

80574 EP SEQ.ST25
SEQUENCE LISTING

<110> Ursapharm Arzneimittel GmbH & Co.; KG
 <120> Recombinant preparation of Bromelain inhibitors and Bromelain inhibitor precursor
 <130> 80574 EP
 <140> EP 08013773.0 / EP 08013773
 <141> 2008-07-31
 <160> 33
 <170> PatentIn version 3.3
 <210> 1
 <211> 55
 <212> PRT
 <213> Artificial

<220>
 <223> Bromelain inhibitor bi-I

<400> 1

Ala Cys Ser Glu Cys Val Cys Pro Leu Arg Ser Ser Ser Asp Glu Glu
 1 5 10 15

Tyr Lys Cys Tyr Cys Thr Asp Thr Tyr Ser Asp Cys Pro Gly Phe Cys
 20 25 30

Lys Lys Cys Lys Ala Glu Phe Gly Lys Tyr Ile Cys Leu Asp Leu Ile
 35 40 45

Ser Pro Asn Asp Cys Val Lys
 50 55

<210> 2
 <211> 56
 <212> PRT
 <213> Artificial

<220>
 <223> Bromelain inhibitor bi-II

<400> 2

Ala Cys Ser Glu Cys Val Cys Pro Leu Arg Thr Ser Ser Ser Asp Glu
 1 5 10 15

Glu Tyr Lys Cys Tyr Cys Thr Asp Thr Tyr Ser Asp Cys Pro Gly Phe
 20 25 30

Cys Lys Lys Cys Lys Ala Glu Phe Gly Lys Tyr Ile Cys Leu Asp Leu
 35 40 45

Ile Ser Pro Asn Asp Cys Val Lys
 50 55

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<210> 3
 <211> 57
 <212> PRT
 <213> Artificial

<220>
 <223> Bromelain inhibitor bi-III

<400> 3

Thr Ala Cys Ser Glu Cys Val Cys Pro Leu Arg Thr Ser Ser Ser Asp
 1 5 10 15

Glu Glu Tyr Lys Cys Tyr Cys Thr Asp Thr Tyr Ser Asp Cys Pro Gly
 20 25 30

Phe Cys Lys Lys Cys Lys Ala Glu Phe Gly Lys Tyr Ile Cys Leu Asp
 35 40 45

Leu Ile Ser Pro Asn Asp Cys Val Lys
 50 55

<210> 4
 <211> 57
 <212> PRT
 <213> Artificial

<220>
 <223> Bromelain inhibitor bi-VI

<400> 4

Thr Ala Cys Ser Glu Cys Val Cys Pro Leu Arg Thr Ser Ser Ser Asp
 1 5 10 15

Glu Glu Tyr Lys Cys Tyr Cys Thr Asp Thr Tyr Ser Asp Cys Pro Gly
 20 25 30

Phe Cys Lys Thr Cys Lys Ala Glu Phe Gly Lys Tyr Ile Cys Leu Asp
 35 40 45

Leu Ile Ser Pro Asn Asp Cys Val Lys
 50 55

<210> 5
 <211> 57
 <212> PRT
 <213> Artificial

<220>
 <223> Bromelain inhibitor bi-VII

<400> 5

Thr Ala Cys Ser Glu Cys Val Cys Pro Leu Gln Thr Ser Ser Ser Asp
 1 5 10 15

Asp Glu Tyr Lys Cys Tyr Cys Ala Asp Thr Tyr Ser Asp Cys Pro Gly
 20 25 30

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Phe Cys Lys Lys Cys Lys Ala Glu Phe Gly Lys Tyr Ile Cys Leu Asp
 35 40 45

Leu Ile Ser Pro Asn Asp Cys Val Lys
 50 55

<210> 6
 <211> 244
 <212> PRT
 <213> Artificial

<220>
 <223> Bromelain inhibitor precursor

<400> 6

Met Leu Leu Leu Phe Leu His Glu Val Ile Asn Gly Glu Arg Val Thr
 1 5 10 15

Leu Thr Ala Cys Ser Glu Cys Val Cys Pro Leu Gln Thr Ser Ser Ser
 20 25 30

Asp Asp Glu Tyr Lys Cys Tyr Cys Ala Asp Thr Tyr Ser Asp Cys Pro
 35 40 45

Gly Phe Cys Lys Lys Cys Lys Ala Glu Phe Gly Lys Tyr Ile Cys Leu
 50 55 60

Asp Leu Ile Ser Pro Asn Asp Cys Val Lys Pro Val Ser Ser Ser Glu
 65 70 75 80

Ala Lys Gln Lys Met Ile Lys Gly Glu Arg Val Thr Leu Thr Ala Cys
 85 90 95

Ser Glu Cys Val Cys Pro Leu Arg Thr Ser Ser Ser Asp Glu Glu Tyr
 100 105 110

Lys Cys Tyr Cys Thr Asp Thr Tyr Ser Asp Cys Pro Gly Phe Cys Lys
 115 120 125

Lys Cys Lys Ala Glu Phe Gly Lys Tyr Ile Cys Leu Asp Leu Ile Ser
 130 135 140

Pro Asn Asp Cys Val Lys Pro Val Ser Ser Leu Glu Ala Lys Gln Asn
 145 150 155 160

Met Ile Lys Glu Glu Arg Val Thr Leu Thr Ala Cys Ser Glu Cys Val
 165 170 175

Cys Pro Leu Arg Thr Ser Ser Ser Asp Glu Glu Tyr Lys Cys Tyr Cys
 180 185 190

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Thr Asp Thr Tyr Ser Asp Cys Pro Gly Phe Cys Lys Thr Cys Lys Ala
195 200 205

Glu Phe Gly Lys Tyr Ile Cys Leu Asp Leu Ile Ser Pro Asn Asp Cys
210 215 220

Val Lys Pro Val Ser Ser Trp Glu Ala Arg Gln Lys Ile Lys Leu Leu
225 230 235 240

Gln Gly Arg Glu

<210> 7
<211> 171
<212> DNA
<213> Artificial

<220>
<223> Bromelain inhibitor bi-I

<400> 7
atggccttgca gcgaatgcgt gtgtccacta cgaacaagtt catctgaaga gtacaaatgc 60
tactgcacgg atacttactc cgactgcccc ggctttttgca agaaatgcaa ggccgagttc 120
ggaaagtaca tatgcctcga cttgatctcg cccaacgatt gcgtaaaata g 171

<210> 8
<211> 174
<212> DNA
<213> Artificial

<220>
<223> Bromelain inhibitor bi-II

<400> 8
atggccttgca gcgaatgcgt gtgtccacta cgaacaagtt catctgatga agagtacaaa 60
tgctactgca cggatactta ctccgactgc ccgggctttt gcaagaaatg caaggccgag 120
ttcggaaagt acatatgcct cgacttgatc tcgccaacg attgcgtaaa atag 174

<210> 9
<211> 177
<212> DNA
<213> Artificial

<220>
<223> Bromelain inhibitor bi-III

<400> 9
atgacagctt gcagcgaatg cgtgtgtcca ctacgaacaa gttcatctga tgaagagtag 60
aaatgctact gcacggatac ttactccgac tgcccgggct tttgcaagaa atgcaaggcc 120
gagttcggaa agtacatatg cctcgacttg atctcgccca acgattgcgt aaaatag 177

<210> 10
<211> 176
<212> DNA
<213> Artificial

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<220>

<223> Bromelain inhibitor bi-VI

<400> 10

atgcagcttg tagcgaatgc gtgtgtccgc tgcgaacaag ttcattctgat gaagagtaca	60
aatgctactg cacggatact tactccgact gcccgggctt ttgcaagaca tgcaaggccg	120
agttcggaaa gtacatatgc ctcgacttga tctcgcccaa cgattgcgta aaatag	176

<210> 11

<211> 174

<212> DNA

<213> Artificial

<220>

<223> Bromelain inhibitor bi-VII

<400> 11

atgacagcct gcagcgaatg cgtgtgtccg ctgacaagtt catctgatga tgagtacaaa	60
tgctactgtg cggatactta ctccgactgc ccgggctttt gcaagaaatg caaggccgag	120
ttcggaaagt acatatgcct cgacttgatc tcgccaacg attgcgtaaa atag	174

<210> 12

<211> 638

<212> DNA

<213> Artificial

<220>

<223> Bromelain inhibitor precursor

<400> 12

atgatgagta caaatgctac tgtgcggata cttactccga ctgcccgggc ttttgcaaga	60
aatgcaaggc cgagttcgga aagtacatat gcctcgactt gatctcgccc aacgattgcg	120
taaaaccggt ctctctctcg gaggcgaagc aaaagatgat caaggagag agagttacac	180
taacagcttg cagcgaatgc gtgtgtccac tacgaacmag ttcattctgat gaagagtaca	240
aatgctactg cacggatact tactccgact gcccgggctt ttgcaagaaa tgcaaggccg	300
agttcggaaa gtacatatgc ctcgacttga tctcgcccaa cgattgcgta aaaccggtct	360
cctccttgga ggcgaagcaa aatatgatca aggaagagag agttacacta acagcttgta	420
gcgaatgcgt gtgtccgctg cgaacaagtt catctgatga agagtacaaa tgctactgca	480
cggatactta ctccgactgc ccgggctttt gcaagacatg caaggccgag ttcggaaagt	540
acatatgcct cgacttgatc tcgccaacg attgcgtaaa accggtctcc tcctgggagg	600
cgaggcaaaa gatcaagttg ctgcagggtc gtgaatga	638

<210> 13

<211> 34

<212> DNA

<213> Artificial

<220>

<223> BI-forward primer

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<400> 13
 aatcaagaat tcatgaacat gttgctgctc tttc 34

<210> 14
 <211> 35
 <212> DNA
 <213> Artificial

<220>
 <223> BI-reverse primer

<400> 14
 tcacttatgc ggccgcactc attcacgacc ctgca 35

<210> 15
 <211> 94
 <212> DNA
 <213> Artificial

<220>
 <223> Bir2 primer

<400> 15
 tagatgcggc cgctatttta cgcaatcggt gggcgagatc aagtcgaggc atatgtactt 60
 tccgaactcg gccttgcatg tcttgcaaaa gccc 94

<210> 16
 <211> 115
 <212> DNA
 <213> Artificial

<220>
 <223> BiVIIP-f primer

<400> 16
 tcagtgaatt catgacagcc tgcagcgaat gcgtgtgtcc gctgcaaaca agttcatctg 60
 atgatgagta caaatgctac tgtgcggata cttactccgc tccgactgcc cgggc 115

<210> 17
 <211> 115
 <212> DNA
 <213> Artificial

<220>
 <223> BiVIp-f primer

<400> 17
 tcagtgaatt catgacagct tgtagcgaat gcgtgtgtcc gctgcgaaca agttcatctg 60
 atgaagagta caaatgctac tgcacggata cttactccgc tccgactgcc cgggc 115

<210> 18
 <211> 110
 <212> DNA
 <213> Artificial

<220>
 <223> BiIII-f primer

<400> 18
 tcagtgaatt catgacagct tgcagcgaat gcgtgtgtcc actacgaaca agttcatctg 60

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atgaagagta caaatgctac tgcacggata cttactccga ctgcccgggc 110

<210> 19
<211> 112
<212> DNA
<213> Artificial

<220>
<223> BiIIP-f primer

<400> 19
tcagtgaatt catggcttgc agcgaatgcg tgtgtccact acgaacaagt tcatctgatg 60

aagagtacaa atgctactgc acggatactt actccgctcc gactgcccgg gc 112

<210> 20
<211> 109
<212> DNA
<213> Artificial

<220>
<223> BiIP-f primer

<400> 20
tcagtgaatt catggcttgc agcgaatgcg tgtgtccact acgaacaagt tcatctgatg 60

agtacaaatg ctactgcacg gatacttact ccgctccgac tgcccgggc 109

<210> 21
<211> 106
<212> DNA
<213> Artificial

<220>
<223> BIR1 primer

<400> 21
atgtagcggc cgctatttta cgcaatcgtt gggcgagatc aagtcgaggc atatgtactt 60

tccgaactcg gccttgcatt tcttgcaaaa gcccgggcag tcggag 106

<210> 22
<211> 57
<212> PRT
<213> Artificial

<220>
<223> bi-III as part of Bromelain inhibitor precursor

<400> 22

Thr Ala Cys Ser Glu Cys Val Cys Pro Leu Arg Thr Ser Ser Ser Asp
1 5 10 15

Glu Glu Tyr Lys Cys Tyr Cys Thr Asp Thr Tyr Ser Asp Cys Pro Gly
20 25 30

Phe Cys Lys Lys Cys Lys Ala Glu Phe Gly Lys Tyr Ile Cys Leu Asp
35 40 45

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Leu Ile Ser Pro Asn Asp Cys Val Lys
50 55

<210> 23
<211> 257
<212> PRT
<213> Artificial

<220>
<223> Bromelain inhibitor precursor with peptides found by peptide mass finger print

<400> 23

Met Tyr Val Glu Phe Met His His His His His Asn Met Leu Leu
1 5 10 15

Leu Phe Leu His Glu Val Ile Asn Gly Glu Arg Val Thr Leu Thr Ala
20 25 30

Cys Ser Glu Cys Val Cys Pro Leu Gln Thr Ser Ser Ser Asp Asp Glu
35 40 45

Tyr Lys Cys Tyr Cys Ala Asp Thr Tyr Ser Asp Cys Pro Gly Phe Cys
50 55 60

Lys Lys Cys Lys Ala Glu Phe Gly Lys Tyr Ile Cys Leu Asp Leu Ile
65 70 75 80

Ser Pro Asn Asp Cys Val Lys Pro Val Ser Ser Ser Glu Ala Lys Gln
85 90 95

Lys Met Ile Lys Gly Glu Arg Val Thr Leu Thr Ala Cys Ser Glu Cys
100 105 110

Val Cys Pro Leu Arg Thr Ser Ser Ser Asp Glu Glu Tyr Lys Cys Tyr
115 120 125

Cys Thr Asp Thr Tyr Ser Asp Cys Pro Gly Phe Cys Lys Lys Cys Lys
130 135 140

Ala Glu Phe Gly Lys Tyr Ile Cys Leu Asp Leu Ile Ser Pro Asn Asp
145 150 155 160

Cys Val Lys Pro Val Ser Ser Leu Glu Ala Lys Gln Asn Met Ile Lys
165 170 175

Glu Glu Arg Val Thr Leu Thr Ala Cys Ser Glu Cys Val Cys Pro Leu
180 185 190

Arg Thr Ser Ser Ser Asp Glu Glu Tyr Lys Cys Tyr Cys Thr Asp Thr
195 200 205

Tyr Ser Asp Cys Pro Gly Phe Cys Lys Thr Cys Lys Ala Glu Phe Gly

210

215

Lys Tyr Ile Cys Leu Asp Leu Ile Ser Pro Asn Asp Cys Val Lys Pro
225 230 235 240

Val Ser Ser Trp Glu Ala Arg Gln Lys Ile Lys Leu Leu Gln Gly Arg
245 250 255

Glu

<210> 24
<211> 44
<212> PRT
<213> Artificial

<220>
<223> Brom-an1

<400> 24

Met Thr Ser Lys Val Gln Leu Val Phe Leu Phe Leu Phe Leu Cys Val
1 5 10 15

Met Trp Ala Ser Pro Ser Ala Ala Ser Cys Asp Glu Pro Ser Asp Pro
20 25 30

Met Met Lys Gln Phe Glu Glu Trp Met Ala Glu Tyr
35 40

<210> 25
<211> 44
<212> PRT
<213> Artificial

<220>
<223> brom_an11

<400> 25

Met Ala Trp Lys Val Gln Val Val Phe Leu Phe Leu Phe Leu Cys Val
1 5 10 15

Met Trp Ala Ser Pro Ser Ala Ala Ser Ala Asp Glu Pro Ser Asp Pro
20 25 30

Met Met Lys Arg Phe Glu Glu Trp Met Val Glu Tyr
35 40

<210> 26
<211> 44
<212> PRT
<213> Artificial

<220>
<223> 91

<400> 26

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Met Ala Trp Lys Val Gln Leu Val Phe Leu Phe Leu Phe Leu Cys Val
1 5 10 15

Met Trp Ala Ser Pro Ser Ala Ala Ser Ala Asp Glu Pro Ser Asp Pro
20 25 30

Met Met Lys Arg Phe Glu Glu Trp Met Val Glu Tyr
35 40

<210> 27
<211> 44
<212> PRT
<213> Artificial

<220>
<223> Brom-fruit

<400> 27

Met Ala Ser Lys Val Gln Leu Val Phe Leu Phe Leu Phe Leu Cys Ala
1 5 10 15

Met Trp Ala Ser Pro Ser Ala Ala Ser Arg Asp Glu Pro Asn Asp Pro
20 25 30

Met Met Lys Arg Phe Glu Glu Trp Met Ala Glu Tyr
35 40

<210> 28
<211> 13
<212> PRT
<213> Artificial

<220>
<223> Inhibitorprecursor

<400> 28

Met Asn Met Leu Leu Leu Phe Leu His Glu Val Ile Asn
1 5 10

<210> 29
<211> 19
<212> PRT
<213> Artificial

<220>
<223> signal peptide of Bromelain inhibitor precursor

<400> 29

Met Asn Met Leu Leu Leu Phe Leu His Glu Val Ile Asn Gly Glu Arg
1 5 10 15

Val Thr Leu

<210> 30

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<211> 76
<212> PRT
<213> Artificial

<220>
<223> Inhibitor protein bi-VII of Bromelain inhibitor precursor
<400> 30

Thr Ala Cys Ser Glu Cys Val Cys Pro Leu Gln Thr Ser Ser Ser Asp
1 5 10 15

Asp Glu Tyr Lys Cys Tyr Cys Ala Asp Thr Tyr Ser Asp Cys Pro Gly
20 25 30

Phe Cys Lys Lys Cys Lys Ala Glu Phe Gly Lys Tyr Ile Cys Leu Asp
35 40 45

Leu Ile Ser Pro Asn Asp Cys Val Lys Pro Val Ser Ser Ser Glu Ala
50 55 60

Lys Gln Lys Met Ile Lys Gly Glu Arg Val Thr Leu
65 70 75

<210> 31
<211> 76
<212> PRT
<213> Artificial

<220>
<223> Inhibitor protein bi-III of Bromelain inhibitor precursor
<400> 31

Thr Ala Cys Ser Glu Cys Val Cys Pro Leu Arg Thr Ser Ser Ser Asp
1 5 10 15

Glu Glu Tyr Lys Cys Tyr Cys Thr Asp Thr Tyr Ser Asp Cys Pro Gly
20 25 30

Phe Cys Lys Lys Cys Lys Ala Glu Phe Gly Lys Tyr Ile Cys Leu Asp
35 40 45

Leu Ile Ser Pro Asn Asp Cys Val Lys Pro Val Ser Ser Leu Glu Ala
50 55 60

Lys Gln Asn Met Ile Lys Glu Glu Arg Val Thr Leu
65 70 75

<210> 32
<211> 68
<212> PRT
<213> Artificial

<220>
<223> Inhibitor protein bi-VI of Bromelain inhibitor precursor
<400> 32

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Thr Ala Cys Ser Glu Cys Val Cys Pro Leu Arg Thr Ser Ser Ser Asp
1 5 10 15

Glu Glu Tyr Lys Cys Tyr Cys Thr Asp Thr Tyr Ser Asp Cys Pro Gly
20 25 30

Phe Cys Lys Thr Cys Lys Ala Glu Phe Gly Lys Tyr Ile Cys Leu Asp
35 40 45

Leu Ile Ser Pro Asn Asp Cys Val Lys Pro Val Ser Ser Trp Glu Ala
50 55 60

Arg Gln Lys Ile
65

<210> 33
<211> 7
<212> PRT
<213> Artificial

<220>
<223> C-terminal of Bromelain inhibitor precursor

<400> 33

Lys Leu Leu Gln Gly Arg Glu
1 5