CHAPTER 1: INTRODUCTION

Protease inhibitors 2
Kunitz inhibitor family 7
Bowman-Birk inhibitor family 7
Occurrence of BBI (Sources) 8
BBIs of Leguminosae 8
BBIs of Compositeae 10
BBIs of monocots 11
Biosynthesis of BBI 12
Screening/Identification 13
Quantitative Assays 14
Molecular properties 15
Isolation and purification of BBIs 17
Inhibitory properties 18
Chemistry and structure of BBIs 20
Homology 26
Heterogeneity and isoforms of BBI 28
Oligomeric status of BBIs 29
Reactive site peptide bond of BBI 32
Chemical modification 35
Enzymatic fragmentation 36
Stability of BBIs 37
Standard Mechanism of action of BBIs 38
Evolution of inhibitors 40
BBI derived synthetic inhibitors 42
Heterologous expression of BBIs 45
Application of BBIs 46
Horsegram 52

Aim and Scope of present investigation 54
CHAPTER 2: MATERIALS AND METHODS

Materials 58
Methods 61

CHAPTER 3: Functional expression of the major Bowman-Birk inhibitor of horsegram-HGI-III

Results 90

Construction of pRSET-rHGI 90
Functional expression of rHGI 96
Purification of rHGI 96
Biochemical characterization of rHGI 98
N-terminal sequence analysis 102
Molecular weight determination 102
Estimation of disulphide bonds 105
pH and thermal stability of rHGI 106
Inhibitory properties of rHGI 107
Simultaneous and independent inhibition of trypsin/chymotrypsin by rHGI 113
Cross reactivity of rHGI with anti HGI-III 115

Discussion 115

CHAPTER 4: Site directed mutagenesis to validate the role of C-terminal tail in the dimerisation of HGI-III

Results 123

Primer designing for the site directed mutagenesis 123
Construction of point mutants of rHGI 124
Mutagenesis of Lys^{24} to Ala 125
Size exclusion chromatography 126
Thermal stability studies of rHGI-K24A 129
Construction of expression plasmids and point mutants of HGI-III as C-terminal (His)_6 tagged proteins. 130

Expression of pET20b-HGI 132
One step purification of the (His)_6-tagged fusion proteins 132
Expression of pET-20bHGI K24A mutant 133
Construction of pET-20bHGI D75A 134
Construction of Δ76 mutant of rHGI 136
CHAPTER 5: Design and molecular engineering of a small trypsin inhibitor based on the binding loop of horsegram seed trypsin inhibitor

Results

- Design of the stable mini inhibitors 158
- Purification and characterisation of recombinant trypsin inhibitory domain (rTID) 161
- Size exclusion chromatography 165
- Inhibitory properties of pET20b-rTID 166
- Stability studies 170
- Thermal stability of rTID 170
- In vitro stability of rTID to gastrointestinal proteases 170

Discussion 171

CHAPTER 6: SUMMARY AND CONCLUSIONS 178

REFERENCES 185