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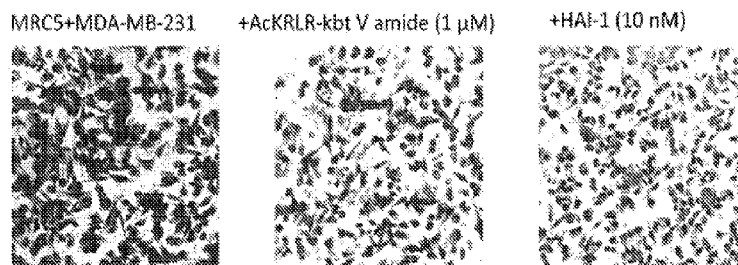
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(54) Title: INHIBITORS OF GROWTH FACTOR ACTIVATION ENZYMES

FIG. 19



(57) Abstract: The present invention generally relates to compounds that are useful for inhibiting one or more of hepatocyte growth factor activator, matriptase, hepsin, Factor Xa, or thrombin. The present invention also relates to various methods of using the inhibitor compounds including treating a malignancy, a pre-malignant condition, or cancer by administering an effective amount of the inhibitor to a subject in need thereof.

INHIBITORS OF GROWTH FACTOR ACTIVATION ENZYMES

FIELD OF THE INVENTION

[0001] The present invention generally relates to compounds that are useful for inhibiting one or more serine proteases including Hepatocyte Growth Factor Activator, matriptase, and hepsin. The present invention also relates to various methods of using the inhibitor compounds including treating a malignancy, a pre-malignant condition, or cancer by administering an effective amount of the inhibitor to a subject in need thereof.

BACKGROUND OF THE INVENTION

[0002] Proteases, also known as proteinases, peptidases, or proteolytic enzymes, are enzymes that degrade proteins by hydrolyzing peptide bonds between amino acid residues. It is known that proteases regulate numerous physiological processes which enable or stimulate the growth and metastasis of tumor cells. This involves the proteolytic degradation of the extracellular matrix proteins surrounding the tumor cells, which enables the invasion of tumor cells metastasizing from the tumors into the surrounding tissue and the lymph system or the blood system. Proteases are also involved in the activation of growth factors that stimulate the proliferation of cancer cells, thus enabling tumors to grow. These proteolytic enzymes include matrix metalloproteases, membrane-bound metalloproteases, lysosomal cysteine proteases, and a large number of serine proteases such as urokinase, plasmin, elastase, thrombin, and cathepsin G. In addition, a family of serine proteases called type II transmembrane serine proteases (TTSPs) has been found to be important in tissue homeostasis and in cancer, in particular with tumor metastasis.

[0003] One member of this enzyme class, matriptase (matriptase-1, MT-SP1, TADG-15, CAP3, epithin, and ST14), is a trypsin-like serine protease expressed by cells of epithelial origin and overexpressed in a wide variety of human cancers. Unlike most proteases, which are either secreted from or retained in the cell, matriptase, as a TTSP, is readily accessible on the cell surface and hence a good target for a variety of therapies, including vaccines, monoclonal antibodies and small molecule compounds. Inhibition of the enzyme results in concomitant inhibition of two crucial mediators of tumorigenesis, hepatocyte growth factor (HGF) and the urokinase-type plasminogen activator (uPA).

[0004] Hepsin is another member of the type II transmembrane serine protease family. Hepsin is thought to play a role in cell growth and is known to be produced at a

particularly high level in the liver as well as in human hepatoma cells, some cancer cells, and nerve cells. Hepatocyte Growth Factor Activator (HGFA) is a trypsin-like protease but is a plasma protease.

[0005] Hepsin, matriptase and HGFA are differentially expressed and have upregulated function in numerous tumor types including multiple myeloma, breast, prostate, lung, liver, and pancreatic. These proteases cleave the single-chain zymogen precursors, pro-HGF (hepatocyte growth factor), and pro-MSP (macrophage stimulating protein) into active two-chain heterodimeric forms. Active HGF and MSP are activating ligands for the oncogenic receptor tyrosine kinases (RTKs), c-MET and RON, respectively.

[0006] Increased activity of hepsin, matriptase, and/or HGFA, resulting from either expression or upregulation of these proteases and/or downregulation of their endogenous serine protease inhibitors (serpins), HAI-1 (SPINT1), HAI-2 (SPINT2), and protein C inhibitor (PCI), has been demonstrated in almost every tumor type driven by c-MET and/or RON pathway signaling. This increased protease function has been clearly associated with increased metastatic cancer phenotypes and direct inhibition of this protease activity has been demonstrated to reduce this metastatic potential in multiple tumor types. The biological reason for the redundancy of activation by these three different proteases and the tight regulation by serpins in cancer is not yet understood. Furthermore, since HGF/c-MET and MSP/RON signaling are necessary for development and normal cell physiology, selective inhibitors of each protease involved in individual tumors need to be identified when developing as therapeutics in order to limit potential toxicities.

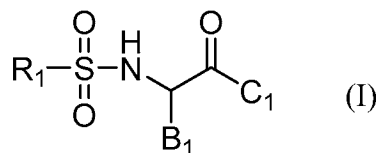
[0007] Matriptase inhibitors are of therapeutic importance, but development has been a challenging task. To date, a number of small molecule inhibitors and inhibitory antibodies have been reported. See, for example, Enyedy et al., *J. Med. Chem.* 2001, 44, 1349-1355; Steinmetzer et al., *J. Med. Chem.*, 2006, 49: 4116-4126, and Farady et al., *J Mol Biol*, 2007, 369: 1041-1051. Also a series of peptide-based matriptase inhibitors was recently described by Marsault et al., *ACS Med Chem. Lett.*, 2012, 3: 530-534.

[0008] As compared to matriptase, inhibitory antibodies have also been reported but relatively few inhibitors are known for hepsin and HFGA. Small molecule hepsin inhibitors were discovered through high-throughput screening (Chevillet, J. R., et al. *Mol. Cancer Ther.* 2008, 7, 3343) but the only reported HGFA inhibitors are the non-selective serine protease inhibitors, Nafamostat and Leupeptin (Shimomura, T., et al., *Cytotechnology*, 1992, 8, 219).

[0009] Although some progress has been made toward inhibitors of matriptase, hepsin, and HGFA, there remains a need for small molecular weight inhibitors of matriptase, hepsin, and HGFA that are both potent and selective for one or more of these enzymes. Such compounds have significant therapeutic value, in particular for the treatment of cancer and other conditions involving tumor progression and migration and abnormal cell differentiation and proliferation or hematological malignancies. Compounds having improved selectivity, solubility, metabolic stability, half-life, and oral bioavailability are particularly desirable.

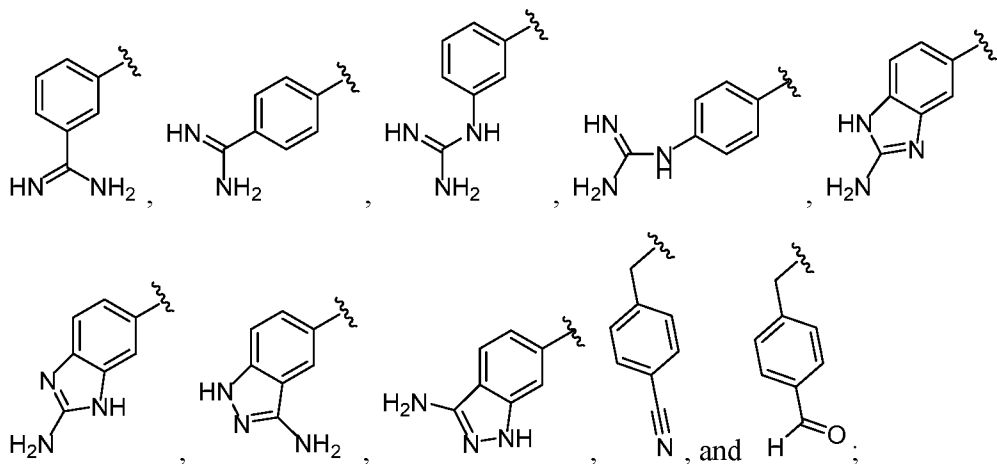
SUMMARY OF THE INVENTION

[0010] Generally, the present invention relates to compounds that are useful for inhibiting one or more serine proteases including Hepatocyte Growth Factor Activator, matriptase, hepsin, thrombin, and Factor Xa along with various methods of use. In various aspects, the present invention is directed to compounds of Formula (I), as a single stereoisomer or as a mixture thereof:

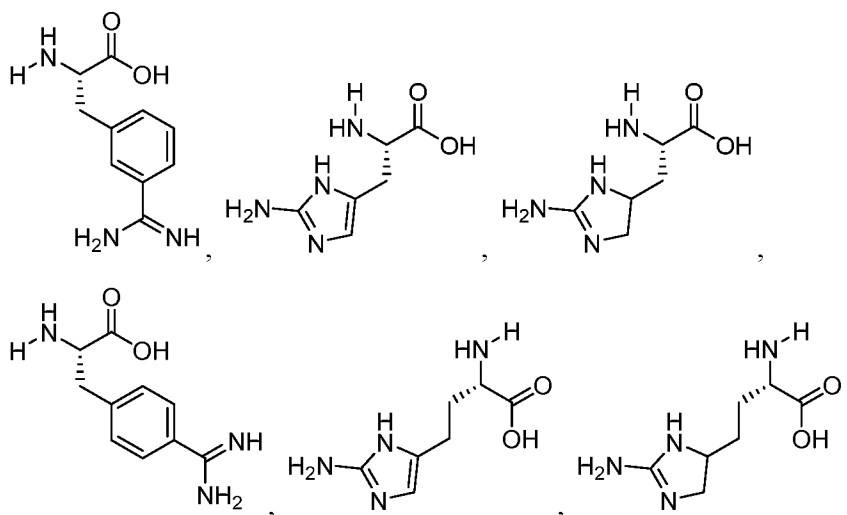


wherein R_1 is substituted or unsubstituted alkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl;

B_1 is selected from the group consisting of:



P₁ is a residue of an amino acid selected from the group consisting of Arg, D-Arg, Lys, substituted Lys, and an alpha-amino acid of the following:



or an unnatural amino acid residue;

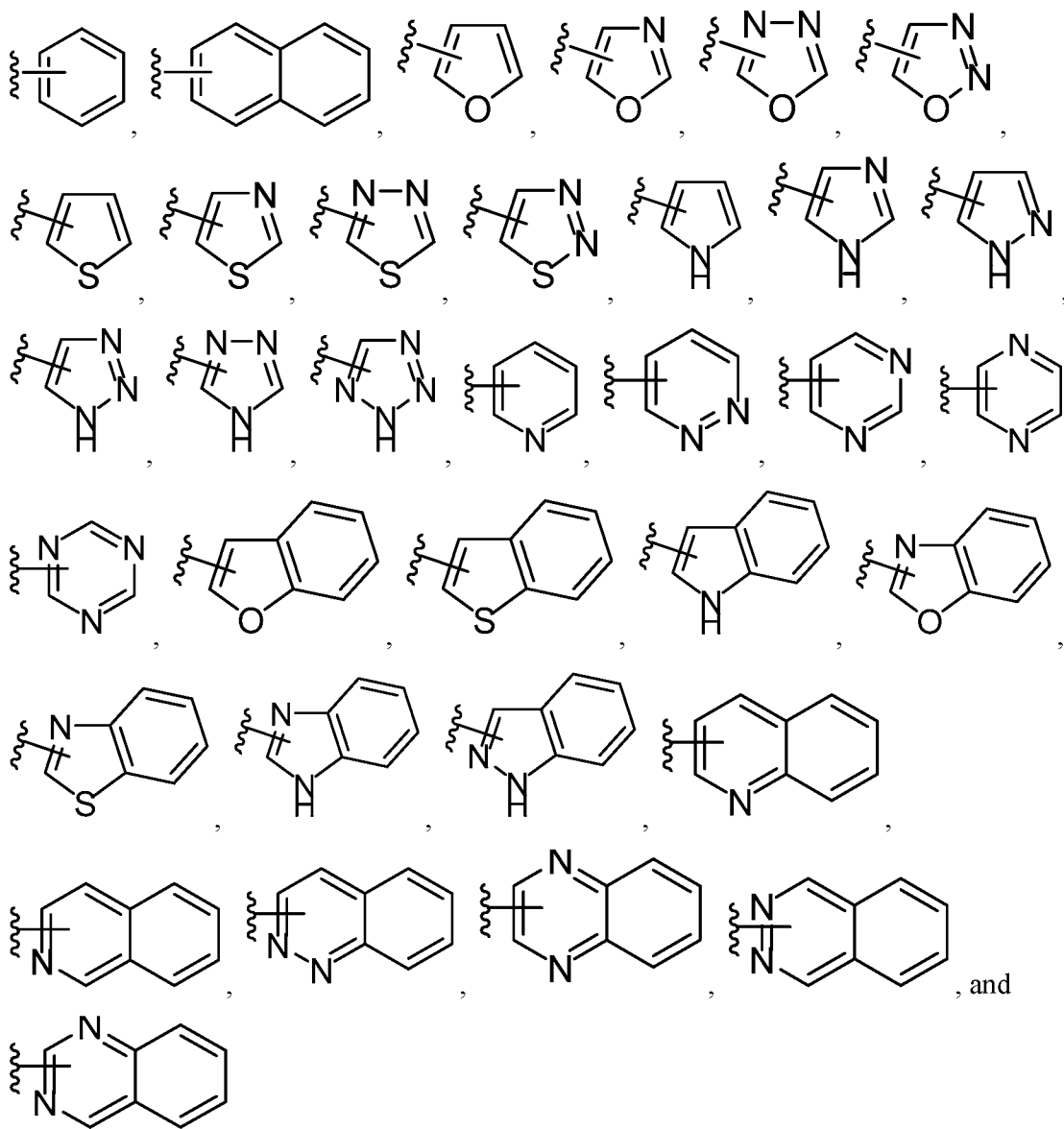
P₂ is a residue of an amino acid selected from the group consisting of Leu, Phe, Met, Thr, Val, Tyr, Trp, Ser, Pro, His, Glu, Gln, Asp, Arg, Lys, Ile, Ala, Gly, Asn, hLeu, NptGly, L-Orn, L-Cha, Nle, hTyr, Nva, Orn, Cha, and an unnatural amino acid residue;

P₃ is a residue of an amino acid selected from the group consisting of Asp, Glu, Arg, Lys, Met, Trp, Leu, Gln, Phe, Tyr, His, hArg, D-Trp, L-Orn, D-Gln, L-Met(O), L-Nle(OBzl), Agp, hCha, hTyr, hPhe, D-Arg, Nle(OBzl), Orn, Met(O), and an unnatural amino acid residue;

P₄ is a residue of an amino acid selected from the group consisting of Arg, Lys, Met, Trp, Trp, Ser, His, Phe, Thr, Asn, Pro, Gln, Asp, Glu, Chg, Idc, dhLeu, Agp, D-Ser, Agp, His(3-Bom), Lys(2-Cl-Z), L-Orn, L-Arg(NO₂), L-Nle(OBzl), L-DAB(Z), and an unnatural amino acid residue;

P₅ is a residue of an amino acid selected from the group consisting of Lys, Arg, Leu, Phe, Met, Thr, Val, Tyr, Trp, Ser, Pro, His, Glu, Gln, Asp, Ile, Ala, Gly, Asn, and an unnatural amino acid residue; and

Z is Val, Ser, Lys, Ala, Gly, Trp, Tyr, Phe, Arg, Thr, Leu, Ile, Met, His, Nle, Phg, Pro, Gln, Asn, $-\text{CH}_2\text{Cl}$, or a substituted or unsubstituted ring substituent selected from the group consisting of:



[0012] The present invention further relates to pharmaceutical compositions comprising a therapeutically effective amount of at least one compound as described herein.

[0013] The present invention also relates to various methods of use including a method of inhibiting matriptase, hepsin, or HGFA comprising administering to a subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of at least one compound as described herein. Another method includes a method of inhibiting

HGF/MET oncogenic signaling comprising administering to a subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of at least one compound as described herein.

[0014] Other methods include a method of inhibiting carcinoma progression and a method of treating a malignancy, a pre-malignant condition, or cancer comprising administering to the subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of at least one compound as described herein.

[0015] Further, the present invention relates to imaging compositions comprising a radiolabeled compound of Formula (I) or (II) as described herein and a method of detecting cancer comprising administering to a subject an imaging composition comprising the radiolabeled compound; employing a nuclear imaging technique for monitoring or visualizing a distribution of the radiolabeled compound within the body or within a portion thereof; and correlating the distribution of the radiolabeled compound to the existence of cancer.

[0016] Additionally, the present invention relates to imaging compositions comprising a fluorescent compound of Formula (I) or (II) as described herein and a method of detecting cancer comprising administering to a subject an imaging composition comprising the fluorescent compound; employing an imaging technique for monitoring or visualizing a distribution of the fluorescent compound within the body or within a portion thereof; and correlating the distribution of the fluorescent compound to the existence of cancer.

[0017] Other objects and features will be in part apparent and in part pointed out hereinafter.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] FIG. 1 presents a plot of the results for the HGFA competition assay of inhibitor Ac-KQLR(SEQ ID NO: 1)-Kt.

[0019] FIG. 2 presents a plot of the results for the fluorescent inhibitor assay for inhibitor Boc-QLR-AMC and HGFA.

[0020] FIG. 3 presents a plot of the results for the fluorescent inhibitor assay for inhibitor Boc-QAR-AMC and hepsin.

[0021] FIG. 4 presents a plot of the results for the fluorescent inhibitor assay for inhibitor Boc-QAR-AMC and matriptase.

[0022] FIG. 5 presents a plot of the results of a dilution recovery experiments with Ac-KQLR(SEQ ID NO: 1)-Kt and HGFA.

[0023] FIG. 6 presents a plot of the results of a dilution recovery experiments with Ac-KQLR(SEQ ID NO: 1)-Kt and HGFA.

[0024] FIG. 7 shows the inhibition of HGFA-mediated scHGF (pro-HGF) cleavage by inhibitors. Immunoblot of scHGF cleavage reactions: Pro-HGF (30 ng) was cleaved with 1 nM HGFA in the presence of inhibitors (5-fold dilutions from 12.5 μ M).

[0025] FIG. 8 shows the inhibition of HGFA-mediated scMSP (pro-MSP) cleavage by inhibitors. Immunoblot of scMSP cleavage reactions: Pro-MSP (50 ng) was cleaved with 10 nM HGFA in the presence of inhibitors (5-fold dilutions from 12.5 μ M).

[0026] FIG. 9 shows MDA-MB-231 c-MET phosphorylation of cells treated with pro-HGF/HGFA reactions (3-fold dilutions of inhibitors starting at 100 μ M). A. Immunoblot of pY1234/135 c-MET; B. % inhibition of c-MET phosphorylation.

[0027] FIG. 10 presents a plot of the results of a chromogenic kinetic enzyme assay for matriptase and selected inhibitor compounds.

[0028] FIG. 11 presents a plot of the results of a chromogenic kinetic enzyme assay for matriptase and selected inhibitor compounds.

[0029] FIG. 12 presents a plot of the results of a chromogenic kinetic enzyme assay for hepsin and selected inhibitor compounds.

[0030] FIG. 13 presents a plot of the results of a chromogenic kinetic enzyme assay for matriptase and selected inhibitor compounds.

[0031] FIG. 14 presents a plot of the results of a chromogenic kinetic enzyme assay for matriptase and selected inhibitor compounds.

[0032] FIG. 15 presents a plot of the results of a chromogenic kinetic enzyme assay for matriptase and selected inhibitor compounds.

[0033] FIG. 16 presents a plot of the results of a chromogenic kinetic enzyme assay for hepsin and selected inhibitor compounds.

[0034] FIG. 17 presents a plot of the results of a chromogenic kinetic enzyme assay for hepsin and selected inhibitor compounds.

[0035] FIG. 18 shows MDA-MB-231 vs T47D breast cancer cells following MSP stimulation. A. Immunoblot of total RON and MAPK of breast cancer cells; B. RON phosphorylation ELISA assay.

[0036] FIG. 19 shows the inhibition of MRC-5 mediated/pro-HGF migration and invasion of triple negative human MDA-MB-231 breast cancer cells using selected inhibitor compounds.

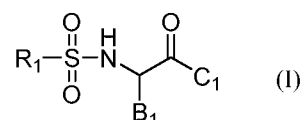
[0037] FIG. 20 shows the MSP-MS profiling for HGFA substrate cleavage and specificity.

[0038] FIG. 21 shows iceLogo figures. A. Shows frequency at which specific amino acids are present at each position. B. Comparison of P4 and P4' amino acid frequency of the 22 peptides that were cleaved versus all possible peptides that contained an arginine.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

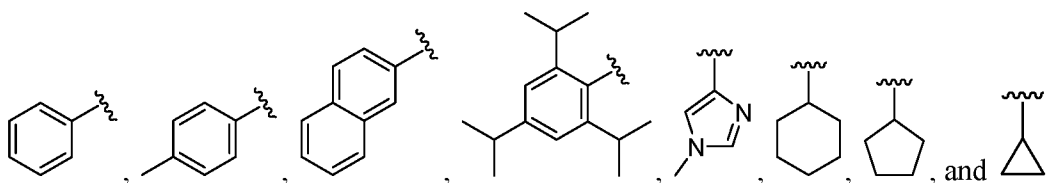
[0039] In various aspects, the present invention generally relates to compounds that are useful for inhibiting one or more serine proteases, including Hepatocyte Growth Factor Activator, matriptase, hepsin, thrombin, and Factor Xa along with various methods of use. In other aspects, the present invention also relates to various methods of using the inhibitor compounds including treating a malignancy, a pre-malignant condition, or cancer by administering an effective amount of the inhibitor to a subject in need thereof.

[0040] In accordance with the present invention, one class of compounds useful for inhibiting one or more of hepatocyte growth factor activator, matriptase, and hepsin includes benzamidine compounds of Formula (I), as a single stereoisomer or as a mixture thereof:

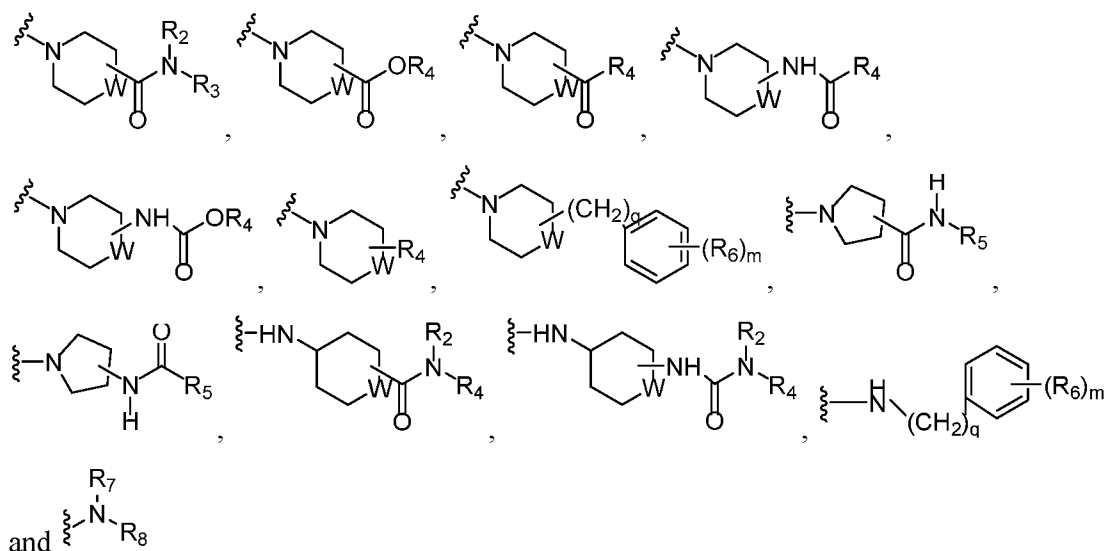


wherein R₁ is substituted or unsubstituted alkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl;

[0041] In various embodiments, R_1 is substituted or unsubstituted C_1 - C_6 alkyl, substituted or unsubstituted C_3 - C_6 cycloalkyl, substituted or unsubstituted phenyl, substituted or unsubstituted naphthyl, or a substituted or unsubstituted nitrogen-containing aromatic ring. For example, the substituted C_1 - C_6 alkyl, substituted C_3 - C_6 cycloalkyl, substituted phenyl, substituted naphthyl, or substituted nitrogen-containing aromatic ring can comprise one or more substituents comprising halo, hydroxy, C_1 - C_6 alkyl, C_1 - C_6 alkoxy, halo-substituted C_1 - C_4 alkyl, or amino. In certain embodiments, R_1 is an group selected from the group consisting of:



[0042] In some embodiments, C_1 is a group selected from the group consisting of:

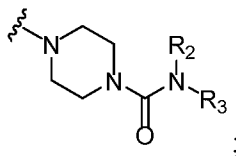


W is CH or N ;

R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , and R_8 are each independently hydrogen, substituted or unsubstituted alkyl or cycloalkyl, substituted or unsubstituted alkylaryl, substituted or unsubstituted heterocyclic ring, substituted or unsubstituted aryl or heteroaryl, with the proviso that when R_2 is methyl, then R_3 cannot also be methyl and vice versa; and m is 0 to 5, or a pharmaceutically acceptable salt thereof.

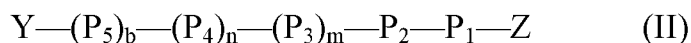
[0043] In some embodiments, R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , and R_8 are each independently hydrogen, substituted or unsubstituted C_1 - C_{10} alkyl or cycloalkyl, substituted or unsubstituted heterocyclic ring, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl. In particular embodiments, R_2 is hydrogen; R_3 is hydrogen, C_1 - C_6 alkyl, benzyl, or halo-substituted benzyl; R_4 and R_5 are each independently hydrogen, C_1 - C_6 alkyl, halo- or alkoxy-substituted C_1 - C_6 alkyl, phenyl, phenethyl, benzyl, halo- or alkoxy-substituted benzyl; substituted or unsubstituted 3-benzothiophenyl, or substituted or unsubstituted 1-morpholinyl; R_6 is hydrogen, C_1 - C_4 alkoxy; and/or R_7 and R_8 are each independently hydrogen or C_1 - C_6 alkyl.

[0044] In certain embodiments, C_1 is



R_2 is hydrogen; and R_3 is hydrogen, C_1 - C_6 alkyl, benzyl, or halo-substituted benzyl. In these and other embodiments, the compound of Formula (I) is a selective inhibitor of hepsin.

[0045] In accordance with the present invention, another class of compounds useful for inhibiting one or more of HGFA, matriptase, and hepsin includes polypeptide of Formula (II):



wherein

n is 0 or 1;

m is 0 or 1;

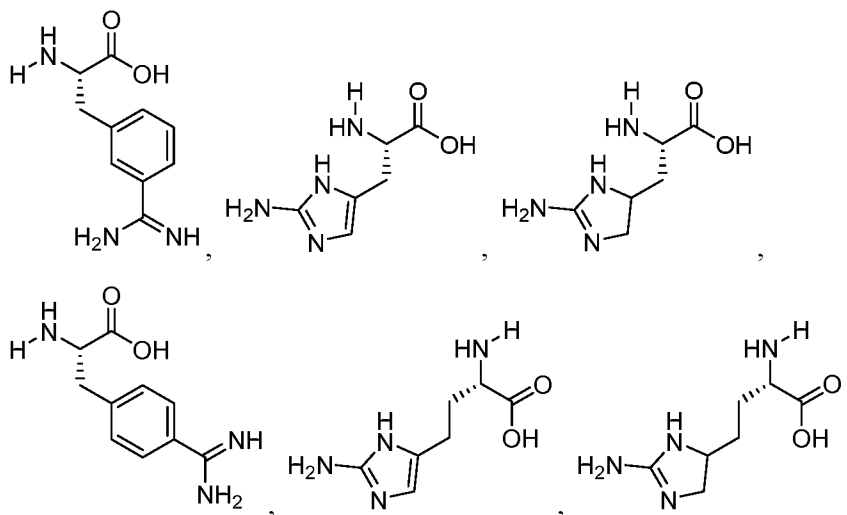
b is 0 or 1;

Y is H, acetyl, *tert*-butyloxycarbonyl, benzyloxymethyl acetyl, carboxybenzyl, fluorene, benzyl, $-C(O)R_9$, $-SOOR_9$, $-COOR_9$, $-C(O)NHR_9$, $-(CH_2)_x$ aryl- R_9 , heteroaryl- R_9 , -cycloalkyl- R_9 , a fluorophore, biotin, or a reporter tag;

x is 0, 1, or 2;

R_9 is C_1 to C_{12} alkyl, cycloalkyl, alkylaryl, or aryl;

P₁ is a residue of an amino acid selected from the group consisting of Arg, D-Arg, Lys, substituted Lys, and an alpha-amino acid of the following:



or an unnatural amino acid residue;

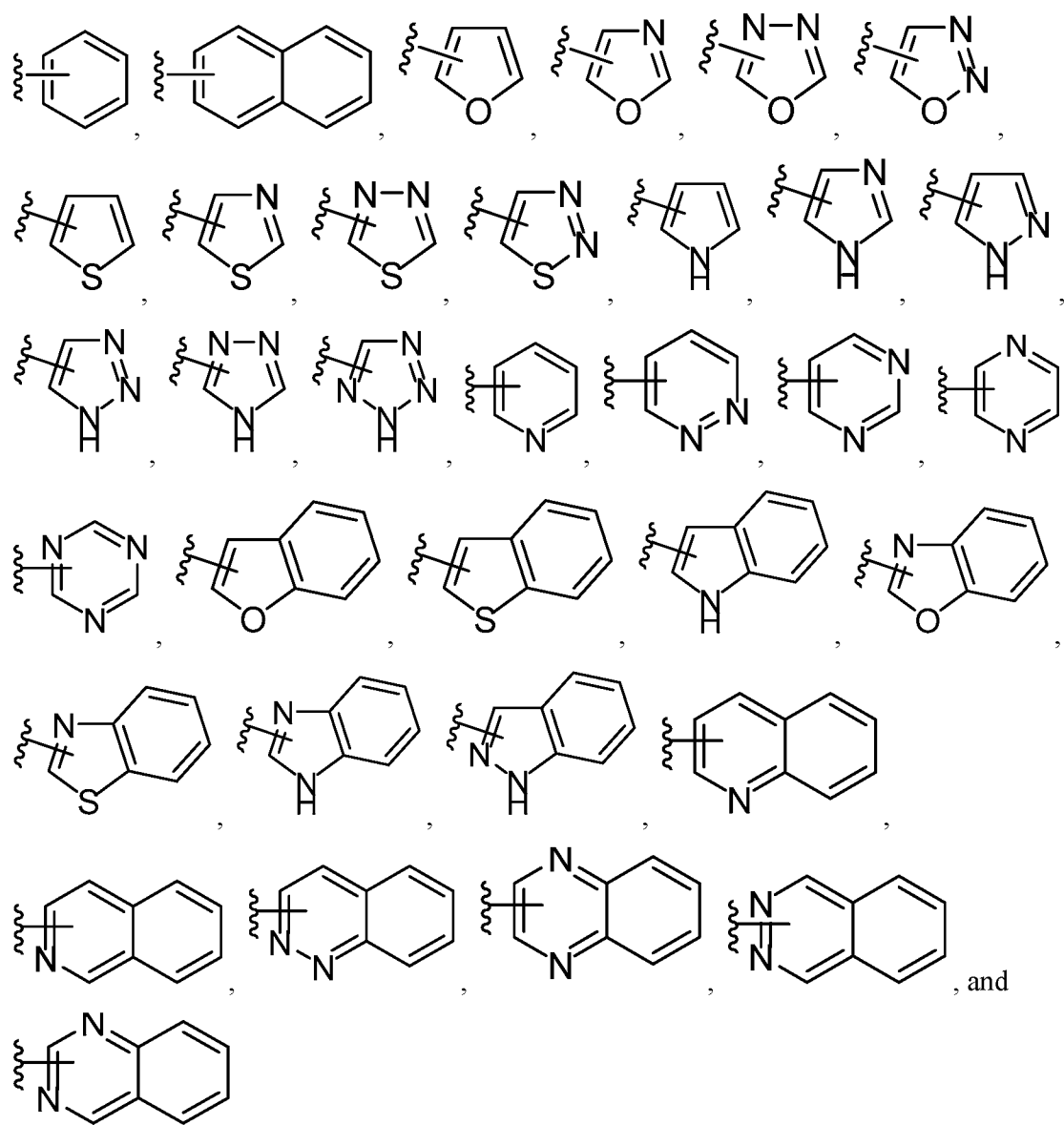
P₂ is a residue of an amino acid selected from the group consisting Leu, Phe, Met, Thr, Val, Tyr, Trp, Ser, Pro, His, Glu, Gln, Asp, Arg, Lys, Ile, Ala, Gly, Asn, hLeu, NptGly, L-Orn, L-Cha, Nle, hTyr, Nva, Orn, Cha, and an unnatural amino acid residue;

P₃ is a residue of an amino acid selected from the group consisting Asp, Glu, Arg, Lys, Met, Trp, Leu, Gln, Phe, Tyr, His, hArg, D-Trp, L-Orn, D-Gln, L-Met(O), L-Nle(OBzl), Agp, hCha, hTyr, hPhe, D-Arg, Nle(OBzl), Orn, Met(O), and an unnatural amino acid residue;

P₄ is a residue of an amino acid selected from the group consisting Arg, Lys, Met, Try, Trp, Ser, His, Phe, Thr, Asn, Pro, Gln, Asp, Glu, Chg, Idc, dhLeu, Agp, D-Ser, Agp, His(3-Bom), Lys(2-Cl-Z), L-Orn, L-Arg(NO₂), L-Nle(OBzl), L-DAB(Z), and an unnatural amino acid residue;

P₅ is a residue of an amino acid selected from the group consisting Lys, Arg, Leu, Phe, Met, Thr, Val, Tyr, Trp, Ser, Pro, His, Glu, Gln, Asp, Ile, Ala, Gly, Asn, and an unnatural amino acid residue; and

Z is Val, Ser, Lys, Ala, Gly, Trp, Tyr, Phe, Arg, Thr, Leu, Ile, Met, His, Nle, Phg, Pro, Gln, Asn, $-\text{CH}_2\text{Cl}$, or a substituted or unsubstituted ring substituent selected from the group consisting of:



[0046] As understood herein, when two or more amino acids combine to form a peptide (e.g., of Formula (II)), the elements of water are removed, and what remains of each amino acid is called an amino-acid residue.

[0047] In various embodiments, the polypeptide of Formula (II) include one or more of the following:

Y is H, acetyl, *tert*-butyloxycarbonyl, benzyloxymethyl acetyl, carboxybenzyl, fluorene, benzyl, -C(O)R₉, -SOOR₉, -COOR₉, -C(O)NHR₉, -(CH₂)_xaryl-R₉, heteroaryl-R₉, -cycloalkyl-R₉, or a fluorophore;

x is 0, 1, or 2;

R₉ is C₁ to C₁₂ alkyl, cycloalkyl, alkylaryl, or aryl;

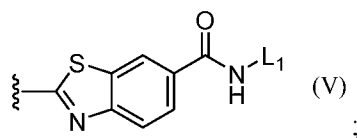
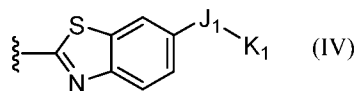
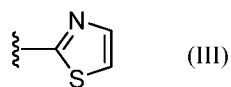
P₂ is a residue of an amino acid selected from the group consisting Leu, Phe, Met, Thr, Val, Tyr, Trp, Ser, Pro, His, Glu, Gln, Asp, Arg, Lys, Ile, Ala, Gly, Asn, hLeu, NptGly, L-Orn, L-Cha, Nle, hTyr, Nva, Orn, Cha, and an unnatural amino acid residue;

P₃ is a residue of an amino acid selected from the group consisting Asp, Glu, Arg, Lys, Met, Trp, Leu, Gln, Phe, Tyr, His, hArg, D-Trp, L-Orn, D-Gln, L-Met(O), L-Nle(OBzl), Agp, hCha, hTyr, hPhe, D-Arg, Nle(OBzl), Orn, Met(O), and an unnatural amino acid residue;

P₄ is a residue of an amino acid selected from the group consisting Arg, Lys, Met, Trp, Trp, Ser, His, Phe, Thr, Asn, Pro, Gln, Asp, Glu, Chg, Idc, dhLeu, Agp, D-Ser, Agp, His(3-Bom), Lys(2-Cl-Z), L-Orn, L-Arg(NO₂), L-Nle(OBzl), L-DAB(Z), and an unnatural amino acid residue;

P₅ is a residue of an amino acid selected from the group consisting Lys, Arg, Leu, Phe, Met, Thr, Val, Tyr, Trp, Ser, Pro, His, Glu, Gln, Asp, Ile, Ala, Gly, Asn, and an unnatural amino acid residue; and

Z is Val, Ser, Lys, Ala, Gly, Trp, Tyr, Phe, Arg, Thr, Leu, Ile, Met, His, Nle, Phg, Pro, Gln, Asn, -CH₂Cl, a thiazole of Formula (III), or a benzothiazole of Formula (IV) or (V)



J₁ is C(O), SO₂, CH₂ or heterocyclo;

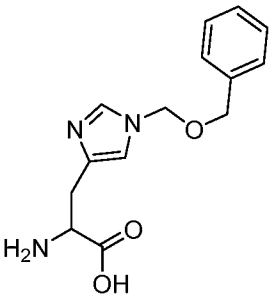
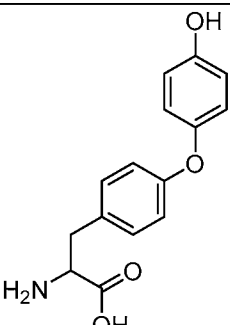
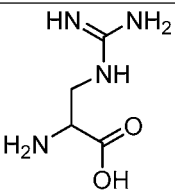
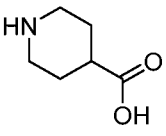
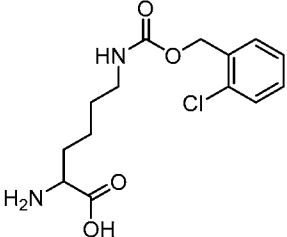
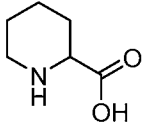
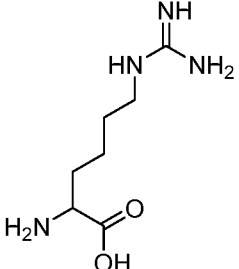
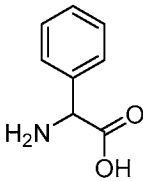
K₁ is a D- or L-amino acid, wherein the C-terminus is -COOH, -C(O)NH₂, -OH, -OR₁₀, -NH₂, -NR₁₁R₁₂, -H or heterocyclo;

R₁₀ is C₁ to C₁₂ alkyl, cycloalkyl, alkylaryl, or aryl;

R₁₁ and R₁₂ are each independently H, C₁ to C₁₂ alkyl, cycloalkyl, alkylaryl, aryl, or heterocyclo; and R₁₁ and R₁₂ together can form a ring; and/or

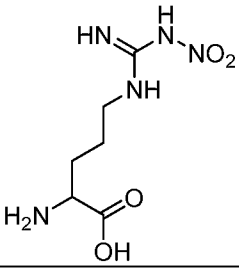
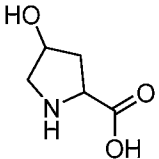
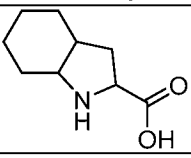
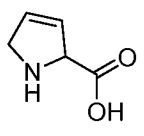
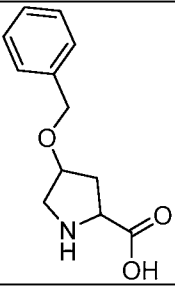
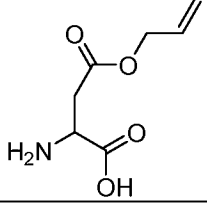
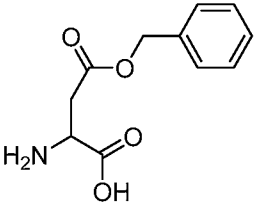
L₁ is H, alkyl, cycloalkyl, alkylaryl, benzyl, substituted benzyl, 2- or 3- or 4-piperdiny, 2- or 3- or 4-pyridiny, alkyl, cycloalkyl, aryl, heterocyclo, or heteroaryl.

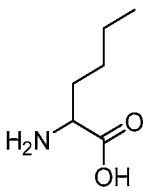
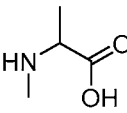
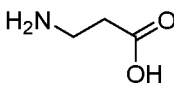
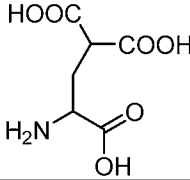
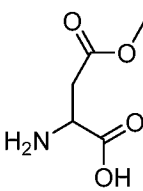
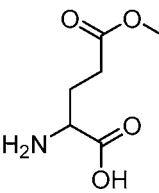
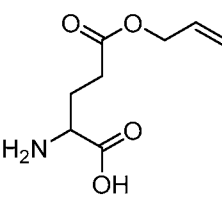
[0048] In various embodiments, P₁, P₂, P₃, P₄ and P₅ can each independently be an unnatural amino acid residue. The unnatural amino acids can be selected from the group listed in the following Table. The unnatural amino acids can be the D- and/or L-isomers.

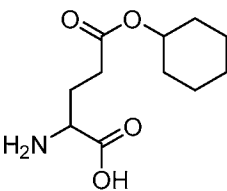
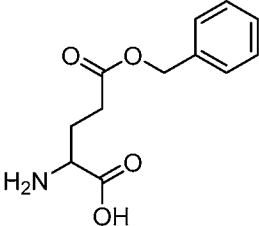
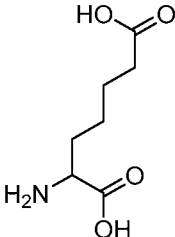
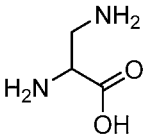
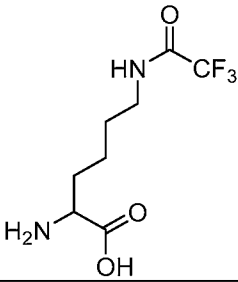
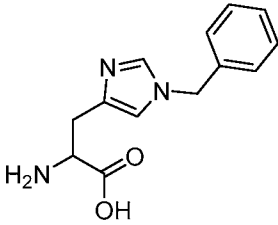
Abbreviation	Structure	Abbreviation	Structure
His(3-Bom)		Thyr	
Agp		Inp	
Lys(2-Cl-Z)		Pip	
hArg		Phg	

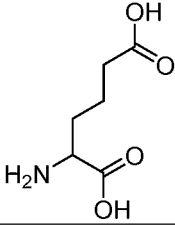
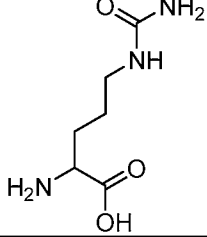
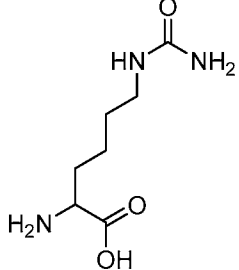
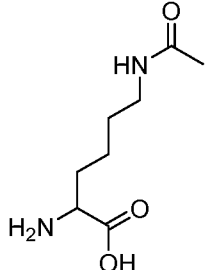
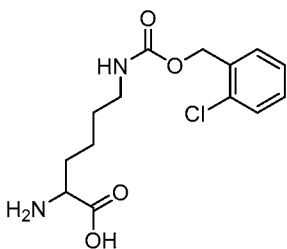
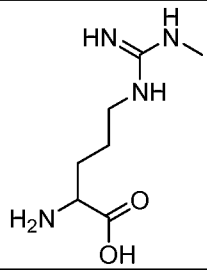
Abbreviation	Structure
hTyr	
hPhe	
hLeu	
NptGly	
DAB(Z)	
Nle(OBzl)	

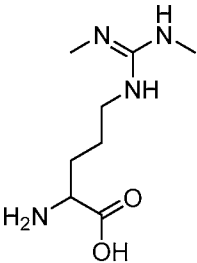
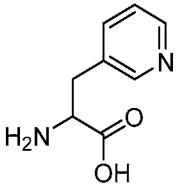
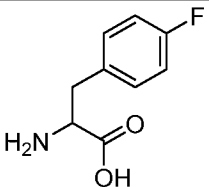
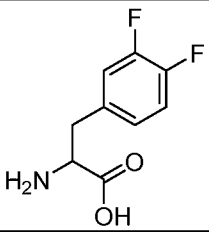
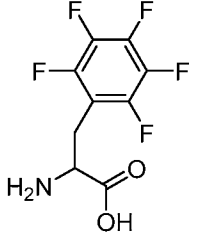
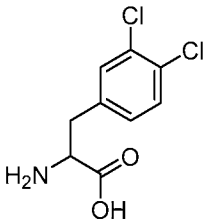
Abbreviation	Structure
3-Pal	
4-Pal	
hCha	
Orn	
Met(O)	
Cha	

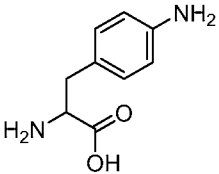
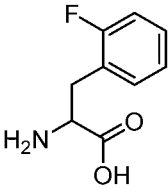
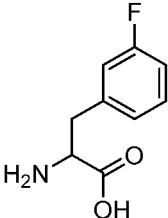
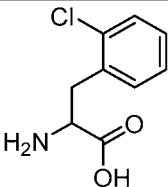
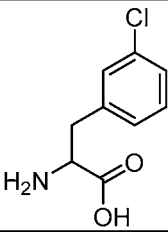
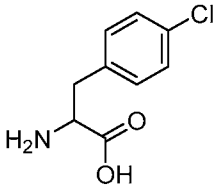
Abbreviation	Structure
Arg(NO ₂)	
Hyp	
Oic	
hPro	
Hyp(Bzl)	
Asp(All)	
Asp(Bzl)	

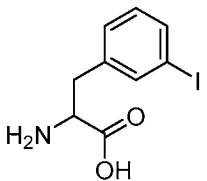
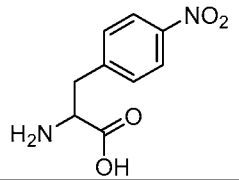
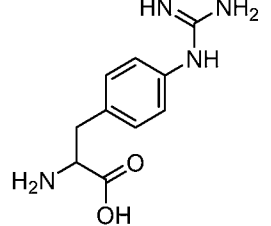
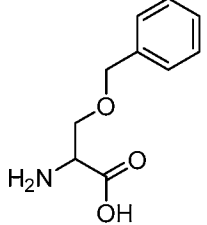
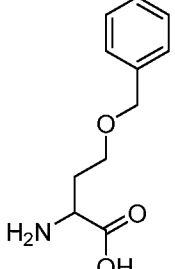
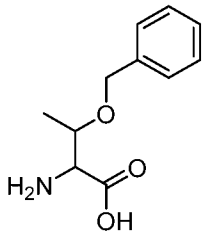
Abbreviation	Structure
Nle	
MeAla	
βAla	
Gla	
Asp(Me)	
Glu(Me)	
Glu(All)	

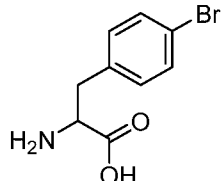
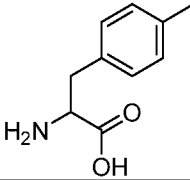
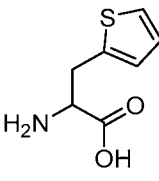
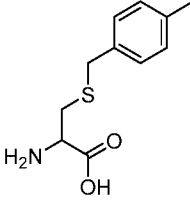
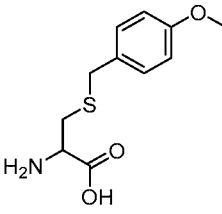
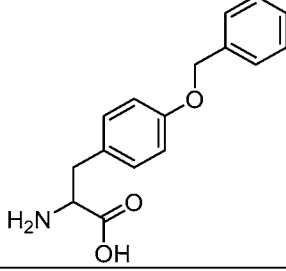
Abbreviation	Structure
Glu(Chx)	
Glu(Bzl)	
Api	
Dap	
Lys(TFA)	
His(Bzl)	

Abbreviation	Structure
Aad	
Cit	
hCit	
Lys(Ac)	
Lys(2Cl-Z)	
Arg(Me)	

Abbreviation	Structure
Arg(Me) ₂	
3-Pal	
4-Pal	
Phe(4-F)	
Phe(3,4-F)	
Phe(F ₅)	
Phe(3,4-Cl)	

Abbreviation	Structure
Phe(4-NH ₂)	
Phe(2-F)	
Phe(3-F)	
Phe(2-Cl)	
Phe(3-Cl)	
Phe(4-Cl)	
Phe(4-I)	

Abbreviation	Structure
Phe(3-I)	
Phe(4-NO ₂)	
Phe(4-guan)	
Ser(Bzl)	
hSer(Bzl)	
Thr(Bzl)	

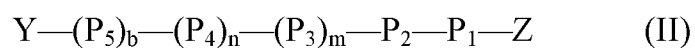
Abbreviation	Structure
Phe(4-Br)	
Phe(4-Me)	
Ala(2-th)	
Cys(4-MeBzl)	
Cys(4-MeOBzl)	
Tyr(Bzl)	

Abbreviation	Structure
Cys(Bzl)	
Tyr(Me)	
hTyr(Me)	
Tyr(2,6-Cl-Bzl)	
Bpa	
1-Nal	

Abbreviation	Structure
Dht	
Trp(Me)	
Abu(Bth)	
Bip	
hSer	
Hnv	

Abbreviation	Structure	Abbreviation	Structure
2-Nal		Met(O ₂)	
Abu		AC5C	
Nva		Chg	
2-Aoc		Tic	
Tle		Ally Gly	
4-NO ₂ -3-F-Phe			

[0049] In accordance with the present invention, another class of compounds useful for inhibiting one or more of HGFA, matriptase, and hepsin includes cyclic peptides of Formula (II):



wherein

n is 0 or 1;

m is 0 or 1;

b is 0 or 1;

Y is H, acetyl, *tert*-butyloxycarbonyl, benzyloxymethyl acetyl, carboxybenzyl, fluorene, benzyl, -C(O)R₉, -SOOR₉, -COOR₉, -C(O)NHR₉, -(CH₂)_xaryl-R₉, heteroaryl-R₉, -cycloalkyl-R₉, or a fluorophore;

x is 0, 1, or 2;

R₉ is C₁ to C₁₂ alkyl, cycloalkyl, alkylaryl, or aryl;

P₁ is a residue of an amino acid selected from the group consisting Arg, D-Arg, Lys, substituted Lys, and an unnatural amino acid residue;

P₂ is a residue of an amino acid selected from the group consisting Leu, Phe, Met, Thr, Val, Tyr, Trp, Ser, Pro, His, Glu, Gln, Asp, Arg, Lys, Ile, Ala, Gly, Asn, hLeu, NptGly, L-Orn, L-Cha, Nle, hTyr, Nva, Orn, Cha, and an unnatural amino acid residue;

P₃ is a residue of an amino acid selected from the group consisting Asp, Glu, Arg, Lys, Met, Trp, Leu, Gln, Phe, Tyr, His, hArg, D-Trp, L-Orn, D-Gln, L-Met(O), L-Nle(OBzl), Agp, hCha, hTyr, hPhe, D-Arg, Nle(OBzl), Orn, Met(O), and an unnatural amino acid residue;

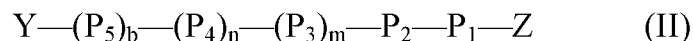
P₄ is a residue of an amino acid selected from the group consisting Arg, Lys, Met, Trp, Trp, Ser, His, Phe, Thr, Asn, Pro, Gln, Asp, Glu, Chg, Idc, dhLeu, Agp, D-Ser, Agp, His(3-Bom), Lys(2-Cl-Z), L-Orn, L-Arg(NO₂), L-Nle(OBzl), L-DAB(Z), and an unnatural amino acid residue;

P₅ is a residue of an amino acid selected from the group consisting Lys, Arg, Leu, Phe, Met, Thr, Val, Tyr, Trp, Ser, Pro, His, Glu, Gln, Asp, Ile, Ala, Gly, Asn, and an unnatural amino acid residue;

P₃ can bond with P₅ and form a cyclic peptide; and

P₂ can bond with P₄ and form a cyclic peptide.

[0050] In various embodiment, a compound useful for inhibiting HGFA includes a compound of Formula (II):



wherein

n is 0 or 1;

m is 0 or 1;

b is 0 or 1;

Y is H, acetyl, *tert*-butyloxycarbonyl, benzyloxymethyl acetyl, carboxybenzyl, fluorene, benzyl, -C(O)R₉, -SOOR₉, -COOR₉, -C(O)NHR₉, -(CH₂)_xaryl-R₉, heteroaryl-R₉, -cycloalkyl-R₉, or a fluorophore;

x is 0, 1, or 2;

R₉ is C₁ to C₁₂ alkyl, cycloalkyl, alkylaryl, or aryl;

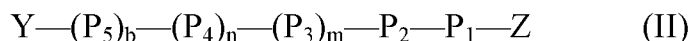
R₁ is Arg;

R₂ is selected from the group consisting of Leu, Met, Phe, Tyr, Trp, hLeu, NptGly, Nle, hTyr, and Nva;

P₃ is selected from the group consisting of His, Gln, Arg, Lys, Leu, Phe, Trp, Tyr, hArg, D-Trp, Agp, hCha, hTyr, hPhe, and D-Arg; and

P₄ is selected from the group consisting of Thr, Asn, Ser, Arg, Lys, Phe, Trp, His(Bom), Agp, Lys(2-Cl-Z), dhLeu, Idc, and Chg.

[0051] In other embodiments, a compound useful for inhibiting matriptase includes a compound of Formula (II):



wherein

n is 0 or 1;

m is 0 or 1;

b is 0 or 1;

Y is H, acetyl, *tert*-butyloxycarbonyl, benzyloxymethyl acetyl, carboxybenzyl, fluorene, benzyl, -C(O)R₉, -SOOR₉, -COOR₉, -C(O)NHR₉, -(CH₂)_xaryl-R₉, heteroaryl-R₉, -cycloalkyl-R₉, or a fluorophore;

x is 0, 1, or 2;

R₉ is C₁ to C₁₂ alkyl, cycloalkyl, alkylaryl, or aryl;

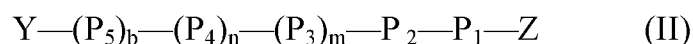
R₁ is selected from the group consisting of Arg, and Lys;

R₂ is selected from the group consisting of Phe, Ala, Arg, Asn, Gln, Glu, Gly, His, Leu, Lys, Met, Pro, and Ser;

R₃ is selected from the group consisting of Arg, Leu, Trp, Phe, His, Gln, Lys, D-Trp, and D-Arg; and

R₄ is selected from the group consisting of Pro, Phe, Thr, Asn, Trp, Gln, Ser, Lys, and Arg, His(Bom).

[0052] In further embodiments, a compound useful for inhibiting hepsin includes a compound of Formula (II):



wherein

n is 0 or 1;

m is 0 or 1;

b is 0 or 1;

Y is H, acetyl, *tert*-butyloxycarbonyl, benzyloxymethyl acetyl, carboxybenzyl, fluorene, benzyl, -C(O)R₉, -SOOR₉, -COOR₉, -C(O)NHR₉, -(CH₂)_xaryl-R₉, heteroaryl-R₉, -cycloalkyl-R₉, or a fluorophore;

x is 0, 1, or 2;

R₉ is C₁ to C₁₂ alkyl, cycloalkyl, alkylaryl, or aryl;

R₁ is Arg;

R₂ is selected from the group consisting of Pro, Arg, Asn, Asp, Gln, Ile, Leu, Lys, Phe, Thr, Trp, Tyr, Orn, Cha, Nle, and Nva;

R₃ is selected from the group consisting of Leu, Trp, Phe, His, Gln, Lys, Arg, D-Gln, Agp, Nle (OBzl), Orn, Met(O), D-Trp, and D-Arg; and

R₄ is selected from the group consisting of Pro, Phe, Thr, Asn, Trp, Gln, Ser, Arg, Lys, Agp, DAB(Z), Nle (OBzl), Orn, Arg(NO₂), and His(Bom).

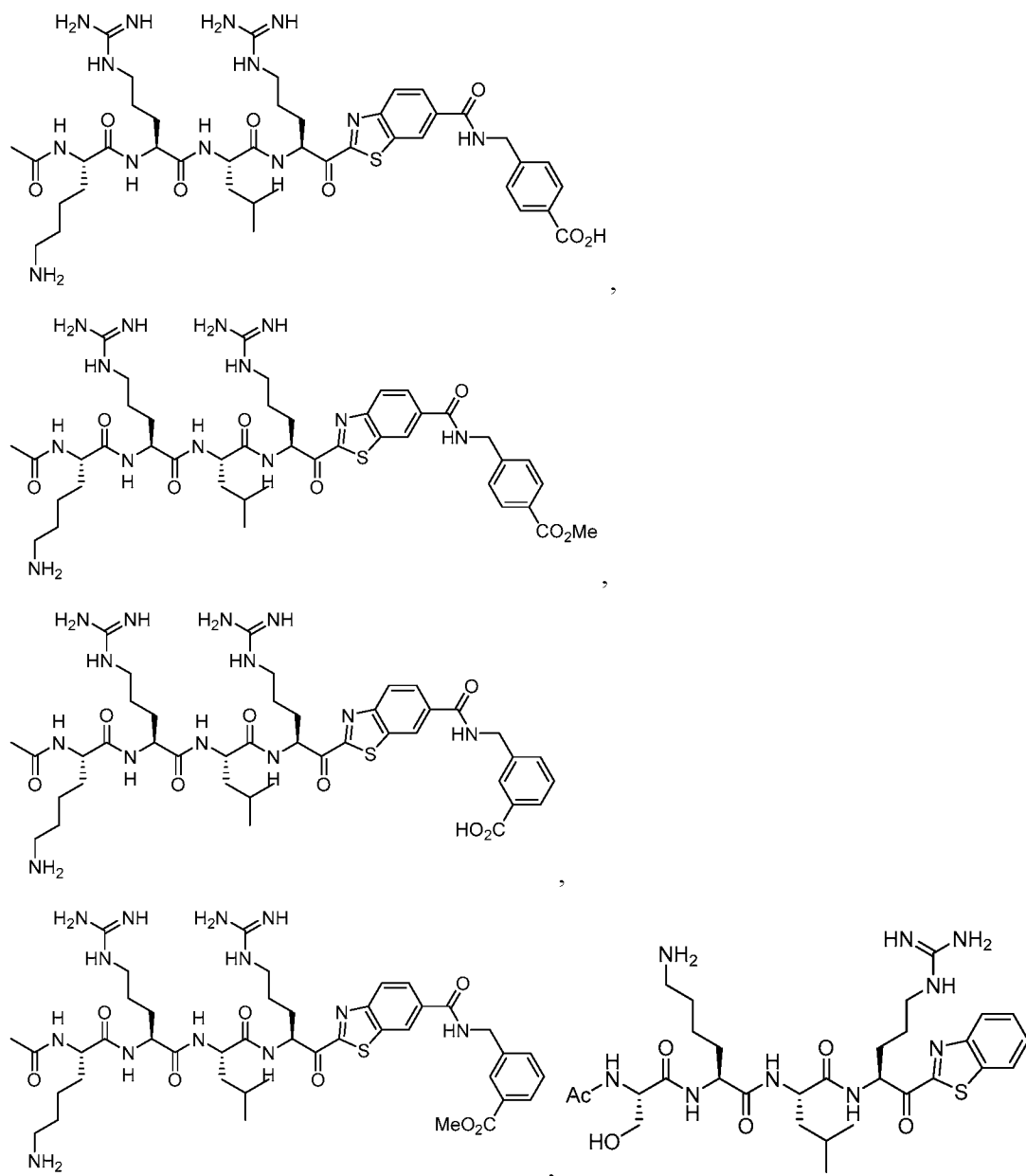
[0053] In various embodiments, P₁ can be an amino acid residue of Arg. P₂ can be an amino acid residue of Leu, Phe or Met. P₃ can be an amino acid residue of Arg, Lys, Met, or Trp. P₄ can be an amino acid residue of Arg, Lys, or Try. In these and other embodiments, the compound of Formula (II) is a selective inhibitor of HGFA.

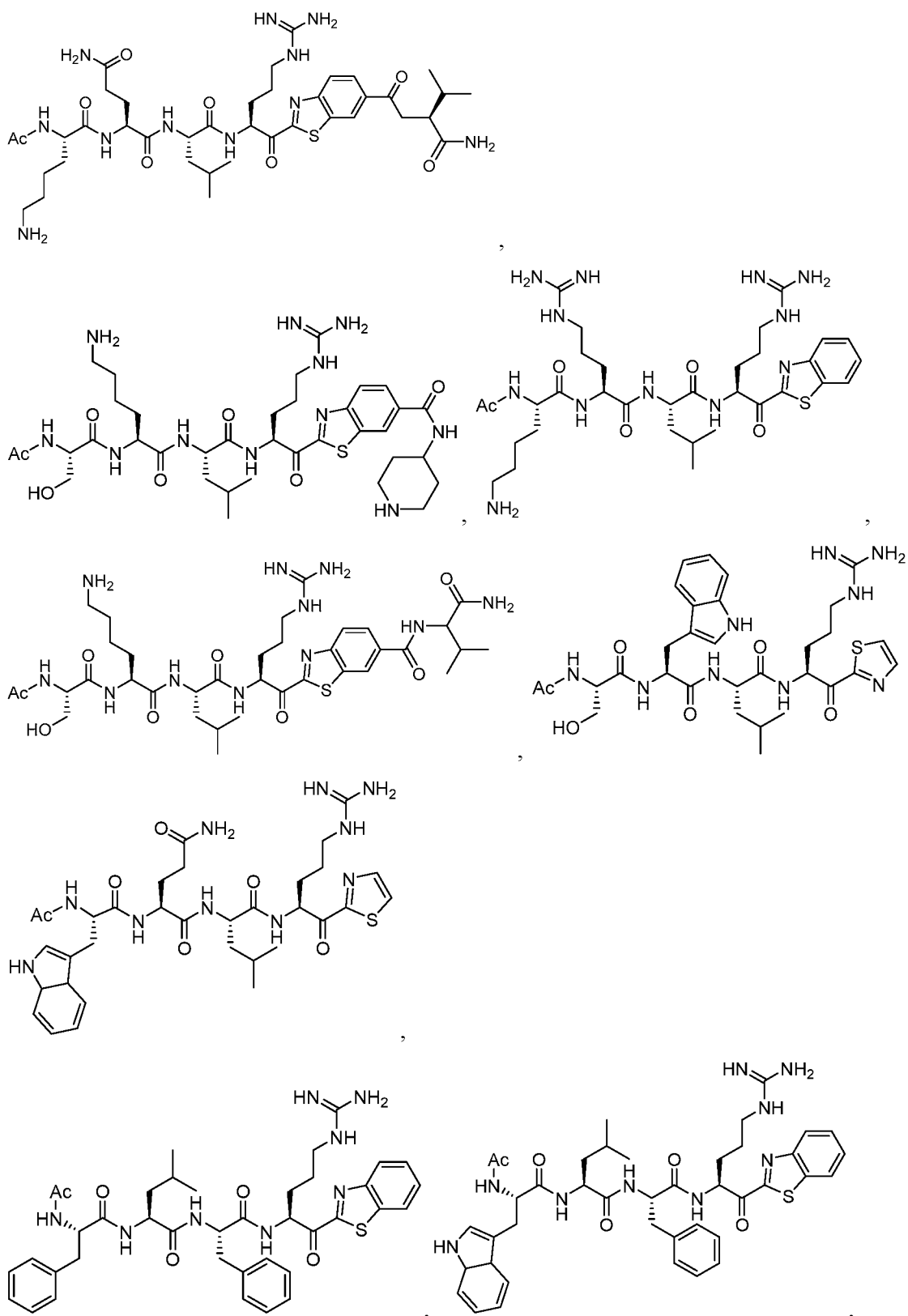
[0054] In various embodiments, Z is a benzothiazole of Formula (IV). In these and other embodiments, J₁ is C(O) and/or K₁ is an amino acid residue of Val.

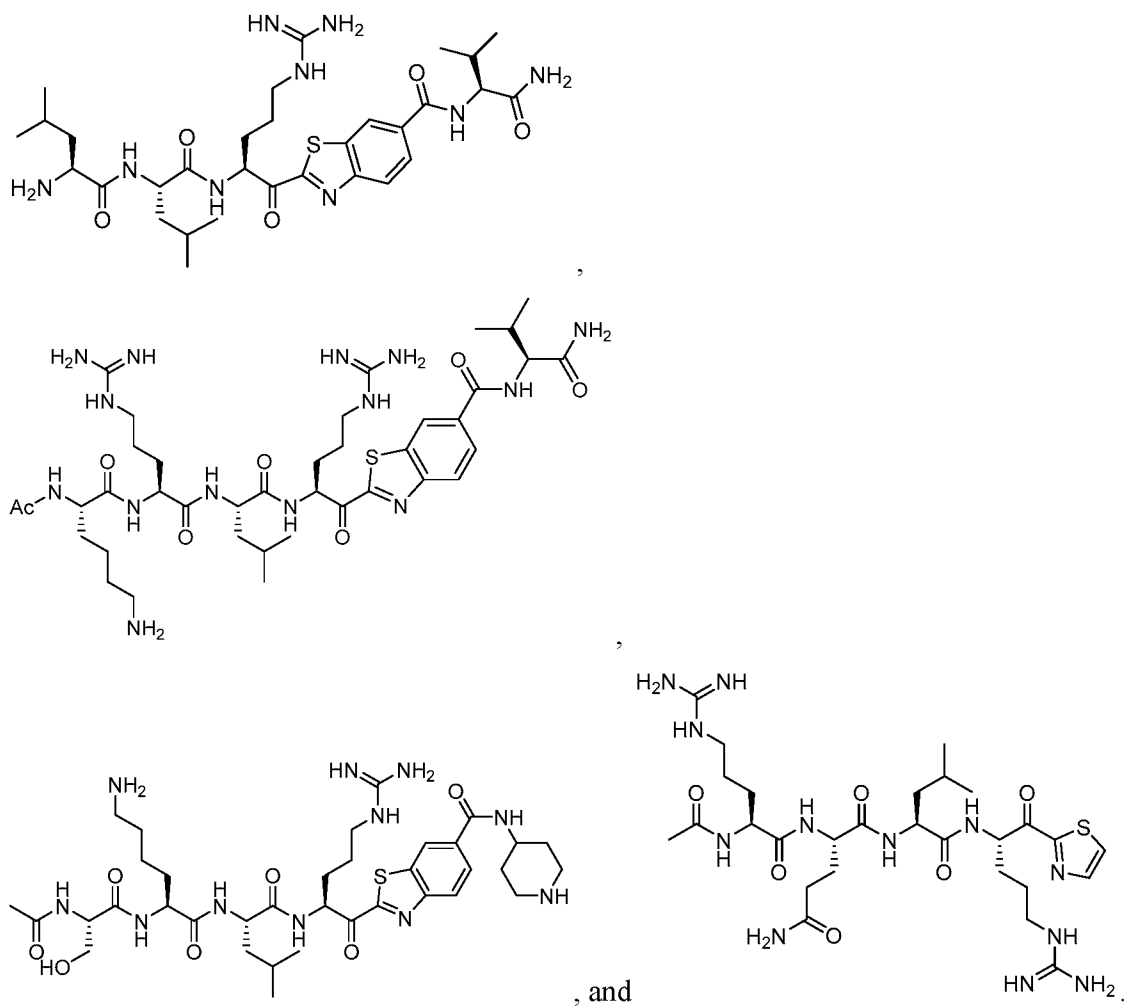
[0055] In various embodiments, when Z is a benzothiazole of Formula (V), then L₁ is a substituted benzyl group. Exemplary substituted benzyl groups include, but are not limited to: 2-, 3-, and 4-carboxybenzyl and the C1-C5 esters thereof.

[0056] In some embodiments, m is 1, n is 1, and P₄-P₃-P₂-P₁ of Formula (II) is a tetrapeptide selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, and mixtures thereof.

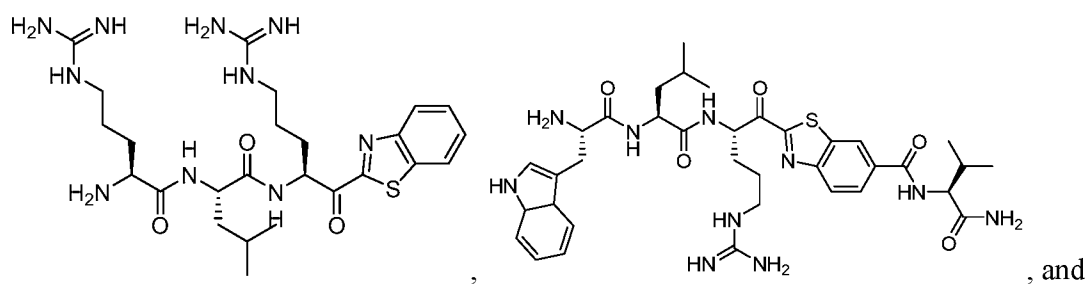
[0057] In some embodiments, the compound of Formula (II) is a tetrapeptide selected from the group consisting of:

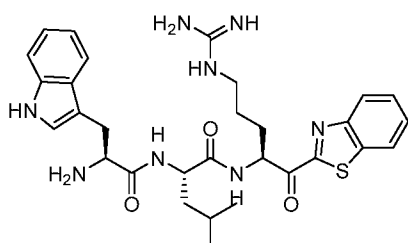




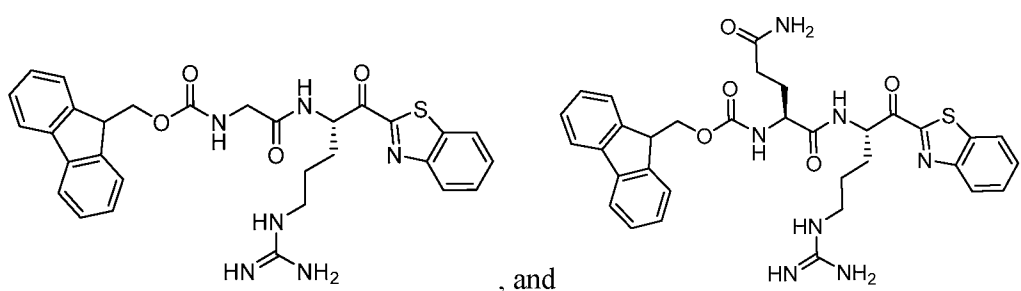


[0058] In some embodiments, the compound of Formula (II) is a tripeptide selected from the group consisting of:

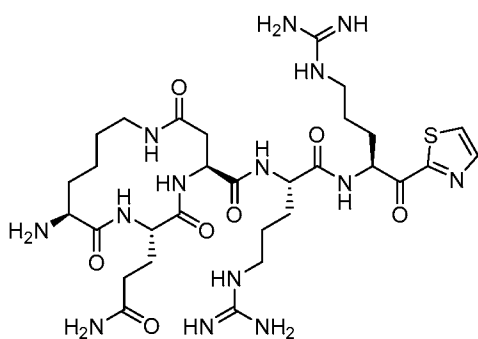




[0059] In some embodiments, the compound of Formula (II) is a dipeptide selected from the group consisting of:

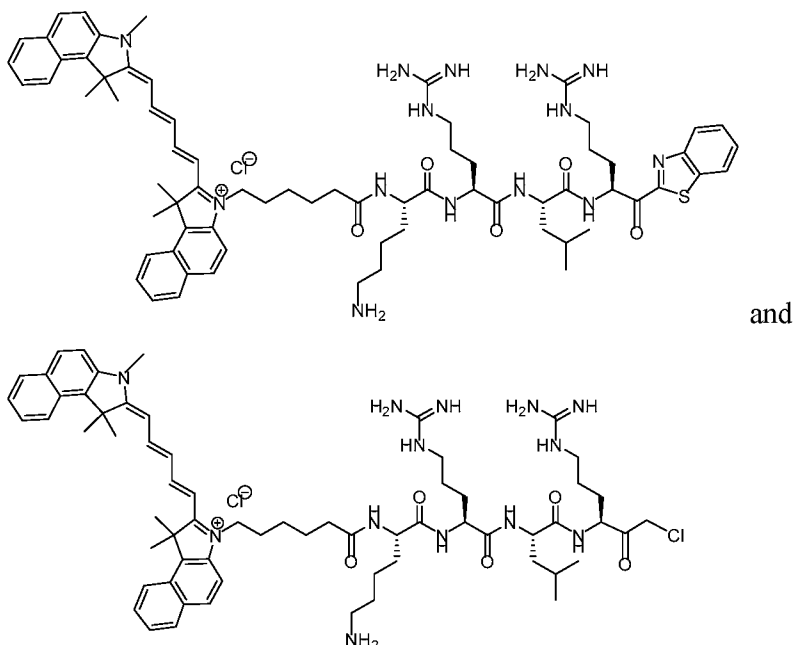


[0060] In some embodiments, the compound of Formula (II) is a cyclic peptide of the following structure:



[0061] In various embodiments, Y is a fluorophore. The fluorophore can be selected from the group consisting of Cy3, Cy3.5, Cy5, Cy5.5, Cy7, and Cy7.5.

[0062] In some embodiments, the compound of Formula (II) is selected from the group consisting of:



[0063] The compounds of Formulas (I) and (II) are useful for inhibiting one or more of matriptase, hepsin, and/or HGFA. Accordingly, the present invention is also directed to a method of inhibiting matriptase, hepsin, and/or HGFA comprising administering to a subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of at least one compound of Formula (I) or (II). In certain, embodiments the compounds are highly selective for one of matriptase, hepsin, or HGFA.

[0064] As noted, matriptase, hepsin, and/or HGFA are involved in various cancerous disease conditions. Thus, the present invention is directed to various methods of using the inhibitor compounds to treat cancer in a subject (e.g., a human). One method includes inhibiting HGF/MET oncogenic signaling by administering to a subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of at least one compound of Formula (I) or (II). Another method includes inhibiting MPS/RON oncogene signaling by administering to a subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of at least one compound of Formula (I) or (II). Yet another method including reversing resistance to a kinase inhibitor by blocking HGF and/or MPS production and/or activation by administering to a subject in need thereof a

pharmaceutical composition comprising a therapeutically effective amount of at least one compound of Formula (I) or (II).

[0065] Another method includes inhibiting carcinoma progression comprising by administering to the subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of at least one compound of Formula (I) or (II).

[0066] A further method includes treating a malignancy, a pre-malignant condition, or cancer in a subject comprising administering to the subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of at least one compound of Formula (I) or (II). The cancer can be selected from the group consisting of breast, ovarian, prostate, endometrial, colon, pancreatic, head and neck, gastric, renal, brain, liver, bladder, kidney, lung, esophageal, leukemia, multiple myeloma, lymphoma, and melanoma. For example, the malignancy and the pre-malignant condition can be a condition of the breast. Also, the pre-malignant condition can be selected from the group consisting of a typical ductal hyperplasia of the breast, actinic keratosis, leukoplakia, Barrett's epithelium (columnar metaplasia) of the esophagus, ulcerative colitis, adenomatous colorectal polyps, erythroplasia of Queyrat, Bowen's disease, bowenoid papulosis, vulvar intraepithelial neoplasia, and dysplastic changes to the cervix. In various methods, the cancer can also be metastasized.

[0067] In the various methods of the present invention, the compounds of Formula (I) or (II) can also be administered in combination with an anticancer compound, radiation therapy, a compound that induces apoptosis, a surgical procedure, or any combination thereof.

[0068] In accordance with the various methods of the present invention, a pharmaceutical composition comprising an inhibitor compound of Formula (I) or (II) is administered to the subject in need thereof. The pharmaceutical composition can be administered by a routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, parenteral, topical, sublingual, or rectal means. In various embodiments, administration is selected from the group consisting of oral, intranasal, intraperitoneal, intravenous, intramuscular, intratumoral, rectal, and transdermal.

[0069] The determination of a therapeutically effective dose for any one or more of the inhibitor compounds described herein is within the capability of those skilled in the art. A therapeutically effective dose refers to that amount of active ingredient which provides the

desired result. The exact dosage will be determined by the practitioner, in light of factors related to the subject that requires treatment. Dosage and administration are adjusted to provide sufficient levels of the active ingredient or to maintain the desired effect. Factors which can be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions can be administered every 3 to 4 days, every week, or once every two weeks depending on the half-life and clearance rate of the particular formulation.

[0070] Typically, the normal dosage amount of the inhibitor can vary from about 0.05 to about 100 mg per kg body weight depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. It will generally be administered so that a daily oral dose in the range, for example, from about 0.1 mg to about 75 mg, from about 0.5 mg to about 50 mg, or from about 1 mg to about 25 mg per kg body weight is given. The active ingredient can be administered in a single dose per day, or alternatively, in divided doses (e.g., twice per day, three times a day, four times a day, etc.). In general, lower doses can be administered when a parenteral route is employed. Thus, for example, for intravenous administration, a dose in the range, for example, from about 0.05 mg to about 30 mg, from about 0.1 mg to about 25 mg, or from about 0.1 mg to about 20 mg per kg body weight can be used.

[0071] A pharmaceutical composition for oral administration can be formulated using pharmaceutically acceptable carriers known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the subject. In certain embodiments, the composition is formulated for parenteral administration. Further details on techniques for formulation and administration can be found in the latest edition of REMINGTON'S PHARMACEUTICAL SCIENCES (Mack Publishing Co., Easton, Pa., which is incorporated herein by reference). After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. Such labeling would include amount, frequency, and method of administration.

[0072] In addition to the active ingredients (e.g., the inhibitor compound), the pharmaceutical composition can contain suitable pharmaceutically acceptable carriers

comprising excipients and auxiliaries that facilitate processing of the active compounds into preparations which can be used pharmaceutically. As used herein, the term "pharmaceutically acceptable carrier" means a non-toxic, inert solid, semi-solid or liquid filler, diluent, encapsulating material, or formulation auxiliary of any type. Some examples of materials which can serve as pharmaceutically acceptable carriers are sugars such as lactose, glucose, and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose, and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil; safflower oil; sesame oil; olive oil; corn oil; and soybean oil; glycols such as propylene glycol; esters such as ethyl oleate and ethyl laurate; agar; detergents such as TWEEN 80; buffering agents such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; artificial cerebral spinal fluid (CSF), and phosphate buffer solutions, as well as other non-toxic compatible lubricants such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, releasing agents, coating agents, sweetening, flavoring, and perfuming agents, preservatives and antioxidants can also be present in the composition, according to the judgment of the formulator based on the desired route of administration.

[0073] The compounds of the present invention can also be used in various nuclear imaging techniques when labeled with a suitable radionuclide. Accordingly, an imaging composition in accordance with the present invention comprises a radiolabeled compound of Formula (I) or (II), wherein the labeled compound comprises a radioisotope selected from the group consisting of ^{11}C , ^{13}N , ^{15}O , ^{18}F , ^{75}Br , ^{124}I , ^{125}I , and ^{131}I . Methods known in the art for radiolabeling the compounds of the present invention may be used.

[0074] Imaging methods in accordance with the present invention include a method of detecting cancer comprising:

administering to a subject a radiolabeled compound of Formula (I) or (II);

employing a nuclear imaging technique for monitoring or visualizing a distribution of the radiolabeled compound within the body or within a portion thereof; and

correlating the distribution of the radiolabeled compound to the existence of cancer.

In various embodiments, the nuclear imaging technique is positron emission tomography (PET) or photon emission computed tomography (SPECT).

[0075] Imaging methods in accordance with the present invention include a method of detecting cancer comprising:

- administering to a subject a fluorescent compound of Formula (I) or (II);
- employing an imaging technique for monitoring or visualizing a distribution of the fluorescent compound within the body or within a portion thereof; and
- correlating the distribution of the fluorescent compound to the existence of cancer.

[0076] As used herein, the abbreviations of the naturally occurring amino acids are as follows:

Amino acid	Three letter code	One letter code	Amino acid	Three letter code	One letter code
alanine	Ala	A	leucine	Leu	L
arginine	Arg	R	lysine	Lys	K
asparagine	Asn	N	methionine	Met	M
aspartic acid	Asp	D	phenylalanine	Phe	F
cysteine	Cys	C	proline	Pro	P
glutamic acid	Glu	E	serine	Ser	S
glutamine	Gln	Q	threonine	Thr	T
glycine	Gly	G	tryptophan	Trp	W
histidine	His	H	tyrosine	Tyr	Y
isoleucine	Ile	I	valine	Val	V

[0077] The naturally occurring amino acids described herein are the L-isomer unless denoted as a D-isomer.

[0078] Having described the invention in detail, it will be apparent that modifications and variations are possible without departing from the scope of the invention defined in the appended claims.

EXAMPLES

[0079] The following non-limiting examples are provided to further illustrate the present invention.

Example 1. General synthesis, purification, and analytical chemistry procedures

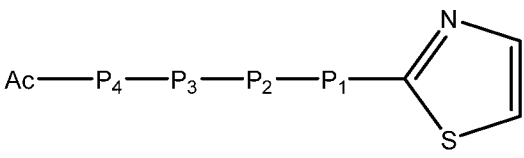
[0080] Starting materials, reagents, and solvents were purchased from commercial vendors unless otherwise noted. ¹H NMR spectra were measured on a Varian 400 MHz NMR instrument. The chemical shifts were reported ppm relative to tetramethylsilane (TMS)

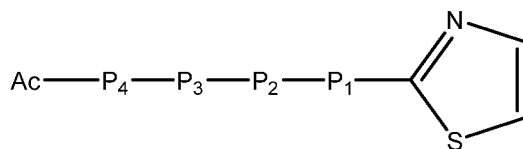
using residual solvent peak as the reference unless otherwise noted. The following abbreviations were used to express the multiplicities: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; br = broad. High-performance liquid chromatography (HPLC) was carried out on GILSON GX-281 using Waters C18 5 μ M, 4.6*50mm and Waters Prep C18 5 μ M, 19*150mm reverse phase columns, eluted with a gradient system of 5:95 to 95:5 acetonitrile:water with a buffer consisting of 0.05% TFA. Purity assessment and mass spectra (MS) data were obtained using a Hewlett-Packard HPLC/MSD using electrospray ionization (ESI) for detection. All reactions were monitored by thin layer chromatography (TLC) carried out on Merck silica gel plates (0.25 mm thick, 60F254), visualized by using UV absorbance (254 nm) or dyes such as ninhydrin, KMnO₄, *p*-anisaldehyde or ceric ammonium molybdate (CAM). Silica gel chromatography was carried out on a Teledyne ISCO CombiFlash purification system using pre-packed silica gel columns (12g~330g sizes). All compounds used for biological assays are greater than 95% purity based on NMR and HPLC by UV absorbance at 210 nm and 254 nm wavelengths.

Example 2. Synthesis of Tetrapeptide Ketothiazole Inhibitor Compounds

[0081] The tetrapeptide ketothiazoles (kt) listed in Table 2.1 were synthesized in accordance with the general schemes 2A and 2B shown below and the procedures described below.

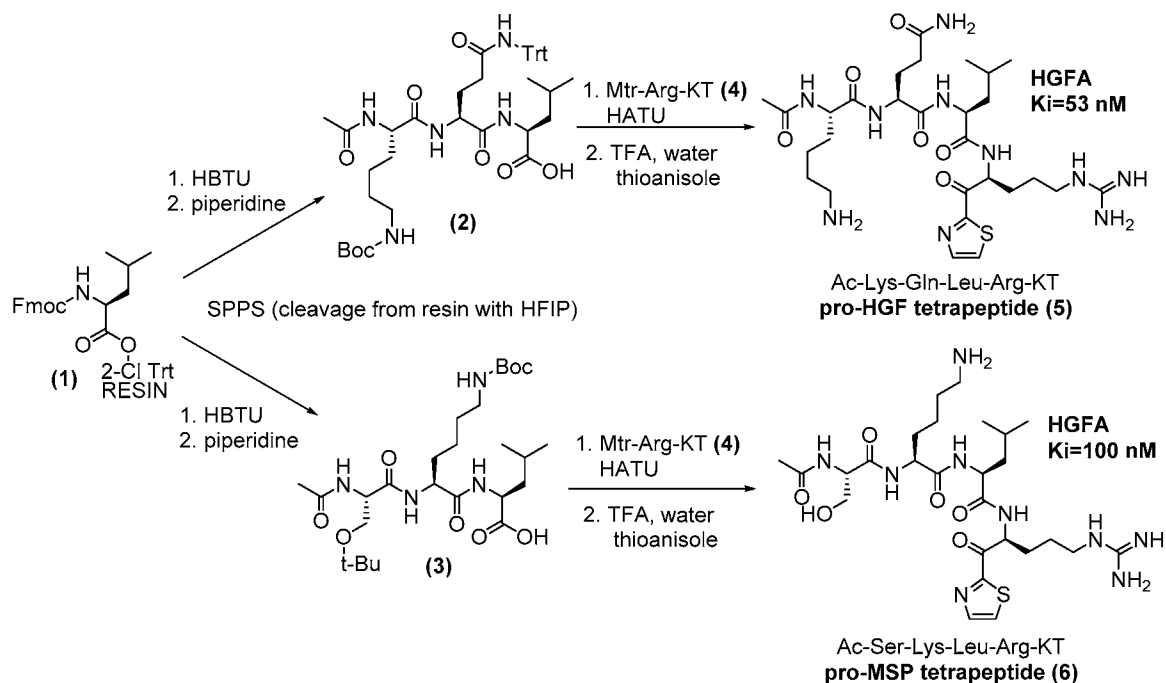
Table 2.1 Tetrapeptide ketothiazoles.

	
Compound No.	Ac-P ₄ -P ₃ -P ₂ -P ₁ -kt
5	Ac-KQLR(SEQ ID NO: 1)-kt
5-1	Ac-KQLdR-kt
5a	Ac-KHLR(SEQ ID NO: 2)-kt
5a-1	Ac-KHLdR-kt
5b	Ac-WQLR(SEQ ID NO: 3)-kt
5b-1	Ac-WQLdR-kt
5c	Ac-KQFR(SEQ ID NO: 4)-kt
5c-1	Ac-KQFdR-kt
5d	Ac-KFLR(SEQ ID NO: 5)-kt
5e	Ac-RQLR(SEQ ID NO: 6)-kt

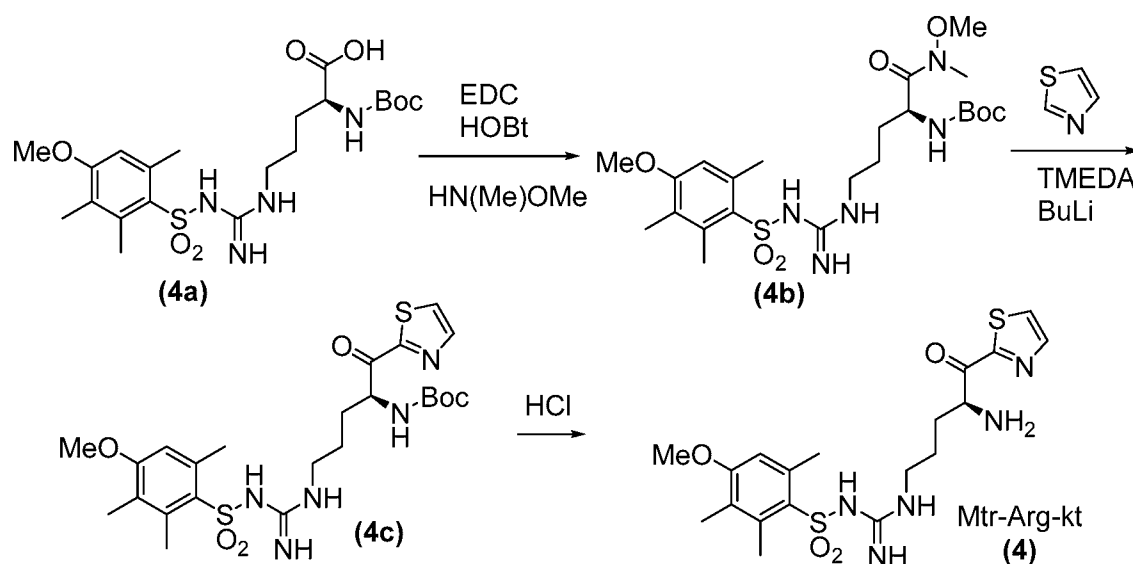


Compound No.	Ac-P ₄ -P ₃ -P ₂ -P ₁ -kt
5e-1	Ac-RQLdR-kt
5f	Ac-SQLR(SEQ ID NO: 7)-kt
5g	Ac-KRLR(SEQ ID NO: 8)-kt
5h	Ac-WRLR(SEQ ID NO: 9)-kt
6	Ac-SKLR(SEQ ID NO: 10)-kt
6-1	Ac-SKLdR-kt
6a	Ac-SHLR(SEQ ID NO: 11)-kt
6a-1	Ac-SHLdR-kt
6b	Ac-WKLR(SEQ ID NO: 12)-kt
6b-1	Ac-WKLdR-kt
6c	Ac-SKFR(SEQ ID NO: 13)-kt
6c-1	Ac-SKFdR-kt
6d	Ac-NKLR(SEQ ID NO: 14)-kt
6e	Ac-SRLR(SEQ ID NO: 15)-kt
6e-1	Ac-SdRLR-kt
6e-2	Ac-SRLdR-kt
6e-3	Ac-SdRLdR-kt
6f	Ac-TKLR(SEQ ID NO: 16)-kt
6g	Ac-SWLR(SEQ ID NO: 17)-kt
6h	Ac-RKLR(SEQ ID NO: 18)-kt

Scheme 2A. Synthesis of pro-HGF and pro-MSP P4-P1 tetrapeptide ketothiazoles



Scheme 2B. Synthesis of protected arginine ketothiazole



[0082] Tripeptide synthesis: Tripeptide intermediates were synthesized on a 0.1 mmol scale using a CEM Liberty Microwave Peptide Synthesizer and Fmoc-amino acid-preloaded 2-Cl-trityl resins. Standard peptide coupling conditions were employed utilizing 5 equiv. of Fmoc-amino acid/4.5 equiv. HBTU/10 equiv. N,N-Diisopropylethylamine

(ⁱPr₂NEt) in DMF was added and the mixture was heated at 75 °C for 5 minutes by microwave. Piperidine/DMF (20% v/v) was employed for the deprotection of the Fmoc protecting group using the microwave for 5 minutes.

[0083] Acetyl (Ac) capping of the tripeptides: The tripeptide resin was suspended in 15 mL of 0.5 M Ac₂O/DMF and 1 M ⁱPr₂NEt/DMF. The mixture was shaken at room temperature for 1 hour. The resin was filtered and washed with DMF (10 mL x 4) followed by CH₂Cl₂ (10 mL x 4).

[0084] Cleavage of tripeptide resin: Ac-capped tripeptide resin was suspended and shaken in 15 mL of 25% v/v HFIP/ CH₂Cl₂ for 1 hour. The mixture was filtered. The filtrate was concentrated then dried in vacuo, giving rise to crude Ac-capped tripeptide product.

[0085] Ac-KQFR(SEQ ID NO: 4) ketothiazole (5c). Under nitrogen atmosphere, at 0 °C anhydrous DMF (5 mL) was added into the round bottom flask containing Ac-capped KQF tripeptide (0.081 g, 0.1 mmol) and HATU (0.042 g, 0.11 mmol). After stirring for 10 minutes, arginine ketothiazole (0.042 g, 0.1 mmol), then *N,N*-diisopropylethylamine (0.065 g, 0.5 mmol) were added. The mixture was stirred overnight while being warmed to room temperature naturally. The majority of DMF was removed and to the resulting residue was added 20 mL of water. The precipitate that formed was filtered and dried. To this precipitate was added 5 mL of TFA/thioanisole/water (95/2.5/2.5(v/v/v)). The mixture was stirred at room temperature for 4 hours and then was added to 40 mL of cold ether. The precipitated crude product was collected by centrifugation, followed by carefully decanting out the ether solvent. The crude product was purified by HPLC (C18, 15*150 mm column; eluent: acetonitrile/water (0.1% TFA)) to give Ac-KQFR(SEQ ID NO: 4) ketothiazole (0.032 g) in 46% yield. At the same time the isomer KQFR(SEQ ID NO: 4)* ketothiazole (0.020 g) was also collected. ¹H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 1.35 (m, 2 H) 1.45 - 1.77 (m, 7 H) 1.78 - 2.04 (m, 6 H) 2.06 - 2.29 (m, 2 H) 2.92 (t, *J*=7.63 Hz, 2 H) 3.00 (m, 2 H) 3.13 (m, 2 H) 4.12 - 4.20 (m, 1 H) 4.21 - 4.29 (m, 1 H) 4.59 (t, *J*=7.63 Hz, 1 H) 5.30 - 5.45 (m, 1 H) 7.17 (m, 5 H) 8.04 (br. s., 1 H) 8.07 (br. s., 1 H). MS (ESI): found: [M + H]⁺, 687.5.

[0086] Ac-SQLR(SEQ ID NO: 7) ketothiazole (5f) (yield: 42%) ¹H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 0.85 (d, *J*=5.87 Hz, 3 H) 0.90 (d, *J*=5.87 Hz, 3 H) 1.48 - 1.76 (m, 5 H) 1.77 - 1.88 (m, 1 H) 1.89 - 2.02 (m, 1 H) 2.02 - 2.20 (m, 5 H) 2.26 - 2.47 (m, 2 H) 3.11 - 3.32 (m, 2 H) 3.84 (tt, *J*=11.44, 5.77 Hz, 2 H) 4.25 - 4.45 (m, 3 H) 5.47 (dd, *J*=9.39,

4.30 Hz, 1 H) 8.06 (d, $J=3.13$ Hz, 1 H) 8.11 (d, $J=3.13$ Hz, 1 H). MS (ESI): found: $[M + H]^+$, 612.5.

[0087] Ac-SRLR(SEQ ID NO: 15) ketothiazole (6e) (yield: 26%) ^1H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 0.85 (d, $J=5.87$ Hz, 3 H) 0.91 (d, $J=5.87$ Hz, 3 H) 1.52 - 1.77 (m, 8 H) 1.79 - 1.89 (m, 2 H) 1.99 - 2.18 (m, 4 H) 3.09 - 3.34 (m, 4 H) 3.83 (tt, $J=11.35$, 5.67 Hz, 2 H) 4.29 - 4.45 (m, 3 H) 5.46 (dd, $J=9.20$, 4.11 Hz, 1 H) 8.06 (d, $J=3.13$ Hz, 1 H) 8.12 (d, $J=3.13$ Hz, 1 H). MS (ESI): found: $[M + H]^+$, 640.5.

[0088] Ac-TKLR(SEQ ID NO: 16) ketothiazole (6f) (yield: 59%) ^1H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 0.85 (d, $J=5.87$ Hz, 3 H) 0.91 (d, $J=5.48$ Hz, 3 H) 1.12 - 1.24 (m, 3 H) 1.32 - 1.90 (m, 12 H) 1.99 - 2.18 (m, 4 H) 2.98 (t, $J=7.43$ Hz, 2 H) 3.15 - 3.30 (m, 2 H) 4.07 - 4.19 (m, 1 H) 4.24 (d, $J=5.48$ Hz, 1 H) 4.34 (td, $J=8.90$, 5.67 Hz, 2 H) 5.45 (dd, $J=9.19$, 4.50 Hz, 1 H) 8.07 (d, $J=3.13$ Hz, 1 H) 8.12 (d, $J=3.13$ Hz, 1 H). MS (ESI): found: $[M + H]^+$, 626.5.

[0089] Ac-KFLR(SEQ ID NO: 5) ketothiazole (5d) (yield: 43%) ^1H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 0.85 (d, $J=6.26$ Hz, 3 H) 0.90 (d, $J=6.26$ Hz, 3 H) 1.13 - 1.38 (m, 2 H) 1.40 - 1.77 (m, 9 H) 1.78 - 1.91 (m, 1 H) 1.99 (s, 3 H) 2.04 - 2.17 (m, 1 H) 2.82 - 3.05 (m, 3 H) 3.09 - 3.32 (m, 3 H) 4.08 - 4.21 (m, 1 H) 4.37 (dd, $J=8.80$, 6.06 Hz, 1 H) 4.65 (dd, $J=8.61$, 6.26 Hz, 1 H) 5.44 (dd, $J=9.19$, 4.50 Hz, 1 H) 7.15 - 7.43 (m, 5 H) 8.08 (d, $J=3.13$ Hz, 1 H) 8.13 (d, $J=3.13$ Hz, 1 H). MS (ESI): found: $[M + H]^+$, 672.5.

[0090] Ac-NKLR(SEQ ID NO: 14) ketothiazole (6d) (yield: 39%) ^1H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 0.85 (d, $J=5.48$ Hz, 3 H) 0.91 (d, $J=5.87$ Hz, 3 H) 1.32 - 1.48 (m, 2 H) 1.48 - 1.91 (m, 10 H) 1.93 - 2.17 (m, 4 H) 2.62 - 2.84 (m, 2 H) 2.98 (t, $J=7.63$ Hz, 2 H) 3.14 - 3.30 (m, 2 H) 4.20 - 4.42 (m, 2 H) 4.63 (t, $J=7.04$ Hz, 1 H) 5.45 (dd, $J=9.39$, 4.30 Hz, 1 H) 8.06 (d, $J=3.13$ Hz, 1 H) 8.11 (d, $J=3.13$ Hz, 1 H). MS (ESI): found: $[M + H]^+$, 639.5.

[0091] Ac-RQLR(SEQ ID NO: 6) ketothiazole (5e) (yield: 13%) ^1H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 0.85 (d, $J=5.87$ Hz, 3 H) 0.91 (d, $J=5.87$ Hz, 3 H) 1.45 - 1.89 (m, 10 H) 1.90 - 2.19 (m, 6 H) 2.35 (td, $J=7.43$, 3.91 Hz, 2 H) 3.14 - 3.28 (m, 4 H) 4.25 (dd, $J=8.02$, 6.06 Hz, 1 H) 4.30 - 4.43 (m, 2 H) 5.47 (dd, $J=9.39$, 4.30 Hz, 1 H) 8.07 (d, $J=3.13$ Hz, 1 H) 8.12 (d, $J=3.13$ Hz, 1 H). MS (ESI): found: $[M + H]^+$, 681.5.

[0092] Ac-WQLR(SEQ ID NO: 3) ketothiazole (5b) (yield: 13%) ^1H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 0.81 (d, $J=5.87$ Hz, 3 H) 0.88 (d, $J=5.48$ Hz, 3 H) 1.37 -

1.69 (m, 6 H) 1.70 - 1.91 (m, 3 H) 1.91 - 2.10 (m, 5 H) 2.98 - 3.35 (m, 4 H) 3.92 - 4.06 (m, 1 H) 4.11 - 4.23 (m, 1 H) 4.48 (t, $J=6.65$ Hz, 1 H) 5.39 (m, 1 H) 7.09 (t, $J=7.24$ Hz, 1 H) 7.14 - 7.26 (m, 2 H) 7.43 (d, $J=8.22$ Hz, 1 H) 7.52 (d, $J=7.43$ Hz, 1 H) 8.00 (d, $J=2.74$ Hz, 1 H) 8.05 (d, $J=2.74$ Hz, 1 H). MS (ESI): found: $[M + H]^+$, 711.5.

[0093] **Ac-KHLR(SEQ ID NO: 2) ketothiazole (5a)** (yield: 52%) ^1H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 0.82 (d, $J=4.30$ Hz, 3 H) 0.87 (d, $J=4.70$ Hz, 3 H) 1.22 - 1.44 (m, 2 H) 1.44 - 1.73 (m, 9 H) 1.74 - 1.87 (m, 1 H) 1.96 (s, 3 H) 2.06 (m, 1 H) 2.83 - 2.99 (m, 2 H) 3.02 - 3.36 (m, 4 H) 4.06 - 4.22 (m, 1 H) 4.34 (m, 1 H) 4.58 - 4.72 (m, 1 H) 5.43 (dd, $J=8.61, 4.30$ Hz, 1 H) 7.23 (s, 1 H) 8.03 (d, $J=2.35$ Hz, 1 H) 8.08 (d, $J=2.35$ Hz, 1 H) 8.58 (s, 1 H). MS (ESI): found: $[M + H]^+$, 662.5.

[0094] **Ac-SKLR(SEQ ID NO: 10) ketothiazole (6)** (yield: 57%) ^1H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 0.81 (d, $J=4.70$ Hz, 3 H) 0.87 (d, $J=4.30$ Hz, 3 H) 1.26 - 1.91 (m, 12 H) 1.93 - 2.16 (m, 4 H) 2.94 (t, $J=7.43$ Hz, 2 H) 3.18 (m, 2 H) 3.79 (t, $J=6.46$ Hz, 2 H) 4.18 - 4.46 (m, 3 H) 5.43 (dd, $J=9.00, 3.91$ Hz, 1 H) 8.03 (d, $J=2.74$ Hz, 1 H) 8.08 (d, $J=2.74$ Hz, 1 H). MS (ESI): found: $[M + H]^+$, 612.5.

[0095] **Ac-SHLR(SEQ ID NO: 11) ketothiazole (6a)** (yield: 32%) ^1H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 0.81 (d, $J=5.09$ Hz, 3 H) 0.86 (d, $J=5.09$ Hz, 3 H) 1.41 - 1.60 (m, 3 H) 1.60 - 1.72 (m, 2 H) 1.73 - 1.88 (m, 1 H) 1.90 - 2.15 (m, 4 H) 2.99 - 3.37 (m, 4 H) 3.63 - 3.83 (m, 2 H) 4.20 - 4.41 (m, 2 H) 4.68 (dd, $J=8.61, 5.48$ Hz, 1 H) 5.44 (dd, $J=8.61, 3.13$ Hz, 1 H) 7.24 (s, 1 H) 8.03 (d, $J=2.35$ Hz, 1 H) 8.08 (d, $J=2.35$ Hz, 1 H) 8.57 (s, 1 H). MS (ESI): found: $[M + H]^+$, 621.5.

[0096] **Ac-SKFR(SEQ ID NO: 13) ketothiazole (6c)** (yield: 46%) ^1H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 1.25 (m, 2 H) 1.42 - 1.81 (m, 7 H) 1.86 - 2.11 (m, 4 H) 2.80 - 3.06 (m, 4 H) 3.13 (m, 2 H) 3.64 - 3.86 (m, 2 H) 4.17 - 4.28 (m, 1 H) 4.32 (t, $J=5.67$ Hz, 1 H) 4.56 (t, $J=7.83$ Hz, 1 H) 5.38 (dd, $J=8.61, 4.30$ Hz, 1 H) 7.00 - 7.36 (m, 5 H) 8.04 (d, $J=2.74$ Hz, 1 H) 8.07 (d, $J=2.74$ Hz, 1 H). MS (ESI): found: $[M + H]^+$, 646.5.

[0097] **Ac-WKLR(SEQ ID NO: 12) ketothiazole (6b)** (yield: 38%) ^1H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 0.86 (d, $J=5.48$ Hz, 3 H) 0.93 (d, $J=5.48$ Hz, 3 H) 1.09 - 1.20 (m, 2 H) 1.39 - 1.71 (m, 9 H) 1.73 - 1.88 (m, 1 H) 1.98 (s, 3 H) 2.01 - 2.14 (m, 1 H) 2.80 - 2.94 (m, 2 H) 3.10 - 3.27 (m, 4 H) 4.04 - 4.13 (m, 1 H) 4.15 - 4.24 (m, 1 H) 4.45 - 4.59 (m, 1 H) 5.42 (dd, $J=9.00, 3.91$ Hz, 1 H) 7.08 - 7.17 (m, 1 H) 7.18 - 7.28 (m, 2 H) 7.47 (d, $J=7.83$

Hz, 1 H) 7.57 (d, $J=7.83$ Hz, 1 H) 7.93 (dd, $J=13.50, 6.85$ Hz, 1 H) 8.05 (d, $J=2.74$ Hz, 1 H) 8.09 (d, $J=2.74$ Hz, 1 H). MS (ESI): found: $[M + H]^+$, 711.8.

[0098] Ac-KQLR(SEQ ID NO: 1)-ketothiazole (5) (yield: 32%) ^1H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 0.80 (d, $J=5.09$ Hz, 3 H) 0.86 (d, $J=5.09$ Hz, 3 H) 1.26 - 1.84 (m, 12 H) 1.84 - 2.13 (m, 6 H) 2.29 (m, 2 H) 2.93 (t, $J=7.43$ Hz, 2 H) 3.17 (m, 2 H) 4.18 (t, $J=7.04$ Hz, 1 H) 4.23 - 4.37 (m, 2 H) 5.35 - 5.51 (m, 1 H) 8.01 (d, $J=2.74$ Hz, 1 H) 8.07 (d, $J=2.74$ Hz, 1 H). MS (ESI): found: $[M + H]^+$, 653.5.

[0099] Ac-KRLR(SEQ ID NO: 8)-ketothiazole (5g) (yield: 44%) ^1H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 0.77 - 0.91 (m, 6 H) 1.26 - 1.45 (m, 3 H) 1.46 - 1.58 (m, 5 H) 1.58 - 1.70 (m, 7 H) 1.70 - 1.85 (m, 4 H) 1.97 (s, 3 H) 1.99 - 2.11 (m, 2 H) 2.93 (t, $J=7.63$ Hz, 2 H) 3.15 (dt, $J=13.79, 6.99$ Hz, 4 H) 4.17 (dd, $J=8.22, 5.87$ Hz, 1 H) 4.24 - 4.30 (m, 1 H) 4.30 - 4.37 (m, 1 H) 5.41 (dd, $J=9.20, 4.11$ Hz, 1 H) 8.02 (d, $J=2.74$ Hz, 1 H) 8.07 (d, $J=3.13$ Hz, 1 H) MS (ESI): found: $[M + H]^+$, 681.7.

[0100] Ac-WRLR(SEQ ID NO: 9)-ketothiazole (5h) (yield: 24%) ^1H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 0.82 (d, $J=5.87$ Hz, 3 H) 0.89 (d, $J=5.87$ Hz, 3 H) 1.10 - 1.32 (m, 3 H) 1.34 - 1.45 (m, 2 H) 1.48 (d, $J=6.65$ Hz, 3 H) 1.53 - 1.68 (m, 3 H) 1.68 - 1.83 (m, 1 H) 1.95 (s, 3 H) 2.02 (d, $J=10.56$ Hz, 1 H) 2.97 (t, $J=5.87$ Hz, 2 H) 3.12 (t, $J=6.85$ Hz, 2 H) 3.15 - 3.22 (m, 2 H) 4.02 (dd, $J=8.02, 5.67$ Hz, 1 H) 4.15 (t, $J=7.63$ Hz, 1 H) 4.47 (t, $J=7.04$ Hz, 1 H) 5.37 (dd, $J=9.00, 4.30$ Hz, 1 H) 7.02 - 7.12 (m, 1 H) 7.17 (t, $J=7.63$ Hz, 1 H) 7.21 (s, 1 H) 7.42 (d, $J=8.22$ Hz, 1 H) 7.51 (d, $J=7.83$ Hz, 1 H) 8.00 (d, $J=3.13$ Hz, 1 H) 8.05 (d, $J=3.13$ Hz, 1 H) MS (ESI): found: $[M + H]^+$, 739.7.

[0101] Ac-SWLR(SEQ ID NO: 17)-ketothiazole (6g) (yield: 20%) ^1H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 0.74 (d, $J=6.65$ Hz, 3 H) 0.71 (d, $J=6.26$ Hz, 3 H) 0.99 - 1.15 (m, 2 H) 1.35 (t, $J=7.04$ Hz, 2 H) 1.51 - 1.64 (m, 3 H) 1.72 (s, 3 H) 1.76 (br. s., 1 H) 2.00 (dd, $J=13.69, 4.70$ Hz, 2 H) 3.00 - 3.18 (m, 3 H) 3.25 (d, $J=5.48$ Hz, 2 H) 3.60 - 3.81 (m, 3 H) 4.11 - 4.26 (m, 2 H) 4.62 (t, $J=5.87$ Hz, 1 H) 5.33 (dd, $J=9.59, 4.11$ Hz, 1 H) 7.08 - 7.15 (m, 1 H) 7.15 (s, 1 H) 7.17 - 7.23 (m, 1 H) 7.43 (d, $J=8.22$ Hz, 1 H) 7.57 (d, $J=8.22$ Hz, 1 H) 7.63 (br. s., 1 H) 7.77 (br. s., 1 H) 8.00 (d, $J=3.13$ Hz, 1 H) 8.06 (d, $J=2.74$ Hz, 1 H) MS (ESI): found: $[M + H]^+$, 670.5.

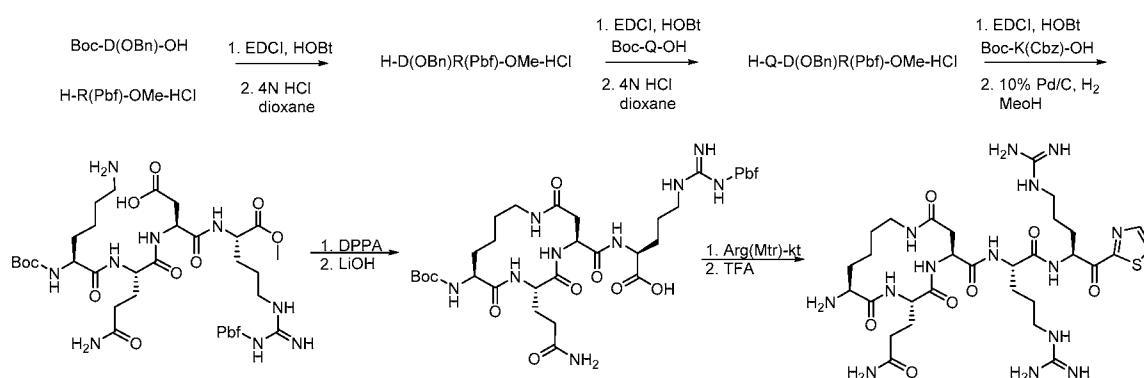
[0102] Ac-RKLR(SEQ ID NO: 18)-ketothiazole (6h) (yield: 31%) ^1H NMR (400 MHz, DEUTERIUM OXIDE) δ ppm 0.76 - 0.91 (m, 6 H) 1.24 - 1.47 (m, 3 H) 1.47 - 1.56 (m, 3 H) 1.56 - 1.70 (m, 7 H) 1.70 - 1.86 (m, 4 H) 1.97 (s, 3 H) 2.00 - 2.12 (m, 1 H) 2.93 (t,

$J=7.63$ Hz, 2 H) 3.06 - 3.24 (m, 4 H) 4.19 (t, $J=7.04$ Hz, 1 H) 4.27 (dd, $J=8.80, 6.06$ Hz, 1 H) 4.33 (d, $J=9.00$ Hz, 1 H) 5.41 (dd, $J=9.00, 4.70$ Hz, 1 H) 8.03 (d, $J=3.13$ Hz, 1 H) 8.08 (d, $J=3.13$ Hz, 1 H) MS (ESI): found: $[M + H]^+$, 681.7.

Example 3. Synthesis of Cyclic Peptides

[0103] The cyclic peptide is listed in Table 3.1 were synthesized in accordance with the general schemes 3A and 3B and cyclization schemes 3C, 3D, 3E, and 3F shown below and the procedures described below.

Scheme 3A



Scheme 3B

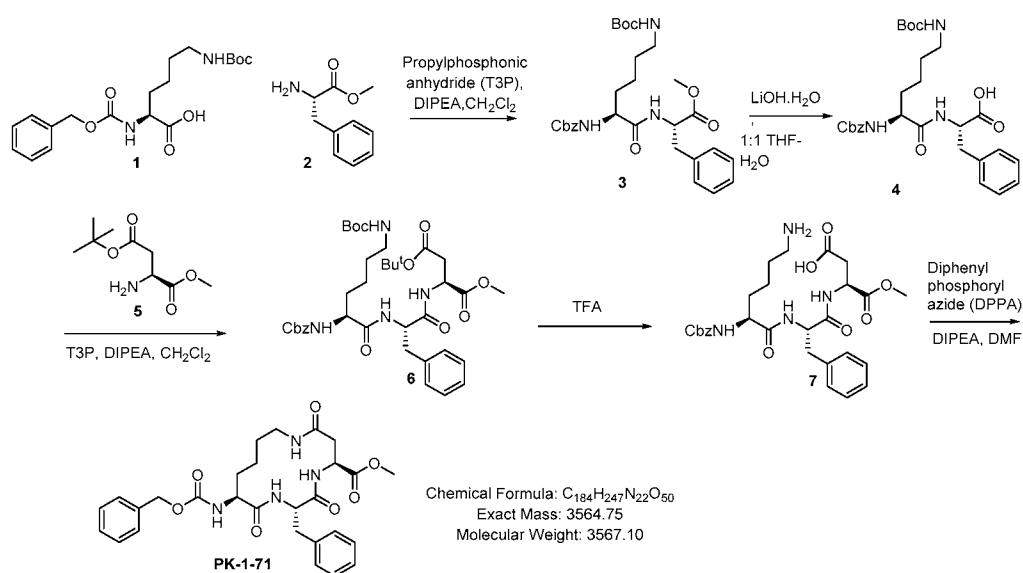


Table 3.1

Y—P ₅ —P ₄ —P ₃ —P ₂ —P ₁ —Z		
Compound No.	Y-cyclo(P ₅ -P ₄ -P ₃)-P ₂ -P ₁ -Z	Cyclization
7074	H-cyclo(KQD)RR-kt	P ₃ -P ₅

[0104] Cbz-K(Boc)F-OMe (3): Compound **1** (0.1 mmol) and compound **2** (0.1 mmol) was taken in dry CH₂Cl₂ (5 mL) and cooled to 0 °C and diisopropylethylamine (DIPEA) (0.3 mmol) and propylphosphonic anhydride (T3P) (0.1 mmol, 50% solution in ethyl acetate) were added dropwise to the reaction mixture respectively. The reaction mixture was stirred for 1 hr at room temperature under nitrogen atmosphere. The completion of the reaction was confirmed by LCMS monitoring. On completion, the reaction mixture was diluted with 5 mL and washed with 10% citric acid, saturated Na₂CO₃ solution and brine respectively. The organic layer was dried over sodium sulfate and concentrated under reduced pressure to obtain the compound **3** as white solid (yield: 80%, Chemical Formula: C₂₉H₃₉N₃O₇, Exact Mass: 541.28, ESIMS: 564.6 [M+Na⁺]).

[0105] Cbz-K(Boc)F-OH (4): Compound **3** (0.1 mmol) was taken in 4 mL THF-H₂O (1:1) mixture and LiOH.H₂O (0.3 mmol) was added to the solution. The reaction mixture was stirred for 15 minutes at room temperature and the completion of the reaction was confirmed by LCMS monitoring. On completion, the THF was evaporated under reduced pressure and the water layer was cooled to 0 °C. The water layer was brought to pH 6.5 by dropwise addition of cold 0.5 M HCl solution and kept on ice for 15 minutes to complete the precipitation of the product as white solid. The precipitate was isolated by filtration and dried under reduced pressure to obtain the compound **4** as white solid (Yield: 83%, Chemical Formula: C₂₈H₃₇N₃O₇, Exact Mass: 527.26, ESIMS: 528.3 [M+H⁺]).

[0106] Cbz-K(Boc)FD(t-Bu)-OMe (6): Compound **4** (0.1 mmol) and compound **5** (0.1 mmol) was taken in dry CH₂Cl₂ (5 mL) and cooled to 0 °C and diisopropylethylamine (DIPEA) (0.3 mmol) and propylphosphonic anhydride (T3P) (0.1 mmol, 50% solution in ethyl acetate) were added dropwise to the reaction mixture respectively. The reaction mixture was stirred for 1 hr at room temperature under nitrogen atmosphere. The completion of the reaction was confirmed by LCMS monitoring. On completion, the reaction mixture was diluted with 5 mL and washed with 10% citric acid, saturated Na₂CO₃ solution and brine

respectively. The organic layer was dried over sodium sulfate and concentrated under reduced pressure to obtain the compound **3** as white solid (yield: 80%, Chemical Formula: $C_{37}H_{52}N_4O_{10}$, Exact Mass: 712.37, ESIMS: 713.4 $[M+H]^+$).

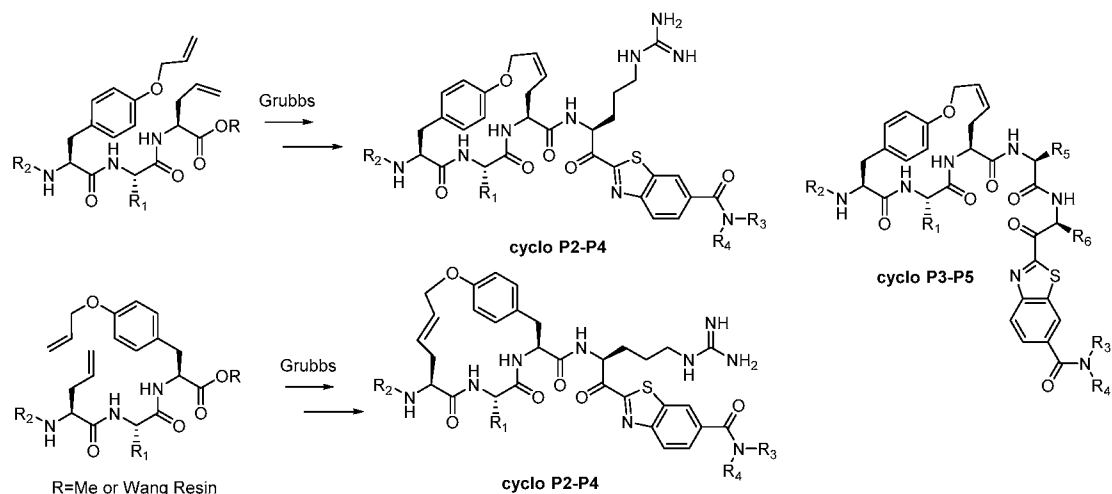
[0107] Cbz-KFD-OMe (7): Trifluoroacetic acid (TFA) (3 mL) was added to the compound **6** and the reaction mixture was stirred for 1 hr. The completion of the reaction was confirmed by LCMS monitoring. On completion, the TFA was evaporated under reduced pressure by azeotrope with toluene and the dry mass was triturated with diethyl ether to obtain the pure compound **7** as white solid (Yield: 92%, Chemical Formula: $C_{28}H_{36}N_4O_8$, Exact Mass: 556.25, ESIMS: 557.3 $[M+H]^+$).

[0108] Cbz-cyclo(KFD)-OH (PK-1-71): Compound **7** (0.09 mmol) was taken in 25 mL DMF and DIPEA (0.27 mmol) and diphenyl phosphoryl azide (0.1 mmol) was added to the solution. The reaction mixture was stirred for 12 hr at room temperature under nitrogen atmosphere and the completion of the reaction was confirmed by LCMS monitoring. The DMF was evaporated under reduced pressure and the crude product was purified by normal phase column chromatography using silica gel column and 5% MeOH in dichloromethane as eluent to obtain the product **PK-1-71** as white solid (yield: 68%, Chemical Formula: $C_{28}H_{34}N_4O_7$, Exact Mass: 538.24, ESIMS: 539.4 $[M+H]^+$.) 1H NMR (400 MHz, METHANOL- d_4) δ 8.03 (s, 1H), 7.97 (s, 1H), 7.42 - 7.49 (m, 2H), 7.29 - 7.35 (m, 6H), 7.17 - 7.23 (m, J = 7.80 Hz, 5H), 7.10 - 7.16 (m, 2H), 3.69 (td, J = 6.65, 13.30 Hz, 1H), 3.19 (q, J = 7.43 Hz, 1H), 2.98 (s, 1H), 2.85 (s, 1H), 2.74 (s, 1H), 1.36 (s, 3H), 1.34 (t, J = 3.33 Hz, 8H).

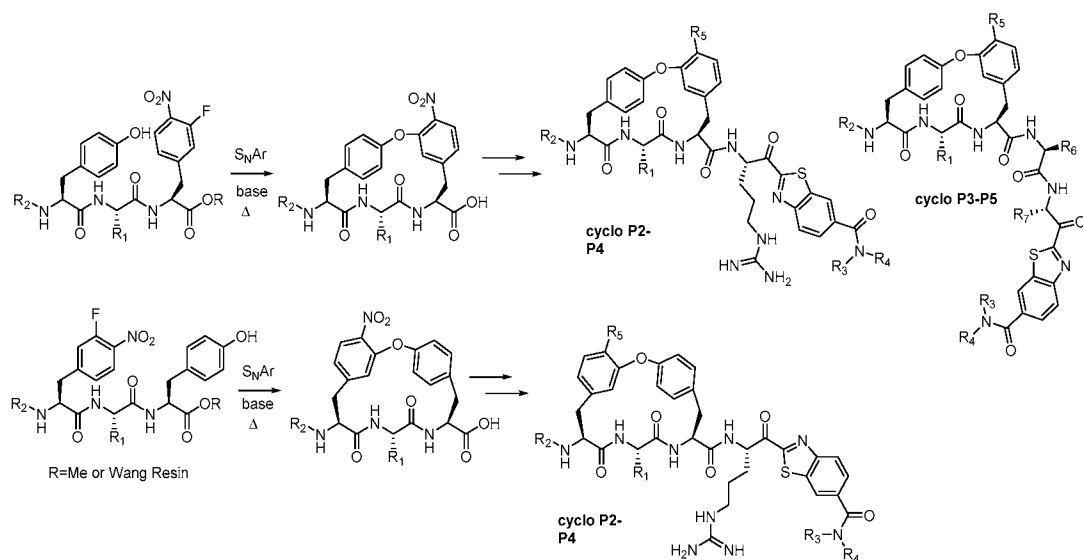
[0109] ((7S,10S,13S,Z)-13-acetamido-10-(3-amino-3-oxopropyl)-9,12-dioxo-2-oxa-8,11-diaza-1(1,4)-benzenacyclotetradecaphan-4-ene-7-carbonyl)-L-arginine (CJ-1-55). methyl N2-((S)-2-((S)-2-((S)-2-acetamido-3-(4-(allyloxy)phenyl)propanamido)-5-amino-5-oxopentanamido)pent-4-enoyl)-Nw-((2,2,4,5,7-pentamethyl-2,3-dihydrobenzofuran-6-yl)sulfonyl)-L-argininate [calculated for Chemical Formula: $C_{44}H_{62}N_8O_{11}S$, Exact Mass: 910.43, MS(ESI): found: $[M + H]^+$, 911.4, (0.18 g, 0.2 mmol)] was dissolved in DCM (125 mL). Grubbs second generation catalyst (50 mg, 0.06 mmol) was added and then the stirred reaction mixture was heated to reflux. The reaction was filtered and purified by silica gel chromatography (17 mg, 0.019 mmol). The product was dissolved in water (1 mL) and THF (1 mL), then LiOH (2 mg, 0.084 mmol) was added, and the reaction stirred for 1 hour. The THF was removed and 2 drops of 3N HCl were added to precipitate the Pbf-protected acid. The solid was dissolved in 3 mL of TFA/water/thioanisole (95:2.5:2.5) and stirred for 3

hours. The solvent was removed under reduced pressure and the residue was purified by C18 reverse phase HPLC (5-95% Acetonitrile/water/0.05% TFA). After lyophilization of pure fractions the title compound (4.4 mg) was obtained as the TFA salt. Calculated for Chemical Formula: $C_{28}H_{40}N_8O_8$, Exact Mass: 616.30, MS(ESI): found: $[M + H]^+$ 617.3.

Scheme 3C



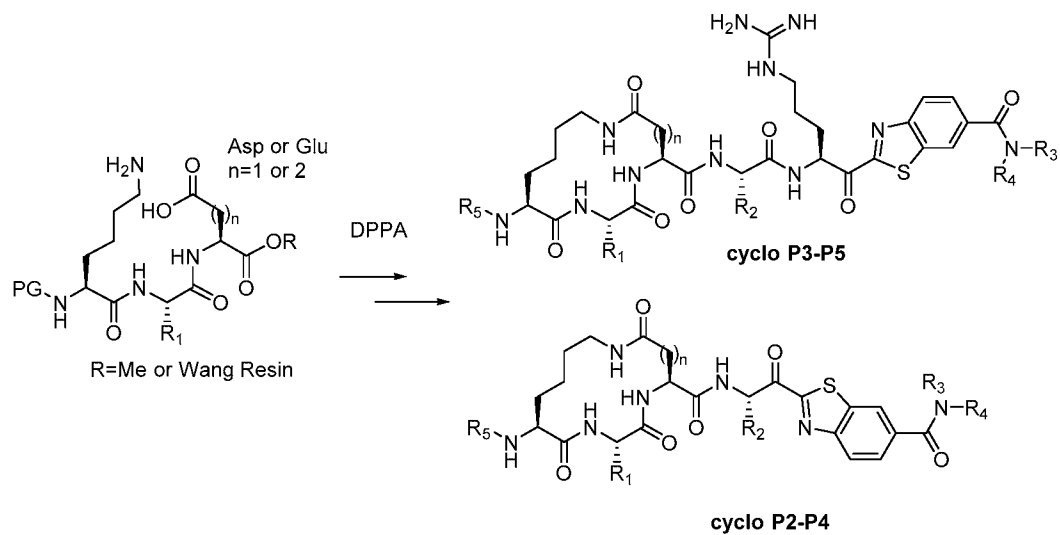
Scheme 3D



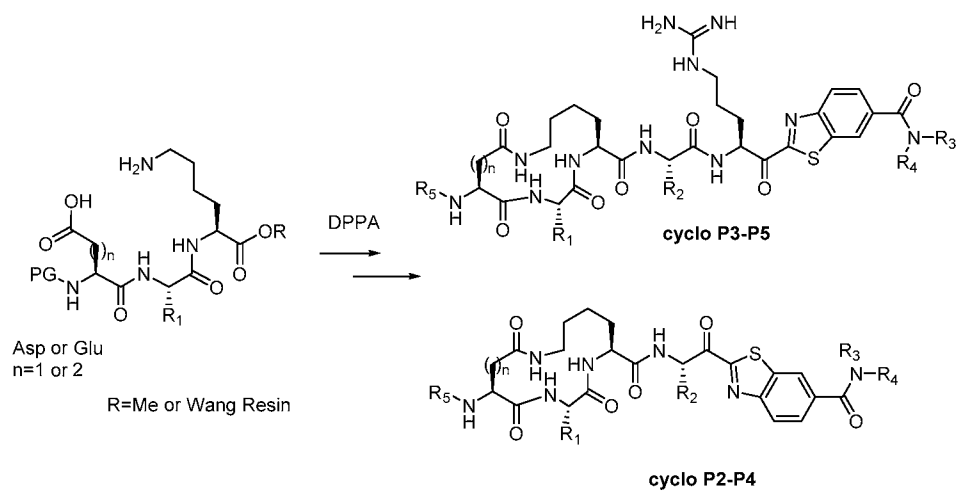
[0110] (2S,5S,13S)-13-amino-2-(3-amino-3-oxopropyl)-N-((S)-5-guanidino-1-(((S)-5-guanidino-1-oxo-1-(thiazol-2-yl)pentan-2-yl)amino)-1-oxopentan-2-yl)-3,7,14-trioxo-1,4,8-triazacyclotetradecane-5-carboxamide (7074). A solution of (6S,9S,12S)-9-

(3-amino-3-oxopropyl)-6-(4-aminobutyl)-12-(((S)-1-methoxy-1-oxo-5-(3-((2,2,4,5,7-pentamethyl-2,3-dihydrobenzofuran-6-yl)sulfonyl)guanidino)pentan-2-yl)carbamoyl)-2,2-dimethyl-4,7,10-trioxo-3-oxa-5,8,11-triazatetradecan-14-oic acid [calculated for Chemical Formula: $C_{40}H_{65}N_9O_{13}S$, Exact Mass: 911.44, MS(ESI): found: $[M + H]^+$, 912.4; (0.3 g, 0.33 mmol)] in DMF (10 mL) was slowly added to a stirred solution of DPPA (0.66 mmol, 0.15 mL) and DIPEA (0.66 mmol, 0.12 mL) in DMF (140 mL). The solvent was concentrated under reduced pressure and the residue purified by silica gel chromatography to give 100 mg of the fully protected cycloamide. This product (56 mg, 0.063 mmol) was dissolved in water (2 mL) and THF (2 mL) and then 2 mL of MeOH. LiOH (5mg, 0.21 mmol) was added and the reaction stirred for 1 hour. 2 drops of 3N HCl was added and the reaction was then concentrated in vacuo to give 52 mg of Boc/Pbf-protected acid. This product (52 mg, 0.059 mmol) was dissolved in 5 mL of DMF, placed under a nitrogen atmosphere and cooled to with an ice-bath. To this stirred solution was added HATU (27 mg, 0.071 mmol), DIPEA (0.36 mmol, 0.063 mL), and then a solution of H-Arg(Mtr)-kt-HCl salt (29 mg, 0.06 mmol) in DMF. The reaction was allowed to come to room temperature and then stirred overnight. The reaction was added to water and the precipitate filtered to give the product as a mixture of two peaks by LCMS (51 mg crude). The solid was dissolved in 5 mL of TFA/water/thioanisole (95:2.5:2.5) and stirred for 6 hours. The solvent was removed under reduced pressure and the residue was purified by C18 reverse phase HPLC (5-95% Acetonitrile/water/ 0.05% TFA). After lyophilization of fractions, the title compound (12 mg) was obtained as a 3:1 ratio of D and L diastereomers (mixture of R and S isomers of the alpha keto carbon). Calculated for Chemical Formula: $C_{30}H_{50}N_{14}O_7S$, Exact Mass: 750.37, MS(ESI): found: $[M + H]^+$, 751.4.

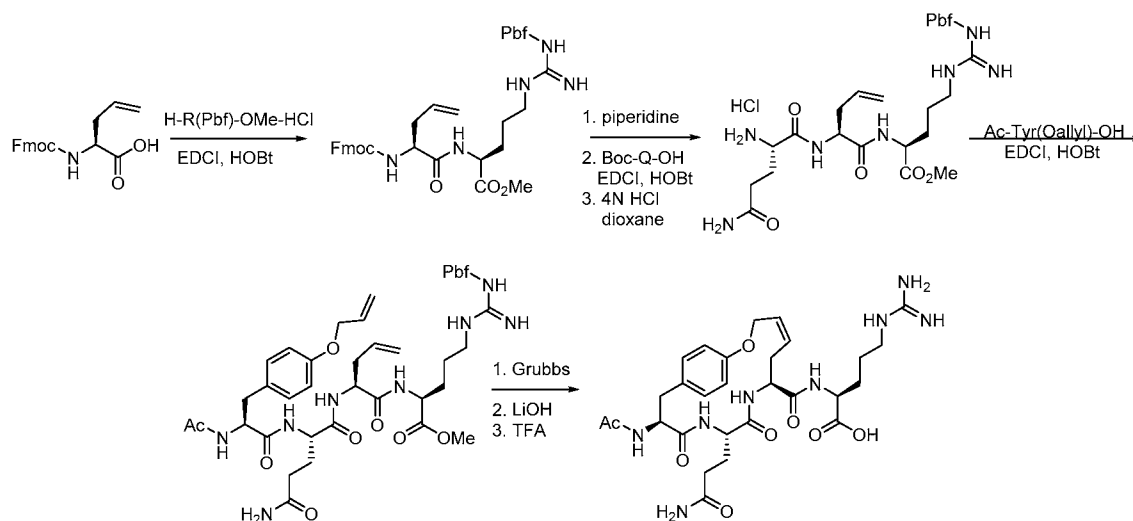
Scheme 3E



Scheme 3F



Scheme 3G



Example 4. Inhibition Studies with Ketothiazoles

[0111] The compounds prepared in Example 2 were subjected to a series of inhibitions studies. The compounds were first tested in an HGFA enzymatic assay using the fluorogenic substrate Boc-QLR-AMC with a recombinant form of the HGFA serine protease domain.

Synthesis of Boc-QLR-AMC fluorogenic substrate

[0112] **Boc-R(NO₂)-AMC.** Under nitrogen atmosphere, pyridine (60 mL) was added into the round bottom flask containing Boc-R(NO₂)-OH (4.653 g, 14.6 mmol) and 7-amino-4-methylcoumarin (3.829 g, 21.9 mmol). Diisopropylcarbodiimide (2.023 g, 16.0 mmol) was added and the mixture was stirred overnight. The mixture was filtered. The filtrate was concentrated then dried in vacuo. The resultant residue was purified by silica gel chromatography with dichloromethane/methanol combinations as eluent giving rise to Boc-R(NO₂)-AMC (2.964 g) in 43% yield. MS (ESI): found $[M + H]^+$, 477.4.

[0113] **HCl.H₂N-R(NO₂)-AMC.** 4 N HCl in dioxane (25 mL) was added into the round bottom flask containing Boc-R(NO₂)-AMC (2.964 g, 6.2 mmol) and the mixture stirred for 2 hours. The dioxane was removed in vacuo and to the resultant residue methanol was added then concentrated in vacuo three times, giving rise to the title compound in quantitative yield. MS (ESI): found $[M + H]^+$, 377.3.

[0114] **Boc-QL-OH.** Under nitrogen atmosphere, anhydrous DMF (10 mL) was added into the round bottom flask containing Boc-Q-OH (0.500 g, 2.0 mmol), H-L-OMeHCl

(0.406 g, 2.2 mmol), EDCI.HCl (0.467 g, 2.4 mmol), and HOBt (0.466 g, 3.1 mmol). *N,N*-diisopropylethylamine (0.787 g, 6.1 mmol) was added and the mixture was stirred overnight. The majority of DMF was removed in vacuo and to the resulting residue was added 20 mL of water. The precipitate was isolated by filtration then purified by silica gel chromatography with dichloromethane/methanol combinations as eluent to give Boc-QL-OMe (0.711 g) in 95% yield. MS (ESI): found $[M + Na]^+$, 396.4. Methanol/water (1:1 v/v, 10 mL) was added into the round bottom flask containing the Boc-QL-OMe (0.711 g, mmol) and LiOH (0.068 g, 2.8 mmol). The reaction was stirred overnight. The mixture was concentrated in vacuo and to the resulting residue was added 30 mL of water. 0.5 M HCl was added dropwise until pH=4.5 was reached, then the mixture was extracted three times with ethyl acetate. The ethyl acetate layers were collected, dried with Na₂SO₄, then concentrated in vacuo to give rise to Boc-QL-OH (0.603 g) in 49% yield. MS (ESI): found $[M + Na]^+$, 373.4.

[0115] Boc-QLR(NO₂)-AMC. Under nitrogen atmosphere, anhydrous DMF (10 mL) was added into the round bottom flask containing Boc-QL-OH (0.603 g, 1.7 mmol), HCl.H₂N-R(NO₂)-AMC (0.406 g, 2.2 mmol), EDCI.HCl (0.322 g, 1.7 mmol), and HOBt (0.257 g, 1.7 mmol). *N,N*-diisopropylethylamine (0.904 g, 7.0 mmol) was added and the mixture was stirred overnight. The majority of DMF was removed in vacuo and to the resulting residue was added 20 mL of water. The mixture was extracted three times with ethyl acetate. The ethyl acetate layers were collected, dried with Na₂SO₄, then concentrated in vacuo. The resultant residue was purified by silica gel chromatography with dichloromethane/methanol combinations as eluent giving rise to Boc-QLR(NO₂)-AMC (0.250 g) in 20% yield. MS (ESI): found $[M + H]^+$, 718.5.

[0116] Boc-QLR-AMC. Into the solution of Boc-QLR(NO₂)-AMC (0.250 g, 0.35 mmol) in MeOH (15 mL) was added Pd/C(10%) (0.111 g) followed by several drops of acetic acid. The mixture was stirred under hydrogen atmosphere for 21 hours. Additional Pd/C(10%) (0.184 g) was added with a few drops of acetic acid. The mixture was stirred for 24 hours, then filtered. The filtrate was concentrated. 1/5 of the resulting residue was purified by HPLC (C18, 15*150 mm column; eluent: acetonitrile/water (0.05% TFA)) to give the title compound (0.037 g) in 78% yield. ¹H NMR (400 MHz, METHANOL-*d*₄) δ ppm 0.92 (d, *J*=6.30 Hz, 3 H) 0.98 (d, *J*=6.26 Hz, 3 H) 1.45 (s, 9 H) 1.54 - 2.14 (m, 9 H) 2.25 - 2.41 (m, 2 H) 2.49 (s, 3 H) 3.17 - 3.29 (m, 2 H) 4.00 - 4.14 (m, 1 H) 4.34 - 4.47 (m, 1 H) 4.49 - 4.61 (m,

1 H) 6.28 (s, 1 H) 7.46 - 7.60 (m, 1 H) 7.71 - 7.80 (m, 1 H) 7.80 - 7.89 (m, 1 H). MS (ESI): found: $[M + H]^+$, 673.6.

[0117] Expression and purification of N-terminal His-tag HGFA serine protease domain: Using primers identified as SEQ ID NO 1, SEQ ID NO 2, and standard PCR protocols, the nucleotide sequence encoding amino acids 373-655 of HGFA was synthesized. This PCR product was cloned into the SfoI-HindIII sites of a modified pFastBac HT baculovirus expression vector (Addgene, Cambridge, MA). This vector contains a six amino His tag followed by a seven amino spacer and a seven amino acid TEV cleavage site placed immediately downstream of the Honey Bee melittin signal peptide. Using a modified Bac to Bac Expression System (Life Technologies, Carlsbad, CA), recombinant HGFA bacmids were obtained by transforming DH10Bac *Escherichia coli* cells. To obtain HGFA containing baculovirus, purified bacmids were transfected into Sf9 insect cells. After 5 days in culture at 27 °C, media was harvested from transfected Sf9 cells. This media was used to prepare baculovirus infected insect cells (BIICs). These BIICs were used to infect High 5 insect cells. Four days post infection, media was harvested and recombinant protein prepared as follows. Media was chilled to 4 °C and spun at 4000xg for 20 minutes (all subsequent steps were performed at 4 °C unless noted). Clarified media was passed first through a Whatman GF/B 1 um (# 1821-047, GE Healthcare Life Sciences, Piscataway, NJ) and then a 0.22 um PES membrane (#99955, TPP Techno Plastic Products AG, Trasadingen, Switzerland) and then concentrated using a Pall Centramate tangential flow system and Centramate T-series Cassette (#OS010T12, Pall Corporation, Port Washington, NY). Concentrated media was then buffer exchanged in two steps, five volumes of 50 mM Na-phosphate, 500 mM NaCl, pH 6.2, followed by five volumes of 50 mM Na-phosphate, 500 mM NaCl, pH 7.5. The concentrated and buffered exchanged insect cell media was again filtered as above and made 25 mM imidazole (#I202, Sigma-Aldrich, St. Louis, MO) and was mixed with nickel agarose beads (# H-321-25, Gold Biotechnology, Inc., St. Louis MO). After mixing this slurry for 12 hours, nickel agarose beads were allowed to settle by gravity and then loaded into a column. Beads were washed with buffer (25 mM Na-phosphate, 500 mM NaCl, 25 mM imidazole, pH 8) and the bound protein eluted using (25 mM Na-phosphate, 500 mM NaCl, 250 mM imidazole, pH 8). Using a Amicon Ultra-4 Centrifugal filters (#UFC801008, Merck Millipore, Ltd., Tullagreen, Ireland), peak protein fractions were concentrated and run over a Superdex-200 10/300 GL column (GE Healthcare Life Sciences, Piscataway, NJ) in 10 mM

Tris, 200 mM NaCl, 0.2 mM EDTA, pH 8. HGFA containing fractions were pooled, concentrated, made 50% glycerol, and stored at minus 20 °C. Protein was quantitated using a modified Lowry protein assay (#500-0006, Bio-Rad Laboratories, Hercules, CA).

[0118] Chromogenic Kinetic Enzyme Inhibitor Assays of HGFA: The inhibitors (0-50 μ M final concentration in reaction) were diluted in DMSO (2% DMSO final concentration in reaction) and then mixed with recombinant HGFA (12.5 nM final concentration in reaction) in TNC buffer (25 mM Tris, 150 mM NaCl, 5 mM CaCl₂, 0.01% TRITON X-100, pH 8). After incubating for thirty minutes at 25° C, chromogenic substrate, Pefachrome FVIIa, (#093-01, Enzyme Research Laboratories, South Bend, IN) was added to a final concentration of 250 μ M in a final reaction volume of 40 microliters. Changes in absorbance at 405 nm were measured over time in a Biotek Synergy 2 plate reader (Winnoski, VT). Using Gen 5 2.00 software program (Biotek, Winnoski, VT), a four parameter curve fit was used to determine the inhibitor IC₅₀s from a plot of the mean reaction velocity versus the inhibitor concentration. The IC₅₀ values represent the average of three separate experimental determinations.

[0119] Chromogenic Kinetic Enzyme Assays of Thrombin and Factor Xa: Inhibitors (11-pt serial dilutions, 0-20 μ M final concentration) were serially diluted in DMSO (2% DMSO final concentration) and then mixed with recombinant thrombin (0.15 nM final concentration) or Factor Xa (0.35 nM final concentration) in TNC buffer (25 mM Tris, 150 mM NaCl, 5 mM CaCl₂, 0.01% TRITON X-100, pH 8) using clear 384 well plates. After incubating for 30 minutes at 25° C, the chromogenic substrate (S2238; D-Phe-Pip-Arg-pNA) for thrombin (K_m = 14.5 μ M) or (S2222; Bz-Ile-Glu-Gly-Arg-pNA) for Factor Xa (K_m = 200 μ M) was added to a final concentration of K_m (4 x K_m (50 μ M) for thrombin) in a final reaction volume of 40 microliters. Changes in absorbance at 405 nm were measured over time in a Biotek Synergy 2 plate (Winnoski, VT). Using GraphPad Prism version 6.04 software program, (GraphPad Software, San Diego, CA, www.graphpad.com), a four parameter curve fit was used to determine the inhibitor IC₅₀s from a plot of the mean reaction velocity versus the inhibitor concentration. K_i values were calculated from the IC₅₀ values using the Cheng and Prusoff equation ($K_i = IC_{50}/(1+[S]/K_m)$).

Table 4.1

Structure	HGFA IC ₅₀ (μ M)	Matriptase IC ₅₀ (μ M)	Hepsin IC ₅₀ (μ M)	Thrombin Ki (nM)	Factor Xa Ki (nM)
Nafamostat	0.80	0.001	0.002		
Leupeptin (Ac-LLR-H	0.535	1.150	0.256		
Ac-KQLR(SEQ ID NO: 1)-kt (5)	0.445	0.005	0.002	>20000	170
Ac-KQLdR-kt (5-1)	5.526	--	--	--	--
Ac-KHLR(SEQ ID NO: 2)-kt (5a)	0.650	--	--	--	--
Ac-KHLdR-kt (5a-1)	10.931	--	--	--	--
Ac-WQLR(SEQ ID NO: 3)-kt (5b)	0.471	0.049	0.001	--	--
Ac-WQLdR-kt (5b-1)	7.123	--	--	--	--
Ac-KQFR(SEQ ID NO: 4)-kt (5c)	0.470	--	--	>20000	78.8
Ac-KQFdR-kt (5c-1)	7.824	--	--	--	--
Ac-KFLR(SEQ ID NO: 5)-kt (5d)	0.330	0.035	0.022	--	--
Ac-RQLR(SEQ ID NO: 6)-kt (5e)	0.270	0.001	0.001	>20000	8.10
Ac-RQLdR-kt (5e-1)	3.118	--	--	--	--
Ac-SQLR(SEQ ID NO: 7)-kt (5f)	0.180	--	--	--	--
Ac-KRLR(SEQ ID NO: 8)-kt (5g)	0.225	0.014	0.005	--	--
Ac-WRLR(SEQ ID NO: 9)-kt (5h)	0.275	0.031	0.0003	--	--
Ac-SKLR(SEQ ID NO: 10)- kt (6)	0.56	0.048	0.00	>20000	3062
Ac-SKLdR-kt (6-1)	6.311	--	--	--	--
Ac-SHLR(SEQ ID NO: 11)- kt (6a)	1.800	0.560	0.005	--	--
Ac-SHLdR-kt (6a-1)	21.218	--	--	--	--
Ac-WKLR(SEQ ID NO: 12)- kt (6b)	0.320	0.097	0.004	--	--
Ac-WKLdR-kt (6b-1)	6.500	--	--	--	--
Ac-SKFR(SEQ ID NO: 13)-kt (6c)	0.520	0.034	0.051	>2000	530.00
Ac-SKFdR-kt (6c-1)	10.795	--	--	--	--
Ac-NKLR(SEQ ID NO: 14)- kt (6d)	0.397	--	--	--	--
Ac-SRLR(SEQ ID NO: 15)-kt (6e)	0.303	0.024	0.005	--	--

Structure	HGFA IC ₅₀ (μ M)	Matriptase IC ₅₀ (μ M)	Hepsin IC ₅₀ (μ M)	Thrombin Ki (nM)	Factor Xa Ki (nM)
Ac-SdRLR-kt (6e-1)	1.420	--	--	--	--
Ac-SRLdR-kt (6e-2)	2.241	--	--	--	--
Ac-SdRLdR-kt (6e-3)	11.920	--	--	--	--
Ac-TKLR(SEQ ID NO: 16)- kt (6f)	0.567	--	--	--	--
Ac-SWLR(SEQ ID NO: 17)- kt (6g)	0.435	0.175	0.009	3698	759
Ac-RKLR(SEQ ID NO: 18)- kt (6h)	0.370	--	--	--	--
Ac-NKLR(SEQ ID NO: 14)- kt	0.4	--	--	--	--
H-KQLdR-kt	11.31	--	--	--	--
H-KQLR(SEQ ID NO: 1)-Kt	0.44	--	--	--	--
Ac-LLR-kt	1.48				

[0120] HGFA Competition Assay of inhibitor Ac-KQLR(SEQ ID NO: 1)-Kt (5) and substrate: To determine whether the ketothiazoles are behaving as competitive inhibitors, different amounts of Ac-KQLR(SEQ ID NO: 1)-Kt (5) (0, 0.25, 0.5, and 1.0 μ M) were mixed with Pefachrome serially diluted in TNC buffer. HGFA was added to 12.5 nM and changes in absorbance at 405 nm were measured over time in a Biotek Synergy 2 plate reader. From the plots of the mean reaction velocity versus substrate concentration and the Michaelis-Menten enzyme kinetics equation within GraphPad Prism version 6.04 for Windows, the reaction $V_{\max}$ and subsequent K_m for each concentration of inhibitor was determined as shown in the FIG 1.

[0121] Fluorescent Kinetic Enzyme Inhibitor Assays of HGFA, matriptase hepsin, and thrombin: Inhibitors (11-pt serial dilutions, 0-20 μ M final concentration in reaction) were serially diluted in DMSO (2% DMSO final concentration) and then mixed with either recombinant serine protease domain of HGFA [Z. Han, P. K. Harris, D. E. Jones, R. Chugani, T. Kim, M. Agarwal, W. Shen, S. A. Wildman, J. W. Janetka, *Acs Med Chem Lett* 2014, 5, 1219-1224], matriptase (Charles Craik, UCSF) or hepsin* (#4776-SE-010, R&D Systems, Minneapolis, MN) in black 384 well plates (Corning # 3575, Corning, NY). The final assay concentration for HGFA, matriptase and hepsin 7.5 nM, 0.2 nM, and 0.3 nM, respectively in TNC buffer (25 mM Tris, 150 mM NaCl, 5 mM CaCl₂, 0.01% TRITON X-100, pH 8). After thirty minutes incubation at room temperature, Boc-QLR-AMC substrate

($K_m = 37 \mu\text{M}$) was added to the HGFA assays and Boc-QAR-AMC substrate was added to the matriptase ($K_m = 93 \mu\text{M}$) and hepsin ($K_m = 156 \mu\text{M}$) assays. The final substrate concentrations for all assays were at the K_m for the respective enzymes. Changes in fluorescence (excitation at 380 nm and emission at 460 nm) were measured at room temperature over time in a Biotek Synergy 2 plate reader (Winnoski, VT). Using GraphPad Prism version 6.04 software program, (GraphPad Software, San Diego, CA, www.graphpad.com), a four parameter curve fit was used to determine the inhibitor IC_{50} s from a plot of the mean reaction velocity versus the inhibitor concentration. The IC_{50} values represent the average of three separate experimental determinations. K_i values were calculated using the Cheng and Prusoff equation ($K_i = \text{IC}_{50}/(1+[S]/K_m)$).

[0122] *Hepsin Activation: Recombinant Hepsin (10 μg , 0.44mg/mL as received from R&D Systems, Catalogue # 4776-SE) was diluted to 2.4 μM in TNC buffer (25 mM Tris, 150 mM NaCl, 5 mM CaCl_2 , 0.01% TRITON X-100, pH 8) and incubated at 37°C. After twenty-four hours, the hepsin was diluted in glycerol to 50%. This stock hepsin (1.2 μM) was stored in a -20°C freezer and diluted in TNC buffer for use in assays.

[0123] As shown in the following table, the pro-HGF and pro-MSP tetrapeptides **5** and **6** showed K_i s of 53 nM and 81 nM, respectively. These results demonstrate that inhibitor **5** was competitive with substrate to the active-site. The majority of compounds evaluated are more selective for matriptase and hepsin; most pronounced with hepsin where some inhibitors having K_i s < 1 nM. There are several compounds which are equipotent for HGFA compared to matriptase but leupeptin is 3-fold selective for HGFA over matriptase and only 3-fold favoring hepsin. The pro-HGF peptide (**5**) is 200-fold more potent for hepsin and 50-fold for matriptase while the pro-MSP peptide (**6**) is only slightly more potent for matriptase and 70-fold potent for hepsin versus HGFA. Ac-SWLR(SEQ ID NO: 17)-Kt (**6g**) is equipotent for both HGFA and matriptase with a K_i =66 nM but K_i =1.2 nM for hepsin. **6g**, Ac-SKFR(SEQ ID NO: 13)-Kt (**6c**), Ac-KFLR(SEQ ID NO: 5)-Kt (**5d**) and Ac-SRLR(SEQ ID NO: 15)-Kt (**6e**) show HGFA selectivity over both hepsin and matriptase.

Table 4.2

Structure	HGFA K _i (nM)	Matriptase K _i (nM)	Hepsin K _i (nM)
Nafamostat	25	0.02	0.53
Leupeptin (Ac-LLR-H)	188	696	61
Ac-KQLR(SEQ ID NO: 1)-kt (5)	70	1.60	0.19
Ac-KQLdR-kt (5-1)	--	--	--
Ac-KHLR(SEQ ID NO: 2)-kt (5a)	96	22.3	0.41
Ac-KHLdR-kt (5a-1)	--	--	--
Ac-WQLR(SEQ ID NO: 3)-kt (5b)	65	32.4	0.21
Ac-WQLdR-kt (5b-1)	--	--	--
Ac-KQFR(SEQ ID NO: 4)-kt (5c)	58	0.69	0.58
Ac-KQFdR-kt (5c-1)	--	--	--
Ac-KFLR(SEQ ID NO: 5)-kt (5d)	80	15	2.1
Ac-RQLR(SEQ ID NO: 6)-kt (5e)	60	0.32	0.28
Ac-RQLdR-kt (5e-1)	--	--	--
Ac-SQLR(SEQ ID NO: 7)-kt (5f)	182	9.2	0.34
Ac-KRLR(SEQ ID NO: 8)-kt (5g)	12	1.1	0.57
Ac-WRLR(SEQ ID NO: 9)-kt (5h)	21	5.5	0.21
Ac-SKLR(SEQ ID NO: 10)(SEQ ID NO: 10)-kt (6)	81	58	1.2
Ac-SKLdR-kt (6-1)	--	--	--
Ac-SHLR(SEQ ID NO: 11)-kt (6a)	332	104.2	0.60
Ac-SHLdR-kt (6a-1)	--	--	--
Ac-WKLR(SEQ ID NO: 12)-kt (6b)	56	8.6	0.55
Ac-WKLDdR-kt (6b-1)	--	--	--
Ac-SKFR(SEQ ID NO: 13)-kt (6c)	57	3.03	8.5
Ac-SKFdR-kt (6c-1)	--	--	--
Ac-NKLR(SEQ ID NO: 14)-kt (6d)	79	12	1.4
Ac-SRLR(SEQ ID NO: 15)-kt (6e)	24	5.8	0.68
Ac-SdRLR-kt (6e-2)	--	--	--
Ac-SRLdR-kt (6e-1)	--	--	--
Ac-SdRLdR-kt (6e-3)	--	--	--
Ac-TKLR(SEQ ID NO: 16)-kt (6f)	103	8.7	0.61
Ac-SWLR(SEQ ID NO: 17)-kt (6g)	63	69	1.2
Ac-RKLR(SEQ ID NO: 18)-kt (6h)	17	0.83	0.47
Ac-NKLR(SEQ ID NO: 14)-kt	79	12.4	1.41
Ac-LLR-kt	253.00	27.950	2.288

-- Not measured. **K_m Determinations:**

[0124] In black 384 well plates (Corning #3575), 12.5 nM HGFA was mixed with various amounts of Boc-QLR-AMC and 1 nM matriptase and 0.3 nM hepsin were mixed with various amounts of Boc-QAR-AMC. Changes in fluorescence (excitation at 380nm and emission at 460nm) were measured at room temperature over time in a Biotek Synergy 2 plate

reader (Winnoski, VT). Using plots of the mean reaction velocity versus substrate concentration and the Michaelis-Menten enzyme kinetics equation within GraphPad Prism version 6.04 for Windows (GraphPad Software, San Diego, CA, www.graphpad.com), the reaction $V_{\max}$ and subsequent K_m for each of the substrates were determined. The plots are presented in FIGS. 2-4.

[0125] Dilution recovery experiments with Ac-KQLR(SEQ ID NO: 1)-Kt (5) and HGFA: To demonstrate reversibility of HGFA ketothiazole inhibitors and to examine the dissociation of the enzyme-inhibitor complex, dilution recovery experiments were performed. High concentration HGFA (7.5 μM) were mixed with different concentrations of Ac-KQLR(SEQ ID NO: 1)-Kt (5), between 0-120 μM . After incubating for 20 minutes at room temperature, these reactions were diluted rapidly (1:350) in TNC buffer containing 250 μM Boc-QLR-AMC substrate (~ 7 times K_m). Activity was monitored by recording the change in fluorescence (excitation at 380 nm and emission at 460 nm) over time in a Biotek Synergy 2 plate reader. GraphPad Prism was used to plot the change in fluorescence over time. The results are provided in FIGS. 5 and 6. These results show that Ac-KQLR(SEQ ID NO: 1)-Kt (5) is a reversible inhibitor of HGFA as the enzyme activity is recovered slowly over time even at an inhibitor concentrations $7 \times K_i$.

[0126] Biochemical Assay for Proteolysis of pro-HGF and pro-MSP by HGFA: A biochemical assay was employed in order to demonstrate that inhibitors can block the proteolytic activation of the endogenous growth factors, pro-HGF and pro-MSP by HGFA in a dose-dependent manner. In order to determine efficiency of our recombinant HGFA we performed a concentration response of HGFA using a fixed concentration of pro-HGF and pro-MSP. Since both the single chain inactive pro-HGF or pro-MSP and the active heterodimers have the same molecular weight, SDS gels were developed under reducing conditions.

[0127] For pro-HGF proteolysis, inhibitors (0-12.5 μM final concentration in reaction) were diluted in DMSO (2% DMSO final concentration in reaction) and then mixed with recombinant HGFA (1.0 nM final concentration) in TNC buffer. After 30 minutes incubation at room temperature (25 $^{\circ}\text{C}$), 25 ng of pro-HGF (R&D Systems, NKG011306A) was added. After 1 hour incubation at 37 $^{\circ}\text{C}$, reactions were stopped by adding SDS gel loading buffer containing DTT (reducing) and then run on 12% PAGE. Proteins were transferred to Millipore Immobilon-P membranes (Billerica, MA) and then immunoblotted at

4 °C with anti-HGF antibody (R&D Systems, AF-294-NA) and HRP conjugated donkey anti-goat IgG (Santa Cruz, SC-2020) diluted 1:500 and 1:5000 in 5% milk/TBST (5% Carnation nonfat dried milk/10 mM Tris, 150 mM NaCl, 0.05% TWEEN 20, pH 8.0), respectively. Membranes were washed in TBST, immersed in Millipore Luminata Crescendo Western-HRP Substrate for 5 minutes, and then exposures made on a BioRad ChemiDoc MP Imaging System (Hercules, CA). For pro-MSP proteolysis assays, the procedures were similar, except that the HGFA concentration was 75 nM, pro-MSP (R&D Systems ZN081306A) concentration was 50 ng per reaction, and 1:500 dilution of anti-MSP antibody (R&D Systems, AF352) was used for MSP detection during immunoblotting.

[0128] The results are shown in FIGS. 7 and 8 below. Lanes with pro-HGF (FIG 7) and pro-MSP (FIG 8) contain one band at 90 and 75 KDa, respectively, whereas activated HGF and MSP appear as two bands as the 60 KDa α -chain and 30 KDa β -chain in HGF and 50 KDa and 25 KDa for MSP (note: MSP Ab only recognizes the α -chain). Shown in Figure 4, we found that nafamostat and the three inhibitors 5, 5h, 5g all showed a dose-dependent inhibition of pro-HGF activation with similar EC50 values in direct correlation with those found from the HGFA enzyme assay. These inhibitors in addition to 6e also all show dose-dependent inhibition of pro-MSP proteolysis by HGFA as shown in FIG 7. While it is difficult to quantitate the level of MSP activation since the pro-MSP has some active two-chain MSP present, the EC50 values correlate with the level of potency seen in the enzyme assay. These results show the inhibitors inhibit the processing of both known protein substrates of HGFA.

[0129] c-MET Phosphorylation (Y1234/1235) in cells treated with HGFA processed pro-HGF and MSP: To show the inhibition provided by the tetrapeptide compounds would have effects on cell signaling through c-MET kinase, a phosphorylation assay using the invasive breast cancer cell line, MDA-MB-231 was developed. This cell line has high expression of c-MET and pro-HGFA but not pro-HGF.

[0130] MDA-MB-231 cells were maintained in RPMI medium (Sigma-Aldrich R8758, St. Louis, MO) containing 10% fetal bovine serum (Sigma F2442) and 1X Penicillin/Streptomycin (Pen/Strep) antibiotics (Thermo Fisher SV30010). For cMet phosphorylation measurements, MDA-MB-231 cells were switched to starve medium (RPMI medium containing 1 mM Sodium Pyruvate (Corning 25-000-ci), 10 mM HEPES (Corning 25-060-ci), 0.225% Glucose (Corning 25-037-ci), 1X PenStrep). After 18 hours, cells were

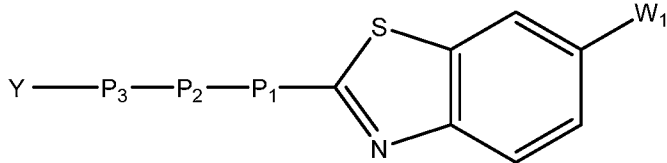
switched to fresh starve media and then treated with HGFA Proteolysis reactions containing 1 nM HGFA, 50 ng pro-HGF and various amounts of inhibitors. After 15 minutes incubation, media was removed by aspiration and the remaining cells were washed twice with cold Dulbeccos Phosphate Buffered Saline (Life Technologies #14190-136). Next cells were scraped into in Lysis Buffer (50 mM Tris, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 0.25% Sodium Deoxcholate, pH 7.5) containing 1mM sodium fluoride, 1 mM sodium orthovanadate, 1X Sigma (P8340) Inhibitor Cocktail, and one Roche Complete Mini, EDTA-free Protease Inhibitor Cocktail tablet per 10 ml buffer and stored frozen. Lysate aliquots were mixed with reducing SDS gel loading buffer and run on 10% PAGE. Immunoblots were performed as described previously, except primary antibodies, anti-phospho cMet (Cell Signaling, CS3077) and anti-total cMet (Cell Signaling, CS3127) were used and diluted in 5% BSA/TBST. Secondary antibodies, HRP-anti rabbit antibody (CS7074) and HRP-anti mouse (CS7076) were used, respectively. All antibodies were used at a 1:1000 dilution. Images were obtained and quantitated using BioRad ChemiDoc MP Imaging System. cMet phosphorylation signals were normalized using the total cMet signals and % inhibition calculated using the ratio of inhibited versus uninhibited HGFA Proteolytic reactions. Results were plotted in GraphPad Prism and a four parameter curve fit was used to determine the inhibitor EC₅₀s.

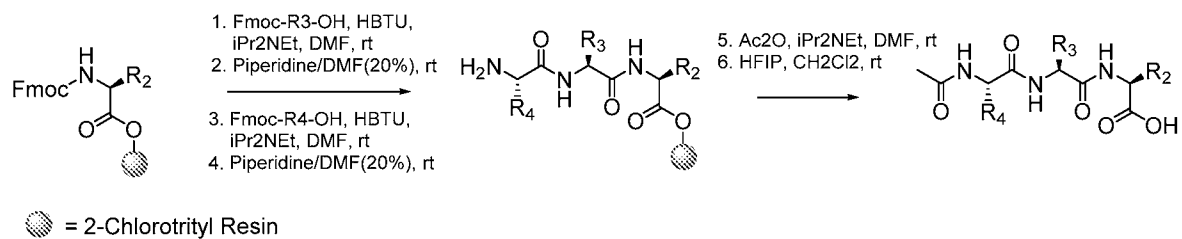
[0131] As shown in FIG. 9, several inhibitors were effective at decreasing c-MET phosphorylation in a dose-dependent manner. Thus, these results show that these inhibitors of HGFA can function as non-kinase inhibitors of HGF-mediated c-MET kinase signaling in cancer. The most potent compound 5g had an EC₅₀ of 180 nM.

Example 5. Synthesis of Ketobenzothiazole Inhibitor Compounds

[0132] The polypeptide ketobenzothiazoles (kbt) listed in Table 5.1 were synthesized in accordance with the general scheme 5A shown below and the procedures described below.

Table 5.1. Polypeptide Compounds

		
Compound No.	Ac-P ₃ -P ₂ -P ₁ -Kt	W ₁
1-13A1	H-WFR-kbt	H
1-18A1	H-dWFR-kbt	H
1-15A1	H-dWLR-kbt	H
1-56A1	H-WLR-kbt-COOH	COOH
1-56A1	H-dWLR-kbt-COOH	COOH
1-54A1	H-His(Bom)WLR-kbt	H
7182	H-LLR-kbt V amide	Val-NH ₂
7185-1	Fmoc-AR-kbt	H
7185-2	Fmoc-RR-kbt	H
7185-3	Fmoc-NR-kbt	H
7185-4	Fmoc-DR-kbt	H
7185-6	Fmoc-QR-kbt	H
7185-7	Fmoc-ER-kbt	H
7185-8	Fmoc-GR-kbt	H
7185-9	Fmoc-HR-kbt	H
7185-10	Fmoc-IR-kbt	H
7185-11	Fmoc-LR-kbt	H
7185-12	Fmoc-KR-kbt	H
7187-13	Fmoc-MR-kbt	H
7187-14	Fmoc-FR-kbt	H
7187-15	Fmoc-PR-kbt	H
7187-16	Fmoc-SR-kbt	H
7188-17	Fmoc-TR-kbt	H
7188-18	Fmoc-WR-kbt	H
7188-19	Fmoc-YR-kbt	H
7188-20	Fmoc-VR-kbt	H

Scheme 5A

[0133] Tripeptides were synthesized in 0.5 mmol scale through consecutive coupling of amino acid-preloaded 2-Cl-trityl resin with Fmoc protected amino acids followed by the deprotection of Fmoc group. In the coupling reaction, 5 equiv. Fmoc-amino acid/5 equiv. HBTU/ 10 equiv. $i\text{Pr}_2\text{NEt}$ was used and the mixture was shaken at room temperature overnight. In the deprotection of Fmoc group, piperidine/DMF (20% v/v) was employed and the mixture was shaken from 1 to 4 hours at room temperature to ensure complete reaction.

[0134] **Peptide coupling and deprotection steps of the Fmoc group:** Into the reaction vial (with a fritted glass resin support) containing H-Leu-2-Cl trityl, H-Phe-2-Cl trityl resin or H-Xxx-2-Cl trityl resin (0.714 g, 0.5 mmol), DMF/ CH_2Cl_2 (15/15 mL) was added. The mixture was shaken at room temperature for 30 minutes, then filtered. The resulting resin was washed with DMF (10 mL) 2 times. Into another vial containing Fmoc-AA-OH (2.5 mmol) in DMF (20 mL), HBTU (0.853 g, 2.25 mmol) and $i\text{Pr}_2\text{NEt}$ was added (0.87 mL, 5 mmol). The mixture was stirred at room temperature for 10 minutes, then added into the reaction vial containing the resin. The mixture was shaken at room temperature overnight, then filtered. The resin was washed with DMF (20 mL x 4). To the resulting resin piperidine/DMF (20% v/v, 30 mL) was added. The mixture was shaken for 1-4 hours at room temperature, then filtered. The resin was washed with DMF (10 mL x 4).

[0135] **Acetyl capping of the tripeptides:** The tripeptide resin was suspended in 30 mL of 0.5 M Ac_2O /DMF and 1 M $i\text{Pr}_2\text{NEt}$ /DMF. The mixture was shaken at room temperature for 1 hour. The resin was filtered and washed with DMF (10 mL x 4) followed by CH_2Cl_2 (10 mL x 4).

[0136] **Cleavage of tripeptide resin:** Ac-capped tripeptide resin was suspended and shaken in 30 mL of 25% v/v HFIP/ CH_2Cl_2 for 1 hour. The mixture was filtered. The filtrate was concentrated then dried in vacuo, giving rise to crude product of Acetyl-capped tripeptides.

[0137] **Boc-WF-OMe:** Boc-Trp-OH (1 g, 3.28 mmol) and HCl.Phe-OMe (0.708g, 3.28 mmol) was taken in dry dichloromethane (10 mL) under nitrogen atmosphere and the reaction mixture was cooled to 0 °C and N,N-diisopropylethylamine (1.7 mL, 9.84 mmol) and propylphosphonic anhydride (1.9 mL, 3.28 mmol, 50% solution in EtOAc) were added to the solution drop wise respectively. The reaction mixture was then stirred at 25 °C under nitrogen atmosphere for 1 hour and the completion of the reaction was confirmed by LC-MS monitoring. On completion the reaction mixture was diluted with 10 mL dichloromethane

and washed with 10% citric acid solution, saturated sodium bicarbonate solution and brine respectively. The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The crude product was triturated with hexane to obtain the title product in pure form as a white solid. Yield: 1.3 g (92.8%). Chemical formula: $C_{23}H_{33}N_3O_5$, Exact Mass: 465.55, MS(ESI): found: $[M + Na]^+$, 488.58.

[0138] Boc-WF-OH: Boc-WF-OMe (0.130 g, 0.279 mmol) was taken in a 1:1 mixture of THF and water and LiOH.H₂O (0.035g, 0.873 mmol) was added to it. The reaction mixture was stirred for 30 minutes at 25 °C and the completion of the reaction was confirmed by LCMS monitoring. On completion, the THF was evaporated under reduced pressure and the remaining water layer was cooled to 0 °C. The water layer was then brought to pH 6.5 by slow addition of 0.5 M HCl solution in water. The crude product precipitates out on addition of HCl and it was isolated by filtration. The crude product was dried under reduced pressure and triturated with diethyl ether to obtain the pure title product in pure form as white solid. Yield: 0.102g (80%). Exact Mass: 451.21, MS(ESI): found: $[M + H]^+$, 452.26.

[0139] Boc-WFR(Mtr)-kbt: Boc-WF-OH (35 mg, .077 mmol) and HATU (43.9 mg, 0.115 mmol) was taken in dry DMF under nitrogen atmosphere and the reaction mixture was cooled to 0 °C. N,N-diisopropylethylamine (0.04mL, 0.231 mmol) was then added drop wise to the reaction mixture and the reaction mixture was allowed to stir for 15 minutes followed by addition of HCl.Arg(Mtr)-kbt (41.75 mg, .077 mmol). The reaction mixture was stirred for 12 hours at 25 °C under nitrogen atmosphere and the completion of the reaction was confirmed by LC-MS monitoring. On completion, the reaction mixture was diluted with EtOAc and washed with 10% citric acid solution, saturated sodium bicarbonate solution and brine respectively. The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The crude product was directly taken to the next step without further purification. Chemical formula: $C_{48}H_{56}N_8O_8S_2$, Exact Mass: 936.37, MS(ESI): found: $[M + H]^+$, 937.17.

[0140] H-WFR-kbt (1-13A1): Boc-WFR(Mtr)-kbt (85 mg, crude product from previous step) was taken in 5 mL TFA:thioanisole:H₂O (95:2.5:2.5) and the reaction mixture was stirred for 6 hours at 25 °C. The completion of the reaction was confirmed by LC-MS monitoring. On completion, the reaction mixture was concentrated under reduced pressure and triturated with diethyl ether to obtain the crude product as brown solid. The crude product was then subjected to reverse phase semi-preparative HPLC (Stationary phase: C18 column,

mobile phase: H₂O-Acetonitrile with 0.1% TFA in each, 15-65% Acetonitrile in H₂O gradient for 20 minutes) to obtain the pure title product as yellow solid. Yield: 20 mg (41% over two steps). ¹H NMR (400 MHz, CHLOROFORM-d) δ ppm 8.08 (br. s., 1H), 7.67 (d, *J* = 8.22 Hz, 1H), 7.37 (d, *J* = 8.22 Hz, 1H), 7.10 - 7.25 (m, 5H), 6.94 - 7.05 (m, 1H), 6.82 (d, *J* = 7.04 Hz, 2H), 6.14 - 6.29 (m, 1H), 5.12 (d, *J* = 2.74 Hz, 1H), 4.69 - 4.82 (m, 1H), 4.45 (br. s., 1H), 3.62 (s, 3H), 3.09 - 3.37 (m, 1H), 2.95 (d, *J* = 5.48 Hz, 2H), 1.62 (s, 1H), 1.43 (br. s., 9H). Chemical formula: C₃₃H₃₆N₈O₃S, Exact Mass: 624.26, MS (ESI): found: [M + H]⁺, 625.5.

[0141] Boc-dWF-OMe: The title compound was synthesized using the same procedure as Boc-WF-OMe starting with Boc-dTrp-OH. Yield: 90%. Chemical formula: C₂₃H₃₃N₃O₅, Exact Mass: 465.55, MS(ESI): found: [M + Na]⁺, 488.58.

[0142] Boc-dWF-OH: The title compound was synthesized using the same procedure as Boc-WF-OH starting with Boc-dWF-OMe. Yield: 80%. Exact Mass: 451.21, MS(ESI): found: [M + H]⁺, 452.26.

[0143] Boc-dWFR(Mtr)-kbt: The title compound was synthesized using the same procedure as Boc-WFR(Mtr)-kbt starting with Boc-dWF-OH. The crude product was directly taken to the next step. Chemical formula: C₄₈H₅₆N₈O₈S₂, Exact Mass: 936.37, MS(ESI): found: [M + H]⁺, 937.17.

[0144] H-dWFR-kbt (1-18A1): The title compound was synthesized using the same procedure as H-WFR(Mtr)-kbt starting with Boc-dWF-OH. Yield: 25 mg (42% over two steps). Chemical formula: C₃₃H₃₆N₈O₃S, ¹H NMR (400 MHz, METHANOL-d₄) δ ppm 8.21 (d, *J* = 6.65 Hz, 1H), 8.11 (d, *J* = 7.43 Hz, 1H), 7.57 - 7.67 (m, 4H), 7.12 - 7.36 (m, 10H), 7.01 - 7.08 (m, 2H), 6.84 (s, 1H), 3.48 (d, *J* = 1.96 Hz, 1H), 3.13 (d, *J* = 6.65 Hz, 4H), 3.01 - 3.07 (m, 1H), 2.75 - 2.84 (m, 1H), 2.65 (s, 2H), 2.03 (s, 5H), 1.41 (d, *J* = 8.61 Hz, 1H). Exact Mass: 624.26, MS (ESI): found: [M + H]⁺, 625.5.

[0145] Boc-dWL-OMe: The title compound was synthesized using the same procedure as Boc-WF-OMe starting with Boc-dTrp-OH and HCl.H-Leu-OMe. Yield: 92%. Chemical formula: C₂₃H₃₃N₃O₅, Exact Mass: 431.24, MS(ESI): found: [M + Na]⁺, 454.3.

[0146] Boc-dWL-OH: The title compound was synthesized using the same procedure as Boc-WF-OH starting with Boc-dWF-OMe. Yield: 83%. Exact Mass: 417.23, MS(ESI): found: [M + H]⁺, 418.26.

[0147] Boc-dWLR(Mtr)kbt: The title compound was synthesized using the same procedure as Boc-WFR(Mtr)-kbt starting with Boc-dWL-OH. The crude product was directly

taken to the next step. . Chemical formula: $C_{45}H_{58}N_8O_8S_2$, Exact Mass: 902.38, MS(ESI): found: $[M + H]^+$, 903.5.

[0148] H-dWLR-kbt (1-15A1): The title compound was synthesized using the same procedure as H-WFR(Mtr)-kbt starting with Boc-dWLR(Mtr)kbt. Yield: 22 mg (41% over two steps). Chemical formula: $C_{30}H_{38}N_8O_3S$, 1H NMR (400 MHz, METHANOL- d_4) δ ppm 10.58 (br. s., 0H), 8.82 (s, 1H), 8.22 - 8.32 (m, 2H), 7.70 (dd, $J = 8.02, 16.24$ Hz, 0H), 7.57 - 7.64 (m, 1H), 7.37 (d, $J = 6.26$ Hz, 1H), 7.22 (d, $J = 13.30$ Hz, 0H), 7.11 - 7.18 (m, 2H), 7.02 - 7.10 (m, 1H), 5.55 - 5.67 (m, 1H), 4.47 - 4.58 (m, 0H), 4.35 (dd, $J = 5.09, 10.17$ Hz, 0H), 4.05 - 4.31 (m, 2H), 3.37 - 3.55 (m, 1H), 3.07 - 3.27 (m, 3H), 2.65 (s, 1H), 2.18 (dd, $J = 6.06, 13.11$ Hz, 1H), 1.57 - 1.95 (m, 3H), 1.16 - 1.52 (m, 2H), 1.06 (td, $J = 7.14, 13.89$ Hz, 1H), 0.91 - 1.00 (m, 2H), 0.65 - 0.81 (m, 6H) Exact Mass: 624.26, MS (ESI): found: $[M + H]^+$, 625.5.

[0149] H-dWFRkbt-COOH (1-45A1): The title compound was synthesized using the same procedure as WFR(Mtr)kbt starting with Boc-dWFR(Mtr)kbt-COOH. Yield: 24 mg (43% over two steps). Chemical formula: $C_{34}H_{36}N_8O_5S$, 1H NMR (400 MHz, METHANOL- d_4) δ ppm 10.58 (br. s., 0H), 8.82 (s, 1H), 8.22 - 8.32 (m, 2H), 7.70 (dd, $J = 8.02, 16.24$ Hz, 0H), 7.57 - 7.64 (m, 1H), 7.37 (d, $J = 6.26$ Hz, 1H), 7.22 (d, $J = 13.30$ Hz, 0H), 7.11 - 7.18 (m, 2H), 7.02 - 7.10 (m, 1H), 5.55 - 5.67 (m, 1H), 4.47 - 4.58 (m, 0H), 4.35 (dd, $J = 5.09, 10.17$ Hz, 0H), 4.05 - 4.31 (m, 2H), 3.37 - 3.55 (m, 1H), 3.07 - 3.27 (m, 3H), 2.65 (s, 1H), 2.18 (dd, $J = 6.06, 13.11$ Hz, 1H), 1.57 - 1.95 (m, 3H), 1.16 - 1.52 (m, 2H), 1.06 (td, $J = 7.14, 13.89$ Hz, 1H), 0.91 - 1.00 (m, 2H), 0.65 - 0.81 (m, 6H) Exact Mass: 668.253, MS (ESI): found: $[M + H]^+$, 669.5.

[0150] H-WLRkbt-COOH (1-56A1): The title compound was synthesized using the same procedure as WLR(Mtr)kbt starting with Boc-WLR(Mtr)kbt-COOH. Yield: 26 mg (44% over two steps). Chemical formula: $C_{31}H_{38}N_8O_5S$, 1H NMR (400 MHz, METHANOL- d_4) δ ppm 8.77 - 8.84 (m, 1H), 8.22 - 8.32 (m, 3H), 7.57 - 7.71 (m, 1H), 7.31 - 7.42 (m, 2H), 7.04 - 7.23 (m, 3H), 4.09 - 4.13 (m, 1H), 3.48 (s, 1H), 3.22 - 3.27 (m, 2H), 3.13 (s, 1H), 2.65 (s, 2H), 1.62 (d, $J = 7.04$ Hz, 1H), 1.17 (s, 1H), 0.90 - 1.00 (m, 6H), 0.65 - 0.80 (m, 7H) Exact Mass: 634.269, MS (ESI): found: $[M + H]^+$, 635.8.

[0151] H-dWLRkbt-COOH (1-56A1): The title compound was synthesized using the same procedure as dWLR(Mtr)kbt starting with Boc-dWLR(Mtr)kbt-COOH. Yield: 25 mg (42% over two steps). Chemical formula: $C_{31}H_{38}N_8O_5S$, 1H NMR (400 MHz,

METHANOL-d₄) δ ppm 10.58 (br. s., 0H), 8.82 (s, 1H), 8.22 - 8.32 (m, 2H), 7.70 (dd, J = 8.02, 16.24 Hz, 0H), 7.57 - 7.64 (m, 1H), 7.37 (d, J = 6.26 Hz, 1H), 7.22 (d, J = 13.30 Hz, 0H), 7.11 - 7.18 (m, 2H), 7.02 - 7.10 (m, 1H), 5.55 - 5.67 (m, 1H), 4.47 - 4.58 (m, 0H), 4.35 (dd, J = 5.09, 10.17 Hz, 0H), 4.05 - 4.31 (m, 2H), 3.37 - 3.55 (m, 1H), 3.07 - 3.27 (m, 3H), 2.65 (s, 1H), 2.18 (dd, J = 6.06, 13.11 Hz, 1H), 1.57 - 1.95 (m, 3H), 1.16 - 1.52 (m, 2H), 1.06 (td, J = 7.14, 13.89 Hz, 1H), 0.91 - 1.00 (m, 2H), 0.65 - 0.81 (m, 6H) Exact Mass: 634.269, MS (ESI): found: $[M + H]^+$, 635.8.

[0152] Fmoc-H(Bom)WL-OMe: The title compound was synthesized using same procedure described as Boc-WF-OMe starting from Fmoc-His(Bom)-OH and HCl.H-WL-OMe. The crude product was directly taken to the next step. Chemical formula: C₄₇H₅₀N₆O₇, Exact Mass: 810.5, MS(ESI): found: $[M + H]^+$, 811.78.

[0153] Fmoc-H(Bom)WL-OH : The title compound was synthesized using same procedure described as Boc-WF-OH starting from Fmoc-His(Bom)WL-OMe. Yield: 76%. Chemical formula: C₄₆H₄₈N₆O₇, Exact Mass: 796.35, MS(ESI): found: $[M + H]^+$, 797.78.

[0154] Fmoc-H(Bom)WLR(Mtr)-Kbt : The title compound was synthesized using same procedure described as Boc-WF-RKbt starting from Fmoc-His(Bom)WL-OH and HCl.H-Arg(Mtr)-Kbt. Yield: 65%. Exact Mass: 1281.514, MS(ESI): found: $[M + H]^+$, 1262.7.

[0155] H(Bom)WLR-Kbt (1-54A1): The title compound was synthesized using same procedure described as H-WF-RKbt starting from Fmoc-His(Bom)WLR(Mtr)Kbt, followed by a treatment with 4M HCl solution. Yield: 20% over three steps. Exact Chemical formula: C₄₄H₅₃N₁₁O₅S, ¹H NMR (400 MHz, METHANOL-d₄) δ ppm 7.89 (d, J = 7.83 Hz, 4H), 7.71 (d, J = 7.43 Hz, 4H), 7.46 - 7.52 (m, 4H), 7.39 - 7.45 (m, 4H), 4.49 (t, J = 5.28 Hz, 2H), 3.83 (d, J = 5.48 Hz, 5H), 3.42 (d, J = 12.13 Hz, 4H), 2.99 (t, J = 11.54 Hz, 4H), 1.88 (br. s., 6H), 1.75 (br. s., 8H), 1.44 - 1.60 (m, 2H) Mass: 847.3, MS(ESI): found: $[M + H]^+$, 848.7.

[0156] H(Bom)dWLR-Kbt (1-58A1): The title compound was synthesized using same procedure described as H-WF-RKbt starting from Fmoc-dHis(Bom)WLR(Mtr)Kbt, followed by a treatment with 4M HCl solution. Yield: 36% over three steps. Exact Chemical formula: C₄₄H₅₃N₁₁O₅S, ¹H NMR (400 MHz, METHANOL-d₄) δ ppm 7.89 (d, J = 7.83 Hz, 4H), 7.71 (d, J = 7.43 Hz, 4H), 7.46 - 7.52 (m, 4H), 7.39 - 7.45 (m, 4H), 4.49 (t, J = 5.28 Hz, 2H), 3.83 (d, J = 5.48 Hz, 5H), 3.42 (d, J = 12.13 Hz, 4H), 2.99 (t, J = 11.54 Hz, 4H), 1.88

(br. s., 6H), 1.75 (br. s., 8H), 1.44 - 1.60 (m, 2H) Mass: 847.3, MS(ESI): found: $[M + H]^+$, 848.7.

[0157] Boc-LL-OMe: The title compound was synthesized using the same procedure as Boc-dWL-OMe starting with Boc-Leu-OH and HCl.H-Leu-OMe.

[0158] Boc-LL-OH: The title compound was synthesized using the same procedure as Boc-dWL-OH starting with Boc-LL-OMe. Exact Mass: 417.5 MS(ESI): found: $[M + H]^+$ 418.6

[0159] H-LLR-kbt V amide (7182). Synthesized in a similar manner to H-WFRkbt from Boc-LLR(Mtr)-Kbt V amide. Yield 4.6 mg. Chemical Formula: $C_{31}H_{49}N_9O_5S$, Exact Mass: 659.36, MS(ESI): found: $[M + H]^+$, 660.6.

[0160] Boc-WL-OH: The title compound was synthesized using the same procedure as Boc-dWL-OH starting with Boc-WL-OMe. Exact Mass: 417.2, MS(ESI): found: $[M + H]^+$, 418.3.

[0161] H-WLR-kbt V amide (7181). Synthesized in a similar manner to H-dWLR-kbt from Boc-WLR(Mtr)-kbt V amide. Yield 4.5 mg. Chemical Formula: $C_{36}H_{48}N_{10}O_5S$, Exact Mass: 732.35, MS(ESI): found: $[M + H]^+$, 733.6.

[0162] Boc-R(Mtr)L-OH: The title compound was synthesized using the same procedure as Boc-dWL-OH starting with Boc-R(Mtr)L-OMe. Exact Mass: 599.7 found: $[M + H]^+$, 600.8.

[0163] H-RLR-kbt V amide (7180). Yield 5 mg. 1H NMR (400 MHz, METHANOL- d_4) δ ppm 8.90 (d, $J = 7.04$ Hz, 0H), 8.65 (s, 1H), 8.35 (d, $J = 8.61$ Hz, 1H), 8.28 (d, $J = 8.61$ Hz, 1H), 8.10 (d, $J = 9.00$ Hz, 1H), 5.53 - 5.61 (m, 1H), 4.42 - 4.55 (m, 2H), 3.92 (t, $J = 5.87$ Hz, 1H), 3.18 (t, $J = 6.85$ Hz, 2H), 2.20 (qd, $J = 6.81, 13.60$ Hz, 2H), 1.86 - 1.98 (m, 3H), 1.70 - 1.82 (m, 3H), 1.56 - 1.68 (m, 3H), 1.07 (dd, $J = 4.30, 6.65$ Hz, 6H), 0.96 (d, $J = 6.26$ Hz, 16H). Chemical Formula: $C_{31}H_{50}N_{12}O_5S$, Exact Mass: 702.37, MS(ESI): found: $[M + H]^+$, 703.6.

[0164] Fmoc-A-R-kbt (7185-1). Under nitrogen atmosphere, Fmoc-A-OH (0.02 mmol) was dissolved in DMF (10 mL) and then HATU (0.02 mmol) was added. After stirring for 10 minutes, H-R(Mtr)-kbt-HCl (0.0185 mmol) and *N,N*-diisopropylethylamine (0.1 mmol) were added. The mixture was stirred at room temperature for 4 hours. The solvent was removed and then 1.5 mL of cleavage cocktail (38:1:1 TFA/water/thioanisole) was added and then the reaction was stirred for 4 hours. After concentration in vacuo, the residue was

purified by C18 reverse phase HPLC to give the title compound as a lyophilized white powder.

[0165] Fmoc-RR-kbt (7185-2). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{34}H_{39}N_9O_4S$, Exact Mass 669.28, MS(ESI): found $[M+H]^+$ 670.4.

[0166] Fmoc-NR-kbt (7185-3). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{32}H_{33}N_7O_5S$, Exact Mass 627.23, MS(ESI): found $[M+H]^+$ 628.3.

[0167] Fmoc-DR-kbt (7185-4). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{32}H_{32}N_6O_6S$, Exact Mass, 628.21, MS(ESI): found $[M+H]^+$ 629.3.

[0168] Fmoc-QR-kbt (7185-6). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{33}H_{35}N_7O_5S$, Exact Mass 641.24, MS(ESI): found $[M+H]^+$ 642.4.

[0169] Fmoc-ER-kbt (7185-7). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{33}H_{34}N_6O_6S$, Exact Mass 642.23, MS(ESI): found $[M+H]^+$ 643.3.

[0170] Fmoc-GR-kbt (7185-8). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{30}H_{30}N_6O_4S$, Exact Mass 570.2, MS(ESI): found $[M+H]^+$ 571.3.

[0171] Fmoc-HR-kbt (7185-9). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{34}H_{34}N_8O_4S$, Exact Mass 650.24, MS(ESI): found $[M+H]^+$ 651.4.

[0172] Fmoc-IR-kbt (7185-10). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{34}H_{38}N_6O_4S$, Exact Mass 626.27, MS(ESI): found $[M+H]^+$ 627.4.

[0173] Fmoc-LR-kbt (7185-11). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{34}H_{38}N_6O_4S$, Exact Mass 626.27, MS(ESI): found $[M+H]^+$ 627.4.

[0174] Fmoc-KR-kbt (7185-12). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{34}H_{39}N_7O_4S$, Exact Mass 641.28, MS(ESI): found $[M+H]^+$ 642.5.

[0175] Fmoc-MR-kbt (7187-13). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{32}H_{34}N_6O_4S_2$, Exact Mass 630.21, MS(ESI): found $[M+H]^+$ 631.3.

[0176] Fmoc-FR-kbt (7187-14). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{37}H_{36}N_6O_4S$, Exact Mass 660.25, MS(ESI): found $[M+H]^+$ 661.4.

[0177] Fmoc-PR-kbt (7187-15). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{33}H_{34}N_6O_4S$, Exact Mass 610.24, MS(ESI): found $[M+H]^+$ 611.5.

[0178] Fmoc-SR-kbt (7187-16). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{31}H_{32}N_6O_5S$, Exact Mass 600.22, MS(ESI): found $[M+H]^+$ 601.3.

[0179] Fmoc-TR-kbt (7188-17). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{32}H_{34}N_6O_5S$, Exact Mass 614.23, MS(ESI): found $[M+H]^+$ 615.4.

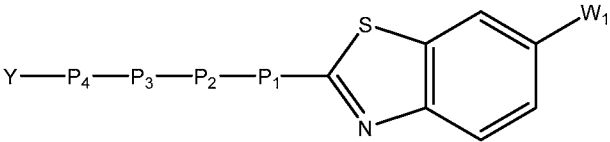
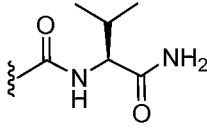
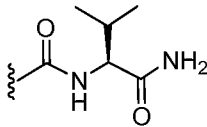
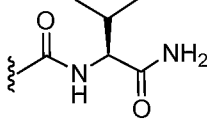
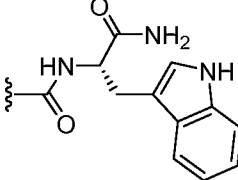
[0180] Fmoc-WR-kbt (7188-18). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{39}H_{37}N_7O_4S$, Exact Mass 699.26, MS(ESI): found $[M+H]^+$ 700.4.

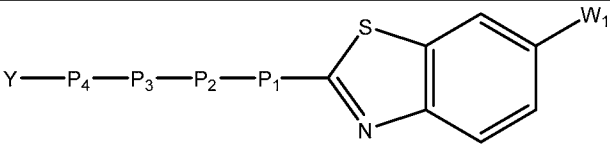
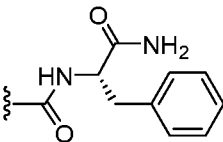
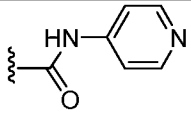
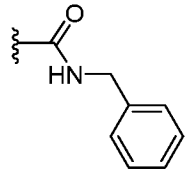
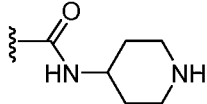
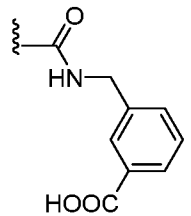
[0181] Fmoc-YR-kbt (7188-19). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{37}H_{36}N_6O_5S$, Exact Mass 676.25, MS(ESI): found $[M+H]^+$ 677.4.

[0182] Fmoc-VR-kbt (7188-20). The title compound was synthesized using the same procedure as (7185-1). Chemical formula $C_{33}H_{36}N_6O_4S$, Exact Mass 612.25, MS(ESI): found $[M+H]^+$ 613.5.

[0183] The tetrapeptide ketothiazoles listed in Table 5.2 were synthesized in accordance with the procedures and schemes described below.

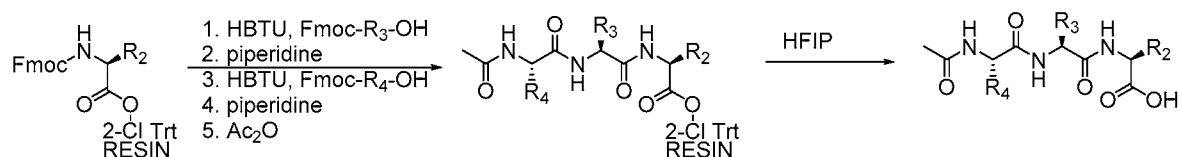
Table 5.2 Tetrapeptide ketobenzothiazoles.

		
Compound No.	Y-P ₄ -P ₃ -P ₂ -P ₁ -Z	W ₁
7115	Ac-KRLR(SEQ ID NO: 8)-Kbt-Val-NH ₂	
7054	Ac-KRLR(SEQ ID NO: 8)-Kbt	H
7117	H-WRLR(SEQ ID NO: 9)-Kbt	H
1-45A1	H-dWFR-kbt-COOH	COOH
7055	Ac-KQLR(SEQ ID NO: 1)-Kbt-Val-NH ₂	
7116	Ac-SKLR(SEQ ID NO: 10)-Kbt-Val-NH ₂	
7124	Ac-SKLR(SEQ ID NO: 10)-Kbt-Trp-NH ₂	

		
Compound No.	Y-P ₄ -P ₃ -P ₂ -P ₁ -Z	W ₁
7125	Ac-SKLR(SEQ ID NO: 10)-Kbt-Phe-NH _s	
7006	Ac-KQLR(SEQ ID NO: 1)-Kbt	H
7126	Ac-SKLR(SEQ ID NO: 10)-Kbt-4-pyridinylamide	
7053	Ac-SKLR(SEQ ID NO: 10)-Kbt	H
7063	Ac-FLFR(SEQ ID NO: 19)-Kbt	H
7118	Ac-SKLR(SEQ ID NO: 10)-Kbt-COOH	
7064	Ac-WLFR(SEQ ID NO: 20)-Kbt	H
7139	Ac-SKLR(SEQ ID NO: 10)-Kbt-benzylamide	
7140	Ac-SKLR(SEQ ID NO: 10)-Kbt-4-piperidinylamide	
7165	Ac-KRLR(SEQ ID NO: 8)-Kbt-5-COOH	

[0184] Procedure for the Preparation of Tripeptide Ketobenzothiazole:

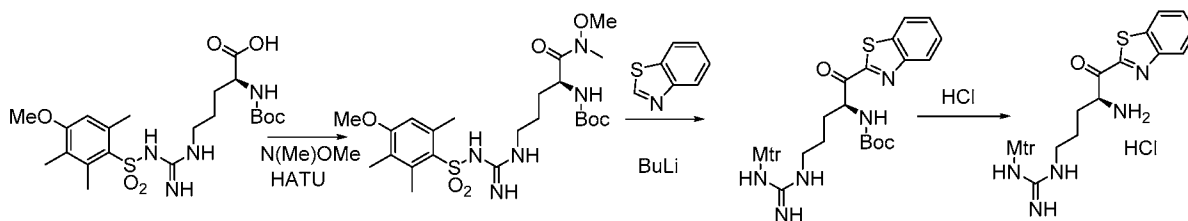
Tripeptides were synthesized in 0.5 mmol scale through consecutive coupling of amino acid-preloaded 2-Cl-trityl resin with Fmoc protected amino acids followed by the deprotection of Fmoc group. In the coupling reaction, 5 equiv. Fmoc-amino acid/4.5 equiv. HBTU/ 10 equiv. i Pr₂NEt was used and the mixture was shaken at room temperature overnight. In the deprotection of Fmoc group, piperidine/DMF (20% v/v) was employed and the mixture was shaken for 4 hours at room temperature.



[0185] Peptide coupling and deprotection of Fmoc: Into the reaction vial (with a fritted glass resin support) containing H-Leu-2-Cl-trityl resin (0.714 g, 0.5 mmol), DMF/CH₂Cl₂ (15/15 mL) was added. The mixture was shaken at room temperature for 30 minutes, then filtered. The resulting resin was washed with DMF (10 mL x 2). Into another vial containing Fmoc-AA-OH (2.5 mmol) in DMF (20 mL), HBTU (0.853 g, 2.25 mmol) and i Pr₂NEt was added (0.87 mL, 5 mmol). The mixture was stirred at room temperature for 10 minutes, then added into the reaction vial containing the resin. The mixture was shaken at room temperature overnight, then filtered. The resin was washed with DMF (20 mL x 4). To the resulting resin piperidine/DMF (20% v/v, 30 mL) was added. The mixture was shaken for 4 hours at room temperature, then filtered. The resin was washed with DMF (10 mL x 4).

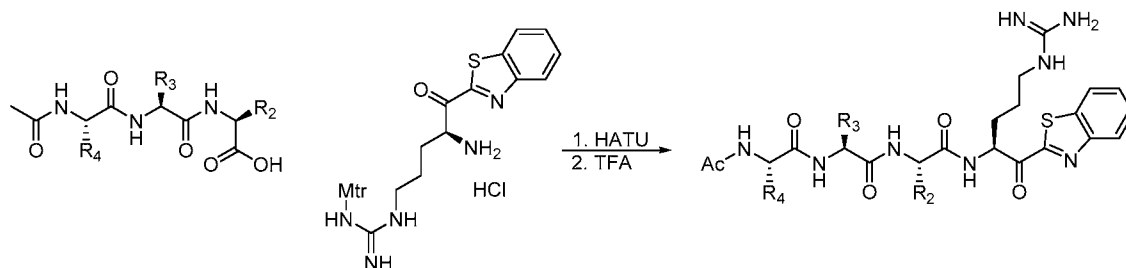
[0186] Acetyl capping of the tripeptides: The tripeptide resin was suspended in 30 mL of 0.5 M Ac₂O/DMF and 1 M i Pr₂NEt/DMF. The mixture was shaken at room temperature for 1 hour. The resin was filtered and washed with DMF (10 mL x 4) followed by CH₂Cl₂ (10 mL x 4).

[0187] Cleavage of tripeptide resin: Ac-capped tripeptide resin was suspended and shaken in 30 mL of 25% v/v HFIP/ CH₂Cl₂ for 1 hour. The mixture was filtered. The filtrate was concentrated then dried in vacuo, giving rise to crude product of Ac-capped tripeptide.



[0188] BocHN-Arg(Mtr) ketobenzothiazole. At -78°C , $n\text{-BuLi/Hex}(2.5\text{M})$ (14 mL, 35 mmol) was added dropwise into the solution of benzothiazole (4.87 g, 36 mmol) in THF (50 mL) over 15 minutes. After the mixture was stirred for additional half an hour, the solution of Boc-HN-Arg(Mtr) Weinreb amide (1.06 g, 2 mmol) in THF (15 mL) was added slowly over 50 min. For synthesis of Weinreb amide, see Han et al., "Inhibitors of HGFA, Matriptase, and Hepsin Serine Proteases: A Nonkinase Strategy to Block Cell Signaling in Cancer, *Acs Med Chem Lett* (2014) 5, 1219-1224. The mixture was stirred at -78°C for additional 2 hours. The reaction was quenched with aqueous NH_4Cl and the aqueous layer was extracted with AcOEt. The organic phase was collected, dried with Na_2SO_4 , then concentrated. The resulting residue was purified by silica gel chromatography with $\text{CHCl}_3/\text{MeOH}$ combination as eluent to give the title compound (1.14 g) in 94% yield. ^1H NMR (400 MHz, $\text{METHANOL-}d_4$) δ ppm 1.43 (s, 9 H) 1.53 - 1.78 (m, 4 H) 2.05 (s, 3 H) 2.55 (s, 3 H) 2.61 (s, 3 H) 3.17 - 3.29 (m, 2 H) 3.82 (s, 3 H) 5.19 - 5.43 (m, 1 H) 6.57 (s, 1 H) 7.53 - 7.71 (m, 2 H) 8.06 - 8.28 (m, 2 H). MS(ESI): found: $[\text{M} + \text{H}]^+$, 604.4.

[0189] $\text{HCl}\cdot\text{H}_2\text{N-Arg(Mtr)}$ ketobenzothiazole. The mixture of BocHN-Arg(Mtr) ketobenzothiazole (1.0 g, 1.66 mmol) in 30 mL of HCl/dioxane (1.5 M) was stirred at room temperature. The reaction was monitored by LCMS until completion. The solvent was removed then the resulting residue was dried in vacuo to the title product in quantitative yield. ^1H NMR (400 MHz, $\text{METHANOL-}d_4$) δ ppm 1.60 - 1.86 (m, 2 H) 2.00 - 2.20 (m, 4 H) 2.24 - 2.36 (m, 1 H) 2.53 (s, 3 H) 2.61 (s, 3 H) 3.84 (s, 3 H) 5.14 - 5.40 (m, 1 H) 6.65 (s, 1 H) 7.59 - 7.78 (m, 2 H) 8.10 - 8.34 (m, 2 H). MS(ESI): found: $[\text{M} + \text{H}]^+$, 504.4.



[0190] Ac-KQLR(SEQ ID NO: 1) ketobenzothiazole (7006). Under nitrogen atmosphere, at 0 °C anhydrous DMF (5 mL) was added into the round bottom flask containing AcHN-K(Boc)-Q(Trt)-L-OH tripeptide (0.064 g, 0.083 mmol) and HATU (0.035 g, 0.091 mmol). After stirring for 10 minutes, Mtr-protected arginine ketobenzothiazole (0.068 g, 0.013 mmol), then *N,N*-diisopropylethylamine (0.053 g, 0.41 mmol) were added. The mixture was stirred at room temperature for 4 hours while being warmed to room temperature naturally. DMF was removed and to the resulting residue water (20 mL) was added. The precipitate formed was filtered and dried. To this precipitate 5 mL of TFA/thioanisole/water (95/2.5/2.5(v/v/v)) was added. The mixture was stirred at room temperature for 4 hours. Then cold ether (40 mL) was added. The resulting precipitate, which is the crude product, was collected by centrifugation, then by decanting out carefully ether solvent. The crude product was purified by HPLC (C18, 15*150 mm column; eluent: acetonitrile/water (0.05% TFA)) to give Ac-KQLR(SEQ ID NO: 1) ketobenzothiazole (0.030 g) in 43% yield. ¹H NMR (400 MHz, METHANOL-*d*₄) δ ppm 0.88 (d, *J*=6.26 Hz, 3 H) 0.92 (d, *J*=6.26 Hz, 3 H) 1.36 - 1.90 (m, 12 H) 1.90 - 2.26 (m, 3 H) 2.02 (s, 3 H) 2.26 - 2.44 (m, 2 H) 2.87 - 2.97 (m, 2 H) 3.20 - 3.40 (m, 2 H) 4.21 - 4.29 (m, 1 H) 4.31 - 4.38 (m, 1 H) 4.39 - 4.48 (m, 1 H) 5.63 - 5.78 (m, 1 H) 7.54 - 7.74 (m, 2 H) 8.08 - 8.16 (m, 1 H) 8.19 - 8.29 (m, 1 H). MS(ESI): found: [M + H]⁺, 703.6.

[0191] Ac-SKLR(SEQ ID NO: 10) ketobenzothiazole (7053). (yield: 23%). Synthesized using the same procedure as for Ac-KQLR(SEQ ID NO: 1) ketobenzothiazole. ¹H NMR (400 MHz, METHANOL-*d*₄) δ ppm 0.87 (d, *J*=6.65 Hz, 3 H) 0.91 (d, *J*=6.65 Hz, 3 H) 1.36 - 2.08 (m, 12 H) 2.01 (s, 3 H) 2.10 - 2.29 (m, 1 H) 2.82 - 3.04 (m, 2 H) 3.22 - 3.38 (m, 2 H) 3.65 - 3.93 (m, 2 H) 4.24 - 4.53 (m, 3 H) 5.58 - 5.74 (m, 1 H) 7.56 - 7.71 (m, 2 H) 8.08 - 8.16 (m, 1 H) 8.18 - 8.25 (m, 1 H). MS(ESI): found: [M + H]⁺, 662.5.

[0192] Ac-KRLR(SEQ ID NO: 8) ketobenzothiazole (7054). (yield: 38%). Synthesized using the same procedure as for Ac-KQLR(SEQ ID NO: 1) ketobenzothiazole. ¹H NMR (400 MHz, METHANOL-*d*₄) δ ppm 0.90 (d, *J*=6.80 Hz, 3 H) 0.94 (d, *J*=6.80 Hz, 3 H) 1.36 - 1.96 (m, 16 H) 1.99 (s, 3 H) 2.09 - 2.34 (m, 1 H) 2.87 - 2.98 (m, 2 H) 3.14 - 3.23 (m, 2 H) 3.25 - 3.33 (m, 2 H) 4.20 - 4.30 (m, 1 H) 4.33 - 4.52 (m, 2 H) 5.54 - 5.76 (m, 1 H) 7.59 - 7.70 (m, 2 H) 8.10 - 8.16 (m, 1 H) 8.18 - 8.23 (m, 1 H). MS(ESI): found: [M + H]⁺, 731.7.

[0193] Ac-FLFR(SEQ ID NO: 19) ketobenzothiazole (7063). (yield: 47%).

Synthesized using the same procedure as for Ac-KQLR(SEQ ID NO: 1) ketobenzothiazole.

^1H NMR (400 MHz, METHANOL- d_4) δ ppm 0.85 (d, $J=6.26$ Hz, 3 H) 0.90 (d, $J=6.26$ Hz, 3 H) 1.36 - 1.63 (m, 3 H) 1.66 - 1.99 (m, 3 H) 1.91 (s, 3 H) 2.05 - 2.25 (m, 1 H) 2.80 - 2.91 (m, 1 H) 2.92 - 3.02 (m, 1 H) 3.02 - 3.18 (m, 2 H) 3.20 - 3.33 (m, 2 H) 4.19 - 4.39 (m, 1 H) 4.47 - 4.70 (m, 2 H) 5.64 - 5.75 (m, 1 H) 7.01 - 7.33 (m, 10 H) 7.57 - 7.71 (m, 2 H) 8.10 - 8.17 (m, 1 H) 8.19 - 8.25 (m, 1 H). MS(ESI): found: $[\text{M} + \text{H}]^+$, 741.6.

[0194] Ac-WLFR(SEQ ID NO: 20) ketobenzothiazole (7064). (yield: 19%).

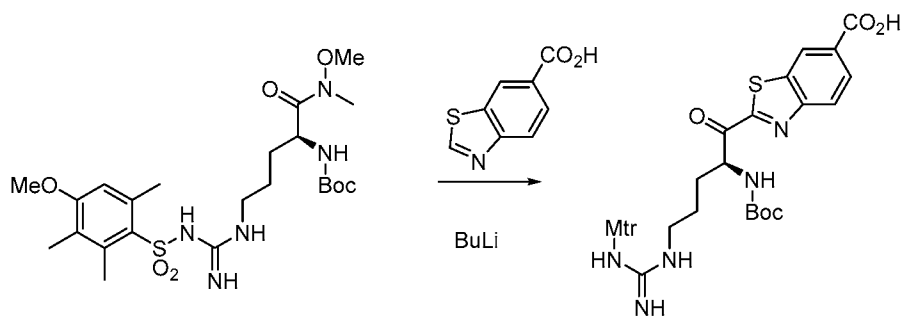
Synthesized using the same procedure as for Ac-KQLR(SEQ ID NO: 1) ketobenzothiazole.

^1H NMR (400 MHz, METHANOL- d_4) δ ppm 0.79 (d, $J=6.26$ Hz, 3 H) 0.84 (d, $J=6.26$ Hz, 3 H) 1.32 - 1.50 (m, 3 H) 1.62 - 1.91 (m, 3 H) 1.95 (s, 3 H) 2.06 - 2.21 (m, 1 H) 2.88 - 3.00 (m, 1 H) 3.05 - 3.29 (m, 5 H) 4.14 - 4.33 (m, 1 H) 4.50 - 4.72 (m, 2 H) 5.61 - 5.77 (m, 1 H) 6.96 - 7.03 (m, 1 H) 7.04 - 7.20 (m, 7 H) 7.29 - 7.37 (m, 1 H) 7.54 - 7.71 (m, 3 H) 7.85 - 7.97 (m, 1 H) 8.10 - 8.16 (m, 1 H) 8.18 - 8.26 (m, 1 H). MS(ESI): found: $[\text{M} + \text{H}]^+$, 780.6.

[0195] WRLR(SEQ ID NO: 9) ketobenzothiazole (7117). (yield: 39%).

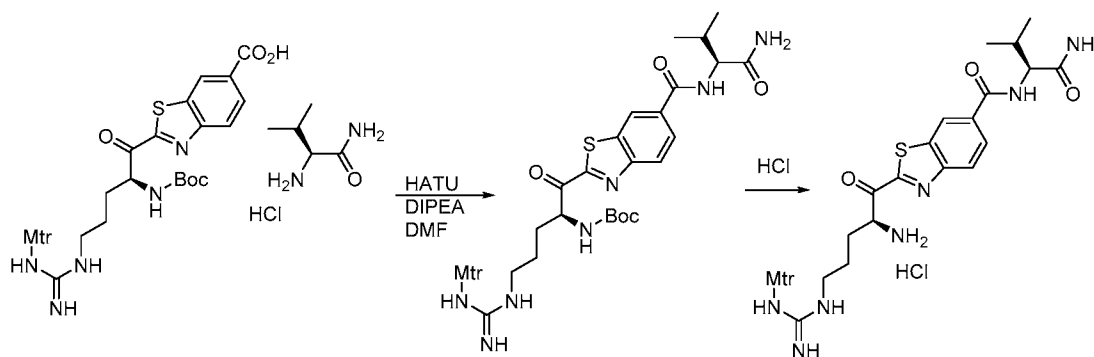
Synthesized using the same procedure as for Ac-KQLR(SEQ ID NO: 1) ketobenzothiazole, starting with the coupling of BocHN-W-R(Mtr)-L-OH tripeptide and Mtr-protected arginine

ketobenzothiazole. ^1H NMR (400 MHz, METHANOL- d_4) δ ppm 0.94 (d, $J=6.65$ Hz, 3 H) 0.95 (d, $J=6.65$ Hz, 3 H) 1.48 - 1.99 (m, 10 H) 2.12 - 2.30 (m, 1 H) 3.14 - 3.23 (m, 3 H) 3.24 - 3.29 (m, 2 H) 3.39 - 3.52 (m, 1 H) 4.18 - 4.29 (m, 1 H) 4.37 - 4.53 (m, 2 H) 5.61 - 5.68 (m, 1 H) 7.01 - 7.09 (m, 1 H) 7.10 - 7.17 (m, 1 H) 7.22 (s, 1 H) 7.34 - 7.41 (m, 1 H) 7.58 - 7.71 (m, 3 H) 8.10 - 8.16 (m, 1 H) 8.17 - 8.24 (m, 1 H) 8.25 - 8.33 (m, 1 H). MS(ESI): found: $[\text{M} + \text{H}]^+$, 747.7.

**[0196] BocHN-Arg(Mtr) ketobenzothiazole-6-COOH.** At -78 °C,

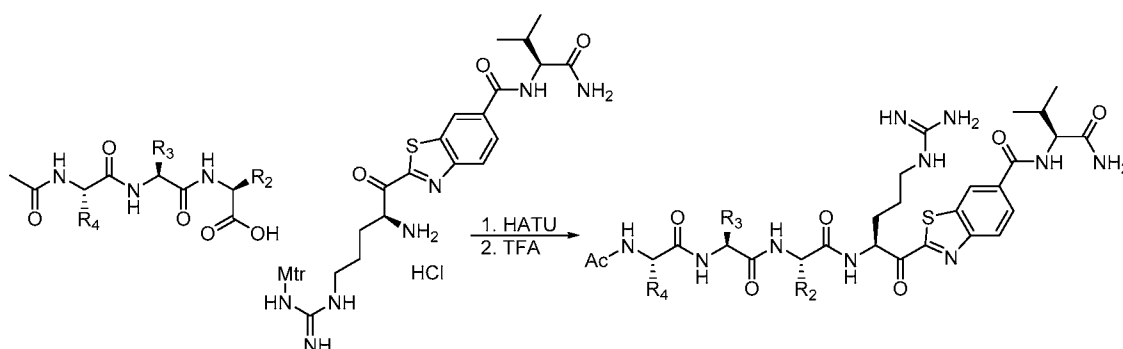
$n\text{BuLi/Hex}(2.5\text{M})$ (28.5 mL, 71.4 mmol) was added dropwise into the solution of 6-

carboxybenzothiazole (6.57 g, 37 mmol) in THF (450 mL) over 25 minutes. After the mixture was stirred for additional half an hour, the solution of Boc-HN-Arg(Mtr) Weinreb amide (1.63 g, 3.08 mmol) in THF (60 mL) was added slowly over 20 minutes at -78 °C. After the addition then the mixture was stirred at -24 °C to -20 °C for 1.5 hours. The reaction was quenched with saturated aqueous NH₄Cl (270 mL). The layers were separated and the aqueous layer was extracted with AcOEt. The organic phase was collected and washed with water, 5% citric acid, then dried with Na₂SO₄ and concentrated. The resulting residue was purified by silica gel chromatography with CH₂Cl₂/MeOH combination as eluent to give the title compound (0.91 g) in 46% yield. ¹H NMR (400 MHz, METHANOL-*d*₄) δ ppm 1.43 (s, 9 H) 1.66 (m, 4 H) 2.03 (s, 3 H) 2.54 (s, 3 H) 2.60 (s, 3 H) 3.22 - 3.29 (m, 2 H) 3.80 (s, 3 H) 5.18 - 5.41 (m, 1 H) 6.55 (s, 1 H) 8.20 (m, 2 H) 8.79 (s, 1 H). MS(ESI): found: [M + H]⁺, 648.4.



[0197] BocHN-Arg(Mtr) ketobenzothiazole-6-CONH-Val-amide. Under nitrogen atmosphere, at 0 °C anhydrous DMF (5 mL) was added into the round bottom flask containing BocHN-Arg(Mtr) ketobenzothiazole-COOH (0.060 g, 0.093 mmol) and HATU (0.042 g, 0.11 mmol). After stirring for 10 minutes, L-valine amide hydrochloride (0.017 g, 0.11 mmol), then *N,N*-diisopropylethylamine (0.062 g, 0.55 mmol) were added. The mixture was stirred overnight while being warmed to room temperature naturally. DMF was removed and to the resulting residue 20 mL water was added. The precipitate formed was filtered and dried. The product was purified by silica gel chromatography with CHCl₃/MeOH combination as eluent to give the title compound (0.049 g) in 70% yield ¹H NMR (400 MHz, METHANOL-*d*₄) δ ppm 1.06 (d, *J*=1.57 Hz, 3 H) 1.08 (d, *J*=1.96 Hz, 3 H) 1.42 (s, 9 H) 1.56 - 1.79 (m, 4 H) 2.04 (s, 3 H) 2.15 - 2.30 (m, 1 H) 2.55 (s, 3 H) 2.60 (s, 3 H) 3.20 - 3.28 (m, 2 H) 3.80 (s, 3 H) 4.45 (d, *J*=7.43 Hz, 1 H) 5.19 - 5.41 (m, 1 H) 6.57 (s, 1 H) 8.00 - 8.12 (m, 1 H) 8.13 - 8.29 (m, 1 H) 8.62 (s, 1 H). MS(ESI): found: [M + H]⁺, 746.5.

[0198] HCl.H₂N-Arg(Mtr) ketobenzothiazole-6-CONH-Val-amide. The mixture of BocHN-Arg(Mtr) ketobenzothiazole-(C=O)-V-amide (0.049 g, 0.066 mmol) in 10 mL of HCl/dioxane (2.5 M) was stirred at room temperature. The reaction was monitored by LCMS until completion. The solvent was removed then the resulting residue was dried in vacuo to the title product in quantitative yield. ¹H NMR (400 MHz, METHANOL-*d*₄) δ ppm 1.06 (d, *J*=3.13 Hz, 3 H) 1.08 (d, *J*=3.13 Hz, 3 H) 1.58 - 1.91 (m, 2 H) 1.95 - 2.41 (m, 6 H) 2.52 (s, 3 H) 2.61 (s, 3 H) 3.85 (s, 3 H) 4.46 (d, *J*=7.40 Hz, 1 H) 5.16 - 5.38 (m, 1 H) 6.66 (bs, 1 H) 7.99 - 8.18 (m, 1 H) 8.22 - 8.39 (m, 1 H) 8.55 - 8.78 (m, 1 H). MS(ESI): found: [M + H]⁺, 646.5.



[0199] Ac-KQLR(SEQ ID NO: 1) ketobenzothiazole-6-CONH-Val-amide (7055).

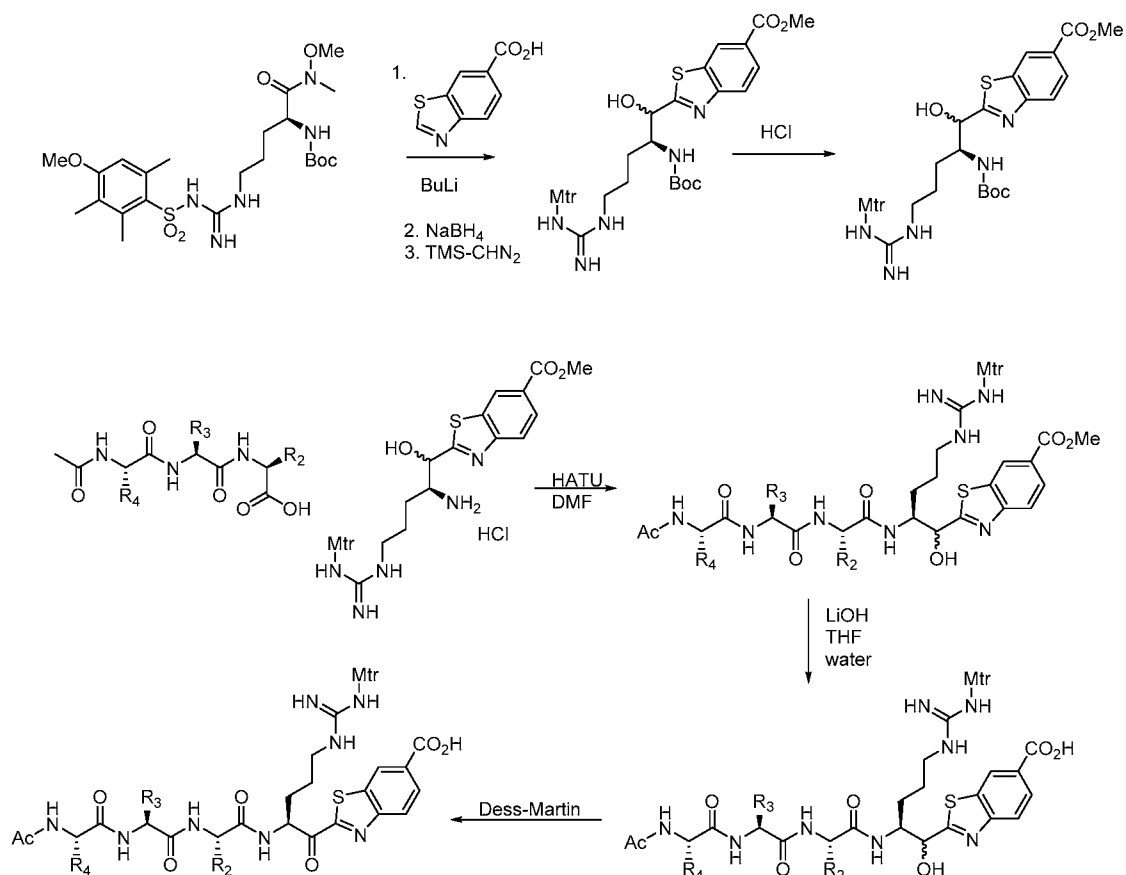
The title compound was synthesized using the same procedure as for the tetrapeptide ketobenzothiazoles, starting with the coupling of protected Ac-K(Boc)-Q(Trt)-L-OH tripeptide and HCl.H₂N-Arg(Mtr) ketobenzothiazole-(C=O)-V-amide. Yield: 11%. ¹H NMR (400 MHz, METHANOL-*d*₄) δ ppm 0.87 (d, *J*=6.00 Hz, 3 H) 0.92 (d, *J*=6.00 Hz, 3 H) 1.06 (d, *J*=2.74 Hz, 3 H) 1.08 (d, *J*=2.74 Hz, 3 H) 1.37 - 2.14 (m, 14 H) 2.02 (s, 3 H) 2.14 - 2.43 (m, 4 H) 2.87 - 3.00 (m, 2 H) 3.22 - 3.38 (m, 2 H) 4.20 - 4.29 (m, 1 H) 4.29 - 4.38 (m, 1 H) 4.39 - 4.50 (m, 2 H) 5.58 - 5.75 (m, 1 H) 8.05 - 8.14 (m, 1 H) 8.24 - 8.33 (m, 1 H) 8.62 - 8.67 (m, 1 H). MS(ESI): found: [M + H]⁺, 845.7.

[0200] Ac-SKLR(SEQ ID NO: 10) ketobenzothiazole-6-CONH-Val-amide (7116).

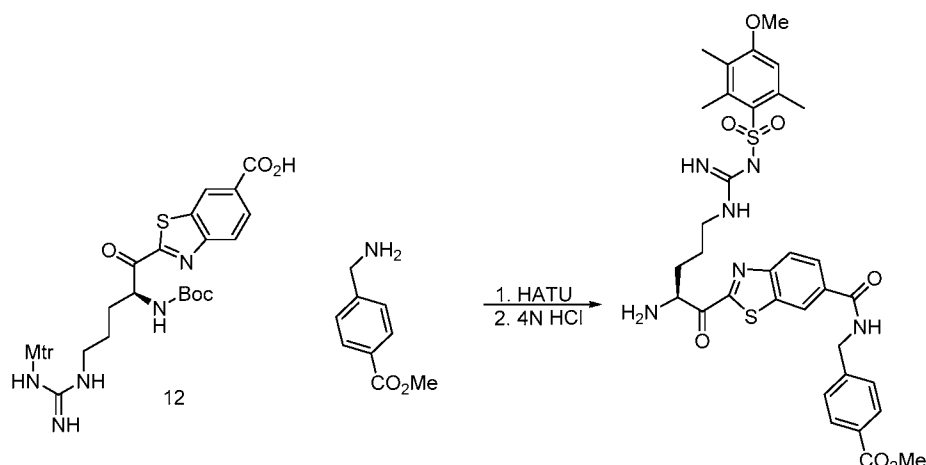
The title compound was synthesized using the same procedure as for Ac-KQLR(SEQ ID NO: 1) ketobenzothiazole-(C=O)-V-amide. Yield: 24%. ¹H NMR (400 MHz, METHANOL-*d*₄) δ ppm 0.87 (d, *J*=6.46 Hz, 3 H) 0.91 (d, *J*=6.46 Hz, 3 H) 1.06 (d, *J*=2.54 Hz, 3 H) 1.07 (d, *J*=2.54 Hz, 3 H) 1.37 - 1.97 (m, 12 H) 2.01 (s, 3 H) 2.13 - 2.28 (m, 2 H) 2.79 - 3.03 (m, 2 H) 3.22 - 3.38 (m, 2 H) 3.67 - 3.98 (m, 2 H) 4.23 - 4.54 (m, 4 H) 5.49 - 5.77 (m, 1 H) 8.07 - 8.12 (m, 1 H) 8.25 - 8.32 (m, 1 H) 8.61 - 8.66 (m, 1 H). MS(ESI): found: [M + H]⁺, 804.7.

[0201] Ac-KRLR(SEQ ID NO: 8) ketobenzothiazole-6-CONH-Val-amide (7115).

The title compound was synthesized using the same procedure as for Ac-KQLR(SEQ ID NO: 1) ketobenzothiazole-(C=O)-V-amide. Yield: 28%. ^1H NMR (400 MHz, METHANOL- d_4) δ ppm 0.92 (dd, $J=11.15$, 6.46 Hz, 6 H) 1.07 (dd, $J=6.65$, 2.74 Hz, 6 H) 1.35 - 1.95 (m, 16 H) 1.99 (s, 3 H) 2.14 - 2.27 (m, 2 H) 2.87 - 2.98 (m, 2 H) 3.14 - 3.21 (m, 2 H) 3.25 - 3.33 (m, 2 H) 4.21 - 4.28 (m, 1 H) 4.33 - 4.39 (m, 1 H) 4.40 - 4.49 (m, 2 H) 5.46 - 5.74 (m, 1 H) 8.07 - 8.12 (m, 1 H) 8.24 - 8.33 (m, 1 H) 8.61 - 8.68 (m, 1 H). MS(ESI): found: $[\text{M} + \text{H}]^+$, 873.7.



[0202] H-R (Mtr) ketobenzothiazole-(C=O)-NHBn(4-COOMe)-HCl. The title compound was synthesized from using the same procedure as HCl.H-Arg(Mtr) ketobenzothiazole-6-CONH-Val-amide. Yield, 85 mg, MS(ESI): found: $[\text{M} + \text{H}]^+$, 695.4.



[0203] Ac-KRLR(SEQ ID NO: 8) ketobenzothiazole-(C=O)-NHBn(4-COOMe)

(23a). The title compound was synthesized from H-R ketobenzothiazole-(C=O)-NHBn(4-COOMe)-HCl using the same procedure as Ac-KQLR(SEQ ID NO: 1) ketobenzothiazole-6-CONH-Val-amide and was purified by HPLC. Yield: 23% over two steps. ^1H NMR (400 MHz, METHANOL- d_4) δ ppm 0.90 (d, $J=6.26$ Hz, 3 H) 0.93 (d, $J=6.65$ Hz, 3 H) 1.37 – 1.95 (m, 16 H) 1.99 (s, 3 H) 2.13 – 2.28 (m, 1 H) 2.87 – 2.97 (m, 2 H) 3.13 – 3.23 (m, 2 H) 3.25 – 3.32 (m, 2 H) 3.89 (s, 3 H) 4.21 – 4.28 (m, 1 H) 4.32 – 4.41 (m, 1 H) 4.41 – 4.50 (m, 1 H) 4.69 (s, 2 H) 5.56 – 5.66 (m, 1 H) 7.50 (d, $J=7.83$ Hz, 2 H) 8.00 (d, $J=8.22$ Hz, 2 H) 8.09 – 8.13 (m, 1 H) 8.27 – 8.32 (m, 1 H) 8.62 – 8.67 (m, 1 H). MS(ESI): found: $[\text{M} + \text{H}]^+$, 922.8.

[0204] H-R (Mtr) ketobenzothiazole-(C=O)-NHBn(3-COOMe)-HCl. The title compound was synthesized from using the same procedure as HCl.H-Arg(Mtr) ketobenzothiazole-6-CONH-Val-amide. Yield, 80 mg, MS(ESI): found: $[\text{M} + \text{H}]^+$, 695.4.

[0205] Ac-KRLR(SEQ ID NO: 8) ketobenzothiazole-(C=O)-NHBn(3-COOMe)

(23b). The title compound was synthesized from H-R ketobenzothiazole-(C=O)-NHBn(3-COOMe)-HCl using the same procedure as Ac-KQLR(SEQ ID NO: 1) ketobenzothiazole-6-CONH-Val-amide and was purified by HPLC. Yield: 19% over two steps. ^1H NMR (400 MHz, METHANOL- d_4) δ ppm 0.90 (d, $J=6.65$ Hz, 3 H) 0.93 (d, $J=6.26$ Hz, 3 H) 1.40 – 1.95 (m, 16 H) 1.99 (s, 3 H) 2.13 – 2.27 (m, 1 H) 2.88 – 2.97 (m, 2 H) 3.13 – 3.23 (m, 2 H) 3.25 – 3.31 (m, 2 H) 3.89 (s, 3 H) 4.21 – 4.28 (m, 1 H) 4.33 – 4.41 (m, 1 H) 4.41 – 4.50 (m, 1 H) 4.68 (s, 2 H) 5.57 – 5.66 (m, 1 H) 7.43 – 7.52 (m, 1 H) 7.61 – 7.69 (m, 1 H) 7.90 – 7.97 (m, 1 H) 8.03 – 8.08 (m, 1 H) 8.09 – 8.13 (m, 1 H) 8.26 – 8.31 (m, 1 H) 8.58 – 8.68 (m, 1 H). MS(ESI): found: $[\text{M} + \text{H}]^+$, 922.8.

[0206] Ac-KRLR(SEQ ID NO: 8) ketobenzothiazole-(C=O)-NHBN(3-COOH)

(23c). Compound **23b** (0.01 g, 0.010 mmol) was taken in 0.5 mL 0.05M LiOH solution and the reaction mixture was stirred for 1.5 hours at room temperature. On completion, the pH of the reaction mixture was brought to 2 by dropwise addition of 1N HCl solution. The crude product was purified by HPLC. Yield: 44%. ¹H NMR (400 MHz, METHANOL-*d*₄) δ ppm 0.90 (d, *J*=6.65 Hz, 3 H) 0.93 (d, *J*=6.65 Hz, 3 H) 1.37 - 1.93 (m, 16 H) 1.99 (s, 3 H) 2.13 - 2.27 (m, 1 H) 2.88 - 2.96 (m, 2 H) 3.14 - 3.21 (m, 2 H) 3.25 - 3.30 (m, 2 H) 4.20 - 4.28 (m, 1 H) 4.32 - 4.41 (m, 1 H) 4.41 - 4.50 (m, 1 H) 4.64 - 4.73 (m, 2 H) 5.56 - 5.65 (m, 1 H) 7.43 - 7.50 (m, 1 H) 7.61 - 7.67 (m, 1 H) 7.91 - 7.97 (m, 1 H) 8.04 - 8.09 (m, 1 H) 8.09 - 8.15 (m, 1 H) 8.26 - 8.31 (m, 1 H) 8.62 - 8.67 (m, 1 H). MS(ESI): found: [M + H]⁺, 908.8.

[0207] Ac-KRLR(SEQ ID NO: 8) ketobenzothiazole-(C=O)-NHBN(4-COOH)

(23d). The title compound was synthesized using the same procedure as **23c** starting with **23a**. Yield: 44%. ¹H NMR (400 MHz, METHANOL-*d*₄) δ ppm 0.91 (d, *J*=6.65 Hz, 3 H) 0.93 (d, *J*=6.26 Hz, 3 H) 1.33 - 1.95 (m, 16 H) 1.99 (s, 3 H) 2.14 - 2.27 (m, 1 H) 2.88 - 2.96 (m, 2 H) 3.14 - 3.21 (m, 2 H) 3.25 - 3.30 (m, 2 H) 4.19 - 4.28 (m, 1 H) 4.33 - 4.41 (m, 1 H) 4.41 - 4.50 (m, 1 H) 4.61 - 4.76 (m, 2 H) 5.54 - 5.69 (m, 1 H) 7.46 - 7.52 (m, 2 H) 7.98 - 8.04 (m, 2 H) 8.09 - 8.15 (m, 1 H) 8.27 - 8.32 (m, 1 H) 8.62 - 8.68 (m, 1 H). MS(ESI): found: [M + H]⁺, 908.8.

[0208] Ac-S(^tBu)-K(Boc)-L-R(Mtr)-ketobenzothiazole-6-COOH. Under nitrogen atmosphere at -78 °C, ⁿBuLi/Hex(2.5M) (28.5 mL, 71.4 mmol) was added dropwise into the solution of 6-carboxybenzothiazole (6.566 g, 37 mmol) in THF (450 mL) over 25 minutes. After the mixture was stirred for additional half an hour, the solution of Boc-HN-Arg(Mtr) Weinreb amide (1.633 g, 3.08 mmol) in THF (60 mL) was added slowly over 20 min at -78 °C. After the addition then the mixture was stirred at -24 °C to -20 °C for 1.5 hours. The reaction was quenched with saturated aqueous NH₄Cl (270 mL). The layers were separated and the aqueous layer was extracted with AcOEt. The organic phase was collected and washed with water and brine, dried with Na₂SO₄, then concentrated in vacuo. To the resulting residue MeOH (50 mL) was added. The mixture was cooled at -25 °C and sodium borohydride (0.706 g, 18.7 mmol) was added. The mixture was stirred at -25 ~ -20 °C for 1 hour. Acetone (10 mL) was added to quench the reaction and the mixture was stirred for 15 minutes then concentrated in vacuo. The residue was suspended in water, acidified to pH 3~4, and extracted with AcOEt. The organic phase was washed with brine, dried with Na₂SO₄,

then concentrated in vacuo. The resulting residue was dissolved in CH₂Cl₂/MeOH (17/3 v/v, 40 mL), cooled to 0 °C. Into it, (trimethylsilyl)diazomethane (2 M in hexane, 9.2 mL, 18.4 mmol) was added dropwise over 25 minutes. The mixture was stirred at 0 °C for 1 hour. MeOH (5 mL) was added and the mixture was concentrated in vacuo. The residue was purified by silica gel chromatography with CHCl₃/MeOH combination as eluent to give BocHN-Arg(Mtr)-CH(OH)benzothiazole-6-COOMe **a** (0.445 g, mixture of diastereomers) in 22% yield. MS(ESI): found: [M + H]⁺, 664.5.

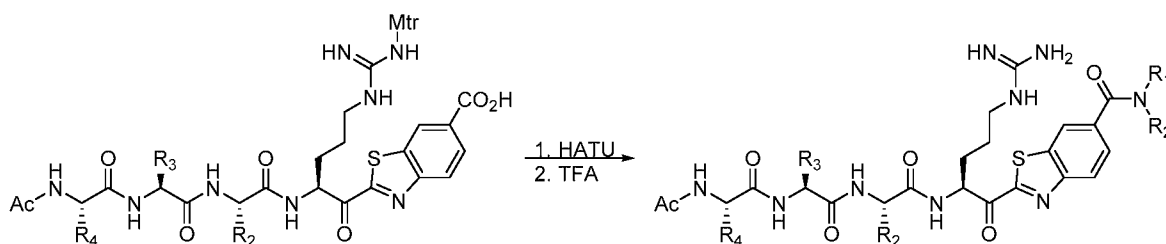
[0209] The mixture of **a** (0.44 g, 0.66 mmol) in TFA/CH₂Cl₂ (1/4 v/v, 18 mL) was stirred at room temperature for 2 hours. The solvents were removed and the residue was dried in vacuo to give TFA·H₂N-Arg(Mtr)-CH(OH)benzothiazole-6-COOMe **b** (0.45 g, mixture of diastereomers) in quantitative yield. MS(ESI): found: [M + H]⁺, 564.5.

[0210] Under nitrogen atmosphere, at 0 °C anhydrous DMF (13 mL) was added into the round bottom flask containing Ac-S(^tBu)-K(Boc)-L-OH (0.3650 g, 0.67 mmol) and HATU (0.255 g, 0.67 mmol). The mixture was stirred for 10 minutes, then **b** (0.40 g, 0.56 mmol) and *N,N*-diisopropylethylamine (0.49 mL, 2.8 mmol) were added sequentially. The mixture was stirred overnight while being warmed to room temperature naturally. DMF was removed and to the resulting residue, water (20 mL) was added. The precipitate formed was collected and purified by silica gel chromatography with CHCl₃/MeOH combination as eluent. The fractions with Ac-S(^tBu)-K(Boc)-L-Arg(Mtr)-CH(OH)benzothiazole-6-COOMe **c** (mixture of diastereomers) confirmed by LCMS [MS(ESI): found: [M + H]⁺, 1090.8], were collected, and concentrated in vacuo. The residue **c** was dissolved in THF/H₂O (15 mL/10 mL) and LiOH (0.036 g, 1.5 mmol) was added. The mixture was stirred overnight, then concentrated. The concentrated mixture was acidified to pH 4.0 with 0.25 N HCl aqueous solution and extracted with AcOEt. The organic phase was collected, dried with Na₂SO₄ and concentrated in vacuo to give Ac-S(^tBu)-K(Boc)-L-Arg(Mtr)-CH(OH)benzothiazole-6-COOH **d** (mixture of diastereomers) (0.52 g) in 86% yield. MS(ESI): found: [M + H]⁺, 1076.8. **d** was used in the next step without further purification.

[0211] Into the solution of **d** (0.30 g, 0.28 mmol) in CH₂Cl₂ (100 mL), Dess-Martin periodinane (DMP) (0.198 g, 0.47 mmol) was added. The mixture was stirred at room temperature for 3 hours, then additional DMP (0.12 g, 0.28 mmol) was added. The mixture was stirred overnight, then quenched with 1 M Na₂S₂O₃ aqueous solution. The organic phase was separated and the aqueous was extracted with CH₂Cl₂ 2 times. The organic phases were

combined, dried with Na_2SO_4 and concentrated in vacuo. The resulting residue was purified by silica gel chromatography with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ combination as eluent to give the title compound $\text{Ac-S}^{\text{(t)Bu}}\text{-K(Boc)-L-R(Mtr)-ketobenzothiazole-COOH}$ (0.186 g) in 62% yield. ^1H NMR (400 MHz, $\text{METHANOL-}d_4$) δ ppm 0.88 (d, $J=5.87$ Hz, 3 H) 0.92 (d, $J=6.26$ Hz, 3 H) 1.18 (s, 9 H) 1.27 - 1.90 (m, 21 H) 1.92 - 2.19 (m, 7 H) 2.54 (s, 3 H) 2.60 (s, 3 H) 2.95 - 3.08 (m, 2 H) 3.23 - 3.33 (m, 2 H) 3.53 - 3.70 (m, 2 H) 3.79 (s, 3 H) 4.18 - 4.54 (m, 3 H) 5.37 - 5.69 (m, 1 H) 6.55 (s, 1 H) 8.14 - 8.25 (m, 2 H) 8.79 (s, 1 H). MS(ESI): found: $[\text{M} + \text{H}]^+$, 1074.9.

[0212] Ac-SKLR(SEQ ID NO: 10)-ketobenzothiazole-6-COOH (7118). To the crude $\text{Ac-S}^{\text{(t)Bu}}\text{-K(Boc)-L-R(Mtr)-ketobenzothiazole-COOH}$ received from the DMP oxidation of **d** (0.053 g, 0.049 mmol), 5 mL of TFA/thioanisole/water (95/2.5/2.5(v/v/v)) was added. The mixture was stirred at room temperature for 4 hours. Then cold ether (40 mL) was added. The resulting precipitate, which is the crude product, was collected by centrifugation, then by decanting out carefully ether solvent. The crude product was purified by HPLC (C18, 15*150 mm column; eluent: acetonitrile/water (0.05% TFA)) to give the title compound (0.01 g) in 29% yield. ^1H NMR (400 MHz, $\text{METHANOL-}d_4$) δ ppm 0.75 - 1.00 (m, 6 H) 1.40 - 2.05 (m, 15 H) 2.13 - 2.27 (m, 1 H) 2.87 - 2.98 (m, 2 H) 3.29 - 3.33 (m, 2 H) 3.73 - 3.91 (m, 2 H) 4.25 - 4.46 (m, 3 H) 5.58 - 5.67 (m, 1 H) 8.25 - 8.29 (m, 2 H) 8.83 (s, 1 H). MS(ESI): found: $[\text{M} + \text{H}]^+$, 706.5.



[0213] Ac-SKLR(SEQ ID NO: 10)-ketobenzothiazole-6-CONH-Phe-amide (7125). The title compound was synthesized using the same procedure as for BocHN-Arg(Mtr) ketobenzothiazole-6-CONH-Val-amide, starting with the coupling of $\text{Ac-S}^{\text{(t)Bu}}\text{-K(Boc)-L-R(Mtr)-ketobenzothiazole-COOH}$ and L-phenylalanine amide hydrochloride. Yield: 25%. ^1H NMR (400 MHz, $\text{METHANOL-}d_4$) δ ppm 0.83 - 0.93 (m, 6 H) 1.35 - 2.05 (m, 15 H) 2.12 - 2.25 (m, 1 H) 2.86 - 2.97 (m, 2 H) 3.02 - 3.12 (m, 1 H) 3.21 - 3.38 (m, 3 H) 3.72 - 3.90 (m, 2 H) 4.25 - 4.45 (m, 3 H) 4.85 - 4.94 (m, 1 H) 5.56 - 5.64 (m, 1 H) 7.17 - 7.22

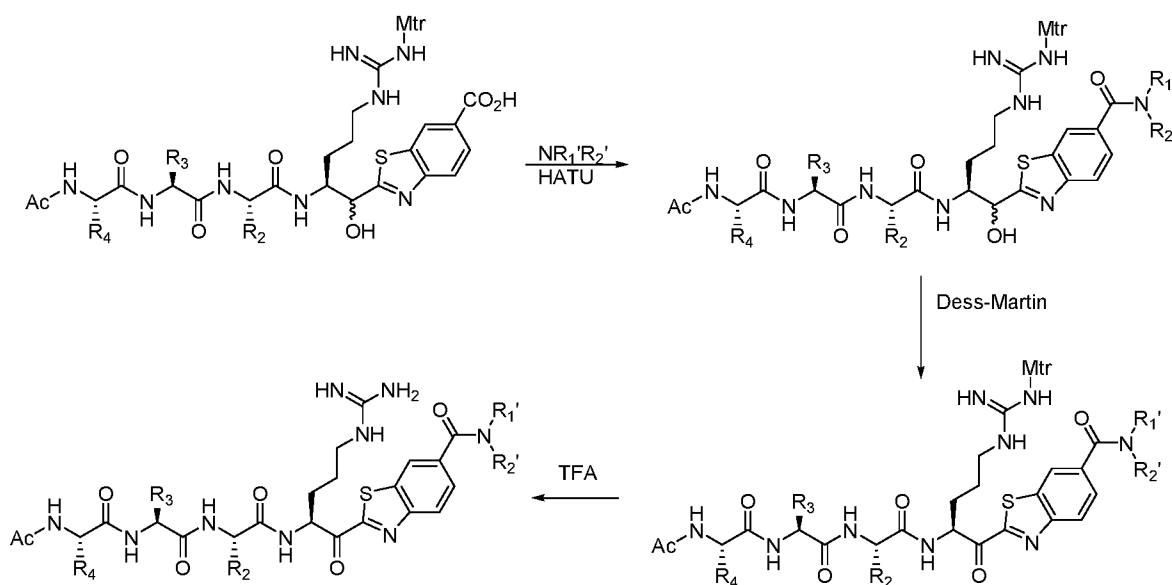
(m, 1 H) 7.24 - 7.35 (m, 4 H) 7.93 - 7.99 (m, 1 H) 8.20 - 8.26 (m, 1 H) 8.47 - 8.51 (m, 1 H). MS(ESI): found: $[M + H]^+$, 852.7.

[0214] Ac-SKLR(SEQ ID NO: 10)-ketobenzothiazole-6-CONH-Trp-amide

(7124). The title compound was synthesized using the same procedure as for Ac-SKLR(SEQ ID NO: 10)-ketobenzothiazole-6-CONH-Phe-amide. Yield: 24%. ^1H NMR (400 MHz, METHANOL- d_4) δ ppm 0.84 - 0.93 (m, 6 H) 1.33 - 2.05 (m, 15 H) 2.11 - 2.25 (m, 1 H) 2.83 - 2.97 (m, 2 H) 3.20 - 3.36 (m, 3 H) 3.40 - 3.50 (m, 1 H) 3.71 - 3.90 (m, 2 H) 4.24 - 4.46 (m, 3 H) 4.91 - 5.00 (m, 1 H) 5.54 - 5.65 (m, 1 H) 6.97 - 7.03 (m, 1 H) 7.06 - 7.11 (m, 1 H) 7.18 (s, 1 H) 7.30 - 7.35 (m, 1 H) 7.65 - 7.71 (m, 1 H) 7.91 - 7.97 (m, 1 H) 8.00 - 8.06 (m, 1 H) 8.18 - 8.23 (m, 1 H) 8.36 - 8.39 (m, 1 H). MS(ESI): found: $[M + H]^+$, 891.7.

[0215] Ac-SKLR(SEQ ID NO: 10)-ketobenzothiazole-6-CONH-4-pyridine

(7126). The title compound was synthesized using the same procedure as for Ac-SKLR(SEQ ID NO: 10)-ketobenzothiazole-6-CONH-Phe-amide. Yield: 18%. ^1H NMR (400 MHz, METHANOL- d_4) δ ppm 0.84 - 0.93 (m, 6 H) 1.35 - 2.05 (m, 15 H) 2.15 - 2.29 (m, 1 H) 2.86 - 2.98 (m, 2 H) 3.24 - 3.37 (m, 2 H) 3.73 - 3.98 (m, 2 H) 4.23 - 4.47 (m, 3 H) 5.57 - 5.69 (m, 1 H) 8.23 - 8.29 (m, 1 H) 8.35 - 8.44 (m, 3 H) 8.67 - 8.72 (m, 2 H) 8.85 (s, 1 H). MS(ESI): found: $[M + H]^+$, 782.7.



[0216] Ac-SKLR(SEQ ID NO: 10)-ketobenzothiazole-6-CONH-4-piperidine

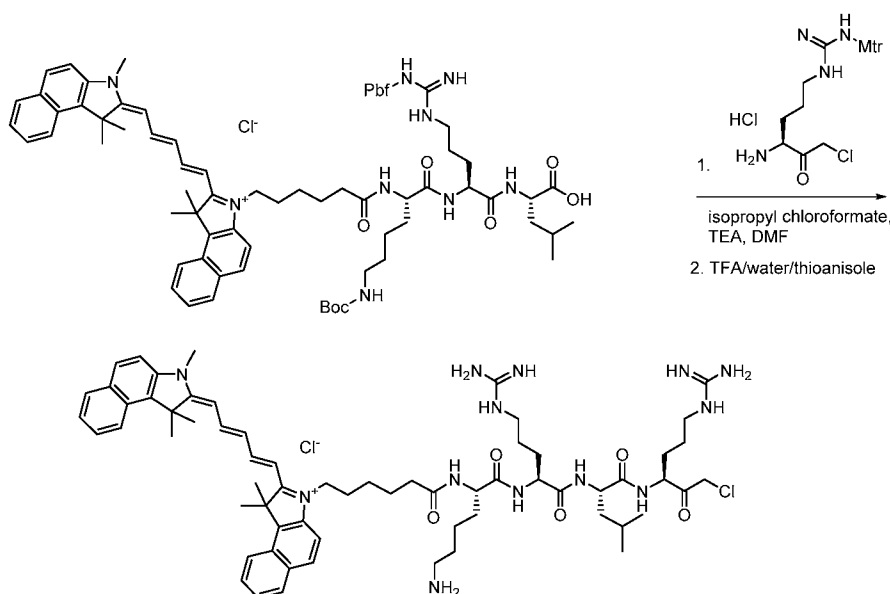
(7140). Under nitrogen atmosphere, at 0 °C anhydrous DMF (5 mL) was added into the RB

flask containing Ac-S(^tBu)-K(Boc)-L-Arg(Mtr)-CH(OH)benzothiazole-6-COOH **d** (0.088 g, 0.081 mmol) and HATU (0.037 g, 0.097 mmol). The mixture was stirred for 10 minutes, then 4-amino-1-Boc-piperidine (0.019 g, 0.097 mmol) and *N,N*-diisopropylethylamine (0.031 g, 0.24 mmol) were added sequentially. The mixture was stirred while being warmed to room temperature naturally. The reaction was monitored by LCMS until completion. DMF was removed and to the resulting residue, water (20 mL) was added. The precipitate formed was collected and purified by silica gel chromatography with CH₂Cl₂/MeOH combination as eluent to give Ac-S(^tBu)-K(Boc)-L-Arg(Mtr)-CH(OH)benzothiazole-6-CONH-4-1-Boc-piperidine **e** (0.088 g, mixture of diastereomers) in 86% yield. MS(ESI): found: [M + H]⁺, 1259.0.

[0217] Into the solution of **e** (0.083 g, 0.066 mmol) in CH₂Cl₂ (15 mL), Dess-Martin periodinane (DMP) (0.042 g, 0.099 mmol) was added. The mixture was stirred at room temperature for 3 hours, then quenched with 1 M Na₂S₂O₃ aqueous solution. The organic phase was separated and the aqueous was extracted with CH₂Cl₂ 2 times. The organic phases were combined, dried with Na₂SO₄ and concentrated in vacuo. Into the resulting residue 3 mL of TFA/thioanisole/water (95/2.5/2.5(v/v/v)) was added. The mixture was stirred at room temperature for 4 hours. Then cold ether (40 mL) was added. The resulting precipitate, which is the crude product, was collected by centrifugation, then by decanting out carefully ether solvent. The crude product was purified by HPLC (C18, 15*150 mm column; eluent: acetonitrile/water (0.05% TFA)) to give the title compound Ac-SKLR(SEQ ID NO: 10)-ketobenzothiazole-6-CONH-4-piperidine (0.022 g) in 42% yield. ¹H NMR (400 MHz, METHANOL-*d*₄) δ ppm 0.87 (d, *J*=6.26 Hz, 3 H) 0.91 (d, *J*=6.26 Hz, 3 H) 1.33 - 2.05 (m, 17 H) 2.15 - 2.30 (m, 3 H) 2.85 - 2.98 (m, 2 H) 3.10 - 3.23 (m, 2 H) 3.23 - 3.38 (m, 2 H) 3.43 - 3.55 (m, 2 H) 3.72 - 3.90 (m, 2 H) 4.15 - 4.46 (m, 4 H) 5.55 - 5.71 (m, 1 H) 8.06 - 8.11 (m, 1 H) 8.25 - 8.30 (m, 1 H) 8.60 - 8.63 (m, 1 H) MS(ESI): found: [M + H]⁺, 788.7.

[0218] Ac-SKLR(SEQ ID NO: 10)-ketobenzothiazole-6-CONH-Bn (7139). The title compound was synthesized using the same procedure as for Ac-SKLR(SEQ ID NO: 10)-ketobenzothiazole-6-CONH-4-piperidine. Yield: 33%. ¹H NMR (400 MHz, METHANOL-*d*₄) δ ppm 0.81 - 0.95 (m, 6 H) 1.31 - 2.09 (m, 15 H) 2.12 - 2.26 (m, 1 H) 2.84 - 2.98 (m, 2 H) 3.20 - 3.40 (m, 2 H) 3.65 - 3.91 (m, 2 H) 4.20 - 4.46 (m, 3 H) 4.59 - 4.66 (m, 2 H) 5.57 - 5.67 (m, 1 H) 7.22 - 7.29 (m, 1 H) 7.30 - 7.41 (m, 4 H) 8.08 - 8.13 (m, 1 H) 8.21 - 8.34 (m, 1 H) 8.57 - 8.67 (m, 1 H). MS(ESI): found: [M + H]⁺, 795.6.

[0219] Cy5.5-KRLR(SEQ ID NO: 8)-OH (RSC-1177). To H-K(Boc)R(Pbf)L-2-Cl Trt resin [(190 mg, 0.067 mmol), synthesized in a similar fashion to 7-AMINE)] was added 5 mL of DMF and 5 mL of DCM. After 30 min, the solvent was filtered and a solution of Cy5.5-NHS ester (71 mg, 0.10 mmol, Lumiprobe) in DMF (5 mL) and Hunig's Base (0.025 mL, 0.13 mmol) were added, then stirred overnight at room temperature. The solvent was filtered off and the resin washed thoroughly with DMF and DCM, then dried under vacuum. To the resin was added a 1:3 mixture of HFIP and DCM (15 mL) and after 45 minutes of gently shaking, the solvent was removed and then replenished. After 30 minutes of additional shaking, the solvent was removed. The combined filtrates were concentrated in vacuo to give 107 mg of crude material. Purification was achieved with 5-50% MeOH/DCM on silica gel. Yield 65 mg, 73%. Chemical Formula: $C_{76}H_{102}N_9O_{10}S+Cl^-$, Exact Mass: 1332.75, MS(ESI): found: $[M]^+$, 1332.9.



[0220] Cy5.5-KRLR(SEQ ID NO: 8)-cmk (RSC-1179). To a cooled (ice bath) solution of Cy5.5-KRLR(SEQ ID NO: 8)-OH (15 mg, 0.011 mmol) in THF (1 mL) was added N-methylmorpholine (0.011 mmol) and isopropyl chloroformate (0.011 mL, 1M solution in toluene). The reaction mixture was stirred for 10-15 minutes and then trimethylamine (0.011 mmol) and (S)-N-(N-(4-amino-6-chloro-5-oxohexyl)carbamidoyl)-4-methoxy-2,3,6-trimethylbenzenesulfonamide hydrochloride [aE. Gherardi, W. Birchmeier, C. Birchmeier, G. Vande Woude, *Nature reviews. Cancer* **2012**, 12, 89-103; bE. C. Smyth, F. Sclafani, D. Cunningham, *Oncotargets Ther* **2014**, 7, 1001-1014.] (5 mg, 0.011 mmol) in

DMF (0.2 mL) were added followed by stirring for an additional 2 hours. The solvent was removed and then 1.5 mL of cleavage cocktail (38:1:1 TFA/water/thioanisole) was added to the resin. After gentle shaking overnight, the solvent was removed in vacuo, triturated in toluene, and then concentrated in vacuo. The residue was purified by C18 reverse phase HPLC to give 10 mg of crude product which was re-purified to give 4 mg of pure title compound as the TFA salt. Chemical Formula: $C_{65}H_{91}ClN_{13}O_5+Cl^-$, Exact Mass: 1168.69, MS(ESI): found: $[M]^+$, 1168.8.

[0221] Cy5.5-KRLR(SEQ ID NO: 8)-kbt (RSC-1178). Synthesized in a similar manner to Ac-KQLR(SEQ ID NO: 1) kbt (**3**) from Cy5.5-KRLR(SEQ ID NO: 8)-OH. Chemical Formula: $C_{71}H_{93}N_{14}O_5S+Cl^-$, Exact Mass: 1253.72, MS(ESI): found: $[M]^+$, 1253.7.

Example 6. Inhibition Studies with Polypeptide Ketobenzothiazoles

[0222] Fluorescent inhibitor and chromogenic proteolytic assays were performed in general accordance with the procedures described in Example 4 for compounds prepared in Examples 5 and 6.

[0223] The results of the assays are presented in Table 6.1. The results show increased potency of 2-fold of the ketobenzothiazoles over thiazole 5. More importantly when we substitute a Val on the benzothiazole ring 36c as in 8, we increase the HGFA potency another two-fold (K_i 13 nM) but decreased matriptase activity >10-fold (K_i 7 nM) and >3-fold for hepsin (K_i 0.3 nM). Ac-SKLR(SEQ ID NO: 10)-kbt V amide (**9**) showed a 4-fold enhancement in HGFA potency with no effect on hepsin or matriptase. We discovered Ac-KQLR(SEQ ID NO: 1)-kbt (**7**) improves potency 2-fold and further substitution of the benzothiazole ring with Valine, Ac-KQLR(SEQ ID NO: 1)-kbt V amide (**8**) increases potency another 2-fold for HGFA (K_i 13 nM) but decreased matriptase activity >10-fold (K_i 7 nM) and >3-fold for hepsin (K_i 0.3 nM). This result demonstrates that these inhibitor compounds have improved selectivity for HGFA.

Table 6.1

Compound No.	Y-P ₄ -P ₃ -P ₂ -P ₁ -Z	Fluorogenic Assay		
		HGFA Ki (nM)	Matriptase Ki (nM)	Hepsin Ki (nM)
7185-1	Fmoc-AR-kbt	7983.5	0.5	272.37
7185-2	Fmoc-RR-kbt	8633.5	2.0	99.92
7185-3	Fmoc-NR-kbt	>20000	84.1	1.92
7185-4	Fmoc-DR-kbt	>20000	397.9	232.92
7185-6	Fmoc-QR-kbt	6827	1.6	170.07
7185-7	Fmoc-ER-kbt	>20000	8.4	337.63
7185-8	Fmoc-GR-kbt	9375	0.1	831.00
7185-9	Fmoc-HR-kbt	6356.19	66.7	268.07
7185-10	Fmoc-IR-kbt	6466.05	657.1	297.32
7185-11	Fmoc-LR-kbt	449.776	425.3	114.90
7185-12	Fmoc-KR-kbt	>20000	0.2	159.25
7187-13	Fmoc-MR-kbt	1208.00	62.1	489.97
7187-14	Fmoc-FR-kbt	963.50	1281.5	777.88
7187-15	Fmoc-PR-kbt	>20000	40.4	776.25
7187-16	Fmoc-SR-kbt	>20000	90.00	885.50
7188-17	Fmoc-TR-kbt	>20000	1217.00	436.75
7188-18	Fmoc-WR-kbt	3197.00	823.5	410.62
7188-19	Fmoc-YR-kbt	2961.00	331.6	217.38
7188-20	Fmoc-VR-kbt	4633.83	1488.0	313.60

Compound No.	Y-P ₄ -P ₃ -P ₂ -P ₁ -Z	Fluorogenic Assay		
		HGFA Ki (nM)	Matriptase Ki (nM)	Hepsin Ki (nM)
1-13A1	H-WFR-kbt	5681.25	1871.5	240.15
1-18A1	H-dWFR-kbt	2250	135.3	369.80
1-15A1	H-dWLR-kbt	127.98	34.6	3.58
1-45A1	H-WFR-kbt-COOH	981	232	1.41
7171	H-RLR-kbt	83.07	3.3	0.50
1-56A1	H-WLR-kbt-COOH	1379	1206.5	446.30
1-57A1	H-dWLR-kbt-COOH	345.9	363.6	136.50
1-54A1	H-His(Bom)-WLR-kbt	3711.83	1549.8	2.08
1-58A1	H-His(Bom)-dWLR-kbt	2282.17	1504.7	1.13
7182	H-LLR-kbt-Val-NH ₂	82.09	23.6	0.88
7181	H-WLR-kbt-Val-NH ₂	73.89	49.2	1.31
7180	H-RLR-kbt-Val-NH ₂	12.50	4.2	0.44
7170	H-WLR-kbt	103.98	33.5	1.52
7115	Ac-KRLR(SEQ ID NO: 8)-kbt-Val-NH ₂	5.83	7.415	0.2265
7054	Ac-KRLR(SEQ ID NO: 8)-kbt	6.00	0.540	0.112
7117	H-WRLR(SEQ ID NO: 9)-kbt	8.75	3.79	0.12
7055	Ac-KQLR(SEQ ID NO: 1)-kbt-Val-NH ₂	12.00	6.98	0.29
7116	Ac-SKLR(SEQ ID NO: 10)-kbt-Val-NH ₂	12.98	10.3	0.19
7124	Ac-SKLR(SEQ ID NO: 10)-kbt-Trp-NH ₂	15.75	3.74	0.098
7125	Ac-SKLR(SEQ ID NO: 10)-kbt-Phe-NH ₂	17.28	6.26	0.14

Compound No.	Y-P ₄ -P ₃ -P ₂ -P ₁ -Z	Fluorogenic Assay		
		HGFA Ki (nM)	Matriptase Ki (nM)	Hepsin Ki (nM)
7006	Ac-KQLR(SEQ ID NO: 1)-kbt	30.00	0.550	0.09
7126	Ac-SKLR(SEQ ID NO: 10)-kbt-4-pyridinylamide	31.76	3.95	0.14
7053	Ac-SKLR(SEQ ID NO: 10)-kbt	33.00	3.05	0.16
7063	Ac-FLFR(SEQ ID NO: 19)-kbt	114.00	3.60	1.44
7118	Ac-SKLR(SEQ ID NO: 10)-kbt-COOH	131.97	54.8	0.173
7064	Ac-WLFR(SEQ ID NO: 20)-kbt	133.00	5.960	0.347
7139	Ac-SKLR(SEQ ID NO: 10)-kbt-benzylamide	9.10	0.360	0.80
7140	Ac-SKLR(SEQ ID NO: 10)-kbt-4-piperidinylamide	7.70	1.28	0.20
7165	Ac-KRLR(SEQ ID NO: 8)-kbt-Phe-COOH	6.07	5.14	0.37
7164	Ac-KRLR(SEQ ID NO: 8)-kbt-Benzyl-3-COOH	3.33	3.60	0.30
7159	Ac-KRLR(SEQ ID NO: 8)-kbt-Benzyl-3-COOMe	7.76	1.80	0.36
7158	Ac-KRLR(SEQ ID NO: 8)-kbt-Benzyl-4-COOMe	5.85	0.98	0.26
1179	Cy5.5-KRLR(SEQ ID NO: 8)-cmk	34.14	172.2	323.53
1178-2	Cy5.5-KRLR(SEQ ID NO: 8)-kbt	608	87.5	3.89

[0224] The results of selectivity assays are presented in Table 6.2.

Table 6.2.

Compound No.	Y-P ₄ -P ₃ -P ₂ -P ₁ -Z	Fluorogenic Assay		
		Thrombin Ki (nM)	Factor Xa Ki (nM)	Trypsin Ki (nM)
7118	Ac-SKLR(SEQ ID NO: 10)-kbt-COOH	>2000	1247	0.03
7006	Ac-KQLR(SEQ ID NO: 1)-kbt	>20000	129	0.24
7055	Ac-KQLR(SEQ ID NO: 1)-kbt-Val-NH ₂	3212	66	0.07
7053	Ac-SKLR(SEQ ID NO: 10)-kbt	>20000	1901	0.16
7116	Ac-SKLR(SEQ ID NO: 10)-kbt-Val-NH ₂	4505	385	0.34
7117	H-WRLR(SEQ ID NO: 9)-kbt	4890	2.4	0.96
7063	Ac-FLFR(SEQ ID NO: 19)-kbt	>20000	13.1	0.27
7054	Ac-KRLR(SEQ ID NO: 8)-kbt	>20000	91.9	0.82
7064	Ac-WLFR(SEQ ID NO: 20)-kbt	>20000	1.0	
7115	Ac-KRLR(SEQ ID NO: 8)-kbt-Val-NH ₂	1560	17.0	
7124	Ac-SKLR(SEQ ID NO: 10)-kbt-Trp-NH ₂	1231	163	0.02
7125	Ac-SKLR(SEQ ID NO: 10)-kbt-Phe--NH ₂	3640	544	0.27
7126	Ac-SKLR(SEQ ID NO: 10)-kbt-pyridine-NH ₂	3431	221	1.06
7139	Ac-SKLR(SEQ ID NO: 10)-kbt-benzyl	2467	190	0.05
7140	Ac-SKLR(SEQ ID NO: 10)-kbt-piperidine	2948	447	0.41
7158	Ac-KRLR(SEQ ID NO: 8)-kbt-benzyl-4-COOMe	20.92	9.0	0.05
7159	Ac-KRLR(SEQ ID NO: 8)-kbt-Benzyl-3-COOMe	85.38	14	0.04
7164	Ac-KRLR(SEQ ID NO: 8)-kbt-benzyl-4-COOH	1618		
7170	H-WLR-kbt	457.00	532.00	0.050
7171	H-RLR-kbt	8198	56	0.13
7182	H-LLR-Val-NH ₂	101.4	159	0.61
7181	H-WLR-kbt-Val-NH ₂	41.04	159	0.01
7180	H-RLR-kbt-Val-NH ₂	548	18	0.31
7165	Ac-KRLR(SEQ ID NO: 8)-kbt-benzyl-3-COOH	1944	20.2	1.06
7187-14	Fmoc-FR-kbt	>2000	28.4	14.350
7185-11	Fmoc-LR-kbt	995	410	19.78

Example 7. Inhibition Studies with Cyclic Peptides

[0225] Fluorescent inhibitor and chromogenic proteolytic assays were performed in general accordance with the procedures described in Example 4 for compounds prepared in Example 3. Table 7.1 presents the results for a cyclic peptide compound 7074.

Table 7.1

Compound	Y-P ₅ -P ₄ -P ₃ -P ₂ -P ₁ -Z	Fluorogenic Assay		
		HGFA Ki (nM)	Matriptase Ki (nM)	HGFA Ki (nM)
7074	H-cyclo(KQD)RR-kt	5941.67	0.71	1.55

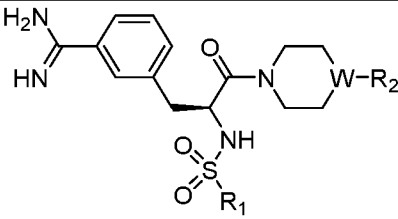
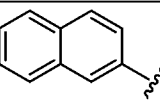
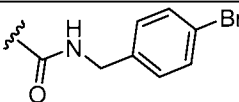
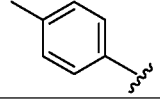
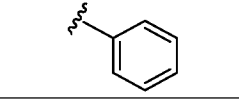
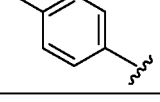
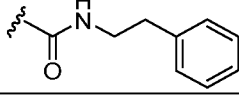
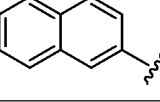
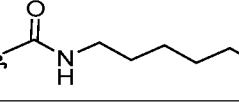
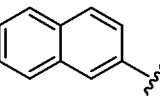
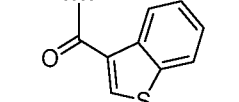
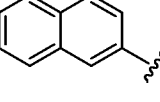
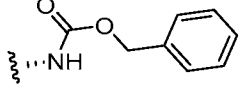
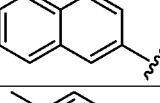
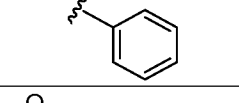
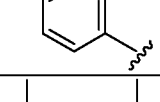
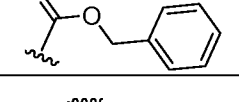
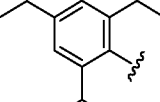
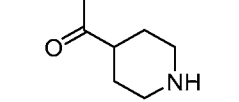
Example 8. Synthesis of Benzamidine Inhibitor Compounds

[0226] The benzamidine inhibitors listed in Table 8.1 were synthesized in accordance with the general scheme 8A shown below and the procedures described below.

Phenylalanine naphthyl sulfonamides were synthesized in accordance with general scheme B and the procedures described below.

Table 8.1 Benzamidine inhibitors.

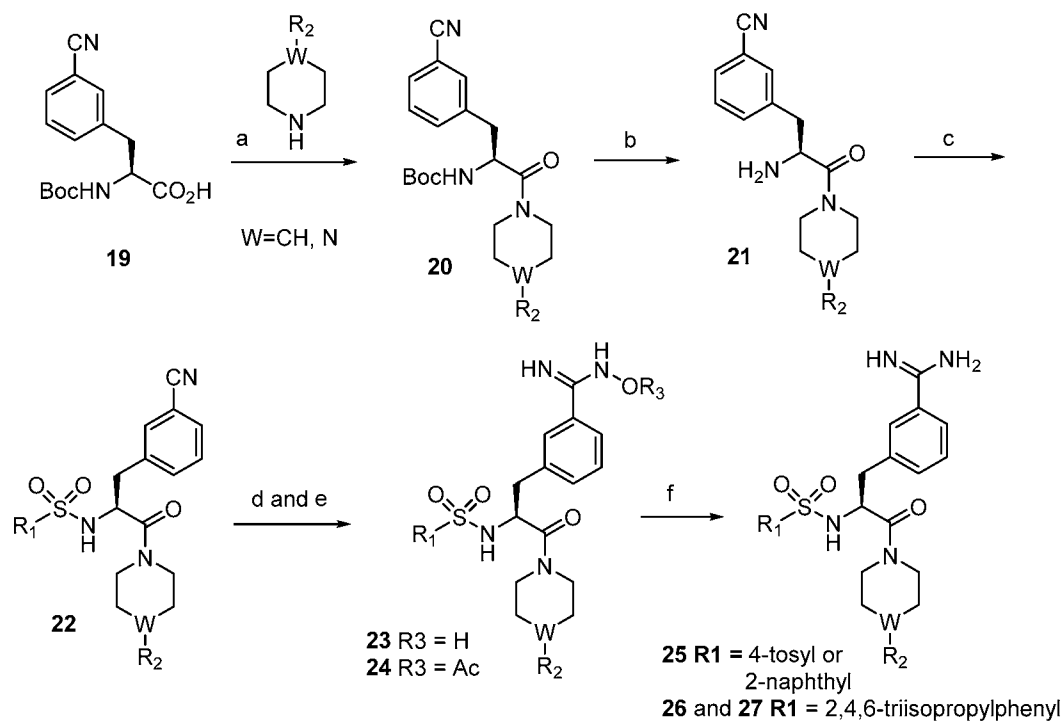
Compound	R ₁	R ₂	W
16			C
17a			N
18*			C

			
Compound	R ₁	R ₂	W
25a			N
25b			C
25c*			C
25d			N
25e			N
25f*			C
25g			N
25h			N
26			N

Compound	R ₁	R ₂	W
27			N

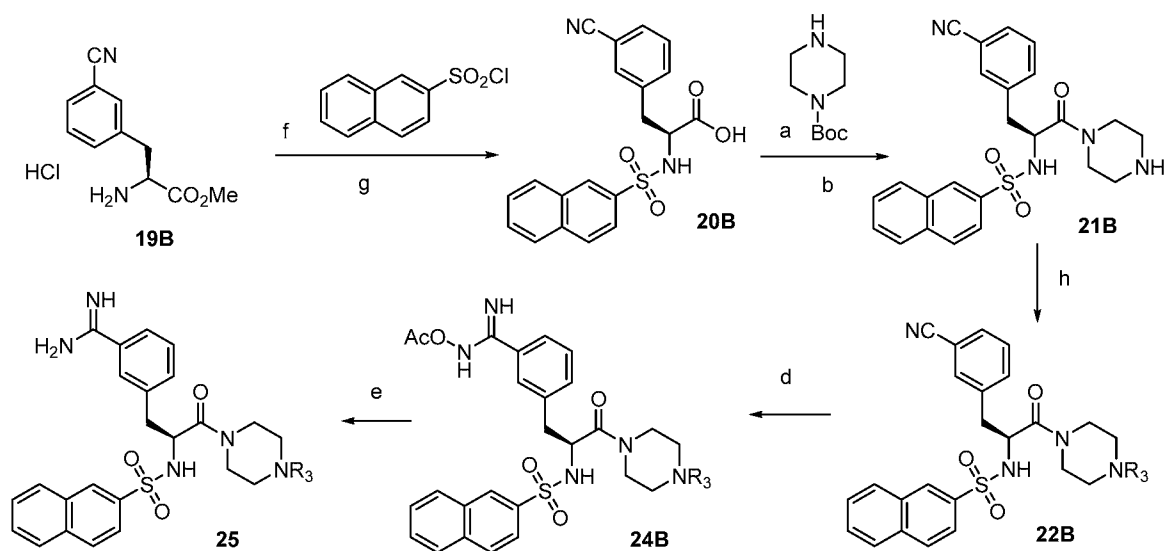
*R₂ is substituted on the 3-position of the piperidine ring.

Scheme 8A. Synthesis of 3-amidinophenylalanine sulfonamide analogs



[0227] Reagents and conditions in Scheme A are as follows a) HATU, THF (or DMF), DIPEA; b) 4N HCl in 1,4-dioxane; c) R₁SO₂Cl, DMAP, pyridine, THF; d) i. NH₂OH-HCl, K₂CO₃ (or DIPEA), EtOH; e) Ac₂O, AcOH; f) Zn, AcOH.

Scheme 8B. Synthesis of 3-amidinophenylalanine sulfonamide analogs



[0228] Reagents and conditions in Scheme B are as follows a) HATU, THF (or DMF), DIPEA; b) 4N HCl in 1,4-dioxane; d) i. NH₂OH-HCl, K₂CO₃ (or DIPEA), EtOH; ii. Ac₂O, AcOH; e) Zn, AcOH; f) DMAP, pyridine, THF; g) LiOH, THF; h) HATU, RCO₂H (R₃ = COR) or R-N=C=O (R₃ = CONHR).

[0229] General Procedure A: General procedure for the synthesis of amides (20):

A solution of N-Boc-3-cyanophenylalanine (19) in dry DMF (0.5 M) was added Hunig's Base (4.0 equiv) followed by HATU (1.3 equiv). The yellow solution was allowed to stir for 10 minutes and was then added amine (1.3 equiv). The resulting mixture was stirred at room temperature. After two hours, the reaction was checked by LCMS and was complete. The reaction mixture was diluted with ethyl acetate, washed with water (1x), brine (2x), dried (MgSO₄), filtered and concentrated to oil. The crude product was purified by MPLC (0-75% hexanes/ethyl acetate) to afford pure product.

[0230] General Procedure B: General procedure for BOC deprotection (21):

A solution of N-Boc protected amide in dioxane (0.4 M) was added 4.0 M HCl in dioxane (10 equiv). The resulting mixture was allowed to stir at room temperature. After 4 hours, the reaction was checked by LCMS and was complete. The solvent was removed under reduced pressure to afford pure product.

[0231] General Procedure C: General procedure for sulfonamide formation (22):

A mixture of amide in THF (0.2 M) was added Hunig's Base (10 equiv) and the mixture was

stirred until all solids dissolved. The solution was then added DMAP (10 mol%) followed by arylsulfonyl chloride (1.1 equiv) and stirred at room temperature. After 2 hours the reaction was checked by LCMS and was complete. The reaction was diluted with ethyl acetate, washed with saturated NaHCO₃ (2x), brine (1x), dried (MgSO₄), filtered and concentrated under reduced pressure. The crude material was purified via MPLC (0-75% ethyl acetate/hexanes) to afford pure product as a solid.

[0232] General Procedure D: General procedure for formation of the amidoxime (23): A suspension of sulfonamide in dry ethanol (0.2M) was added hydroxylamine hydrochloride (2.2 equiv) followed by Hunig's base (2.3 equiv). The resulting mixture was heated to 75°C for approximately 3 hours which resulted in a homogeneous solution. The reaction mixture was allowed to cool to room temperature, checked by LCMS and was complete. The solvent was removed under reduced pressure to afford the crude amidoxime intermediate as a white solid.

[0233] General Procedure E: General procedure for acetylation of amidoxime (24). A solution of crude amidoxime in acetic acid (0.2 M) was added acetic anhydride at room temperature. The resulting mixture was allowed to stir at room temperature. After approximately 2 hours the reaction was checked by LCMS and was complete. The solvent was removed under reduced pressure to afford the crude product.

[0234] General Procedure F: General procedure for preparation of amidines (25) and (26): A solution of O-acetylamidoxime in acetic acid (0.2 M) was added zinc dust (15 equiv) at room temperature. The resulting suspension was stirred for 3 hours at room temperature. The reaction was checked by LCMS and was complete. The mixture was filtered and concentrated under reduced pressure to give the crude product as an oil. The crude product was purified via reverse phase HPLC (5-65 % acetonitrile/water/0.05% trifluoroacetic acid) to afford pure product as a white solid after lyophilization.

[0235] (S)-3-(2-(Naphthalene-2-sulfonamido)-3-oxo-3-(4-phenylpiperidin-1-yl)propyl)benzimidamide (16). Prepared from *N*-acetoxy-3-[(2*S*)-2-[(2-naphthylsulfonyl)amino]-3-oxo-3-(4-phenylpiperidin-1-yl)propyl]benzenecarboximidamide using **General Procedure F**. Purified by reversed-phase HPLC (5-75% acetonitrile/water/0.05% TFA), Yield (0.092 g, 52.5%). ¹H NMR (300 MHz, DMSO-*d*₆) δ 9.40 (s, 2H), 9.26 (s, 2H), 8.23 - 8.50 (m, 2H), 7.94 - 8.10 (m, 2H), 7.56 - 7.78 (m, 4H), 7.35 - 7.56 (m, 2H), 7.07 - 7.33 (m, 3H), 6.96 (d, *J* = 7.42 Hz, 1H), 6.78 (d, *J* = 7.14 Hz, 1H), 4.49

- 4.79 (m, 1H), 3.82 - 4.21 (m, 2H), 3.61 - 3.79 (m, 1H), 3.41 - 3.56 (m, 2H), 2.89 - 3.06 (m, 1H), 2.72 - 2.89 (m, 1H), 2.59 (t, $J = 12.22$ Hz, 1H), 1.31 - 1.64 (m, 2H), 0.75 - 1.08 (m, 1H), 0.38 - 0.67 (m, 1H). LRMS (ESI) $C_{31}H_{32}N_4O_3S$ found $[M+H]^+$: 541.2.

[0236] *N*-Acetoxy-3-[(2*S*)-2-[(2-naphthylsulfonyl)amino]-3-oxo-3-(4-phenylpiperidin-1-yl)propyl]benzenecarboximidamide. Prepared from *N*-hydroxy-3-[(2*S*)-2-[(2-naphthylsulfonyl)amino]-3-oxo-3-(4-phenylpiperidin-1-yl)propyl]benzenecarboximidamide using **General Procedure E**. Purification by MPLC (0-90% EtOAc/hexanes). Yield (0.17 g, 47.7%). LRMS (ESI) $C_{33}H_{34}N_4O_5S$ found $[M+H]^+$: 599.2.

[0237] *N*-Hydroxy-3-[(2*S*)-2-[(2-naphthylsulfonyl)amino]-3-oxo-3-(4-phenylpiperidin-1-yl)propyl]benzenecarboximidamide. Prepared from tert-butyl (S)-(3-(3-cyanophenyl)-1-oxo-1-(4-phenylpiperazin-1-yl)propan-2-yl)carbamate (0.312 g, 0.589 mmol) using **General Procedure D**. LRMS (ESI) $C_{31}H_{32}N_4O_4S$ found $[M+H]^+$: 557.2.

[0238] *N*-[(2*S*)-3-(3-Cyanophenyl)-1-oxo-1-(4-phenylpiperidin-1-yl)propan-2-yl]naphthalene-2-sulfonamide. Prepared from 3-[(2*S*)-2-amino-3-oxo-3-(4-phenylpiperidin-1-yl)propyl]benzonitrile (4.1 g, 20.1 mmol) using **General Procedure C**. Yield (0.312 g, 79%). LRMS (ESI) $C_{31}H_{29}N_3O_3S$ found $[M+H]^+$: 524.2.

[0239] 3-[(2*S*)-2-Amino-3-oxo-3-(4-phenylpiperidin-1-yl)propyl]benzonitrile. Prepared from tert-butyl [(2*S*)-3-(3-cyanophenyl)-1-oxo-1-(4-phenylpiperidin-1-yl)propan-2-yl]carbamate (0.67 g, 1.54 mmol) using **General Procedure B**. LRMS (ESI) $C_{21}H_{23}N_3O$ found $[M+H]^+$: 334.2.

[0240] tert-butyl [(2*S*)-3-(3-Cyanophenyl)-1-oxo-1-(4-phenylpiperidin-1-yl)propan-2-yl]carbamate. Prepared from N-Boc-3-cyanophenylalanine (**19**) (0.56 g, 1.81 mmol) and N-phenylpiperidine (0.32 g, 2.01 mmol) using **General Procedure A**. Purified by MPLC (0-75% EtOAc/Hexanes). Yield (0.67 g, 85%). LRMS (ESI) $C_{26}H_{31}N_3O_3$ found $[M+H]^+$: 434.2.

[0241] (S)-3-(2-(Naphthalene-2-sulfonamido)-3-oxo-3-(4-phenylpiperazin-1-yl)propyl)benzimidamide (25g). Prepared from (S)-N-acetoxy-3-(2-(naphthalene-2-sulfonamido)-3-oxo-3-(4-phenylpiperazin-1-yl)propyl)benzimidamide (0.123 g, 0.205 mmol) using **General Procedure F**. Purified by reversed-phase HPLC (5-60% acetonitrile/water/0.05% TFA), Yield (0.069 g, 54.7%). 1H NMR (300 MHz, DMSO- d_6) δ 9.42 (s, 2H), 9.29 (s, 2H), 8.22 - 8.51 (m, 2H), 7.85 - 8.12 (m, 3H), 7.36 - 7.74 (m, 7H), 7.08 - 7.28 (m, 2H), 6.61 - 6.90 (m, 3H), 4.49 - 4.75 (m, 1H), 3.37 - 3.61 (m, 1H), 3.19 - 3.37 (m,

1H), 3.03 - 3.18 (m, 1H), 2.96 (dd, $J = 7.00, 13.32$ Hz, 2H), 2.52 - 2.89 (m, 4H), 2.25 - 2.45 (m, 1H). LRMS (ESI) $C_{30}H_{31}N_5O_3S$ found $[M+H]^+$: 542.2.

[0242] (S)-N-Acetoxy-3-(2-(naphthalene-2-sulfonamido)-3-oxo-3-(4-phenylpiperazin-1-yl)propyl)benzimidamide. Prepared from *N*-hydroxy-3-[(2*S*)-2-[(2-naphthylsulfonyl)amino]-3-oxo-3-(4-phenylpiperidin-1-yl)propyl]benzenecarboximidamide (0.26 mmol) using **General Procedure E**. Purification by MPLC (0-40% EtOAc/hexanes). Yield (0.123 g, 79%). LRMS (ESI) $C_{32}H_{33}N_5O_5S$ found $[M+H]^+$: 600.2.

[0243] (S)-N-Hydroxy-3-(2-(naphthalene-2-sulfonamido)-3-oxo-3-(4-phenylpiperazin-1-yl)propyl)benzimidamide. Prepared from (S)-N-(3-(3-cyanophenyl)-1-oxo-1-(4-phenylpiperazin-1-yl)propan-2-yl)naphthalene-2-sulfonamide (0.312 g, 0.589 mmol) using **General Procedure D**. LRMS (ESI) $C_{30}H_{31}N_5O_4S$ found $[M+H]^+$: 558.2.

[0244] (S)-N-(3-(3-Cyanophenyl)-1-oxo-1-(4-phenylpiperazin-1-yl)propan-2-yl)naphthalene-2-sulfonamide. Prepared from (S)-3-(2-amino-3-oxo-3-(4-phenylpiperazin-1-yl)propyl)benzonitrile (0.121 g, 0.327 mmol) using **General Procedure C**. Purified by MPLC (0-75% EtOAc/Hexanes). Yield (0.133 g, 79.5%). LRMS (ESI) $C_{30}H_{28}N_4O_3S$ found $[M+H]^+$: 525.2.

[0245] (S)-3-(2-Amino-3-oxo-3-(4-phenylpiperazin-1-yl)propyl)benzonitrile. Prepared from *tert*-butyl [(2*S*)-3-(3-cyanophenyl)-1-oxo-1-(4-phenylpiperidin-1-yl)propan-2-yl]carbamate (0.347 g, 0.80 mmol) using **General Procedure B**. Purified product obtained by trituration in ether followed by filtration and drying. Yield (0.29 g, 97%). LRMS (ESI) $C_{20}H_{22}N_4O$ found $[M+H]^+$: 335.2.

[0246] *tert*-butyl (S)-3-(3-(3-Cyanophenyl)-1-oxo-1-(4-phenylpiperazin-1-yl)propan-2-yl)carbamate. Prepared from *N*-Boc-3-cyanophenylalanine (**19**) (0.24 g, 0.83 mmol) and *N*-phenylpiperazine (0.92 mmol) using **General Procedure A**. Purified by MPLC (0-75% EtOAc/Hexanes). Yield (0.35 g, 96%). LRMS (ESI) $C_{25}H_{30}N_4O_3$ found $[M+H]^+$: 435.2.

[0247] (S)-Benzyl 4-(3-(3-carbamimidoylphenyl)-2-(4-methylphenylsulfonamido)propanoyl)piperazine-1-carboxylate (25h**).** Prepared from benzyl (S)-4-(3-(3-(*N*-acetoxy carbamimidoyl)phenyl)-2-(4-methylphenyl)sulfonamido)propanoyl)piperazine-1-carboxylate (0.141 g, 0.228 mmol) using **General Procedure F**. Purified by reversed-phase HPLC (5-75% acetonitrile/water/0.05% TFA), Yield (0.058 g). 1H NMR (300 MHz, DMSO- d_6) δ 9.39 (br. s., 2H), 9.29 (br. s., 2H), 8.15 (d, $J = 9.34$ Hz, 1H), 7.63 - 7.82 (m, 2H), 7.22 - 7.62 (m, 10H), 5.07 (s, 2H), 4.37 - 4.63

(m, 1H), 3.44 - 3.71 (m, 2H), 3.10 - 3.29 (m, 3H), 2.84 - 3.10 (m, 3H), 2.60 - 2.84 (m, 2H), 2.32 (s, 3H). LRMS (ESI) $C_{29}H_{33}N_5O_5S$ found $[M+H]^+$: 564.2.

[0248] Benzyl (S)-4-(3-(3-(N-acetoxycarbamimidoyl)phenyl)-2-((4-methylphenyl)sulfonamido)propanoyl)piperazine-1-carboxylate. Prepared from benzyl (S)-4-(3-(3-(N-hydroxycarbamimidoyl)phenyl)-2-((4-methylphenyl)sulfonamido)propanoyl)piperazine-1-carboxylate (0.132 g, 0.228 mmol) using **General Procedure E**. LRMS (ESI) $C_{31}H_{35}N_5O_7S$ found $[M+H]^+$: 622.2.

[0249] Benzyl (S)-4-(3-(3-(N-hydroxycarbamimidoyl)phenyl)-2-((4-methylphenyl)sulfonamido)propanoyl)piperazine-1-carboxylate. Prepared from benzyl (S)-4-(3-(3-cyanophenyl)-2-((4-methylphenyl)sulfonamido)propanoyl)piperazine-1-carboxylate (0.24 g, 0.228 mmol) using **General Procedure D**. LRMS (ESI) $C_{29}H_{33}N_5O_6S$ found $[M+H]^+$: 580.2.

[0250] Benzyl (S)-4-(3-(3-cyanophenyl)-2-((4-methylphenyl)sulfonamido)propanoyl)piperazine-1-carboxylate. Prepared from benzyl (S)-4-(2-amino-3-(3-cyanophenyl)propanoyl)piperazine-1-carboxylate (0.243 g, 0.522 mmol) using **General Procedure C**. Purified by MPLC (0-75% EtOAc/Hexanes). Yield (0.124 g, 45.6%). LRMS (ESI) $C_{29}H_{30}N_4O_5S$ found $[M+H]^+$: 547.2.

[0251] Benzyl (S)-4-(2-amino-3-(3-cyanophenyl)propanoyl)piperazine-1-carboxylate. Prepared from benzyl (S)-4-(2-((tert-butoxycarbonyl)amino)-3-(3-cyanophenyl)propanoyl)piperazine-1-carboxylate (0.257 g, 0.522 mmol) using **General Procedure B**. LRMS (ESI) $C_{22}H_{24}N_4O_3$ found $[M+H]^+$: 393.2.

[0252] Benzyl (S)-4-(2-((tert-butoxycarbonyl)amino)-3-(3-cyanophenyl)propanoyl)piperazine-1-carboxylate. Prepared from N-Boc-3-cyanophenylalanine (**19**) (1.02 g, 3.51 mmol) and N-carboxybenzyl piperazine (3.89 mmol) using **General Procedure A**. Purified by MPLC (0-80% EtOAc/Hexanes). Yield (0.257 g). LRMS (ESI) $C_{27}H_{32}N_4O_5$ found $[M+H]^+$: 493.2.

[0253] (S)-3-(3-Cyanophenyl)-2-(naphthalene-2-sulfonamido)propanoic acid (**20B**). Prepared from methyl (S)-2-amino-3-(3-cyanophenyl)propanoate (**19B**) (4.1 g, 20.1 mmol) using **General Procedure C** to give methyl (S)-3-(3-cyanophenyl)-2-(naphthalene-2-sulfonamido)propanoate. Yield (8.19 g). LRMS (ESI) $C_{21}H_{18}N_2O_4S$ found $[M+H]^+$: 395.1. A solution of the crude ester in 1M HCl/AcOH (2:1) was refluxed for ~8 hours until no starting material was remaining by LCMS. After cooling to room temperature, the precipitate was

filtered to give the title compound, Yield (8.0 g). LRMS (ESI) $C_{20}H_{16}N_2O_4S$ found $[M+H]^+$: 381.1.

[0254] (S)-N-(3-(3-Cyanophenyl)-1-oxo-1-(piperazin-1-yl)propan-2-yl)naphthalene-2-sulfonamide (21B). Using **General Procedure A**, the crude **20B** (2.0 g, 5.26 mmol) and N-tert-butoxycarbonyl-piperazine (0.98 g, 5.26 mmol) were combined to give tert-butyl (S)-4-(3-(3-cyanophenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperazine-1-carboxylate after MPLC (0-75% EtOAc/hexanes), Yield (2.15 g). LRMS (ESI) $C_{29}H_{32}N_4O_5S$ found $[M+H]^+$: 549.2. To a solution of the product in 1,4-dioxane was added HCl and stirred at room temperature overnight. After concentrating in vacuo, the residue was dissolved in MeOH and then concentrated to dryness. Purification by MPLC (0-10% MeOH/DCM gave the title product, Yield (0.8 g). 1H NMR (300 MHz, *dms**o*-*d*₆) δ 2.30 - 2.47 (m, 2 H) 2.71 (dd, *J*=13.46, 9.07 Hz, 2 H) 2.87 (dd, *J*=13.46, 5.77 Hz, 2 H) 3.09 - 3.21 (m, 1 H) 3.31 - 3.43 (m, 3 H) 4.51 (dd, *J*=8.65, 6.46 Hz, 1 H) 7.33 (t, *J*=7.83 Hz, 1 H) 7.49 (d, *J*=7.69 Hz, 2 H) 7.57 - 7.74 (m, 5 H) 7.93 - 8.10 (m, 3 H) 8.24 (s, 1 H) ppm. LRMS (ESI) $C_{24}H_{24}N_4O_3S$ found $[M+H]^+$: 449.2.

[0255] (S)-N-Benzyl-4-(3-(3-carbamimidoylphenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperazine-1-carboxamide (17a). Prepared from (S)-4-(3-(3-(N-acetoxycarbamidoyl)phenyl)-2-(naphthalene-2-sulfonamido)propanoyl)-N-benzylpiperazine-1-carboxamide using **General Procedure F**. Purification by reverse phase HPLC (10-60% acetonitrile/water/0.05% TFA). Yield: 24.2 mg (44%). 1H NMR (300 MHz, *dms**o*-*d*₆) δ ppm 2.56 - 2.83 (m, 3 H) 2.86 - 3.10 (m, 3 H) 3.18 (br. s., 2 H) 3.79 - 4.02 (m, 1 H) 4.17 (m, 2 H) 4.56 (m, 2 H) 6.96 (s, 1 H) 7.02 - 7.08 (m, 2 H) 7.13 - 7.22 - 7.31 (m., 3 H) 7.43 (m, 2 H) 7.51 - 7.77 (m, 4 H) 7.97 (br. s., 1 H) 8.00 - 8.21 (m, 2 H) 8.22 - 8.49 (m, 2 H) 8.92 (m, 2 H) 8.63 (br. S. 1 H) 9.29 (br s, 1 H). LRMS (ESI) $C_{32}H_{34}N_6O_4S$ found $[M+H]^+$: 599.2.

[0256] (S)-4-(3-(3-(N-Acetoxycarbamidoyl)phenyl)-2-(naphthalene-2-sulfonamido)propanoyl)-N-benzylpiperazine-1-carboxamide. Prepared from (S)-N-benzyl-4-(3-(3-(N-hydroxycarbamidoyl)phenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperazine-1-carboxamide using **General Procedure E**. LRMS (ESI) $C_{34}H_{36}N_6O_6S$ found $[M+H]^+$: 657.2.

[0257] (S)-N-Benzyl-4-(3-(3-(N-hydroxycarbamidoyl)phenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperazine-1-carboxamide. Prepared from (S)-N-benzyl-4-(3-(3-

cyanophenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperazine-1-carboxamide (54.4 mg, 0.094 mmol) using **General Procedure D**. LRMS (ESI) $C_{34}H_{36}N_6O_6S$ found $[M+H]^+$: 615.2.

[0258] (S)-N-Benzyl-4-(3-(3-cyanophenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperazine-1-carboxamide. To a solution of **21B** (50 mg, 0.119 mmol) in THF (1 mL) under a nitrogen atmosphere was added benzyl isocyanate (41 μ L, 0.335 mmol). The reaction mixture was stirred overnight at room temperature. The reaction was concentrated in vacuo and the residue purified by MPLC (10-75 % EtOAc/hexanes) to give the title compound. Yield (54.4 mg). LRMS (ESI) $C_{32}H_{31}N_5O_4S$ found $[M+H]^+$: 582.2.

[0259] (S)-N-(4-Bromobenzyl)-4-(3-(3-carbamimidoylphenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperazine-1-carboxamide (25a). Prepared from (S)-4-(3-(3-(N-acetoxycarbamidoyl)phenyl)-2-(naphthalene-2-sulfonamido)propanoyl)-N-(4-bromobenzyl)piperazine-1-carboxamide using **General Procedure F**. Purification by reverse phase HPLC (25-75% acetonitrile/water/0.05% TFA). Yield: 9.8 mg (12%). 1H NMR (300 MHz, *dms**o*-*d*₆) δ ppm 2.56 - 2.83 (m, 3 H) 2.83 - 3.00 (m, 2 H) 3.04 (d, *J*=9.39 Hz, 1 H) 3.11 - 3.28 (m, 2 H) 3.39 - 3.51 (m, 1 H) 3.57 - 3.76 (m, 1 H) 4.11 (d, *J*=5.48 Hz, 1 H) 4.42 - 4.61 (m, 1 H) 6.99 - 7.27 (m, 3 H) 7.27 - 7.52 (m, 4 H) 7.52 - 7.74 (m, 4 H) 7.82 (s, 1 H) 7.89 - 8.17 (m, 3 H) 8.17 - 8.43 (m, 2 H) 9.09 (s, 2 H) 9.32 (s, 2 H). LRMS (ESI) $C_{32}H_{33}BrN_6O_4S$ found $[M+H]^+$: 677.1/679.1.

[0260] (S)-4-(3-(3-(N-Acetoxycarbamidoyl)phenyl)-2-(naphthalene-2-sulfonamido)propanoyl)-N-(4-bromobenzyl)piperazine-1-carboxamide. Prepared from (S)-N-(4-bromobenzyl)-4-(3-(3-(N-hydroxycarbamidoyl)phenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperazine-1-carboxamide using **General Procedure E**. LRMS (ESI) $C_{34}H_{35}BrN_6O_6S$ found $[M+H]^+$: 635.1/637.1.

[0261] (S)-N-(4-Bromobenzyl)-4-(3-(3-(N-hydroxycarbamidoyl)phenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperazine-1-carboxamide. Prepared from (S)-N-(4-bromobenzyl)-4-(3-(3-cyanophenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperazine-1-carboxamide (78 mg, 0.112 mmol) using **General Procedure D**. LRMS (ESI) $C_{32}H_{33}BrN_6O_5S$ found $[M+H]^+$: 693.1/695.1.

[0262] (S)-N-(4-Bromobenzyl)-4-(3-(3-cyanophenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperazine-1-carboxamide. To a solution of **21B** (50 mg, 0.115 mmol) in THF (1 mL) under a nitrogen atmosphere was added 4-bromobenzyl isocyanate (32 μ L, 0.230 mmol). The reaction mixture was stirred overnight at room temperature. The

reaction was concentrated in vacuo and the triturated with THF, filtered, and dried to give the title compound. Yield (78 mg). LRMS (ESI) $C_{32}H_{30}BrN_5O_4S$ found $[M+H]^+$: 660.1/662.1.

[0263] (S)-4-(3-(3-Carbamimidoylphenyl)-2-(naphthalene-2-sulfonamido)propanoyl)-N-hexylpiperazine-1-carboxamide (25d). Prepared from (S)-4-(3-(3-(N-acetoxycarbamimidoyl)phenyl)-2-(naphthalene-2-sulfonamido)propanoyl)-N-hexylpiperazine-1-carboxamide using **General Procedure F**. The crude product was purified using MPLC (0-10% MeOH/DCM) to give the title compound. Yield: 20 mg (50%). 1H NMR (300 MHz, $dmsO-d_6$) δ ppm 0.75 - 0.93 (m, 3 H) 1.12 - 1.31 (m, 5 H) 1.72 (s, 3 H) 2.54 (s, 4 H) 2.62 - 2.74 (m, 2 H) 2.86 (d, $J=7.43$ Hz, 1 H) 2.93 (d, $J=7.04$ Hz, 2 H) 3.00 (s, 1 H) 3.50 (s, 1 H) 4.28 (br. s., 1 H) 4.45 (br. s., 1 H) 4.56 (d, $J=7.43$ Hz, 1 H) 6.41 (br. s., 2 H) 6.54 - 6.79 (m, 2 H) 6.95 (d, $J=8.61$ Hz, 1 H) 7.14 (d, $J=7.04$ Hz, 1 H) 7.23 (br. s., 1 H) 7.34 (br. s., 1 H) 7.54 (br. s., 1 H) 7.57 - 7.75 (m, 2 H) 7.89 - 8.13 (m, 2 H) 8.22 - 8.38 (m, 1 H) 8.49 (s, 1 H). LRMS (ESI) $C_{31}H_{40}N_6O_4S$ found $[M+H]^+$: 593.3.

[0264] (S)-4-(3-(3-(N-Acetoxycarbamimidoyl)phenyl)-2-(naphthalene-2-sulfonamido)propanoyl)-N-hexylpiperazine-1-carboxamide. Prepared from (S)-N-hexyl-4-(3-(3-(N-hydroxycarbamimidoyl)phenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperazine-1-carboxamide using **General Procedure E**. LRMS (ESI) $C_{33}H_{42}N_6O_6S$ found $[M+H]^+$: 651.3.

[0265] (S)-N-Hexyl-4-(3-(3-(N-hydroxycarbamimidoyl)phenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperazine-1-carboxamide. Prepared from (S)-4-(3-(3-cyanophenyl)-2-(naphthalene-2-sulfonamido)propanoyl)-N-hexylpiperazine-1-carboxamide (40 mg, 0.069 mmol) using **General Procedure D**. LRMS (ESI) $C_{31}H_{40}N_6O_5S$ found $[M+H]^+$: 609.3.

[0266] (S)-4-(3-(3-Cyanophenyl)-2-(naphthalene-2-sulfonamido)propanoyl)-N-hexylpiperazine-1-carboxamide. To a solution of **21B** (50 mg, 0.115 mmol) in THF (1 mL) under a nitrogen atmosphere was added hexyl isocyanate (49 μ L, 0.335 mmol). The reaction mixture was stirred overnight at room temperature. The reaction was concentrated in vacuo and the residue purified by MPLC (10-70% EtOAc/hexanes) to give the title compound. Yield (40 mg). LRMS (ESI) $C_{31}H_{37}N_5O_4S$ found $[M+H]^+$: 576.3.

[0267] (S)-3-(3-(4-(Benzo[b]thiophene-3-carbonyl)piperazin-1-yl)-2-(naphthalene-2-sulfonamido)-3-oxopropyl)benzimidamide (25e). Prepared from (S)-N-acetoxy-3-(3-(4-(benzo[b]thiophene-3-carbonyl)piperazin-1-yl)-2-(naphthalene-2-sulfonamido)-3-oxopropyl)benzimidamide using **General Procedure F**. Purification by

reverse phase HPLC (5-60% acetonitrile/water/0.05% TFA). Yield: 11 mg (21%). ^1H NMR (300 MHz, $\text{dms}\text{-}d_6$) δ ppm 2.71 (br. s., 1 H) 2.74 - 2.86 (m, 1 H) 2.87 - 2.96 (m, 1 H) 3.00 (br. s., 1 H) 3.15 (d, $J=19.17$ Hz, 2 H) 3.92 (s, 2 H) 4.54 (s, 2 H) 6.57 (s, 2 H) 7.04 - 7.20 (m, 1 H) 7.20 - 7.34 (m, 1 H) 7.34 - 7.54 (m, 2 H) 7.59 (br. s., 1 H) 7.63 - 7.77 (m, 2 H) 7.87 (s, 2 H) 7.97 - 8.22 (m, 3 H) 8.33 (br. s., 1 H) 8.39 (m, 1 H) 8.60 (m, 1 H) 8.90 (m, 2 H) 9.25 (m, 2 H). LRMS (ESI) $\text{C}_{33}\text{H}_{31}\text{N}_5\text{O}_4\text{S}_2$ found $[\text{M}+\text{H}]$: 626.2.

[0268] (S)-N-Acetoxy-3-(3-(4-(benzo[b]thiophene-3-carbonyl)piperazin-1-yl)-2-(naphthalene-2-sulfonamido)-3-oxopropyl)benzimidamide. Prepared from (S)-1-(3-(4-(benzo[b]thiophene-3-carbonyl)piperazin-1-yl)-2-(naphthalene-2-sulfonamido)-3-oxopropyl)-N-hydroxy-114-pyran-3-carboximidamide using **General Procedure E**. LRMS (ESI) $\text{C}_{35}\text{H}_{33}\text{N}_5\text{O}_6\text{S}_2$, found $[\text{M}+\text{H}]$: 684.2.

[0269] (S)-1-(3-(4-(Benzo[b]thiophene-3-carbonyl)piperazin-1-yl)-2-(naphthalene-2-sulfonamido)-3-oxopropyl)-N-hydroxy-114-pyran-3-carboximidamide. Prepared from (S)-N-(1-(4-(benzo[b]thiophene-3-carbonyl)piperazin-1-yl)-3-(3-cyanophenyl)-1-oxopropan-2-yl)naphthalene-2-sulfonamide (51 mg, 0.0139 mmol) using **General Procedure D**. LRMS (ESI) $\text{C}_{33}\text{H}_{31}\text{N}_5\text{O}_5\text{S}_2$, found $[\text{M}+\text{H}]$: 642.2.

[0270] (S)-N-(1-(4-(Benzo[b]thiophene-3-carbonyl)piperazin-1-yl)-3-(3-cyanophenyl)-1-oxopropan-2-yl)naphthalene-2-sulfonamide. To a solution of 3-carboxybenzothiophene (18 mg, 0.101 mmol), HBTU (42 mg, 0.115 mmol), and DIPEA (88 μL , 0.506 mmol) in THF (1.5 mL) under a nitrogen atmosphere, was added **21B** (50 mg, 0.115 mmol). The reaction mixture was stirred overnight at room temperature. The reaction was concentrated in vacuo and the residue purified by MPLC (10-70% EtOAc/hexanes) to give the title compound. Yield (51 mg). LRMS (ESI) $\text{C}_{33}\text{H}_{28}\text{N}_4\text{O}_4\text{S}_2$ found $[\text{M}+\text{H}]$: 609.2.

[0271] (S)-3-(3-Oxo-3-(4-phenylpiperazin-1-yl)-2-((2,4,6-triisopropylphenyl)sulfonamido)propyl)benzimidamide (27). Prepared from (S)-N-acetoxy-3-(3-oxo-3-(4-phenylpiperazin-1-yl)-2-((2,4,6-triisopropylphenyl)sulfonamido)propyl)benzimidamide using **General Procedure F**. Purified by reversed-phase HPLC (5-80% acetonitrile/water/0.05% TFA), Yield (0.166g, 42.8%). ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 9.08-9.47 (m, 4H), 8.15-7.91 (d, 1H), 7.57-7.74 (m, 2H), 7.39-7.56 (m, 2H), 7.07 - 7.29 (m, 4H), 6.71-6.91 (m, 3H), 4.41 - 4.64 (m, 1H), 3.93 - 4.20 (m, 2H), 3.27 - 3.52 (m, 2H), 3.15-3.27 (m, 2H), 2.99 - 3.15 (m, 2H), 2.78 - 2.99 (m, 4H), 2.59-2.78 (m, 2H), 0.98-1.31 (m, 18H). LRMS (ESI), $\text{C}_{35}\text{H}_{47}\text{N}_5\text{O}_3\text{S}$, $[\text{M}+\text{H}]$: 618.3.

[0272] (S)-N-Acetoxy-3-(3-oxo-3-(4-phenylpiperazin-1-yl)-2-((2,4,6-triisopropylphenyl)sulfonamido)propyl)benzimidamide. Prepared from (S)-N-hydroxy-3-(3-oxo-3-(4-phenylpiperazin-1-yl)-2-((2,4,6-triisopropylphenyl)sulfonamido)propyl)benzimidamide using **General Procedure E**. LRMS (ESI), $C_{37}H_{49}N_5O_5S$, [M+H]: 676.3.

[0273] (S)-N-Hydroxy-3-(3-oxo-3-(4-phenylpiperazin-1-yl)-2-((2,4,6-triisopropylphenyl)sulfonamido)propyl)benzimidamide. Prepared from (S)-N-(3-(3-cyanophenyl)-1-oxo-1-(4-phenylpiperazin-1-yl)propan-2-yl)-2,4,6-triisopropylbenzenesulfonamide using **General Procedure D**. LRMS (ESI), $C_{35}H_{47}N_5O_4S$, [M+H]: 634.3

[0274] (S)-N-(3-(3-Cyanophenyl)-1-oxo-1-(4-phenylpiperazin-1-yl)propan-2-yl)-2,4,6-triisopropylbenzenesulfonamide. Prepared from (S)-3-(3-cyanophenyl)-2-((2,4,6-triisopropylphenyl)sulfonamido)propanoic acid (0.36 g, 0.96 mmol)³⁵ and 4-phenylpiperazine (1.19 mmol) using **General Procedure A**. Purification by MPLC (0-50% EtOAc/hexanes), Yield (0.34 g, 72%). LRMS (ESI), $C_{35}H_{44}N_4O_3S$, [M+H]: 601.3

[0275] (S)-3-(2-(4-Methylphenylsulfonamido)-3-oxo-3-(4-phenylpiperidin-1-yl)propyl)benzimidamide (25b). Prepared from using **General Procedure F**. Yield (0.13 g, 55%). ¹H NMR (300 MHz, DMSO-*d*₆) δ 9.32 (s, 2H), 9.37 (s, 2H), 8.00 - 8.36 (m, 1H), 7.63 - 8.00 (m, 2H), 7.40 - 7.63 (m, 4H), 7.06 - 7.40 (m, 5H), 7.00 (d, *J* = 7.42 Hz, 1H), 4.41 - 4.73 (m, 1H), 4.21 - 4.39 (m, 1H), 3.92 (t, *J* = 9.75 Hz, 1H), 3.63 - 3.81 (m, 1H), 2.90 - 3.02 (m, 1H), 2.49 - 2.88 (m, 4H), 2.36 (s, 2H), 1.95 - 2.33 (m, 1H), 1.65 (d, *J* = 11.81 Hz, 1H), 1.39 - 1.60 (m, 1H), 1.08 - 1.39 (m, 1H), 0.78 - 1.08 (m, 1H). LRMS (ESI), $C_{28}H_{32}N_4O_3S$, [M+H]: 505.2.

[0276] (S)-N-Acetoxy-3-(2-((4-methylphenyl)sulfonamido)-3-oxo-3-(4-phenylpiperidin-1-yl)propyl)benzimidamide. Prepared from (S)-N-hydroxy-3-(2-((4-methylphenyl)sulfonamido)-3-oxo-3-(4-phenylpiperidin-1-yl)propyl)benzimidamide using **General Procedure E**. Purification by MPLC (0-90% EtOAc/hexanes). Yield (0.24 g, 89%). LRMS (ESI), $C_{30}H_{34}N_4O_5S$, [M+H]: 563.2.

[0277] (S)-N-Hydroxy-3-(2-((4-methylphenyl)sulfonamido)-3-oxo-3-(4-phenylpiperidin-1-yl)propyl)benzimidamide. Prepared from (S)-N-(3-(3-cyanophenyl)-1-oxo-1-(4-phenylpiperidin-1-yl)propan-2-yl)-4-methylbenzenesulfonamide using **General Procedure D**. LRMS (ESI), $C_{28}H_{32}N_4O_4S$, [M+H]: 521.2.

[0278] (S)-N-(3-(3-Cyanophenyl)-1-oxo-1-(4-phenylpiperidin-1-yl)propan-2-yl)-4-methylbenzenesulfonamide. Prepared from 3-[(2S)-2-amino-3-oxo-3-(4-phenylpiperidin-1-yl)propyl]benzonitrile (0.256 g, 0.69 mmol) using **General Procedure C**. Yield (0.23 g, 68%). LRMS (ESI), $C_{28}H_{29}N_3O_3S$, $[M+H]^+$: 488.2.

[0279] Benzyl (S)-1-((S)-3-(3-carbamimidoylphenyl)-2-(4-methylphenylsulfonamido)propanoyl)piperidin-3-ylcarbamate (18). Prepared from benzyl ((S)-1-((S)-3-(3-(N-acetoxycarbamimidoyl)phenyl)-2-(4-methylphenyl)sulfonamido)propanoyl)piperidin-3-yl)carbamate using **General Procedure F**. Yield (0.14 g, 44%). 1H NMR (300 MHz, DMSO- d_6) δ 9.13 - 9.47 (m, 4H), 7.60 - 7.77 (m, 2H), 7.40 - 7.59 (m, 4H), 7.12 - 7.40 (m, 7H), 5.00 (s, 1H), 4.29 - 4.60 (m, 1H), 3.95 - 4.15 (m, 1H), 3.62 - 3.85 (m, 2H), 3.56 (d, $J = 1.10$ Hz, 1H), 3.29 - 3.46 (m, 2H), 2.64 - 2.99 (m, 3H), 2.53 - 2.63 (m, 1H), 2.34 (s, 3H), 1.69 - 1.98 (m, 1H), 1.56 - 1.69 (m, 1H), 1.26 - 1.49 (m, 1H), 1.08 - 1.24 (m, 1H). LRMS (ESI), $C_{30}H_{35}N_5O_5S$, $[M+H]^+$: 578.2.

[0280] Benzyl ((S)-1-((S)-3-(3-(N-acetoxycarbamimidoyl)phenyl)-2-(4-methylphenyl)sulfonamido)propanoyl)piperidin-3-yl)carbamate. Prepared from benzyl ((S)-1-((S)-3-(3-(N-hydroxycarbamimidoyl)phenyl)-2-(4-methylphenyl)sulfonamido)propanoyl)piperidin-3-yl)carbamate using **General Procedure E**. LRMS (ESI), $C_{32}H_{37}N_5O_7S$, $[M+H]^+$: 636.2.

[0281] Benzyl ((S)-1-((S)-3-(3-(N-hydroxycarbamimidoyl)phenyl)-2-(4-methylphenyl)sulfonamido)propanoyl)piperidin-3-yl)carbamate. Prepared from benzyl ((S)-1-((S)-3-(3-cyanophenyl)-2-(4-methylphenyl)sulfonamido)propanoyl)piperidin-3-yl)carbamate using **General Procedure D**. LRMS (ESI), $C_{30}H_{35}N_5O_6S$, $[M+H]^+$: 594.2.

[0282] Benzyl ((S)-1-((S)-3-(3-cyanophenyl)-2-(4-methylphenyl)sulfonamido)propanoyl)piperidin-3-yl)carbamate. Prepared from benzyl ((S)-1-((S)-2-amino-3-(3-cyanophenyl)propanoyl)piperidin-3-yl)carbamate using **General Procedure C**. Purification by MPLC (0-75% EtOAc/hexanes). Yield (0.27 g, 69%). LRMS (ESI), $C_{30}H_{32}N_4O_5S$, $[M+H]^+$: 561.2.

[0283] Benzyl ((S)-1-((S)-2-amino-3-(3-cyanophenyl)propanoyl)piperidin-3-yl)carbamate. Prepared from benzyl ((S)-1-((S)-2-((tert-butoxycarbonyl)amino)-3-(3-cyanophenyl)propanoyl)piperidin-3-yl)carbamate using **General Procedure B**. LRMS (ESI), $C_{23}H_{26}N_4O_3$, $[M+H]^+$: 407.2.

[0284] Benzyl ((S)-1-((S)-2-((tert-butoxycarbonyl)amino)-3-(3-cyanophenyl)propanoyl)piperidin-3-yl)carbamate. Prepared from **19** (0.46 g, 1.59 mmol) and benzyl (S)-piperidin-3-ylcarbamate (0.48 g, 2.04 mmol) using **General Procedure A**. LRMS (ESI), $C_{28}H_{34}N_4O_5$, $[M+H]^+$: 507.2.

[0285] Benzyl (R)-1-((S)-3-(3-carbamimidoylphenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperidin-3-ylcarbamate (25f). Prepared from benzyl ((R)-1-((S)-3-(3-(N-acetoxycarbamimidoyl)phenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperidin-3-yl)carbamate using **General Procedure F**. Yield (0.136 g, 53%). 1H NMR (300 MHz, DMSO- d_6) δ 9.20 - 9.48 (m, 3H), 9.15 (br. s., 1H), 8.15 - 8.43 (m, 2H), 7.85 - 8.15 (m, 3H), 7.77 (br. s., 1H), 7.55 - 7.69 (m, 3H), 7.29 - 7.53 (m, 8H), 4.86 - 5.23 (m, 3H), 4.35 - 4.68 (m, 1H), 3.87 - 4.17 (m, 1H), 3.57 - 3.87 (m, 1H), 2.81 - 3.09 (m, 2H), 2.52 - 2.81 (m, 3H), 1.74 - 1.94 (m, 1H), 1.58 - 1.74 (m, 1H), 1.34 - 1.58 (m, 1H), 0.90 - 1.29 (m, 2H). LRMS (ESI), $C_{33}H_{35}N_5O_5S$, $[M+H]^+$: 614.2.

[0286] Benzyl ((R)-1-((S)-3-(3-(N-acetoxycarbamimidoyl)phenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperidin-3-yl)carbamate. Prepared from benzyl ((R)-1-((S)-3-(3-(N-hydroxycarbamimidoyl)phenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperidin-3-yl)carbamate using **General Procedure E**. LRMS (ESI), $C_{35}H_{37}N_5O_7S$, $[M+H]^+$: 672.2.

[0287] Benzyl ((R)-1-((S)-3-(3-(N-hydroxycarbamimidoyl)phenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperidin-3-yl)carbamate. Prepared from benzyl ((R)-1-((S)-3-(3-cyanophenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperidin-3-yl)carbamate using **General Procedure D**. LRMS (ESI), $C_{33}H_{35}N_5O_6S$, $[M+H]^+$: 630.2.

[0288] Benzyl ((R)-1-((S)-3-(3-cyanophenyl)-2-(naphthalene-2-sulfonamido)propanoyl)piperidin-3-yl)carbamate. Prepared from benzyl ((R)-1-((S)-2-amino-3-(3-cyanophenyl)propanoyl)piperidin-3-yl)carbamate using **General Procedure C**. Purification by MPLC (0-85% EtOAc/hexanes). Yield (0.22 g, 57%). LRMS (ESI), $C_{33}H_{32}N_4O_5S$, $[M+H]^+$: 597.2.

[0289] Benzyl ((R)-1-((S)-2-amino-3-(3-cyanophenyl)propanoyl)piperidin-3-yl)carbamate. Prepared from benzyl ((R)-1-((S)-2-((tert-butoxycarbonyl)amino)-3-(3-cyanophenyl)propanoyl)piperidin-3-yl)carbamate using **General Procedure B**. Yield (0.29 g). LRMS (ESI), $C_{23}H_{26}N_4O_3$, $[M+H]^+$: 407.2.

[0290] Benzyl ((R)-1-((S)-2-((tert-butoxycarbonyl)amino)-3-(3-cyanophenyl)propanoyl)piperidin-3-yl)carbamate. Prepared from **19** (0.42 g, 1.46 mmol) and

benzyl (R)-piperidin-3-ylcarbamate (0.48 g, 2.04 mmol) using **General Procedure A**. Purification by MPLC (5-80% EtOAc/hexanes), Yield (0.346 g, 47%). LRMS (ESI), $C_{28}H_{34}N_4O_5$, [M+H]: 507.2.

[0291] 1-((S)-3-(3-Carbamimidoylphenyl)-2-(4-methylphenyl)sulfonamido)propanoyl)-N-phenethylpiperidine-3-carboxamide (25c). Prepared from 1-((S)-3-(3-(N-acetoxycarbamimidoyl)phenyl)-2-(4-methylphenyl)sulfonamido)propanoyl)-N-phenethylpiperidine-3-carboxamide using **General Procedure F**. Yield (0.28 g, 39%). 1H NMR (300 MHz, DMSO- d_6) δ 9.52 - 9.78 (m, 2H), 9.30-9.52 (m, 2H), 8.03 - 8.40 (m, 2H), 7.76 - 7.98 (m, 2H), 7.49 - 7.76 (m, 4H), 7.24 - 7.49 (m, 7H), 4.51 - 4.78 (m, 1H), 3.90 - 4.36 (m, 1H), 3.78 - 3.90 (m, 1H), 3.59 - 3.68 (m, 1H), 3.29 - 3.46 (m, 2H), 3.00 - 3.19 (m, 1H), 2.69 - 3.00 (m, 4H), 2.56 - 2.69 (m, 1H), 2.01 - 2.25 (m, 1H), 1.80 - 2.01 (m, 1H), 1.23 - 1.80 (m, 3H), 0.30 - 1.21 (m, 1H). LRMS (ESI), $C_{31}H_{37}N_5O_4S$, [M+H]: 576.3.

[0292] 1-((S)-3-(3-(N-Acetoxycarbamimidoyl)phenyl)-2-(4-methylphenyl)sulfonamido)propanoyl)-N-phenethylpiperidine-3-carboxamide. Prepared from 1-((S)-3-(3-(N-hydroxycarbamimidoyl)phenyl)-2-(4-methylphenyl)sulfonamido)propanoyl)-N-phenethylpiperidine-3-carboxamide using **General Procedure E**. LRMS (ESI), $C_{33}H_{39}N_5O_6S$, [M+H]: 634.3.

[0293] 1-((S)-3-(3-(N-Hydroxycarbamimidoyl)phenyl)-2-(4-methylphenyl)sulfonamido)propanoyl)-N-phenethylpiperidine-3-carboxamide. Prepared from 1-((S)-3-(3-cyanophenyl)-2-(4-methylphenyl)sulfonamido)propanoyl)-N-phenethylpiperidine-3-carboxamide using **General Procedure D**. LRMS (ESI), $C_{31}H_{37}N_5O_5S$, [M+H]: 592.3.

[0294] 1-((S)-3-(3-Cyanophenyl)-2-(4-methylphenyl)sulfonamido)propanoyl)-N-phenethylpiperidine-3-carboxamide. Prepared from (S)-3-(3-cyanophenyl)-2-(4-methylphenyl)sulfonamido)propanoic acid (0.67 g, 1.95 mmol) and N-phenethylpiperidine-3-carboxamide (0.68 g, 2.53 mmol) using **General Procedure A**. Yield (0.96 g, 88%). LRMS (ESI), $C_{31}H_{34}N_4O_4S$, [M+H]: 559.2.

[0295] (S)-3-(3-Cyanophenyl)-2-(4-methylphenyl)sulfonamido)propanoic acid. To a solution of methyl (S)-3-(3-cyanophenyl)-2-(4-methylphenyl)sulfonamido)propanoate (1.85g, 5.17 mmol) in THF (30 mL) was added LiOH (0.80 g) in water (10 mL). The reaction was stirred at room temperature for 2 h. The organic layer was extracted with water and the

combined aqueous fractions were adjusted to pH 3 with conc. HCl and then extracted with EtOAc. The combined organic layers were dried over MgSO₄, filtered and the filtrate concentrated in vacuo to give the title compound. Yield (1.68 g, 95%). LRMS (ESI), C₁₇H₁₆N₂O₄S, [M+H]⁺: 345.1.

[0296] Methyl (S)-3-(3-cyanophenyl)-2-((4-methylphenyl)sulfonamido)propanoate.

Prepared from **19B** (1.53 g, 6.36 mmol) using **General Procedure C**. Yield (1.85 g, 81%). LRMS (ESI), C₁₈H₁₈N₂O₄S, [M+H]⁺: 359.1.

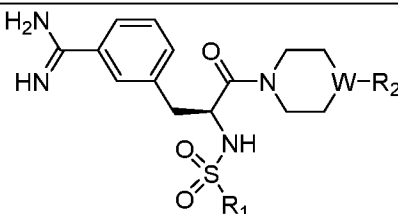
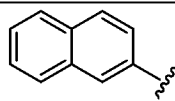
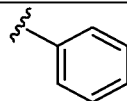
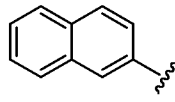
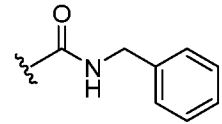
Example 9 Inhibition Studies with Benzamidine Compounds

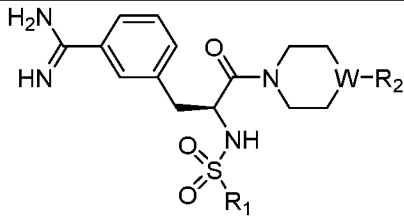
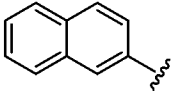
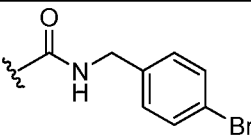
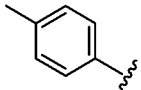
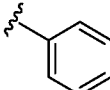
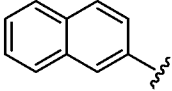
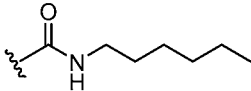
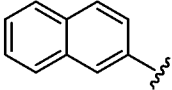
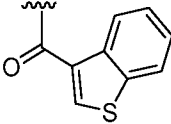
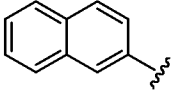
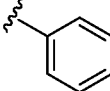
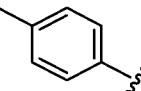
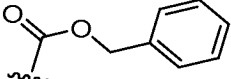
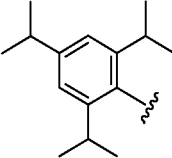
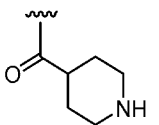
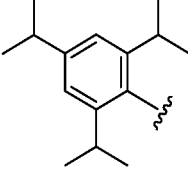
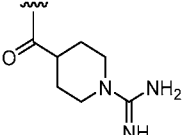
[0297] Fluorescent inhibitor and chromogenic proteolytic assays were performed in general accordance with the procedures described in Example 4 for compounds prepared in Example 8.

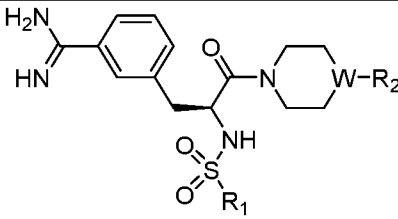
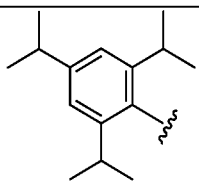
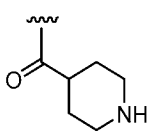
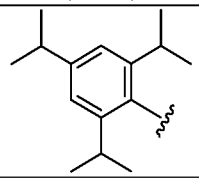
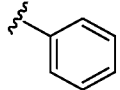
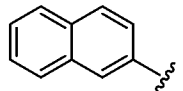
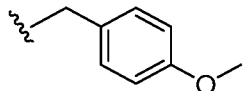
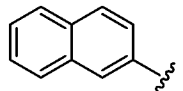
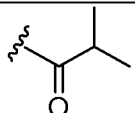
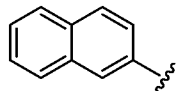
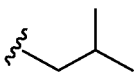
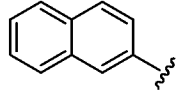
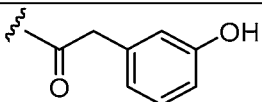
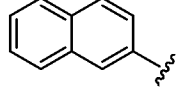
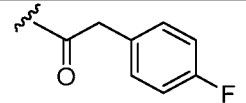
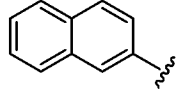
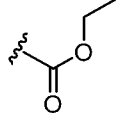
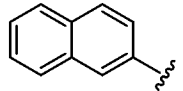
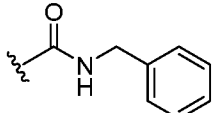
[0298] The results for these studies are presented in Tables 9.1, 9.2, and 9.3 and FIGS. 10-17.

[0299] The results for selective studies are presented in Table 9.4.

Table 9.1

						
Compound No.	R ₁	R ₂	W	Fluorogenic Assay		
				HGFA K _i (μM)	Matriptase K _i (μM)	Hepsin K _i (μM)
16 (DEJ-1-69-002)			C	10.1	0.3	3.28
17a (FMF-1-279-003)			N	14.0	2.06	1.0

						
Compound No.	R ₁	R ₂	W	Fluorogenic Assay		
				HGFA K _i (μM)	Matriptase K _i (μM)	Hepsin K _i (μM)
25a (FMF-I-236-003)			N	16.2	4.2	0.048
25b (DEJ-1-65-001)			C	19.85	0.57	4.8
25d (FMF-1-278-003)			N	>50	14.9	1.3
25e (FMF-1-281-003)			N	>50	1.2	3.75
25g (DEJ-1-63-001)			N	18.3	1.8	7.0
25h (DEJ-1-112-004)			N	>50	0.43	2.4
26 (JJ-I-134-2)			N	5.5	0.12	8.14
JWJ-I-135			N	17.84	0.0043	1.58

						
Compound No.	R ₁	R ₂	W	Fluorogenic Assay		
				HGFA K _i (μM)	Matriptase K _i (μM)	Hepsin K _i (μM)
JJ-I-134-2			N	5.45	0.12	8.13
27 (DEJ-1-173-004)			N	22.2	0.76	7.4
28 (FMF-1-238-003)			N		2.286-002	
29 (FMF-1-276-003)			N		>20	>20
30 (FMF-1-277-004)			N		12.4	4.9
31 (FMF-1-280-003)			N		4.4	1.7
32 (FMF-1-237-003)			N		2.8	1.0
FMF-1-275-004			N		2.78	39.5
FMF-1-279-003			N	14.03	2.56	1.04

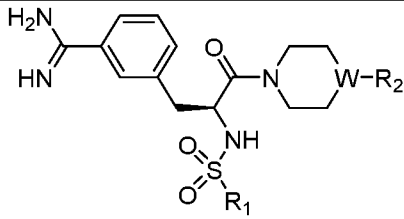
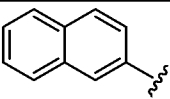
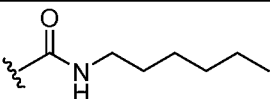
						
Compound No.	R ₁	R ₂	W	Fluorogenic Assay		
				HGFA K _i (μM)	Matriptase K _i (μM)	Hepsin K _i (μM)
FMF-1-278-003			N	>50	14.87	1.28

Table 9.2

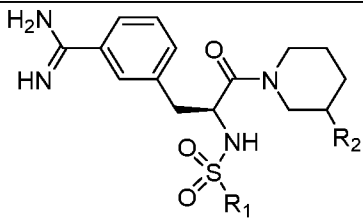
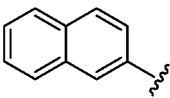
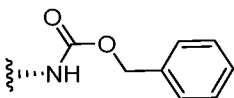
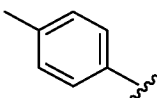
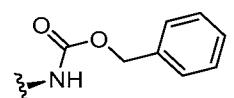
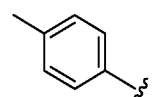
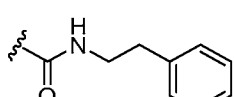
					
Compound No.	R ₁	R ₂	Fluorogenic Assay		
			HGFA K _i (μM)	Matriptase K _i (μM)	Hepsin K _i (μM)
25f* (DEJ-1-86-002)			>50	4.9	2.8
18* (DEJ-1-85-002)			>50	1	2.3
25c* (DEJ-1-194-002)			>50	1.3	0.67

Table 9.3

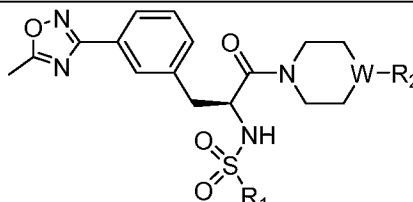
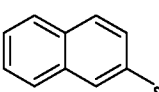
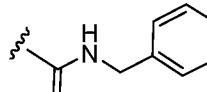
						
Compound No.	R ₁	R ₂	W	Fluorogenic Assay		
				HGFA K _i (μM)	Matriptase K _i (μM)	Hepsin K _i (μM)
(FMF-1-254-004)			N	>50	>10	>10

Table 9.4

Compound No.	Thrombin K _i (nM)	Factor Xa K _i (nM)	Trypsin K _i (nM)
FMF-1-236-003	3106.0	4564.0	2.20
JWJ-1-135	>2000	--	1.93

Example 10. Tumor microenvironment and drug sensitivity

[0300] The microenvironment of solid tumors is characterized by the presence of activated stromal cells, which produce an abundance of inflammatory mediators and growth factors that have been shown to play a critical role in tumor progression. Macrophages and myofibroblasts are two major components of the tumor microenvironment. HGF and other factors produced by macrophages and myofibroblasts that modulate oncogenic signaling pathways in tumor cells, include MET were investigated. An objective of this example is to understand the nature of tumor-derived factors that are responsible for activation of stromal cells and determine how the presence of genetic alterations in tumor cells alters communication of tumor cells with the stroma. In addition to their effect on tumor growth, HGF or MSP from the tumor microenvironment are also important regulators of responsiveness of cancer cells to antineoplastic therapies.

[0301] RON phosphorylation and migration and invasion assays of breast cancer cells: RON receptor tyrosine kinase activation confers resistance to tamoxifen in the MCF7

and T47D breast cancer cell lines. MDA-MB-231 express RON but we have not been able to detect phosphorylated RON (pRON) following MSP stimulation.

[0302] Detection and quantification of pRON in T47D cells was performed using a sandwich ELISA assay. RON capture antibody (R&D Systems, AF691) was immobilized on high protein binding 96-well plates. Next, cell lysates are added to wells, followed by incubation with an anti-phosphotyrosine-HRP antibody and then addition of a TMB substrate. Optical density of wells was determined by reading plates at 450 nm in a plate reader.

[0303] It can be seen in FIG. 18 that RON phosphorylation in T47D cells following active MSP stimulation, or HGFA-mediated pro-MSP stimulation, results in similar levels of RON phosphorylation. Inhibition with the triplex HGFA, matriptase, hepsin inhibitor Nafamostat, reduces pRON to background levels.

[0304] Triple-negative MDA-MB-231 breast cancer cells co-express MET, hepsin and HGFA but lower levels of RON and matriptase while T47D cells have good expression of RON.

[0305] MRC5 fibroblasts were plated in the bottom well of a Boyden chamber, and incubated overnight to condition the attractant media. The following day, MDA-MB-231 or T47D cells +/- inhibitors were plated above on Matrigel coated inserts (8.0 μ m membrane inserts, 12-well; Corning 3422) and incubated in FBS media overnight. Inhibitors were added to fresh media and then cells were plated above on the inserts. The cells were fixed and stained at the 24 hour time point. The inserts were then removed and mounted on glass plates where the number of migrated cells were imaged and counted at 4 fields per membrane using a light microscope at 20x.

[0306] It can be seen in FIG. 19 that the two triplex inhibitors of HGFA, matriptase, and hepsin, 7115 and 7054 (1 μ M) abrogate the MRC5-mediated migration and invasion of MDA-MB-231 cells through Matrigel by blocking active HGF production. The inhibitors tested are as effective as HAI-1, an endogenous inhibitor of matriptase, hepsin, and HGFA.

Example 11. PS-SCL and MSP-MS substrate screening of natural and unnatural amino acids

[0307] **Characterization of hepsin and HGFA P4-P2 substrate specificity using HyCoSuL.** Each of the P₄, P₃, and P₂ sublibraries were screened at the 100 μ M concentration with hepsin either HGFA in a 100 μ L final volume per well (99 μ L of protease

in buffer + 1 μ L of 10 mM substrates mixture). The total time of the assay was 30 minutes, however only linear portions of the progress curve (5-15 minutes) were used for velocity (RFU/s) calculations. Each sublibrary screening was repeated at least 3-times and the average value calculated from each measurement was used to create the substrate specificity matrix – the best recognized amino acid in each position was set to 100%, and other amino acids were adjusted accordingly.

[0308] Recombinant human hepsin (10 μ g in 20 μ L, 4776-SE-010, R&D Systems) was added to the 100 μ L of activation buffer (0.1 M Tris, 0.15 M NaCl, 0.01 M CaCl₂, 0.05% TRITON-X, pH=8.0) and incubated at 37 °C for 24 hours (hepsin concentration in activation buffer 2 μ M). Once activated hepsin was diluted in assay buffer (0.05 M Tris, pH=9.0) to the final concentration of 4 nM and incubated at 37 °C for 15 minutes before added to a substrate.

[0309] HGFA enzyme was diluted in the assay buffer (0.15 M NaCl, 0.025 M Tris, 0.005 M CaCl₂, pH=8.0) to the final concentration of 10-20 nM (depending of sublibrary) and incubated at 25 °C for 30 minutes before added to a substrate.

[0310] Characterization of hepsin and HGFA P₁ substrate specificity using Ac-Ala-Arg-Leu-P₁-ACC individual substrate library: The 142-membered Ac-Ala-Arg-Leu-P₁-ACC fluorogenic substrate library containing 19 natural (except cysteine) and 123 unnatural amino acids was used to determine hepsin and HGFA preferences in P₁ position. The protocol for both enzymes activation and incubation are as the same as in HyCoSuL screening. The P₁ individual substrate library was used at the final concentration of 5 μ M. The total time of the assay was 30min, however only linear portions of the progress curve (5-15 minutes) were used for velocity (RFU/s) calculations. Each screening for hepsin either HGFA was repeated 3-times and the average value for each measurement was used to create the substrate specificity matrix.

[0311] The detailed protocol for the use of ACC-labeled combinatorial peptide libraries in protease substrate specificity screening can be found in: Poręba M, Szalek A, Kasperkiewicz P, Drag M., *Methods Mol Biol.* **2014**, 1133, 41-59, which is incorporated herein by reference.

[0312] Results for MSP-MS profiling for HGFA substrate cleavage and specificity can be seen in FIG. 20 and Tables 11.1-11.4. IceLogo graphs can be seen in FIG. 21A and 21B.

Table 11.1

P4-P4'-All	P4-P4'-Cleaved by HGFA (4hr)
ADARKYWN	AMFRKYPI
AGKRRDWX	ASMRIYIE
AMFRKYPI	EIFRKWHX
AMWRLDII	ESARDXXX
ASHRMGKN	EYFRMIRW
ASMRIYIE	FLVRTWKM
DAQRWKNI	FRIRSGTX
DEPRGHMY	FTQRAGIL
DQVRRMNX	IDLRWMAY
DSIRHQGP	KLFRFNWX
EIFRKWHX	NFLRGPXX
EKQRFHPX	NQMRGFXX
ELGRSANA	PQRRGMXX
ESARDXXX	RMIRWAVL
EVARPLGX	SLYRMIRQ
EYFRMIRW	SYMRWPXX
EYPRPQEW	TYFRAWXX
FDNRVGKW	VQHRLFTY
FHWRIMQG	WAFRSRYH
FLVRTWKM	WPQRRGMX
FPVRPTEX	XKARSAFA
FRIRSGTX	YAFRSTMV

Table 11.2

Amino Acid	P1 position	
	% activity	
	Hepsin	HGFA
L-Ala (A)	0.49	0.00
L-Arg (R)	100.00	100.00
L-Asn (N)	0.00	0.00
L-Asp (D)	0.00	0.00
L-Gln (Q)	0.00	0.00
L-Glu (E)	0.13	0.00
Gly (G)	0.07	0.00
L-His (H)	0.17	0.00
L-Ile (I)	0.08	0.00
L-Leu (L)	0.03	0.00
L-Lys (K)	2.34	0.99
L-Nle	0.07	0.00
L-Phe (F)	0.12	0.00
L-Pro (P)	0.04	0.00
L-Ser (S)	0.20	0.00
L-Thr (T)	0.45	0.00
L-Trp (W)	0.70	0.73
L-Tyr (Y)	0.30	0.00
L-Val (V)	0.00	0.00
L-MeAla	0.09	0.00
B-Ala	0.00	0.00
dhPro 2/2	0.00	0.00
L-Oic	0.00	2.10
L-Hyp	0.00	0.00
L-Hyp(Bzl)	0.00	0.30
L-Gla	0.00	0.00
L-Asp(Me)	2.77	3.11

Amino Acid	P1 position	
	% activity	
	Hepsin	HGFA
L-Asp(Oall)	2.34	0.00
L-Asp(Bzl)	0.93	12.28
L-Glu(Me)	0.41	0.00
L-Glu(All)	1.26	0.00
L-Glu(Ochx)	0.17	0.00
L-Glu(Bzl)	0.09	0.00
L-Aad	0.00	0.00
L-API	0.00	0.00
L-Dap	0.03	0.00
L-Orn	0.00	0.00
L-Cit	0.42	0.00
L-hCit	0.13	0.00
L-Lys(Ac)	0.00	0.00
L-Lys(tfa)	0.00	0.00
L-Lys(2-Cl-Z)	0.09	0.00
His(Bzl)	0.12	0.00
Arg(Me)	0.00	0.00
Arg(Me)₂ 1/2 sym	0.00	0.00
L-hArg	0.24	0.00
L-3-Pal	0.00	0.00
L-4-Pal	0.00	0.00
L-Phe(4-NH₂)	0.08	0.00
L-Phe(2-F)	0.04	0.00
L-Phe(3-F)	0.01	0.00
L-Phe(4-F)	0.08	0.00
L-Phe(3,4-F₂)	0.25	0.00
L-Phe(F₅)	0.00	0.00
L-Phe(2-Cl)	0.04	0.00

Amino Acid	P1 position	
	% activity	
	Hepsin	HGFA
L-Phe(3-Cl)	0.21	0.00
L-Phe(4-Cl)	0.07	0.00
L-Phe(3,4-Cl₂)	0.02	0.00
L-Phe(3-I)	0.00	0.00
L-Phe(4-I)	0.34	0.00
L-Phe(4-Br)	0.10	0.00
L-Phe(NO₂)	0.39	1.37
L-Phe(guan)	9.45	0.00
L-Phe(4-Me)	0.00	0.00
L-hPhe	0.16	0.00
L-Ala(2th)	0.28	0.42
L-Ser(Bzl)	0.30	0.00
L-Hse(Bzl)	0.06	0.00
L-Thr(Bzl)	0.04	0.00
L-Cys(Bzl)	0.00	0.00
L-Cys(Me-Bzl)	0.13	0.00
L-Cys(4-MeOBzl)	0.25	0.00
L-Tyr(Bzl)	0.45	0.00
L-Dht	0.00	0.00
L-Trp(Me)	0.13	0.48
L-Tyr(Me)	0.14	0.00
L-hTyr(Me)	0.00	0.56
L-Tyr(2,6-Cl-Bzl)	0.41	0.00
L-Abu(Bth)	0.00	0.00
L-Bip	0.13	0.00
L-Bpa	0.56	1.57
Nle(Obzl)	0.15	0.00
L-1-Nal	0.20	0.00

Amino Acid	P1 position	
	% activity	
	Hepsin	HGFA
L-2-Nal	0.61	0.00
L-Hse	0.00	0.00
L-Hnv	1.55	0.00
L-Met (M)	0.00	0.00
L-Met(O)	0.00	0.00
L-Met(O₂)	0.00	0.00
L-2-Abu	0.00	0.00
L-Nva	0.14	0.00
L-Tle	0.42	0.00
L-hLeu	0.00	0.00
L-2-Aoc	2.82	0.00
AC5C	0.15	6.43
L-Chg	0.00	0.00
L-Cha	0.00	0.00
L-hCha	0.21	0.00
L-Thyr	0.28	0.00
L-Inp	0.07	0.00
D-Ala	0.00	0.00
D-Asn	0.00	0.00
D-Asp	0.05	0.00
D-Gln	0.00	0.00
D-Glu	0.00	0.00
D-Leu	0.00	0.00
D-Lys	0.23	0.00
D-Phe	0.38	0.00
D-Pro	1.08	0.00
D-Phe	0.09	0.00
D-Ser	0.02	0.00

Amino Acid	P1 position	
	% activity	
	Hepsin	HGFA
D-Thr	0.24	0.00
D-Trp	0.50	0.00
D-Tyr	0.23	0.00
D-Pip	0.42	5.57
D-Tic	0.14	1.12
D-Gla	0.00	0.00
D-Chg	0.60	0.65
D-Cha	0.13	0.00
D-Phg	0.09	0.00
D-3-Pal	0.00	0.00
D-4-Pal	0.00	0.00
D-Phe(4-Me)	0.48	0.00
D-Phe(2-F)	0.00	0.00
D-Phe(3-F)	0.44	0.00
D-Phe(4-F)	0.00	0.00
D-Phe(3,4-F2)	0.31	0.00
D-Phe(F5)	0.34	0.00
D-Phe(2-Cl)	0.66	0.00
D-Phe(3-Cl)	0.12	0.00
D-Phe(4-Cl)	0.00	0.00
D-Phe(3,4-Cl2)	0.07	0.00
D-Phe(4-I)	0.32	0.00
D-Phe(4-Br)	0.11	4.00
D-Phe(4-NO2)	1.45	0.74
D-Ser(Bzl)	0.00	0.00
D-Thr(Bzl)	0.00	0.00
D-hPhe	0.04	0.00
D-Bip	0.30	0.00

Amino Acid	P1 position	
	% activity	
	Hepsin	HGFA
D-Bpa	0.34	0.00
D-1-Nal	0.23	0.49
D-2-Nal	0.11	0.00

Table 11.3

Amino Acid	P4 Position		P3 Position		P2 Position	
	% Activity		% Activity		% Activity	
	Hepsin	HGFA	Hepsin	HGFA	Hepsin	HGFA
L-Ala (A)	41.30	6.68	28.07	8.97	17.85	2.37
L-Arg (R)	80.32	23.62	70.75	43.98	57.23	2.08
L-Asn (N)	25.25	8.20	24.47	6.12	63.72	4.79
L-Asp (D)	0.22	1.90	3.89	2.81	0.33	1.21
L-Gln (Q)	29.82	9.01	58.69	15.98	12.19	3.35
L-Glu (E)	0.76	1.96	16.72	3.47	0.00	0.89
Gly (G)	29.19	10.84	26.14	10.42	0.00	1.14
L-His (H)	25.35	8.90	43.05	11.93	31.24	4.00
L-Ile (I)	47.02	14.09	11.37	9.06	45.68	4.94
L-Leu (L)	33.10	10.18	19.51	13.71	100.00	100.00
L-Lys (K)	75.43	18.29	80.71	44.63	46.70	1.04
L-Nle	40.93	11.76	24.09	19.15	62.04	45.52
L-Phe (F)	30.80	9.42	2.91	13.51	25.22	16.60
L-Pro (P)	56.00	10.30	2.09	2.04	13.10	2.21
L-Ser (S)	31.63	7.04	38.70	14.57	18.40	3.24
L-Thr (T)	29.29	7.67	35.34	14.51	70.75	8.87
L-Trp (W)	6.82	8.78	0.00	17.50	6.00	4.04
L-Tyr (Y)	22.39	16.47	7.75	11.59	22.79	3.17
L-Val (V)	43.08	11.66	16.77	9.51	51.85	8.33
D-Ala	18.44	7.11	48.09	9.84	0.00	0.00

Amino Acid	P4 Position		P3 Position		P2 Position	
	% Activity		% Activity		% Activity	
	Hepsin	HGFA	Hepsin	HGFA	Hepsin	HGFA
D-Arg	44.37	23.20	66.08	29.10	0.00	0.00
D-Asn	18.48	7.38	38.75	15.16	0.00	0.00
D-Asp	1.98	2.25	17.18	5.37	0.00	0.00
D-Gln	20.13	8.03	97.80	13.84	0.00	0.00
D-Glu	2.98	2.62	34.55	3.61	0.00	0.00
D-His	19.33	7.79	40.09	11.09	0.00	0.00
D-Leu	24.76	10.56	20.14	7.08	0.00	0.00
D-Lys	49.84	17.66	64.56	21.11	0.00	0.00
D-Phe	13.15	8.13	24.92	15.22	0.00	0.00
D-Pro	20.45	10.30	38.88	4.96	0.00	0.00
D-Ser	21.84	7.94	54.84	10.86	11.55	0.00
D-Phg	22.48	9.06	57.07	40.01	13.53	4.40
D-Thr	25.74	8.61	36.69	6.45	0.00	0.00
D-Trp	1.48	7.65	24.90	87.59	0.00	0.00
D-Tyr	12.66	10.09	29.94	17.32	0.00	0.00
D-Val	22.58	8.25	27.04	5.76	0.00	0.00
β-Ala	30.74	10.07	22.78	15.95	0.00	0.24
L-Hyp	37.81	10.66	4.70	2.18	38.67	3.95
L-Hyp(Bzl)	33.33	8.91	1.29	4.34	32.68	4.39
L-Thz	45.46	12.65	9.93	7.76	6.16	2.80
L-Oic	53.72	16.76	2.92	2.78	0.67	20.71
L-Idc	0.00	32.64	0.00	6.43	0.00	0.96
L-Pip	34.68	9.25	1.64	0.83	10.66	1.75
L-Tic	13.31	10.84	8.10	25.13	2.45	3.58
dhAbu	44.51	24.45	7.29	9.44	7.72	14.12
dhLeu	52.44	42.50	5.85	11.02	0.00	0.48
L-Dap	35.65	13.31	48.71	24.27	10.01	1.49
L-Dab	47.53	16.76	48.55	29.40	13.49	0.50

Amino Acid	P4 Position		P3 Position		P2 Position	
	% Activity		% Activity		% Activity	
	Hepsin	HGFA	Hepsin	HGFA	Hepsin	HGFA
L-Dab(Z)	96.34	16.55	31.58	21.38	20.86	4.84
L-Cit	36.68	6.44	29.53	13.41	32.27	6.32
L-hCit	40.51	10.53	27.31	19.71	36.25	7.59
L-Orn	84.52	20.23	79.70	54.04	78.48	0.00
L-Lys(TFA)	50.71	11.66	35.84	21.11	38.29	5.16
L-Lys(Ac)	35.02	8.71	29.15	16.02	23.04	7.36
L-Lys(2-ClZ)	41.91	47.56	2.55	14.21	21.20	29.53
L-Agp	98.08	80.01	97.35	74.18	35.36	11.83
L-Agb	37.96	11.59	26.80	19.62	3.53	1.05
L-Arg(NO2)	82.11	8.33	41.82	16.01	17.44	2.76
L-Arg(Z)2	61.73	16.44	50.93	33.31	52.91	4.93
L-hArg	56.24	22.07	47.00	100.00	57.96	5.83
L-His(3-Bom)	38.40	100.00	15.18	13.20	0.00	0.43
L-Phe(NH2)	24.64	9.11	11.65	17.32	30.51	2.45
L-Phe(guan)	35.43	20.91	9.10	42.11	34.36	11.74
L-Trp(Me)	0.00	2.49	0.00	18.14	7.81	1.12
L-Dht	1.56	10.19	0.00	58.65	13.91	20.10
L-Asp(Me)	18.55	9.31	13.81	10.52	0.00	0.75
L-Asp(Chx)	27.32	10.34	19.99	22.46	0.79	2.16
L-Asp(Bzl)	19.34	9.60	7.29	6.92	0.00	1.35
L-Glu(Me)	36.79	9.13	64.70	32.95	13.49	6.65
L-Glu(Chx)	45.93	11.19	43.91	30.08	14.72	6.42
L-Glu(Bzl)	60.31	13.89	50.15	33.35	36.33	6.88
L-Phe(2-F)	24.33	10.49	7.80	13.39	1.78	5.98
L-Phe(3-F)	24.03	10.21	10.43	15.12	30.55	11.72
L-Phe(4-F)	27.78	11.22	5.16	15.01	32.86	4.84
L-Phe(3,4-F2)	19.65	8.79	0.71	15.06	38.16	3.73
L-Phe(F5)	26.60	12.25	5.13	22.36	0.36	1.55

Amino Acid	P4 Position		P3 Position		P2 Position	
	% Activity		% Activity		% Activity	
	Hepsin	HGFA	Hepsin	HGFA	Hepsin	HGFA
L-Phe(2-Cl)	14.92	10.91	0.98	10.78	2.71	11.87
L-Phe(3-Cl)	9.26	10.25	0.35	21.45	33.86	12.49
L-Phe(4-Cl)	26.01	12.60	0.31	13.32	2.85	1.74
L-Phe(3,4-Cl₂)	2.40	9.92	0.00	16.15	0.00	2.22
L-Phe(4-Br)	5.08	13.65	0.00	13.96	2.42	2.41
L-Phe(4-Me)	15.51	11.98	2.39	16.82	6.44	0.97
L-3-Pal	31.27	7.69	28.37	11.00	17.32	4.29
L-4-Pal	33.68	9.27	21.91	14.57	0.11	4.34
L-Ala(2th)	35.30	11.16	11.54	20.51	10.54	11.48
L-Ala(Bth)	14.64	10.26	20.97	23.90	6.52	2.16
L-Bta	13.63	9.79	0.13	24.11	0.00	3.71
L-Abu	38.68	9.87	28.98	15.31	0.00	1.81
L-Abu(Bth)	38.68	11.84	4.21	21.63	0.64	14.29
L-Ser(Ac)	37.96	7.56	42.09	21.69	6.73	1.67
L-Ser(Bzl)	21.57	14.49	11.56	19.45	20.43	5.27
L-hSer	26.55	7.34	33.48	16.59	31.76	2.12
L-hSer(Bzl)	40.00	12.03	21.24	20.03	46.09	17.39
L-Thr(Bzl)	40.13	13.14	16.36	21.72	36.14	7.19
L-Cys(Bzl)	23.44	16.89	3.06	25.14	1.50	7.46
L-Cys(MeBzl)	15.53	13.63	0.87	17.21	3.49	6.24
L-Cys(4-MeOBzl)	12.84	13.88	0.10	16.76	7.07	9.44
L-Nle(O-Bzl)	91.83	16.90	82.17	30.67	0.00	0.00
L-Phg	34.01	11.24	28.27	23.25	48.33	14.18
L-hPhe	44.79	11.14	10.63	61.77	4.71	32.14
L-Chg	46.64	25.12	6.60	18.69	10.88	27.27
L-Cha	25.44	13.42	3.64	22.61	74.49	6.03
L-hCha	5.44	12.92	0.13	73.74	0.00	2.90

Amino Acid	P4 Position		P3 Position		P2 Position	
	% Activity		% Activity		% Activity	
	Hepsin	HGFA	Hepsin	HGFA	Hepsin	HGFA
L-Igl	25.47	10.62	0.00	52.15	2.20	19.79
L-1-Nal	5.18	6.36	0.00	23.07	3.00	4.19
L-2-Nal	1.34	11.72	0.00	12.75	0.00	5.10
L-Bpa	0.86	12.77	0.00	8.16	0.00	1.57
L-2-Aoc	19.98	12.15	12.74	33.06	8.90	14.37
L-hLeu	41.61	14.86	13.84	23.44	14.44	72.69
L-NptGly	27.66	8.34	9.92	12.95	37.46	53.86
L-Hnv	33.75	7.92	33.40	15.07	34.62	5.49
L-Tle	38.05	9.71	7.48	6.70	0.00	0.00
L-Tyr(Me)	31.38	9.54	4.55	17.41	10.89	1.79
L-Tyr(2,6Cl2-Z)	0.08	9.94	0.09	4.47	0.00	2.83
L-Tyr(Bzl)	3.10	9.39	0.00	14.03	0.28	3.71
L-Tyr(2-Br-Z)	22.51	12.67	8.05	17.09	7.72	2.39
L-hTyr	31.56	13.69	7.46	73.59	0.00	42.92
L-hTyr(Me)	36.30	10.62	10.00	25.48	0.00	23.13
L-Nva	41.99	11.78	25.85	17.60	58.40	39.80
L-Met(O)	22.86	6.64	68.16	16.63	11.40	1.68

Table 11.4

	Ac-Ala-Arg-Leu-Arg-ACC	NH ₂ -Leu-DTrp-Nle-Arg-ACC	Ac-His(3Bom)-Agp-hLeu-Arg-ACC	Ac-Agp-hArg-Leu-Arg-ACC
K_M	107.2	73.4	52.1	37.8
k_{cat}	0.337	1.30	1.47	1.56
k_{cat}/K_M	3,144	17,662	28,140	41,254

[0313] When introducing elements of the present invention or the preferred embodiments(s) thereof, the articles "a", "an", "the" and "said" are intended to mean that there are one or more of the elements. The terms "comprising", "including" and "having" are

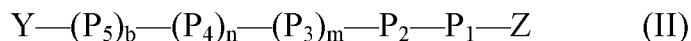
intended to be inclusive and mean that there may be additional elements other than the listed elements.

[0314] In view of the above, it will be seen that the several objects of the invention are achieved and other advantageous results attained.

[0315] As various changes could be made in the above compositions and methods without departing from the scope of the invention, it is intended that all matter contained in the above description and shown in the accompanying figures shall be interpreted as illustrative and not in a limiting sense.

WHAT IS CLAIMED IS:

1. A compound of Formula (II):



wherein

n is 0 or 1;

m is 0 or 1;

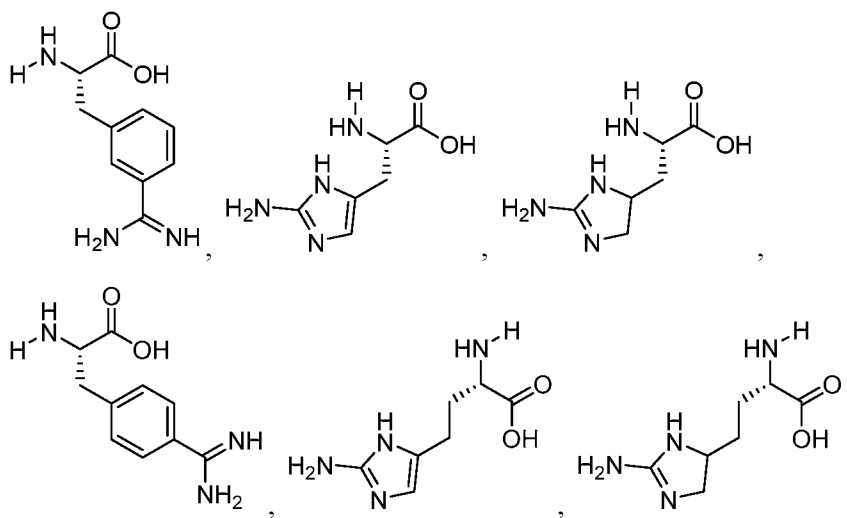
b is 0 or 1;

Y is H, acetyl, *tert*-butyloxycarbonyl, benzyloxymethyl acetyl, carboxybenzyl, fluorene, benzyl, -C(O)R₉, -SOOR₉, -COOR₉, -C(O)NHR₉, -(CH₂)_xaryl-R₉, heteroaryl-R₉, -cycloalkyl-R₉, or a fluorophore;

x is 0, 1, or 2;

R₉ is C₁ to C₁₂ alkyl, cycloalkyl, alkylaryl, or aryl;

P₁ is a residue of an amino acid selected from the group consisting of Arg, D-Arg, Lys, substituted Lys, and an alpha-amino acid of the following:



or an unnatural amino acid residue;

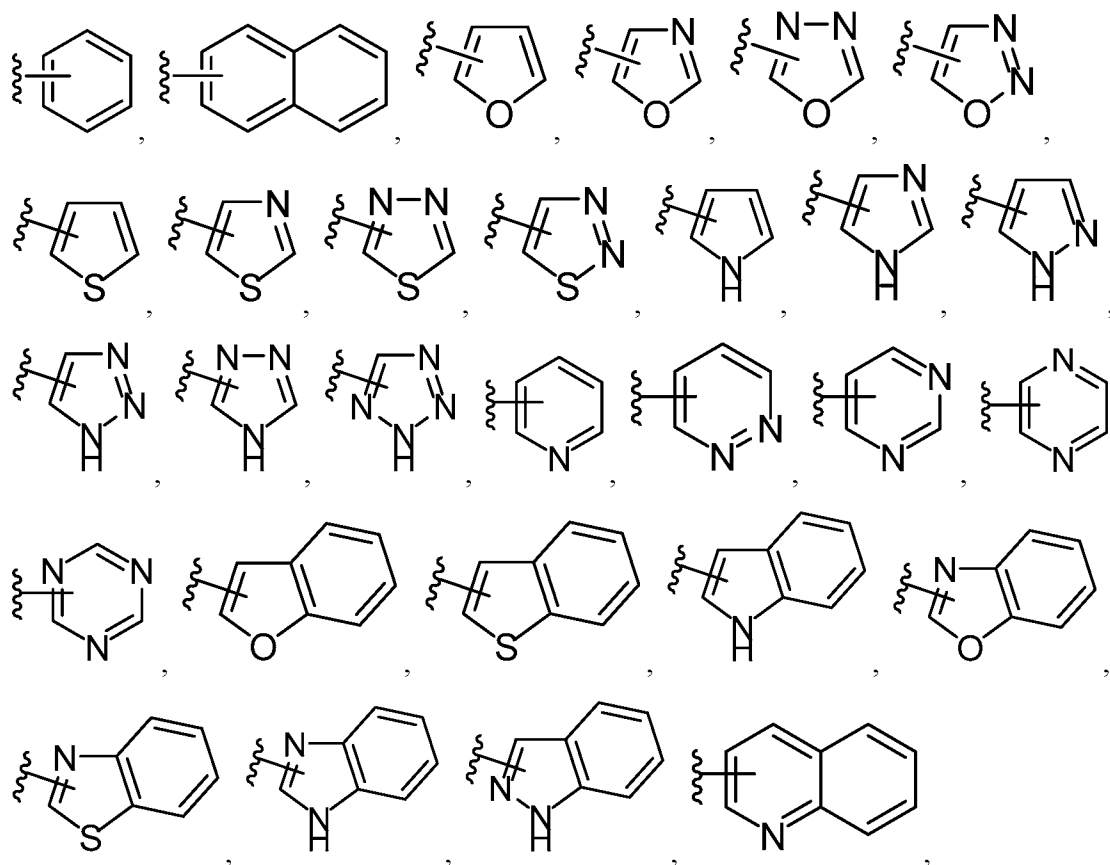
P₂ is a residue of an amino acid selected from the group consisting Leu, Phe, Met, Thr, Val, Tyr, Trp, Ser, Pro, His, Glu, Gln, Asp, Arg, Lys, Ile, Ala, Gly, Asn, hLeu, NptGly, L-Orn, L-Cha, Nle, hTyr, Nva, Orn, Cha, and an unnatural amino acid residue;

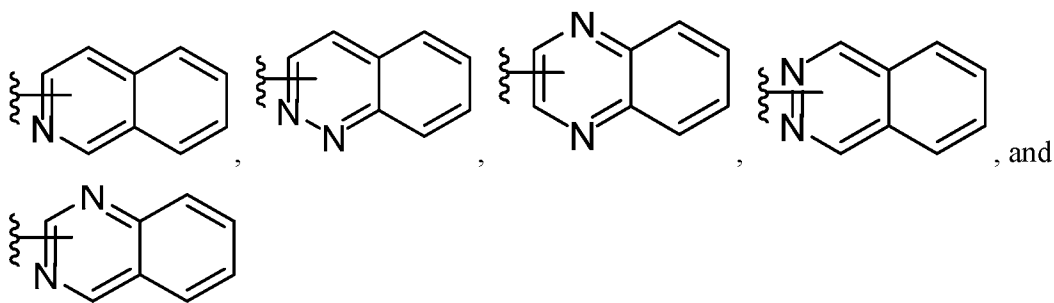
P₃ is a residue of an amino acid selected from the group consisting Asp, Glu, Arg, Lys, Met, Trp, Leu, Gln, Phe, Tyr, His, hArg, D-Trp, L-Orn, D-Gln, L-Met(O), L-Nle(OBzl), Agp, hCha, hTyr, hPhe, D-Arg, Nle(OBzl), Orn, Met(O), and an unnatural amino acid residue;

P₄ is a residue of an amino acid selected from the group consisting Arg, Lys, Met, Try, Trp, Ser, His, Phe, Thr, Asn, Pro, Gln, Asp, Glu, Chg, Idc, dhLeu, Agp, D-Ser, Agp, His(3-Bom), Lys(2-Cl-Z), L-Orn, L-Arg(NO₂), L-Nle(OBzl), L-DAB(Z) and an unnatural amino acid residue;

P₅ is a residue of an amino acid selected from the group consisting Lys, Arg, Leu, Phe, Met, Thr, Val, Tyr, Trp, Ser, Pro, His, Glu, Gln, Asp, Ile, Ala, Gly, Asn, and an unnatural amino acid residue; and

Z is Val, Ser, Lys, Ala, Gly, Trp, Tyr, Phe, Arg, Thr, Leu, Ile, Met, His, Nle, Phg, Pro, Gln, Asn, -CH₂Cl, or a substituted or unsubstituted ring substituent selected from the group consisting of:





2. The compound of claim 1 wherein the compound of Formula (II) includes one or more of the following:

Y is H, acetyl, *tert*-butoxycarbonyl, benzyloxymethyl acetyl, carboxybenzyl, fluorene, benzyl, -C(O)R₉, -SOOR₉, -COOR₉, -C(O)NHR₉, -(CH₂)_xaryl-R₉, heteroaryl-R₉, -cycloalkyl-R₉, or a fluorophore;

x is 0, 1, or 2;

R₉ is C₁ to C₁₂ alkyl, cycloalkyl, alkylaryl, or aryl;

P₁ is a residue of an amino acid selected from the group consisting Arg, D-Arg, Lys, and substituted Lys;

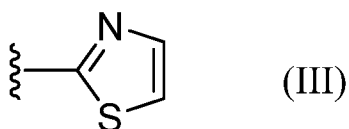
P₂ is a residue of an amino acid selected from the group consisting Leu, Phe, Met, Thr, Val, Tyr, Trp, Ser, Pro, His, Glu, Gln, Asp, Arg, Lys, Ile, Ala, Gly, Asn, hLeu, NptGly, L-Orn, L-Cha, Nle, hTyr, Nva, Orn, and Cha;

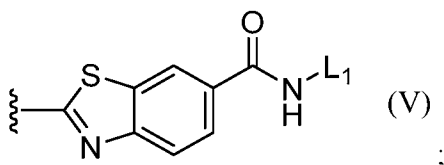
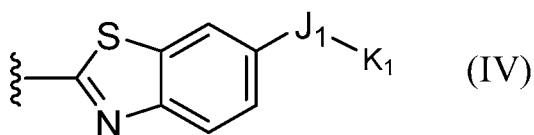
P₃ is a residue of an amino acid selected from the group consisting Asp, Glu, Arg, Lys, Met, Trp, Leu, Gln, Phe, Tyr, His, hArg, D-Trp, L-Orn, D-Gln, L-Met(O), L-Nle(OBzl), Agp, hCha, hTyr, hPhe, D-Arg, Nle(OBzl), Orn, and Met(O);

P₄ is a residue of an amino acid selected from the group consisting Arg, Lys, Met, Trp, Trp, Ser, His, Phe, Thr, Asn, Pro, Gln, Asp, Glu, Chg, Idc, dhLeu, Agp, D-Ser, Agp, His(3-Bom), Lys(2-Cl-Z), L-Orn, L-Arg(NO₂), L-Nle(OBzl), and L-DAB(Z);

P₅ is a residue of an amino acid selected from the group consisting Lys, Arg, Leu, Phe, Met, Thr, Val, Tyr, Trp, Ser, Pro, His, Glu, Gln, Asp, Ile, Ala, Gly, and Asn; and

Z is Val, Ser, Lys, Ala, Gly, Trp, Tyr, Phe, Arg, Thr, Leu, Ile, Met, His, Nle, Phg, Pro, Gln, Asn, -CH₂Cl, a thiazole of Formula (III), or a benzothiazole of Formula (IV) or (V)





J₁ is C(O), SO₂, CH₂, or heterocyclo;

K₁ is a D- or L-amino acid, wherein the C-terminus is -COOH, -C(O)NH₂, -OH, -OR₁₀, -NH₂, -NR₁₁R₁₂, -H, or heterocyclo;

R₁₀ is C₁ to C₁₂ alkyl, cycloalkyl, alkylaryl, or aryl;

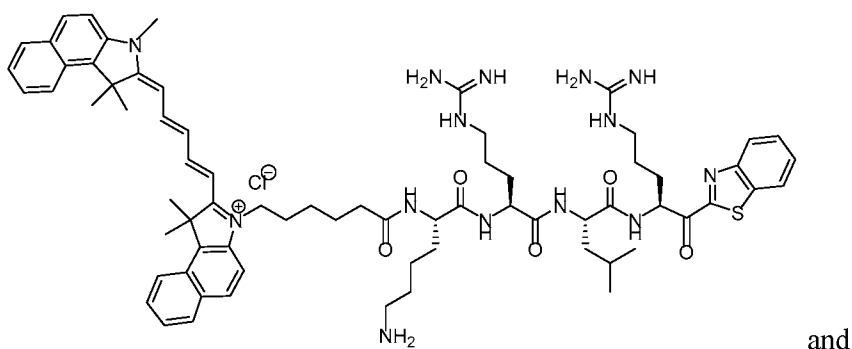
R₁₁ and R₁₂ are each independently H, C₁ to C₁₂ alkyl, cycloalkyl, alkylaryl, aryl, or heterocyclo; and R₁₁ and R₁₂ together can form a ring; and/or

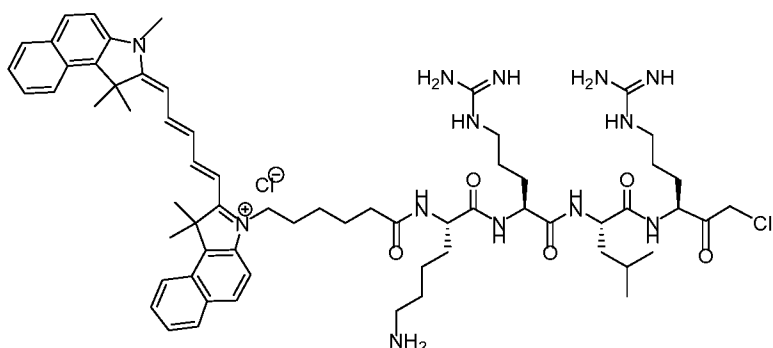
L₁ is H, alkyl, cycloalkyl, alkylaryl, benzyl, substituted benzyl, 2- or 3- or 4-piperdiny, 2- or 3- or 4-pyridiny, alkyl, cycloalkyl, aryl, heterocyclo, or heteroaryl.

3. The compound of claim 1 or 2 wherein P₁ is an amino acid residue of Arg.
4. The compound of any one of claims 1 to 3 wherein P₂ is an amino acid residue of Leu, Phe, Met, Tyr, Trp, hLeu, NptGly, Nle, hTyr, or Nva.
5. The compound of any one of claims 1 to 4 wherein P₃ is an amino acid residue of His, Gln, Arg, Lys, Leu, Phe, Trp, Tyr, hArg, D-Trp, Agp, hCha, hTyr, hPhe, or D-Arg.
6. The compound of any one of claims 1 to 5 wherein P₄ is an amino acid residue of Thr, Asn, Ser, Arg, Lys, Phe, Trp, His(Bom), Agp, Lys(2-Cl-Z), dhLeu, Idc, or Chg.
7. The compound of any one of claims 1 to 6 wherein P₁ is an amino acid residue of Arg or Lys.
8. The compound of any one of claims 1 to 7 wherein P₂ is an amino acid residue of Phe, Ala, Arg, Asn, Gln, Glu, Gly, His, Leu, Lys, Met, Pro, or Ser.

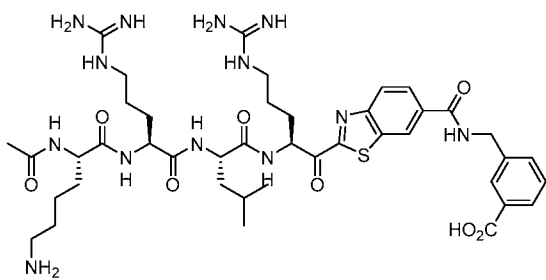
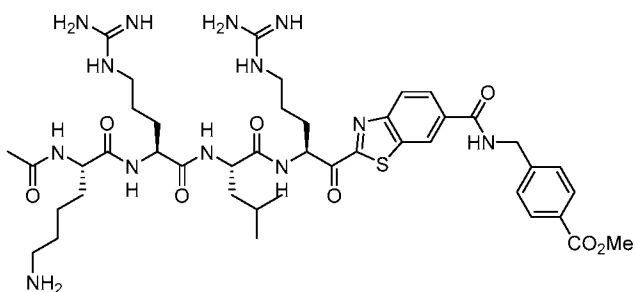
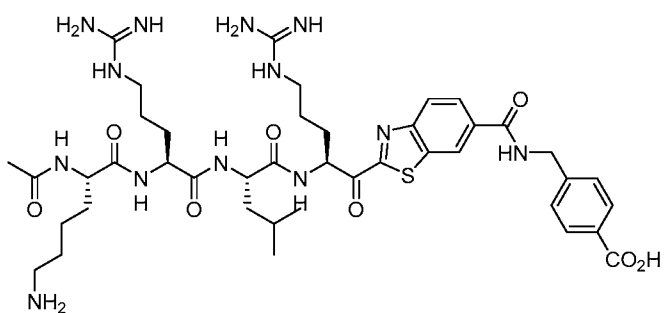
9. The compound of any one of claims 1 to 8 wherein P_3 is an amino acid residue of Arg, Leu, Trp, Phe, His, Gln, Lys, D-Trp, or D-Arg.
10. The compound of any one of claims 1 to 9 wherein P_4 is an amino acid residue of Pro, Phe, Thr, Asn, Trp, Gln, Ser, Lys, Arg, or His (Bom).
11. The compound of any one of claims 1 to 10 wherein P_1 is an amino acid residue of Arg.
12. The compound of any one of claims 1 to 11 wherein P_2 is an amino acid residue of Pro, Arg, Asn, Asp, Gln, Ile, Leu, Lys, Phe, Thr, Trp, Tyr, Orn, Cha, Nle, or Nva.
13. The compound of any one of claims 1 to 12 wherein P_3 is an amino acid residue of Leu, Trp, Phe, His, Gln, Lys, Arg, D-Gln, Agp, Nle (OBzl), Orn, Met(O), D-Trp, or D-Arg.
14. The compound of any one of claims 1 to 13 wherein P_4 is an amino acid residue of Pro, Phe, Thr, Asn, Trp, Gln, Ser, Arg, Lys, Agp, DAB(Z), Nle (OBzl), Orn, Arg(NO₂), or His(Bom).
15. The compound of any one of claims 1 to 14 wherein m is 1, n is 1, and P_4 - P_3 - P_2 - P_1 of Formula (II) is a tetrapeptide selected from the group consisting of SEQ ID NO 1, SEQ ID NO 2, SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 11, SEQ ID NO 12, SEQ ID NO 13, SEQ ID NO 14, SEQ ID NO 15, SEQ ID NO 16, SEQ ID NO 17, SEQ ID NO 18, SEQ ID NO 19, SEQ ID NO 20, and mixtures thereof.
16. The compound of any one of claims 1 to 15 wherein P_3 can form a bond with P_5 and form a cyclic peptide.
17. The compound of any one of claims 1 to 16 wherein P_2 can form a bond with P_4 and form a cyclic peptide.

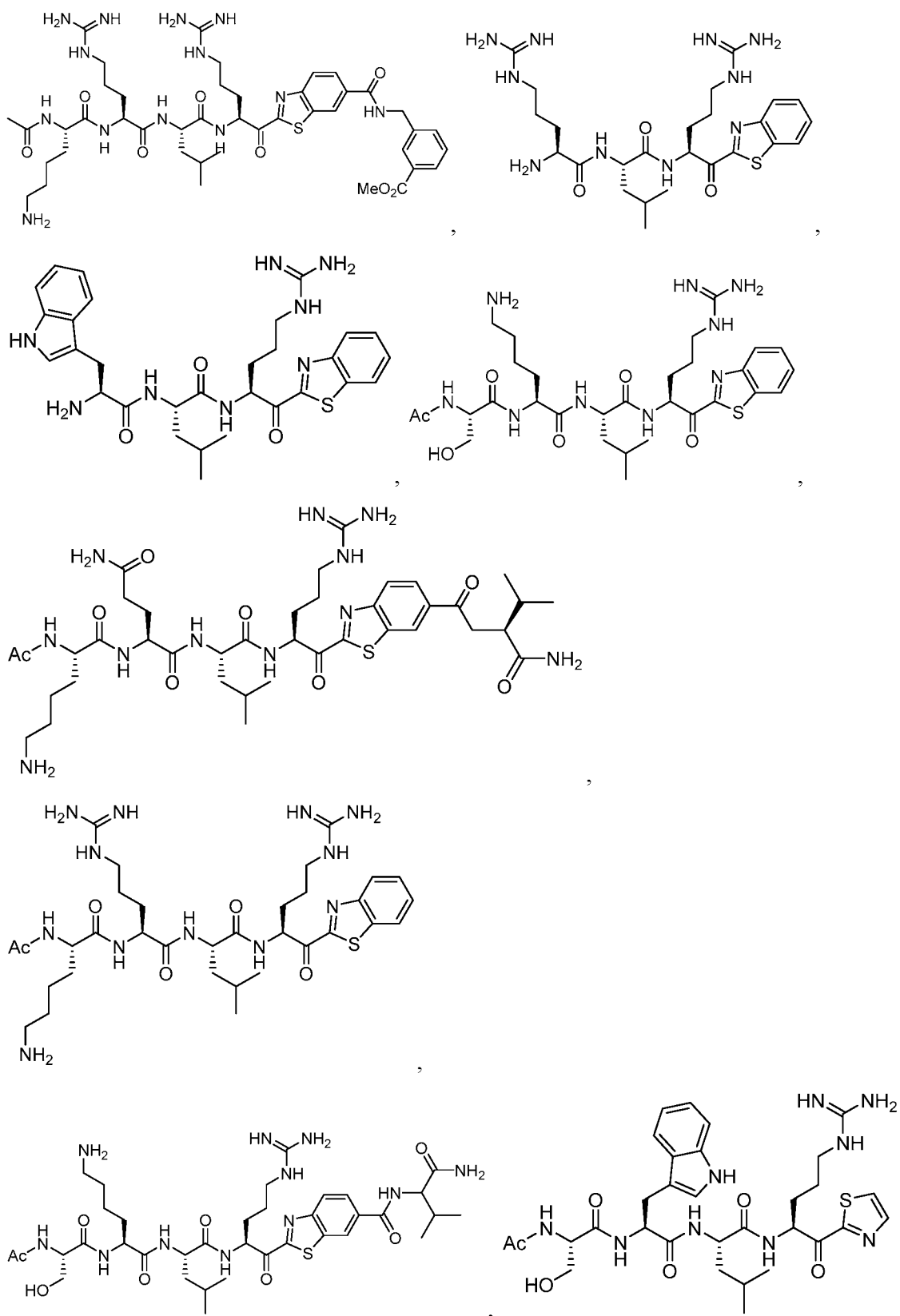
18. The compound of any one of claims 1 to 17 in P₂, P₃, P₄, and P₅ are independently selected from the group consisting of Asp, Glu, Lys, Tyr, 4-NO₂-3-F-Phe, or allyGly.
19. The compound of any one of claims 1 to 18 wherein Z is a benzothiazole of Formula (IV).
20. The compound of any one of claims 1 to 19 wherein Z is a benzothiazole of Formula (V).
21. The compound of any one of claims 1 to 20 wherein L₁ is a substituted benzyl group.
22. The compound of any one of claims 1 to 21 wherein Y is acetyl; J₁ is C(O); and/or K₁ is amino acid residue of Val.
23. The compound of any one of claims 1 to 22 wherein Y is a fluorophore, biotin, or a reporter tag.
24. The compound of any one of claims 1 to 23 wherein the fluorophore is selected from the group consisting of Cy3, Cy3.5, Cy5, Cy5.5, Cy7, and Cy7.5.
25. The compound of any one of claims 1 to 24 wherein the compound of Formula (II) is selected from the group consisting of:

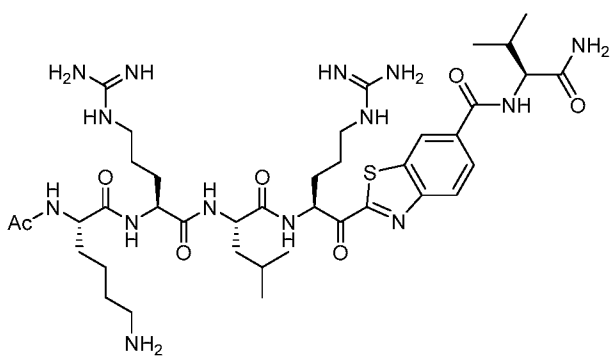
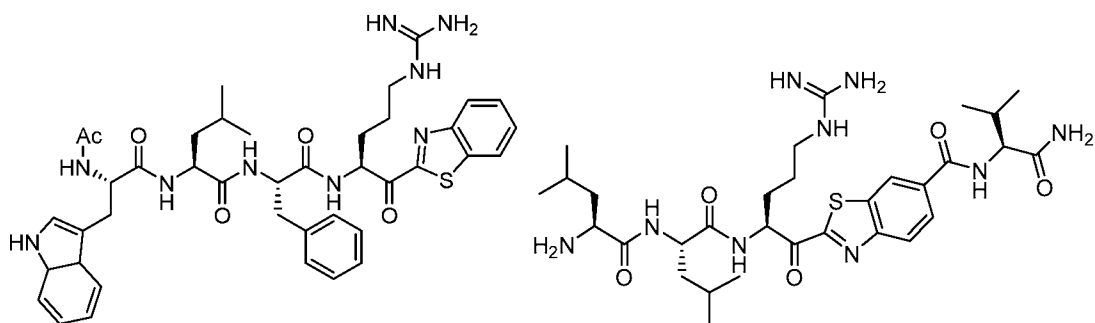
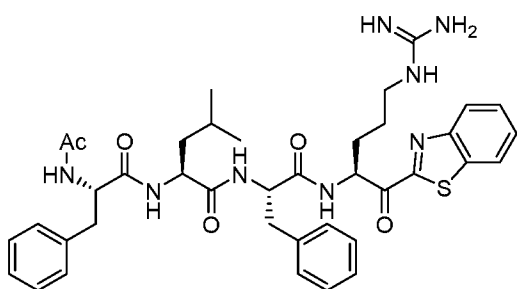
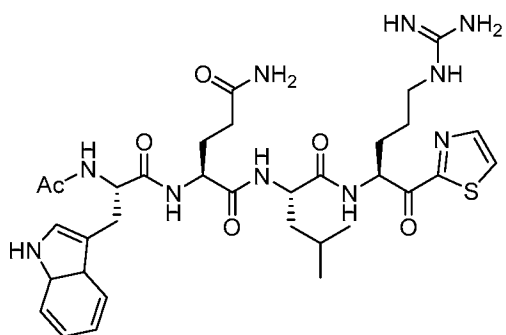


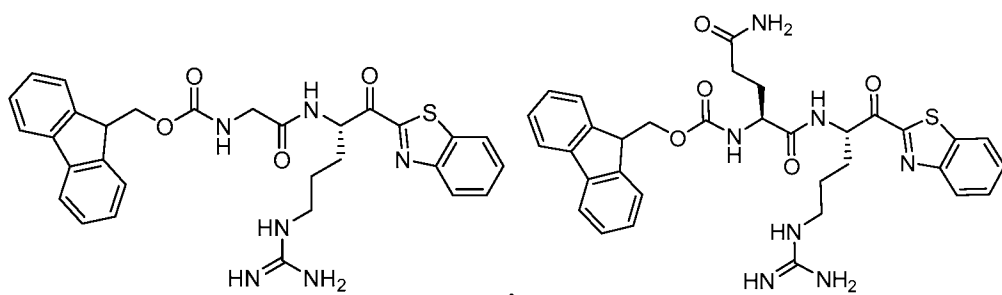
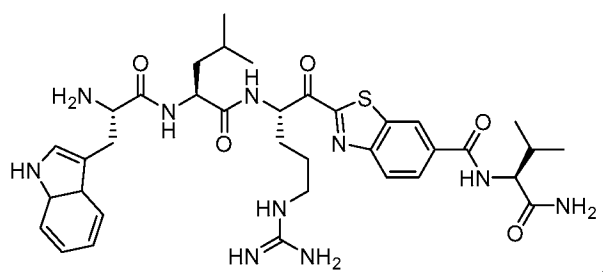
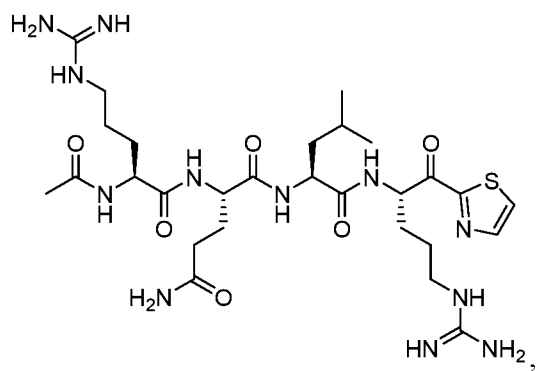
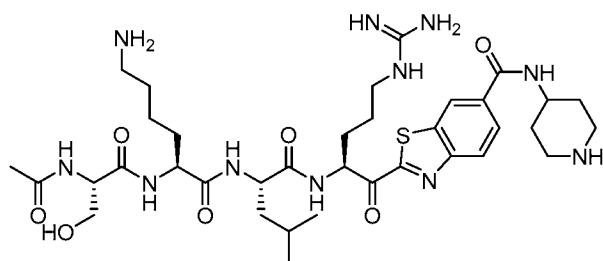


26. The compound of any one of claims 1 to 25 wherein Formula (II) is a compound selected from the group consisting of:

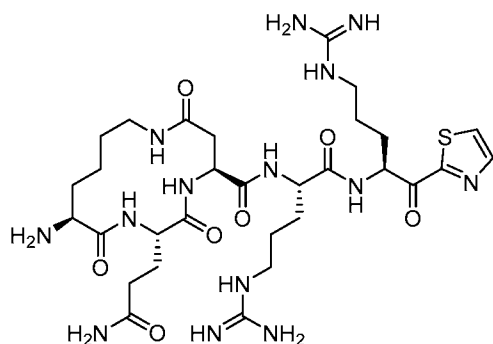




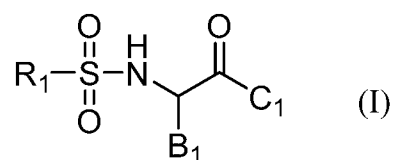




and

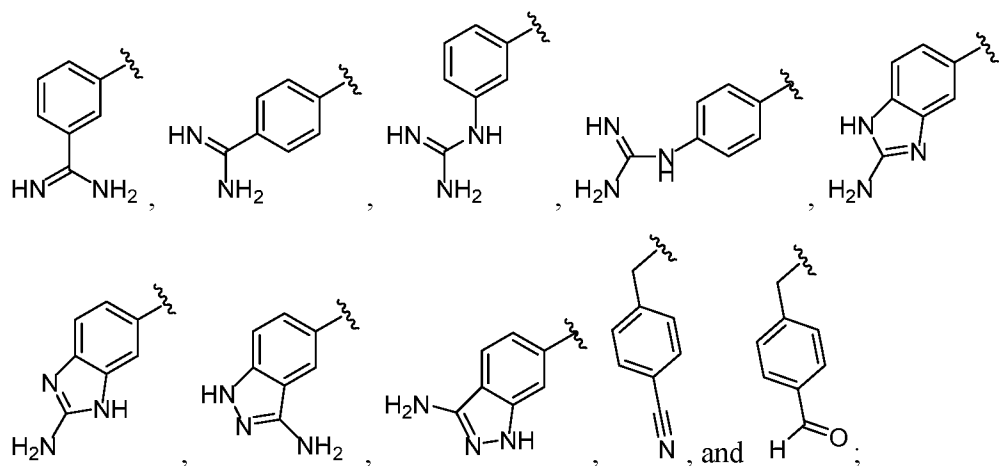


27. A compound of Formula (I), as a single stereoisomer or as a mixture thereof:

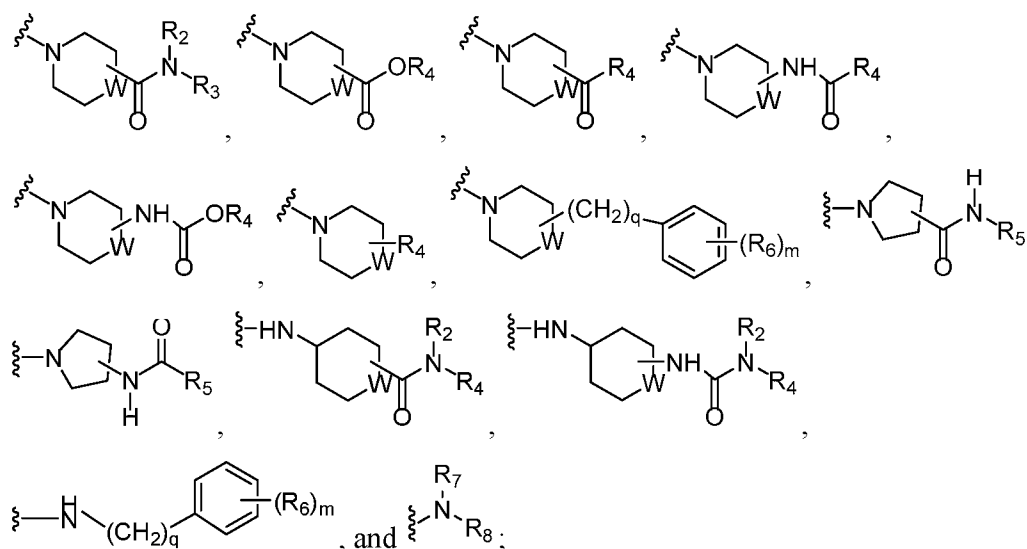


wherein R_1 is substituted or unsubstituted alkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl;

B_1 is selected from the group consisting of:



C_1 is a group selected from the group consisting of:



W is CH, CH₂, N, or NH;

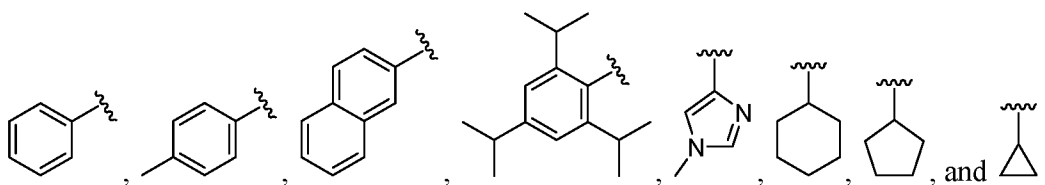
R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , and R_8 are each independently hydrogen, substituted or unsubstituted alkyl or cycloalkyl, substituted or unsubstituted alkylaryl, substituted or unsubstituted heterocyclic ring, substituted or unsubstituted aryl or heteroaryl, with the proviso that when R_2 is methyl, then R_3 cannot also be methyl and vice versa; and m is 0 to 5, or a pharmaceutically acceptable salt thereof.

28. The compound of claim 27 wherein R_1 is substituted or unsubstituted C₁-C₆ alkyl, substituted or unsubstituted C₃-C₆ cycloalkyl, substituted or unsubstituted phenyl, substituted or unsubstituted naphthyl, or a substituted or unsubstituted nitrogen-containing aromatic ring.

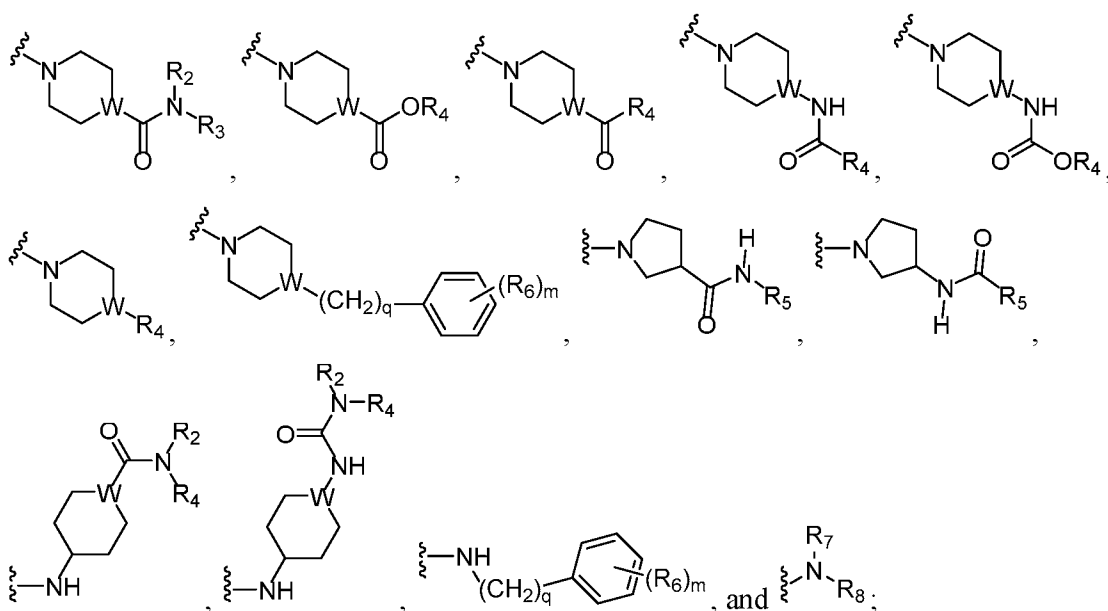
29. The compound of claim 27 or 28 wherein R_1 is substituted or unsubstituted phenyl or substituted or unsubstituted naphthyl.

30. The compound of any one of claims 27 to 29 wherein the substituted C₁-C₆ alkyl, substituted C₃-C₆ cycloalkyl, substituted phenyl, substituted naphthyl, or substituted nitrogen-containing aromatic ring comprise one or more substituents comprising halo, hydroxy, C₁-C₆ alkyl, C₁-C₆ alkoxy, halo-substituted C₁-C₄ alkyl, or amino.

31. The compound of any one of claims 27 to 30 wherein R_1 is a group selected from the group consisting of:



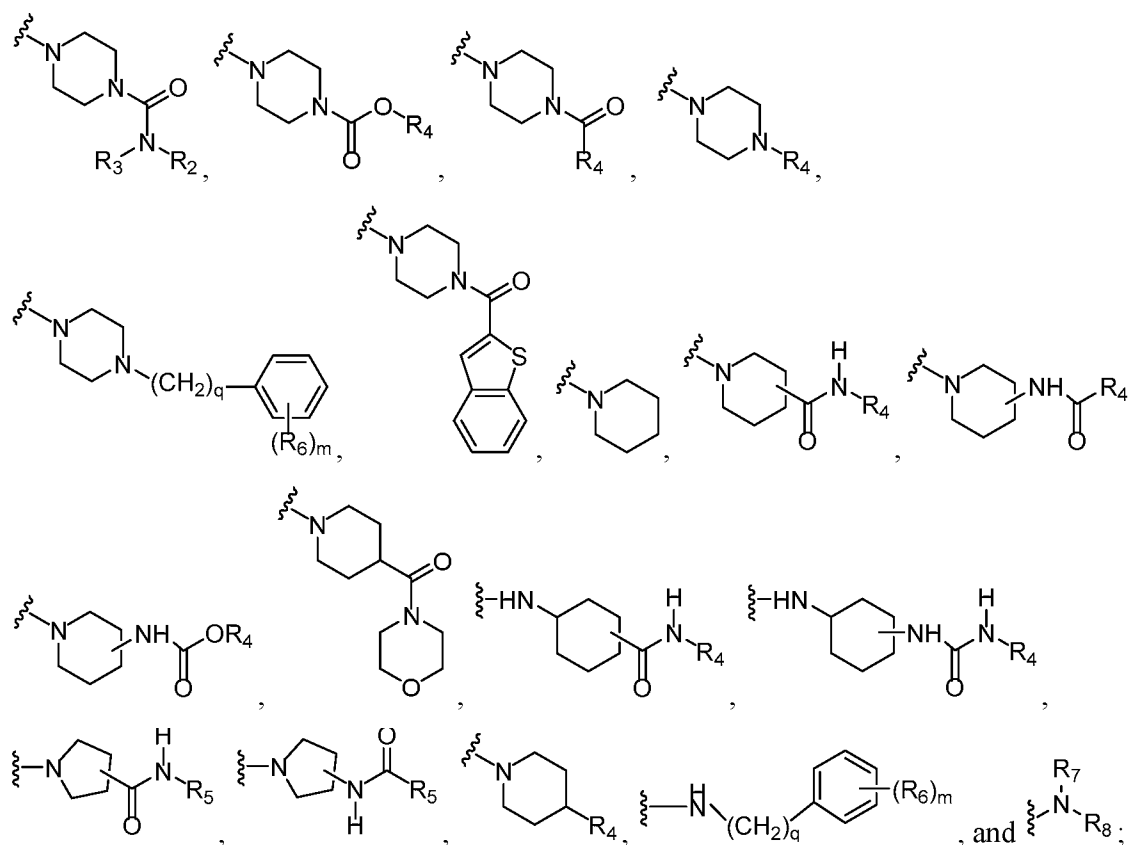
32. The compound of any one of claims 27 to 31 wherein C_1 is a group selected from the group consisting of:



W is CH or N;

R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , and R_8 are each independently hydrogen, substituted or unsubstituted alkyl or cycloalkyl, substituted or unsubstituted alkylaryl, substituted or unsubstituted heterocyclic ring, substituted or unsubstituted aryl or heteroaryl, with the proviso that when R_2 is methyl, then R_3 cannot also be methyl and vice versa; and m is 0 to 5, or a pharmaceutically acceptable salt thereof.

33. The compound of any one of claims 27 to 32 wherein C_1 is a group selected from the group consisting of:



R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , and R_8 are each independently hydrogen, substituted or unsubstituted alkyl or cycloalkyl, substituted or unsubstituted alkylaryl, substituted or unsubstituted heterocyclic ring, substituted or unsubstituted aryl or heteroaryl, with the proviso that when R_2 is methyl, then R_3 cannot also be methyl and vice versa; and m is 0 to 5, or a pharmaceutically acceptable salt thereof.

34. The compound of any one of claims 27 to 33 wherein R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , and R_8 are each independently hydrogen, substituted or unsubstituted C_1 - C_{10} alkyl or cycloalkyl, substituted or unsubstituted heterocyclic ring, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl.

35. The compound of any one of claims 27 to 34 wherein:

R_2 is hydrogen;

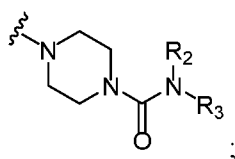
R_3 is hydrogen, C_1 - C_6 alkyl, benzyl, or halo-substituted benzyl;

R₄ and R₅ are each independently hydrogen, C₁-C₆ alkyl, halo- or alkoxy-substituted C₁-C₆ alkyl, phenyl, phenethyl, benzyl, halo- or alkoxy-substituted benzyl; substituted or unsubstituted 3-benzothiophenyl, or substituted or unsubstituted 1-morpholinyl;

R₆ is hydrogen, C₁-C₄ alkoxy; and/or

R₇ and R₈ are each independently hydrogen or C₁-C₆ alkyl.

36. The compound of any one of claims 27 to 35 wherein C₁ is



R₂ is hydrogen; and

R₃ is hydrogen, C₁-C₆ alkyl, benzyl, halo-substituted benzyl, aryl, cycloalkyl, alkylaryl, or heterocyclo.

37. A method of inhibiting matriptase, hepsin, or hepatocyte growth factor activator (HGFA) comprising administering to a subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of at least one compound of any one of claims 1 to 36.

38. A method of inhibiting HGF/MET oncogenic signaling comprising administering to a subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of at least one compound of any one of claims 1 to 36.

39. A method of inhibiting MSP/RON oncogenic signaling comprising administering to a subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of at least one compound of any one of claims 1 to 36.

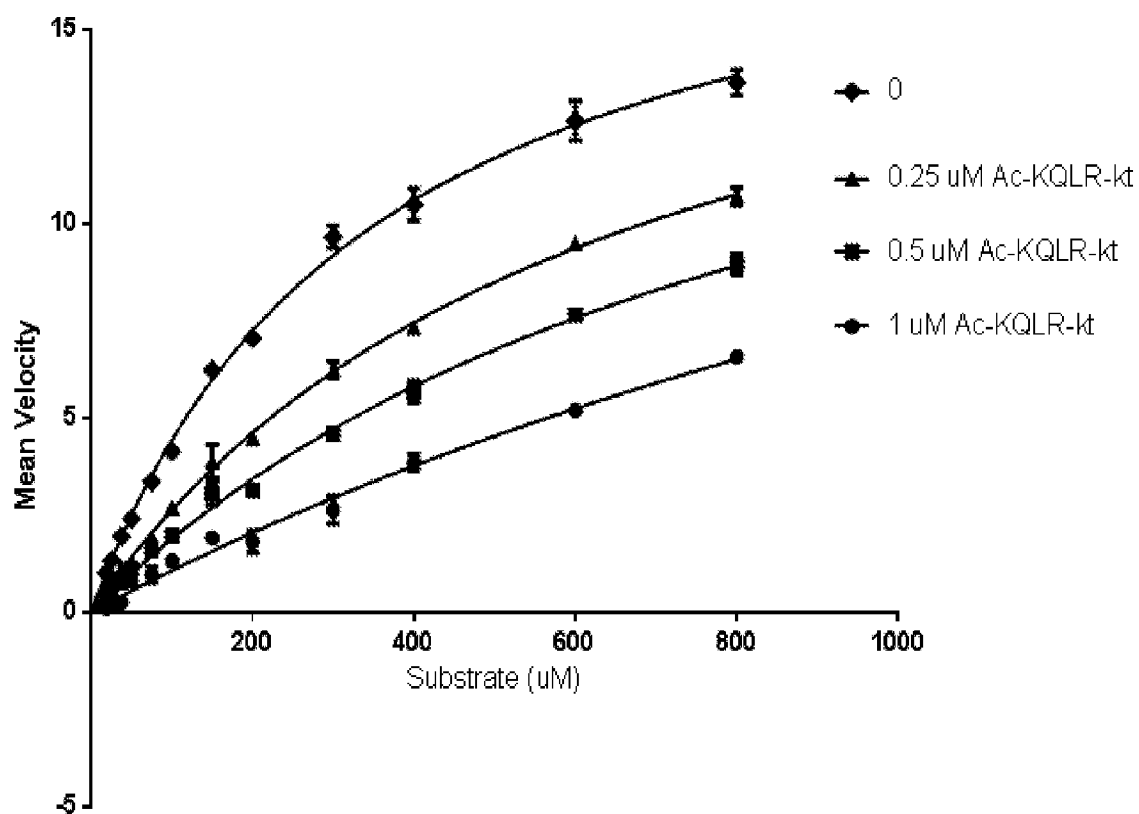
40. A method of inhibiting HGF/MET and MPS/RON oncogenic signaling comprising administering to a subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of at least one compound of any one of claims 1 to 36.

41. A method of reversing resistance to a kinase inhibitor by blocking HGF and MSP production or activation comprising administering to a subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of at least one compound of any one of claims 1 to 36.
42. A method of inhibiting tumor progression comprising administering to the subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of at least one compound of any one of claims 1 to 36.
43. A method of treating a malignancy, a pre-malignant condition, or cancer in a subject comprising administering to the subject in need thereof a pharmaceutical composition comprising a therapeutically effective amount of at least one compound of any one of claims 1 to 36.
44. The method of claim 43, wherein the cancer is selected from the group consisting of breast, ovarian, prostate, endometrial, colon, pancreatic, head and neck, gastric, renal, brain, liver, bladder, kidney, lung, esophageal, leukemias, multiple myeloma, lymphoma, and melanoma.
45. The method of claim 44, wherein the malignancy and the pre-malignant condition is a condition of the breast, prostate, bladder, multiple myeloma, leukemia, glioma, liver, lung, and colon.
46. The method of claim 45, wherein the malignancy and the pre-malignant condition is a condition of the breast.
47. The method of claim 46, wherein the pre-malignant condition is selected from the group consisting of a typical ductal hyperplasia of the breast, actinic keratosis, leukoplakia, Barrett's epithelium (columnar metaplasia) of the esophagus, ulcerative colitis, adenomatous colorectal polyps, erythroplasia of Queyrat, Bowen's disease, bowenoid papulosis, vulvar intraepithelial neoplasia, and dysplastic changes to the cervix.

48. The method of any one of claims 43 to 47 wherein the cancer has progressed or metastasized.
49. The method of any one of claims 37 to 48, wherein the at least one compound is administered by a method selected from the group consisting of oral, intranasal, intraperitoneal, intravenous, intramuscular, intratumoral, rectal, and transdermal.
50. The method of any one of claims 37 to 49, wherein the effective amount ranges from 0.01 mg to 500 mg for each kilogram of subject body weight.
51. The method of any one of claims 37 to 50, further comprising administration of an anticancer compound, radiation therapy, a compound that induces apoptosis, a surgical procedure, or any combination thereof.
52. The method of any one of claims 37 to 51 wherein the subject is human.
53. A pharmaceutical composition comprising a therapeutically effective amount of at least one compound of any one of claims 1 to 36.
54. An imaging composition comprising a fluorescent compound of Formula (I) or (II) of any one of claims 1 to 36, wherein the labeled compound comprises a fluorophore selected from the group consisting of Cy3, Cy3.5, Cy5, Cy5.5, Cy7, and Cy7.5.
55. A method of detecting cancer comprising:
- (i) administering to a subject an imaging composition of claim 54;
 - (ii) employing a fluorescence imaging technique for monitoring or visualizing a distribution of the fluorescent compound within the body or within a portion thereof; and
 - (iii) correlating the distribution of the fluorescent compound to the existence of cancer.

56. An imaging composition comprising a radiolabeled compound of Formula (I) or (II) of any one of claims 1 to 36, wherein the labeled compound comprises a radioisotope selected from the group consisting of ^{11}C , ^{13}N , ^{15}O , ^{18}F , ^{75}Br , ^{124}I , ^{125}I , and ^{131}I .
57. A method of detecting cancer comprising:
- (iv) administering to a subject an imaging composition of claim 56;
 - (v) employing a nuclear imaging technique for monitoring or visualizing a distribution of the radiolabeled compound within the body or within a portion thereof; and
 - (vi) correlating the distribution of the radiolabeled compound to the existence of cancer.
58. The method of claim 57, wherein the nuclear imaging technique is positron emission tomography (PET) or photon emission computed tomography (SPECT).

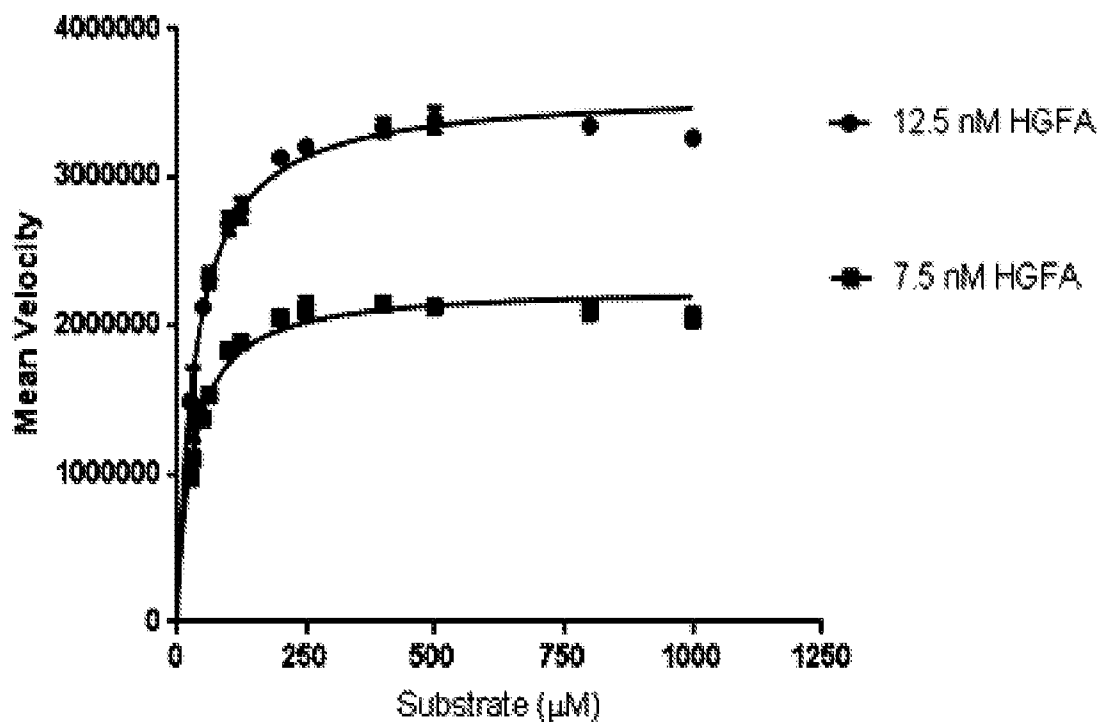
FIG. 1



	1 uM Ac-KQLR-kt	0.5 uM Ac-KQLR-kt	0.25 uM Ac-KQLR-kt	0
Vmax	23.93	19.17	19.27	19.79
Km	2137	917.7	632.9	345.7

	1 uM Ac-KQLR-kt	0.5 uM Ac-KQLR-kt	0.25 uM Ac-KQLR-kt	0
R square	0.9862	0.9944	0.9973	0.9962

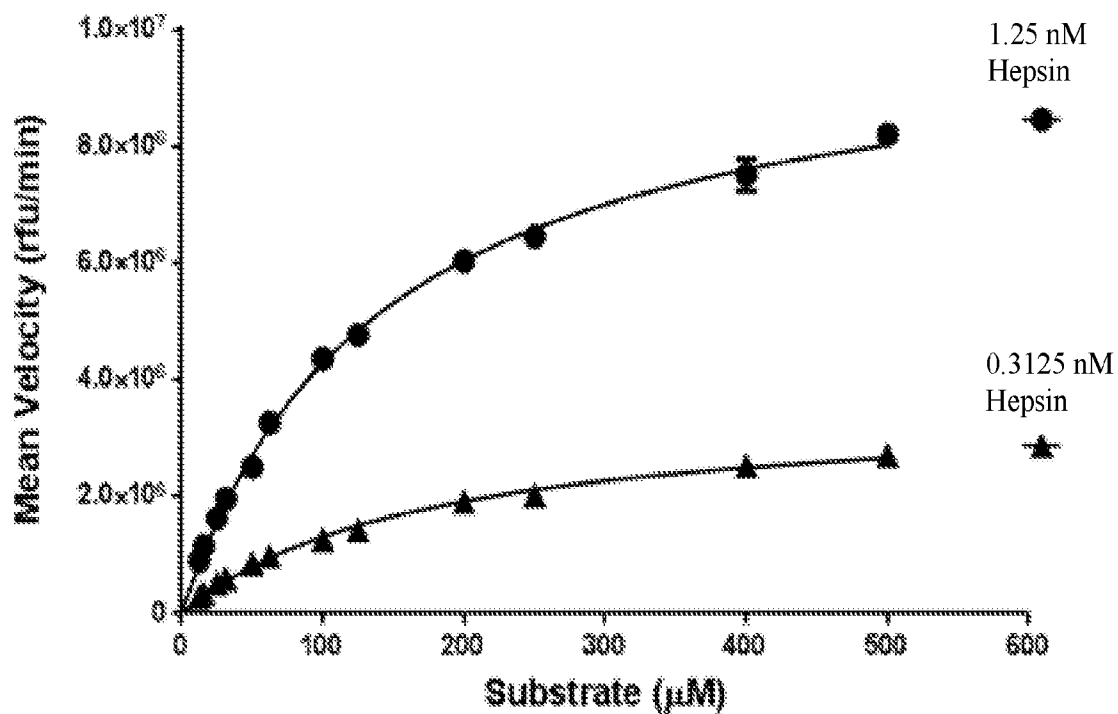
FIG. 2



	12.5 nM HGFA	7.5 nM HGFA
Km	36.06	29.88

	12.5 nM HGFA	7.5 nM HGFA
R square	0.9716	0.9610

FIG. 3

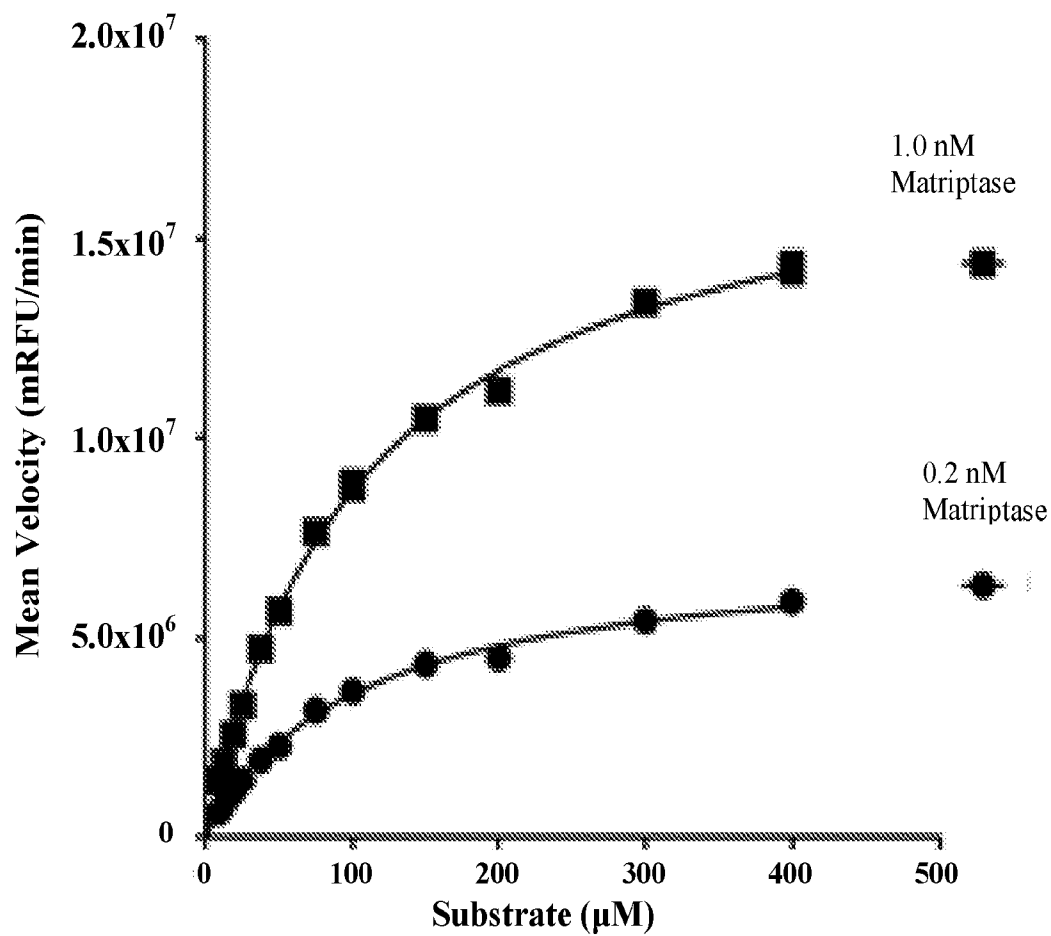


	1.25 nM Hepsin	0.3125 nM Hepsin
Km	139.7	173.0

	1.25 nM Hepsin	0.3125 nM Hepsin
Vmax	133264	61821

	1.25 nM Hepsin	0.3125 nM Hepsin
R square	0.9965	0.9952

FIG. 4



	0.2 nM Matriptase	1.0 nM Matriptase
Vmax	7.256e+006	1.793e+007
Km	101.6	106.4

	0.2 nM Matriptase	1.0 nM Matriptase
R square	0.9950	0.9969

FIG. 5

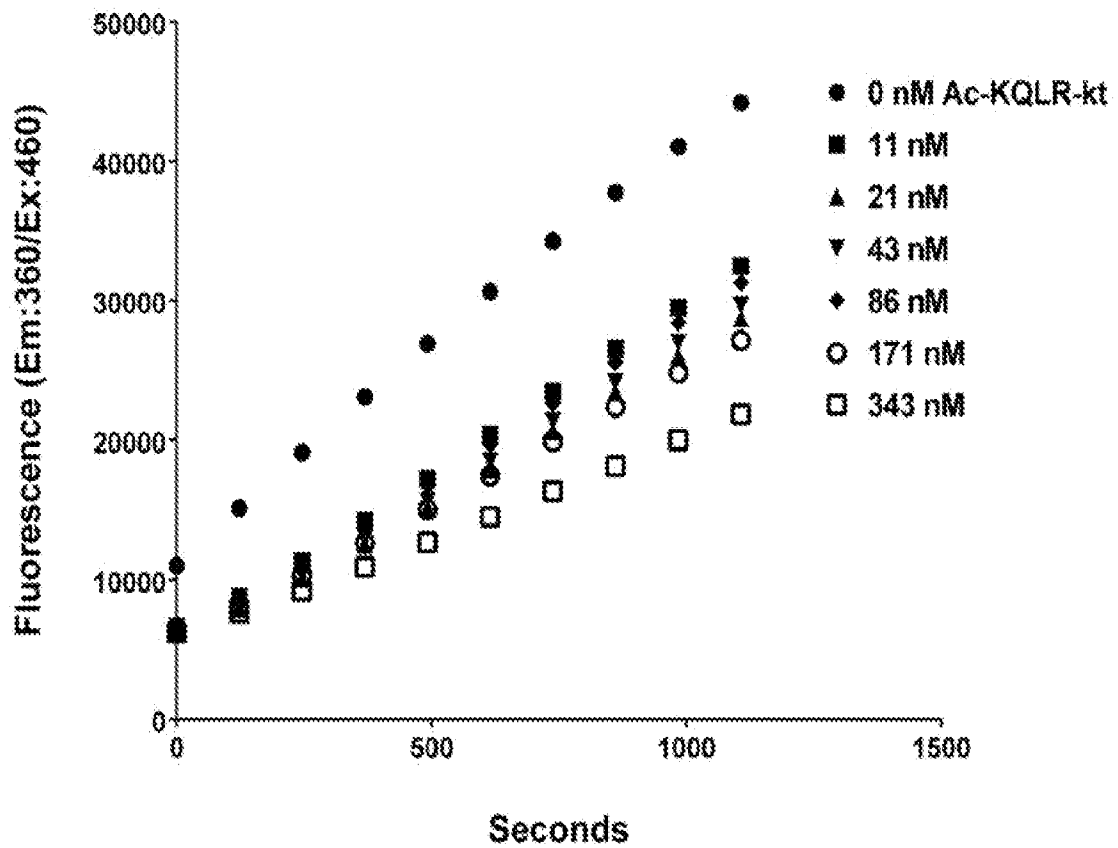


FIG. 6

**7.5 μ M HGFA protease incubated for 20 minutes
in the presence of various amount of Inhibitor.
Reactions then diluted 350-fold with 250 μ M Boc-QLR-AMC
and perform kinetic read in Synergy 2, Ex 360 nm, Em 460 nm.**

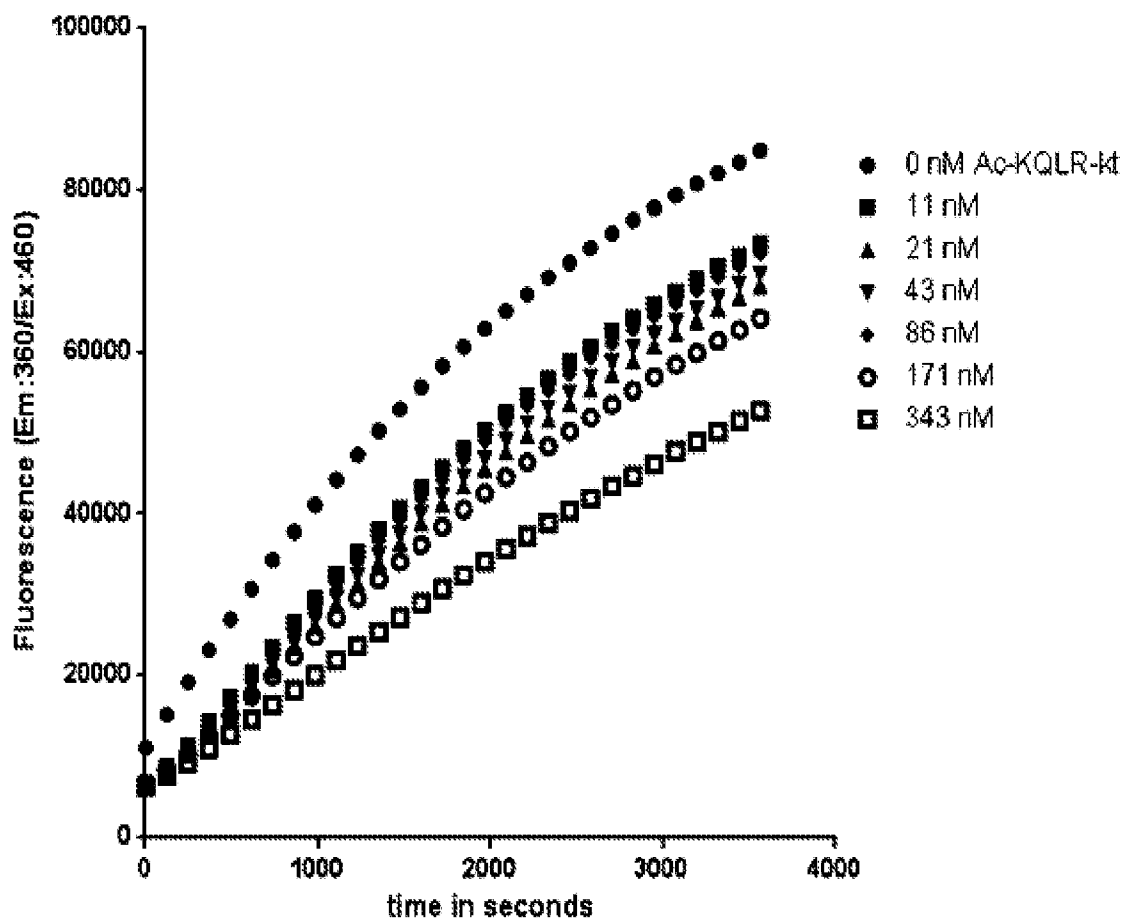


FIG. 7

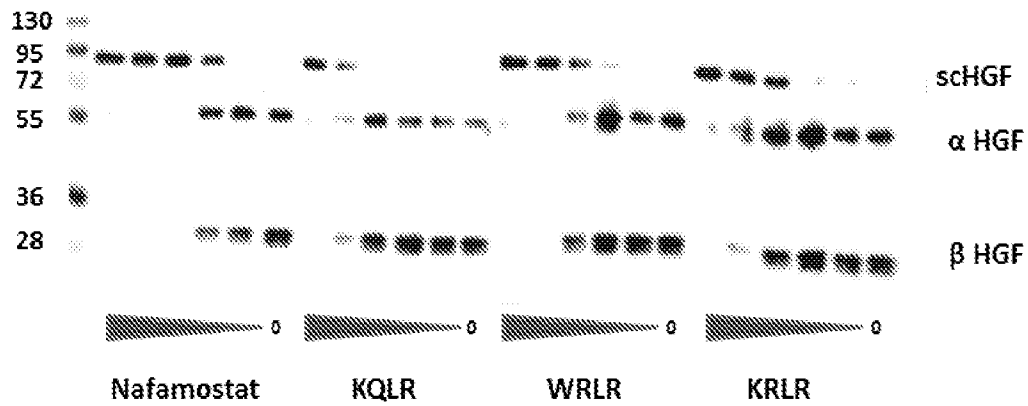


FIG. 8

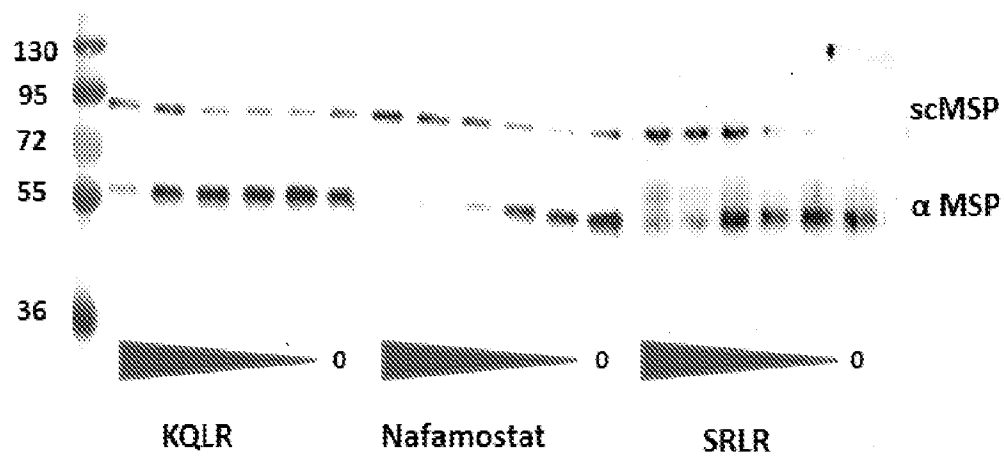


FIG. 9

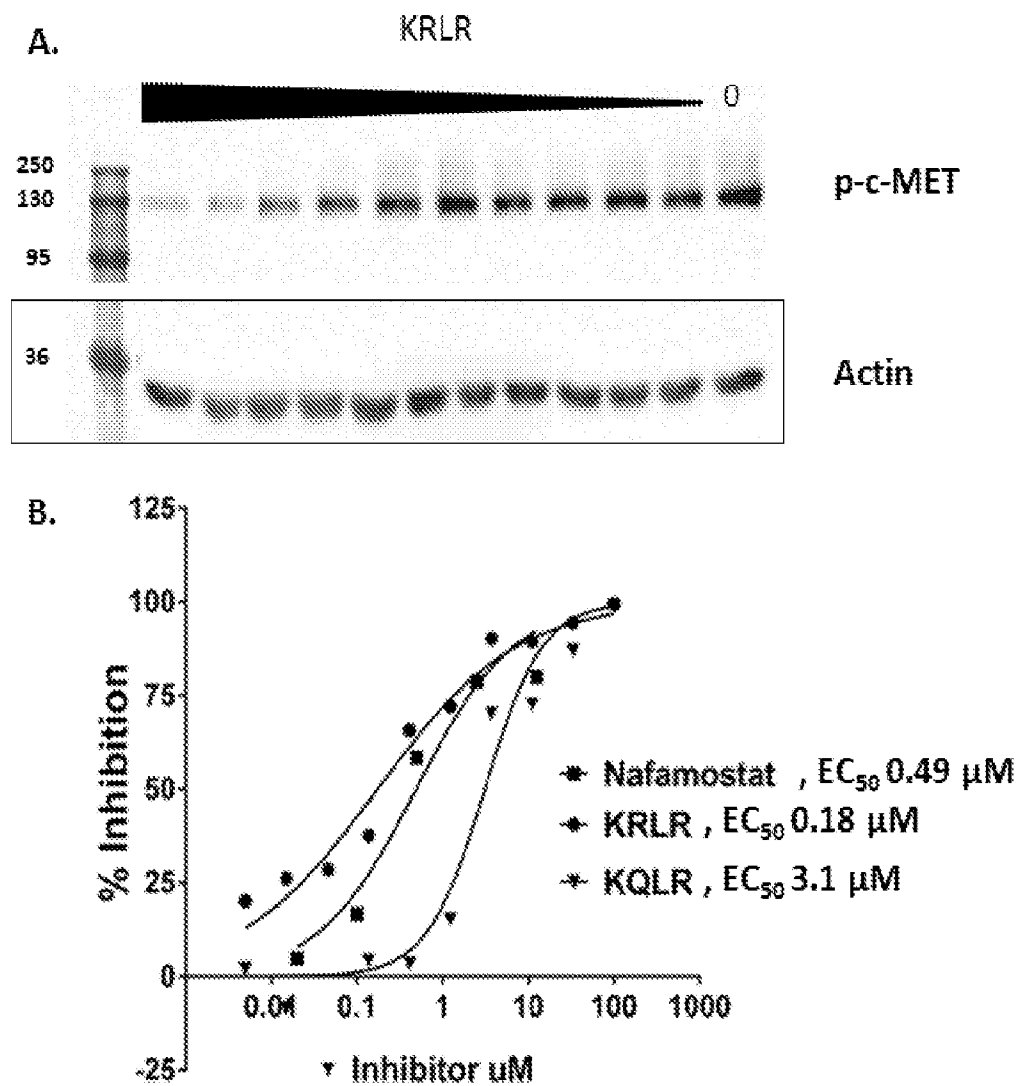
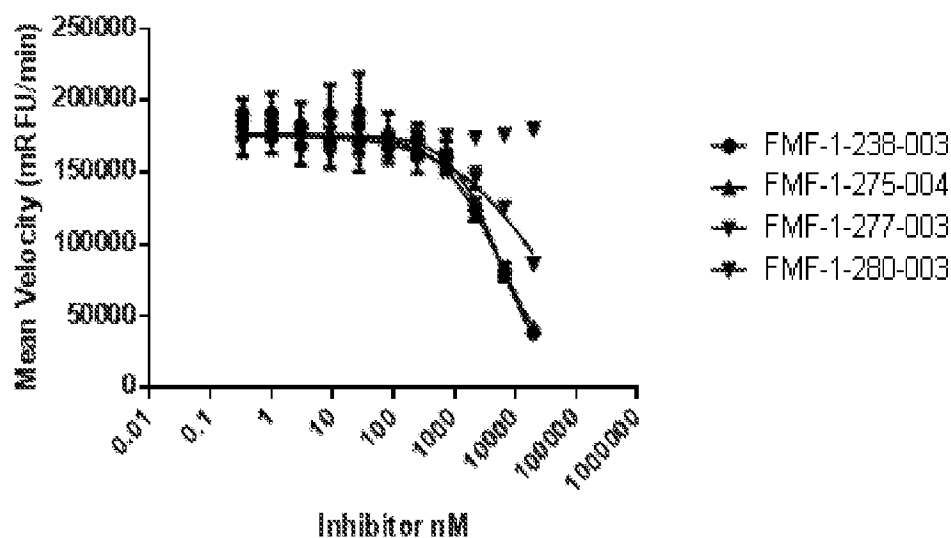


FIG. 10

Transform of Matriptase assay cpds 1-4 constrained

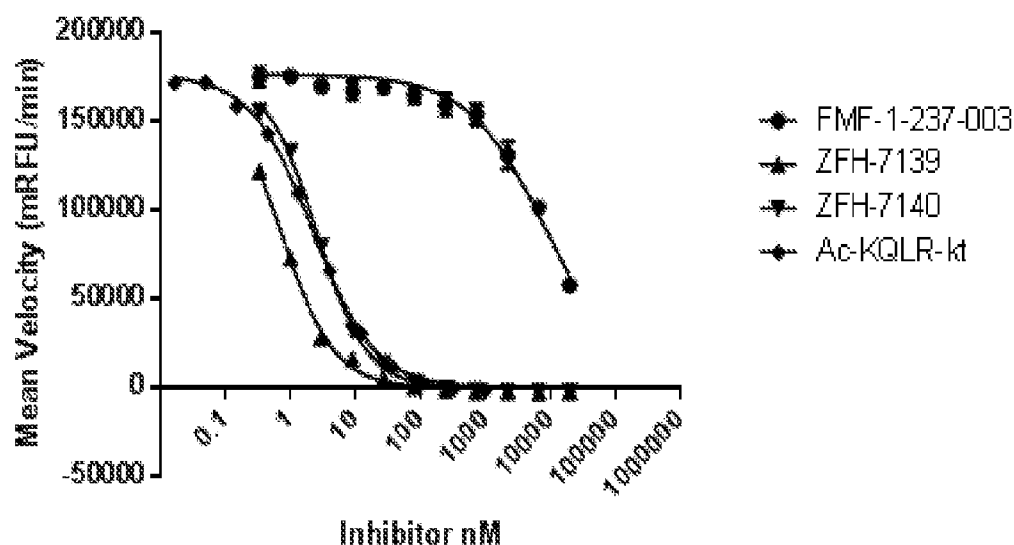


	FMF-1-238-003	FMF-1-275-004	FMF-1-277-003	FMF-1-280-003
IC50	5645	5554		24744

	FMF-1-238-003	FMF-1-275-004	FMF-1-277-003	FMF-1-280-003
R square	0.9220	0.9915		0.8630

FIG. 11

Transform of Matriptase assay cpds 5-8 constrained

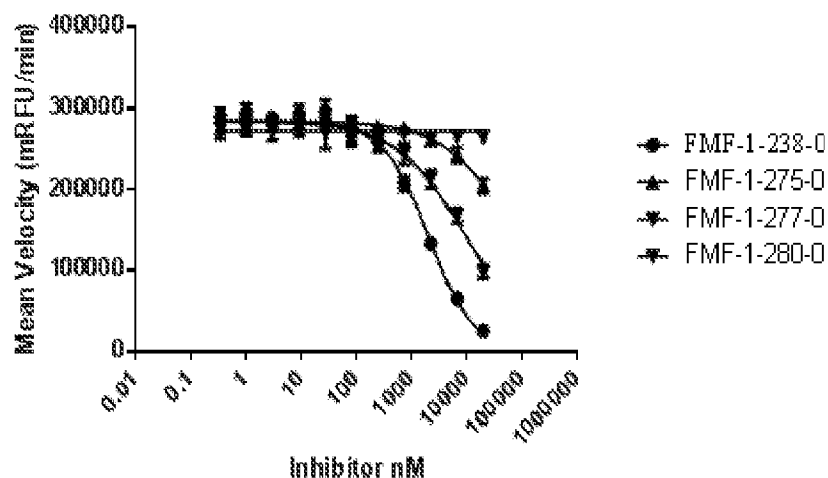


	FMF-1-237-003	ZFH-7139	ZFH-7140	Ac-KQLR-kt
IC50	8884	0.7198	2.556	2.265

	FMF-1-237-003	ZFH-7139	ZFH-7140	Ac-KQLR-kt
R square	0.9745	0.9959	0.9981	0.9992

FIG. 12

Transform of Hepsin assay cpds 9-12 constrained

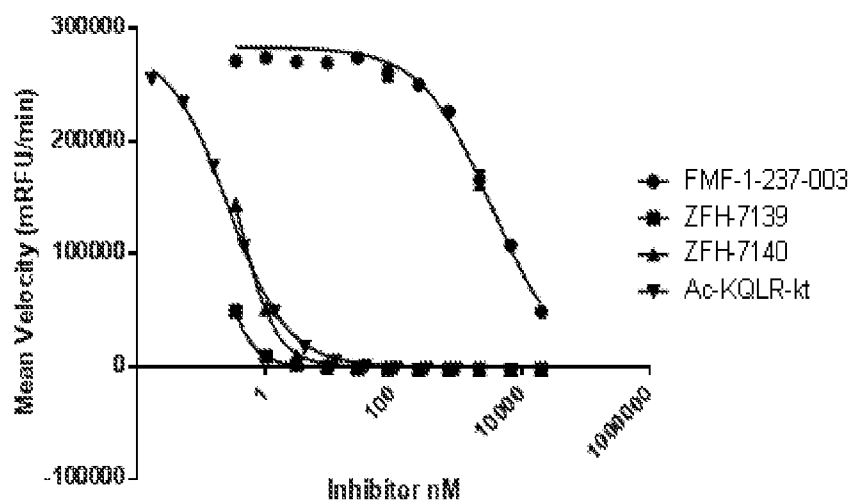


	FMF-1-238-003	FMF-1-275-004	FMF-1-277-003	FMF-1-280-003
IC50	2026	79036	0.0	9764

	FMF-1-238-003	FMF-1-275-004	FMF-1-277-003	FMF-1-280-003
R square	0.9895	0.9176		0.9591

FIG. 13

Transform of Hepsin assay cpds 12-16 constrained

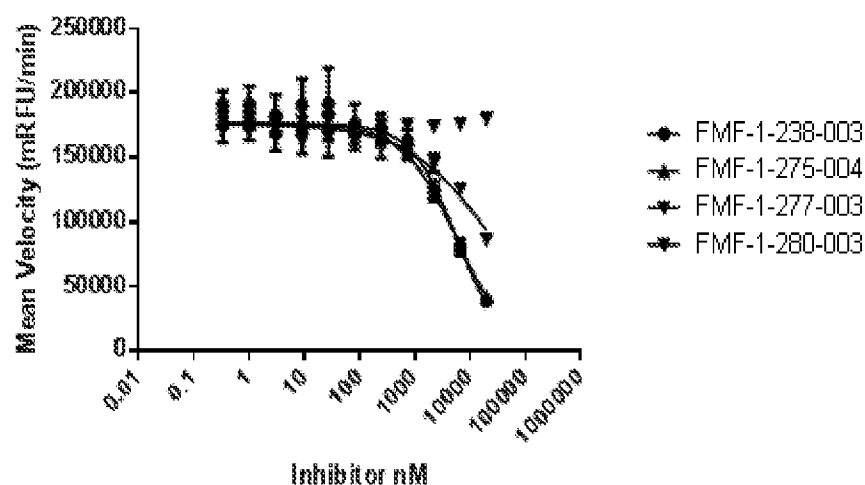


	FMF-1-237-003	ZFH-7139	ZFH-7140	Ac-KQLR-kt
IC50	3396	0.1357	0.3477	0.2597

	FMF-1-237-003	ZFH-7139	ZFH-7140	Ac-KQLR-kt
R square	0.9864	0.9820	0.9975	0.9987

FIG. 14

Transform of Matriptase assay cpds 1-4 constrained

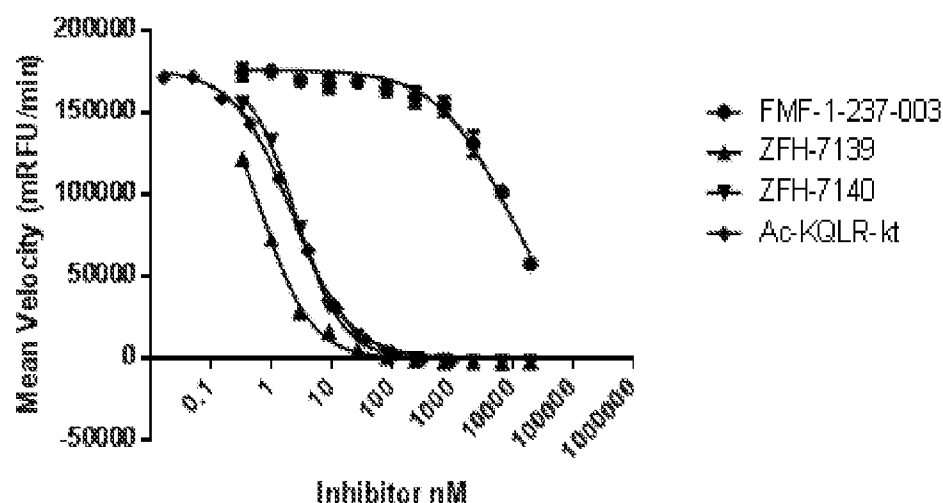


	FMF-1-238-003	FMF-1-275-004	FMF-1-277-003	FMF-1-280-003
IC50	5645	5554		24744

	FMF-1-238-003	FMF-1-275-004	FMF-1-277-003	FMF-1-280-003
R square	0.9220	0.9915		0.8630

FIG. 15

Transform of Matriptase assay cpds 5-8 constrained

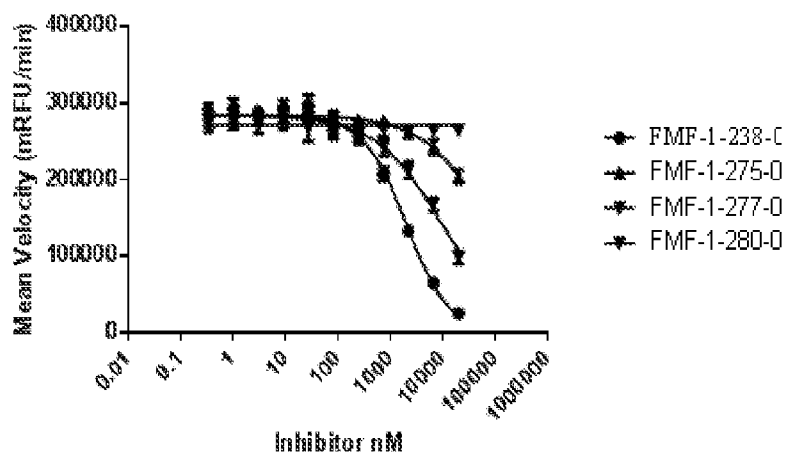


	FMF-1-237-003	ZFH-7139	ZFH-7140	Ac-KQLR-kt
IC50	8884	0.7198	2.556	2.265

	FMF-1-237-003	ZFH-7139	ZFH-7140	Ac-KQLR-kt
R square	0.9745	0.9959	0.9981	0.9992

FIG. 16

Transform of Hepsin assay cpds 9-12 constrained

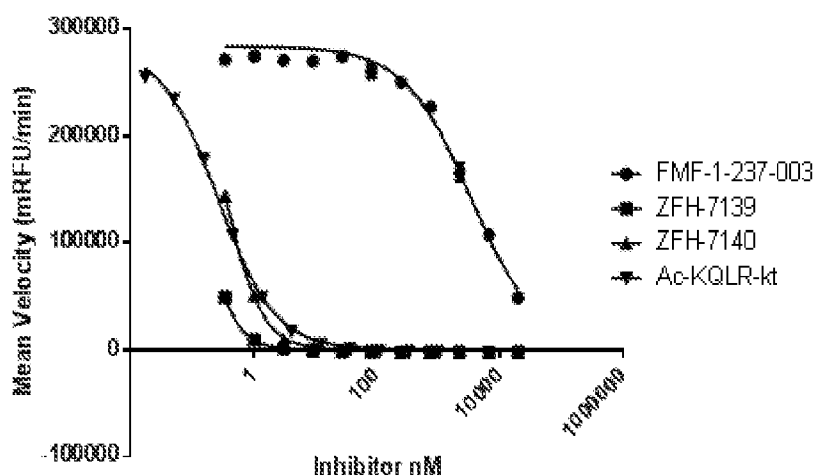


	FMF-1-238-003	FMF-1-275-004	FMF-1-277-003	FMF-1-280-003
IC50	2026	79036	0.0	9764

	FMF-1-238-003	FMF-1-275-004	FMF-1-277-003	FMF-1-280-003
R square	0.9895	0.9176		0.9591

FIG. 17

Transform of Hepsin assay cpds 12-16 constrained



	FMF-1-237-003	ZFH-7139	ZFH-7140	Ac-KQLR-kt
IC50	3396	0.1357	0.3477	0.2597

	FMF-1-237-003	ZFH-7139	ZFH-7140	Ac-KQLR-kt
R square	0.9864	0.9820	0.9975	0.9987

FIG. 18

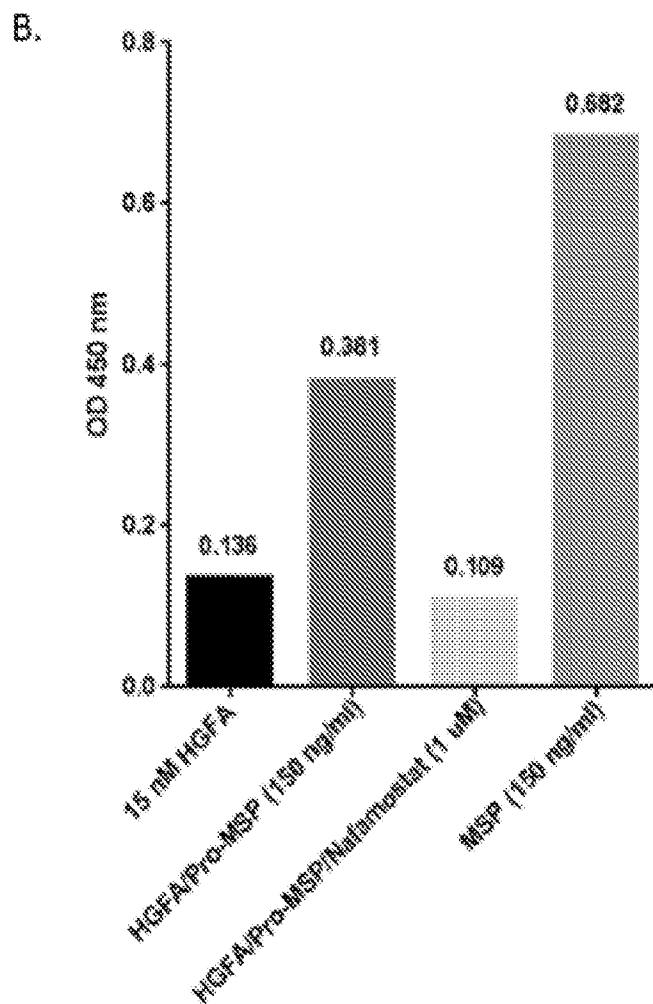
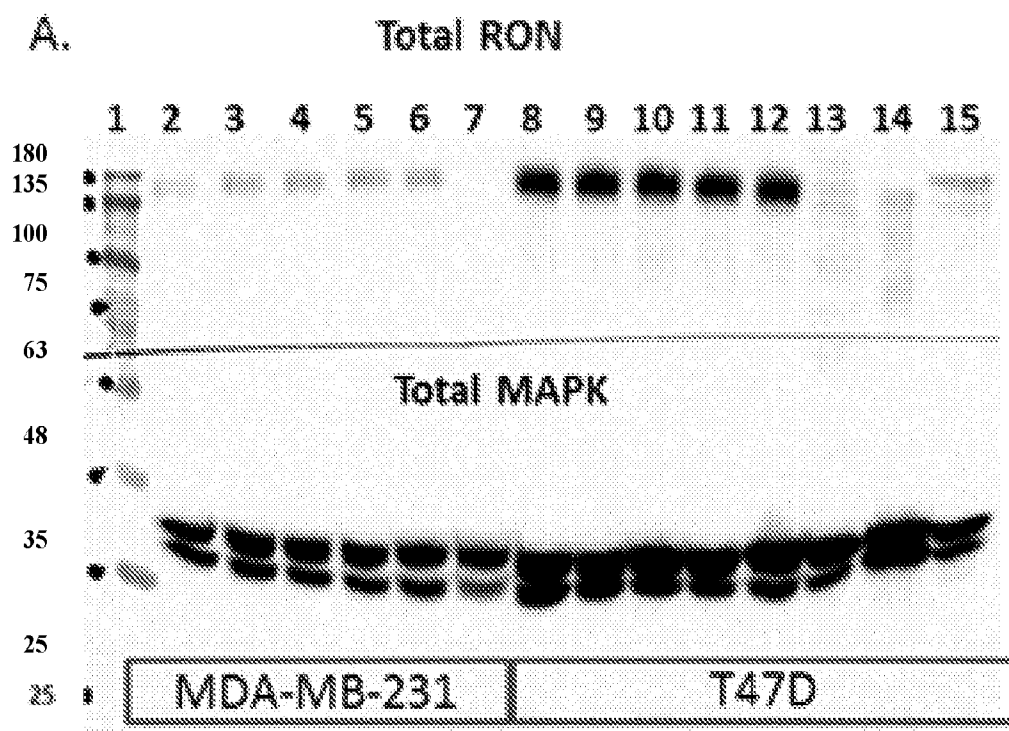


FIG. 19

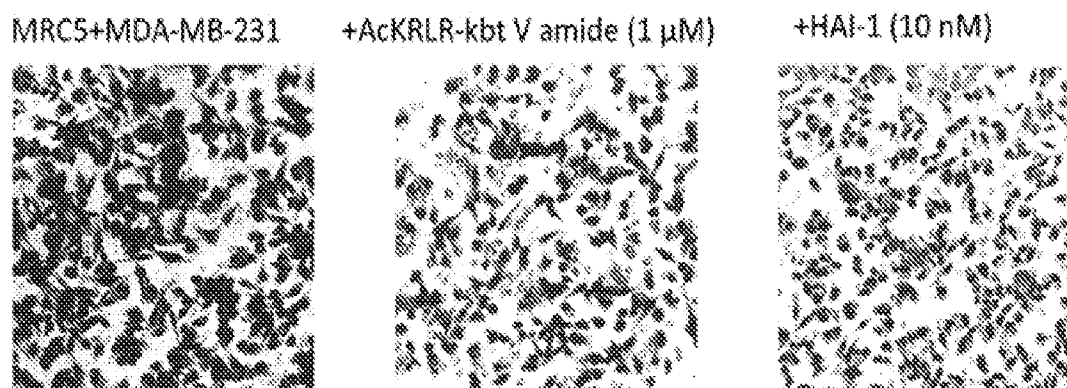


FIG. 20

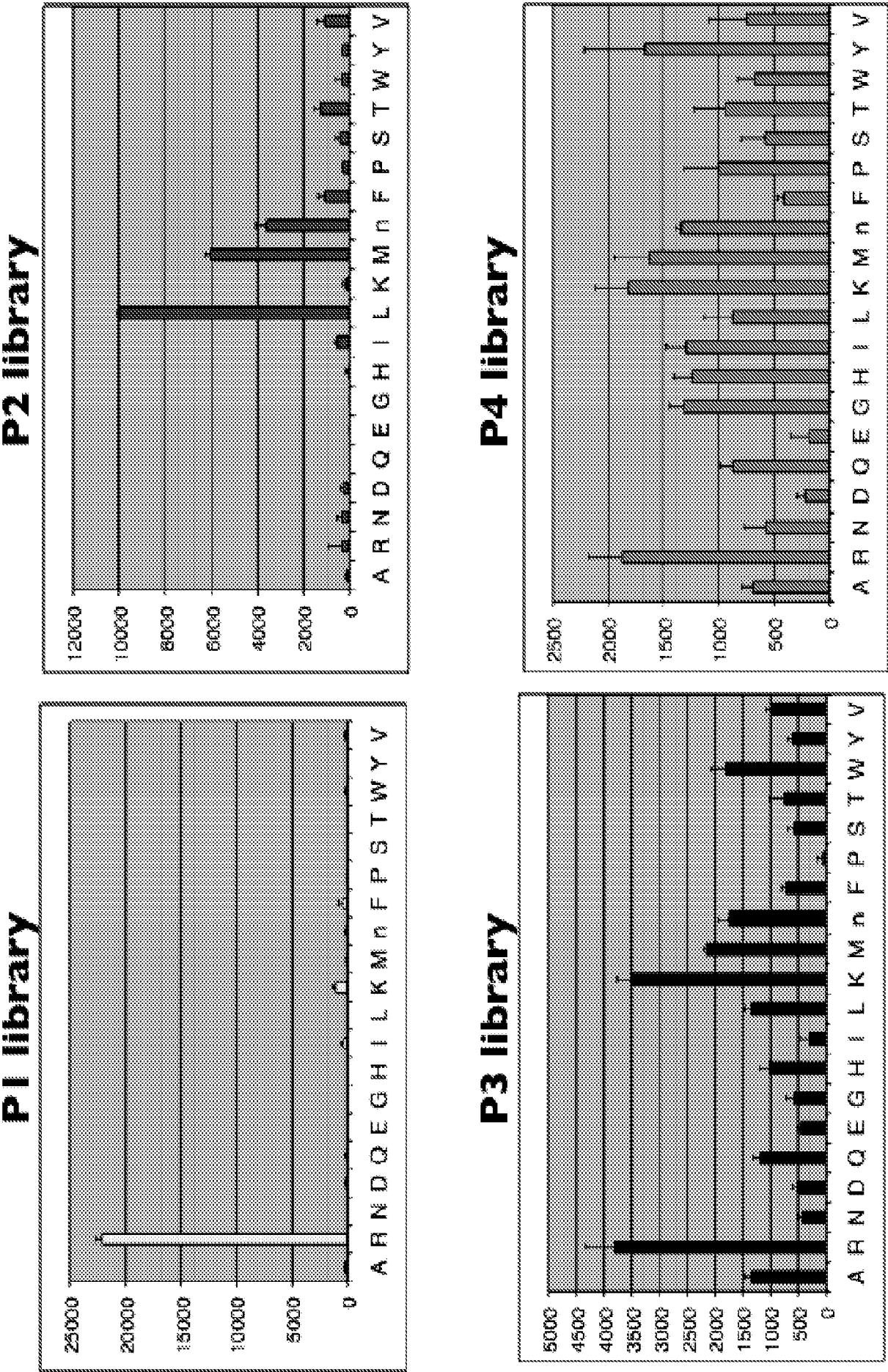


FIG. 21 A.

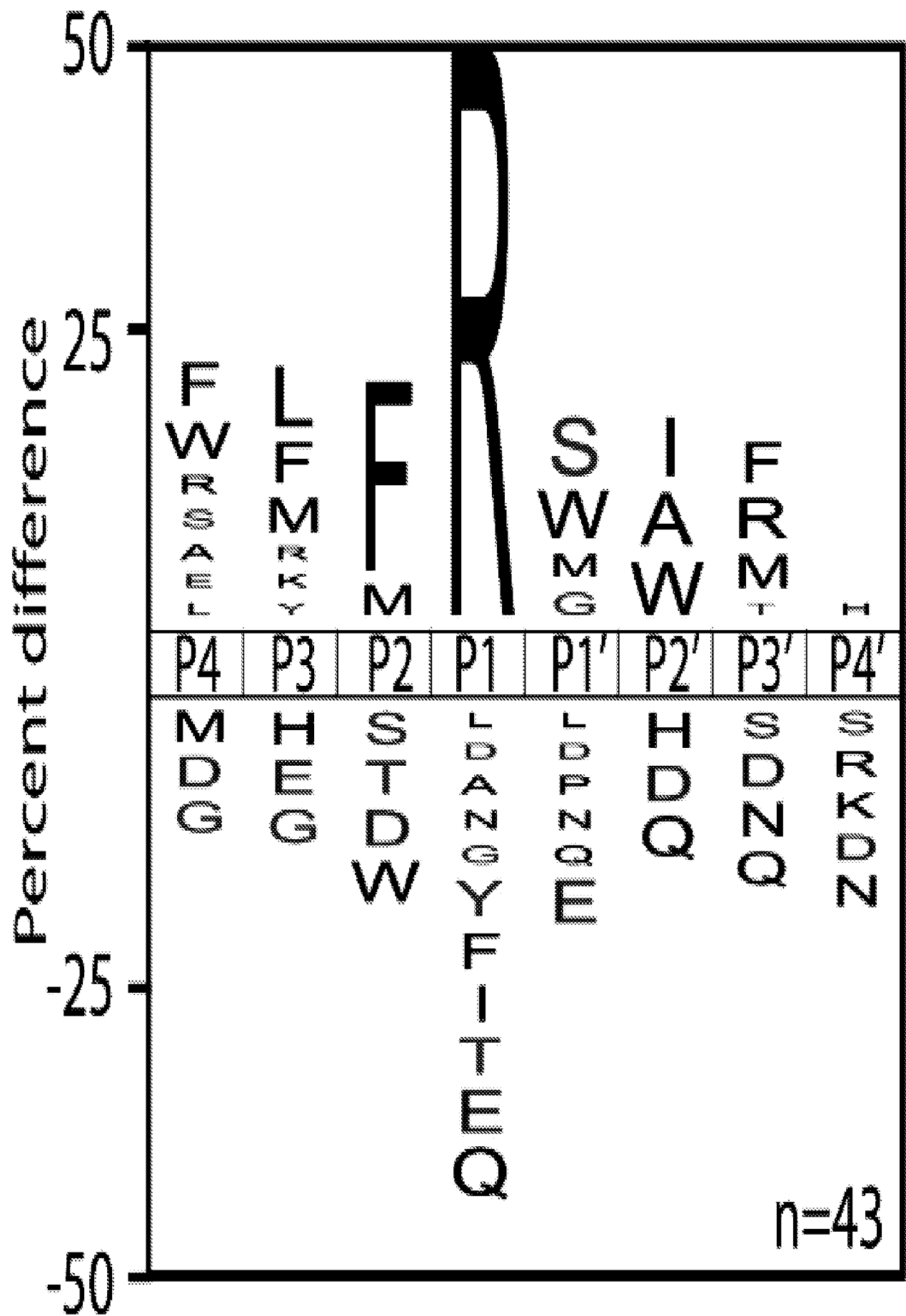
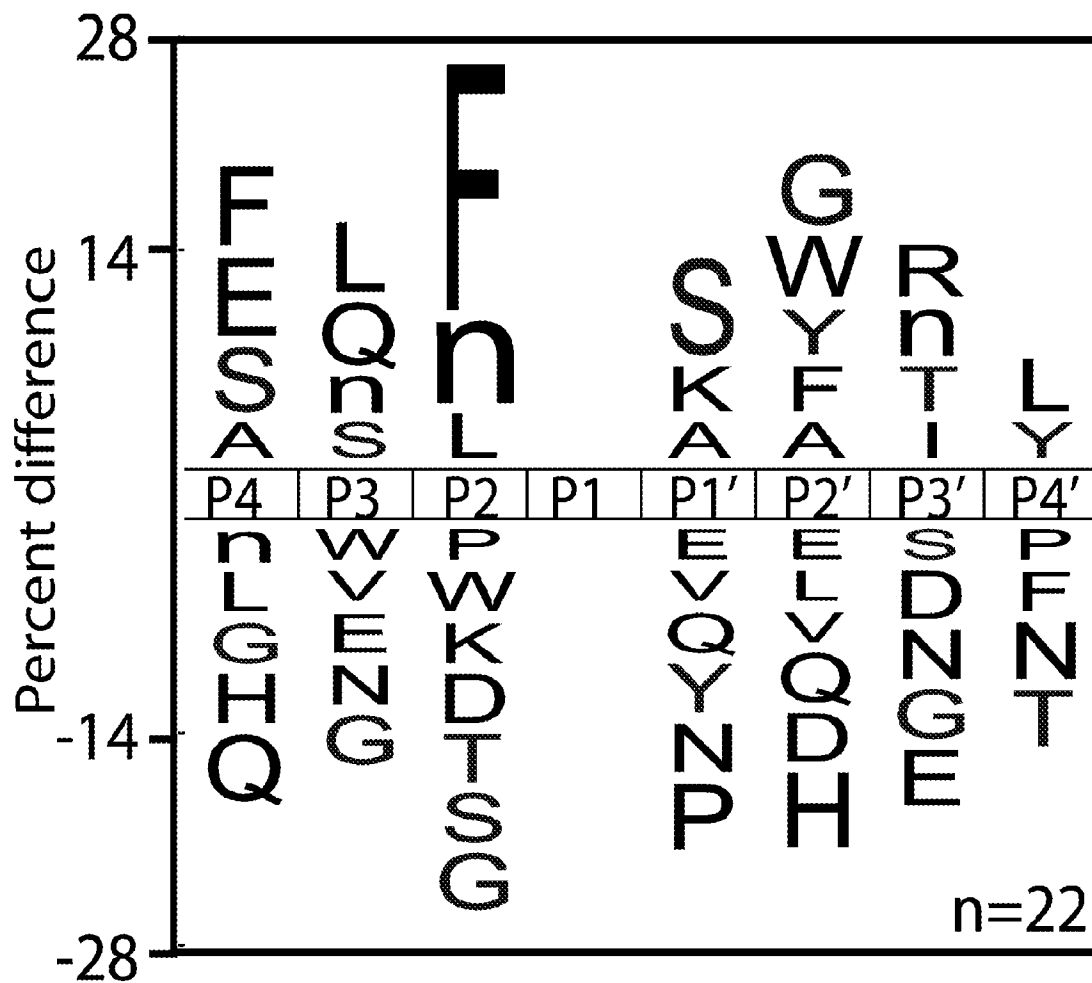


FIG. 21 B.



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/020516

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 38/04; A61K 38/06; C07K 5/08; C07K 5/09; C07K 5/083 (2016.01)

CPC - A61K 38/04; A61K 38/06; C07K 5/08; C07K 5/09; C07K 5/804; C07K 5/815 (2016.05)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - A61K 38/04; A61K 38/06; C07K 5/08; C07K 5/09; C07K 5/083 (2016.01)

CPC - A61K 38/04; A61K 38/06; C07K 5/08; C07K 5/09; C07K 5/804; C07K 5/815 (2016.05)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

IPC(8) - A61K 38/04; A61K 38/06; C07K 5/08; C07K 5/09; C07K 5/083 (2016.1); CPC - A61K 38/04; A61K 38/06; C07K 5/08; C07K 5/09; C07K 5/804; C07K 5/815 (2016.05); USPC - 514/2; 514/18; 530/331 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PatBase, STN, PubChem, Google Patents, Google Scholar

Search terms used: tripeptide Leu-Arg-Val

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PUBCHEM, Substance Record for SID 29871797, Create Date: 2007-12-04. [retrieved on 08 June 2016]. Retrieved from the Internet. < https://pubchem.ncbi.nlm.nih.gov/substance/29871797/version/1#section=Top >. entire document	1-3
A	PUBCHEM, Substance Record for SID 231910502, Create Date: 2015-02-12. [retrieved on 14 April 2016]. Retrieved from the Internet. < https://pubchem.ncbi.nlm.nih.gov/substance/231910502/version/1#section=Top >. entire document	1-3
A	PUBCHEM, Substance Record for SID 162750732, Create Date: 2013-05-22. [retrieved on 14 April 2016]. Retrieved from the Internet. < https://pubchem.ncbi.nlm.nih.gov/substance/162750732/version/1#section=Top >. entire document	1-3



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

09 June 2016

Date of mailing of the international search report

15 JUL 2016

Name and mailing address of the ISA/

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, VA 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Blaine R. Copenheaver

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/020516

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-26, 30-58
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Extra Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-3

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/020516

Continued from Box No. III Observations where unity of invention is lacking

Claims 1-3 have been analyzed subject to the restriction that the claims read on the compound of Formula (II) as described in the Lack of Unity of Invention (See Box IV). The claims are restricted to a compound of Formula (II); wherein n is 0; m is 0; b is 0; Y is H; P1 is a residue of an amino acid selected from Arg; P2 is a residue of an amino acid selected from Leu; P3 is absent; P4 is absent; P5 is absent; and Z is Val.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-3 are drawn to compounds of Formula (II).

Group II+: claims 27-29 are drawn to compounds of Formula (I).

The first invention of Group I+ is restricted to a compound of Formula (II); wherein n is 0; m is 0; b is 0; Y is H; P1 is a residue of an amino acid selected from Arg; P2 is a residue of an amino acid selected from Leu; P3 is absent; P4 is absent; P5 is absent; and Z is Val. It is believed that claims 1-3 read on this first named invention and thus these claims will be searched without fee to the extent that they read on the above embodiment.

The first invention of Group II+ is restricted based on the proviso that when R2 is methyl, then R3 cannot also be methyl and vice versa; and is restricted to a compound of Formula (I), as a single stereoisomer or as a mixture thereof; wherein R1 is substituted alkyl, wherein the alkyl is substituted with halo; B1 is the first shown structure; and C1 is the first shown structure, wherein the amide is attached to the ring through W; W is CH; R2 and R3 are each independently hydrogen; or a pharmaceutically acceptable salt thereof.

Applicant is invited to elect additional formula(e) for each additional compound to be searched in a specific combination by paying an additional fee for each set of election. Each additional elected formula(e) requires the selection of a single definition for each compound variable. An exemplary election would be a compound of Formula (II); wherein n is 1; m is 1; b is 1; Y is carboxybenzyl; P1 is an alpha-amino acid of the first shown structure; P2 is a residue of an amino acid selected from Ala; P3 is hArg; P4 is Arg; P5 is Arg; and Z is Arg. Additional formula(e) will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ and II+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The special technical features of Group I+, compounds of Formula (II), are not present in Group II+; and the special technical features of Group II+, compounds of Formula (I), are not present in Group I+.

The Groups I+ and II+ formulae do not share a significant structural element requiring the selection of alternatives for the compound variables P1, P2, P3, P4, P5, Y, Z, m, n, b, R1, B1, and C1.

The Groups I+ and II+ share the technical features of a compound having the core structure of Formula (II); and a compound of Formula (I); as a single stereoisomer or as a mixture thereof; or a pharmaceutically acceptable salt thereof. However, these shared technical features do not represent a contribution over the prior art.

Specifically, Substance Record for SID 231910502 to PubChem teaches a compound having the core structure of Formula (II); wherein P1 is Arg; P2 is Glu; and P3 is Arg (See Pg. 3;...see shown structure...).

Additionally, Substance Record for SID 162750732 to PubChem teaches a compound of Formula (I); as a single stereoisomer or as a mixture thereof; wherein R1 is substituted aryl; B1 is the second to last shown structure; C1 N(R7)(R8); R7 is substituted alkyl and R8 is hydrogen (See Pg. 3;...see shown structure...).

The inventions listed in Groups I+ and II+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical feature.