CHAPTER I

INTRODUCTION AND BACKGROUND

Neutral proteases from leukocytes and macrophages are currently thought to be the agents responsible for the destruction in diseases such as pulmonary emphysema (1-3). In addition, human leukocytic elastase (HLE) is implicated in the growth of tumors, degradation of tissues in arthritis and the destruction of lung tissue in emphysema (4-5). Therefore synthetic elastase inhibitors should be useful for studying the role of elastolytic enzymes both in chronic destructive and connective tissue diseases. These inhibitors might be important as potential drugs. This research is involved in a study of synthetic elastase inhibitors and their related serine proteases. There is evidence to show that the neutral proteases of human leukocytes (polymorphonuclear leukocytes) are involved in the degradation of cartilage in both rheumatoid and osteoarthritis. These enzymes are generally called human leukocyte elastase, cathepsin G and other specific collagenases. The chronic destruction of the elastic component of lung connective tissue by HLE and cathepsin G is currently believed to result in the onset of chronic obstructive lung disease. These proteolytic enzymes are primarily inhibited by alpha-1-proteinase inhibitor (α 1-PI) which is the major serum protease inhibitor and also a normal constituent in the fluid of the bronchioalveolar system.

However, the inhibitor α1-PI is unstable (6). It is readily inactivated by either an oxidizing agent such as the one present in the cigarette smoke or an oxidase such as myeloperoxidase. This particular oxidative enzyme is normally active in the phagacytic cells during inflammatory states. In addition, the presence of α1-PI in human is genetically dependent. Some persons are found to have the inhibitor levels as low as 25% of normal due to their genetically heritage. Thus, persons with lower concentration of α1-PI, resulting in high concentrations of HLE are prone to chronic obstructive lung disease. In this particular case a drug which is active as an elastase inhibitor to supplement α1-PI would be a good candidate to cure or prevent such diseases.

Many anti-inflammatory drugs, including phenylbutazone, indomethacin, myochrysin and arteparon are fairly potent inhibitors of HLE. Unfortunately, this useful property must be weighed against the known deleterious side-effects of these drugs. The most serious one in term of cartilage integrity is the suppression of proteoglycan synthesis.

There are many classes of compounds reported in the literatures, which inhibit HLE. Some of these are listed in Table 1.1.

Table 1.1 Some HLE inhibitors.

Class of compound	Ref.
chloro methyl ketones	7,8
sulfonyl fluorides	8
imidazole-N-carboxamides	9
aza-peptides	8
cyclohexylamide	10
cis-unsaturated fatty acid	11
adamantane sulphenyl	12
gold thiomalate	13

All the compounds in Table1.1 have one or more reactive functional group. They are difficult, if not impossible, to target onto receptor sites, as they are likely to react with the many components of the body available between the point of administration and the target at the receptor site.

Oleic acid, a cis-unsaturated carboxylic acid, has been shown to be reasonably good specific inhibitor of HLE, but not of PPE (Porcine pancreatic elastase), trypsin, chymotrypsin and cathepsin G, which has indicated that the principal difference between HLE and the other serine proteases could be due to the different hydrophobic character of a site near the active site. These proteases show preferences for the hydrolysis of peptide bonds next to certain amino acids or short peptide sequences (7). It is not surprising therefore that some workers in this field have sought to utilize these preferences, either by using the synthetic substrates as competitive inhibitors (14) or by introducing functional

groups into these substrates so as to covalently inactivate the enzyme (7,15).

A certain degree of substrate specificity is apparent in all those proteolytic enzymes. This property has, in the past, been utilized mostly in the design of irreversible inhibitors. In these cases the amino acid, or short peptide is functionalized so that when the peptide is "recognised" by the active site, the catalytic site is inactivated by covalent bond formation. The design of this type of inhibitors is not the aim of this project. However, rather than completely inactivate the essential, but over abundant enzymes, our aim is to synthesis and try to control the extent of the active form present.

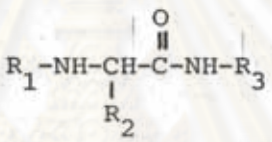
The enzymes in both the pancreas and the human polymorphonuclear leukocytes are mainly serine proteases with catalytic machanism and sequence homologies are related to trypsin and chymotrypsin.

Sometime ago the idea has been conceived of combining a preferred amino acid or short peptide, which on its own is ubiquitous physiological component without toxicity, with a hydrocarbon chain of appropriate length, which is likewise a natural physiological component, but in this case provides the required inhibition. This combination of a "recognition site" with a hydrophobic chain is a logical extension of the observation that some hydrocarbons of appropriate lenghth, even without functional groups, are themselves inhibitors of some of these proteases. The novelty and advantage of this concept resides in that enzyme cleavage of the inhibitors would merely result in end products which are physiologically innocuous compounds.

It was considered that the potential of these compounds as effective specific inhibitors of HLE could be improved by introducing

an extended peptide sequence of alanine and or valine amino acids. At this stage it was thought that the peptide sequence would impart some preference for HLE to bind the compound in the active site, as it does for natural peptide sequence, and in doing so, the alkyl chain would impart some mode of inhibition of the enzyme (16).

Utilization of this concept, together with the empirical structural activity relationships, as the stereochemistry and molecular parameters of the area around the active site of HLE are unknown, requires the synthesis and testing of alkylated amino acid compounds of the general formula I and II.

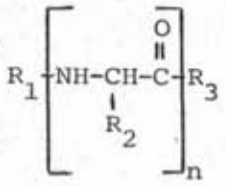


Formula I

where R_1 is selected from hydrogen, benzyloxy, tertiary butoxy carbonyl, succinyl or other suitable protective group

R_2 is methyl or isoproply or other suitable alkyl group

R_3 is $-(CH_2)_7CH_3$, $-(CH_2)_9CH_3$, $-(CH_2)_{11}CH_3$, $-(CH_2)_{13}CH_3$ or other suitable alkyl group



Formula II

where R_1 is benzyloxy or tertiary butoxy carbonyl or $CH_3(CH_2)_{13}$- or other suitable protective or suitable alkyl group

R_2 is methyl or isopropyl or other suitable alkyl group

R_3 is $-(CH_2)_{11}CH_3$ or methoxy or ethoxy or other suitable alkyl group or other suitable protective group

These R_1, R_2 and R_3 groups in each of the two formula can be similar or different. The individual compounds to be made and tested are listed in Table 1.2 and Table 1.3

Table 1.2 Compounds made at the Department of Chemistry, La Trobe University, Australia

No.	Name	Short formula[a]
1.	Benzyloxy-L-valylamido-dodecane	Z-V-NH-C_{12}
2.	Benzyloxy-L-valine-L-valylamido-dodecane	Z-V-V-NH-C_{12}
3.	Tetradecanoyl-L-alanyl-L-valyl-methyl ester	C_{14}-A-V-OMe
4.	Tetradecanoyl-L-valyl-L-valyl methyl ester	C_{14}-V-V-OMe
5.	Tetradecanoyl-L-valyl-L-alanyl ethyl ester	C_{14}-V-A-OEt
6.	Tetradecanoyl-L-valyl-L-valyl-L-alanyl ethyl ester	C_{14}-V-V-A-OEt
7.	Dodecyl-L-valyl-L-valyl-methyl ester	C_{12}-V-V-OMe
8.	L-alanylamido dodecane	NH_2-A-NH-C_{12}
9.	tert-butoxy carbonyl-L-alanylamido dodecane	t-BOC-A-NH-C_{12}
10.	tetradecanoyl-L-valyl-L-valyl-L-valyl methyl ester	C_{14}-V-V-V-OMe

Table 1.3 Compounds made at the Department of Chemistry, Faculty of Science Chulalongkorn University

No.	Name	Short formula[(a)]
11.	tert-butoxy carbonyl-L-alanylamido octane	t-BOC-A-NH-C_8
12.	tert-butoxy carbonyl-L-alanylamido decane	t-BOC-A-NH-C_{10}
13.	tert-butoxy carbonyl-L-alanylamido tetradecane	t-BOC-A-NH-C_{14}
14.	tert-butoxy carbonyl-L-valylamido dodecane	t-BOC-V-NH-C_{12}
15.	tert-butoxy carbonyl-L-valyl-L-valylamido dodecane	t-BOC-V-V-NH-C_{12}
16.	Succinyl-L-alanylamido dodecane	Suc-A-NH-C_{12}
17.	Succinyl-L-valylamido dodecane	Suc-V-NH-C_{12}

(a) The abbreviation in the short formulas are represented as follows :

Z = benzyloxy carbonyl

t-BOC = tertiary butyloxy carbonyl

A = L-alanine

V = L-valine

Suc = succinyl

C_8 = $-(CH_2)_7-CH_3$

C_{10} = $-(CH_2)_9-CH_3$

C_{12} = $-(CH_2)_{11}-CH_3$

C_{14} = $-(CH_2)_{13}-CH_3$

It must be emphasised that the synthesis making all possible combinations of substituents would require an effort beyond our capabilities, therefore only a few compounds were selected for study.

It is also important to note that this investigation consists of two parts, the chemical synthesis and the enzyme kinetic studies. It is carried out in cooperation with a group of workers at La Trobe University, Australia. The La Trobe group has provided some compounds as listed in Table 1.2 and tested all compounds against HLE and PPE, while other compounds as listed in Table 1.3 were made at Chulalongkorn University but all compounds including those made at La Trobe, are tested here against trypsin and chymotrypsin.

In summary, the aim of this investigation are :

1. To synthesise and test the compounds, with respect to percentage inhibition against the enzymes HLE, PPE, trypsin and chymotrypsin in order to ascertain their mode of actions.

2. To synthesise and test compounds with various hydrocarbon chains, to study the effect of chain length on some dipeptide inhibitors.

3. To study the effect of the protecting group in various inhibitors.

4. To determine the type of inhibition of these compounds with respect to specifec enzymes.