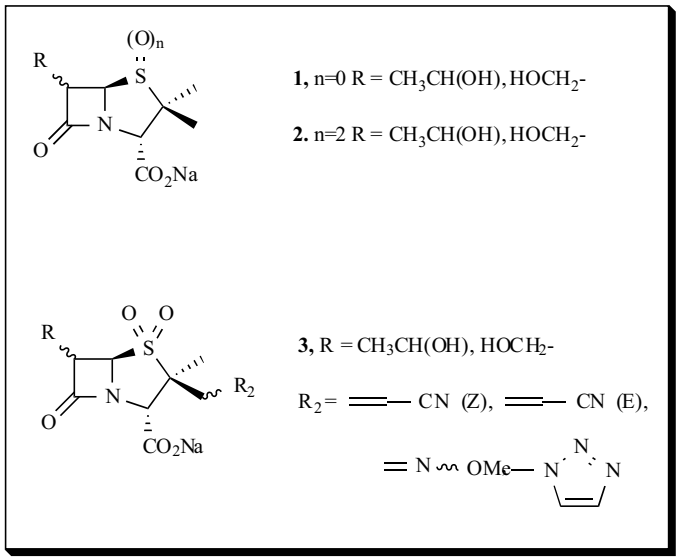

Fig. (4).

substrate turnover. Evolutionarily, this indicates a highly conserved mechanism of acylation with an array of metabolic processes controlled by compounds, including serpins, able to acylate a serine enzyme. The promise that β-lactams exhibit as serine protease inhibitors resides in the finding that since they appear to be acylating agents of serine three families of bacterial enzymes that specifically recognize β-lactam ring containing antibiotics, i.e. transpeptidases β-lactam synthases, and β-lactamases, it is the latter which contain serine proteases. β-Lactamases are generally divided into serine- and zinc-dependent enzymes [14]. β-Lactamases of the classes A, C, and D are a distinct family, which have

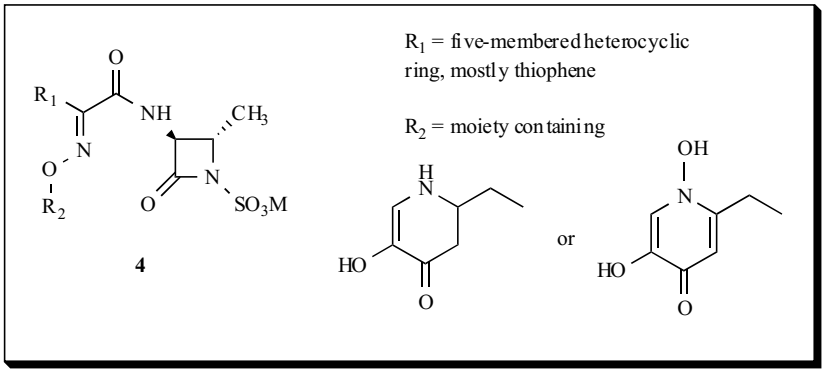

Fig. (5).

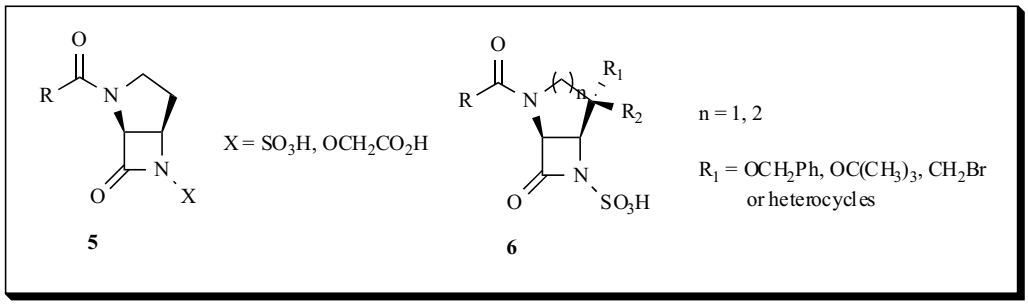

Fig. (6).

serine in the active-site. This is a property makes them part of the larger class serine proteases, which are currently known to span four of the five taxonomic kingdoms, Animalia, Plantae, Monera, Protista, and Fungi. Class B β-lactamases represent the bacterial metallo-β-lactamases. An "active site" model of the PBPs can be represented kinetically:

$$E + C \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EC \xrightarrow{k_2} EC^* \xrightarrow{k_3} E + P(s)$$

where E is the enzyme, C the antibiotic, EC a non-covalent complex, EC* a covalent acyl-enzyme and P(s) the inactivated product(s) of degradation of the antibiotic. Efficient inactivation of the enzyme depends on the rapid and nearly quantitative accumulation of the EC* complex, which is the result of both of its stability (a low k_3 value) and its rapid formation (generally due to high k_2 values). This model for depicting how a large number of β-lactamases accomplish the initial acylation of the active site serine also works for depicting how β-lactams interact with transpeptidases [15]. It is the more rapid deacylation behavior of the β-lactamases that separates them from the PBPs. Thus, nature appears to have taken a basic conserved protein template and through mutation and selection, to have produced two types of bacterial enzymes. These enzymes differ from one another but both have vital functions for the survival of the bacteria.

A strategy used against the action of the β-lactamases is the design of β-lactamase inhibitors e.g. clavulanic acid, tazobactam and sulbactam [16], which only have activity against the class A β-lactamases, the most common class at this time [17a]. β-Lactamase inhibitors feature a short acylamino chain at the carbon atom adjacent to the β-lactam carbonyl (C6-β in the penams, C7-β in the cephems and C3-β in the monocyclic structures) [18], but there are "exceptions", where the substituents are with an α-orientation [19]. Another important feature is the presence of a free carboxylic acid, which acts as an electron withdrawing group, and it is necessary for interaction with carboxypeptidases. The difficulties involved in the successful design of inhibitors for the serine β-lactamases appear to be related to the complexity of these enzymes. The variations on the theme of the 6-(1-hydroxyalkyl) penam nucleus as inhibitors of and C β-lactamases has led to the synthesis of 6-(hydroxymethyl) penams (**1**), 6-(1-hydroxyalkyl)- (**2**) and 3,6-disubstituted penam sulfone derivative (**3**), with both C6α and C6β stereochemistry [20]. Penam sulfone derivatives, both mono- and di-substituted C6β-hydroxymethyl group, have demonstrated good IC_{50} values against both TEM-1 and AmpC β-lactamases. From the monocyclic structures, the monobactams have proved successful as antibacterial agents. Metal salts of their sulfonic acid derivatives (**4**) have been recently described in the patent literature [21]. Bridged sulfactams (**5**) and (**6**) have been found to be effective inhibitors of class A and class C β-lactamases [22]. The acetate elimination from the 3' position in the cephem sulfones was proposed to be a necessary step for the irreversible enzyme inhibition, which was observed in the inhibition of PC1 β-lactamase of *Staphylococcus aureus* by Pratt *et al*. [23]. Interestingly, the biological evaluation of sodium 3'-substituted cephalosporinate sulfones (**7**), synthesized by analogy with sulbactam devoid of substituted at the 7-position, has demonstrated that the leaving groups (LG) ability of the group at 3'-position does not contribute to biological potency of these compounds. Moreover, the absence of substituents at that position leads to the only compound with activity comparable to that of sulbactam against *E. cloacae* P99 β-lactamase [24]. However, some of the bacteria differed from others in the action of the inhibitors, clavulanate, sulbactam and tazobactam against their β-lactamases. Growth of the mycobacteria was suppressed by

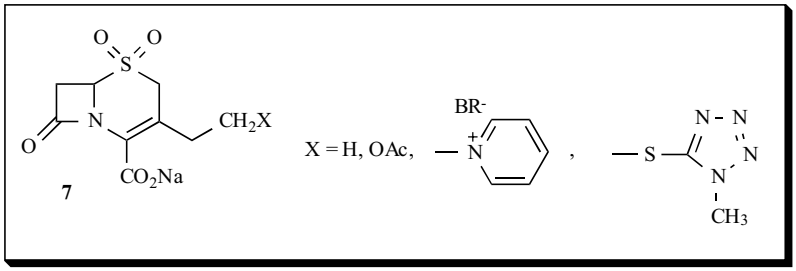

Fig. (7).

novel combinations of the β-lactam/β-lactamase-inhibitors, and by a new β-lactamase-stable cephalosporin, Cefepime (aminothiazolyl methoxyimino cephalosporin). The results presented, as well as reports of previous studies *in vivo*, suggest that the intracellular growth of the bacilli or the high partition coefficient of a β-lactamase inhibitor such as sulbactam does not impede the antimycobacterial action of these compounds.

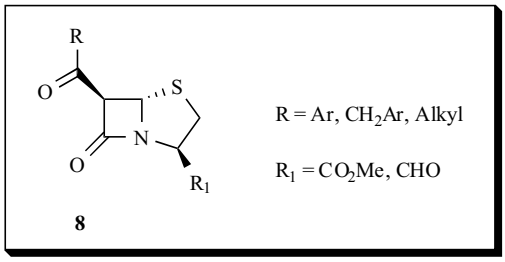

Fig. (8).

It is important to note that the activity of the β-lactams in control of bacteria does not appear to be confined to inhibition of peptidoglycan synthesis, but extends to the fundamental mechanism of inhibition of bacterial colonization, i.e. prevention of bacterial adherence. In order to cause disease, bacteria need to adhere to host tissue. Many pathogenic species of bacteria exhibit pili -extracellular protein organelles in order to attach themselves to host epithelial cells. Pilus assembly is accomplished by the action of periplasma chaperons, which bring pilin subunits to the outer cell membrane where they are incorporated into the growing pilus [25]. Inhibition of pilus formation by a pilicide could be used to prevent disease caused by piliated bacteria. Toward this end, penams with stereochemistry different than that of the original penicillins (**8**) have been designed to act as chaperone inhibitors [26]. This stereochemistry has been chosen in order to give these β-lactams the chance to withstand enzymatic degradation by penicillin-resistant bacteria. *In vitro*, the rigid β-lactam framework appears to mimic the peptides which are found to inhibit complex formation between PapD chaperone and the adhesin PapG [27]. However, *in situ* action awaits demonstration. β-Lactams have also been shown to be inhibitors of *E. coli* leader peptidase [28b]. This enzyme is an integral membrane protein, suggested to be a novel serine enzyme, which catalyzes the removal of the signal of leader sequence as one of the last steps of translocation of proteins across membranes [28a]. This transport process is similar in both prokaryotes and eucaryotes. Monocyclic β-lactams (**9**) have been demonstrated to be time dependent inhibitors of this enzyme [28b]. The core structure of evaluated compounds contains substituents at C3-position - short alkyl chains, a para-hydroxybenzoic acid at C4-position, and N-1 substituent, thus making them compatable to the inhibitory requirements for porcine pancreatic elastase and human leukocyte elastase (**10**). However, the C3- diethyl substituted β-lactams do not demonstrate activity as inhibitors of *E. coli* leader peptidase.

Inhibitors in the Kingdom Monera are not confined to the prokaryotes (bacteria). Cytomegalovirus (CMV) belongs to the family of human herpes viruses and is the major cause of blindness in HIV/ AIDS patients. The crystal structure of CMV protease [29], whose activity is essential for the proper assembly of the viral capsid [30 -32], indicates the presence of a unique protein fold and a catalytic triad previously unseen among serine proteases. NMR and mass spectrometry [33b, 34] suggest that β-lactams, which are hydrolyzed by CMV, could act as competitive inhibitors. The design and synthesis of monocyclic β-lactam derivatives as inhibitors of this enzyme presents an opportunity for the design of agents with a novel mechanism, which should lack the toxicity and limited effectiveness of current antiviral treatment, i.e. nucleoside and phosphonate CMV protease substrate analogs [35,36b, c, d, e]. Work toward this goal led to synthesis of peptidyl derivatives of monocyclic β-lactams (**11, 12**) which have been evaluated for their inhibitory activity [37a]. Based on the Structure Activity Relationships (SAR) that, resulted novel non-peptide series (**13-17**) of monocyclic β-lactams with an urea moiety at the N-atom of the β-lactam ring have been prepared [36b, c, d, e]. These appear to be inhibitors with good aqueous stability and good selectivity. Monocyclic β-lactams that contain a heterocycle such as 2-furyl, 2-thiophenyl, 4-methyl-2-tetrazole and 2-benzothiazole were found to possess activity in plaque reduction assays. It is interesting to note that in one member of the series (**13**),

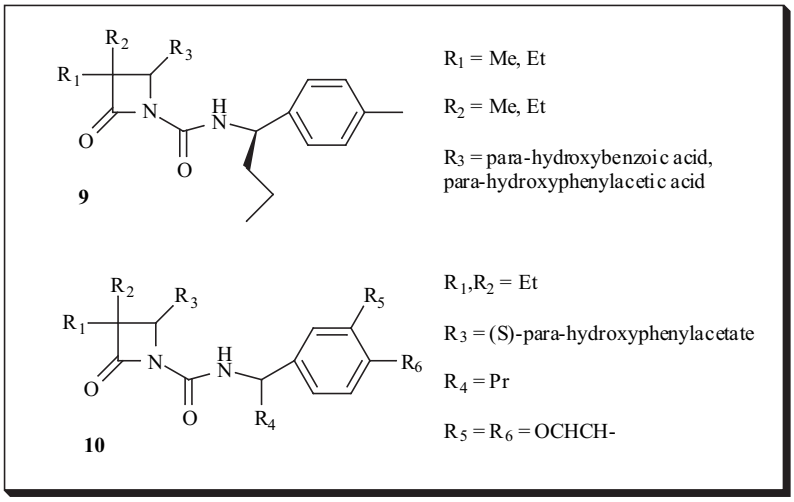

Fig. (9).

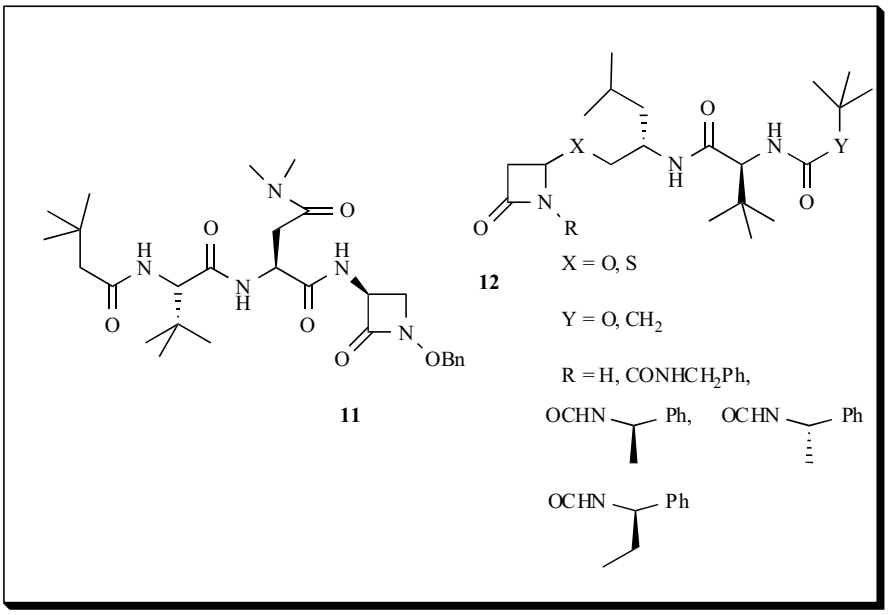

Fig. (10).

the C3α-position of the methyl group substituent led to the more active inhibitors (C4 substituent is β) [36b], while in the other series, the C3β-position of the methyl substituent led to inhibitors with high selectivity toward CMV protease [36c, d].

The antifungal activity of some cephalosporins was noted in the early 70's [38]. A series of 4-aryloxy- and 4-arylthioazetidin-2-ones, with no substituents at C3-position have been prepared and have proved effective against various genera of fungi, such as *Microsporon*, *Trichophyton, Candida* and *Phycomycetes* [39a, b]. In addition, some 3-halo- and 3-alkylazetidin-2- inhibitors have been reported effective against plant pathogenic fungi [39c, d]. Recently, synthetically prepared monocyclic and bicyclic β-lactams

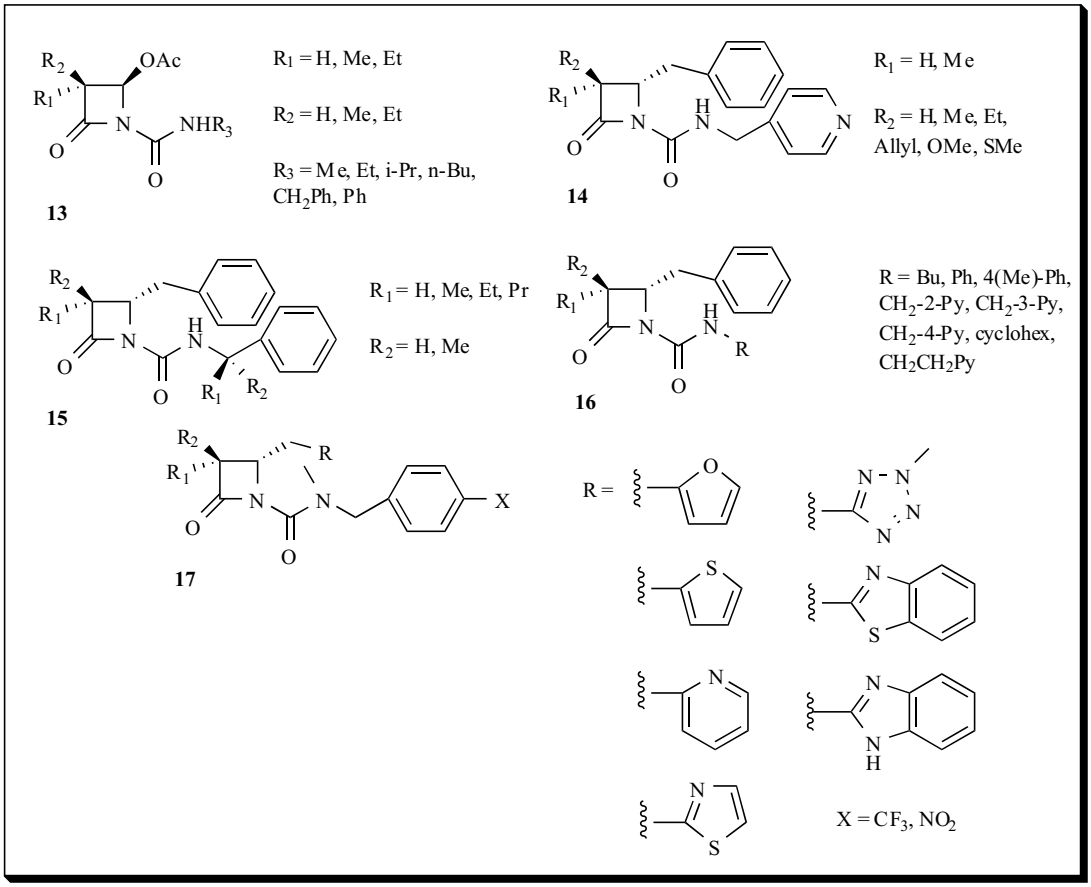

Fig. (11).

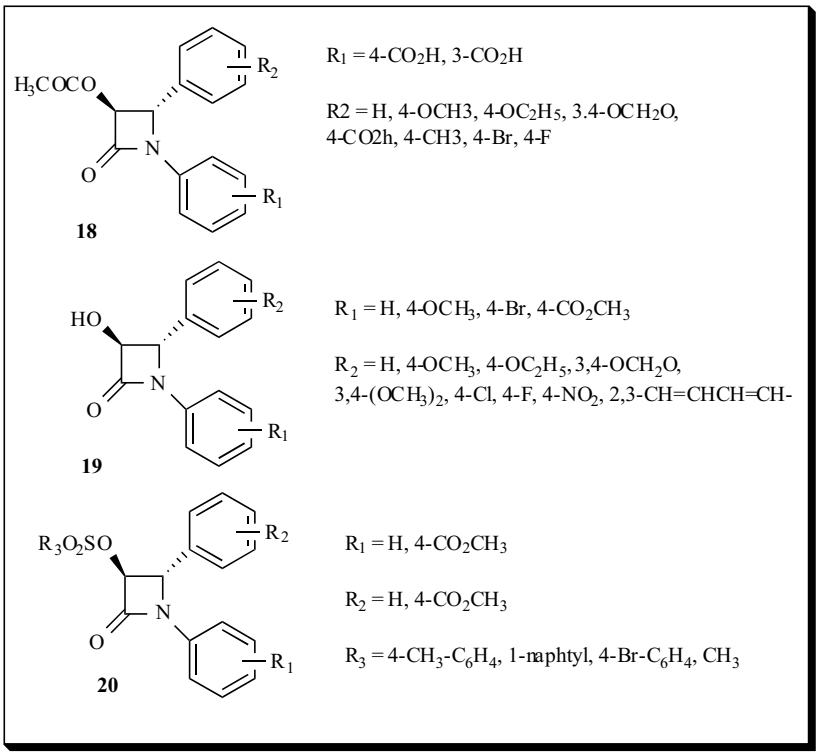

Fig. (12).

have shown promising antifungal activity [40]. The monocyclic structures contain an acetoxy-(**18**) or an hydroxy-(**19**) or sulfonyl (**20**) group at the C3-position and an aryl ring at N-1 position. While the C3-acetoxy-2-ones (**18**) with a free carboxylic acid on the aromatic ring at N-1 demonstrated broad spectrum antimicrobial activity. The monocyclic and bicyclic compounds containing an oxygen function at C3- position showed antifungal activity against several species of *Candida*. The mode of action of monocyclic β-lactam inhibitors appears to be through binding to the protease active site without inducing structural reorganization [41a]. In the monocyclic β-lactam series of inhibitors, in addition to the necessity for having a chemically reactive β-lactam ring, for which an electron-withdrawing group (EWG) appears to be necessary, the potential presence of a leaving group (LG) is designed to promote suicide-type irreversible inhibition of the corresponding serine enzyme [42]. Substituents at C3 that could provide specific enzyme recognition are also required. Monocyclic β-lactams have been functionalized with EWG at C4 [43, 44] and LG an N-1 [43c, 45] as well as at reverse positions types (**21**) and (**22**). Structures having both EWG and LG at position N-1 (**23**) and (**24**) have been synthesized and their inhibition potential examined [43b]. Monocyclic β-lactams with an aryl substituent at N-1 position, which are capable of releasing a latent quinonimmonium methide function into the enzymatic cavity upon opening of the azetidinone ring, have been proposed as human leukocyte elastase (HLE) inhibitors [46]. Similar structures, but with a methyl group at the ortho position to the β-lactam nitrogen instead of a potential LG have been shown to exhibit β-lactamase inhibition [46d].

HLE is produced to degrade structural proteins, such as elastin, fibronectin, and collagen [50]. Normally, the lungs are protected from excessive HLE expression by endogenous inhibitors. Lack of inhibition of HLE results in diseases, which include rheumatoid arthritis, pulmonary emphysema and cystic fibrosis [51]. Suicide HLE inhibitors of types (**25**) and (**26**) have been constructed [47, 48] in which the nucleophilic attack of the active site serine onto the β-lactam

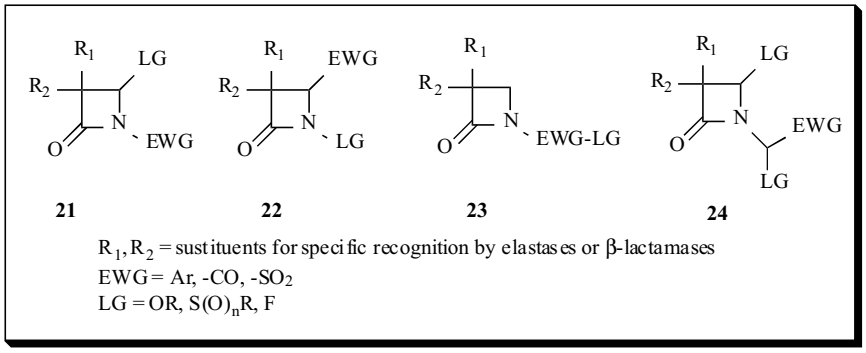

Fig. (13).

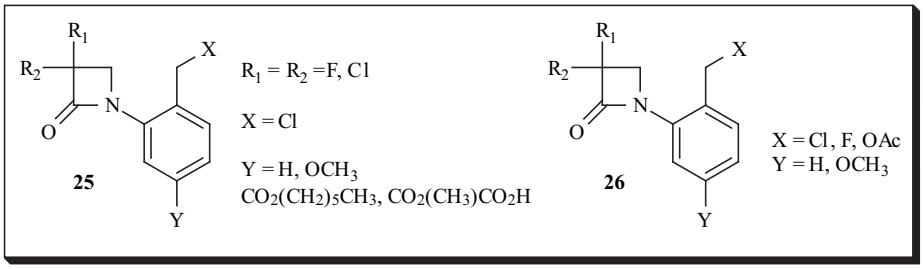

Fig. (14).

carbonyl, after the elimination of the LG, creates a Schiff base. The latter could react with a nucleophilic residue in the active site. Monocyclic β-lactams with a methyl or methoxy group in the para-position of the benzene ring connected to the urea nitrogen atom (of type **27**, **28** and **31**) have also been demonstrated to be potent inhibitors of HLE both *in vitro*, and *in vivo* [49a]. Recently, monocyclic structures with a methyleneaminoxy (MAOAs) LG at C4-position have been evaluated as HLE inhibitors [52]. Compounds with a diethyl-substituent at C3 have demonstrated activity *in vitro* and *in vivo*. This is yet a further confirmation of the importance of the presence and size of the substituent at C3-position in the monocyclic structures for molecular recognition by HLE.

Bicyclic β-lactams with therapeutic application for HLE operate by means of mechanisms resulting in the formation of hydrolytically stable-enzyme complexes. For HLE the requirements for molecular recognition are more defined in comparison to those for β-lactamases due to the mutation

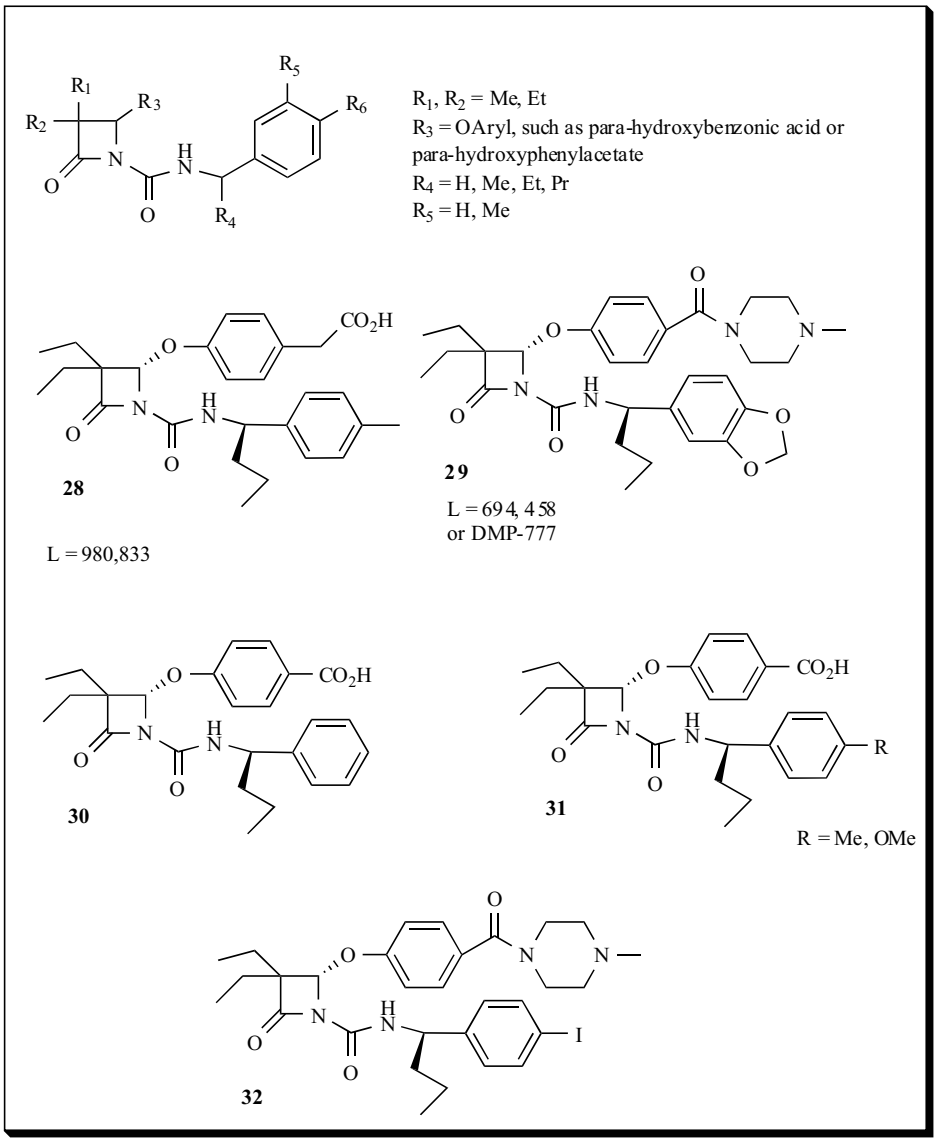

Fig. (15).

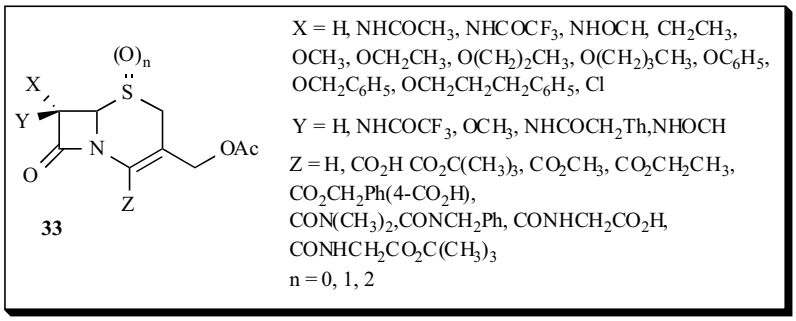

Fig. (16).

rate of the latter. SAR have revealed certain requirements for molecular recognition by HLE with regard to the type and stereochemistry of the substituents on the β-lactam ring. Generally, small groups no more than three carbon atoms long adjacent to the β-lactam carbonyl (at C7-α position in cephalosporins, C6-α position in penems and C3-α in monocyclic β-lactams, respectively) are necessary for specific binding in the active site of HLE [34a, 38]. Increasing the length of the alkyl group at this position leads to a rapid decrease in activity. This is possibly due to the preference of HLE to cleave proteins at sites having a relatively small alkyl substituent. This preference for α-over β-substituents in the C7- position for HLE is presumed to be due to the difference in the substrates of the PBPs and HLE. HLE cleaves L-L-amino acid peptide linkages, whereas the target enzymes for β-lactam antibiotics cleave at the D-D-amino-acid peptide bond [34a]. Another distinctive structural feature of the HLE inhibitors is the presence of a lipophilic ester as EWG [34a, 38], as compared to the carboxylic acid associated with the β-lactamase inhibitors [39]. As an endopeptidase HLE prefers to cleave amide bonds between non-ionic AA. In the series of type (**33**) HLE inhibitors, the sulfones have generally been found the most active members, with the sulfoxides and sulfides being considerably less active. Recently, evaluation of N-substituted piperazines and esters of hydrophobic amino acids as the required hydrophobic ester for HLE activity in both penam (**34**) and cephem (**35**) core structures has led to identification of novel inhibitors [40]. Selected 7-alkylidenecephalosporin esters have been reported to be potent HLE inhibitors [42b]. As early as 1965 [34f], certain side chains in the cephalosporins at the C3'-position were observed to depart spontaneously from the molecule upon hydrolysis of the β- lactam ring. In order to leave, the substituent must be able to accept electrons readily, so that it becomes a good leaving group (LG). The presence of free acetate during the hydrolysis of cephalosporin C or cephalotin, and the presence of pyridine from cephaloridine are due to their good LG ability. A necessary step for irreversible HLE inhibition proposed by Doherty *et al*. [34e] was the acetate elimination from the 3' position in the cephem sulfones. Interestingly, the biological evaluation of sodium 3'-substituted cephalosporinate sulfones (**36**), synthesized by analogy with sulbactam devoid of groups at the 7-position, has demonstrated that the LG ability of the group at 3'-position does not contribute to biological potency of these compounds. In another series of cephem sulfones without a good LG at the 3' position, but which have substituents such as spirocyclopropyl [48] (**37**) or thioethyl [49] (**38**) at the C-2 position did demonstrate elastase inhibition. Other possible variations of groups in the periphery of the β-lactam ring have been demonstrated by the elastase inhibitory activity of 7α-methoxy- (**39**) and 7α-chloro derivatives (**40**) of the cephem sulfones [50]. Reaction of clavams, ester derivatives of clavulanic acid, with elastase has been recently examined using nuclear magnetic resonance (NMR) and electrospray ionization mass spectrometry (ESIMS) techniques [37]. This analysis has shown that clavams form stable malonyl semi-aldehyde derivatives, which are analogs to the ones formed in the inhibition of β-lactamases by clavulanic acid. This provides yet another insight into possible ways to inhibit enzymes with active site serines. β-Lactams can also inhibit other eukaryotic serine proteases. β-Lactams that inhibit porcine pancreatic elastase (PPE) [52c], for example, have no β-lactamase or elastase activity [52a]. N-peptidyl derivatives of 4-phenylazetidin-2-one (**41**, **42**) as inhibitors of PPE and papain have been also synthesized [40]. The enzymatic assays have shown that some derivatives are effective inhibitors of PPE and /or papain. The derivatives with N-BOC protected amino acids (**41**), which lacked a spacer proved to be irreversible inhibitors of PPE. The presence of spacers for these compounds is detrimental to the PPE inhibitory activity, and the compounds show either weak or no inhibition of the enzyme. Papain is inactivated irreversibly by compounds with a spacer group, such as ethyl (*RS*)-2-oxo-4-phenylazetidin-1-acetate. The diastereomers of N-(2-oxo-4-phenylazetidin-1-acetyl)-L-alanyl- L-valine benzyl ester show the highest inhibitory activity for papain [65]. Recently, a C4 unsubstituted monocyclic 3-amino-β-lactam analog (**43**) of the dipeptide Phe-Gly methyl ester has been synthesized [63] and demonstrated to behave as a non-time-dependent inhibitor of α-Chym, carboxypeptidase Y and cathepsin G [64].

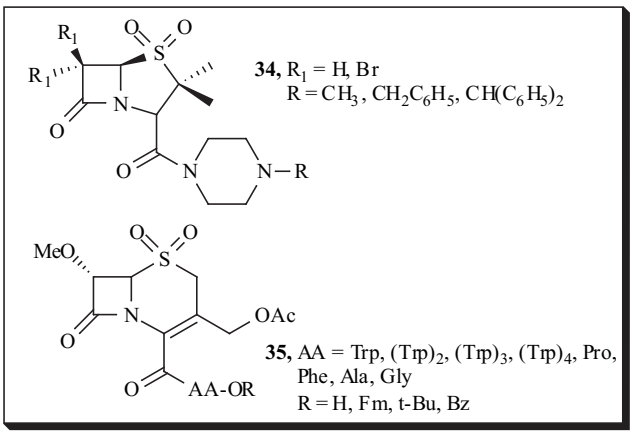

Fig. (17).

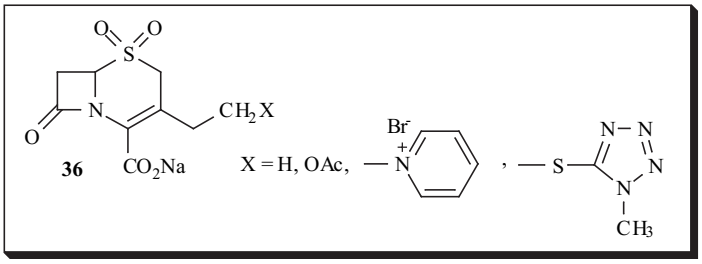

Fig. (18).

In the lymphoreticular and circulatory systems a variety of serine proteases and pathways for synthesis of serum components have been demonstrated to be affected by the β-lactams. Thrombin, the final product of the coagulation cascade, is activated by factor Xa-mediated cleavage of prothrombin [66]. Thrombin participates in both blood coagulation, as well as clot dissolution [67]. Because of its role in both venous and arterial thrombosis, inhibitors of thrombin are of clinical interest for the prevention and treatment of both types of thrombosis. The majority of thrombin inhibitors are arginine or lysine peptide derivatives [68]. Recently, monocyclic β-lactams derivatives (**44**), designed to contain structural elements that can be recognized by thrombin have been prepared [69]. Many of the compounds proved to be potent, time-dependent inhibitors of thrombin *in vitro*. In the presence of human plasma, a higher concentration of the compounds is necessary in order to achieve comparable inhibition. The main characteristic of these structures is that they contain an arginine-like substituent at C3, with an α-orientation. Compounds without this substituent are not active as thrombin inhibitors. The arginine-like side chain appears to act as the structural element necessary for molecular recognition by the trypsin-like serine enzymes. Future studies are directed toward modification of these monocyclic β-lactams in order to achieve enhanced plasma stability and greater enzyme specificity [69a].

The reduction of low-density plasma lipoproteins (LDL) is the current first-line therapy for atherosclerosis, the major cause of death in the western world [79]. This can be achieved by cholesterol biosynthesis inhibitors (HMG-CoA reductase inhibitors, the statins), bile acid sequestrates, HDL-elevating agents [80], and cholesterol absorbing agents [81]. In the last several years monocyclic β-lactams have been evaluated as members of the last category [82-87]. β-Lactams involved in cholesterol absorption are highly substituted with aromatic rings either directly attached to the β-lactam ring, or through an S-atom. The oxidation state of sulfur is either a sulfide or sulfoxide. In addition, most known cholesterol absorption inhibitors (**45-49**) have aromatic substituents at the C3, C4, and N-1 positions [83-87]. Usually, (**45, 46, 48, 49**) the aromatic substituent is attached though a carbon chain to the C3α-position [84, 86, 87]. In cases where the aromatic ring is directly attached to the C3-position (**47**), its orientation is β to the four-membered ring [82c, 85]. The specific stereochemistry has

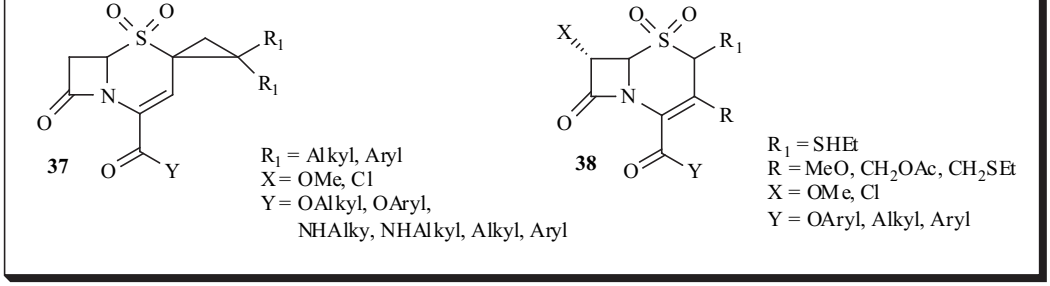

Fig. (19).

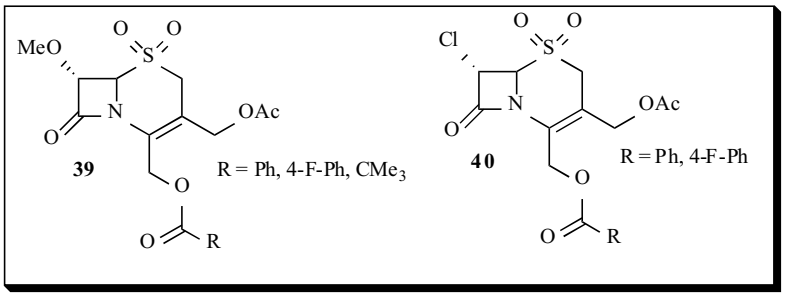

Fig. (20).

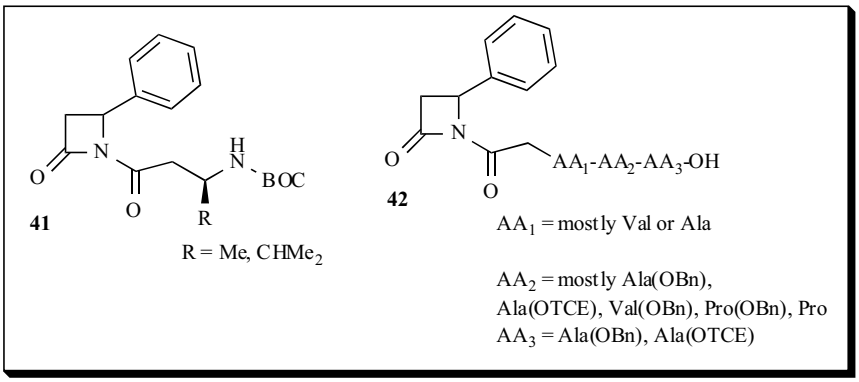

Fig. (21).

been achieved by employing different asymmetric approaches [85]. EWG substituents, such as Cl or F-atoms at para -position (**46, 49**) of the aromatic rings appear to increase the potency of the compounds as inhibitors. Monocyclic structures not containing substituents at C3, or having halogen(s) or alkyl group(s) at C3-position show good inhibitory activity against phospholipase A2 enzyme Lp-PLA2 and have been proposed for treatment of atherosclerosis [83].

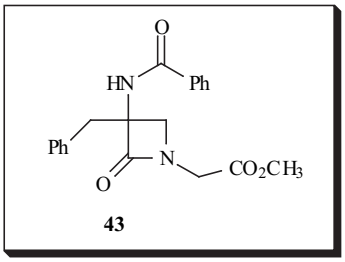

Fig. (22).

β-Lactams have also been demonstrated to exhibit analgesic and anti-inflammatory activity due, in part, to their ability to inhibit coenzyme A-independent transacylase and cytosolic phospholipase A2 which would enhance their activity as anti-atherogenic agents [84c, 90, 95]. The coenzymeA-independent transacylase (CoA-IT) is an enzyme responsible for remodeling of arachidonate between different phospholipids [88] and seems to specifically remodel arachidonate and other long-chained unsaturated fatty acids. It has been shown that the CoA-IT mediates arachidonate movement. This is important for several functions of inflammatory cells, including platelet activating factor (PAF), release of free arachidonic acid and production of prostanoids [89a]. A hypothesis exists that a CoA-IT is a member of a family of transacylases, typified by lectin cholesterol acyl-transferase [89b]. According to this hypothesis, an active site nucleophile in CoA-IT attacks an acyl carbonyl of the sn-2 arachidonate of phospholipids, leading to formation of a covalent interaction between arachidonate and CoA-IT. The covalently attached arachidonate can later be donated to a lyso phospholipid acceptor. Based on the proposed catalytic mechanism it is possible that monocyclic β-lactams may be utilized as acylating agents toward this enzyme. A library of monocyclic compounds has been synthesized and tested as CoA-IT inhibitors. Those compounds found to be inhibitors (**50-54**) feature triphenyl substituent at C3-position and an ester or carboxylic acid attached to the C4- position via an ether linkage [84c]. These monocyclic structures interact with CoA-IT as irreversible inhibitors, thus supporting the proposed mechanism of action of CoA-IT. A bicyclic penam with a peptide chain at C6β-position (**53**) has been found to be a cytosolic phospholipase A2 (cPLA2) non-time-dependent inhibitor [90]. cPLA2 catalyzes the hydrolysis of the sn-2 arachidonate of pospholipids. The enzyme is calcium-dependent and is located in several types of tissues and cells, such as monocytes, neutrophils, and platelets [91]. The catalytic mechanism of cPLA2 is believed to be similar to that of serine proteases. The enzyme forms an acyl enzyme intermediate between arachidonate of the phospholids and the active-site serine [92]. Compound (**53**) shows high specificity toward the cPLA2 phospholipase.

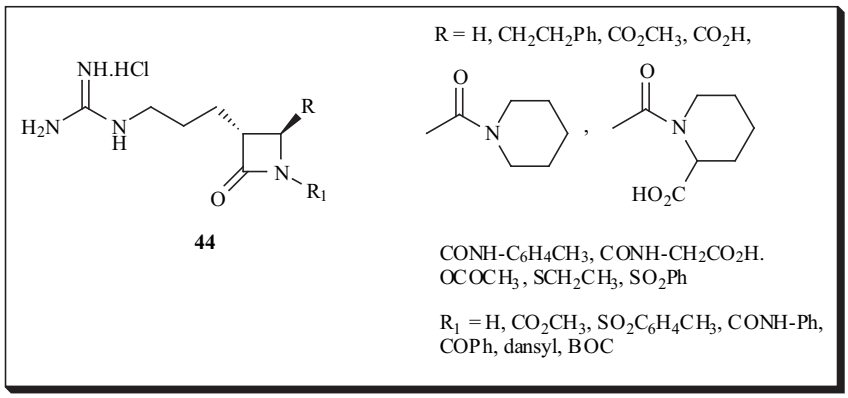

Fig. (23).

The observation that the degradation of this inactivator was not catalyzed by the enzyme led to the assumption that the inhibition does not result from the formation of acyl-enzyme intermediate. Interestingly, the ring-opened form of (**53**) inactivated the enzyme with similar potency. Another type of monocyclic β-lactam (**54**) with anti-inflammatory and analgesic activity has also been reported [93]. These β-lactams do not contain the C4-substituent, but instead have two substituents at C3-position. One is a methyl group and the second either methyl or chloromethyl. They also have chlorosubstituted phenyl ring(s) with or without a spacer at N-1, respectively. All compounds tested have shown *in vivo* anti-inflammatory and analgesic activity in rats. The two most active compounds have displayed efficacy (15mg/kg) at a dose above that of the indomethacin (5mg/kg) the reference compound. The mechanism of anti-inflammatory action of this type of monocyclic β-lactams is not known. One hypothesis is that the activity is due to anti- HLE activity. Developing specific inhibitors might help to differentiate between these two effects and lead to a new generation of anti-inflammatory agents.

Monocyclic β-lactams with no substituent at C3-position, a thiophenyl substituent at C4 position (**55 - 57**), and a benzyl group connected through a ketone to the β-lactam N-atom, have been recently synthesized and evaluated as inhibitors of LDL phospholipase A2 [95] as racemic mixtures and pure *R* and *S* enantiomers. LDL phospholipase A2, also known as platelet activating factor (PAF) acetyl hydrolase, is involved in degrading PAF and hydrolyzing phosphatidylcholines [94]. It appears that this enzyme acts both as an anti-inflammatory and a pro-inflammatory enzyme, respectively. Findings indicate that the *R*-enantiomer is a better inhibitor than the *S*-enantiomer. It seems that the mechanism of inhibition of this enzyme by β-lactams is similar to the mechanism of inhibition of β-lactamases by β-lactams in that both involve slow hydrolysis of substrates through a branched pathway leading to a more stable covalent enzyme intermediate. Beside the lymphoreticular and circulatory systems being impacted other organ systems can be targeted by the β-lactams including the central nervous system, and the urogenital tract.

γ-Aminobutyric acid (GABA) is an inhibitory transmitter in the mammalian central nervous system. Many studies have been performed in order to better understand the role of GABA in causing convulsions [70]. It is known that inhibition of GABA aminotransferase, the enzyme that catalyzes the degradation of GABA, can produce an anticonvulsant effect [71]. Currently, an irreversible inhibitor of this enzyme, vigabartin (γ-vynyl GABA) is used in the treatment of convulsions, but not without side effects. There exist structural similarities between monocyclic and bicyclic β-lactams to γ-aminobutyric acid (**58**) and to the known substrates and inhibitors of GABA aminotransferase (**60**). A series of β-lactam compounds (**59, 61**) have been evaluated as GABA aminotransferase inhibitors [72]. One characteristic of this type of β-lactam is the presence of a positively charged amino group at the carbon adjacent to the β-lactam carbonyl. Another is the presence of EWG, directly attached to the N-atom, as in monobactams, or a carboxyl group, attached to N-1-position through one carbon spacer. The combination of a positively charged amine and sulfate group mimics the time-dependent inactivator of GABA, vigabartin [73], and the combination of positively charged amine and carboxylic acids mimics GABA itself. In testing, all of the β-lactams have been demonstrated to be competitive

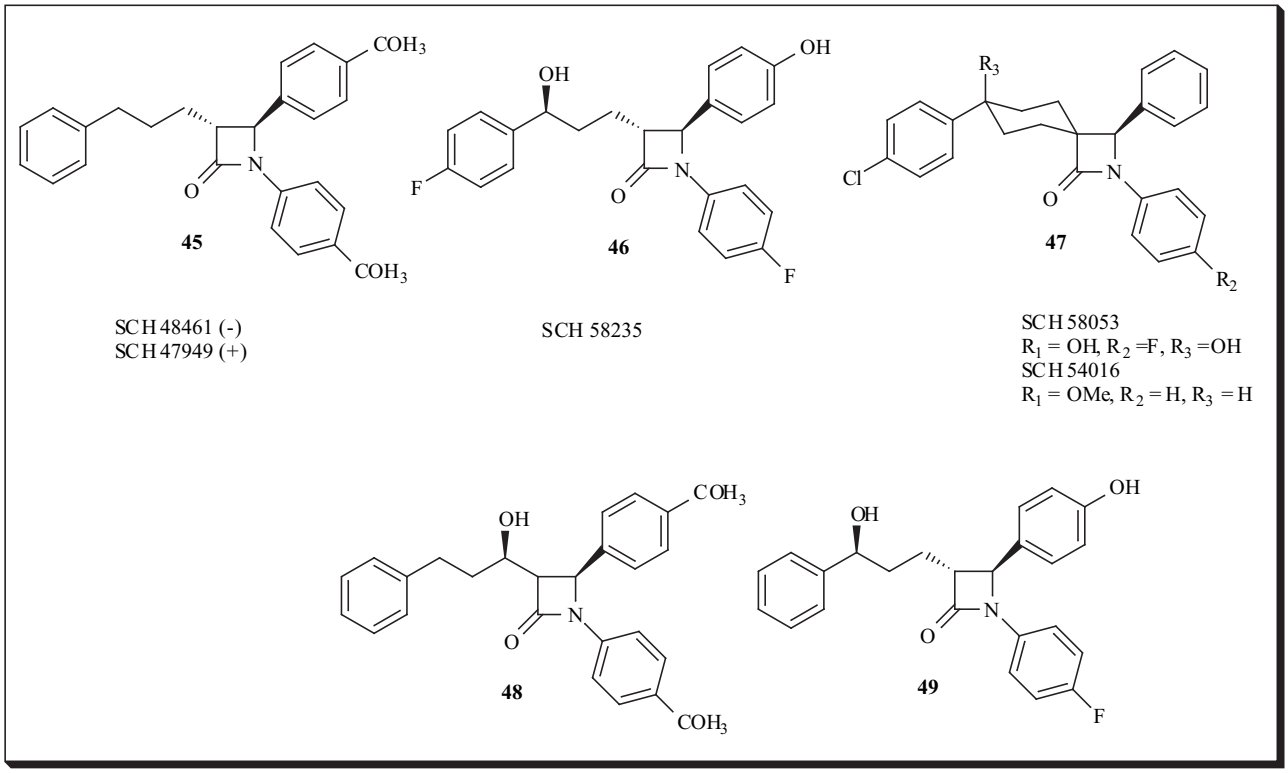

Fig. (24).

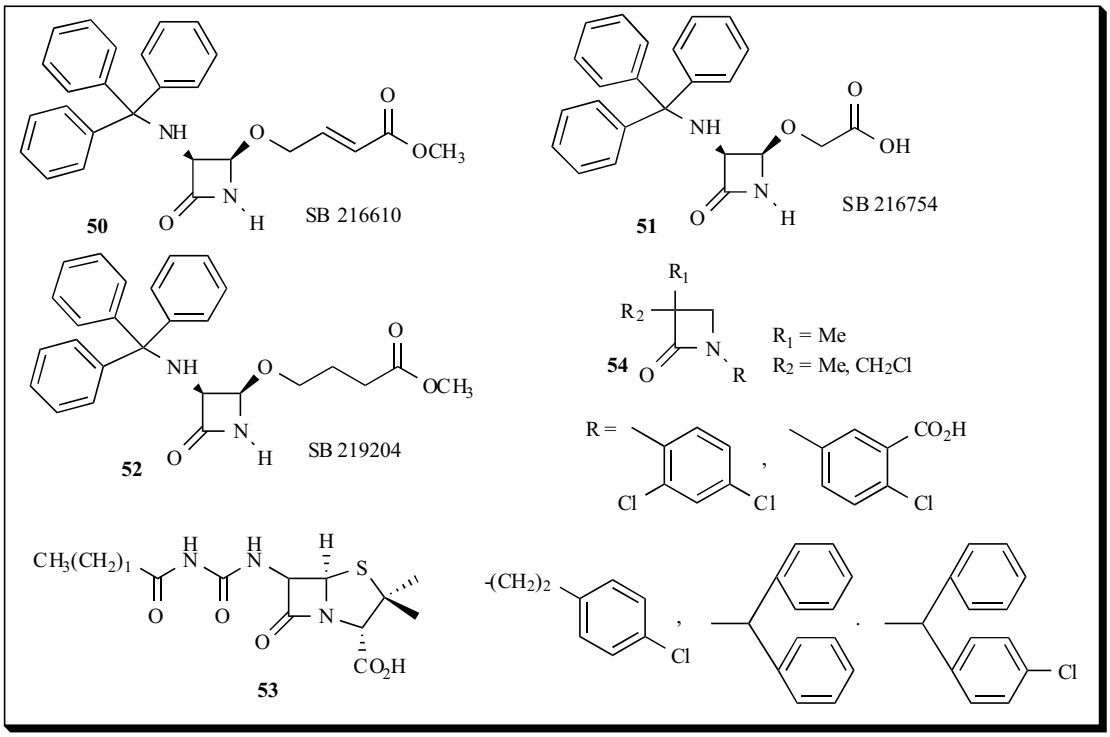

Fig. (25).

inhibitors of GABA aminotransferase. The stereochemistry of aminosubstituent in both series is important, as the compounds with a β-aminosubstituent are more potent GABA inhibitors in comparison to either α-enantiomer or α-diastereomer, respectively. None of the β-lactams act as irreversible inactivators. One explanation as to why the examined structures do not irreversibly inhibit the enzyme is that they bind to the GABA aminotransferase active site but do not acetylate the enzyme, possibly because of the lack of a nucleophile properly situated to attack the β-lactam carbonyl. It is worth noting that cephalosporins have been well known to induce convulsions. Recently, it has been proposed that cephalosporins bind to the GABA receptor [74], rendering the inhibitory transmitter GABA incapable of binding to its receptor, resulting in convulsions.

In the urinary tract, prostate specific antigen (PSA) is a serine protease that has been used as a diagnostic marker for prostate carcinoma [75]. PSA exerts proteolytic activity on insulin-like growth factor binding protein-3, which might contribute to the malignant growth in the prostate in males [76]. In addition it has been shown that PSA may play a role in breast tumors [77]. Therefore, development of inhibitors of PSA is of great interest from a standpoint of organic synthesis. Monocyclic β-lactams have been designed (based on peptide mapping and Electrospray Ionization Mass Spectrometry, ESI-MS, studies), as well as synthesized and evaluated as PSA inhibitors [78]. These inhibitors (**62**) feature a benzyl substituent at C3-position, a benzyl ester at C4-position and a substituent containing two aromatic rings at N-1. The extended N-1 side chain has been found to be essential for PSA inhibition. The absence of the second aromatic ring leads to inactive compounds. The para-hydroxyl group on the benzyl moiety has led to the development of improved PSA inhibitors.

PEPTIDE INHIBITORS: SERPINS AND OTHER PEPTIDE INHIBITORS

Serpins (serine protease inhibitors) and other peptide protease inhibitors such as the macrocycles and subtilisin act

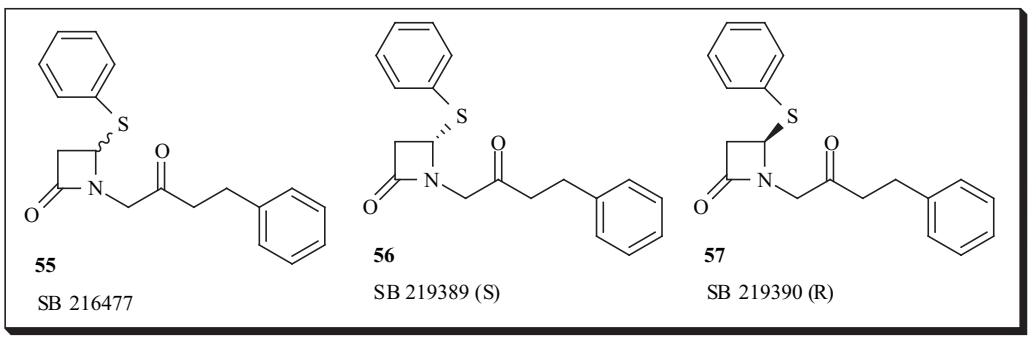

Fig. (26).

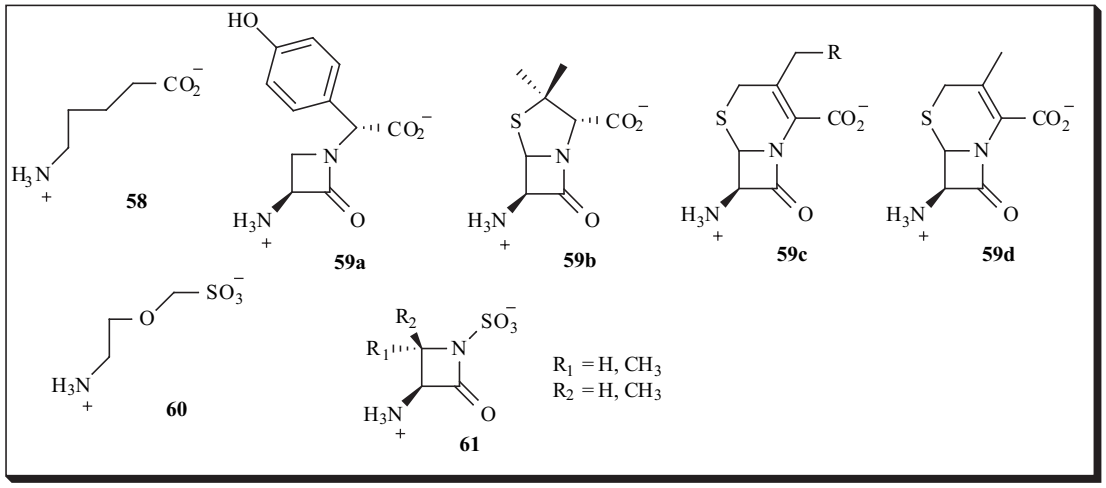

Fig. (27).

by binding to serine proteases in an extended conformation, e.g. the antiparallel beta-sheet structure formed between caspases and inhibitor. Therapeutically, peptide inhibitors may have utility in the treatment of pulmonary destructive diseases including emphysema, type I hypersensitivity, atherosclerosis, cancers, neurodegenerative disorders and certain viral infections. Serpins inhibit the activity of both circulating and tissue proteases by acylating the serine hydroxyl at the active site [1]. The work of Irving *et al*. [2] suggests phylogenetic relationships and conserved residues in the mechanism of conformational change, which are triggered in the serpin while maintaining the covalent linkage between the protease and serpin. In a scenario parallel to that described for the β-lactam protease inhibitors, peptide-based inhibitors, which exhibit activity against a diverse range of proteases, have the best activity when configured in an entropically advantaged rigid conformation.

The superfamily of serpins is exceptionally broad paralleling in that respect the activity of the β-lactams. There is evidence of some 500 serpins divided into ~16 clans based on phylogenetic analysis [6]. Serpins have been demonstrated to regulate a variety of mammalian processes. The pharmacological agents 4-(2-aminoethyl)-benzenesulfonylfluoride hydrochloride (AEBSF) and Nα-p-tosyl-L-lysine chloromethylketone (TLCK), inhibit trypsin-like serine proteases, and prevent the death of trophic factor-deprived PC12 cells and sympathetic neurons by inhibiting caspase activation. These serine protease inhibitors act at a point upstream in the apoptotic pathway, prior to p53 induction and the mitochondrial checkpoint, to delay neuronal death in this model, and do not act at the level of the caspases. Therapeutic strategies based on this serine protease inhibition may be useful in preventing neuronal cell death [95].

The complement system, a major mediator of innate immune defense, functions in the recognition, then opsonization or lysis, of microorganisms including bacteria and yeast, as well as host cell debris and altered host cells. After recognition, which occurs by the binding of complement proteins to charge or saccharide arrays, a series of serine proteases is activated, culminating in the assembly of complex unstable proteases called C3/C5 convertases. When activated, the proteases are regulated, like many plasma serine proteases, by a serpin, C1-inhibitor, C2 and Factor B [96].

Serpins have also been isolated from pathogens. The swinepox virus is the etiologic agent of a worldwide disease specific for swine [97]. Another viral zoonotic agent, myxoma virus the causative agent of myxomatosis, a fatal disease of the European rabbit has been demonstrated to produce three serpins. SERP-1 binds to several targets and is an anti-inflammatory molecule. SERP-2 is essential for virus virulence and has both anti-inflammatory and anti-apoptotic effects, while SERP-3 exhibits a similarity to poxvirus late promoters. Regardless of viral origin, the presence of serpins appears to be essential for virulence [98].

The essential nature of serpins to virulence in pathogens is not limited to the viruses. In addition to organisms in the Kingdom Monera affected by serine protease inhibitors, those of the Kingdom Protista are likewise impacted. Molecular cloning has resulted in the characterization of a

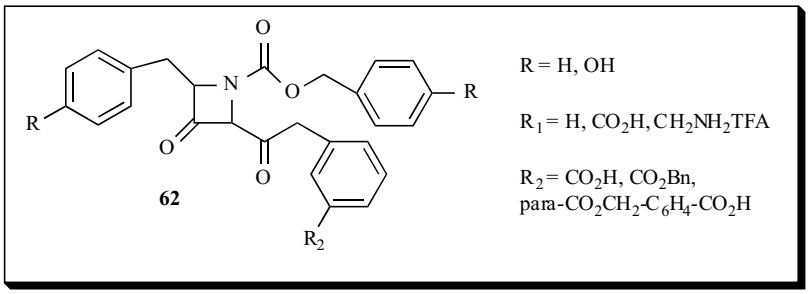

Fig. (28).

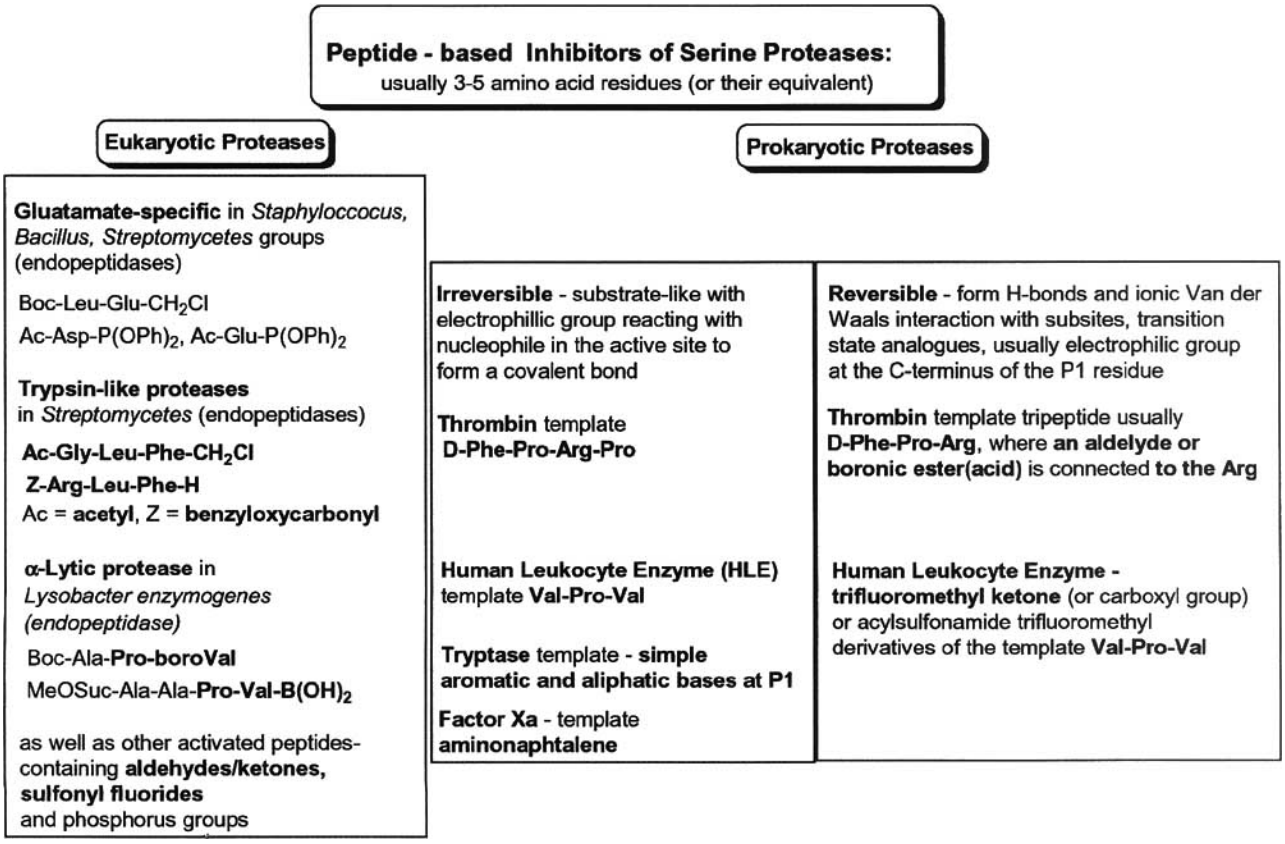

Fig. (29).

serine proteinase inhibitor from *Trichinella spiralis* [99]. For both the parasites *Plasmodium*, the etiologic agent of malaria, and *Toxoplasma gondii* serine protease inhibitors have been demonstrated to play a role in morphogenesis. Infection of mice with the parasite *Plasmodium* leads to a thrombocytopenia, eventually associated with a syndrome of severe or cerebral malaria. Treatment of infected mice with the caspases inhibitor ZVAD-fmk decreased the thrombocytopenia, and a decrease in the cerebral malaria associated mortality [100].

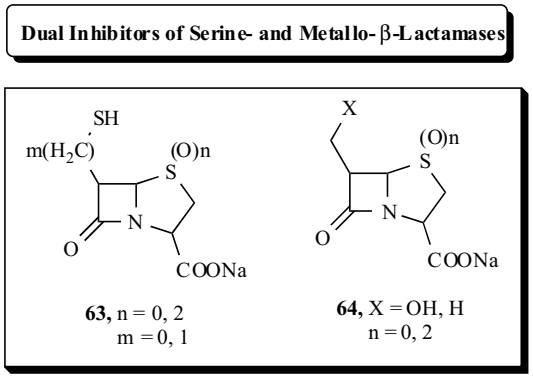

Fig. (30).

Toxoplasma gondii has a broad host-range including man and a variety of warm-blooded animals. The ability to infect and survive in this wide spectrum of hosts suggests highly evolved mechanisms to handle the harsh environments encountered. The high survival rate of the parasite in the upper gastrointestinal tract may be enhanced by the presence of the TgTI-molecule, an inhibitor of trypsin associated with the surface of *T. gondii*, TgTI. However, this becomes a disadvantage and appears to be down-regulated during the intracellular stages of its life cycle since a number of serine protease inhibitors have been demonstrated to block the intracellular growth and replication of *Toxoplasma gondii* tachyzoites. These include cathepsin inhibitor III, TPCK and subtilisin inhibitor III, which caused a breakdown of the parasite surface membrane, and disrupted rhoptry formation [101, 102]. Thus, organisms can posses peptide protease inhibitors of a variety of chemical configurations including serpins and subtilisins.

Subtilisins, as measured by the inhibitor constant, are an example of the basic conformational flexibility of a protein core and the degree of its tolerance of an amino acid replacement. This was amply demonstrated by *Streptomyces* subtilisin inhibitor, where single amino acid mutations of Met103 in the hydrophobic core caused little change in the inhibitory activity. *Streptomyces* are known to produce a family of dimeric serine protease inhibitors, called the SSI family inhibitors [103]. They have a common structural design but consist of a variety of amino acid sequences, which have a unique homodimeric structure with 11.5-kDa subunits. The results also reveal the crucially designed structural relationship between the core of the inhibitor and the enzyme-binding segment with the reactive site in a serine protease inhibitor. [104] This is in contrast to the dual inhibitors, which have the same function (proteases) yet can be inhibited by inhibitors that can combine features for recognition by both serine and metallo-enzymes. However, regardless of the fact that the active sites of serine and

metallo-proteases are quite dissimilar (the MMP active sites are broad and shallow, while the serine enzymes have a deeper narrow cleft), it has been demonstrated that it is possible to design an inhibitor for both type of enzymes, more specifically for both types of β-lactamases [105].

CONCLUSION

It has been demonstrated in the last several years that aspartic, serine, cysteine, and metallo-proteases bind their inhibitors/substrates in extended or beta-strand conformations - the peptide backbone or equivalent is drawn out in a linear arrangement. This common conformational requirement for recognition by proteases suggests a fundamental platform for the building of inhibitors regardless of their resultant structure (β-lactam based or peptide based) is dependent on developing conformationally restricted inhibitors that adopt receptor-binding conformation, and are therefore entropically advantaged for binding to a protease. Most of the many thousands of protease inhibitors that have been developed so far are relatively flexible molecules that have to use energy to rearrange into a protease-binding conformation. Entropically advantaged compounds, containing adequate levels of potential energy required to drive the refolding of the structures to confirmations adequate for active site fit, appear to be the fundamental requirement for serine protease inhibitors regardless of class. A rigid confirmation is one of the common threads for both β-lactam and peptide-based inhibitors because they are entropically advantaged. However, given this basic requirement selectivity has been difficult to achieve based on the similarity in the active sites. This double-edged sword will make the future development of inhibitors at once both easier, and more difficult because of the potential for inflicting the very damage the use of these inhibitors is designed to prevent.

ABBREVIATIONS

EWG	=	Electron Withdrawing Group
ESI-MS	=	Electrospray Ionization Mass Spectrometry
GABA	=	γ-Aminobutyric Acid
HCMV	=	Human Cytomegalovirus Protease
HDL	=	High-density Plasma Lipoproteins
HLE	=	Human Leukocyte Elastase
LDL	=	Low-density Plasma Lipoproteins
LG	=	Leaving Group
MDR	=	Multidrug Resistance
PMP	=	para- MethoxyPhenyl
PPE	=	Porcine Pancreatic Elastase
PSA	=	Prostate Specific Antigen
SAR	=	Structure Activity Relationship

REFERENCES

[1] Ye, S.; Goldsmith, E.J. *Curr. Opin. Struct. Biol*. **2001,** *11*, 740.

[2] Irving, J.A.; Pike, R.N.; Lesk, A.M.; Wisstock, J.C. *Genome Res.* **2000**, *10*, 1845.

[3] Powers, J.C.; Asgian, J.L.; Ekici, O.D.; James, K.E. *Chem. Rev*. **2002**, *102*, 4639.

[4] Copley, R.R.; Schultz, J.; Ponting, C. P.; Bork, P. *Curr. Opin. Struct. Biol.* **1999,** *9*, 408.

[5] Ponting, C.P.; Aravind, L.; Schultz, J.; Bork, P.; Koonin, E.V. *J. Mol. Biol.* **1999,** *289*, 729.

[6] Whelan, K.F.; Sherburne, R.K.; Taylor, D.E. *J. Bacteriol.* **1997,** *179*, 63.

[7] (a) Silverman, G.A.; Bird, P.I.; Carrell, R.W.; Church, F.C.; Coughlin, P.B.; Gettings, P.G.W.; Irving, J.A.; Lomas, D.A.; Luke, C.J.; Moyer, R.W.; Pemberton, P.A.; Remold-O'Donnell, E.; Salvesen, G.S.; Travis, J.; Whisstock, J.C. *J. Biol. Chem.* **2001**, *276,* 33293. (b) Gettings, P.G.W. *Chem. Rev*. **2002**, *102*, 4751.

[8] (a) Massova, I.; Mobashery, S. *Antimicrob. Agents Chemother*. **1998**, *42*, 1. (b) Massova, I.; Mobashery, S. *Acc. Chem. Res*.. **1997**, *30*, 162. (c) Bulychev, A.; Massova, I.; Miyashita, K.; Mobashery, S. *J. Am. Chem. Soc.* **1997**, *119*, 7619. (d) Golemi, D.; Maveyraud, L.; Vakulenko, S.; Tranier, S.; Ishiwata, A.; Kotra, L.P.; Samam, J.-P.; Mobashery, S. *J. Am. Chem. Soc.* **2000**, *122*, 6132.

[9] (a) Barrett, A.J.; Rawlings, N.D. *Archiv. Biochem. Biophys*. **1995**, *318*, 247. (b) Rawlings, N.D.; Barrett, A. J. *Biochem. J.* **1993**, *290*, 205.

[10] (a) Krem, M.M.; Di Cera, E. *EMBO J*. **2001**, *20*, 3036. (b) Krem, M.M.; Rose, T.; Di Cera, E. *TCM*, **2000**, *10*, 171.

[11] (a) Nduwimana, J.; Guenet, L.; Dorval. I.; Blayau, M.; Le Gall, J.Y.; Le Treut, A. *Ann. Biol. Clin*. **1995**, *53*, 251. (b) Gojobori, T.; Ikeo, K. *Phil. Trans. R. Soc. Lond.(B)* **1994**, *344*, 411. (c) Ikeo, K. Takahashi K.; Gojobori, T. *J. Mol. Evol*. **1995**, *40*, 331. (d) Murzin, A.G. *Curr. Opin. Struct. Biol*. **1998**, *8*, 380. (e) Tong, L.; Qian, C.; Massariol, M.-J.; Deziel, R.; Yoakim, C.; Lagace, L. *Nature Struct. Biol*. **1998**, *5*, 819. (f) Otlewski, J.; Apostoluk, W. *Acta Biochimica Polonica* **1997**, *44*, 367. (g) Otlewski, J.; Krowarsch, D.; Apostoluk, W. *Acta Biochimica Polonica* **1999**, *46*, 531.

[12] Leung, D.; Abbenante, G.; Fairlie, D.P. *J. Med. Chem*. **2000**, *43*, 305.

[13] Sanders, C.C.; Sanders, W.E. *Clin. Inf. Dis.* **1992**, *15*, 824.

[14] Knott-Hunziker *Biochem. J*. **1982**, *207*, 315.

[15] Bush, K. *Antimicrob. Chemoth.* **1989**, *24*, 831-840.

[16] Knowels, J.R. *Acc. Chem. Res*. **1985**, *18*, 97.

[17] (a) Bush, K.; Mobashery, S. In *Resolving the antibiotic paradox: Progress in Understanding Drug Resistance and Development of new antibiotics*; Rosen, B. P.; Mobashery, S. Eds.; Plenum Press: New York, **1998**; pp. 71-98. (b) Bush, K.; Jacoby, G.A.; Medeiros, A.A. *Antimicrob. Agents Chemother*. **1995**, *39*, 1211.

[18] Herzberg, O.; Moult, J. *Curr. Opin. Struct. Biol*. **1991**, *1*, 946; *Science* **1987**, *236*, 694.

[19] Maiti, S.N.; Phillips, O.A.; Micetich, R.G.; Livermore, D.M. *Curr. Med. Chem*. **1998**, *5*, 441.

[20] Bitha, P.; Li, Z.; Fransisco, G.D.; Rasmussen, B.A.; Lin, Y-I. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 991; *Bioorg. Med. Chem. Lett.* **1999**, *9*, 997.

[21] WO 98/47895, Maiti, S.; Setti, E.L.; Phillips, O.; Reddy, A.V.; Micetich, R.; Singh, R.; Higashitani, F.; Kunugita, C.; Nishida, K.

[22] (a) Heinze-Krauss, I.; Angehrn, P.; Charnas, R.L.; Gubernator, K.; Gutknecht, E.M.; Hubschwerlen, C.; Kania, M.; Oefner, C.; Page, M.G.P.; Sogabe, S.; Specklin, J.L. Winkler, F. *J. Med. Chem*. **1998**, *41*, 3961. (b) Hubschwerlen, C.; Angehrn, P.; Gubernator, K.; Page, M.G.P.; Specklin, J.L. *J. Med. Chem*. **1998**, *41*, 3972.

[23] Faraci, S.W.; Pratt, R.F. *Biochemistry* **1985**, *24*, 903.

[24] De Angelis, F.; Attorrese, G.; Cavicchio, G.; Ciampa, S.; Di Tullio, A.; Fattori, D.; Nicoletti, R.; Domenici, E. *Eur. J. Org. Chem*. **2001**, 3075.

[25] Hultgren, S.J.; Abraham, S.; Caparon, M.; Falk, P.; St. Geme, J.W. III; Normark, S. *Cell* **1993**, *73*, 887.

[26] Emtenas, H.; Soto, G.; Hultgren, S.J.; Marshall, G.R.; Almqvist, F. *Org. Lett*. **2000**, *2*, 2065.

[27] Flemmer Karlsson, K.; Walse, B.; Drakenberg, T.; Roy, S.; Bergqust, K.-E.; Pinkner, J.S.; Hultgren, S.J.; Kihlberg, J. *Bioorg. Med. Chem*. **1998**, *6*, 2085.

[28] (a) Randall, L.L.: Hardy, J.S.; Thom, J.R. *Annu. Rev. Microb*. **1987**, *41*, 507. (b) Kuo, D.; Weidner, J.; Griffin, P.; Shah, S.K.; Knight, W.B. *Biochemistry* **1994**, *33*, 8347.

[29] (a) Tong, L.; Qian, C.; Massariol, M.-J.; Bommeau, P.; Cordinglry, M.; Lagace, L. *Nature* **1996**, *383*, 272. (b) Qiu, X.; Culp, J.S.; DiLella, A.G.; Hellmig, B.; Hoog, S.S.; Janson, C.A.; Smith, W.W.; Abdel-Mequid, S.S. *Nature* **1996**, *383*, 275. (c) Shieh, H.-S.; Kurumball, R.G.; Stevens, A.M.; Stegeman, R.A.; Sturman, E.J.; Pak, J.Y.; Wittwer, A.J.; Palmier, M.O.; Wiegand, R.C.; Holwerda, B.C.; Stallings, W.C. *Nature* **1996**, *383*, 279. (d) Chen, P.; Tsuge, H.; Almassy, R.J.; Gribskov, C.L.; Katoh, S.; Vanderpool, D.L.; Margosiak, S.A.; Pimnko, C.; Kan, C.-C. *Cell* **1996**, *86*, 835.

[30] (a) Howerda, B.C. *Antiviral Res*. **1997**, *35*, 1. (b) Gibson, W.; Welch, A.R.; Hall, M.R.T. *Perspect. Drug. Discov. Des*. **1994**, *2*, 413.

[31] Trus, B.L.; Booy, F.P.; Newcomb, W.W.; Brown, J.C.; Homa, F.L.; Thomsen, D.R.; Steven, A.C. *J. Mol. Biol.* **1996**, *263*, 447.

[32] (a) Gao, M.; Matusick-Kumar, L.; Hurlburt, W.; DiTusa, S.F.; Newcomb, W.W.; Brown, J.C.; McCann, P.J. III; Deckman, I.; Colonno, R.J. *J. Virol*. **1994**, *68*, 3702. (b) Matusick-Kumar, L.; McCann, P.J. III; Robertson, B.J.; Newcomb, W.W.; Brown, J.C.; Gao, M. *J. Virol*. **1995**, *69*, 7113.

[33] (a) LaPlante, S.R.; Bonneau, P.R.; Norman, A.; Cameron, D.R.; Deziel, R.; Grand-Maitre, C.; Plouffe, C.; Kawai, S.H. *J. Am. Chem. Soc*. **1999**, *121*, 2974. (b) Bonneau, P.R.; Hasani, F.; Plouffe, C.; Malenfant, E.; LaPlante, S.R.; Guse, I.; Ogilvie, W.W.; Davidson, W.C.; Hopkins, J.L.; Morelock, M.M.; Cordingley, M.C.; Deziel, R. *J. Am. Chem. Soc*. **1999**, *121*, 2965.

[34] Haley, T.M.; Angier, S.J.; Borthwick, A.D.; Montgomery, D.S.; Purvis, I.J.; Smart, D.H.; Bessant, C.; Van Wely, C.; Hart, G.H. *J. Mass Spectrom*. **1998**, *33*, 1246.

[35] Goodgame, R. W. *Ann. Intern. Med*. **1993**, *119*, 924.

[36] (a) Ogilvie, W.W.; Bailey, M.; Poupart, M.A.; Abraham, A. Bonneau, P.; Bordeleau, J.; Bousquet, Y.; Chabot, C.; Duceppe, J.S.; Fazal, G.; Goublet, S.; Grand-Maitre, C.; Guse, I.; Halmos, T.; Lavallee, P.; Leach, M.; Malenfant, E.; O. Meara, J.; Plante, R.; Plouffe, C.; Poirier, M.; Soucy, F.; Yoakim, C.; Deziel, R. *J. Med. Chem*. **1997**, *40*, 4113. (b) Borthwick, A.D.; Weingarten, G.; Haley, T.M.; Tomaszewski, M.; Wang, W.; Hu, Z.; Bedard, J.; Jin, H.; Yuen, L.; Mansour, T.S. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 365. (c) Deziel, R.; Malenfant, E. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1437. (d) Yoakim, C.; Ogilvie, W.W.; Cameron, D.R.; Chabot, C. Guse, I.; Hache, B.; Naud, J.; O'Meara, J.; Plante, R.; Deziel, R. *J. Med. Chem*. **1998**, *41*, 2882. (e) Ogilvie, W.W.; Yoakim, C.; Do, F.; Hache, B.; Lagace, L.; Naud, J.; O'Meara, J.; Deziel, R. *Bioorg. Med. Chem.* **1999**, *7*, 1521.

[37] (a) Tew, D.G.; Southan, C.; Rice, S.Q.; Lawrence, M.P.; Li, H.; Boyd, H.F.; Moores, K.; Gloger, I.s.; Macphee, C.H. *Atheroscler. Thromb. Vasc. Biol*. **1996**, *16*, 591. (b) Tjoelker, L.W.; Wilder, C.; Eberhardt, C.; Stafforini, D.M.; Dietsch, G.; Schimpf, B.; Hooper, S.; Trong, H.L.; Cousens, L.S.; Zimmerman, G.A.; Yamada, Y.; McIntire, T.M.; Prescott, S.M.; Gray, P.W. *Nature* **1995**, *374*, 549. (c) Stemler, K.E.; Stafforini, D.M.; Prescott, S.M.; McIntire, T.M. *J. Biol. Chem*. **1991**, *266*, 11095.

[38] Gottstein, W.J.; Eachus, A.H.; Misco, P.F.; Cheney, L.C.; Misiek, M.; Price, K.E. *J. Med. Chem*. **1971**, *14*, 770.

[39] (a) Arnoldi, A.; Grasso, S.; Mainardi, G.; Merlini, L. *Eur. J. Med. Chem*. **1988**, *23*, 149. (b) Arnoldi, A.; Cabrini, R.M.; Farina, G.; Merlini, L. *J. Agric. Food Chem*. **1990**, *38*, 2197. (c) Fahmy, A.M.; Hasssan, K.M.; Khalaf, A.A.; Ahmed, R.A. *Indian J. Chem. Sect. B*, **1987**, *26*, 884. (d) Ube Industries and Hokko Chemical Industry (1981), Japanese Patent 8140662; *Chem. Abstr*. **1981**, *95*, 132655.

[40] Walsh, O.M.; Meegan, M.J.; Prendergast, R.M.; Al Nakib, T. *Eur. J. Med. Chem*. **1996**, *31*, 989.

[41] (a) LaPlante, S.R.; Bonneau, P.R.; Norman, A.; Cameron, D.R.; Deziel, R.; Grand-Maitre, C.; Plouffe, C.; Kawai, S.H. *J. Am. Chem. Soc*. **1999**, *121*, 2974. (b) Bonneau, P.R.; Hasani, F.; Plouffe, C.; Malenfant, E.; LaPlante, S.R.; Guse, I.; Ogilvie, W.W.; Davidson, W.C.; Hopkins, J.L.; Morelock, M.M.; Cordingley, M.C.; Deziel, R. *J. Am. Chem. Soc*. **1999**, *121*, 2965.

[42] Ator, M.A.; Ortiz de Montellano, P.R. *The Enzymes*, Vol. *XIX*, Academica Press, New York, **1980**.

[43] (a) Marchand-Brynaert, J.; Iaub, R.; De Meester, F.; Frere, J.M. *Eur. J. Med. Chem*. **1998**, *23*, 561. (b) Beauve, C.; Bouchet, M.; Touillaux, R.; Fastrez, J.; Marchand-Brynaert, J. *Tetrahedron* **1999**, *55*, 13301.; c) Beauve, C.; Tjoens, G.; Touillaux, R.; Lamotte-Brasseur, J.; Marchand-Brynaert, J. *Eur. J. Org. Chem.* **1999**, 1441.

[44] Ahluwalia, R.; Day, R.A.; Nauss, J. *Biochem. Biophys. Res. Commun*. **1995**, *206*, 577.

[45] (a) Bulychev, A.; Bellettini, J.R.; O'Brien, M.E.; Crocker, P.J.; Samama, J.P.; Miller, M.J.; Mobashery, S. *Tetrahedron* **2000**, *56*, 5719. (b) Swaren, P.; Massova, I.; Bellettini, J.R.; Bulychev, A.; Maveyraud, L.; Kotra, L.P.; Miller, M.J.; Mobashery, S.; *J. Am. Chem. Soc.* **1999**, *121*, 5353. (c) Bulychev, A.; O'Brien, M.E.; Massova, I.; Teng, M.; Gibson, T.A.; Miller, M.J.; Mobashery, S.; *J. Am. Chem. Soc.* **1995**, *117*, 5938.

[46] (a) Doucet, C.; Vergely, I.; Rebound-Ravaux, M.; Guilhem, J.; Kobaiter, R.; Joyeau, R.; Wakselman, M. *Tetrahedron Assymm*. **1997**, *8*, 759. (b) Joyeau, R.; Felk, A.; Guilaume, S.; Wakselman, M.; Vergely, I.; Doucet, C.; Boggetto, N.; Rebound-Ravaux, M. *J. Pharm. Pharmacol*. **1996**, *48*, 1218. (c) Vergely, I.; Bogetto, N.; Okochi, V.; Golpayegani, S.; Rebound-Ravaux, M.; Kobaiter, R.; Joyeau, R.; Wakselman, M. *Eur. J. Med. Chem*. **1995**, *30*, 199. (d) Joyeau, R.; Molines, H.; Labia, R.; Wakselman, M. *J. Med. Chem.* **1988**, *31*, 370.

[47] Firestone, R.A.; Barker, P.L.; Pirano, J.M.; Ashe, B.M.; Dahlgreen, M.E. *Tetrahedron* **1990**, *46*, 2255.

[48] Hagmann, W.K.; Shah, S.K.; Dorn, C.P.; O'Grady, L.A.; Hale, J.J.; Finke, P.E.; Thompson,K.R.; Brause, K.A.; Ashe, B.M.; Weston, H.; Dahlgreen, M.E.; Maycock, A.L.; Dellea, P.S.; Hand, K.M.; Osinga, D.G.; Bonney, R.J.; Davies, P.; Fletcher, D.S.; Doherty, J.B. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 545.

[49] (a) Shah, S.K.; Dorn, C.P. Jr.; Finke, P.E.; Hale, J.J.; Hagmann, W.K.; Brause, K.A.; Chandler, G.O.; Kissinger, A.L.; Ashe, B.M.; Weston, H.; Knight, W.B.; Maycock, A.L.;Dellea, P.S.; Fletcher, D.;Hand, K.M.; Mumford, R.A.; Underwood, D.J.; Doherty, J.B. *J. Med. Chem*. **1992**, *35*, 3745. (b) Skiles, J.W.; Jeng, A.Y. *Exp. Opin. Ther. Patents* **1999**, *9*, 869.

[50] Starkey, P.M.; Barrett, A.J. *Biochem J.* **1976**, *155*, 265.

[51] (a) Crystal, R.G. *J. Clin. Invest*. **1990**, *85*, 1343. (b) Campbell, E.J.; Campbell, M.A.; *J. Cell. Biol*. **1988**, *106*, 667. (c) Campbell, E.J.; Senior, R.M.; McDonald, J.A.;Cox, D. L. *J. Clin. Invest*. **1982**, *70*, 845.

[52] Macchia, B.; Gentili, D.; Macchia, M.; Mamone, F.; Martinelli, A.; Orlandini, E.; Rossello, A.; Cercignani, G.; Pierotti, R.; Allegretti, M.; Asti, C.; Caselli, G. *Eur. J. Med. Chem*. **2000**, *35*, 53.

[53] (a) Doherty, J.B.; Ashe, B.M.; Argenbright, L.W.; Barker, P.L.; Bonney, R.J.; Chandler, G.O.; Dahlgren, M.E.; Davies, P.; Dorn, C.P. Jr.; Finke, P.E.; Firestone, R.A.; Fletcher, D.; Hagmann, W.K.; Mumford, R.; O'Grady, L.; Maycock, A.L.; Pisano, J.M.; Shah, S.K.; Thompson, K.R.; Zimmerman, M. *Nature (London)* **1986**, *192*, 322. (b) Doherty, J.B.; Ashe, B.M.; Barker, P.L.; Blacklock, T.J.; Butcher, J.W.; Chandler, G.O.; Dahlgren, M.E.; Davies, P.; Dorn, C.P. Jr.; Finke, P.E.; Firestone, R.A.; Hagmann, W.K.; Halgren, T.; Knight, W.B.; Maycock, A.L.; Navia, M.A.; O'Grady, L.; Pisano, J.M.; Shah, S.K.; Thompson, K.R.; Weston, H.; Zimmerman, M. *J. Med. Chem*. **1990**, *33*, 2513. (c) Finke, P.E.; Ashe, B.M.; Knight, W.B.; Maycock, A.L.; Navia, M.A.; Shah, S.K.; Thompson, K.R.; Underwood, D.J.; Weston, H.; Zimmerman, M.; Doherty, J.B. *J. Med. Chem*. **1990**, *33*, 2522. (d) Shah, S.K.; Brause, K.A.; Chandler, G.O.; Finke, P.E.; Ashe, B.M.; Weston, H.; Knight, W.B.; Maycock, A.L.; Doherty, J.B. *J. Med. Chem*. **1990**, *33*, 2529. (e) Knight, W.B.; Maycock, A.L.; Green, B.G.; Ashe, B,M.; Gale, P.; Weston, H.; Finke, P.E.; Hagmann, W.K.; Shah, S.K.; Doherty, J.B. *Biochemistry*. **1992**, *31*, 4980. (f) Sabath, L.D.; Jago, M.; Abraham, E.P. *Biochemical J*. **1965**, *96*, 739.

[54] Bode, W.; Meyer, F.; Powers, J.C. *Biochemistry* **1989**, *28*, 1951.

[55] Koteva, K.P.; Cantin, A.M.; Neugebauer, W.A.; Escher, E. *Can. J. Chem*. **2001**, *79*, 377

[56] (a) Buynak, J.D.; Khasnis, D.; Bachmann, B.; Wu, K.; Lamb, G.*J. Am. Chem. Soc.* **1994**, *116,* 10955. (b) Buynak, J.D.; Rao, A.S.; Ford, G.P.; Carver, C.; Adam, G.; Geng, B.; Bachmann, B.; Shobassy, S.; Lackey, S. *J. Med. Chem*. **1997**, *40*, 3423.

[57] (a) Maiti, S.N.; Czajkowski, D.P.; Narender, A.V.; Reddy, A.V.; Spevak, P.; Kaleta, J.; Micetich, G. *Bioorg. Med. Chem. Lett.* **1996**, *7*, 823. (b) Khan, J.K.; Bu, H-Z.; Samarendra Z.Z.; Maiti, N.; Micetich, R.G. *J. Pharm. Biomed. Analysis* **1999**, *20*, 697.

[58] Alpegiani, M.; Bissolino, P.; Corigli, R.; Del Nero, S.; Perrone, E.; Rizzo, V.; Sacchi, N.; Cassinelli, G.; Franceschi, G. *J. Med. Chem*. **1994**, *37*, 4003.

[59] Balsamo, A.; Cercignani, G.; Gentili, D.; Lapucci, A.; Macchia, M.; Orlandini, E.; Rapposelli, S.; Rossello, A. *Eur. J. Med. Chem.* **2001**, *36*, 185.

[60] Wilmouth, R.C.; Li, Y.-H.; Wright, P.A.; Claridge, T.D.W.; Aplin, R.T.; Schofield, C.J. *Tetrahedron* **2000**, *56*, 5729.
[61] Hultgren, S.J.; Abraham, S.; Caparon, M.; Falk, P.; St. Geme, J.W. III; Normark, S. *Cell* **1993**, *73*, 887.
[62] Wu, Z.; Georg, G.I.; Cathers, B.E.; Schloss, J.V. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 983.
[63] (a) Knight, W.B.; Chabin, R.; Green, B. *Arch. Biochem. Bioph*. **1992**, *296*, 704. (b) Knight, W.B.; Green, B.; Chabin, R.; Gale, P.; Maycock, A.L.; Weston, H.; Kuo, D.W.; Westler, W.M.; Dorn, C.P.; Finke, P.E.; Hagmann, W.K.; Hale, J.J.; Liesch, J.; MacCoss, M.; Navia, M.A.; Shah, S.K.; Underwood, D.; Doherty, J.B. *Biochemistry* **1992**, *31*, 8186.
[64] (a) *Thrombin structure and Function*, Berliner, L.T.E. Ed.; Plenum Press; New York, **1992**. (b) *Bioregulatory Functions of Thrombin*, Vol. 485, Walz, D.A.; Fenton, II J.W.; Shuman, M.A. Eds.; *Ann. New York Acad. Sci.* **1986**. (c) Fenton, J.W.; Ofosu, F.A.; Moon, D.G.; Maraganore, J.M. *Blood Coagulation and Fibrinolysis* **1991**, *2*, 69.
[65] Taylor, F.B. Jr. *Surv. Synth. Pat. Res*. **1983**, *1*, 251.
[66] (a) Wallis, R.B. *Drugs Today* **1989**, *25*, 597. (b) Brundish, D.E. *Curr. Opin. Ther. Pat.* **1992**, 1457.
[67] (a) Tapparelli, C.; Metternich, R.; Ehrhardt, C.; Cook, N.S. *Trends Pharmacol. Sci.* **1993**, *14*, 366. (b) Lyle, T.A. *Perspectives Drug Disc. Design* **1993**, *1*, 453.
[68] (a) Han, W.T.; Trehan, A.K.; Kim Wright, J.J.; Federici, M.E.; Seiler, S.M.; Meanwell, N.A. *Bioorg. Med. Chem.* **1995**,*8*, 1123. (b) Han, W.T. U.S. Patent 5, 037, 819, 6 August 1991.; c) Han, W.T. U.S. Patent 5, 250, 677, 5 October 1993.
[69] McGill, H.C. Jr. *Geographical Pathology of Atherosclerosis*; Williams and Wilken Co.: Baltimore, **1985**.
[70] (a) Wirebaugh, S.R.; Shapiro, M.L.; McIntyre, T.H.; Whitney, E.J. *Pharmacotherapy* **1992**, *12*, 445. (b) Krause, B.R.; Sliskovic, D.R.; Bocan, T.M.A. *Exp. Opin. Invest. Drugs* **1995**, *4*, 353.
[71] (a) Dujovne, C.A.; harris, W.S.; Gelfand, R.A.; Morehouse, L.A.; McCarthy, P.A.; Chandler, C.E.; DeNinno, M.P.; Harwood, H.J. Jr.; Newton, F.A.; Shear, C.L. In *Atherosclerosis X*; Woodford, F.P.; Davignon, J.; Sniderman, A. Eds.; Elsevier Scince: New York, pp. 298-306, **1995**. (b) Bergmann, M.; Morales, H.; Mellars, L.; Kosoglou, T.; Burrier, R.; Davies, H.R.; Sybertz, E.J.; Pollare, T. The Clinical Development of a Novel Cholesterol Absorption Inhibitor. Proceedings of the XII International Symposium of Drugs Affecting Lipid Metabolism, Nov. 7-10, **1995**, Houston, TX.
[72] (a) Burnett, D.A; Caplen, M.A.; Davis, H.R. Jr.; Burrier, R.E.; Clader, J.W. *J. Med. Chem*. **1994**, *37*, 1733. (b) Salisbury, B.G.; Davis, H.R. Jr.; Burrier, R.E.; Burnett, D.A; Boykow, G.; Caplen, M.A.; Clemmons, A.L.; Compton, D.S.; Hoos, L.M.; McGregor, D.G.; Schnitzer-Polokoff, R.; Smith, A.A.; Wig, B.C.; Zilli, D.L.; Clader, J.W.; Sybertz, E. *J. Atherosclerosis* **1995**, *115*, 45. (c) Dugar, S.; Clader, J.W.; Chan, T.-M.; Davis, H. Jr. *J. Med. Chem*. **1995**, *38*, 4875.
[73] (a) Hickey, D.M.B.; Dhanak, D.; Ife, R.J.; Leach, C.A. WO 97/21675. (b) Hickey, D.M.B.; Dhanak, D.; Ife, R.J.; Leach, C.A.; Tew, D.G. WO 97/21676. (c) Tew, D.A.; Boyd, H.F.; Ashman, S.; Theobald, C.; Leach, C.A. *Biochemistry* **1997**, *37*, 10087.
[74] Rosenblum, S.T.; Huynh, T.; Afonso, A.; Davis, H.R. Jr.; Yumobe, N.; Clader, J.W.; Burnett, D.A. *J. Med. Chem*. **1998**, *41*, 973.
[75] Kambara, T; Tomioka, K. *J. Med. Chem*. **1999**, *64*, 9282.
[76] Annunziata, R.; Benaglia, M.; Cinquini, M.; Cozzi, F. *Tetrahydron: Asymmetry* **1999**, *10*, 4841.
[77] Rosenblum, S.T.; Huynh, T.; Afonso, A.; Davis, H.R. Jr.; *Tetrahedron*, **2000**, *56*, 5735.
[78] (a) Snyder, F.; Lee, T.-C.; Blank, M.L. *Prog. Lipid Res*. **1992**, *31*, 65. (b) Winkler, J.D.; Chilton, F.H. In *Inflammation: Mediators and Pathways* (Ruffolo, R.; Holinger, J. eds.) **1995**, pp.147-171, CRC Press, Boca Raton, FL.
[79] (a) Winkler, J.D.; Fonteh, A.N.; Sung, C.-M.; Heravi, J.D.; Nixon, A.B.; Chabot-Fletcher, M.; Griswold, D.; Marshall, L.A.; Chilton, F.H. *J. Pharmacol. Exp. Ther.* **1995**, *274*, 1338. (b) Winkler, J.D.; Chilton, F.H. *Drug News Perspect*. **1993**, *6*, 133. (c) Winkler, J.D.; Sung, C.-M.; Chabot-Fletcher, M.; Griswold, D.; Marshall, L.A.; Chilton, F.H.; Bondinell, W.; Mayer, R.J. *Mol. Pharmacol*. **1998**, *53*, 322.
[80] Burke, J.R.; Gregor, K.R.; Padmanabha, R.; Banville, J.; Witmer, M.R.; Davern, L.B.; Manly, S.P.; Tramposch, K.M. *J. Enzyme Inhibition* **1998**, *13*, 195.
[81] (a) Diez, E.; Louis-Flamberg, P.; Hall, R.H.; Mayer, R.J. *J. Biol. Chem*. **1992**, *267*, 18342. (b) Diez, E.; Chilton, F.H.; Stroup, G.; Mayer, R.J.; Winkler, J.D.; Fonteh, A.N. *Biochem. J*. **1994**, *301*, 721.
[82] (a) Burke, J.R.; Witmer, M.R.; Tredup, J.; Micanovic, R.; Gregor, K.; Lahiri, J.; Tramposch, K.M.; Villafranca, J.J. *Biochemistry* **1995**, *34*, 15165. (b) Sharp, J.D.; Pickard, R.T.; Chiou, X.G.; Manetta, J.V.; Kovacevic, S.; Miller, J.R.; Varshavsky, A.D.; Roberts, E.F.; Strifler, B.A.; Brems, D.N.; Kramer, R.M. *J. Biol. Chem*. **1994**, *269*, 23250.
[83] Saturnino, C.; Fusco, B.; Saturnino, P.; De Martino, G.; Rocco, F.; Lancelot, J.-C. *Biol. Pharm. Bull.* **2000**, *23*, 654.
[84] (a) Tew, D.G.; Southan, C.; Rice, S.Q.; Lawrence, M.P.; Li, H.; Boyd, H.F.; Moores, K.; Gloger, I.s.; Macphee, C.H. *Atheroscler. Thromb. Vasc. Biol*. **1996**, *16*, 591. (b) Tjoelker, L.W.; Wilder, C.; Eberhardt, C.; Stafforini, D.M.; Dietsch, G.; Schimpf, B.; Hooper, S.; Trong, H.L.; Cousens, L.S.; Zimmerman, G.A.; Yamada, Y.; McIntire, T.M.; Prescott, S.M.; Gray, P.W. *Nature* **1995**, *374*, 549. (c) Stemler, K.E.; Stafforini, D.M.; Prescott, S.M.; McIntire, T.M. *J. Biol. Chem*. **1991**, *266*, 11095.
[85] Tew, D.G.; Boyd, H.F.; Ashman, S.; Theobald, C.; Leach, C.A. *Biochemistry* **1998**, *37*, 10087.
[86] (a) Baxter, C. *Handbook of Nerochemistry*, Laijtha, A. ed. Vol. *3*, pp. 289-353; New York; Plenum Press, **1970**. (b) Meldrum, B. *Int. Rev. Neurobiol*. **1975**, *17*, 1.
[87] (a) Leach, M.J.; Walker, J.M.G. *Biochem. Pharmacol*. **1977**, *26*, 1569. (b) Phillips, N.I.; Fowler, L. *J. Biochem. Pharmacol*. **1982**, *31*, 2257.
[88] Hopkins, M.H.; Silverman, R.B. *J. Enzyme Inhibition*, **1992**, *6*, 125.
[89] Fowler, L.J.; John, R.A. *Biochem. J*. **1981**, *197*, 149.
[90] Hori, S.; Kanemitsu, K.; Shimada, J. *J. Antibiot*. **1993**, *46*, 1145.
[91] (a) Stamey, T.A.; Yang, N.; Hay, A.R.; McNeal, J.E.; Freiha, F.S.; Redwine, E. *N. Engl. J. Med*. **1987**, *317*, 909. (b) Oesterling, J.E. *J. Urol*. **1991**, *145*, 907.
[92] (a) Cohen, P.; Graves, H.C.B.; Peehl, D.M.; Kamarei, M.; Guidice, L.C.; Rosenfield, R.G. *J. Clin. Endocrinol. Metab*. **1992**, *75*, 1046. (b) Cohen, P.; Peehl, D.M.; Graves, H.C.B.; Rosenfield, R.G. *J. Clin. Endocrinol.* **1994**, *142*, 407.
[93] Diamandis, E.P.; Yu, H. *J. Clin. Endocrinol. Metab*. **1995**, *80*, 1515.
[94] Adlington, R.M.; Baldwin, J.E.; Chen, B.; Cooper, S.L.; McCoull, W.; Pritchard, G.J. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1689.
[95] Rideout, H.J.; Zang, E.; Yeasmin, M.; Gordon, R.; Jabado, O.; Park, D.S.; Stefanis, L. *Neuroscience* **2001**, *107*, 339.
[96] Sim, R.B.; Laich, A. *Biochem. Soc. Trans*. **2000**, *28*, 545.
[97] Afonso, C.L.; Tulman, E.R.; Lu, Z.; Zsak, L.; Osorio, F.A.; Balinsky, C.; Kutish, G.F.; Rock, D.L. *J. Virol*. **2002**, *76*, 783.
[98] Guerin, J.L.; Gelfi, G.; Camus, C.; Delverdier, M.; Whisstock, J.C.; Amardeihl, M.F.; Py, R.; Betagnoli, S.; Messud-Pettit, F. *J. Gen. Virol*. **2001**, *82*, 1407.
[99] Nagano, I.; Wu, Z.; Nakada, T.; Matsuo, A.; Takahashi, Y. *Parasitology* **2001**, *123*, 77.
[100] Piguet, P.F.; Kan, C.D.; Vesin, C. *Appoptosis* **2002**, *7*, 91.
[101] Shaw, M.K.; Roos, S.D.; Tilney, L.G. *Microbes Infect*. **2002**, *4*, 119.
[102] Lindh, J.G.; Botero-Kleiven, S.; Arboleda, J.I.; Wahlgren, M.A. *Mol. Biochem. Parasitol*. **2001**, *116*, 137.
[103] Hiraga, K.; Suzuki, T.; Oda, K. *J. Biol. Chem*. **2000,** *275*, 25173.
[104] Oda, M.; Tamura, A.; Kanaori, K.; Kojima, S.; Miura, K.-i.; Momma, K.; Tonomuta, B.; Kazuyuki, A. *J. Biochem*. **2002**, *132*, 991.
[105] Buynak, J.D.; Chen, H.; Vogeti, L.; Gadhachanda, V.R.; Zheng, M.; Shaw, R. *224th ACS National Meeting,* **2002,** *August 18-22 Boston, MA.*