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(54) Title: EGFR TARGETING COMPOUNDS AND METHODS OF USE THEREOF

(57) Abstract: Disclosed herein are compounds having activity as EGFR inhibitors. In some examples, the compounds can comprise an EGFR binding moiety; a linker; and a detectable moiety, therapeutic moiety, or a combination thereof. Also provided herein are methods of treating, preventing, detecting or ameliorating cancer in a subject. The disclosed subject matter also concerns methods for treating a subject having an inflammatory disorder or condition.



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EGFR TARGETING COMPOUNDS AND METHODS OF USE THEREOF

STATEMENT REGARDING FEDERALLY FUNDED RESEARCH

This invention was made with government support under grant number 1K22CA154600 awarded by the National Institutes of Health. The government has certain rights in this invention.

BACKGROUND

The epidermal growth factor receptor (EGFR) is a transmembrane receptor tyrosine kinase that performs key roles in cell regulation including proliferation and differentiation (Yarden Y and Sliwkowski MX, *Nat. Rev. Mol. Cell. Biol.*, 2001, 2, 127-137). As such, aberrant EGFR activity is associated with a variety of cancers and is a major target for development of cancer therapeutics. There are numerous limitations with current EGFR therapies including acquired resistance, off-target effects of tyrosine kinase inhibitors, serious side effects of monoclonal antibodies such as obstruction of airways and cardiac arrest, and the high cost of antibody production (Hynes NE and Lane HA, *Nat. Rev. Cancer*, 2005, 5, 341-354; Carter PJ, *Nat. Rev. Immunol.*, 2006, 6, 343-357; Elbakri A *et al.*, *Hum. Immunol.*, 2010, 71, 1243-1250).

The extracellular receptor of EGFR undergoes considerable conformational changes between the inactive and active states. In its inactive form, the receptor is folded to bury the dimerization arm. Once activated, EGFR undergoes a significant rearrangement that projects the dimerization arm outward to engage in receptor dimerization. Dimerization is largely dependent on dimerization arm interactions, and this allosteric change is followed by intracellular kinase domain dimerization and phosphorylation (Dawson JP *et al.*, *Mol. Cell. Biol.*, 2005, 25, 7734-7742; Burgess AT *et al.*, *Mol. Cell*, 2003, 12, 541-552; Lemmon MA *et al.*, *EMBO J.*, 1997, 16, 281-294; Schlessinger J, *Cell*, 2002, 110, 669-672; Zhang X *et al.*, *Cell*, 2006, 125, 1137-1149). Previous studies showed that the dimerization arm of EGFR forms a large part of the dimer interface and contributes a substantial share of the driving energy for receptor dimerization (FIG. 1) (Dawson JP *et al.*, *Mol. Cell. Biol.*, 2005, 25, 7734-7742; Ogiso H *et al.*, *Cell*, 2002, 110, 775-787).

What are needed are new therapies and diagnostics that target EGFR activation, and the disclosed methods and compositions target EGFR using a dimerization arm mimic. This synthetic mimic can bind a ligand-activated receptor monomer, thereby preventing receptor dimerization and EGFR activation.

SUMMARY

Disclosed herein are compositions and methods of making and using compositions. Also disclosed are compounds having activity as EGFR inhibitors. In some examples, the compounds

can comprise an EGFR binding moiety, a linker, and a detectable moiety. In other examples, the compounds can comprise an EGFR binding moiety, a linker, and a therapeutic moiety. In still other examples, the compounds can comprise an EGFR binding moiety, a linker, a detectable moiety, and a therapeutic moiety. The detectable moiety can also serve as the therapeutic moiety.

5 In some examples, the EGFR binding moiety can also serve as the therapeutic moiety.

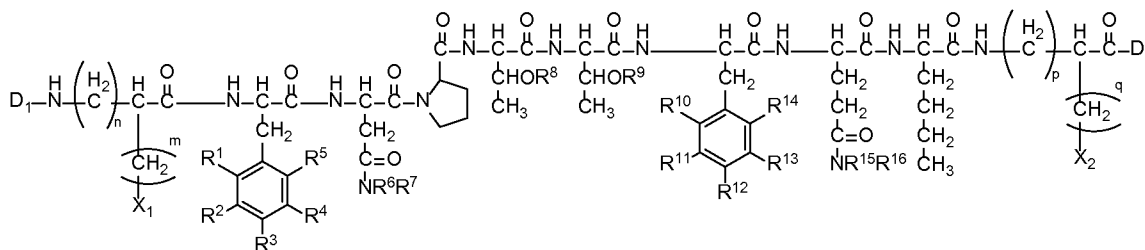
The EGFR binding moiety can, for example, comprise at least 8 amino acids. Each amino acid can be a natural or non-natural amino acid. Examples of suitable amino acids include, but are not limited to, alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine,
10 threonine, tryptophan, tyrosine, valine, a derivative, or combinations thereof.

The detectable moiety can comprise any detectable label. Examples of suitable detectable labels include, but are not limited to, a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a magnetic spin resonance label, a photosensitizer, a photocleavable moiety, a chelating center, a heavy atom, a radioactive isotope, a isotope detectable spin
15 resonance label, a paramagnetic moiety, a chromophore, or any combination thereof. In some specific examples, the detectable moiety can comprise fluorenylmethyloxycarbonyl chloride (Fmoc) or 5(6)-carboxyfluorescein (FAM).

The disclosed compounds can also comprise a therapeutic moiety. The therapeutic moiety can be linked to the detectable moiety. Therapeutic moiety refers to a molecule or
20 molecule portion that when administered to a subject in an effective amount, will reduce one or more symptoms of a disease or disorder. The therapeutic moiety can comprise a wide variety of drugs. The therapeutic moiety can, for example, comprise an anticancer agent, antiviral agent, antimicrobial agent, anti-inflammatory agent, immunosuppressive agent, anesthetics, or any combination thereof.

25 The compounds described herein contain a linker. The term "linker," as used herein, refers to one or more polyfunctional (*e.g.*, bi-functional, tri-functional, etc.) molecules which can be used to covalently couple the EGFR binding moiety and the one or more detectable and/or therapeutic moieties of the disclosed compounds. The linker can be attached to any part of the EGFR binding moiety so long as the point of attachment does not interfere with the biological
30 activity, for example, the anti-tumor and/or anti-inflammatory activity, or the detectability of the compounds described herein.

In some examples, the disclosed compounds are peptides having Formula II:



II

wherein R^1 , R^2 , R^3 , R^4 , R^5 , R^6 , R^7 , R^8 , R^9 , R^{10} , R^{11} , R^{12} , R^{13} , R^{14} , R^{15} , and R^{16} (*i.e.*, R^1 - R^{16}) are independently chosen from hydrogen, halogen, hydroxyl, substituted or unsubstituted amino, substituted or unsubstituted amido, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted alkoxy, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, or wherein, as valence and stability permit, any combination of R^1 - R^5 and R^{10} - R^{14} , together with the atoms to which they are attached, form a 3-10 membered substituted or unsubstituted cyclic moiety optionally including between 1 and 3 heteroatoms;

X^1 and X^2 are independently chosen from halogen, hydroxyl, azide, substituted or unsubstituted amino, silyl, selenol, selenanyl, thiol, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted alkoxy, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl;

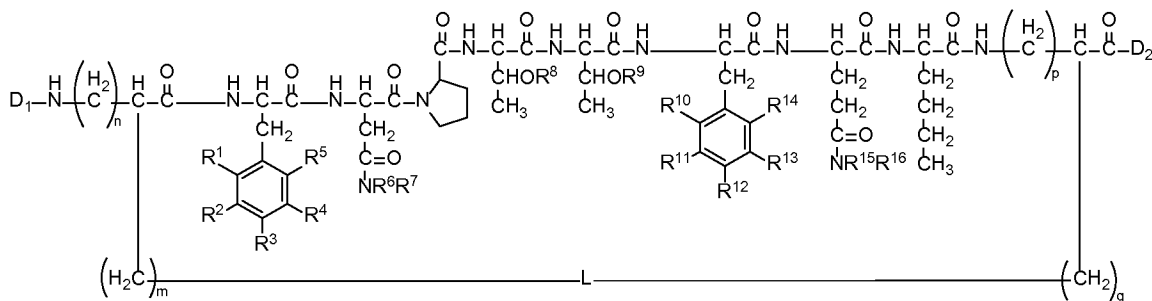
with the proviso that X^1 and X^2 are capable of reacting together and thereby forming a covalently bonded bridge moiety (also termed "linker" herein) having a number of backbone atoms, and the number of backbone atoms in the covalently bonded bridge moiety plus m and q is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10;

n and p are independently selected from 0, 1, and 2;

m and q are independently selected from 0, 1, 2, 3, and 4; and

D^1 and D^2 are each independently selected from a hydrogen, hydroxyl, acid protecting group, amine protecting group, detectable moiety, therapeutic moiety, and combination thereof, with the proviso that at least one of D^1 and D^2 is a detectable moiety, therapeutic moiety, or combination thereof. In other examples D^1 is hydrogen or an amine protecting group, D^2 is hydroxyl, amine, or acid protecting group, and one or more R^1 - R^{16} is a detectable moiety, therapeutic moiety, or combination thereof.

Some examples of Formula II are compounds wherein X^1 and X^2 have undergone a reaction to form a covalently bonded bridge moiety, and the resulting compounds have Formula IIa:



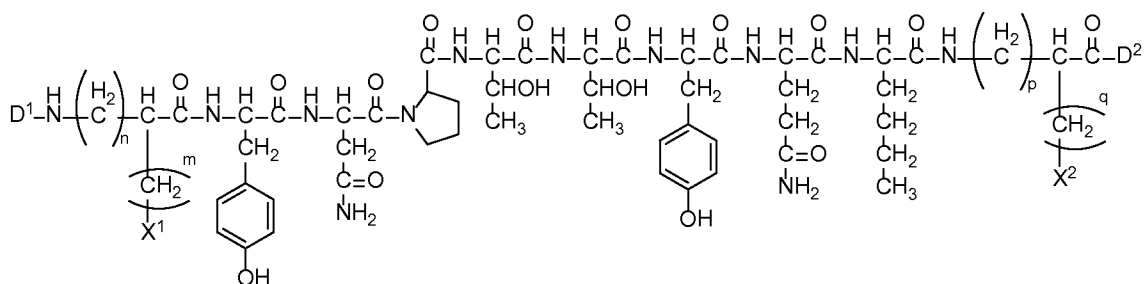
IIa

wherein R^1 - R^{16} , n , m , p , q , D^1 and D^2 are as defined in Formula II; and

L is the covalently bonded bridge moiety formed by the reaction of X^1 and X^2 in Formula II and is selected from substituted or unsubstituted amino, silyl, sulfide, disulfide, selenysulfide, diselenide, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted alkoxy, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted cycloalkenyl, substituted or unsubstituted heterocycloalkenyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl;

with the proviso that L has a number of atoms in its backbone and the sum of the number of atoms forming the backbone of L , m , and q is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10.

In some examples of Formula II, R^1 , R^2 , R^4 - R^{11} , and R^{13} - R^{16} are all hydrogen and R^3 and R^{12} are both hydroxyl, resulting in compounds of Formula III:



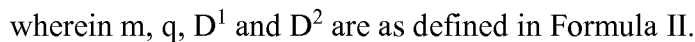
III

wherein n , m , p , q , D^1 and D^2 are as defined in Formula II.

In some examples of Formula III, X^1 and X^2 have undergone a reaction to form a covalently bonded bridge moiety (L), and the compounds have Formula IIIa:



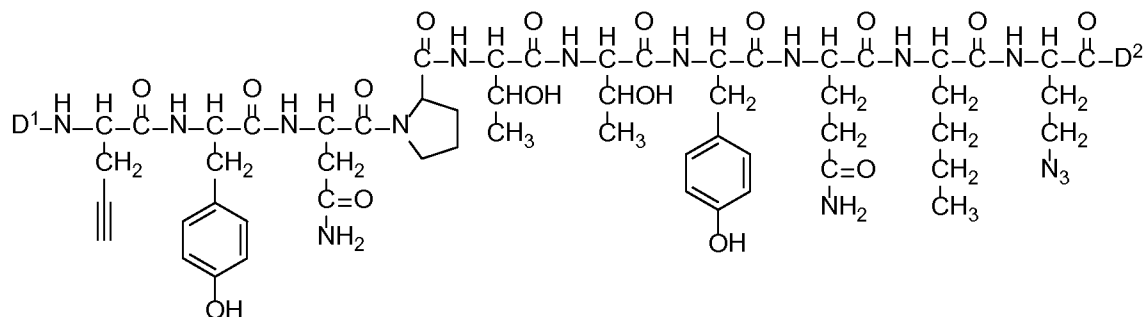
5 IV:



10



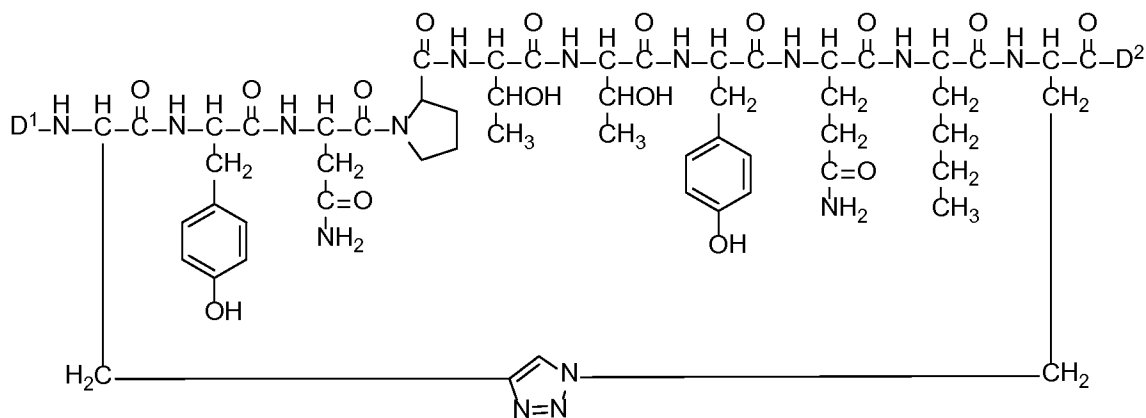
15



V

wherein D^1 and D^2 are as defined in Formula II.

In some examples of Formula V, the alkyne and azide undergo a cycloaddition reaction to form a covalently bonded triazole containing bridge, and the compounds are of Formula Va:

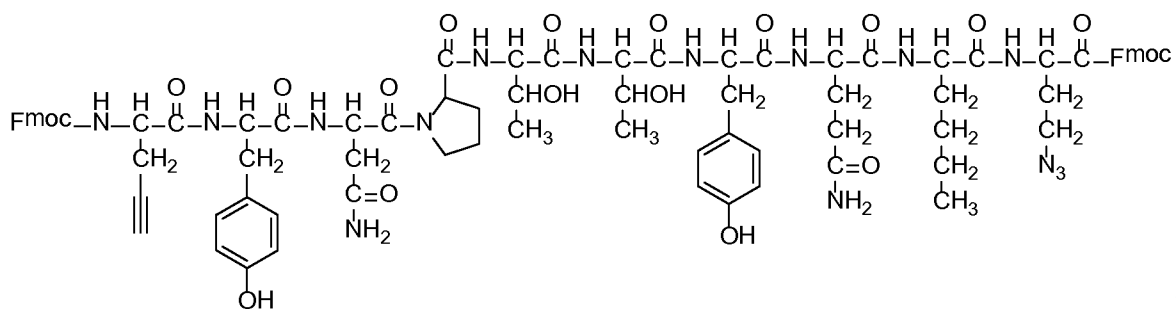


Va

wherein D^1 and D^2 are as defined in Formula II.

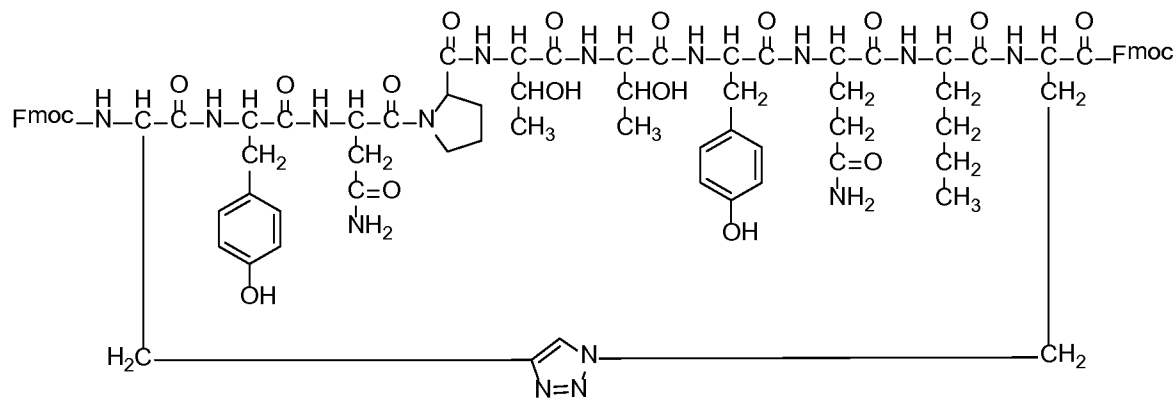
In some examples of Formula V, D^1 and D^2 are Fmoc, and compounds are of Formula

VI:



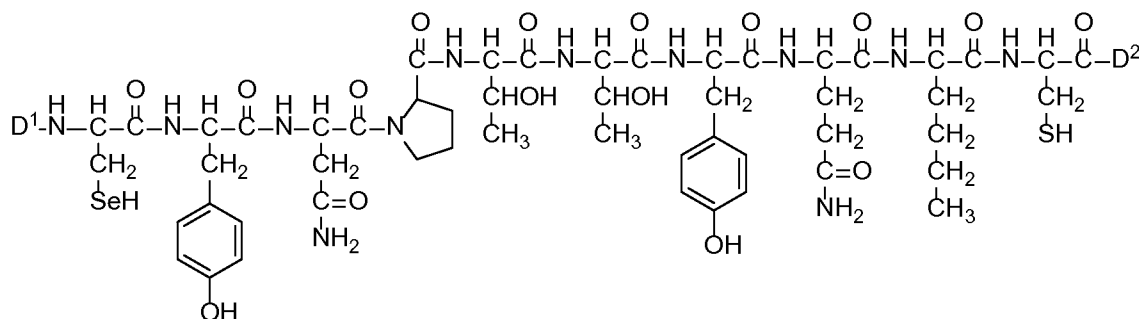
VI

In some examples of Formula VI, the alkyne and azide undergone a cycloaddition reaction to form a covalently bonded triazole containing bridge, and the compounds are of Formula VIa:



VIa

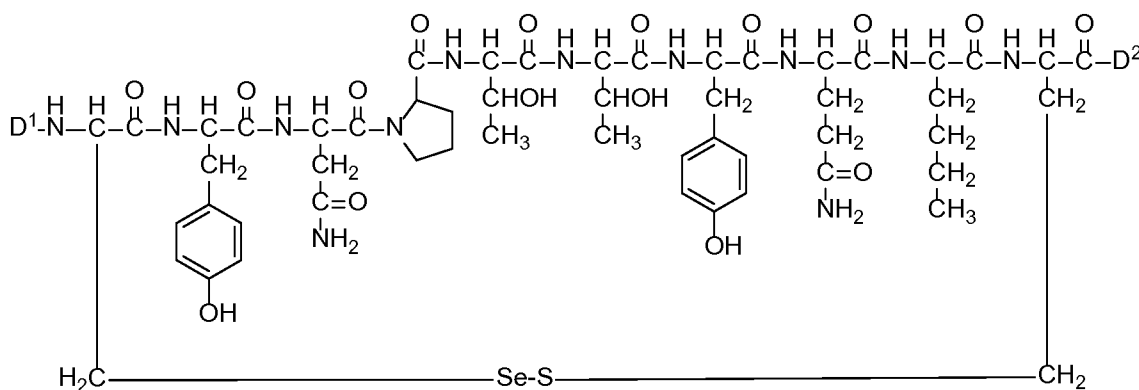
In some examples of Formula IV, m is 1, q is 2, X¹ is selenol or selenanyl, X² is a thiol or selenol. An example of such a compound is shown in Formula VII:



VII

wherein D¹ and D² are as defined in Formula II.

In some examples of Formula VII, the thiol and selenol or selenanyl undergo a reaction to form a covalently bonded selenylsulfide containing bridge. An example of such a compound is shown in Formula VIIa:



VIIa

wherein D¹ and D² are as defined in Formula II.

Also disclosed herein are pharmaceutically-acceptable salts and prodrugs of the disclosed compounds.

Also disclosed are pharmaceutical compositions that comprise a compound disclosed herein in combination with a pharmaceutically acceptable carrier.

Pharmaceutical compositions adapted for oral, topical or parenteral administration, comprising an amount of a compound constitute a preferred aspect. The dose administered to a patient, particularly a human, should be sufficient to achieve a therapeutic response in the patient over a reasonable time frame, without lethal toxicity, and preferably causing no more than an acceptable level of side effects or morbidity. One skilled in the art will recognize that dosage will depend upon a variety of factors including the condition (health) of the subject, the body weight of the subject, kind of concurrent treatment, if any, frequency of treatment, therapeutic ratio, as well as the severity and stage of the pathological condition.

Also provided herein are methods of treating, preventing, or ameliorating cancer in a subject. The methods include administering to a subject an effective amount of one or more of the compounds or compositions described herein, or a pharmaceutically acceptable salt thereof. The methods of treatment or prevention of cancer described herein can further include treatment with one or more additional agents (*e.g.*, an anti-cancer agent and/or ionizing radiation). In some examples, the compounds described herein can be used to treat or prevent lung cancer, anal cancers (including colon cancer), glioblastoma multiforme, epithelial cancers, or combinations thereof in a subject. In still other examples, disclosed are methods of diagnosing cancer or an overexpression of EGFR in a subject.

Also described herein are methods of killing a tumor cell in a subject. The method includes contacting the tumor cell with an effective amount of a compound or composition as described herein, and optionally includes the step of irradiating the tumor cell with an effective amount of ionizing radiation. Additionally, methods of radiotherapy of tumors are provided herein. The methods include contacting the tumor cell with an effective amount of a compound or composition as described herein, and irradiating the tumor with an effective amount of ionizing radiation.

The disclosed subject matter also concerns methods for treating a subject having an inflammatory disorder or condition. In one embodiment, an effective amount of one or more compounds or compositions disclosed herein is administered to a subject having an inflammatory disorder and who is in need of treatment thereof. In some examples, the compounds or compositions described herein can be used to treat or prevent psoriasis, eczema, atherosclerosis, or combinations thereof.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF FIGURES

5 The accompanying Figures, which are incorporated in and constitute a part of this specification, illustrate several aspects described below.

FIG. 1 displays the use of triazolyl-bridged peptides to inhibit EGFR activation. (A) EGF-induced activation of the extracellular receptor of EGFR. In the inactive state, the dimerization arm of the extracellular receptor is buried within domain IV. In the active state, the
10 receptor undergoes a conformational change to promote intermolecular interactions of the dimerization arm for receptor dimerization. Other features of the receptor include domain I, domain II, domain III and EGF. (B) Triazolyl-bridged peptides disclosed herein mimic the dimerization arm, thereby blocking receptor activation through occlusion of the dimerization arm binding pocket. Structures were rendered using PyMol (PDB files: 1NQL and 3NJP).

15 FIG. 2 displays the (A) non-natural amino acids used for the triazole cross-link: N-Fmoc-L-propargylglycine (Pg), N-Fmoc-4-azido-L-homoalanine (Aha), N-Fmoc-5-azido-L-norvaline (Anv), N-Fmoc-6-azido-L-norleucine (Anl). (B) Dimerization arm mimics were synthesized by incorporating non-natural amino acids into the peptide sequence using solid phase peptide synthesis (SPPS). Peptides were cyclized on solid support via copper (I)-catalyzed azide-alkyne
20 cycloaddition prior to resin cleavage. (C) Peptide sequences were derived from the dimerization arm sequence of EGFR. The overall linker length and positioning of the azide and alkyne amino acids were varied. Non-natural amino acids are show in grey. (D) Molecular dynamics simulations were performed to predict the overall structure of the EDA peptides. Cluster centers for EDA2, EDA4, and EDA6. Arrows indicate the position of the linker. Widths between the β -
25 sheets were measured between residues Y246 and Nle253.

FIG. 3 shows the molecular dynamics simulations performed to predict the overall structure of the peptides. Cluster analysis of the molecular dynamics trajectories using the g_cluster program in the Gromacs suite is shown for EDA1, 3, and 5. The triazole cross-link appears to fold back onto the non-binding surface of each peptide, potentially limiting binding
30 interactions of EGFR with the triazole. EDA1 and 5 maintain a narrower loop structure, while EDA3 adopts a more open conformation, suggesting greater conformational flexibility for EDA3. Images were generated in Pymol from the PDB files written from the cluster centers.

FIG. 4 contains results from molecular dynamics simulations where the g_hbond program in Gromacs suite was used to determine the stability of hydrogen bond characteristic of

the β -loop conformation. A query of the number of frames in which the hydrogen bond between N247 and Y251 is present predicts that this hydrogen bond is largely maintained in EDA2 and EDA4. Data is plotted as the percent of molecular dynamics trajectory frames in which the hydrogen bond between N247 and Y251 is present.

FIG. 5 contains RMSD plots for 40 ns simulations. The RMSD was calculated for all atoms in each peptide.

FIG. 6 shows that EDA2 inhibits EGFR activation in the EGFR-overexpressing cell line MDA-MB-231. (A) Serum starved cells were stimulated with 50 ng/mL EGF for 5 min in the presence or absence of gefitinib or EDA peptides, followed by western blotting. (B)

Quantification of phosphorylation of EGFR Tyr1068 shows that EDA2 inhibited phosphorylation by greater than 50%. (C) EDA2 was found to block phosphorylation of Akt by approximately 60%. (D) EGFR phosphorylation was also monitored in the presence of an EDA2 scrambled control (EDA2-Scr). (E) Quantification of phosphorylation of EGFR Tyr1068 shows that EDA2-Scr does not inhibit EGFR phosphorylation. Data is plotted as the average of at least three experiments, where errors bars represent SEM.

FIG. 7 shows the effect of EDA3-6 on EGFR phosphorylation in MDA-MB-231. Serum starved MDA-MB-231 cells were treated with gefitinib, peptide, or vehicle for 30 minutes, after which cells were stimulated for 5 minutes with 50 ng/mL EGF. Cells were immediately lysed following stimulation and proteins were separated by 8% SDS-PAGE. Western blot analysis showed that EDA3-6 do not inhibit EGFR phosphorylation at Tyr1068. Data is plotted as the average of three experiments, where error bars represent SEM.

FIG. 8 shows that EDA2 inhibits EGFR dimerization in MDA-MB-231 cells and is resistant to proteolysis. (A) MDA-MB-231 cells were stimulated with 10 ng/mL EGF for 5 min in the presence or absence of 5 μ M EDA2 and the scrambled control peptide (EDA2-Scr). The dimer species of EGFR was detected using a fluorescent Duolink assay and is shown as an overlay of the PLA signal and DAPI. Images were obtained with a 40x objective and scale bars represent 50 μ m scale. (B) The fluorescent signals of individual cells were measured for each condition tested (n=100 per condition). Data is plotted as the average signal count per cell, where error bars represent SEM. (C) Immunoprecipitation of the fluorescein-labeled EDA2 was performed with A431 cell lysates treated with 5 μ M peptide, followed by western blot analysis. (D) Proteolytic stability was measured in the presence of 50% mouse serum over a time range of 0-4 hours at 37 °C. The relative amount of loss as compared to that of the parent peptide at t=0 was quantified by LC/MS using an internal standard. (E) The stability of EDA2 was also monitored in 50% mouse serum under the same conditions in (D).

FIG. 9 displays data on the stability of cross-linked peptides. (A) Proteolytic stability was evaluated by incubating peptides in a cocktail of trypsin and chymotrypsin for 0-4 hours at 37°C. The relative amount of parent peptide was quantified by LC/MS using an internal standard. (B) Peptide stability in RPMI-1640 was measured using the same conditions above. (C) Serum

5 stability was evaluated by incubating the peptides in fresh 50% mouse serum using the same conditions above. All peptides are stable in the tissue culture media, but only the cross-linked and disulfide peptides are stable in the presence of proteases or serum.

FIG. 10 contains CD spectra of EDA2 and control peptides at physiologic and hypoxic pH. CD spectra were obtained on a Jasco J-710 CD Spectrometer at 25°C in 10 mM sodium

10 phosphate buffer of pH 7.4 and pH 6.5. Peptide secondary structure was predicted using the SOMCD neural network algorithm. Under slightly acidic pH, EDA2 maintains its β -sheet and β -turn conformation, while the disulfide bridged control loses a majority of its structure presumably as a result of reduction of the disulfide bond.

FIG. 11 is a table of peptide sequences designed from the native EGFR dimerization arm

15 with cysteine or selenocysteine added to the termini for cyclization. Cysteine and selenocysteine were replaced with alanine in the uncyclized control. 2a = FAM-PEG-(SEQ ID NO:12); 2b = FAM-PEG-(SEQ ID NO:13); 2c = FAM-PEG-(SEQ ID NO:14); 3a = (SEQ ID NO:15)-PEG-K(FAM); 3b = (SEQ ID NO:16)-PEG-K(FAM); 3c = (SEQ ID NO:17)-PEG-K(FAM); 4a = (SEQ ID NO:18)-PEG; 4b = (SEQ ID NO:19)-PEG; 4c = (SEQ ID NO:20)-PEG; U =

20 Selenocysteine; FAM = 5(6)-carboxyfluorescein; PEG = 11-amino-3,6,9-trioxaundecanoic acid.

FIG. 12 is a pair of chemical reaction schemes showing the synthesis of peptides disclosed herein. (A) Displays the synthesis of selenylsulfide peptide 2c. (i) Fmoc-based solid phase peptide synthesis on rink amide MBHA resin. Deprotection: 25% piperidine in NMP, 25 min. Coupling: 10 equiv amino acid, 9.9 equiv HCTU, 20 equiv DIPEA in NMP, P45 min. (ii)

25 Deprotection: 25% piperidine in NMP, 2 x 5 min. Coupling: 4 equiv amino acid, 4 equiv Oxyma Pure, 4 equiv DIC in DMF, 135-160 min. (iii) 2 equiv 5(6)-carboxyfluorescein, 2 equiv Oxyma Pure, 2 equiv DIC in DMF, overnight. (iv) 97.5% TFA, 2.5% thioanisole, 0.4 equiv DTNP, 1.5 h. FAM = 5(6)-carboxyfluorescein, U = selenocysteine, Dha = dehydroalanine. (B) Displays the synthesis of selenylsulfide peptide 3c. (i) Fmoc-based solid phase peptide synthesis on rink

30 amide MBHA resin. Deprotections: 25% piperidine in NMP, 25 min. Couplings: 10 equiv amino acid, 9.9 equiv HCTU, 20 equiv DIPEA in NMP, P45 min. (ii) 1% TFA in DCM, 30 x 2 min. (iii) 2 equiv 5(6)-carboxyfluorescein, 1.8 equiv HCTU, and 4.6 equiv DIPEA in DMF, overnight. (iv) 6 equiv Trityl chloride and 6 equiv DIPEA in DCM, overnight. (v) Deprotection: 25% piperidine in NMP 25 min. Coupling: 5 equiv Fmoc-Sec(PMB)-OH, 4.95 equiv HCTU, 10

equiv DIPEA in NMP, 95 min. (vi) 25% piperidine in NMP, 3 x 5 min. (vii) 95% TFA, 2.5% water, 2.5% Triisopropyl silane, 3 h. (viii) 97.5% TFA, 2.5% thioanisole, 0.25 equiv DTNP, 1.5 h.

FIG. 13 shows that the selenylsulfide peptide is stable to serum proteases. Peptides 2a–c were incubated in the presence of 50% mouse serum over a time course of 12 h. Degradation was monitored by LC–MS. The 280 nm absorbance of each peptide and their corresponding degradation products was measured. Degradation was measured as compared to the initial time point. Error bars represent SD of triplicates.

FIG. 14 shows that the selenylsulfide-bridged peptide is resistant to the reducing effects of DTT. By circular dichroism analysis, the selenylsulfide showed a notable shift in the minimum from 201 nm to 198 nm after the addition of 400 μ M DTT (32 equiv), demonstrating the stability of the peptide in the presence of a reducing agent. The dashed line indicates the minima at 0 μ M DTT.

FIG. 15 shows that selenylsulfide 3c is non-toxic. Cells were incubated with unlabeled peptide for 6 h, followed by cell viability analysis using an MTT assay. Percent viability relative to the untreated control is reported as the average of quadruplicates, where error bars represent SEM. A two-way ANOVA was performed for all cell lines with Tukey's multiple comparison test. For all conditions, $p > 0.5$ as compared to the untreated control.

DETAILED DESCRIPTION

The epidermal growth factor receptor is a cell-surface receptor for members of the epidermal growth factor family of extracellular protein ligands. Mutations affecting EGFR expression or activity could result in cancer.

EGFR exists on the cell surface and is activated by binding of its specific ligands, including, but not limited to, epidermal growth factor and transforming growth factor α . Upon activation by its growth factor ligands, EGFR undergoes a transition from an inactive monomeric form to an active homodimer. In addition to forming homodimers after ligand binding, EGFR can pair with another member of the ErbB receptor family to create an activated heterodimer. EGFR dimerization stimulates its intracellular protein-tyrosine kinase activity. As a result, autophosphorylation of several tyrosine (Y) residues in the C-terminal domain of EGFR occurs. This autophosphorylation elicits downstream activation and signaling by several other proteins that associate with the phosphorylated tyrosines through their own phosphotyrosine-binding SH2 domains. These downstream signaling proteins initiate several signal transduction cascades. Activation and upregulation of such proteins modulate phenotypes such as cell

migration, adhesion and proliferation. Activation of the receptor is important for the innate immune response in human skin.

Mutations that lead to EGFR overexpression (*e.g.*, upregulation) or overactivity have been associated with a number of epithelial cancers, including lung cancer, anal cancers (including colon cancer) and glioblastoma multiforme. These somatic mutations involving EGFR lead to its constant activation, which produces uncontrolled cell division. Mutations, amplifications or misregulations of EGFR or family members are implicated in about 30% of all epithelial cancers. Aberrant EGFR signaling has also been implicated in inflammatory diseases, such as psoriasis, eczema, and atherosclerosis.

The compounds, compositions, and methods described herein seek to provide new therapies and diagnostics that target EGFR activation, in particular by using a dimerization arm mimic. This synthetic mimic can bind a ligand-activated receptor monomer, thereby preventing receptor dimerization and EGFR activation. The details of the disclosed compounds, compositions, and methods can be understood more readily by reference to the following detailed description of specific aspects of the disclosed subject matter and the Examples and Figures included therein.

Before the present compounds, compositions, and methods are disclosed and described, it is to be understood that the aspects described below are not limited to specific synthetic methods or specific reagents, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only and is not intended to be limiting.

Also, throughout this specification, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which the disclosed matter pertains. The references disclosed are also individually and specifically incorporated by reference herein for the material contained in them that is discussed in the sentence in which the reference is relied upon.

General Definitions

In this specification and in the claims that follow, reference will be made to a number of terms, which shall be defined to have the following meanings.

Throughout the description and claims of this specification the word “comprise” and other forms of the word, such as “comprising” and “comprises,” means including but not limited to, and is not intended to exclude, for example, other additives, components, integers, or steps.

As used in the description and the appended claims, the singular forms “a,” “an,” and

“the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “a composition” includes mixtures of two or more such compositions, reference to “an agent” includes mixtures of two or more such agents, reference to “the component” includes mixtures of two or more such components, and the like.

5 “Optional” or “optionally” means that the subsequently described event or circumstance can or cannot occur, and that the description includes instances where the event or circumstance occurs and instances where it does not.

10 Ranges can be expressed herein as from “about” one particular value, and/or to “about” another particular value. By “about” is meant within 5% of the value, e.g., within 4, 3, 2, or 1% of the value. When such a range is expressed, another aspect includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent “about,” it will be understood that the particular value forms another aspect. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint.

15 As used herein, by a “subject” is meant an individual. Thus, the “subject” can include domesticated animals (*e.g.*, cats, dogs, etc.), livestock (*e.g.*, cattle, horses, pigs, sheep, goats, etc.), laboratory animals (*e.g.*, mouse, rabbit, rat, guinea pig, etc.), and birds. “Subject” can also include a mammal, such as a primate or a human. Thus, the subject can be a human or veterinary patient. The term “patient” refers to a subject under the treatment of a clinician, *e.g.*, physician.

20 The term “inhibit” refers to a decrease in an activity, response, condition, disease, or other biological parameter. This can include but is not limited to the complete ablation of the activity, response, condition, or disease. This can also include, for example, a 10% reduction in the activity, response, condition, or disease as compared to the native or control level. Thus, the reduction can be a 10, 20, 30, 40, 50, 60, 70, 80, 90, 100%, or any amount of reduction in
25 between as compared to native or control levels.

By “reduce” or other forms of the word, such as “reducing” or “reduction,” is meant lowering of an event or characteristic (*e.g.*, tumor growth). It is understood that this is typically in relation to some standard or expected value, in other words it is relative, but that it is not always necessary for the standard or relative value to be referred to. For example, “reduces tumor growth” means reducing the rate of growth of a tumor relative to a standard or a control.

30 By “prevent” or other forms of the word, such as “preventing” or “prevention,” is meant to stop a particular event or characteristic, to stabilize or delay the development or progression of a particular event or characteristic, or to minimize the chances that a particular event or characteristic will occur. Prevent does not require comparison to a control as it is typically more

absolute than, for example, reduce. As used herein, something could be reduced but not prevented, but something that is reduced could also be prevented. Likewise, something could be prevented but not reduced, but something that is prevented could also be reduced. It is understood that where reduce or prevent are used, unless specifically indicated otherwise, the use of the other word is also expressly disclosed. For example, the terms “prevent” or “suppress” can refer to a treatment that forestalls or slows the onset of a disease or condition or reduced the severity of the disease or condition. Thus, if a treatment can treat a disease in a subject having symptoms of the disease, it can also prevent or suppress that disease in a subject who has yet to suffer some or all of the symptoms.

The term “treatment” refers to the medical management of a patient with the intent to cure, ameliorate, stabilize, or prevent a disease, pathological condition, or disorder. This term includes active treatment, that is, treatment directed specifically toward the improvement of a disease, pathological condition, or disorder, and also includes causal treatment, that is, treatment directed toward removal of the cause of the associated disease, pathological condition, or disorder. In addition, this term includes palliative treatment, that is, treatment designed for the relief of symptoms rather than the curing of the disease, pathological condition, or disorder; preventative treatment, that is, treatment directed to minimizing or partially or completely inhibiting the development of the associated disease, pathological condition, or disorder; and supportive treatment, that is, treatment employed to supplement another specific therapy directed toward the improvement of the associated disease, pathological condition, or disorder.

The term “anticancer” refers to the ability to treat or control cellular proliferation and/or tumor growth at any concentration.

The term “therapeutically effective” refers to the amount of the composition used is of sufficient quantity to ameliorate one or more causes or symptoms of a disease or disorder. Such amelioration only requires a reduction or alteration, not necessarily elimination.

The term “pharmaceutically acceptable” refers to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problems or complications commensurate with a reasonable benefit/risk ratio.

The term “carrier” means a compound, composition, substance, or structure that, when in combination with a compound or composition, aids or facilitates preparation, storage, administration, delivery, effectiveness, selectivity, or any other feature of the compound or

composition for its intended use or purpose. For example, a carrier can be selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject.

The terms “peptide,” “protein,” and “polypeptide” are used interchangeably to refer to a natural or synthetic molecule comprising two or more amino acids linked by the carboxyl group of one amino acid to the alpha amino group of another.

Chemical Definitions

As used herein, the term “substituted” is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, and aromatic and nonaromatic substituents of organic compounds. Illustrative substituents include, for example, those described below. The permissible substituents can be one or more and the same or different for appropriate organic compounds. For purposes of this disclosure, the heteroatoms, such as nitrogen, can have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the heteroatoms. This disclosure is not intended to be limited in any manner by the permissible substituents of organic compounds. Also, the terms “substitution” or “substituted with” include the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, *e.g.*, a compound that does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc.

“Z¹,” “Z²,” “Z³,” and “Z⁴” are used herein as generic symbols to represent various specific substituents. These symbols can be any substituent, not limited to those disclosed herein, and when they are defined to be certain substituents in one instance, they can, in another instance, be defined as some other substituents.

The term “aliphatic” as used herein refers to a non-aromatic hydrocarbon group and includes branched and unbranched, alkyl, alkenyl, or alkynyl groups.

The term “alkyl” as used herein is a branched or unbranched saturated hydrocarbon group of 1 to 24 carbon atoms, for example 1 to 3, 1 to 4, 1 to 5, 1 to 6, 1 to 7, 1 to 8, 1 to 9, 1 to 10, or 1 to 15 carbon atoms, such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, t-butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, dodecyl, tetradecyl, hexadecyl, eicosyl, tetracosyl, and the like. The alkyl group can also be substituted or unsubstituted. The alkyl group can be substituted with one or more groups including, but not limited to, alkyl, halogenated alkyl, alkoxyl, alkenyl, alkynyl, aryl, heteroaryl, aldehyde, amino, amido, carboxylic acid, ester, ether, halide, hydroxyl, ketone, nitro, silyl, sulfo-oxo, sulfonyl, sulfone, sulfoxide, thiol, or azide as described below.

Throughout the specification “alkyl” is generally used to refer to both unsubstituted alkyl groups and substituted alkyl groups; however, substituted alkyl groups are also specifically referred to herein by identifying the specific substituent(s) on the alkyl group. For example, the term “halogenated alkyl” specifically refers to an alkyl group that is substituted with one or more halide, *e.g.*, fluorine, chlorine, bromine, or iodine. The term “alkoxyalkyl” specifically refers to an alkyl group that is substituted with one or more alkoxy groups, as described below. The term “alkylamino” specifically refers to an alkyl group that is substituted with one or more amino groups, as described below, and the like. When “alkyl” is used in one instance and a specific term such as “alkylalcohol” is used in another, it is not meant to imply that the term “alkyl” does not also refer to specific terms such as “alkylalcohol” and the like.

This practice is also used for other groups described herein. That is, while a term such as “cycloalkyl” refers to both unsubstituted and substituted cycloalkyl moieties, the substituted moieties can, in addition, be specifically identified herein; for example, a particular substituted cycloalkyl can be referred to as, *e.g.*, an “alkylcycloalkyl.” Similarly, a substituted alkoxy can be specifically referred to as, *e.g.*, a “halogenated alkoxy,” a particular substituted alkenyl can be, *e.g.*, an “alkenylalcohol,” and the like. Again, the practice of using a general term, such as “cycloalkyl,” and a specific term, such as “alkylcycloalkyl,” is not meant to imply that the general term does not also include the specific term.

The term “alkoxyl” as used herein is an alkyl group bound through a single, terminal ether linkage; that is, an “alkoxyl” group can be defined as —OZ^1 where Z^1 is alkyl as defined above.

The term “alkenyl” as used herein is a hydrocarbon group of from 2 to 24 carbon atoms, for example, 2 to 5, 2 to 10, 2 to 15, or 2 to 20 carbon atoms, with a structural formula containing at least one carbon-carbon double bond. Asymmetric structures such as $(\text{Z}^1\text{Z}^2)\text{C}=\text{C}(\text{Z}^3\text{Z}^4)$ are intended to include both the *E* and *Z* isomers. This can be presumed in structural formulae herein wherein an asymmetric alkene is present, or it can be explicitly indicated by the bond symbol $\text{C}=\text{C}$. The alkenyl group can be substituted with one or more groups including, but not limited to, alkyl, halogenated alkyl, alkoxy, alkenyl, alkynyl, aryl, heteroaryl, aldehyde, amino, amido, carboxylic acid, ester, ether, halide, hydroxyl, ketone, nitro, silyl, sulfo-oxo, sulfonyl, sulfone, sulfoxide, thiol, or azide, as described below.

The term “alkynyl” as used herein is a hydrocarbon group of 2 to 24 carbon atoms, for example 2 to 5, 2 to 10, 2 to 15, or 2 to 20 carbon atoms, with a structural formula containing at least one carbon-carbon triple bond. The alkynyl group can be substituted with one or more groups including, but not limited to, alkyl, halogenated alkyl, alkoxy, alkenyl, alkynyl, aryl,

heteroaryl, aldehyde, amino, amido, carboxylic acid, ester, ether, halide, hydroxyl, ketone, nitro, silyl, sulfo-oxo, sulfonyl, sulfone, sulfoxide, thiol, or azide, as described below.

The term “aryl” as used herein is a group that contains any carbon-based aromatic group including, but not limited to, benzene, naphthalene, phenyl, biphenyl, phenoxybenzene, and the like. The term “heteroaryl” is defined as a group that contains an aromatic group that has at least one heteroatom incorporated within the ring of the aromatic group. Examples of heteroatoms include, but are not limited to, nitrogen, oxygen, sulfur, and phosphorus. The term “non-heteroaryl,” which is included in the term “aryl,” defines a group that contains an aromatic group that does not contain a heteroatom. The aryl or heteroaryl group can be substituted or unsubstituted. The aryl or heteroaryl group can be substituted with one or more groups including, but not limited to, alkyl, halogenated alkyl, alkoxyl, alkenyl, alkynyl, aryl, heteroaryl, aldehyde, amino, amido, carboxylic acid, ester, ether, halide, hydroxyl, ketone, nitro, silyl, sulfo-oxo, sulfonyl, sulfone, sulfoxide, thiol, or azide, as described herein. The term “biaryl” is a specific type of aryl group and is included in the definition of aryl. Biaryl refers to two aryl groups that are bound together *via* a fused ring structure, as in naphthalene, or are attached *via* one or more carbon-carbon bonds, as in biphenyl.

The term “cycloalkyl” as used herein is a non-aromatic carbon-based ring composed of at least three carbon atoms. Examples of cycloalkyl groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, etc. The term “heterocycloalkyl” is a cycloalkyl group as defined above where at least one of the carbon atoms of the ring is substituted with a heteroatom such as, but not limited to, nitrogen, oxygen, sulfur, or phosphorus. The cycloalkyl group and heterocycloalkyl group can be substituted or unsubstituted. The cycloalkyl group and heterocycloalkyl group can be substituted with one or more groups including, but not limited to, alkyl, alkoxyl, alkenyl, alkynyl, aryl, heteroaryl, aldehyde, amino, amido, carboxylic acid, ester, ether, halide, hydroxyl, ketone, nitro, silyl, sulfo-oxo, sulfonyl, sulfone, sulfoxide, thiol, or azide, as described herein.

The term “cycloalkenyl” as used herein is a non-aromatic carbon-based ring composed of at least three carbon atoms and containing at least one double bond, *i.e.*, C=C. Examples of cycloalkenyl groups include, but are not limited to, cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclopentadienyl, cyclohexenyl, cyclohexadienyl, and the like. The term “heterocycloalkenyl” is a type of cycloalkenyl group as defined above, and is included within the meaning of the term “cycloalkenyl,” where at least one of the carbon atoms of the ring is substituted with a heteroatom such as, but not limited to, nitrogen, oxygen, sulfur, or phosphorus. The cycloalkenyl group and heterocycloalkenyl group can be substituted or unsubstituted. The cycloalkenyl group

and heterocycloalkenyl group can be substituted with one or more groups including, but not limited to, alkyl, alkoxyl, alkenyl, alkynyl, aryl, heteroaryl, aldehyde, amino, amido, carboxylic acid, ester, ether, halide, hydroxyl, ketone, nitro, silyl, sulfo-oxo, sulfonyl, sulfone, sulfoxide, thiol, or azide, as described herein.

5 The term “cyclic group” is used herein to refer to either aryl groups, non-aryl groups (*i.e.*, cycloalkyl, heterocycloalkyl, cycloalkenyl, and heterocycloalkenyl groups), or both. Cyclic groups have one or more ring systems that can be substituted or unsubstituted. A cyclic group can contain one or more aryl groups, one or more non-aryl groups, or one or more aryl groups and one or more non-aryl groups.

10 The term “carbonyl” as used herein is represented by the formula $-C(O)Z^1$ where Z^1 can be a hydrogen, hydroxyl, alkoxyl, alkyl, halogenated alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocycloalkyl, or heterocycloalkenyl group described above.

Throughout this specification “C(O)” or “CO” is a short hand notation for $C=O$.

The term “azide” as used herein is represented by the formula $-N=N=N$.

15 The term “aldehyde” as used herein is represented by the formula $-C(O)H$.

The terms “amine” or “amino” as used herein are represented by the formula $-NZ^1Z^2$, where Z^1 and Z^2 can each be substitution group as described herein, such as hydrogen, an alkyl, halogenated alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocycloalkyl, or heterocycloalkenyl group described above. “Amido” is

20 $-C(O)NZ^1Z^2$.

The term “carboxylic acid” as used herein is represented by the formula $-C(O)OH$. A “carboxylate” or “carboxyl” group as used herein is represented by the formula $-C(O)O^-$.

25 The term “ester” as used herein is represented by the formula $-OC(O)Z^1$ or $-C(O)OZ^1$, where Z^1 can be an alkyl, halogenated alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocycloalkyl, or heterocycloalkenyl group described above.

The term “ether” as used herein is represented by the formula Z^1OZ^2 , where Z^1 and Z^2 can be, independently, an alkyl, halogenated alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocycloalkyl, or heterocycloalkenyl group described above.

30 The term “ketone” as used herein is represented by the formula $Z^1C(O)Z^2$, where Z^1 and Z^2 can be, independently, an alkyl, halogenated alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocycloalkyl, or heterocycloalkenyl group described above.

The term “halide” or “halogen” as used herein refers to the fluorine, chlorine, bromine, and iodine.

The term “hydroxyl” as used herein is represented by the formula —OH.

The term “nitro” as used herein is represented by the formula —NO₂.

The term “selenol” as used herein is represented by the formula —SeH. The term “selenanyl” as used herein is represented by the term —SeZ¹, where Z¹ can be hydrogen, an alkyl, halogenated alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocycloalkyl, or heterocycloalkenyl group described above. The term “diselenide” is used herein to represent the —Se-Se—.

The term “silyl” as used herein is represented by the formula —SiZ¹Z²Z³, where Z¹, Z², and Z³ can be, independently, hydrogen, alkyl, halogenated alkyl, alkoxy, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocycloalkyl, or heterocycloalkenyl group described above.

The term “sulfonyl” is used herein to refer to the sulfo-oxo group represented by the formula —S(O)₂Z¹, where Z¹ can be hydrogen, an alkyl, halogenated alkyl, alkenyl, alkynyl, aryl, heteroaryl, cycloalkyl, cycloalkenyl, heterocycloalkyl, or heterocycloalkenyl group described above. The term “disulfide” is used herein to represent —S-S—.

The term “sulfonylamino” or “sulfonamide” as used herein is represented by the formula —S(O)₂NH—.

The term “thiol” as used herein is represented by the formula —SH.

The term “thio” as used herein is represented by the formula —S—.

“R¹,” “R²,” “R³,” “Rⁿ,” etc., where n is some integer, as used herein can, independently, possess one or more of the groups listed above. For example, if R¹ is a straight chain alkyl group, one of the hydrogen atoms of the alkyl group can optionally be substituted with a hydroxyl group, an alkoxy group, an amine group, an alkyl group, a halide, and the like. Depending upon the groups that are selected, a first group can be incorporated within second group or, alternatively, the first group can be pendant (*i.e.*, attached) to the second group. For example, with the phrase “an alkyl group comprising an amino group,” the amino group can be incorporated within the backbone of the alkyl group. Alternatively, the amino group can be attached to the backbone of the alkyl group. The nature of the group(s) that is (are) selected will determine if the first group is embedded or attached to the second group.

Unless stated to the contrary, a formula with chemical bonds shown only as solid lines and not as wedges or dashed lines contemplates each possible isomer, *e.g.*, each enantiomer, diastereomer, and meso compound, and a mixture of isomers, such as a racemic or scalemic mixture.

Reference will now be made in detail to specific aspects of the disclosed materials,

compounds, compositions, articles, and methods, examples of which are illustrated in the accompanying Examples and Figures.

Compounds

Disclosed herein are compounds having activity as EGFR inhibitors. In some examples, the compounds can comprise an EGFR binding moiety, a linker, and one or more detectable moieties. In other examples, the compounds can comprise an EGFR binding moiety, a linker, and one or more therapeutic moieties. In still other examples, the compounds can comprise an EGFR binding moiety, a linker, one or more detectable moieties, and one or more therapeutic moieties. The detectable moiety can also serve as the therapeutic moiety. In some examples, the EGFR binding moiety can also serve as the therapeutic moiety.

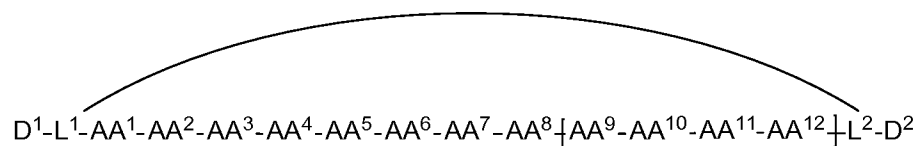
The EGFR binding moiety can, for example, comprise from eight to twelve amino acids (shown as AA¹ through AA¹²), and the compounds can be of Formula I:



I

wherein AA¹, AA², AA³, AA⁴, AA⁵, AA⁶, AA⁷, AA⁸, AA⁹, AA¹⁰, AA¹¹, and AA¹² (*i.e.*, AA¹-AA¹²) are each independently an amino acid; L¹, L² are each independently a linker moiety, with the proviso that L¹ and L² are capable of reacting with one another to form a covalently bonded bridge moiety; and D¹, D² are each independently selected from a detectable moiety, therapeutic moiety, and a combination thereof, and any of amino acids AA⁹ through AA¹², which are shown in brackets are optional.

In some examples, L¹ and L² have undergone a reaction to form a covalently bonded bridge moiety, and the compounds are of Formula Ia:



Ia

wherein AA¹-AA¹², D¹, D², L¹ and L² are as defined in Formula I, though here they have reacted and formed a bridge moiety shown as a curved line.

EGFR Binding Moiety

The EGFR binding moiety comprises at least 8 amino acids, more specifically from 8 to 12, from 8 to 11, from 8 to 10, from 8 to 9, from 10 to 12, and more specifically 10 amino acids. Each amino acid can be a natural or non-natural amino acid. The term “non-natural amino acid” refers to an organic compound that is a congener of a natural amino acid in that it has a structure similar to a natural amino acid so that it mimics the structure and reactivity of a natural amino

acid. The non-natural amino acid can be a modified amino acid, and/or amino acid analog, that is not one of the 20 common naturally occurring amino acids or the rare natural amino acids selenocysteine or pyrrolysine. Examples of suitable amino acids include, but are not limited to, alanine, allosoleucine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, pyroglutamic acid, serine, threonine, tryptophan, tyrosine, valine, N-Fmoc-L-propargylglycine (Pg), N-Fmoc-4-azido-L-homoalanine (Aha), N-Fmoc-5-azido-L-norvaline (Anv), N-Fmoc-6-azido-L-norleucine (Anl), selenocystine, a derivative, or combinations thereof. These are listed in the Table 1 along with their abbreviations used herein.

TABLE 1: Amino Acid Abbreviations

Amino Acid	Abbreviations
alanine	Ala (A)
allosoleucine	Alle
arginine	Arg (R)
asparagine	Asn (N)
aspartic acid	Asp (D)
cysteine	Cys (C)
glutamic acid	Glu (E)
glutamine	Gln (Q)
glycine	Gly (G)
histidine	His (H)
isoleucine	Ile (I)
leucine	Leu (L)
lysine	Lys (K)
phenylalanine	Phe (F)
proline	Pro (P)
pyroglutamic acid	PGlu
serine	Ser (S)
threonine	Thr (T)
tyrosine	Tyr (Y)
tryptophan	Trp (W)
valine	Val (V)
Norleucine	Nle
Methionine	Met (M)
N-Fmoc-L-propargylglycine	Pg
N-Fmoc-4-azido-L-homoalanine	Aha
N-Fmoc-5-azido-L-norvaline	Anv
N-Fmoc-6-azido-L-norleucine	Anl
Selenocystine	Sec (U)
Selenomethionine	Mse

The amino acids can be coupled by a peptide bond. The amino acids can be coupled to the linker at the amino group, the carboxylate group, or the side chain.

In some examples of Formula I, AA¹ comprises cysteine, methionine, aspartic acid, leucine, norleucine, N-Fmoc-L-propargylglycine, N-Fmoc-4-azido-L-homoalanine, N-Fmoc-5-azido-L-norvaline, N-Fmoc-6-azido-L-norleucine, selenocystine, selenomethionine, or an analogue or derivative thereof. In some examples of Formula I, AA² comprises tyrosine or an analogue or derivative thereof. In some examples of Formula I, AA³ comprises asparagine or an analogue or derivative thereof. In some examples of Formula I, AA⁴ comprises proline or an analogue or derivative thereof. In some examples of Formula I, AA⁵ comprises threonine or an analogue or derivative thereof. In some examples of Formula I, AA⁶ comprises threonine or an analogue or derivative thereof. In some examples of Formula I, AA⁷ comprises tyrosine or an analogue or derivative thereof. In some examples of Formula I, AA⁸ comprises glutamine or an analogue or derivative thereof. In some examples of Formula I, AA⁹, if present, comprises methionine or norleucine or an analogue or derivative thereof. In some examples of Formula I, AA¹⁰, if present, comprises cysteine, methionine, aspartic acid, leucine, N-Fmoc-L-propargylglycine, N-Fmoc-4-azido-L-homoalanine, N-Fmoc-5-azido-L-norvaline, N-Fmoc-6-azido-L-norleucine, selenocystine, selenomethionine, or an analogue or derivative thereof. Further it is contemplated that one or two amino acids chosen from any of the twenty natural amino acids or a non-natural amino acid, can be inserted before any one of AA¹-AA¹⁰, as just defined.

In some examples of Formula I, AA¹ comprises tyrosine or an analogue or derivative thereof. In some examples of Formula I, AA² comprises asparagine or an analogue or derivative thereof. In some examples of Formula I, AA³ comprises proline or an analogue or derivative thereof. In some examples of Formula I, AA⁴ comprises threonine or an analogue or derivative thereof. In some examples of Formula I, AA⁵ comprises threonine or an analogue or derivative thereof. In some examples of Formula I, AA⁶ comprises tyrosine or an analogue or derivative thereof. In some examples of Formula I, AA⁷ comprises glutamine or an analogue or derivative thereof. In some examples of Formula I, AA⁸, if present, comprises methionine or norleucine or an analogue or derivative thereof. In some examples of Formula I, L¹ and L² can be comprises cysteine, methionine, aspartic acid, leucine, N-Fmoc-L-propargylglycine, N-Fmoc-4-azido-L-homoalanine, N-Fmoc-5-azido-L-norvaline, N-Fmoc-6-azido-L-norleucine, selenocystine, selenomethionine, or an analogue or derivative thereof.

In some examples, the EGFR binding moiety can be or can comprise YNPTTYQM (SEQ ID NO:1). In other examples, the EGFR binding moiety can be or can comprise

LYNPPTYQMD (SEQ ID NO:2). In other examples, the EGFR binding moiety can be or can comprise a variant of SEQ ID NO:1 or SEQ ID NO:2. Peptide variants are well understood to those of skill in the art and can involve amino acid sequence modifications. For example, amino acid sequence modifications typically fall into one or more of three classes: substitutional, insertional, or deletional variants. Insertions include amino and/or carboxyl terminal fusions, side chain modifications/additions as well as intrasequence insertions of single or multiple amino acid residues. Insertions ordinarily will be smaller insertions than those of amino or carboxyl terminal fusions, for example, on the order of 1 to 3 residues. Deletions are characterized by the removal of one or more amino acid residues from the peptide sequence. Typically, no more than from 1 to 3 residues are deleted at any one site within the peptide. Amino acid substitutions are typically of single residues, but can occur at a number of different locations at once; insertions usually will be on the order of about from 1 to 3 amino acid residues; and deletions will range about from 1 to 3 residues. Deletions or insertions preferably are made in adjacent pairs, *i.e.* a deletion of 2 residues or insertion of 2 residues. Substitutions, deletions, insertions or any combination thereof can be combined to arrive at a final compound. Substitutional variants are those in which at least one residue has been removed and a different residue inserted in its place. Such substitutions can be made in accordance with Table 2 and are referred to as conservative substitutions. Conservative substitutions can also be made using non-natural amino acids.

TABLE 2: Amino Acid Substitutions

Exemplary Conservative Substitutions	
Ala replaced by ser	Leu replaced by ile or val
Arg replaced by lys or gln	Lys replaced by arg or gln
Asn replaced by gln or his	Met replaced by leu or ile
Asp replaced by glu	Phe replaced by met or leu; tyr
Cys replaced by ser	Ser replaced by thr
Gln replaced by asn or lys	Thr replaced by ser
Glu replaced by asp	Trp replaced by tyr
Gly replaced by pro	Tyr replaced by trp or phe
His replaced by asn or gln	Val replaced by ile or leu
Ile replaced by leu or val	

Substantial changes in function are made by selecting substitutions that are less conservative than those in Table 2, *i.e.*, selecting residues that differ more significantly in their effect on maintaining (a) the structure of the peptide backbone in the area of the substitution, for example as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at

the target site or (c) the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in the protein properties will be those in which (a) a hydrophilic residue, e.g., seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g., leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine, in this case, (e) by increasing the number of sites for sulfation and/or glycosylation.

For example, the replacement of one amino acid residue with another that is biologically and/or chemically similar is known to those skilled in the art as a conservative substitution. For example, a conservative substitution would be replacing one hydrophobic residue for another, or one polar residue for another. The substitutions include combinations such as, for example, Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr. Such conservatively substituted variations of each explicitly disclosed sequence are included within the peptides provided herein.

It is understood that one way to define the variants of the disclosed EGFR binding moieties is through defining the variants in terms of homology/identity to specific known sequences. For example, SEQ ID NO:1 sets forth a particular sequence. Specifically disclosed are variants of this peptide that have at least, 85%, 90%, 95%, or 97% homology to SEQ ID NO:1. Those skilled in the art may readily understand how to determine the homology of two proteins. For example, the homology can be calculated after aligning the two sequences so that the homology is at its highest level.

In addition to variants of SEQ ID NO:1 are derivatives of this peptide which also function in the disclosed methods and compositions. Derivatives are formed by replacing one or more residues with a modified residue, where the side chain of the residue has been modified. For example, in Formula II modifications are described where R^1 - R^{16} are not H but rather one or more are independently selected from halogen, hydroxyl, substituted or unsubstituted amino, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted alkoxyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, or wherein, as valence and stability permit, any combination of R^1 - R^5 and R^{10} - R^{14} , together with the atoms to which they are attached, form a 3-10 membered substituted or unsubstituted cyclic moiety optionally including between 1 and 3

heteroatoms. In another example, one or more of R^1 - R^{16} is a detectible moiety, therapeutic moiety, or combination thereof.

Detectable moiety

The detectable moiety can comprise any detectable label. In some examples, the detectable moiety can further comprise a spacer moiety. A “spacer moiety” as used herein, can comprise a substituted or unsubstituted alkyl group, a substituted or unsubstituted polyalkoxy group, one to 6 additional amino acids, and the like. The spacer moiety can, in some examples, add length to the compounds described herein. The spacer moiety can also be used to improve the solubility of the compounds, *e.g.*, using polyalkyleneoxide spacers can improve the water solubility. In other examples the spacer moiety can be an amino acid such as dehydroalanine, which can be used alone or with another spacer moiety like polyalkeneoxides.

In some preferred examples, the detectable moiety can be at the C-terminus of the disclosed compounds (*i.e.*, D^2 in the various formula used herein).

Examples of suitable detectable labels include, but are not limited to, a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a magnetic spin resonance label, a photosensitizer, a photocleavable moiety, a chelating center, a heavy atom, a radioactive isotope, a isotope detectable spin resonance label, a paramagnetic moiety, a chromophore, or any combination thereof. In some embodiments, the label is detectable without the addition of further reagents. In some examples, the detectable moiety can further comprise a spacer moiety. A “spacer moiety” as used herein, can comprise a substituted or unsubstituted alkyl group, a substituted or unsubstituted polyalkoxy group, one to 6 additional amino acids, and the like. The spacer moiety can, in some examples, add length to the compounds described herein.

In some embodiments, the detectable moiety is a biocompatible detectable moiety, such that the compounds can be suitable for use in a variety of biological applications. “Biocompatible” and “biologically compatible”, as used herein, generally refer to compounds that are, along with any metabolites or degradation products thereof, generally non-toxic to cells and tissues, and which do not cause any significant adverse effects to cells and tissues when cells and tissues are incubated (*e.g.*, cultured) in their presence.

The detectable moiety can contain a luminophore such as a fluorescent label or near-infrared label. Examples of suitable luminophores include, but are not limited to, metal porphyrins; benzoporphyrins; azabenzoporphyrine; naphthoporphyrin; phthalocyanine; polycyclic aromatic hydrocarbons such as perylene, perylene diimine, pyrenes; azo dyes; xanthene dyes; boron dipyrromethene, aza-boron dipyrromethene, cyanine dyes, metal-ligand complex such as bipyridine, bipyridyls, phenanthroline, coumarin, and acetylacetonates of ruthenium and iridium;

acridine, oxazine derivatives such as benzophenoxazine; aza-annulene, squaraine; 8-hydroxyquinoline, polymethines, luminescent producing nanoparticle, such as quantum dots, nanocrystals; carbostyryl; terbium complex; inorganic phosphor; ionophore such as crown ethers affiliated or derivatized dyes; or combinations thereof. Specific examples of suitable luminophores include, but are not limited to, Pd (II) octaethylporphyrin; Pt (II)-octaethylporphyrin; Pd (II) tetraphenylporphyrin; Pt (II) tetraphenylporphyrin; Pd (II) meso-tetraphenylporphyrin tetrabenzoporphine; Pt (II) meso-tetraphenyl metrylbenzoporphyrin; Pd (II) octaethylporphyrin ketone; Pt (II) octaethylporphyrin ketone; Pd (II) meso-tetra(pentafluorophenyl)porphyrin; Pt (II) meso-tetra (pentafluorophenyl) porphyrin; Ru (II) tris(4,7-diphenyl-1,10-phenanthroline) (Ru (dpp)₃); Ru (II) tris(1,10-phenanthroline) (Ru(phen)₃), tris(2,2'-bipyridine)ruthenium (II) chloride hexahydrate (Ru(bpy)₃); erythrosine B; fluorescein; eosin; iridium (III) ((N-methyl-benzimidazol-2-yl)-7-(diethylamino)-coumarin)); indium (III) ((benzothiazol-2-yl)-7-(diethylamino)-coumarin))-2-(acetylacetonate); Lumogen dyes; Macroflex fluorescent red; Macrolex fluorescent yellow; Texas Red; rhodamine B; rhodamine 6G; sulfur rhodamine; m-cresol; thymol blue; xlenol blue; cresol red; chlorophenol blue; bromocresol green; bromocresol red; bromothymol blue; Cy2; a Cy3; a Cy5; a Cy5.5; Cy7; 4-nitrophenol; alizarin; phenolphthalein; o-cresolphthalein; chlorophenol red; calmagite; bromo-xlenol; phenol red; neutral red; nitrazine; 3,4,5,6-tetrabromophenolphthalein; congo red; fluorescein; eosin; 2',7'-dichlorofluorescein; 5(6)-carboxy-fluorescein; carboxynaphthofluorescein; 8-hydroxypyrene-1,3,6-trisulfonic acid; semi-naphthorhodafluor; semi-naphthofluorescein; tris (4,7-diphenyl-1,10-phenanthroline) ruthenium (II) dichloride; (4,7-diphenyl-1,10-phenanthroline) ruthenium (II) tetraphenylboron; platinum (II) octaethylporphyrin; dialkylcarbocyanine; dioctadecylcycloxacarbocyanine; fluorenylmethyloxycarbonyl chloride; 5(6)-carboxytetramethylrhodamine, and derivatives or combinations thereof.

The detectable moiety can contain a radiolabel, also referred to herein as radioisotope. The radiolabel can also be a therapeutic moiety, *i.e.*, a radiolabel comprising a therapeutic radionuclide such as, ⁹⁰Y or ¹⁷⁷Lu. Other examples of suitable radiolabels include, but are not limited to, metal ¹⁸F, ⁶⁴Cu, ⁶⁷Cu, ⁸⁹Zr, ¹¹¹In, ¹²⁴I, ¹²³I, and ^{99m}Tc. In some embodiments, the radiolabel can be chelated by a macrocyclic molecule. Examples of such macrocyclic molecules include, but are not limited to, 2,2',2''-(10-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (DOTA) -based chelators, diethylene triamine pentaacetic acid (DTPA)-based chelators, and a derivative or a combination thereof.

The detectable moiety can contain a magnetic spin resonance label. Examples of suitable spin resonance label include free radicals such as nitroxide-stable free radicals. Suitable

nitroxides include, but are not limited to, those derived from 2,2,6,6-tetramethylpiperidine-N-oxyl (TEMPO), 2,2,5,5-tetramethylpyrroline-N-oxyl, and 4,4-dimethyloxazolidine-N-oxyl which is a doxyl nitroxide. All of these compounds are paramagnetic and hence capable of excitation or changes in magnetic resonance energy levels and therefore provide imaging. Other nitroxides include, but are not limited to, doxyl nitroxides, proxyl nitroxides, azethoxyl nitroxides, imidazoline derived nitroxides, tetrahydrooxazine derived nitroxides, and steroid nitroxides, and the like.

In some examples, the detectable moiety can comprise biotin. In some examples, biotin can be detected in the presence of HABA-Avidin.

The detectible moiety can be in the disclosed compounds at D^1 and/or D^2 . Alternatively, the detectible moiety can be attached to the disclosed compounds at one or more of R^1 - R^{16} . When the detectible moiety is at one or more of R^1 - R^{16} , D^1 is hydrogen or an amine protecting group, and D^2 is hydroxyl, amine or an acid protecting group.

Therapeutic moiety

The disclosed compounds can also comprise a therapeutic moiety. The detectable moiety can be linked to a therapeutic moiety or the detectable can also serve as the therapeutic moiety. Therapeutic moiety refers to a group that when administered to a subject will reduce one or more symptoms of a disease or disorder. In some examples, the therapeutic moiety can further comprise a spacer moiety. A “spacer moiety” as used herein, can comprise a substituted or unsubstituted alkyl group, a substituted or unsubstituted polyalkyleneoxide group, one to 6 additional amino acids, and the like. The spacer moiety can, in some examples, add length to the compounds described herein. The space moiety can also be used to improve the solubility of the compounds, *e.g.*, using polyalkyleneoxide spacers can improve the water solubility.

In some preferred examples, the therapeutic moiety can be at the C-terminus of the disclosed compounds (*i.e.*, D^2 in the various formula used herein).

The therapeutic moiety can comprise a wide variety of drugs, including antagonists, for example enzyme inhibitors, and agonists, for example a transcription factor which results in an increase in the expression of a desirable gene product (although as will be appreciated by those in the art, antagonistic transcription factors can also be used), are all included. In addition, therapeutic moiety includes those agents capable of direct toxicity and/or capable of inducing toxicity towards healthy and/or unhealthy cells in the body. Also, the therapeutic moiety can be capable of inducing and/or priming the immune system against potential pathogens.

The therapeutic moiety can, for example, comprise an anticancer agent, antiviral agent, antimicrobial agent, anti-inflammatory agent, immunosuppressive agent, anesthetics, or any combination thereof.

The therapeutic moiety can comprise an anticancer agent. Example anticancer agents
5 include 13-cis-Retinoic Acid, 2-Amino-6-Mercaptopurine, 2-CdA, 2-Chlorodeoxyadenosine, 5-fluorouracil, 6-Thioguanine, 6-Mercaptopurine, Accutane, Actinomycin-D, Adriamycin, Atracuril, Agrylin, Ala-Cort, Aldesleukin, Alemtuzumab, Alitretinoin, Alkaban-AQ, Alkeran, All-transretinoic acid, Alpha interferon, Altretamine, Amethopterin, Amifostine, Aminoglutethimide, Anagrelide, Anandron, Anastrozole, Arabinosylcytosine, Aranesp, Aredia,
10 Arimidex, Aromasin, Arsenic trioxide, Asparaginase, ATRA, Avastin, BCG, BCNU, Bevacizumab, Bexarotene, Bicalutamide, BiCNU, Blenoxane, Bleomycin, Bortezomib, Busulfan, Busulfex, C225, Calcium Leucovorin, Campath, Camptosar, Camptothecin-11, Capecitabine, Carac, Carboplatin, Carmustine, Carmustine wafer, Casodex, CCNU, CDDP, CeeNU, Cerubidine, cetuximab, Chlorambucil, Cisplatin, Citroforum Factor, Cladribine,
15 Cortisone, Cosmegen, CPT-11, Cyclophosphamide, Cytadren, Cytarabine, Cytarabine liposomal, Cytosar-U, Cytosan, Dacarbazine, Dactinomycin, Darbepoetin alfa, Daunomycin, Daunorubicin, Daunorubicin hydrochloride, Daunorubicin liposomal, DaunoXome, Decadron, Delta-Cortef, Deltasone, Denileukin diftitox, DepoCyt, Dexamethasone, Dexamethasone acetate, Dexamethasone sodium phosphate, Dexasone, Dexrazoxane, DHAD, DIC, Diodes, Docetaxel,
20 Doxil, Doxorubicin, Doxorubicin liposomal, Droloxia, DTIC, DTIC-Dome, Duralone, Efudex, Eligard, Ellence, Eloxatin, Elspar, Emcyt, Epirubicin, Epoetin alfa, Erbitux, Erwinia L-asparaginase, Estramustine, Ethyol, Etopophos, Etoposide, Etoposide phosphate, Eulexin, Evista, Exemestane, Fareston, Faslodex, Femara, Filgrastim, Floxuridine, Fludara, Fludarabine, Fluoroplex, Fluorouracil, Fluorouracil (cream), Fluoxymesterone, Flutamide, Folinic Acid,
25 FUDR, Fulvestrant, G-CSF, Gefitinib, Gemcitabine, Gemtuzumab ozogamicin, Gemzar, Gleevec, Lupron, Lupron Depot, Matulane, Maxidex, Mechlorethamine, -Mechlorethamine Hydrochlorine, Medralone, Medrol, Megace, Megestrol, Megestrol Acetate, Melphalan, Mercaptopurine, Mesna, Mesnex, Methotrexate, Methotrexate Sodium, Methylprednisolone, Mylocel, Letrozole, Neosar, Neulasta, Neumega, Neupogen, Nilandron, Nilutamide, Nitrogen
30 Mustard, Novaldex, Novantrone, Octreotide, Octreotide acetate, Oncospar, Oncovin, Ontak, Onxal, Oprevelkin, Orapred, Orasone, Oxaliplatin, Paclitaxel, Pamidronate, Panretin, Paraplatin, Pediapred, PEG Interferon, Pegaspargase, Pegfilgrastim, PEG-INTRON, PEG-L-asparaginase, Phenylalanine Mustard, Platinol, Platinol-AQ, Prednisolone, Prednisone, Prelone, Procarbazine, PROCRIT, Proleukin, Prolifeprosan 20 with Carmustine implant, Purinethol, Raloxifene,

Rheumatrex, Rituxan, Rituximab, Roveron-A (interferon alfa-2a), Rubex, Rubidomycin hydrochloride, Sandostatin, Sandostatin LAR, Sargramostim, Solu-Cortef, Solu-Medrol, STI-571, Streptozocin, Tamoxifen, Targretin, Taxol, Taxotere, Temodar, Temozolomide, Teniposide, TESP, Thalidomide, Thalomid, TheraCys, Thioguanine, Thioguanine Tabloid,

5 Thiophosphoamide, Thioplex, Thiotepa, TICE, Toposar, Topotecan, Toremifene, Trastuzumab, Tretinoin, Trexall, Trisenox, TSPA, VCR, Velban, Velcade, VePesid, Vesanoide, Viadur, Vinblastine, Vinblastine Sulfate, Vincasar Pfs, Vincristine, Vinorelbine, Vinorelbine tartrate, VLB, VP-16, Vumon, Xeloda, Zanosar, Zevalin, Zinecard, Zoladex, Zoledronic acid, Zometa, Gliadel wafer, Glivec, GM-CSF, Goserelin, granulocyte colony stimulating factor, Halotestin,

10 Herceptin, Hexadrol, Hexalen, Hexamethylmelamine, HMM, Hycamtin, Hydrea, Hydrocort Acetate, Hydrocortisone, Hydrocortisone sodium phosphate, Hydrocortisone sodium succinate, Hydrocortone phosphate, Hydroxyurea, Ibritumomab, Ibritumomab Tiuxetan, Idamycin, Idarubicin, Ifex, IFN-alpha, Ifosfamide, IL 2, IL-11, Imatinib mesylate, Imidazole Carboxamide, Interferon alfa, Interferon Alfa-2b (PEG conjugate), Interleukin 2, Interleukin-11, Intron A

15 (interferon alfa-2b), Leucovorin, Leukeran, Leukine, Leuprolide, Leurocristine, Leustatin, Liposomal Ara-C, Liquid Pred, Lomustine, L-PAM, L-Sarcolysin, Meticorten, Mitomycin, Mitomycin-C, Mitoxantrone, M-Prednisol, MTC, MTX, Mustargen, Mustine, Mutamycin, Myleran, Iressa, Irinotecan, Isotretinoin, Kidrolase, Lanacort, L-asparaginase, and LCR. The therapeutic moiety can also comprise a biopharmaceutical such as, for example, an antibody.

20 In some examples, the therapeutic moiety can comprise an antiviral agent, such as ganciclovir, azidothymidine (AZT), lamivudine (3TC), *etc.*

In some examples, the therapeutic moiety can comprise an antibacterial agent, such as acedapsone; acetosulfone sodium; alamecin; alexidine; amdinocillin; amdinocillin pivoxil; amicycline; amifloxacin; amifloxacin mesylate; amikacin; amikacin sulfate; aminosalicic acid;

25 aminosalicylate sodium; amoxicillin; amphomycin; ampicillin; ampicillin sodium; apalcillin sodium; apramycin; aspartocin; astromicin sulfate; avilamycin; avoparcin; azithromycin; azlocillin; azlocillin sodium; bacampicillin hydrochloride; bacitracin; bacitracin methylene disalicylate; bacitracin zinc; bambermycins; benzoylpas calcium; berythromycin; betamicin sulfate; biapenem; biniramycin; biphenamine hydrochloride; bispyrithione magsulfex; butikacin;

30 butirosin sulfate; capreomycin sulfate; carbadox; carbenicillin disodium; carbenicillin indanyl sodium; carbenicillin phenyl sodium; carbenicillin potassium; carumonam sodium; cefaclor; cefadroxil; cefamandole; cefamandole nafate; cefamandole sodium; cefaparole; cefatrizine; cefazaflur sodium; cefazolin; cefazolin sodium; cefbuperazone; cefdinir; cefepime; cefepime hydrochloride; cefetecol; cefixime; cefmenoxime hydrochloride; cefmetazole; cefmetazole

sodium; cefonicid monosodium; cefonicid sodium; cefoperazone sodium; ceforanide; cefotaxime sodium; cefotetan; cefotetan disodium; cefotiam hydrochloride; cefoxitin; cefoxitin sodium; cefpimizole; cefpimizole sodium; cefpiramide; cefpiramide sodium; cefpirome sulfate; cefpodoxime proxetil; cefprozil; cefroxadine; cefsulodin sodium; ceftazidime; ceftibuten; 5 ceftizoxime sodium; ceftriaxone sodium; cefuroxime; cefuroxime axetil; cefuroxime pivoxetil; cefuroxime sodium; cephacetrile sodium; cephalixin; cephalixin hydrochloride; cephaloglycin; cephaloridine; cephalothin sodium; cephapirin sodium; cephradine; cetocycline hydrochloride; cetophenicol; chloramphenicol; chloramphenicol palmitate; chloramphenicol pantothenate complex; chloramphenicol sodium succinate; chlorhexidine phosphanilate; chloroxylenol; 10 chlortetracycline bisulfate; chlortetracycline hydrochloride; cinoxacin; ciprofloxacin; ciprofloxacin hydrochloride; cirolemycin; clarithromycin; clinafloxacin hydrochloride; clindamycin; clindamycin hydrochloride; clindamycin palmitate hydrochloride; clindamycin phosphate; clofazimine; cloxacillin benzathine; cloxacillin sodium; cloxyquin; colistimethate sodium; colistin sulfate; coumermycin; coumermycin sodium; cyclacillin; cycloserine; 15 dalfopristin; dapsone; daptomycin; demeclocycline; demeclocycline hydrochloride; demecycline; denofungin; diaveridine; dicloxacillin; dicloxacillin sodium; dihydrostreptomycin sulfate; dipyrithione; dirithromycin; doxycycline; doxycycline calcium; doxycycline fosfatex; doxycycline hyclate; droxacin sodium; enoxacin; epicillin; epitetracycline hydrochloride; erythromycin; erythromycin acistrate; erythromycin estolate; erythromycin ethylsuccinate; 20 erythromycin gluceptate; erythromycin lactobionate; erythromycin propionate; erythromycin stearate; ethambutol hydrochloride; ethionamide; fleroxacin; floxacillin; fludalanine; flumequine; fosfomycin; fosfomycin tromethamine; fumoxicillin; furazolium chloride; furazolium tartrate; fusidate sodium; fusidic acid; gentamicin sulfate; gloximonam; gramicidin; haloprogin; hetacillin; hetacillin potassium; hexedine; ibafloxacin; imipenem; isoconazole; 25 isepamicin; isoniazid; josamycin; kanamycin sulfate; kitasamycin; levofuraltadone; levopropylcillin potassium; lexithromycin; lincomycin; lincomycin hydrochloride; lomefloxacin; Lomefloxacin hydrochloride; lomefloxacin mesylate; loracarbef; mafenide; meclocycline; meclocycline sulfosalicylate; megalomicin potassium phosphate; mequidox; meropenem; methacycline; methacycline hydrochloride; methenamine; methenamine hippurate; methenamine mandelate; methicillin sodium; metioprime; metronidazole hydrochloride; metronidazole 30 phosphate; mezlocillin; mezlocillin sodium; minocycline; minocycline hydrochloride; mirincamycin hydrochloride; monensin; monensin sodium; nafcillin sodium; nalidixate sodium; nalidixic acid; nатаinycin; nebramycin; neomycin palmitate; neomycin sulfate; neomycin undecylenate; netilmicin sulfate; neutramycin; nifuradene; nifuraldezone; nifuratel; nifuratrone;

nifurdazil; nifurimide; nifiupirinol; nifurquinazol; nifurthiazole; nitrocyline; nitrofurantoin; nitromide; norfloxacin; novobiocin sodium; ofloxacin; onnetoprim; oxacillin; oxacillin sodium; oximonam; oximonam sodium; oxolinic acid; oxytetracycline; oxytetracycline calcium; oxytetracycline hydrochloride; paldimycin; parachlorophenol; paulomycin; pefloxacin; pefloxacin mesylate; penamecillin; penicillin G benzathine; penicillin G potassium; penicillin G procaine; penicillin G sodium; penicillin V; penicillin V benzathine; penicillin V hydrabamine; penicillin V potassium; pentizidone sodium; phenyl aminosalicylate; piperacillin sodium; pirbenicillin sodium; piridicillin sodium; pirlimycin hydrochloride; pivampicillin hydrochloride; pivampicillin pamoate; pivampicillin probenate; polymyxin B sulfate; porfiromycin; propikacin; pyrazinamide; pyridithione zinc; quindecamine acetate; quinupristin; racephenicol; ramoplanin; ranimycin; relomycin; repromicin; rifabutin; rifametan; rifamexil; rifamide; rifampin; rifapentine; rifaximin; rolitetracycline; rolitetracycline nitrate; rosaramicin; rosaramicin butyrate; rosaramicin propionate; rosaramicin sodium phosphate; rosaramicin stearate; rosoxacin; roxarsone; roxithromycin; sancycline; sanfetrinem sodium; sarmoxicillin; sarpicillin; scopafungin; sisomicin; sisomicin sulfate; sparfloxacin; spectinomycin hydrochloride; spiramycin; stallimycin hydrochloride; steffimycin; streptomycin sulfate; streptonicozid; sulfabenz; sulfabenzamide; sulfacetamide; sulfacetamide sodium; sulfacytine; sulfadiazine; sulfadiazine sodium; sulfadoxine; sulfalene; sulfamerazine; sulfameter; sulfamethazine; sulfamethizole; sulfamethoxazole; sulfamonomethoxine; sulfamoxole; sulfanilate zinc; sulfanitran; sulfasalazine; sulfasomizole; sulfathiazole; sulfazamet; sulfisoxazole; sulfisoxazole acetyl; sulfisboxazole diolamine; sulfomyxin; sulopenem; sultamricillin; suncillin sodium; talampicillin hydrochloride; teicoplanin; temafloxacin hydrochloride; temocillin; tetracycline; tetracycline hydrochloride; tetracycline phosphate complex; tetroxoprim; thiamphenicol; thiphencillin potassium; ticarcillin cresyl sodium; ticarcillin disodium; ticarcillin monosodium; ticlatone; tiodonium chloride; tobramycin; tobramycin sulfate; tosufloxacin; trimethoprim; trimethoprim sulfate; trisulfapyrimidines; troleandomycin; trospectomycin sulfate; tyrothricin; vancomycin; vancomycin hydrochloride; virginiamycin; or zorbamycin.

In some examples, the therapeutic moiety can comprise an anti-inflammatory agent.

The therapeutic moiety can be in the disclosed compounds at D¹ and/or D². Alternatively, the therapeutic moiety can be attached to the disclosed compounds at one or more of R¹-R¹⁶. When the therapeutic moiety is at one or more of R¹-R¹⁶, D¹ is hydrogen or an amine protecting group, and D² is hydroxyl, amine, or an acid protecting group.

Linker

The compounds described herein contain a linker. The term “linker,” as used herein, refers to one or more polyfunctional (*e.g.*, bi-functional, tri-functional, etc.) molecules which can be used to covalently couple the EGFR binding moiety and the one or more detectable and/or therapeutic moieties of the disclosed compounds. The linker can be attached to any part of the EGFR binding moiety so long as the point of attachment does not interfere with the biological activity, for example, the anti-tumor and/or anti-inflammatory activity of the compounds described herein.

The linker moiety can be of varying lengths, such as from 1 to 20 atoms in length. For example, the linker moiety can be from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 atoms in length, where any of the stated values can form an upper and/or lower end point of a range. Further, the linker moiety can be substituted or unsubstituted. When substituted, the linker moiety can contain substituents attached to the backbone of the linker or substituents embedded in the backbone of the linker. For example, an amine substituted linker moiety can contain an amine group attached to the backbone of the linker moiety or a nitrogen in the backbone of the linker.

In some examples, the linker moiety X^1 can be covalently coupled to the linker moiety X^2 . In some examples, covalently coupling the linker moiety X^1 to the linker moiety X^2 can be used to covalently constrain the EGFR binding moiety in a loop.

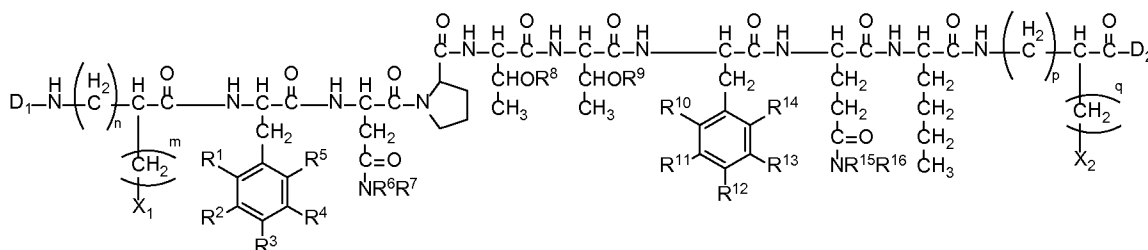
Suitable linkers include, but are not limited to, substituted or unsubstituted amino, substituted or unsubstituted amido, substituted or unsubstituted alkylamino, silyl, sulfide, disulfide, selenylsulfide, diselenide, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted haloalkyl, substituted or unsubstituted alkoxy, substituted or unsubstituted cycloalkyl, substituted or unsubstituted alkylcycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted alkylheterocycloalkyl, substituted or unsubstituted cycloalkenyl, substituted or unsubstituted alkylaryl, substituted or unsubstituted aryl, substituted or unsubstituted alkylheteroaryl, or substituted or unsubstituted heteroaryl, or derivatives or combinations thereof.

For example, X^1 and X^2 can independently comprise an azide and an alkyne capable of cycloaddition and forming a triazole. The triazole can be any triazole isomer. In other examples, X^1 and X^2 can independently comprise an acid or ester and an amine capable of forming an amide. In other examples, X^1 and X^2 can independently comprise an acid or ester and a hydroxyl capable of forming an ester. In other examples, X^1 and X^2 can independently comprise an

isocyanate and a hydroxyl capable of forming a carbamate. In other examples, X^1 and X^2 can independently comprise an isocyanate and amine capable of forming a urea. In other examples, X^1 and X^2 can independently comprise a halogen and a hydroxyl capable of forming an ether. In other examples, X^1 and X^2 can both be thiols capable of forming a disulfide. In other examples, X^1 and X^2 can independently comprise a thiol, selenol, or selenanyl capable of forming a selenylsulfide.

Specific Examples

In some examples, the compounds have Formula II:



II

wherein R^1 , R^2 , R^3 , R^4 , R^5 , R^6 , R^7 , R^8 , R^9 , R^{10} , R^{11} , R^{12} , R^{13} , R^{14} , R^{15} , and R^{16} (*i.e.*, R^1 - R^{16}) are independently chosen from hydrogen, halogen, hydroxyl, substituted or unsubstituted amino, substituted or unsubstituted amido, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted alkoxy, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, or wherein, as valence and stability permit, any combination of R^1 - R^5 and R^{10} - R^{14} , together with the atoms to which they are attached, form a 3-10 membered substituted or unsubstituted cyclic moiety optionally including between 1 and 3 heteroatoms;

X^1 and X^2 are independently chosen from halogen, hydroxyl, azide, substituted or unsubstituted amino, silyl, selenol, selenanyl, thiol, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted alkoxy, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl;

with the proviso that X^1 and X^2 are capable of reacting together and thereby forming a covalently bonded bridge moiety (also termed "linker" herein) having a number of backbone atoms, and the number of backbone atoms in the covalently bonded bridge moiety plus m and q is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10;

n and p are independently selected from 0, 1, 2;

m and q are independently selected from 0, 1, 2, 3, and 4; and

D¹ and D² are each independently selected from a hydrogen, hydroxyl, acid protecting group, amine protecting group, detectable moiety, therapeutic moiety, and combination thereof, with the proviso that at least one of D¹ and D² is a detectable moiety, therapeutic moiety, or combination thereof.

In other examples D¹ is hydrogen or an amine protecting group, D² is hydroxyl, amine, or acid protecting group, and one or more R¹-R¹⁶ is a detectable moiety, therapeutic moiety, or combination thereof.

In some examples of Formula II, m is 1. In some examples of Formula II, q is chosen from 2, 3, and 4. In some examples of Formula II, m is 1 and q is chosen from 2, 3, or 4. In some examples of Formula II, m is 1 and q is 2. In some examples of Formula II, m is 1 and q is 3. In some examples of Formula II, m is 1 and q is 4.

In some examples of Formula II, m is chosen from 2, 3, and 4. In some examples of Formula II, q is 1. In some examples of Formula II, q is 1 and m is chosen from 2, 3 and 4. In some examples of Formula II, q is 1 and m is 2. In some examples of Formula II, q is 1 and m is 3. In some examples of Formula II, q is 1 and m is 4.

In some examples of Formula II, n is 0. In some examples of Formula II, p is 0.

The covalently bonded bridge moiety can be prepared by reacting X¹ and X² in a variety of ways known to one skilled in the art of organic synthesis or variations thereon as appreciated by those skilled in the art.

In some examples, X¹ and X² capable of cycloaddition to form linker/bridge containing a 3-10 membered substituted or unsubstituted cyclic moiety optionally including between 1 and 3 heteroatoms. Examples of cycloadditions include, but are not limited to, Diels-Alder reactions, Huisgen cycloadditions, [2+3]cycloadditions, Nitron-olefin cycloadditions, [3+2]cycloadditions, [4+3]cycloadditions, [6+4] cycloadditions, 1,3-dipolar cycloadditions, aza-Diels-Alder reactions, azide-alkyne Huisgen cycloadditions, diazalkane 1,3-dipolar cycloadditions, McCormack reactions, oxo-Diels-Alder reactions, Povarov reactions, Staudinger Ketene-Imine cycloadditions.

In some examples, X¹ and X² are chosen from azide and substituted or unsubstituted alkyne, wherein X¹ and X² capable of an azide-alkyne [3+2] Huisgen cycloaddition to form a triazole type bridge. This configuration can result when the residues containing X¹ and X² are selected from N-Fmoc-L-propargylglycine (Pg), N-Fmoc-4-azido-L-homoalanine (Aha), N-

Fmoc-5-azido-L-norvaline (Anv), and N-Fmoc-6-azido-L-norleucine (Anl). In other examples, X^1 and X^2 are chosen from nitro and substituted or unsubstituted alkyne or alkyne.

In some examples, X^1 and X^2 are chosen thiol, selenol, and/or selenanyl, and can react to form a disulfide, selenylsulfide, or diselenide linker. This configuration can result when the residues containing X^1 and X^2 are selected from cysteine, methionine, selenocysteine, and selenomethionine or other S- or Se-containing non-natural amino acids.

In some examples of Formula II, R^1 - R^{16} are not all hydrogen. In some examples of Formula II, R^1 - R^5 are not all hydrogen. In some examples of Formula II, R^{10} - R^{14} are not all hydrogen.

In some examples of Formula II, R^1 , R^2 , R^4 , and R^5 are all hydrogen. In some examples of Formula II, R^3 comprises OR^{17} , wherein R^{17} is chosen from hydrogen, halogen, hydroxyl, substituted or unsubstituted amino, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted alkoxy, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl. In some examples of Formula II, R^3 comprises a hydroxyl group.

In some examples of Formula II, R^6 is hydrogen. In some examples of Formula II, R^7 is hydrogen. In some examples of Formula II, R^6 and R^7 are both hydrogen.

In some examples of Formula II, R^8 is hydrogen. In some examples of Formula II, R^9 is hydrogen. In some examples of Formula II, R^8 and R^9 are both hydrogen.

In some examples of Formula II, R^{10} , R^{11} , R^{13} and R^{14} are all hydrogen. In some examples of Formula II, R^{12} comprises OR^{18} , wherein R^{18} is chosen from hydrogen, halogen, hydroxyl, substituted or unsubstituted amino, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted alkoxy, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl. In some examples of Formula II, R^{12} comprises a hydroxyl group.

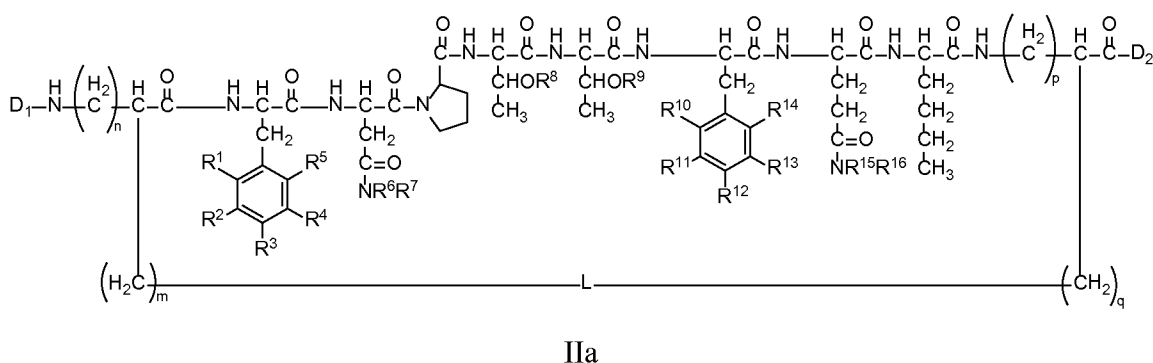
In some examples of Formula II, R^{15} comprises hydrogen. In some examples of Formula II, R^{16} comprises hydrogen. In some examples of Formula II, R^{15} and R^{16} are both hydrogen.

In some examples of Formula II, D^1 comprises a detectable moiety. In some examples of Formula II, D^1 comprises a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a chromophore, or any combination thereof. In some examples of Formula II, D^1 comprises a fluorescent label. In some examples of Formula II, D^1 comprises fluorenylmethyloxycarbonyl chloride (*i.e.*, Fmoc) or 5(6)-carboxyfluorescein (*i.e.*, FAM).

In some examples of Formula II, D² comprises a detectable moiety. In some examples of Formula II, D² comprises a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a chromophore, or any combination thereof. In some examples of Formula II, D² comprises a fluorescent label. In some examples of Formula II, D² comprises fluorenylmethyloxycarbonyl chloride (*i.e.*, Fmoc) or 5(6)-carboxyfluorescein (*i.e.*, FAM).

In some examples of Formula II, D¹ and D² both independently comprise a detectable moiety. In some examples of Formula II, D¹ and D² both independently comprise a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a chromophore, or any combination thereof. In some examples of Formula II, D² comprises a fluorescent label. In some examples of Formula II, D¹ and D² both comprise fluorenylmethyloxycarbonyl chloride (*i.e.*, Fmoc) and/or 5(6)-carboxyfluorescein (*i.e.*, FAM).

In some examples of Formula II, are compounds wherein X¹ and X² have undergone a reaction to form a covalently bonded bridge moiety, and the compounds are of Formula IIa:



wherein R¹-R¹⁶, n, m, p, q, D¹ and D² are as defined in Formula II; and

L is selected from substituted or unsubstituted amino, silyl, sulfide, disulfide, selenylsulfide, diselenide, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted alkoxy, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted cycloalkenyl, substituted or unsubstituted heterocycloalkenyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl;

with the proviso that L has a number of atoms in its backbone and the sum of the number of atoms forming the backbone of L, m, and q is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10.

In some examples of Formula IIa, m is 1. In some examples of Formula IIa, q is chosen from 2, 3, and 4. In some examples of Formula IIa, m is 1 and q is chosen from 2, 3, or 4. In some examples of Formula IIa, m is 1 and q is 2. In some examples of Formula IIa, m is 1 and q is 3. In some examples of Formula IIa, m is 1 and q is 4.

In some examples of Formula IIa, m is chosen from 2, 3, and 4. In some examples of Formula IIa, q is 1. In some examples of Formula IIa, q is 1 and m is chosen from 2, 3 and 4. In some examples of Formula IIa, q is 1 and m is 2. In some examples of Formula IIa, q is 1 and m is 3. In some examples of Formula IIa, q is 1 and m is 4.

5 In some examples of Formula IIa, n is 0. In some examples of Formula IIa, p is 0.

In some examples of Formula IIa, L comprises a 3-10 membered substituted or unsubstituted cyclic moiety optionally including between 1 and 3 heteroatoms. In some examples of Formula IIa, L comprises an unsubstituted heteroaryl. In some examples of Formula IIa, L comprises a triazole. In some examples of Formula IIa, L comprises a disulfide,
10 selenysulfide, or diselenide.

In some examples of Formula IIa, R¹-R¹⁶ are not all hydrogen. In some examples of Formula IIa, R¹-R⁵ are not all hydrogen. In some examples of Formula IIa, R¹⁰-R¹⁴ are not all hydrogen.

In some examples of Formula IIa, R¹, R², R⁴, and R⁵ are all hydrogen. In some examples
15 of Formula IIa, R³ comprises OR¹⁷, wherein R¹⁷ is chosen from hydrogen, halogen, hydroxyl, substituted or unsubstituted amino, silyl, thiol, substituted or unsubstituted thioalkyl, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted haloalkyl, substituted or unsubstituted alkoxy, substituted or
20 unsubstituted cycloalkyl, substituted or unsubstituted alkylcycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted alkylheterocycloalkyl, substituted or unsubstituted alkylaryl, substituted or unsubstituted aryl, substituted or unsubstituted alkylheteroaryl, or substituted or unsubstituted heteroaryl. In some examples of Formula IIa, R³ comprises a hydroxyl group.

In some examples of Formula IIa, R⁶ is hydrogen. In some examples of Formula IIa, R⁷
25 is hydrogen. In some examples of Formula IIa, R⁶ and R⁷ are both hydrogen.

In some examples of Formula IIa, R⁸ is hydrogen. In some examples of Formula IIa, R⁹ is hydrogen. In some examples of Formula IIa, R⁸ and R⁹ are both hydrogen.

In some examples of Formula IIa, R¹⁰, R¹¹, R¹³ and R¹⁴ are all hydrogen. In some examples of Formula IIa, R¹² comprises OR¹⁸, wherein R¹⁸ is chosen from hydrogen, halogen,
30 hydroxyl, substituted or unsubstituted amino, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted alkoxy, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl. In some examples of Formula IIa, R¹² comprises a hydroxyl group.

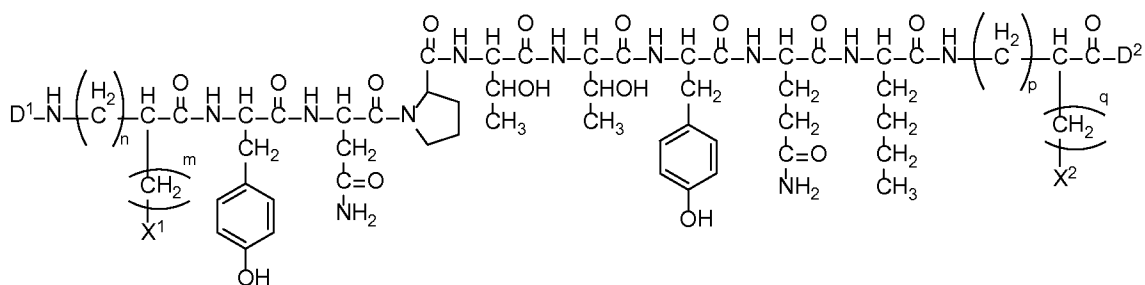
In some examples of Formula IIa, R¹⁵ comprises hydrogen. In some examples of Formula IIa, R¹⁶ comprises hydrogen. In some examples of Formula IIa, R¹⁵ and R¹⁶ are both hydrogen.

In some examples of Formula IIa, D¹ comprises a detectable moiety. In some examples of Formula IIa, D¹ comprises a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a chromophore, or any combination thereof. In some examples of Formula IIa, D¹ comprises a fluorescent label. In some examples of Formula IIa, D¹ comprises fluorenylmethoxycarbonyl chloride (*i.e.*, Fmoc) or 5(6)-carboxyfluorescein (*i.e.*, FAM).

In some examples of Formula IIa, D² comprises a detectable moiety. In some examples of Formula IIa, D² comprises a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a chromophore, or any combination thereof. In some examples of Formula IIa, D² comprises a fluorescent label. In some examples of Formula IIa, D² comprises fluorenylmethoxycarbonyl chloride (*i.e.*, Fmoc) or 5(6)-carboxyfluorescein (*i.e.*, FAM).

In some examples of Formula IIa, D¹ and D² both independently comprise a detectable moiety. In some examples of Formula IIa, D¹ and D² both independently comprise a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a chromophore, or any combination thereof. In some examples of Formula IIa, D² comprises a fluorescent label. In some examples of Formula IIa, D¹ and D² both comprise fluorenylmethyloxycarbonyl chloride (*i.e.*, Fmoc) or 5(6)-carboxyfluorescein (*i.e.*, FAM).

In some examples of Formula II, R¹, R², R⁴-R¹¹, and R¹³-R¹⁶ are all hydrogen and R³ and R¹² are both hydroxyl, resulting in compounds of Formula III:



III

wherein n , m , p , q , D^1 and D^2 are as defined in Formula II.

In some examples of Formula III, m is 1. In some examples of Formula III, q is chosen from 2, 3, and 4. In some examples of Formula III, m is 1 and q is chosen from 2, 3, or 4. In some examples of Formula III, m is 1 and q is 2. In some examples of Formula III, m is 1 and q is 3. In some examples of Formula III, m is 1 and q is 4.

In some examples of Formula III, m is chosen from 2, 3, and 4. In some examples of Formula III, q is 1. In some examples of Formula III, q is 1 and m is chosen from 2, 3 and 4. In

some examples of Formula III, q is 1 and m is 2. In some examples of Formula III, q is 1 and m is 3. In some examples of Formula III, q is 1 and m is 4.

In some examples of Formula III, n is 0. In some examples of Formula III, p is 0.

In some examples of Formula III, X^1 and X^2 are chosen from azide and substituted or unsubstituted alkyne. In some examples of Formula III, X^1 and X^2 capable of an azide-alkyne [3+2] Huisgen cycloaddition to form a triazole type bridge. This configuration can result when the residues containing X^1 and X^2 are selected from N-Fmoc-L-propargylglycine (Pg), N-Fmoc-4-azido-L-homoalanine (Aha), N-Fmoc-5-azido-L-norvaline (Anv), and N-Fmoc-6-azido-L-norleucine (Anl). In other examples, X^1 and X^2 are chosen from nitron and substituted or unsubstituted alkyne or alkyne.

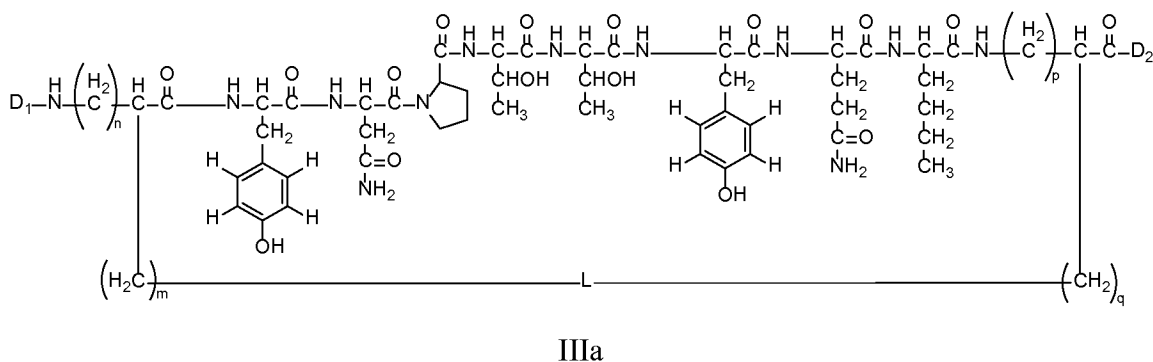
In some examples, X^1 and X^2 are chosen thiol, selenol, and/or selenanyl, and can react to form a disulfide, selenylsulfide, or diselenide linker. This configuration can result when the residues containing X^1 and X^2 are selected from cysteine, methionine, selenocysteine, and selenomethionine or other non-natural amino acids bearing reactive S- or Se- groups.

In some examples of Formula III, D^1 comprises a detectable moiety. In some examples of Formula III, D^1 comprises a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a chromophore, or any combination thereof. In some examples of Formula III, D^1 comprises a fluorescent label. In some examples of Formula III, D^1 comprises fluorenylmethyloxycarbonyl chloride (*i.e.*, Fmoc).

In some examples of Formula III, D^2 comprises a detectable moiety. In some examples of Formula III, D^2 comprises a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a chromophore, or any combination thereof. In some examples of Formula III, D^2 comprises a fluorescent label. In some examples of Formula III, D^2 comprises fluorenylmethyloxycarbonyl chloride (*i.e.*, Fmoc) or 5(6)-carboxyfluorescein (*i.e.*, FAM).

In some examples of Formula III, D^1 and D^2 both independently comprise a detectable moiety. In some examples of Formula III, D^1 and D^2 both independently comprise a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a chromophore, or any combination thereof. In some examples of Formula III, D^2 comprises a fluorescent label. In some examples of Formula III, D^1 and D^2 both comprise fluorenylmethyloxycarbonyl chloride (*i.e.*, Fmoc) or 5(6)-carboxyfluorescein (*i.e.*, FAM).

In some examples of Formula III, X^1 and X^2 have undergone a reaction to form a covalently bonded bridge, and the compounds are of Formula IIIa:



wherein n , m , p , q , D^1 and D^2 are as defined in Formula II, and L is as defined in Formula IIa.

In some examples of Formula IIIa, m is 1. In some examples of Formula IIIa, q is chosen from 2, 3, and 4. In some examples of Formula IIIa, m is 1 and q is chosen from 2, 3, or 4. In some examples of Formula IIIa, m is 1 and q is 2. In some examples of Formula IIIa, m is 1 and q is 3. In some examples of Formula IIIa, m is 1 and q is 4.

In some examples of Formula IIIa, m is chosen from 2, 3, and 4. In some examples of Formula IIIa, q is 1. In some examples of Formula IIIa, q is 1 and m is chosen from 2, 3 and 4. In some examples of Formula IIIa, q is 1 and m is 2. In some examples of Formula IIIa, q is 1 and m is 3. In some examples of Formula IIIa, q is 1 and m is 4.

In some examples of Formula IIIa, n is 0. In some examples of Formula IIIa, p is 0.

In some examples of Formula IIIa, L comprises a 3-10 membered substituted or unsubstituted cyclic moiety optionally including between 1 and 3 heteroatoms. In some examples of Formula IIIa, L comprises an unsubstituted heteroaryl. In some examples of Formula IIIa, L comprises a triazole. In other examples of Formula IIIa, L comprises a disulfide, selenylsulfide, or diselenide.

In some examples of Formula IIIa, D¹ comprises a detectable moiety. In some examples of Formula IIIa, D¹ comprises a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a chromophore, or any combination thereof. In some examples of Formula IIIa, D¹ comprises a fluorescent label. In some examples of Formula IIIa, D¹ comprises fluorenylmethyloxycarbonyl chloride (*i.e.*, Fmoc) or 5(6)-carboxyfluorescein (*i.e.*, FAM).

In some examples of Formula IIIa, D² comprises a detectable moiety. In some examples of Formula IIIa, D² comprises a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a chromophore, or any combination thereof. In some examples of Formula IIIa, D² comprises a fluorescent label. In some examples of Formula IIIa, D² comprises fluorenylmethyloxycarbonyl chloride (*i.e.*, Fmoc) or 5(6)-carboxyfluorescein (*i.e.*, FAM).

In some examples of Formula IIIa, D¹ and D² both independently comprise a detectable moiety. In some examples of Formula IIIa, D¹ and D² both independently comprise a UV-Vis

In some examples of Formula IV, X² comprises a substituted or unsubstituted alkyne. In some examples of Formula IV, X² comprises an unsubstituted alkyne. In some examples of Formula IV, X² comprises C≡CH. In some examples of Formula IV, q is 1 and X² comprises C≡CH. In other examples of Formula IV, X² comprises selenol, selenanyl, or thiol.

In some examples of Formula IV, X^2 comprises an azide. In some examples of Formula IV, q is chosen from 2, 3, or 4 and X^2 comprises an azide. In some examples of Formula IV, q is 2 and X^2 comprises an azide. In some examples of Formula IV, q is 3 and X^2 comprises an azide. In some examples of Formula IV, q is 4 and X^2 comprises an azide.

5 In some examples of Formula IV, m is 1, X^1 comprises $C\equiv CH$, q is chosen from 2, 3, or 4 and X^2 comprises an azide. In some examples of Formula IV, m is 1, X^1 comprises $C\equiv CH$, q is 2 and X^2 comprises an azide. In some examples of Formula IV, m is 1, X^1 comprises $C\equiv CH$, q is 3 and X^2 comprises an azide. In some examples of Formula IV, m is 1, X^1 comprises $C\equiv CH$, q is 4 and X^2 comprises an azide. In other examples of Formula IV, X^1 comprises selenol or selenanyl
10 and X^2 comprises thiol. In other examples of Formula IV, X^1 comprises thiol and X^2 comprises selenol or selenanyl.

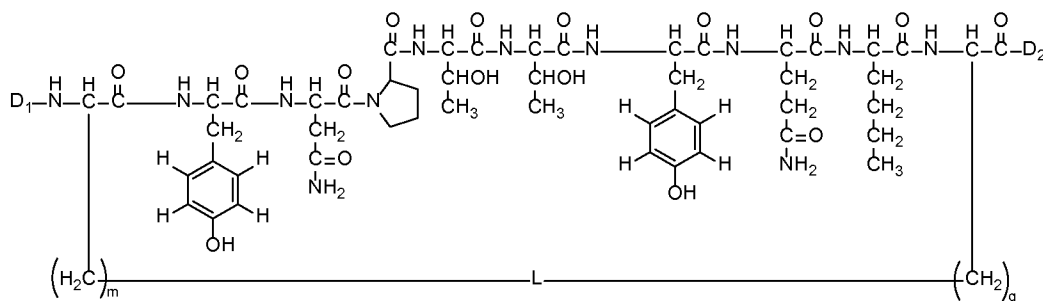
In some examples of Formula IV, m is chosen from 2, 3, or 4, X^1 comprises an azide, q is 1, and X^2 comprises $C\equiv CH$. In some examples of Formula IV, m is 2, X^1 comprises an azide, q is 1, and X^2 comprises $C\equiv CH$. In some examples of Formula IV, m is 3, X^1 comprises an azide,
15 q is 1, and X^2 comprises $C\equiv CH$. In some examples of Formula IV, m is 4, X^1 comprises an azide, q is 1, and X^2 comprises $C\equiv CH$.

In some examples of Formula IV, D^1 comprises a detectable moiety. In some examples of Formula IV, D^1 comprises a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a chromophore, or any combination thereof. In some examples of
20 Formula IV, D^1 comprises a fluorescent label. In some examples of Formula IV, D^1 comprises fluorenylmethyloxycarbonyl chloride (*i.e.*, Fmoc) or 5(6)-carboxyfluorescein (*i.e.*, FAM).

In some examples of Formula IV, D^2 comprises a detectable moiety. In some examples of Formula IV, D^2 comprises a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a chromophore, or any combination thereof. In some examples of
25 Formula IV, D^2 comprises a fluorescent label. In some examples of Formula IV, D^2 comprises fluorenylmethyloxycarbonyl chloride (*i.e.*, Fmoc) or 5(6)-carboxyfluorescein (*i.e.*, FAM).

In some examples of Formula IV, D^1 and D^2 both independently comprise a detectable moiety. In some examples of Formula IV, D^1 and D^2 both independently comprise a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a chromophore, or any
30 combination thereof. In some examples of Formula IV, D^2 comprises a fluorescent label. In some examples of Formula IV, D^1 and D^2 both comprise fluorenylmethyloxycarbonyl chloride (*i.e.*, Fmoc) or 5(6)-carboxyfluorescein (*i.e.*, FAM).

In some examples of Formula IV, X^1 and X^2 have undergone a reaction to form a covalently bonded bridge, and the compounds are of Formula IVa:



IVa

wherein m , q , D^1 and D^2 are as defined in Formula II, and L is as defined in Formula IIa.

In some examples of Formula IVa, L comprises a 3-10 membered substituted or unsubstituted cyclic moiety optionally including between 1 and 3 heteroatoms. In some examples of Formula IVa, L comprises an unsubstituted heteroaryl. In some examples of Formula IVa, L comprises a triazole. In other examples of Formula IVa, L comprises a disulfide, selenysulfide, or diselenide.

In some examples of Formula IVa, m is 1. In some examples of Formula IVa, q is chosen from 2, 3, and 4. In some examples of Formula IVa, m is 1 and q is chosen from 2, 3, or 4. In some examples of Formula IVa, m is 1 and q is 2. In some examples of Formula IVa, m is 1 and q is 3. In some examples of Formula IVa, m is 1 and q is 4.

In some examples of Formula IVa, m is chosen from 2, 3, and 4. In some examples of Formula IVa, q is 1. In some examples of Formula IVa, q is 1 and m is chosen from 2, 3 and 4. In some examples of Formula IVa, q is 1 and m is 2. In some examples of Formula IVa, q is 1 and m is 3. In some examples of Formula IVa, q is 1 and m is 4.

In some examples of Formula IVa, m is 1 and L comprises a triazole. In some examples of Formula IV, q is chosen from 2, 3, and 4 and L comprises a triazole. In some examples of Formula IVa, m is 1, q is chosen from 2, 3, or 4 and L comprises a triazole. In some examples of Formula IVa, m is 1, q is 2, and L comprises a triazole. In some examples of Formula IVa, m is 1, q is 3, and L comprises a triazole. In some examples of Formula IVa, m is 1, q is 4, and L comprises a triazole.

In some examples of Formula IVa, m is chosen from 2, 3, and 4 and L comprises a triazole. In some examples of Formula IVa, q is 1 and L comprises a triazole. In some examples of Formula IVa, q is 1, m is chosen from 2, 3 and 4, and L comprises a triazole. In some examples of Formula IVa, q is 1, m is 2, and L comprises a triazole. In some examples of Formula IVa, q is 1, m is 3, and L comprises a triazole. In some examples of Formula IVa, q is 1, m is 4, and L comprises a triazole.

In some examples of Formula IVa, m is 1 and L comprises a disulfide, selenysulfide, or diselenide. In some examples of Formula IV, q is chosen from 2, 3, and 4 and L comprises a disulfide, selenysulfide, or diselenide. In some examples of Formula IVa, m is 1, q is chosen from 2, 3, or 4 and L comprises a disulfide, selenysulfide, or diselenide. In some examples of Formula IVa, m is 1, q is 2, and L comprises a disulfide, selenysulfide, or diselenide. In some examples of Formula IVa, m is 1, q is 3, and L comprises a disulfide, selenysulfide, or diselenide. In some examples of Formula IVa, m is 1, q is 4, and L comprises a disulfide, selenysulfide, or diselenide.

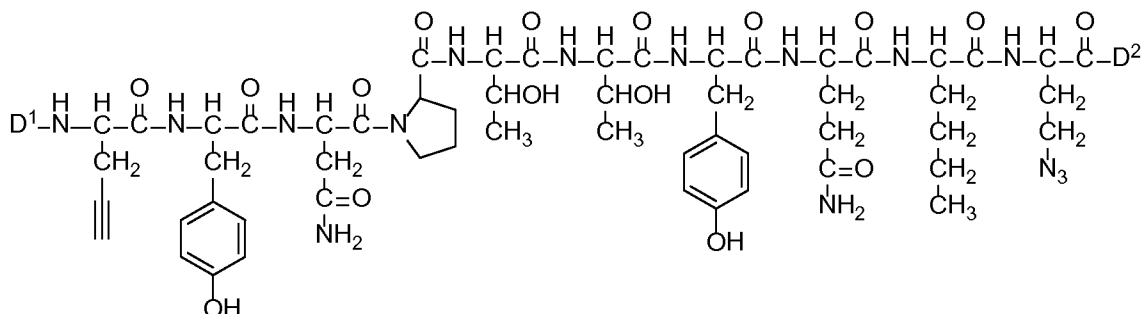
In some examples of Formula IVa, m is chosen from 2, 3, and 4 and L comprises a disulfide, selenysulfide, or diselenide. In some examples of Formula IVa, q is 1 and L comprises a disulfide, selenysulfide, or diselenide. In some examples of Formula IVa, q is 1, m is chosen from 2, 3 and 4, and L comprises a disulfide, selenysulfide, or diselenide. In some examples of Formula IVa, q is 1, m is 2, and L comprises a disulfide, selenysulfide, or diselenide. In some examples of Formula IVa, q is 1, m is 3, and L comprises a disulfide, selenysulfide, or diselenide. In some examples of Formula IVa, q is 1, m is 4, and L comprises a disulfide, selenysulfide, or diselenide.

In some examples of Formula IVa, D¹ comprises a detectable moiety. In some examples of Formula IVa, D¹ comprises a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a chromophore, or any combination thereof. In some examples of Formula IVa, D¹ comprises a fluorescent label. In some examples of Formula IVa, D¹ comprises fluorenylmethyloxycarbonyl chloride (*i.e.*, Fmoc) or 5(6)-carboxyfluorescein (*i.e.*, FAM).

In some examples of Formula IVa, D² comprises a detectable moiety. In some examples of Formula IVa, D² comprises a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a chromophore, or any combination thereof. In some examples of Formula IVa, D² comprises a fluorescent label. In some examples of Formula IVa, D² comprises fluorenylmethyloxycarbonyl chloride (*i.e.*, Fmoc) or 5(6)-carboxyfluorescein (*i.e.*, FAM).

In some examples of Formula IVa, D¹ and D² both independently comprise a detectable moiety. In some examples of Formula IVa, D¹ and D² both independently comprise a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a chromophore, or any combination thereof. In some examples of Formula IVa, D² comprises a fluorescent label. In some examples of Formula IVa, D¹ and D² both comprise fluorenylmethyloxycarbonyl chloride (*i.e.*, Fmoc) or 5(6)-carboxyfluorescein (*i.e.*, FAM).

In some examples of Formula IV, m is 1, q is 2, X¹ is C≡CH, X² is an azide, and the compounds are of Formula V:



V

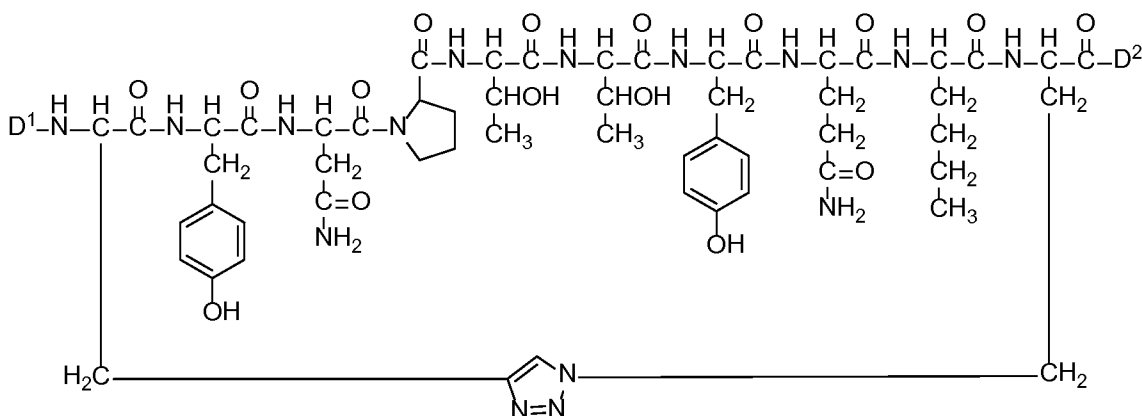
wherein D^1 and D^2 are as defined in Formula II.

In some examples of Formula V, D^1 comprises a detectable moiety. In some examples of Formula V, D^1 comprises a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a chromophore, or any combination thereof. In some examples of Formula V, D^1 comprises a fluorescent label. In some examples of Formula V, D^1 comprises fluorenylmethoxycarbonyl chloride (*i.e.*, Fmoc) or 5(6)-carboxyfluorescein (*i.e.*, FAM).

In some examples of Formula V, D^2 comprises a detectable moiety. In some examples of Formula V, D^2 comprises a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a chromophore, or any combination thereof. In some examples of Formula V, D^2 comprises a fluorescent label. In some examples of Formula V, D^2 comprises fluorenylmethoxycarbonyl chloride (*i.e.*, Fmoc) or 5(6)-carboxyfluorescein (*i.e.*, FAM).

In some examples of Formula V, D^1 and D^2 both independently comprise a detectable moiety. In some examples of Formula V, D^1 and D^2 both independently comprise a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a chromophore, or any combination thereof. In some examples of Formula V, D^2 comprises a fluorescent label. In some examples of Formula V, D^1 and D^2 both comprise fluorenylmethoxycarbonyl chloride (*i.e.*, Fmoc) or 5(6)-carboxyfluorescein (*i.e.*, FAM).

In some examples of Formula V, the alkyne and azide undergo a cycloaddition reaction to form a covalently bonded triazole containing bridge, and the compounds are of Formula Va:



$$V_a$$

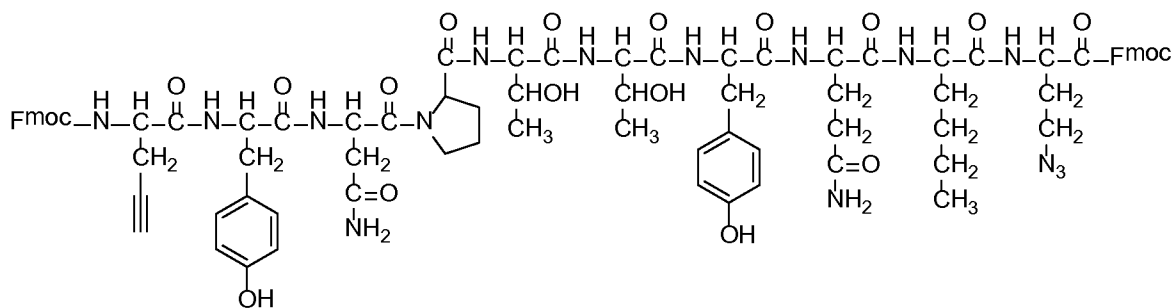
wherein D^1 and D^2 are as defined in Formula II.

In some examples of Formula Va, D¹ comprises a detectable moiety. In some examples of Formula Va, D¹ comprises a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a chromophore, or any combination thereof. In some examples of Formula Va, D¹ comprises a fluorescent label. In some examples of Formula Va, D¹ comprises fluorenylmethyloxycarbonyl chloride (*i.e.*, Fmoc) or 5(6)-carboxyfluorescein (*i.e.*, FAM).

In some examples of Formula Va, D² comprises a detectable moiety. In some examples of Formula Va, D² comprises a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a chromophore, or any combination thereof. In some examples of Formula Va, D² comprises a fluorescent label. In some examples of Formula Va, D² comprises fluorenylmethyloxycarbonyl chloride (*i.e.*, Fmoc) or 5(6)-carboxyfluorescein (*i.e.*, FAM).

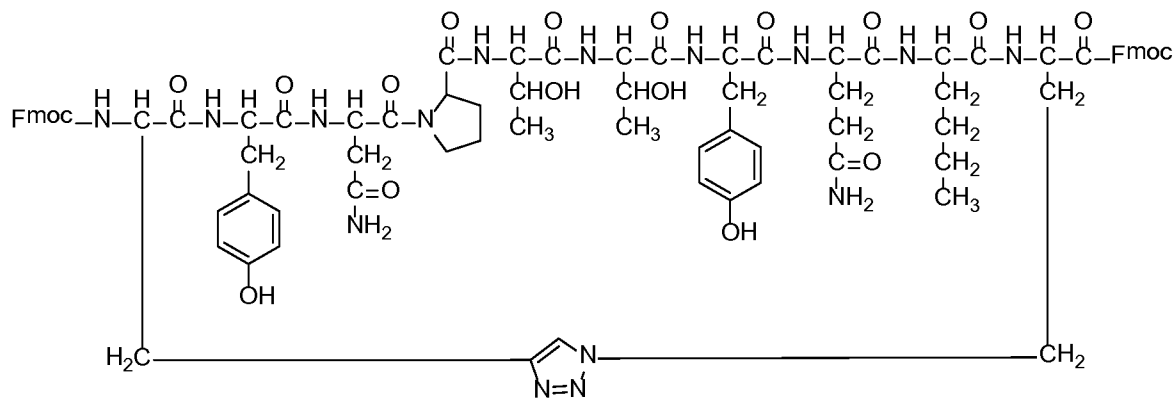
In some examples of Formula Va, D¹ and D² both independently comprise a detectable moiety. In some examples of Formula Va, D¹ and D² both independently comprise a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a chromophore, or any combination thereof. In some examples of Formula Va, D² comprises a fluorescent label. In some examples of Formula Va, D¹ and D² both comprise fluorenylmethyloxycarbonyl chloride (*i.e.*, Fmoc) or 5(6)-carboxyfluorescein (*i.e.*, FAM).

In some examples of Formula V, D¹ and D² are Fmoc, and compounds are of Formula VI:



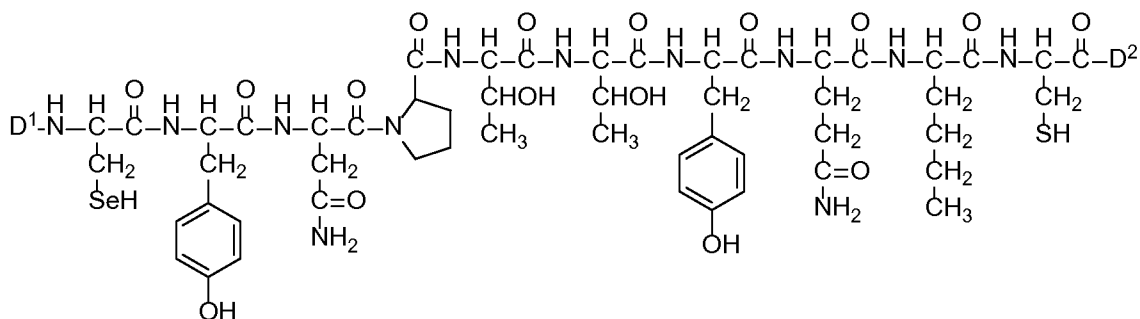
VI

In some examples of Formula VI, the alkyne and azide undergone a cycloaddition reaction to form a covalently bonded triazole containing bridge, and the compounds are of Formula VIa:



Vla

In some examples of Formula IV, m is 1, q is 2, X¹ is selenol or selenanyl, X² is a thiol or selenol. An example of such a compound is shown in Formula VII:



VII

wherein D¹ and D² are as defined in Formula II. Variations of this molecule include two selenols, selenanyl and thiol, two thiols, thiol and selanyl, or two selanyl groups at the respective location where the selenol and thiol are shown.

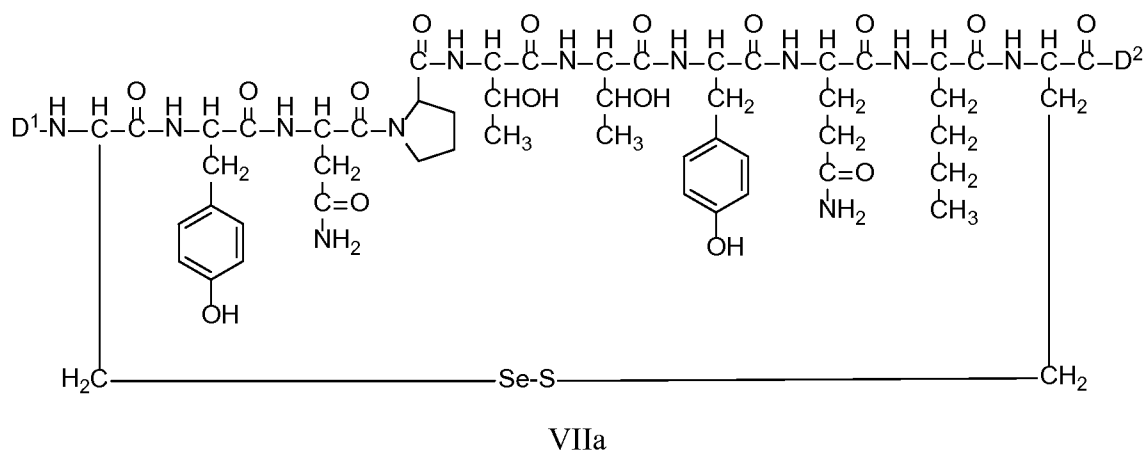
In some examples of Formula VII, D¹ comprises a detectable moiety. In some examples of Formula VII, D¹ comprises a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a chromophore, or any combination thereof. In some examples of Formula VII, D¹ comprises a fluorescent label. In some examples of Formula VII, D¹ comprises fluorenylmethyloxycarbonyl chloride (*i.e.*, Fmoc) or 5(6)-carboxyfluorescein (*i.e.*, FAM).

In some examples of Formula VII, D² comprises a detectable moiety. In some examples of Formula VII, D² comprises a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a chromophore, or any combination thereof. In some examples of Formula VII, D² comprises a fluorescent label. In some examples of Formula VII, D² comprises fluorenylmethyloxycarbonyl chloride (*i.e.*, Fmoc) or 5(6)-carboxyfluorescein (*i.e.*, FAM).

In some examples of Formula VII, D¹ and D² both independently comprise a detectable moiety. In some examples of Formula VII, D¹ and D² both independently comprise a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a chromophore, or any

combination thereof. In some examples of Formula VII, D² comprises a fluorescent label. In some examples of Formula VII, D¹ and D² both comprise fluorenylmethyloxycarbonyl chloride (*i.e.*, Fmoc) or 5(6)-carboxyfluorescein (*i.e.*, FAM).

In some examples of Formula VII, the thiol and selenol or selenanyl undergo a reaction to form a covalently bonded selenylsulfide containing bridge, and the compounds are of Formula VIIa:



wherein D¹ and D² are as defined in Formula II. When two selenols and/or selenanyl are used a diselenide will be present at the location where Se-S is shown above.

In some examples of Formula VIIa, D¹ comprises a detectable moiety. In some examples of Formula VII a, D¹ comprises a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a chromophore, or any combination thereof. In some examples of Formula VIIa, D¹ comprises a fluorescent label. In some examples of Formula VIIa, D¹ comprises fluorenylmethyloxycarbonyl chloride (*i.e.*, Fmoc) or 5(6)-carboxyfluorescein (*i.e.*, FAM).

In some examples of Formula VIIa, D² comprises a detectable moiety. In some examples of Formula VII a, D² comprises a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a chromophore, or any combination thereof. In some examples of Formula VIIa, D² comprises a fluorescent label. In some examples of Formula VIIa, D² comprises fluorenylmethyloxycarbonyl chloride (*i.e.*, Fmoc) or 5(6)-carboxyfluorescein (*i.e.*, FAM).

In some examples of Formula VIIa, D¹ and D² both independently comprise a detectable moiety. In some examples of Formula VIIa, D¹ and D² both independently comprise a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a chromophore, or any combination thereof. In some examples of Formula VIIa, D² comprises a fluorescent label. In some examples of Formula VIIa, D¹ and D² both comprise fluorenylmethyloxycarbonyl chloride (*i.e.*, Fmoc) or 5(6)-carboxyfluorescein (*i.e.*, FAM).

Also disclosed herein are pharmaceutically-acceptable salts and prodrugs of the disclosed compounds. Pharmaceutically-acceptable salts include salts of the disclosed compounds that are prepared with acids or bases, depending on the particular substituents found on the compounds. Under conditions where the compounds disclosed herein are sufficiently basic or acidic to form stable nontoxic acid or base salts, administration of the compounds as salts can be appropriate. Examples of pharmaceutically-acceptable base addition salts include sodium, potassium, calcium, ammonium, or magnesium salt. Examples of physiologically-acceptable acid addition salts include hydrochloric, hydrobromic, nitric, phosphoric, carbonic, sulfuric, and organic acids like acetic, propionic, benzoic, succinic, fumaric, mandelic, oxalic, citric, tartaric, malonic, ascorbic, alpha-ketoglutaric, alpha-glycophosphoric, maleic, tosyl acid, methanesulfonic, and the like. Thus, disclosed herein are the hydrochloride, nitrate, phosphate, carbonate, bicarbonate, sulfate, acetate, propionate, benzoate, succinate, fumarate, mandelate, oxalate, citrate, tartarate, malonate, ascorbate, alpha-ketoglutarate, alpha-glycophosphate, maleate, tosylate, and mesylate salts. Pharmaceutically acceptable salts of a compound can be obtained using standard procedures well known in the art, for example, by reacting a sufficiently basic compound such as an amine with a suitable acid affording a physiologically acceptable anion. Alkali metal (for example, sodium, potassium or lithium) or alkaline earth metal (for example calcium) salts of carboxylic acids can also be made.

Methods of Making

The compounds described herein can be prepared in a variety of ways known to one skilled in the art of organic synthesis or variations thereon as appreciated by those skilled in the art. The compounds described herein can be prepared from readily available starting materials. Optimum reaction conditions can vary with the particular reactants or solvents used, but such conditions can be determined by one skilled in the art.

Variations on the compounds described herein include the addition, subtraction, or movement of the various constituents as described for each compound. Similarly, when one or more chiral centers are present in a molecule, the chirality of the molecule can be changed. Additionally, compound synthesis can involve the protection and deprotection of various chemical groups. The use of protection and deprotection, and the selection of appropriate protecting groups can be determined by one skilled in the art. The chemistry of protecting groups can be found, for example, in Wuts and Greene, *Protective Groups in Organic Synthesis*, 4th Ed., Wiley & Sons, 2006, which is incorporated herein by reference in its entirety.

The starting materials and reagents used in preparing the disclosed compounds and compositions are either available from commercial suppliers such as Aldrich Chemical Co.,

(Milwaukee, WI), Acros Organics (Morris Plains, NJ), Fisher Scientific (Pittsburgh, PA), Sigma (St. Louis, MO), Pfizer (New York, NY), GlaxoSmithKline (Raleigh, NC), Merck (Whitehouse Station, NJ), Johnson & Johnson (New Brunswick, NJ), Aventis (Bridgewater, NJ), AstraZeneca (Wilmington, DE), Novartis (Basel, Switzerland), Wyeth (Madison, NJ), Bristol-Myers-Squibb (New York, NY), Roche (Basel, Switzerland), Lilly (Indianapolis, IN), Abbott (Abbott Park, IL), Schering Plough (Kenilworth, NJ), or Boehringer Ingelheim (Ingelheim, Germany), or are prepared by methods known to those skilled in the art following procedures set forth in references such as Fieser and Fieser's Reagents for Organic Synthesis, Volumes 1-17 (John Wiley and Sons, 1991); Rodd's Chemistry of Carbon Compounds, Volumes 1-5 and Supplementals (Elsevier Science Publishers, 1989); Organic Reactions, Volumes 1-40 (John Wiley and Sons, 1991); March's Advanced Organic Chemistry, (John Wiley and Sons, 4th Edition); and Larock's Comprehensive Organic Transformations (VCH Publishers Inc., 1989). Other materials, such as the pharmaceutical carriers disclosed herein can be obtained from commercial sources.

Reactions to produce the compounds described herein can be carried out in solvents, which can be selected by one of skill in the art of organic synthesis. Solvents can be substantially nonreactive with the starting materials (reactants), the intermediates, or products under the conditions at which the reactions are carried out, *i.e.*, temperature and pressure. Reactions can be carried out in one solvent or a mixture of more than one solvent. Product or intermediate formation can be monitored according to any suitable method known in the art. For example, product formation can be monitored by spectroscopic means, such as nuclear magnetic resonance spectroscopy (*e.g.*, ^1H or ^{13}C) infrared spectroscopy, spectrophotometry (*e.g.*, UV-visible), or mass spectrometry, or by chromatography such as high performance liquid chromatography (HPLC) or thin layer chromatography.

The disclosed compounds can be prepared by solid phase peptide synthesis wherein the amino acid α -N-terminal is protected by an acid or base protecting group. Such protecting groups should have the properties of being stable to the conditions of peptide linkage formation while being readily removable without destruction of the growing peptide chain or racemization of any of the chiral centers contained therein. Suitable protecting groups are 9-fluorenylmethyloxycarbonyl (Fmoc), t-butyloxycarbonyl (Boc), benzyloxycarbonyl (Cbz), biphenylisopropyloxycarbonyl, t-amylloxycarbonyl, isobornyloxycarbonyl, α,α -dimethyl-3,5-dimethoxybenzyloxycarbonyl, o-nitrophenylsulfenyl, 2-cyano-t-butyloxycarbonyl, and the like. The 9-fluorenylmethyloxycarbonyl (Fmoc) protecting group is particularly preferred for the synthesis of the disclosed compounds. Other preferred side chain protecting groups are, for side

chain amino groups like lysine and arginine, 2,2,5,7,8-pentamethylchroman-6-sulfonyl (pmc), nitro, p-toluenesulfonyl, 4-methoxybenzene-sulfonyl, Cbz, Boc, and adamantyloxycarbonyl; for tyrosine, benzyl, o-bromobenzyloxy-carbonyl, 2,6-dichlorobenzyl, isopropyl, t-butyl (t-Bu), cyclohexyl, cyclopentyl and acetyl (Ac); for serine, t-butyl, benzyl and tetrahydropyranyl; for histidine, trityl, benzyl, Cbz, p-toluenesulfonyl and 2,4-dinitrophenyl; for tryptophan, formyl; for aspartic acid and glutamic acid, benzyl and t-butyl and for cysteine, triphenylmethyl (trityl). In the solid phase peptide synthesis method, the α -C-terminal amino acid is attached to a suitable solid support or resin. Suitable solid supports useful for the above synthesis are those materials which are inert to the reagents and reaction conditions of the stepwise condensation-deprotection reactions, as well as being insoluble in the media used. Solid supports for synthesis of α -C-terminal carboxy peptides is 4-hydroxymethylphenoxymethyl-copoly(styrene-1% divinylbenzene) or 4-(2',4'-dimethoxyphenyl-Fmoc-aminomethyl)phenoxyacetamidoethyl resin available from Applied Biosystems (Foster City, Calif.). The α -C-terminal amino acid is coupled to the resin by means of N,N'-dicyclohexylcarbodiimide (DCC), N,N'-diisopropylcarbodiimide (DIC), O-benzotriazol-1-yl-N,N,N',N'-tetramethyluroniumhexafluorophosphate (HBTU), HATU, N,N-diisopropylethylamine (DIEA), or O-(1H-6-Chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HCTU) with or without 4-dimethylaminopyridine (DMAP), 1-hydroxybenzotriazole (HOBT), benzotriazol-1-yloxy-tris(dimethylamino)phosphoniumhexafluorophosphate (BOP) or bis(2-oxo-3-oxazolidinyl)phosphine chloride (BOPCl), mediated coupling for from about 1 to about 24 hours at a temperature of between 10°C and 50°C in a solvent such as dichloromethane, dimethylformamide (DMF), or N-methyl-2-pyrrolidone (NMP). When the solid support is 4-(2',4'-dimethoxyphenyl-Fmoc-aminomethyl)phenoxy-acetamidoethyl resin, the Fmoc group is cleaved with a secondary amine, preferably piperidine, prior to coupling with the α -C-terminal amino acid as described above. One method for coupling to the deprotected 4-(2',4'-dimethoxyphenyl-Fmoc-aminomethyl)phenoxy-acetamidoethyl resin is O-(1H-6-Chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HCTU, 1 equiv.) and N,N-diisopropylethylamine (DIEA, 1 equiv.) in NMP. The coupling of successive protected amino acids can be carried out in an automatic polypeptide synthesizer. In one example, the α -N-terminal in the amino acids of the growing peptide chain are protected with Fmoc. The removal of the Fmoc protecting group from the α -N-terminal side of the growing peptide is accomplished by treatment with a secondary amine, preferably piperidine. Each protected amino acid is then introduced in about 3-fold molar excess, and the coupling is preferably carried out in DMF. The coupling agent can be O-benzotriazol-1-yl-N,N,N',N'-tetramethyluroniumhexafluorophosphate

(HBTU, 1 equiv.) and 1-hydroxybenzotriazole (HOBT, 1 equiv.). At the end of the solid phase synthesis, the polypeptide is removed from the resin and deprotected, either successively or in a single operation. Removal of the polypeptide and deprotection can be accomplished in a single operation by treating the resin-bound polypeptide with a cleavage reagent that may comprise
5 thianisole or triisopropylsilane, water, ethanedithiol, and trifluoroacetic acid. In many cases, trifluoroacetic acid, triisopropylsilane, and water can be used. In cases wherein the α -C-terminal of the polypeptide is an alkylamide, the resin is cleaved by aminolysis with an alkylamine. Alternatively, the peptide can be removed by transesterification, *e.g.* with methanol, followed by aminolysis or by direct transamidation. The protected peptide can be purified at this point or
10 taken to the next step directly. The removal of the side chain protecting groups can be accomplished using the cleavage cocktail described above. The fully deprotected peptide can be purified by a sequence of chromatographic steps employing any or all of the following types: ion exchange on a weakly basic resin (acetate form); hydrophobic adsorption chromatography on underivatized polystyrene-divinylbenzene (for example, Amberlite XAD); silica gel adsorption
15 chromatography; ion exchange chromatography on carboxymethylcellulose; partition chromatography, *e.g.* on Sephadex G-25, LH-20 or countercurrent distribution; high performance liquid chromatography (HPLC), especially reverse-phase HPLC on octyl- or octadecylsilyl-silica bonded phase column packing.

In some examples, azido derivatives of amino acids can be synthesized as described by
20 Nyffeler PT *et al.* (Nyffeler *et al.* *JACS*, 2002, 124, 10773). For example, azido derivatives of amino acids can be prepared by adding a solution of triflic anhydride in dichloromethane to a solution of sodium azide in water. The solution can, for example, be combined with sodium bicarbonate. In some examples, the organic layer of the solution can be collected and reacted with an amine-containing amino acid.

25 In some examples, the Fmoc-labeled compounds can be prepared by combining an aqueous solution containing zinc chloride and either Fmoc-Dab-OH, Fmoc-Orn-OH or Fmoc-Lys-OH or any primary amine side chain. The solution can then be combined with triethylamine, methanol, triflic azide solutions.

30 In a preferred example, the amino acid couplings can be performed by combining a solution of the appropriate amino acid(s) with a coupling agent (*e.g.*, 2-(6-chloro-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU)) and a base (*e.g.*, N,N-diisopropylethylamine (DIEA)).

In some examples, wherein L₁ and L₂ comprise an azide and an alkyne, they can be covalently coupled via a copper(I)-catalyzed azide-alkyne cycloaddition (*e.g.*, CuSO₄ or CuI in

H₂O) or a ruthenium catalyzed azide-alkyne cycloaddition (*e.g.*, CpRuCl(PPh₃) in dioxane, CpRuCl(COD) in toluene, or CpRuCl₄ in DMF).

Methods of Use

Mutations that lead to EGFR overexpression (*e.g.*, upregulation) or overactivity have been associated with a number of cancers, including lung cancer, anal cancers (including colon cancer) and glioblastoma multiforme. These somatic mutations involving EGFR can lead to constitutive activation, which can produce uncontrolled cell division. Mutations, amplifications or misregulations of EGFR or family members are implicated in about 30% of all epithelial cancers. Aberrant EGFR signaling has also been implicated in inflammatory diseases, such as psoriasis, eczema and atherosclerosis.

Also provided herein are methods of treating, preventing, or ameliorating cancer in a subject. The methods include administering to a subject an effective amount of one or more of the compounds or compositions described herein, or a pharmaceutically acceptable salt thereof. The compounds and compositions described herein or pharmaceutically acceptable salts thereof are useful for treating cancer in humans, *e.g.*, pediatric and geriatric populations, and in animals, *e.g.*, veterinary applications. The disclosed methods can optionally include identifying a patient who is or can be in need of treatment of a cancer. Examples of cancer types treatable by the compounds and compositions described herein include bladder cancer, brain cancer, breast cancer, colorectal cancer, cervical cancer, gastrointestinal cancer, genitourinary cancer, head and neck cancer, lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, skin cancer, and testicular cancer. Further examples include cancer and/or tumors of the anus, bile duct, bone, bone marrow, bowel (including colon and rectum), eye, gall bladder, kidney, mouth, larynx, esophagus, stomach, testis, cervix, mesothelioma, neuroendocrine, penis, skin, spinal cord, thyroid, vagina, vulva, uterus, liver, muscle, blood cells (including lymphocytes and other immune system cells). Further examples of cancers treatable by the compounds and compositions described herein include carcinomas, Kaposi's sarcoma, melanoma, mesothelioma, soft tissue sarcoma, pancreatic cancer, lung cancer, leukemia (acute lymphoblastic, acute myeloid, chronic lymphocytic, chronic myeloid, and other), lymphoma (Hodgkin's and non-Hodgkin's), and multiple myeloma.

In some examples, the compounds described herein can be used to treat or prevent lung cancer, anal cancers (including colon cancer), glioblastoma multiforme, epithelial cancers, or combinations thereof in a subject.

The methods of treatment or prevention of cancer described herein can further include treatment with one or more additional agents (*e.g.*, an anti-cancer agent or ionizing radiation).

The one or more additional agents and the compounds and compositions or pharmaceutically acceptable salts thereof as described herein can be administered in any order, including simultaneous administration, as well as temporally spaced order of up to several days apart. The methods can also include more than a single administration of the one or more additional agents and/or the compounds and compositions or pharmaceutically acceptable salts thereof as described herein. The administration of the one or more additional agents and the compounds and compositions or pharmaceutically acceptable salts thereof as described herein can be by the same or different routes. When treating with one or more additional agents, the compounds and compositions or pharmaceutically acceptable salts thereof as described herein can be combined into a pharmaceutical composition that includes the one or more additional agents.

For example, the compounds or compositions or pharmaceutically acceptable salts thereof as described herein can be combined into a pharmaceutical composition with an additional anti-cancer agent, such as 13-cis-Retinoic Acid, 2-Amino-6-Mercaptopurine, 2-CdA, 2-Chlorodeoxyadenosine, 5-fluorouracil, 6-Thioguanine, 6-Mercaptopurine, Accutane, Actinomycin-D, Adriamycin, Adrucil, Agrylin, Ala-Cort, Aldesleukin, Alemtuzumab, Alitretinoin, Alkaban-AQ, Alkeran, All-transretinoic acid, Alpha interferon, Altretamine, Amethopterin, Amifostine, Aminoglutethimide, Anagrelide, Anandron, Anastrozole, Arabinosylcytosine, Aranesp, Aredia, Arimidex, Aromasin, Arsenic trioxide, Asparaginase, ATRA, Avastin, BCG, BCNU, Bevacizumab, Bexarotene, Bicalutamide, BiCNU, Bleomycin, Bortezomib, Busulfan, Busulfex, C225, Calcium Leucovorin, Campath, Camptosar, Camptothecin-11, Capecitabine, Carac, Carboplatin, Carmustine, Carmustine wafer, Casodex, CCNU, CDDP, CeeNU, Cerubidine, cetuximab, Chlorambucil, Cisplatin, Citrovorum Factor, Cladribine, Cortisone, Cosmegen, CPT-11, Cyclophosphamide, Cytadren, Cytarabine, Cytarabine liposomal, Cytosar-U, Cytoxan, Dacarbazine, Dactinomycin, Darbepoetin alfa, Daunomycin, Daunorubicin, Daunorubicin hydrochloride, Daunorubicin liposomal, DaunoXome, Decadron, Delta-Cortef, Deltasone, Denileukin diftitox, DepoCyt, Dexamethasone, Dexamethasone acetate, Dexamethasone sodium phosphate, Dexasone, Dexrazoxane, DHAD, DIC, Diodex, Docetaxel, Doxil, Doxorubicin, Doxorubicin liposomal, Droxia, DTIC, DTIC-Dome, Duralone, Efudex, Eligard, Ellence, Eloxatin, Elspar, Emcyt, Epirubicin, Epoetin alfa, Erbitux, Erwinia L-asparaginase, Estramustine, Ethyol, Etopophos, Etoposide, Etoposide phosphate, Eulexin, Evista, Exemestane, Fareston, Faslodex, Femara, Filgrastim, Floxuridine, Fludara, Fludarabine, Fluoroplex, Fluorouracil, Fluorouracil (cream), Fluoxymesterone, Flutamide, Folinic Acid, FUDR, Fulvestrant, G-CSF, Gefitinib, Gemcitabine, Gemtuzumab ozogamicin, Gemzar, Gleevec, Lupron, Lupron Depot, Matulane, Maxidex,

Mechlorethamine, -Mechlorethamine Hydrochlorine, Medralone, Medrol, Megace, Megestrol, Megestrol Acetate, Melphalan, Mercaptopurine, Mesna, Mesnex, Methotrexate, Methotrexate Sodium, Methylprednisolone, Mylocel, Letrozole, Neosar, Neulasta, Neumega, Neupogen, Nilandron, Nilutamide, Nitrogen Mustard, Novaldex, Novantrone, Octreotide, Octreotide acetate, Oncospar, Oncovin, Ontak, Onxal, Oprevelkin, Orapred, Orasone, Oxaliplatin, Paclitaxel, Pamidronate, Panretin, Paraplatin, Pediapred, PEG Interferon, Pegaspargase, Pegfilgrastim, PEG-INTRON, PEG-L-asparaginase, Phenylalanine Mustard, Platinol, Platinol-AQ, Prednisolone, Prednisone, Prelone, Procarbazine, PROCRIT, Proleukin, Prolifeprospan 20 with Carmustine implant, Purinethol, Raloxifene, Rheumatrex, Rituxan, Rituximab, Roveron-A (interferon alfa-2a), Rubex, Rubidomycin hydrochloride, Sandostatin, Sandostatin LAR, Sargramostim, Solu-Cortef, Solu-Medrol, STI-571, Streptozocin, Tamoxifen, Targretin, Taxol, Taxotere, Temodar, Temozolomide, Teniposide, TESP, Thalidomide, Thalomid, TheraCys, Thioguanine, Thioguanine Tabloid, Thiophosphoamide, Thioplex, Thiotepa, TICE, Toposar, Topotecan, Toremifene, Trastuzumab, Tretinoin, Trexall, Trisenox, TSPA, VCR, Velban, Velcade, VePesid, Vesanoid, Viadur, Vinblastine, Vinblastine Sulfate, Vincasar Pfs, Vincristine, Vinorelbine, Vinorelbine tartrate, VLB, VP-16, Vumon, Xeloda, Zanosar, Zevalin, Zinecard, Zoladex, Zoledronic acid, Zometa, Gliadel wafer, Glivec, GM-CSF, Goserelin, granulocyte colony stimulating factor, Halotestin, Herceptin, Hexadrol, Hexalen, Hexamethylmelamine, HMM, Hycamtin, Hydrea, Hydrocort Acetate, Hydrocortisone, Hydrocortisone sodium phosphate, Hydrocortisone sodium succinate, Hydrocortone phosphate, Hydroxyurea, Ibritumomab, Ibritumomab Tiuxetan, Idamycin, Idarubicin, Ifex, IFN-alpha, Ifosfamide, IL 2, IL-11, Imatinib mesylate, Imidazole Carboxamide, Interferon alfa, Interferon Alfa-2b (PEG conjugate), Interleukin 2, Interleukin-11, Intron A (interferon alfa-2b), Leucovorin, Leukeran, Leukine, Leuprolide, Leurocristine, Leustatin, Liposomal Ara-C, Liquid Pred, Lomustine, L-PAM, L-Sarcosine, Meticorten, Mitomycin, Mitomycin-C, Mitoxantrone, M-Prednisol, MTC, MTX, Mustargen, Mustine, Mutamycin, Myleran, Iressa, Irinotecan, Isotretinoin, Kidrolase, Lanacort, L-asparaginase, and LCR. The additional anti-cancer agent can also include biopharmaceuticals such as, for example, antibodies.

Many tumors and cancers have viral genome present in the tumor or cancer cells. For example, Epstein-Barr Virus (EBV) is associated with a number of mammalian malignancies. The compounds disclosed herein can also be used alone or in combination with anticancer or antiviral agents, such as ganciclovir, azidothymidine (AZT), lamivudine (3TC), *etc.*, to treat patients infected with a virus that can cause cellular transformation and/or to treat patients having a tumor or cancer that is associated with the presence of viral genome in the cells. The

compounds disclosed herein can also be used in combination with viral based treatments of oncologic disease.

Also described herein are methods of killing a tumor cell in a subject. The method includes contacting the tumor cell with an effective amount of a compound or composition as described herein, and optionally includes the step of irradiating the tumor cell with an effective amount of ionizing radiation. Additionally, methods of radiotherapy of tumors are provided herein. The methods include contacting the tumor cell with an effective amount of a compound or composition as described herein, and irradiating the tumor with an effective amount of ionizing radiation. As used herein, the term ionizing radiation refers to radiation comprising particles or photons that have sufficient energy or can produce sufficient energy via nuclear interactions to produce ionization. An example of ionizing radiation is x-radiation. An effective amount of ionizing radiation refers to a dose of ionizing radiation that produces an increase in cell damage or death when administered in combination with the compounds described herein. The ionizing radiation can be delivered according to methods as known in the art, including administering radiolabeled antibodies and radioisotopes.

The methods and compounds as described herein are useful for both prophylactic and therapeutic treatment, and also as diagnostics. As used herein the term treating or treatment includes prevention; delay in onset; diminution, eradication, or delay in exacerbation of signs or symptoms after onset; and prevention of relapse. For prophylactic use, a therapeutically effective amount of the compounds and compositions or pharmaceutically acceptable salts thereof as described herein are administered to a subject prior to onset (*e.g.*, before obvious signs of cancer), during early onset (*e.g.*, upon initial signs and symptoms of cancer), or after an established development of cancer. Prophylactic administration can occur for several days to years prior to the manifestation of symptoms of an infection. Prophylactic administration can be used, for example, in the chemopreventative treatment of subjects presenting precancerous lesions, those diagnosed with early stage malignancies, and for subgroups with susceptibilities (*e.g.*, family, racial, and/or occupational) to particular cancers. Therapeutic treatment involves administering to a subject a therapeutically effective amount of the compounds and compositions or pharmaceutically acceptable salts thereof as described herein after cancer is diagnosed.

The disclosed subject matter also concerns methods for treating a subject having an inflammatory disorder or condition. In one embodiment, an effective amount of one or more compounds or compositions disclosed herein is administered to a subject having an inflammatory disorder and who is in need of treatment thereof. Inflammatory disorders include, but are not limited to, acne vulgaris, asthma, autoimmune diseases, celiac disease, chronic

prostatitis, glomerulonephritis, inflammatory bowel diseases, pelvic inflammatory disease, reperfusion injury, rheumatoid arthritis, sarcoidosis, vasculitis, interstitial cystitis, type 1 hypersensitivities, systemic sclerosis, dermatomyositis, polymyositis, and inclusion body myositis.

5 In some examples, the compounds or compositions described herein can be used to treat or prevent psoriasis, eczema, atherosclerosis, or combinations thereof.

Also disclosed are methods of detecting EGFR overexpression. For example a subject or cell sample could be contacted with a compound or composition as disclosed herein, having a detectable moiety, and a signal can be detected. This signal can be compared to a control or
10 other standard to detect EGFR expression or overexpression.

Compositions, Formulations and Methods of Administration

In vivo application of the disclosed compounds, and compositions containing them, can be accomplished by any suitable method and technique presently or prospectively known to those skilled in the art. For example, the disclosed compounds can be formulated in a
15 physiologically- or pharmaceutically-acceptable form and administered by any suitable route known in the art including, for example, oral, nasal, rectal, topical, and parenteral routes of administration. As used herein, the term parenteral includes subcutaneous, intradermal, intravenous, intramuscular, intraperitoneal, and intrasternal administration, such as by injection. Administration of the disclosed compounds or compositions can be a single administration, or at
20 continuous or distinct intervals as can be readily determined by a person skilled in the art.

The compounds disclosed herein, and compositions comprising them, can also be administered utilizing liposome technology, slow release capsules, implantable pumps, and biodegradable containers. These delivery methods can, advantageously, provide a uniform dosage over an extended period of time. The compounds can also be administered in their salt
25 derivative forms or crystalline forms.

The compounds disclosed herein can be formulated according to known methods for preparing pharmaceutically acceptable compositions. Formulations are described in detail in a number of sources which are well known and readily available to those skilled in the art. For example, *Remington's Pharmaceutical Science* by E.W. Martin (1995) describes formulations
30 that can be used in connection with the disclosed methods. In general, the compounds disclosed herein can be formulated such that an effective amount of the compound is combined with a suitable carrier in order to facilitate effective administration of the compound. The compositions used can also be in a variety of forms. These include, for example, solid, semi-solid, and liquid dosage forms, such as tablets, pills, powders, liquid solutions or suspension, suppositories,

injectable and infusible solutions, and sprays. The preferred form depends on the intended mode of administration and therapeutic application. The compositions also preferably include conventional pharmaceutically-acceptable carriers and diluents which are known to those skilled in the art. Examples of carriers or diluents for use with the compounds include ethanol, dimethyl sulfoxide, glycerol, alumina, starch, saline, and equivalent carriers and diluents. To provide for the administration of such dosages for the desired therapeutic treatment, compositions disclosed herein can advantageously comprise between about 0.1% and 100% by weight of the total of one or more of the subject compounds based on the weight of the total composition including carrier or diluent.

Formulations suitable for administration include, for example, aqueous sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient; and aqueous and nonaqueous sterile suspensions, which can include suspending agents and thickening agents. The formulations can be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and can be stored in a freeze dried (lyophilized) condition requiring only the condition of the sterile liquid carrier, for example, water for injections, prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powder, granules, tablets, *etc.* It should be understood that in addition to the ingredients particularly mentioned above, the compositions disclosed herein can include other agents conventional in the art having regard to the type of formulation in question.

Compounds disclosed herein, and compositions comprising them, can be delivered to a cell either through direct contact with the cell or via a carrier means. Carrier means for delivering compounds and compositions to cells are known in the art and include, for example, encapsulating the composition in a liposome moiety. Another means for delivery of compounds and compositions disclosed herein to a cell comprises attaching the compounds to a protein or nucleic acid that is targeted for delivery to the target cell. U.S. Patent No. 6,960,648 and U.S. Application Publication Nos. 2003/0032594 and 20020120100 disclose amino acid sequences that can be coupled to another composition and that allows the composition to be translocated across biological membranes. U.S. Application Publication No. 2002/0035243 also describes compositions for transporting biological moieties across cell membranes for intracellular delivery. Compounds can also be incorporated into polymers, examples of which include poly (D-L lactide-co-glycolide) polymer for intracranial tumors; poly[bis(p-carboxyphenoxy) propane:sebacic acid] in a 20:80 molar ratio (as used in GLIADEL); chondroitin; chitin; and chitosan.

For the treatment of oncological disorders, the compounds disclosed herein can be administered to a patient in need of treatment in combination with other antitumor or anticancer substances and/or with radiation and/or photodynamic therapy and/or with surgical treatment to remove a tumor. These other substances or treatments can be given at the same as or at different times from the compounds disclosed herein. For example, the compounds disclosed herein can be used in combination with mitotic inhibitors such as taxol or vinblastine, alkylating agents such as cyclophosphamide or ifosfamide, antimetabolites such as 5-fluorouracil or hydroxyurea, DNA intercalators such as adriamycin or bleomycin, topoisomerase inhibitors such as etoposide or camptothecin, antiangiogenic agents such as angiostatin, antiestrogens such as tamoxifen, and/or other anti-cancer drugs or antibodies, such as, for example, GLEEVEC (Novartis Pharmaceuticals Corporation) and HERCEPTIN (Genentech, Inc.), respectively, or an immunotherapeutic such as ipilimumab and bortezomib.

In certain examples, compounds and compositions disclosed herein can be locally administered at one or more anatomical sites, such as sites of unwanted cell growth (such as a tumor site or benign skin growth, *e.g.*, injected or topically applied to the tumor or skin growth), optionally in combination with a pharmaceutically acceptable carrier such as an inert diluent. Compounds and compositions disclosed herein can be systemically administered, such as intravenously or orally, optionally in combination with a pharmaceutically acceptable carrier such as an inert diluent, or an assimilable edible carrier for oral delivery. They can be enclosed in hard or soft shell gelatin capsules, can be compressed into tablets, or can be incorporated directly with the food of the patient's diet. For oral therapeutic administration, the active compound can be combined with one or more excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, aerosol sprays, and the like.

The tablets, troches, pills, capsules, and the like can also contain the following: binders such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, fructose, lactose or aspartame or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring can be added. When the unit dosage form is a capsule, it can contain, in addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials can be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules can be coated with gelatin, wax, shellac, or sugar and the like. A syrup or elixir can contain the active compound, sucrose or fructose as a sweetening agent,

methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any unit dosage form should be pharmaceutically acceptable and substantially non-toxic in the amounts employed. In addition, the active compound can be incorporated into sustained-release preparations and devices.

5 Compounds and compositions disclosed herein, including pharmaceutically acceptable salts or prodrugs thereof, can be administered intravenously, intramuscularly, or intraperitoneally by infusion or injection. Solutions of the active agent or its salts can be prepared in water, optionally mixed with a nontoxic surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, triacetin, and mixtures thereof and in oils. Under ordinary conditions of
10 storage and use, these preparations can contain a preservative to prevent the growth of microorganisms.

 The pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the active ingredient, which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or
15 dispersions, optionally encapsulated in liposomes. The ultimate dosage form should be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can be
20 maintained, for example, by the formation of liposomes, by the maintenance of the required particle size in the case of dispersions or by the use of surfactants. Optionally, the prevention of the action of microorganisms can be brought about by various other antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers or
25 sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the inclusion of agents that delay absorption, for example, aluminum monostearate and gelatin.

 Sterile injectable solutions are prepared by incorporating a compound and/or agent disclosed herein in the required amount in the appropriate solvent with various other ingredients enumerated above, as required, followed by filter sterilization. In the case of sterile powders for
30 the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze drying techniques, which yield a powder of the active ingredient plus any additional desired ingredient present in the previously sterile-filtered solutions.

 For topical administration, compounds and agents disclosed herein can be applied in as a liquid or solid. However, it will generally be desirable to administer them topically to the skin as

compositions, in combination with a dermatologically acceptable carrier, which can be a solid or a liquid. Compounds and agents and compositions disclosed herein can be applied topically to a subject's skin to reduce the size (and can include complete removal) of malignant or benign growths, or to treat an infection site. Compounds and agents disclosed herein can be applied
5 directly to the growth or infection site. Preferably, the compounds and agents are applied to the growth or infection site in a formulation such as an ointment, cream, lotion, solution, tincture, or the like.

Useful solid carriers include finely divided solids such as talc, clay, microcrystalline cellulose, silica, alumina and the like. Useful liquid carriers include water, alcohols or glycols or
10 water-alcohol/glycol blends, in which the compounds can be dissolved or dispersed at effective levels, optionally with the aid of non-toxic surfactants. Adjuvants such as fragrances and additional antimicrobial agents can be added to optimize the properties for a given use. The resultant liquid compositions can be applied from absorbent pads, used to impregnate bandages and other dressings, or sprayed onto the affected area using pump-type or aerosol sprayers, for
15 example.

Thickeners such as synthetic polymers, fatty acids, fatty acid salts and esters, fatty alcohols, modified celluloses or modified mineral materials can also be employed with liquid carriers to form spreadable pastes, gels, ointments, soaps, and the like, for application directly to the skin of the user.

Useful dosages of the compounds and agents and pharmaceutical compositions disclosed
20 herein can be determined by comparing their *in vitro* activity, and *in vivo* activity in animal models. Methods for the extrapolation of effective dosages in mice, and other animals, to humans are known to the art.

The dosage ranges for the administration of the compositions are those large enough to
25 produce the desired effect in which the symptoms or disorder are affected. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of the disease in the patient and can be determined by one of skill in the art. The dosage can be adjusted by the individual physician in the event of any counterindications. Dosage can vary, and
30 can be administered in one or more dose administrations daily, for one or several days.

Also disclosed are pharmaceutical compositions that comprise a compound disclosed herein in combination with a pharmaceutically acceptable carrier. Pharmaceutical compositions adapted for oral, topical or parenteral administration, comprising an amount of a compound constitute a preferred aspect. The dose administered to a patient, particularly a human, should be

sufficient to achieve a therapeutic response in the patient over a reasonable time frame, without lethal toxicity, and preferably causing no more than an acceptable level of side effects or morbidity. One skilled in the art will recognize that dosage will depend upon a variety of factors including the condition (health) of the subject, the body weight of the subject, kind of concurrent treatment, if any, frequency of treatment, therapeutic ratio, as well as the severity and stage of the pathological condition.

Also disclosed are kits that comprise a compound disclosed herein in one or more containers. The disclosed kits can optionally include pharmaceutically acceptable carriers and/or diluents. In one embodiment, a kit includes one or more other components, adjuncts, or adjuvants as described herein. In another embodiment, a kit includes one or more anti-cancer agents, such as those agents described herein. In one embodiment, a kit includes instructions or packaging materials that describe how to administer a compound or composition of the kit. Containers of the kit can be of any suitable material, *e.g.*, glass, plastic, metal, *etc.*, and of any suitable size, shape, or configuration. In one embodiment, a compound and/or agent disclosed herein is provided in the kit as a solid, such as a tablet, pill, or powder form. In another embodiment, a compound and/or agent disclosed herein is provided in the kit as a liquid or solution. In one embodiment, the kit comprises an ampoule or syringe containing a compound and/or agent disclosed herein in liquid or solution form.

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.

EXAMPLES

The following examples are set forth to illustrate the methods and results according to the disclosed subject matter. These examples are not intended to be inclusive of all aspects of the subject matter disclosed herein, but rather to illustrate representative methods and results. These examples are not intended to exclude equivalents and variations which are apparent to one skilled in the art.

Efforts have been made to ensure accuracy with respect to numbers (*e.g.*, amounts, temperature, *etc.*) but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C or is at ambient temperature, and pressure is at or near atmospheric. There are numerous variations and combinations of reaction conditions, *e.g.*, component concentrations, temperatures, pressures and other reaction ranges and conditions that can be used to optimize the product purity and yield obtained from the

described process. Only reasonable and routine experimentation will be required to optimize such process conditions.

All protected amino acids, Rink amide MBHA resin and NovaPEG Rink Amide resin were purchased from Novabiochem unless otherwise noted. HCTU was purchased from Peptides International. 2-(6-chloro-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU) and Fmoc-11-amino-3,6,9-trioxaundecanoic acid were purchased from ChemPep. All synthesis reagents and solvents were purchased from Fisher, Sigma-Aldrich, or Acros and used without further purification. Cell culture media and phosphate buffer saline was obtained from Lonza, fetal bovine serum and horse serum from Fischer, penicillin/streptomycin and bovine serum albumin from Amresco, trypsin EDTA from Corning, and EGF from Abcam. Antibody to p-Akt was purchased from Cell Signaling, EGFR p-Y1068 from Pierce, total EGFR from Santa Cruz Biotech, FITC from Abcam, and α -tubulin from the University of Iowa. Chemiluminescent substrate and PVDF membrane were purchased from Millipore. Immobilized chymotrypsin was obtained from Proteochem, and immobilized trypsin was obtained from Pierce. Peptide characterization and purification were performed on a Zorbax SB-C18, 5 μ m HPLC column using an Agilent 1200 series HPLC system coupled to the Agilent 6120 quadrupole LC/MS. Peptides were quantified using the Biotek Synergy 2 microplate reader. C57BL/6J mice obtained from Jackson Laboratories were maintained in a local breeder colony. All mouse housing and handling procedures were approved by the University of Georgia Institutional Animal Care and Use Committee.

Cells were cultured at 37 °C with 5% carbon dioxide. BxPC-3 cells were cultured in RPMI-1640 (Lonza) with 10% fetal bovine serum (Fisher) and penicillin/streptomycin (Amresco). HCT-116 cells were cultured in DMEM (Lonza) with glucose and L-glutamine, 10% fetal bovine serum, and penicillin/streptomycin. Mia Paca-2 cells were cultured in DMEM with glucose and L-glutamine, 10% fetal bovine serum, 2.5% horse serum (Fisher), and penicillin/streptomycin.

Example 1: Synthesis of Azido Amino Acids

Azido derivatives of amino acids were synthesized as previously described (Nyffeler PT *et al.*, *JACS*, 2002, 124, 10773). Fresh triflic azide was prepared immediately before the azido transfer reaction. Briefly, a 1M solution of triflic anhydride in dichloromethane (DCM) (6 ml, 6 mmol, 3 equiv.) was added to a solution of sodium azide (0.781 g, 12 mmol, 6 equiv.) in 5 ml water at 0°C. The solution was stirred for 2.5 hours at 0°C, after which saturated sodium bicarbonate was added until the evolution of CO₂ stopped. The solution was transferred to a separatory funnel and the organic layer was collected. The aqueous layer was washed two times

with DCM. The organic layers were returned to the separatory funnel to be added dropwise to the amine-containing amino acid.

An aqueous solution containing zinc chloride (18.5 mg, 0.14 mmol) and 1 equivalent (2 mmol) of either Fmoc-Dab-OH (Bachem), Fmoc-Orn-OH (Bachem) or Fmoc-Lys-OH (CHEM-
5 IMPEX) was added to a roundbottom flask. The solution was stirred and triethylamine (0.837 ml, 6 mmol, 3 equiv.) was slowly added. Methanol (40 ml) was added dropwise to create a final ratio of water/methanol/DCM at 3:10:3. After complete addition of methanol, the freshly prepared triflic azide solution was added dropwise from the separatory funnel. The solution was stirred at ambient temperature for 2-3 hours and the reaction was monitored by TLC. The
10 organic solvents were removed by rotary evaporation, and the remaining aqueous solution was acidified with 1% citric acid (20 ml). The aqueous layer was extracted with DCM and the combined organic layers were purified by silica gel chromatography using a 0.5-7% methanol gradient in DCM. Final products were confirmed by NMR and mass spectrometry.

Example 2: Peptide Synthesis

15 Peptides were prepared using Fmoc-based solid-phase peptide synthesis. The non-modified and disulfide peptide controls were synthesized on rink amide MBHA resin. Peptides that were engineered to contain a triazole crosslinker were synthesized on NovaPEG resin. Resin (25 μ mol) was loaded into a fritted peptide synthesis column and equilibrated in N-methylpyrrolidinone (NMP). Deprotections were performed using a solution of 25% piperidine
20 in NMP. Amino acid couplings were performed using a 0.5 M solution of amino acid (0.5 ml, 0.25 mmol, 10 equiv) along with 0.5 M solution of HCTU (0.495 ml, 0.248 mmol, 9.9 equiv) and DIEA (87 μ l, 0.5 mmol, 20 equiv). All couplings containing the azido amino acids or propargyl glycine (Peptech) were performed using 4 equiv of amino acid (0.1 mmol, 0.2 ml), 3.96 equiv of HCTU (0.099 mmol, 0.25 ml), and 8 equiv of DIEA (0.2 mmol, 43.5 ml).

Example 3: On-Resin Cyclization of EDA Peptides

25 The 1,3-triazole crosslinker was formed on-resin using copper(I)-catalyzed azide-alkyne cycloaddition chemistry. The resin (25 μ mol) was suspended in a 1:2 solution of t-butanol (Alfa Aesar) and water. Sodium ascorbate (160 mg, 750 μ mol, 30 equiv.) and copper sulfate (55 mg, 375 μ mol, 15 equiv.) were added, causing the mixture to turn bright orange. The mixture was
30 left to stir overnight.

Example 4: N-terminal 5(6)-Carboxyfluorescein Labeling

Unless otherwise stated, all peptides used in this study were labeled with 5(6)-carboxyfluorescein (Acros) after on-resin cyclization or after the addition of β -Ala. After equilibrating the resin in DMF, 5(6)-carboxyfluorescein (19 mg, 50 μ mol, 2 equiv), HCTU (19

mg, 45 μ mol, 1.8 equiv) and DIEA (20 μ L, 115 μ mol, 4.6 equiv) were dissolved in 1 mL DMF. The 5(6)-carboxyfluorescein solution was added to the resin and bubbled overnight while protected from light.

Example 5: Peptide Cleavage

Resin cleavage was performed by incubating the resin for 4-5 hours in a cleavage solution containing 95:2.5:2.5 trifluoroacetic acid/water/triisopropylsilane. After cleavage, the peptide was filtered through glass wool into ice cold methyl t-butyl ether. The precipitate was collected by centrifugation at 4°C. The supernatant was decanted and the pellet was air-dried. The peptide product was dissolved in methanol and products were confirmed by LC/MS. Non-modified control molecular weight = 1568.6 (expected 1569.62); Disulfide Control = 1632.6 (expected 1631.74); EDA1 = 1648.0 (expected 1648.68); EDA2 = 1648.0 (expected 1648.68); EDA3 = 1662.2 (expected 1662.71); EDA4 = 1662.2 (expected 1662.71); EDA5 = 1676.2 (expected 1676.74); EDA6 = 1676.2 (expected 1676.74); EDA2-Scr = 1647.6 (expected 1648.68).

The peptides were purified by reverse phase HPLC on a Zorbax C18 column using a flow rate of 4 ml/min and a gradient of 10%-100% acetonitrile containing 0.1% TFA over 24 minutes. Fluorescein labeled peptides were quantified using the extinction coefficient of 68,000 L $\cdot$ mol⁻¹ $\cdot$ cm⁻¹ for fluorescein in 10 mM Tris pH 8. Biotinylated peptides were quantified using the extinction coefficient of the HABA-avidin complex in 10 mM Tris pH 8. Normalized stocks were made by dissolving the peptides in DMSO at a 10 mM.

Example 6: EGFR Phosphorylation in MDA-MB-231 cells

MDA-MB-231 cells were seeded in 24 well plates in RPMI 1640 supplemented with 10% FBS and penicillin/streptomycin. Cells were incubated at 37°C until approximately 80% confluent. The media was then replaced with RPMI 1640 supplemented with 0.1% BSA, and the cells were incubated overnight. Serum starved cells were then pretreated with 0.05, 0.5, or 5 μ M peptide for 30 min, followed by a 5 min stimulation with 50 ng/mL EGF. Cells were immediately lysed in 1x Laemmli buffer.

Proteins were separated by 8% SDS-PAGE then transferred onto PVDF membrane. Identical membranes were probed for EGFR p-Y1068, p-Akt and α -Tubulin. Bands were visualized by chemiluminescence and quantified using Licor Image Studio Lite. Results were normalized to α -Tubulin and reported as a ratio relative to EGF-stimulated cells in the absence of peptide. The average of three experiments was plotted in GraphPad Prism, where error bars represent SEM. Total EGFR was quantified independently from four experiments using the same methods above.

Example 7: Proteolytic Degradation of Peptides

Immobilized trypsin and chymotrypsin were added to 5 µg peptide in digestion buffer (100 mM Tris, 200 mM NaCl, 100 mM CaCl₂, pH 8.2) where E/S for each protease was ~375. Benzyl alcohol was used as an internal standard. Peptides were incubated at 37°C for 0, 0.5, 1, 2 and 4 hours with gentle agitation, after which methanol was added to a final concentration of 50%. The immobilized protease was pelleted by centrifugation at 15,000 rpm, and the supernatant was collected and analyzed by LC/MS. After incubation with protease, the disulfide and triazole peptide spectra displayed a peak with a mass equivalent to the methylated peptide. Data represents the combined absorbance of both methylated and unmethylated peptides. Peptide absorbance at 220 nm was normalized relative to the benzyl alcohol peak. Data was plotted in GraphPad Prism as percentage of parent peptide relative to T₀. Normalized curves represent one phase decay where Y₀ equals 100, the plateau equals 0, and K is greater than 0.

Example 8: Peptide Stability in Tissue Culture Media

A solution of 0.2 mM peptide and 0.1% benzyl alcohol in RPMI supplemented with 0.1% BSA was incubated at 37°C with mixing at 300 rpm. Aliquots were collected in duplicate and quenched with an equal volume of acetonitrile with 0.1% TFA. The samples were centrifuged at 14,000 rpm and the supernatant was analyzed by LC/MS. The peptide absorbances at 220 nm were normalized relative the benzyl alcohol standard and the percent of remaining peptide was calculated relative to T₀. The resulting data was plotted in GraphPad Prism.

Example 9: Serum Stability of Peptides

Fresh blood was obtained from isoflurane-anesthetized C57BL/6J male or female mice by terminal cardiac puncture according to standard procedures. Serum was separated from clotted blood by 5 min centrifugation at 10,000 rpm. A solution of 50% fresh mouse serum, 0.1% benzyl alcohol, and 0.2 mM peptide in PBS was incubated at 37°C in a thermomixer with shaking at 300 rpm. Aliquots were collected in duplicate at 0, 0.5, 1, 2 and 4 hours and quenched by the addition of an equal volume of 99.9% acetonitrile and 0.1% TFA. The proteins were pelleted by centrifugation at 14,000 rpm for 5 min. The supernatant was collected and analyzed by LC/MS. Peptide and benzyl alcohol absorbance at 220 nm was recorded, and the percent parent peptide remaining was calculated relative to the benzyl alcohol internal standard. Data was plotted in GraphPad Prism as percent parent peptide relative to T₀. Normalized curves represent one phase decay where Y₀ equals 100, the plateau equals 0, and K is greater than 0.

Example 10: Circular Dichroism

CD spectra were obtained on a Jasco J-710 CD Spectrometer at 25°C using a 0.1 mm cuvette. Solutions of the non-modified control, disulfide control, and EDA2 in 10 mM sodium

phosphate buffer at pH 6.5 and pH 7.4 were prepared. Spectra were recorded over 190-260 nm at 0.5 nm intervals using a 2 nm bandwidth, 100 msec time constant, 50 nm/min scanning speed, and 3 responses. The units were converted to molar ellipticity in Jasco Spectra Manager after baseline subtraction. The Savitzky-Golay smoothing filter was applied with a convolution width of 21. The relative abundance of secondary structures was predicted using the SOMCD neural network algorithm server (Unneberg P *et al.*, *Proteins*, **2001**, 42, 460).

Example 11: Immunoprecipitation of Labeled Peptides

A431 cells were grown to confluence on 10 cm plates then serum starved overnight. The cells were collected from 4 plates and lysed in 1.25 mL NP-40 lysis buffer (20 mM Tris HCl pH 8, 137 mM NaCl, 1% Nonidet P-40 (NP-40), 2 mM EDTA). Proteins were solubilized with gentle agitation for 30 min at 4°C. Cellular debris was removed by centrifugation, and the lysates were precleared for 1 hr using 100 µL Protein A/G Plus-Agarose (Santa Cruz). A 12.5 µM peptide solution in NP-40 lysis buffer (200 µL) was added to 400 µL of the lysate. The solution was incubated at 4°C with gentle agitation for 1.5 hr. Following incubation, 50 µL was combined with 50 µL 2x laemmli buffer to denature the lysate. 400 µL of the remaining lysate was combined with 10 µL of anti-FITC antibody (Abcam) and incubated for 1.5 hr at 4°C. 50 µL of Protein A/G Plus-Agarose was added to the samples and incubated overnight at 4°C with gentle agitation. The mixture was then centrifuged for 2 minutes at 2000 g and 4°C and the supernatant was discarded. The beads were washed 5 times with NP-40 lysis buffer. After the fifth wash the mixture was centrifuged at 14000 g and 4°C for 2 minutes. The supernatant was discarded and 75 µL 2x laemmli buffer was added. The samples were boiled for 10 min, then centrifuged at 14000 g for 10 min, and the supernatant was collected. The proteins were separated by 8% SDS-PAGE and transferred to PVDF for detection of total EGFR. Bands were detected by chemiluminescence.

Example 12: EGFR Dimerization Assay

MDA-MB-231 cells were seeded into 8 well chamber slides in RPMI supplemented with 10% FBS and 1x penicillin/streptomycin. The cells were grown to 80-90% confluence then serum starved for 24 hours in RPMI supplemented with 0.1% BSA. The cells were then treated with 5 µM peptide for 30 minutes then stimulated for 5 minutes with 10 ng/mL EGF. The cells were then washed with PBS and fixed with 2% paraformaldehyde and permeabilized in Triton-X 100. The Duolink In Situ Assay was performed according to the manufacturer's instructions. Wells were blocked using the Duolink Blocking Solution. Equal volumes of the Plus and Minus PLA probes conjugated to the EGFR monoclonal antibody (Millipore) were added to the wells and incubated at 4°C overnight. The probes were then ligated and amplified using the Duolink

Orange Detection Kit. The slide was mounted using Permafluor mounting medium with Dapi. Images were obtained at 40x magnification using TRITC and DAPI filters on an IX71 inverted fluorescent microscope with Cell Sens software (Olympus). Fluorescent signals per cell were counted from five representative 40X magnification microscope fields per condition. The averages of duplicate experiments were plotted using GraphPad Prism. Error bars represent SEM.

Example 13: Modeling of EDA peptides and molecular dynamics simulation

The peptide coordinates corresponding to EDA wild-type were taken from the EGFR dimer structure 3NJP (residues 246-253). Avogadro was used to model the triazolyl linker between Y246 and M253. The geometry of the cross-linked peptide was minimized using the GAFF force-field implemented within Avogadro. The topological parameters, partial charges and other force field parameters were estimated with programs in the AmberTools 13 suite (Case *et al.*, 2012, AMBER 12, University of California, San Francisco). The amber generated force field values were converted to gromacs format using acpype (Sousa da Silva AW and Vranken WF, *BMC Res Notes*, 2012, 5, 367). Molecular dynamics simulations were carried out using Gromacs v4.6.2.

The peptide structures were solvated in a periodic dodecahedron box with at least 1nm space on all sides of the peptide. Energy minimization using steepest descent was carried out till Fmax reached 10 kJ/mol/nm. An NVT simulation with position restraints on the peptide atoms were performed with a V-rescale thermostat used for temperature equilibration. Long range electrostatics were treated using PME, with a short range cutoff of 0.9 nm. Pressure equilibration was done under similar conditions (including position restraints) with an additional Berendsen pressure coupling algorithm. Production runs for 40ns were carried out under the NPT conditions after removing the position restraints. The frames of the trajectory were saved to disk every 2ps.

The g_hbond program in the Gromacs suite was used to query the number of frames in which the hydrogen bond between N247 and Y251 was observed. The hydrogen bond between N247 and Q252 was seen in all peptides with equal occupancy and hence is not discussed in the text. Cluster analysis of the trajectories was carried out with the g_cluster program in the Gromacs suite with a RMSD cutoff of 1.0 angstrom. The cluster centers were written to a PDB file and analyzed.

A panel of EGFR Dimerization Arm (EDA) peptides was designed using the native sequence of human EGFR (residues 245-254, FIG. 2, C). The dimerization arm mimics were initially designed without modification of the amino acid sequence since many of these residues

make contacts with the other receptor half-site. As a strategy to covalently constrain the dimerization arm, cycloaddition chemistry was utilized to introduce a 1,4-disubstituted [1,2,3] triazolyl-containing bridge between the terminal residues of the sequence.

Using this strategy, the dimerization arm was covalently cross-linked while on solid support to link the terminal residues using copper (I)-catalyzed azide-alkyne [3+2] Huisgen cycloaddition chemistry (FIG. 2, B) (Rostovtsev VV *et al.*, *Angew. Chem. Int. Ed.*, 2002, 41, 2596-2599; Kolb HC *et al.*, *Angew. Chem. Int. Ed.*, 2001, 40, 2004-2021; Tornoe CW *et al.*, *J. Org. Chem.*, 2002, 67, 3057-3064). The azide- or alkyne-containing amino acids were incorporated into terminal positions of the sequence to minimize modifications within the dimerization arm itself. Since the optimal bridge length was not known, this length was modified by incorporating different azido-amino acid derivatives (FIG. 2, A) that were synthesized as previously described (Nyffeler PT *et al.*, *JACS*, 2002, 124, 10773-10778). The methylene units of the azido-amino acids were varied between 2 to 4 units (azido-L-homoalanine, azido-norvaline, or azido-norleucine) to alter the overall length of the triazole linker while the alkyne (propargyl glycine) remained fixed. Since the linker asymmetrically connects the triazole, the peptides were synthesized in pairs by swapping the positions of the azido- and alkynyl-amino acids. This was done to evaluate the effects of the triazole orientation on inhibitory activity. In addition, two peptide controls were used: one containing the non- modified sequence of the dimerization arm, and the other containing a labile disulfide linkage in the same position where the triazole was introduced (FIG. 2, C) (Mizuguchi T *et al.*, *Bioorg. Med. Chem. Lett.*, 2009, 19, 3279-3282).

In order to ascertain the impact of the introduced triazolyl bridges on the overall structure of the dimerization arm, molecular dynamics simulations were performed. In these studies, the effects of the linker length were studied in relation to the hydrogen-bonding network and the overall structure of the cyclized peptides (FIG. 2 D, FIG. 3, FIG. 4, and FIG. 5). A query of the number of molecular dynamics frames containing the hydrogen bond between N247 and Y251 predicts that EDA2 and EDA4 largely retain the hydrogen bond supporting β -loop formation throughout the duration of the simulation, whereas the other peptides do not. However, cluster analysis predicts that EDA4 will adopt a more open conformation, while EDA2 maintains the β -loop conformation. Although, EDA6 is also predicted to adopt a β -loop conformation, the triazolyl-linker folds back on the non-binding surface of the peptide, while the linker of EDA2 adopts a more planar conformation relative to the β -strands and can allow for more extensive contacts with the binding surface of Domain II. Additionally, EDA2 appears to have less conformational flexibility as only a single cluster was identified, whereas 2-3 clusters were

identified for EDA4 and EDA6. Taken together, the simulations suggest that the conformation of EDA2 is relatively stable as compared to the other peptides and can mimic the desired binding conformation more closely.

Next, the EDA peptides were tested to see if they could inhibit EGFR activation.

5 Receptor activation was monitored as a function of EGFR autophosphorylation on Tyr1068 in intact MDA-MB-231 cells (FIG. 6 A, B, D, E and FIG. 7). After overnight serum-starvation to decrease autophosphorylation levels, the receptor was stimulated with EGF for 5 min following a 25 min pretreatment with either the EDA peptides or gefitinib as a positive control (Fry DW *et al.*, *Science*, 1994, 265, 1093-1095). While all the EDA peptides containing longer length linkers
10 (EDA3-6) showed little to no inhibitory activity (FIG. 7), the peptides containing the shortest linkers inhibited EGFR activation. EDA1 was found to moderately knock down phosphorylation at the highest dosing tested, while EDA2 reduced phosphorylation by approximately half that of untreated cells at the relatively low dose of 0.5 μ M (FIG. 6 B). In contrast, the EDA2 scramble control (EDA2-Scr) had little to no inhibitory effects on EGFR phosphorylation (FIG. 6 D, E).
15 The total expression levels of EGFR were also measured and were found to remain unchanged during this time course (FIG. 6 A). In addition, phosphorylation of a downstream substrate, Akt, was also monitored (FIG. 6 A, C). A similar trend was noted where EDA2 reduced Akt phosphorylation by nearly 50%. This demonstrates that EDA2 can successfully downregulate EGFR activation and signaling.

20 Although EDA2 could downregulate EGFR phosphorylation, the ability of EDA2 to inhibit EGFR dimerization was also investigated. A quantitative proximity ligation assay was performed using a Duolink fluorescence assay in intact cells (FIG. 8 A, B). MDA-MB-231 cells were stimulated with EGF in the presence or absence of EDA2 or its scramble control (EDA2-Scr). The cells were then fixed and probed with equivalent amounts of the plus and minus PLA
25 probes conjugated to an anti-EGFR monoclonal antibody to highlight EGFR dimers. Fluorescence microscopy was used to visualize the DAPI and PLA signals. EDA2-treated cells displayed a notable decrease in EGFR dimers as compared to the untreated and EDA2-Scr control. Quantification of the fluorescent signals in individual cells (n=100) indicates that EDA2 caused a reduction in EGFR dimerization by over of 50% (FIG. 8 B). Thus, it appears that EDA2
30 substantially inhibits EGFR dimerization.

To further verify that EDA2 directly binds to the EGF receptor, Immunoprecipitation experiments were also performed (FIG. 8 C). Cells were either mock treated or treated with EDA2, followed by Immunoprecipitation of the peptide and probed using anti-EGFR. Western

blot analysis showed that EGFR co-precipitated with EDA2 but not in the mock treatment, demonstrating direct binding of EDA2 to EGFR.

Since the synthetic constraint should promote proteolytic stability, degradation of the EDA peptides was measured in the presence of purified proteases, culture media, and serum. First, the rate of peptide degradation in the presence of purified proteases was measured (FIG. 8 D and FIG. 9 A). EDA peptides were incubated with a cocktail of immobilized trypsin and chymotrypsin over a time course of four hours. The amount of remaining peptide was quantified by LC/MS relative to an internal standard. The non-modified control peptide was rapidly degraded, where 50% was lost within one hour. However, all bridged peptides showed significantly enhanced proteolytic resistance with little to no degradation over the same time course, demonstrating that introduction of the linker provided substantial resistance to proteolytic degradation. To determine the extent of peptide hydrolysis in tissue culture media, peptide stability was measured in RPMI over the same four-hour time course (FIG. 9 B). All peptides remained intact in RPMI throughout the time course, showing that the peptides are stable in cell culture media for the duration of the cell-based assays.

Although multiple proteases are present in serum, peptide stability was also assessed using fresh mouse serum over a time course of four hours (FIG. 8 E and FIG. 9 C). The non-modified peptide was nearly completely degraded in one hour, but EDA2 showed minimal loss during the entire time course tested. Because the extracellular space of the tumor microenvironment and the endosomal compartments where internalized EGFR sorting occurs are both acidic, the structural stability of EDA2 at physiologic and slightly acidic pH conditions was also evaluated using circular dichroism (FIG. 10) (Wojtkowiak JW *et al.*, *Mol. Pharmaceutics*, 2011, 8, 2032-2038; Sorkin A and von Zastrow M, *Nat. Rev. Mol. Cell Biol.* 2002, 3, 600-614; Ceresa BP, *Int. J. Mol. Sci.*, 2013, 14, 72-87). Under these conditions, EDA2 largely retained its relative abundance of β -turn structure. However, nearly all of the relative abundance of β -sheet and β -turn structure was lost in the disulfide-linked control, likely due to increased reduction of the disulfide bond at pH 6.5.

Covalently bridged peptides were developed that mimic the dimerization arm of EGFR and effectively inhibit EGFR activation in cell-based assays. EDA2 displays notable inhibitory activity against EGFR dimerization. The inhibitory effect of EDA2 against EGFR represents a new targeting strategy for allosteric inhibition of ErbB dimerization. This alternative targeting strategy can circumvent shortcomings of gefitinib and other ATP-competitive compounds as it does not require sensitizing point mutations and is not susceptible to secondary resistance mutations that often arise in the kinase domain (Chmielecki J *et al.*, *Sci. Transl. Med.*, 2011, 3,

90ra59; Zhang J *et al.*, *Nat. Rev. Cancer*, 2009, 9, 28-39). Additionally, by targeting the dimer interface, the peptides can retain activity in cells with resistance involving Her2 overexpression. Although the native sequence of the dimerization arm was used in this study, this represents a starting point for sequence optimization so as to improve target inhibition and optimize binding interactions. Further, this study highlights a synthetic strategy to probe allosteric regulatory mechanisms that govern kinase activation and demonstrates utility of intrastrand crosslinking to generate biologically active peptide-based macrocycles for EGFR inhibition.

Example 14: Synthesis of selenylsulfide peptide 2c

The peptide sequence was synthesized on a 25 μ mol scale using general coupling and deprotection procedures. Fmoc-Sec(PMB)-OH and Fmoc-11-amino-3,6,9-trioxaundecanoic acid were coupled using 0.5 M amino acid (0.2 mL, 0.1 mmol, 4 equiv) and 0.2 mL of a pre-activated solution of Oxyma Pure (14 mg, 0.1 mmol, 4 equiv), DIC (15.5 μ L, 0.1 mmol, 4 equiv) in DMF for at least 2 h. Deprotections were performed using two 5 min. reactions with 25% piperidine in NMP. The peptide was labeled using a pre-activated solution of 5(6)-carboxyfluorescein (19 mg, 50 μ mol, 2 equiv), Oxyma Pure (7 mg, 50 μ mol, 2 equiv) and DIC (7.7 μ L, 50 μ mol, 2 equiv) in 1 mL DMF. The peptide was cleaved in a solution of 97.5% TFA, 2.5% thioanisole and DTNP (3 mg, 10 μ mol, 0.4 equiv) for 1.5 h. The peptide was collected and purified using general procedures. Overall yield: 0.2%. Molecular weight = 1797.6 (expected = 1796.8).

Example 15: Synthesis of peptides 3a–c

Peptides 3a–c were synthesized on a 25 μ mol scale using rink amide MBHA resin. The resin was deprotected in 25% piperidine in NMP. Fmoc-Lys(Mtt)-OH and Fmoc-11-amino-3,6,9-trioxaundecanoic acid were coupled using a solution containing 0.5 M amino acid (0.2 mL, 0.1 mmol, 4 equiv), 0.5 MHCTU, (0.248 mL, 124 μ mol, 4.96 equiv) and DIPEA (43.5 μ L, 250 μ mol, 10 equiv) in NMP for at least 1.5 h. The peptide sequence preceding the final alanine, cysteine, or selenocysteine was then synthesized using general procedures. With the N-terminus Fmoc-protected, the Mtt-protecting group of lysine was removed by performing multiple washes with 1% TFA in DCM for a total of 30 min. The peptide was then labeled overnight using a solution of 5(6)-carboxyfluorescein (19 mg, 50 μ mol, 2 equiv), HCTU (19 mg, 45 μ mol, 1.8 equiv) and DIPEA (20 μ L, 115 μ mol, 4.6 equiv) in DMF. The 5(6)-carboxyfluorescein was then tritylated using two overnight reactions of 12.5 μ mol peptide with a solution of trityl chloride (21 mg, 75 μ mol, 6 equiv) and DIEA (13 μ L, 75 μ mol, 6 equiv) in 1.5 mL DCM. The N-terminus was then deprotected using 25% piperidine in NMP for 25 min. Fmoc-Sec(PMB)-OH was then coupled using 0.5 M amino acid (0.25 mL, 0.125 mmol, 5 equiv), 0.5 MHCTU (0.248 mL, 124 μ mol, 4.96 equiv) and DIPEA (43.5 μ L, 250 μ mol, 10 equiv) in NMP, while cysteine and

alanine were coupled using standard procedures. Three 5 min reactions or a 25 min reaction with 25% piperidine in NMP were used to deprotect the final residue of the selenylsulfide or uncyclized/disulfide peptides, respectively. Peptides were cleaved using general procedures. The PMB-protecting group of the selenylsulfide was removed using 97.5% TFA, 2.5% thioanisole, and DTNP (1.9 mg, 6.25 μ mol, 0.25 equiv) for 1.5 hr. The peptides were characterized, purified, and quantified using general procedures. Uncyclized 3a molecular weight = 1815.0 (expected = 1815.9). Disulfide 3b molecular weight = 1879.0 (expected = 1878.0). Selenylsulfide 3c overall yield: 5.1%; molecular weight = 1925.6 (expected = 1924.9).

Example 16: Synthesis of peptides 4a–c

Unlabeled peptides 4a–c were synthesized by coupling Fmoc-11-amino-3,6,9-trioxaundecanoic acid directly to the resin using a solution containing 0.5 M amino acid (0.2 mL, 0.1 mmol, 4 equiv), 0.5 M HCTU (0.248 mL, 124 μ mol, 4.96 equiv) and DIPEA (43.5 μ L, 250 μ mol, 10 equiv) in NMP for at least 1.5 h. The peptide sequence was completed using standard procedures and Fmoc-Sec(PMB)-OH was coupled and deprotected using the conditions above. Uncyclized and disulfide peptides were cleaved in 95% TFA, 2.5% water, and 2.5% triisopropylsilane for 4 h. The selenylsulfide peptide was cleaved in a solution of 97.5% TFA, 2.5% thioanisole and DTNP (1.94 mg, 6.25 μ mol, 0.25 equiv) for 2.5 h. Peptides were characterized and purified according to general procedures. The peptides were quantified in 6 M guanidine HCl using the molar extinction coefficient of tyrosine (1280 M⁻¹) and cysteine (120 M⁻¹). Uncyclized 4a overall yield: 11%; molecular weight = 1328.6 (expected = 1329.5). Disulfide 4b overall yield: 12%; molecular weight = 1392.6 (expected = 1391.6). Selenylsulfide 4c overall yield: 5.1%; molecular weight = 1439.2 (expected = 1438.5).

Example 17: Serum stability

Fresh mouse serum was collected from pooled blood obtained by terminal cardiac puncture from isoflurane-anesthetized C57BL/6J mice; blood collection and mouse husbandry procedures were approved by the University of Georgia IACUC committee. Peptides 3a–c were incubated at a concentration of 0.2 mM in a solution containing 50% mouse serum, 0.4% benzyl alcohol, and 15% dimethylsulfoxide (DMSO) in PBS, pH 7.2 at 37 °C. Aliquots were drawn in triplicate at 0, 1.5, 3, 6, and 12 h and proteins were precipitated with an equal volume of acetonitrile containing 0.1% TFA. The precipitate was pelleted by centrifugation at 14,000 rpm for 5 min, and the supernatant was collected. Degradation was monitored at 280 nm by LC–MS using a Zorbax Eclipse XDB-C18, 5 μ m column, with a gradient of 0–100% acetonitrile in water containing 0.1% TFA and a 1.0 mL/min flow rate at 45 °C. Absorbance was integrated between 9 and 10.75 min using the ChemStation software to determine absorbance of degradation

products. The parent peptide peak was also integrated. The percent composition of degradation products was calculated relative to the combined absorbance of degradation products and parent peptide. The percent composition at T0 was subtracted from all values to determine the increase in percent composition and is plotted over time, where error bars represent SD of triplicate experiments.

Example 18: Circular dichroism

Circular dichroism spectra were obtained for peptides 3a–c with a C-terminal label in 10 mM sodium phosphate buffer, pH 7.0 at concentrations of approximately 10–20 μ M, using a Jasco J-710 CD Spectrometer. Peptides were treated with 16, 80, or 400 μ M DTT for 10 min prior to reading. Blanks were obtained for each concentration of DTT and subtracted from the spectra. Spectra were obtained with a 0.1 cm path length using 100 mdeg sensitivity, 1 nm data pitch, continuous scanning mode with a speed of 50 nm/min, 4 sec response, 2 nm bandwidth, and 3 accumulations. Savitzky–Golay smoothing was applied with a convolution width of 17. Mean residue ellipticity was calculated for the peptides using the equation: $[\theta] = (\theta * 0.1 * \text{MRW}) / (P * C)$

where θ is the theta machine units in mdeg, MRW is the mean residue weight (molecular weight of the peptide/total number of residues), P is the path length in cm, and C is the concentration in mg/mL. The final concentration of peptide was confirmed following the experiment using the Biotek Synergy 2 microplate reader.

Example 19: MTT toxicity assay

Cells were seeded at 10,000 cells per well in a 96 well plate in complete media and allowed to grow for 48 h at 37 °C. The media was replaced with a 0, 1, 5 or 10 μ M solution of unlabeled peptide 4a–c in complete media and incubated for 6 h. Following treatment, the media was replaced with 110 μ L media containing 0.45 mg/mL 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) and incubated for 2 h at 37 °C. The solution was removed and 100 μ L DMSO was added and rocked on an orbital shaker for 15 min protected from light. The absorbance was measured at 570 nm. Percent viability was calculated relative to the vehicle control. The average of quadruplicates was plotted in GraphPad Prism, where error bars represent SEM. For each peptide, a two-way ANOVA was performed across all cell lines with Tukey's multiple comparison test.

Peptides were designed using residues 270–277 (UniProtKB P00533) of the native hEGFR dimerization arm sequence as a template. All peptide sequences were modified with a short polyethylene glycol linker (PEG) to increase solubility and a 5(6)-carboxyfluorescein label. Peptides were prepared using Fmoc-based solid phase peptide synthesis. Disulfide peptide

controls were designed by inserting cysteine at the C- and N-terminus of the peptide, while the uncyclized peptide controls were similarly designed with alanine residues at these positions. Since p-methoxybenzyl (PMB)-protected selenocysteine has a propensity to undergo β -elimination in the presence of piperidine and diisopropylethylamine (DIPEA) during deprotection and coupling, the selenylsulfide peptides with selenocysteine at the N-terminus were prepared so as to minimize exposure of the protected selenol to additional coupling and deprotection steps. Selenylsulfide peptides were cleaved in a solution of trifluoroacetic acid (TFA) and thioanisole, using 2,20-dithiobis(5-nitropyridine) (DTNP) to remove the PMB-protecting group from selenocysteine.

The addition of PEG and fluorescein after selenocysteine coupling is sufficient to cause elimination of the PMB-protected selenol. However, an initial screening of disulfide and selenylsulfide mixture demonstrated that both peptides were capable of inhibiting phosphorylation in a dose-dependent manner. Thus, replacement of the disulfide bond does not appear to interfere with the ability of the peptide to inhibit EGFR phosphorylation. Further, it appears that the selenylsulfide-bridged compound is stabilized in a favorable conformer for targeting the dimerization arm binding pocket.

To improve peptide yield, formation of dehydroalanine was prevented by minimizing the use of bases such as piperidine and diisopropylethylamine during and after selenocysteine coupling (Figure 12 A). Oxyma Pure and diisopropylcarbodiimide (DIC) were used as coupling reagents as a means to circumvent addition of piperidine during coupling of PMB-protected selenocysteine. After coupling the selenocysteine residue, two brief 5-minute deprotections were performed using 25% piperidine to remove the Fmoc group. (PEG)₃ and 5(6)-carboxyfluorescein were then coupled using similar conditions to minimize exposure to base. Although selenopeptide 2c was observed, the dehydroalanine product was also present to a large extent (approximately 1–2 times that of the selenylsulfide product). The elimination of the selenol also resulted in a molar increase for the ratio of DTNP to selenocysteine during the cleavage step. Thus, the excess DTNP resulted in Npys-protected selenol and thiol side chains as illustrated in Figure 12 A. The overall yield of the synthesis was <1%.

Since the two additional deprotections after incorporation of the selenocysteine contributed to elimination of the selenol group, the peptide were redesigned so that (PEG)₃ and 5(6)-carboxyfluorescein moieties were incorporated at the C-terminus of the peptides prior to selenocysteine coupling (Figure 12 B) so as to reduce exposure of PMB-protected selenocysteine to piperidine and DIPEA. This was accomplished by first coupling Fmoc-Lys(Mtt)-OH directly to the resin followed by a short (PEG)₃ linker and the peptide sequence preceding

selenocysteine. While the N-terminus of the peptide remained Fmoc-protected, the Mtt group of lysine was selectively removed using 1% TFA in dichloromethane (DCM) to expose the ϵ -amine, and 5(6)-carboxyfluorescein was coupled to the lysine side chain. To prevent reactivity of the hydroxyls present in the 5(6)-carboxyfluorescein, these hydroxyl groups were first protected with trityl chloride. The peptide was then deprotected with piperidine prior to addition of the selenocysteine. To minimize exposure of PMB-protected selenocysteine to piperidine, the final Fmoc group was removed with three brief 5-minute exposures to piperidine.

Since an excess of DTNP resulted in Npys-protected selenol and thiol side chains and since the DTNP may be recycled during the deprotection reaction, DTNP was reduced from 0.4 to 0.25 mol equiv to deprotect the selenocysteine during cleavage. Further, the selenol was found to readily oxidize to form the selenylsulfide bridge, therefore an additional oxidation reaction was unnecessary. Peptide 3c was synthesized in an improved overall yield that was greater than 20 times that of peptide 2c.

Next, proteolytic stability was measured. Peptides 3a–c were incubated with fresh mouse serum over a time course of 12 h (Figure 13). Degradation was quantified as the change in percent composition of the degradation products. After 12 h, uncyclized peptide 3a was degraded by 70%, while disulfide 3b and selenylsulfide 3c were less than 15% degraded. Additionally, chromatographic peaks corresponding to cleavage between (PEG)₃ and Ala278 at the C-terminus and between N-terminal residues Ala269, Tyr270 and Asn271 of the uncyclized peptide 3a were observed. In contrast, minimal peaks corresponding to the degradation of disulfide 3b and selenylsulfide 3c peptides were observed. Interestingly, the dehydroalanine product of selenylsulfide 3c was observed after incubation with the serum.

The significant reduction of degradation products at the end of the time course demonstrates that the selenylsulfide-bridge appears to prevent proteolytic cleavage due to incorporation of the structural constraint.

Peptide stability was also measured in the presence of the reducing reagent dithiothreitol (DTT). Circular dichroism spectra were obtained over a 0–400 μ M concentration range of DTT (Figure 14). The uncyclized peptide 3a maintained a minimum at 198 nm. However, an apparent shift in the minimum to 198 nm for selenylsulfide 3c was not observed until 32 equiv of DTT was added (400 μ M), demonstrating the stability of the secondary structure of the constrained peptide to this reducing agent over a broader concentration range.

Although selenium is an essential trace nutrient, higher concentrations are toxic. To measure whether the selenopeptide was toxic to cells, a variety of diverse cell lines were treated with unlabeled peptides 4a–c for 6 h at three concentrations ranging from 1 to 10 μ M. Viability

was measured for peptides 4a–c using an MTT assay (Figure 15). No toxicity was observed over the concentration range tested, demonstrating that the selenylsulfide-bridged peptide is not toxic over this concentration range.

5 A selenylsulfide-bridged peptide mimicking the EGFR dimerization arm using Fmoc chemistry was prepared. By labeling the peptide at the C-terminus prior to selenocysteine coupling, the elimination of the PMB-protected selenol during Fmoc-based solid phase peptide was overcome, thereby dramatically improving the yield of the synthesis. Although Boc-based synthesis is often used to prepare selenylsulfidecontaining peptides to circumvent the basic conditions of deprotection that cause elimination of PMB-protected selenium, Fmoc-based
10 synthesis provides a more facile approach for peptide synthesis by eliminating the need for harsh cleavage conditions and specialized equipment for handling hydrofluoric acid. Additionally, pentafluorophenyl esters have been used in selenopeptide synthesis to eliminate the use of base in coupling steps, yet these amino acids are more costly and do not eliminate the use of base during subsequent deprotection steps. The synthetic design strategy shown in this study provides
15 a useful alternative for efficient Fmoc-based synthesis of labeled selenylsulfide-bridged peptides. This peptide also demonstrated resistance to proteolytic degradation as well as structural stability in the presence of the reducing agent DTT. Thus, this redox stable bond may act as a useful tool for the generation of b-loop peptides to target PPIs.

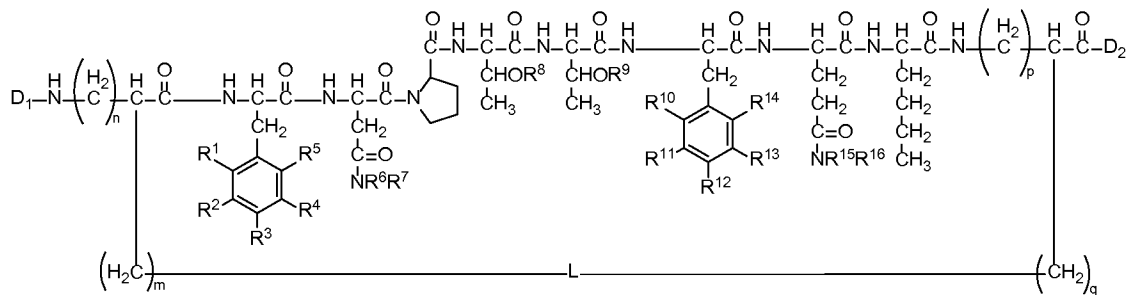
20 Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of skill in the art to which the disclosed invention belongs. Publications cited herein and the materials for which they are cited are specifically incorporated by reference.

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described
25 herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS

What is claimed is:

1. A compound of Formula IIa:



IIa

wherein R¹, R², R³, R⁴, R⁵, R⁶, R⁷, R⁸, R⁹, R¹⁰, R¹¹, R¹², R¹³, R¹⁴, R¹⁵, and R¹⁶ (*i.e.*, R¹-R¹⁶) are independently chosen from hydrogen, halogen, hydroxyl, substituted or unsubstituted amino, substituted or unsubstituted amido, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted alkoxy, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, or wherein, as valence and stability permit, any combination of R¹-R⁵ and R¹⁰-R¹⁴, together with the atoms to which they are attached, form a 3-10 membered substituted or unsubstituted cyclic moiety optionally including between 1 and 3 heteroatoms;

n and p are independently selected from 0, 1, 2;

m and q are independently selected from 0, 1, 2, 3, and 4;

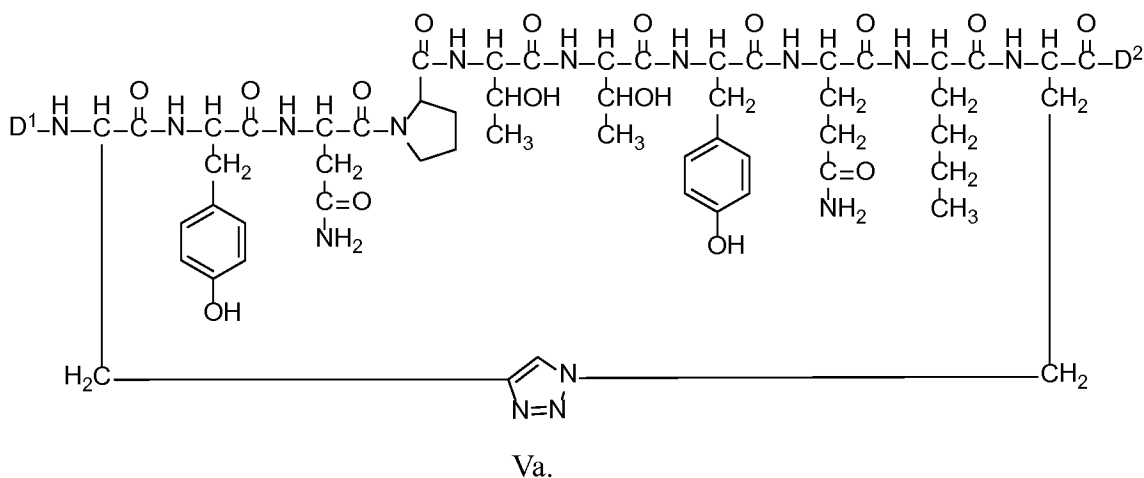
L is selected from substituted or unsubstituted amino, silyl, sulfide, disulfide, selenysulfide, diselenide, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted alkoxy, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted cycloalkenyl, substituted or unsubstituted heterocycloalkenyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl;

with the proviso that L has a number of atoms in its backbone and the sum of the number of atoms forming the backbone of L, m, and q is selected from 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10;

D¹ and D² are each independently selected from a hydrogen, hydroxyl, acid protecting group, amine protecting group, detectable moiety, therapeutic moiety, and combination thereof, with the proviso that at least one of D¹ and D² is a detectable moiety, therapeutic moiety, or combination thereof; or D¹ is hydrogen or an amine protecting group, D² is hydroxyl, amine, or acid protecting group, and one or more R¹-R¹⁶ is a detectable moiety, therapeutic moiety, or combination thereof.

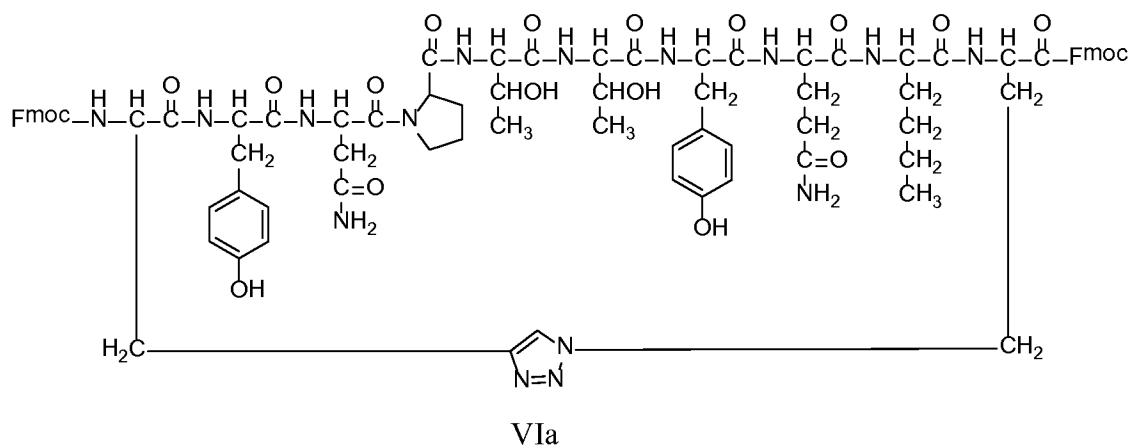
2. The compound of any of claim 1, wherein R¹-R¹⁶ are not all hydrogen.
3. The compound of any of claims 1-2, wherein R¹-R⁵ are not all hydrogen.
4. The compound of any of claims 1-3, wherein R¹⁰-R¹⁴ are not all hydrogen.
5. The compound of any of claims 1-4, wherein R¹, R², R⁴, and R⁵ are all hydrogen.
6. The compound of any of claims 1-5, wherein R³ comprises OR¹⁷ and wherein R¹⁷ is chosen from hydrogen, halogen, hydroxyl, substituted or unsubstituted amino, silyl, thiol, substituted or unsubstituted thioalkyl, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted alkoxy, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted cycloalkenyl, substituted or unsubstituted heterocycloalkenyl, substituted or unsubstituted aryl, or substituted or unsubstituted heteroaryl.
7. The compound of any of claims 1-6, wherein R³ comprises a hydroxyl group.
8. The compound of any of claims 1-7, wherein R⁶ is hydrogen.
9. The compound of any of claims 1-8, wherein R⁷ is hydrogen.
10. The compound of any of claims 1-9, wherein R⁸ is hydrogen.
11. The compound of any of claims 1-10, wherein R⁹ is hydrogen.
12. The compound of any of claims 1-11, wherein R¹⁰, R¹¹, R¹³ and R¹⁴ are all hydrogen.
13. The compound of any of claims 1-12, wherein R¹² comprises OR¹⁸, wherein R¹⁸ is chosen from hydrogen, halogen, hydroxyl, substituted or unsubstituted amino, silyl, thiol, substituted or unsubstituted thioalkyl, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted alkoxy, substituted or unsubstituted cycloalkyl,

31. The compound of any of claims 1-30, wherein L comprises a 3-10 membered substituted or unsubstituted cyclic moiety optionally including between 1 and 3 heteroatoms.
32. The compound of any of claims 1-31, wherein L comprises an unsubstituted heteroaryl.
33. The compound of any of claims 1-32, wherein L comprises a triazole.
34. The compound of any of claims 1-31, wherein L comprises a disulfide, selenyldisulfide, or diselenide.
35. The compound of any of claims 1-23 or 31-34, wherein the compound is of Formula Va:



36. The compound of any of claims 1-35, wherein D¹ comprises a detectable moiety.
37. The compound of any of claims 1-36, wherein D¹ comprises a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a chromophore, or any combination thereof.
38. The compound of any of claims 1-37, wherein D¹ comprises a fluorescent label.
39. The compound of any of claims 1-38, wherein D¹ comprises fluorenylmethyloxycarbonyl chloride (Fmoc) or 5(6)-carboxyfluorescein (FAM).
40. The compound of any of claims 1-39, wherein D² comprises a detectable moiety.
41. The compound of any of claims 1-40, wherein D² comprises a UV-Vis label, a near-infrared label, a luminescent group, a phosphorescent group, a chromophore, or any combination thereof.
42. The compound of any of claims 1-41, wherein D² comprises a fluorescent label.
43. The compound of any of claims 1-42, wherein D² comprises fluorenylmethyloxycarbonyl chloride (Fmoc) or 5(6)-carboxyfluorescein (FAM).

44. The compound of any of claims 1-43, wherein either or both of D1 and D2 comprise a spacer moiety.
45. The compound claims 44, wherein the spacer moiety is a polyalkyleneoxide.
46. The compound of any of claims 1-23 or 31-45, wherein the compound is of Formula VIa:



47. A pharmaceutical composition comprising a compound of any of claims 1-46.
48. A method for treating a disease or pathology in a subject in need thereof comprising administering to the subject an effective amount of a compound or composition of any of claims 1-46.
49. A method of treating or preventing cancer in a subject, comprising administering to the subject an effective amount of a compound or composition of any of claims 1-46.
50. The method of claim 49, wherein the cancer is selected from the group consisting of lung cancer, anal cancers, colon cancer, glioblastoma multiforme, epithelial cancers, or combinations thereof.
51. The method of any of claims 49-50, further comprising administering a second compound or composition, wherein the second compound or composition comprises an anticancer agent.
52. The method of any of claims 49-51, further comprising administering an effective amount of ionizing radiation to the subject.
53. A method of killing a tumor cell in a subject, comprising: contacting the tumor cell with an effective amount of a compound or composition of any of claims 1-47.

54. The method of claim 53, further comprising contacting the tumor cell with a second compound or composition, wherein the second compound or composition comprises an anticancer agent.
55. The method of any of claims 49-54, further comprising irradiating the tumor cell with an effective amount of ionizing radiation.
56. A method of treating or preventing an inflammatory disease in a subject, comprising administering to a subject an effective amount of a compound or composition of any of claims 1-47.
57. The method of claim 56, wherein the inflammatory disease is selected from the group of psoriasis, eczema, atherosclerosis, or combinations thereof.
58. The method of any of claims 56-57, further comprising administering a second compound or composition, wherein the second compound or composition comprises an anti-inflammatory agent.

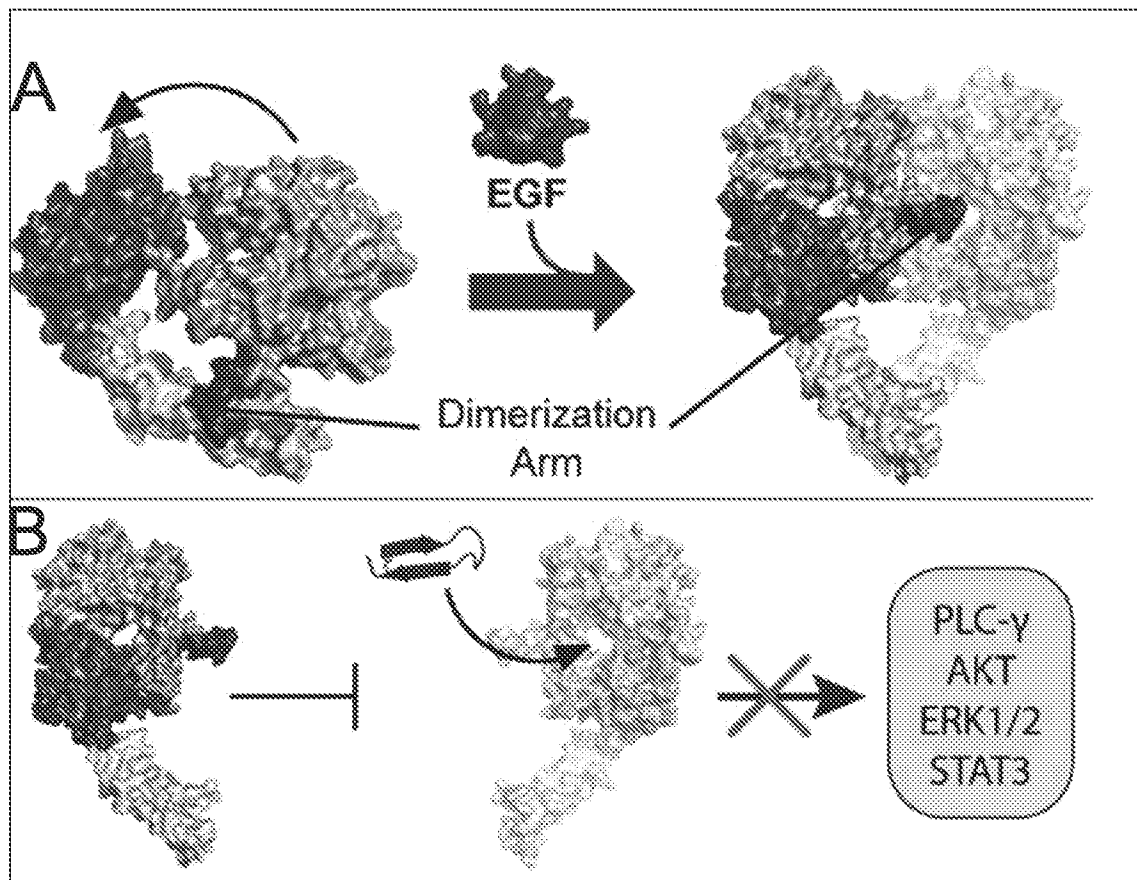
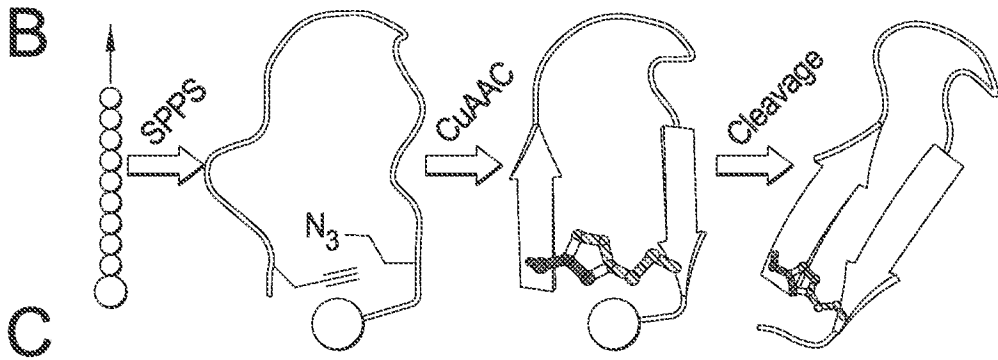
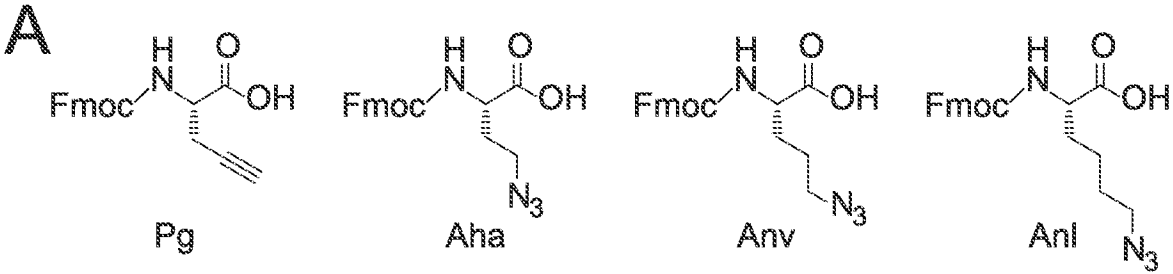


FIG. 1



C

	SEQ ID NO										
Native Sequence	245	L	Y	N	P	T	T	Y	Q	M	D 254 2
Non-mod. Control	A	Y	N	P	T	T	Y	Q	Nle	A	A 3
Disulfide Control	C	Y	N	P	T	T	Y	Q	Nle	C	A 4
EDA1	Aha	Y	N	P	T	T	Y	Q	Nle	Pg	A 5
EDA2	Pg	Y	N	P	T	T	Y	Q	Nle	Aha	A 6
EDA3	Anv	Y	N	P	T	T	Y	Q	Nle	Pg	A 7
EDA4	Pg	Y	N	P	T	T	Y	Q	Nle	Anv	A 8
EDA5	Anl	Y	N	P	T	T	Y	Q	Nle	Pg	A 9
EDA6	Pg	Y	N	P	T	T	Y	Q	Nle	Anl	A 10
EDA2-Scr	Pg	Q	Y	Nle	N	T	T	P	Y	Aha	A 11

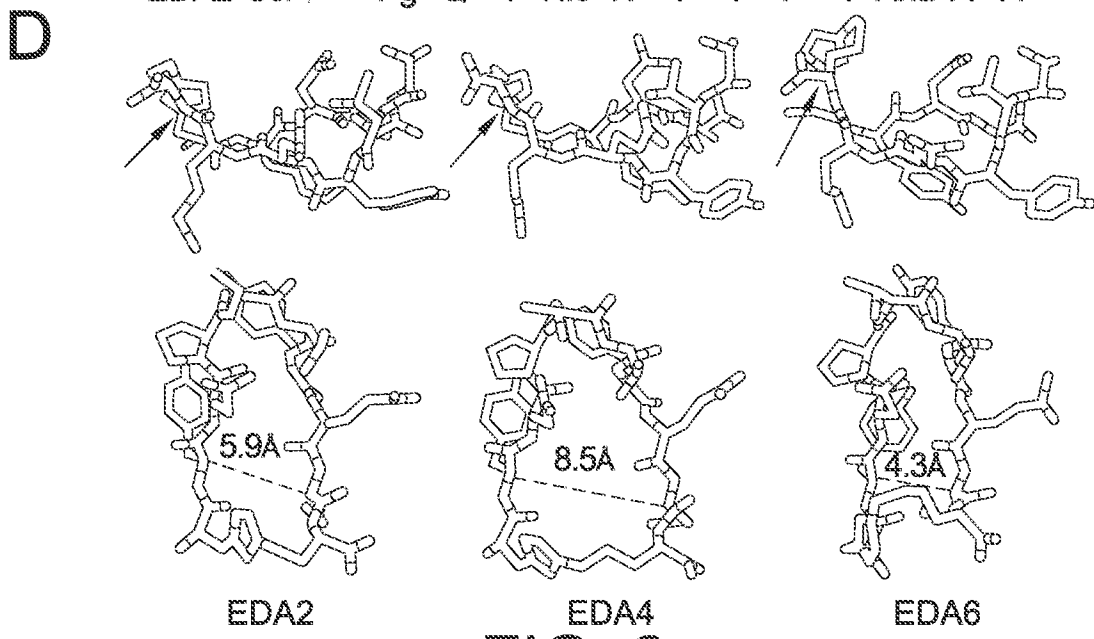


FIG. 2

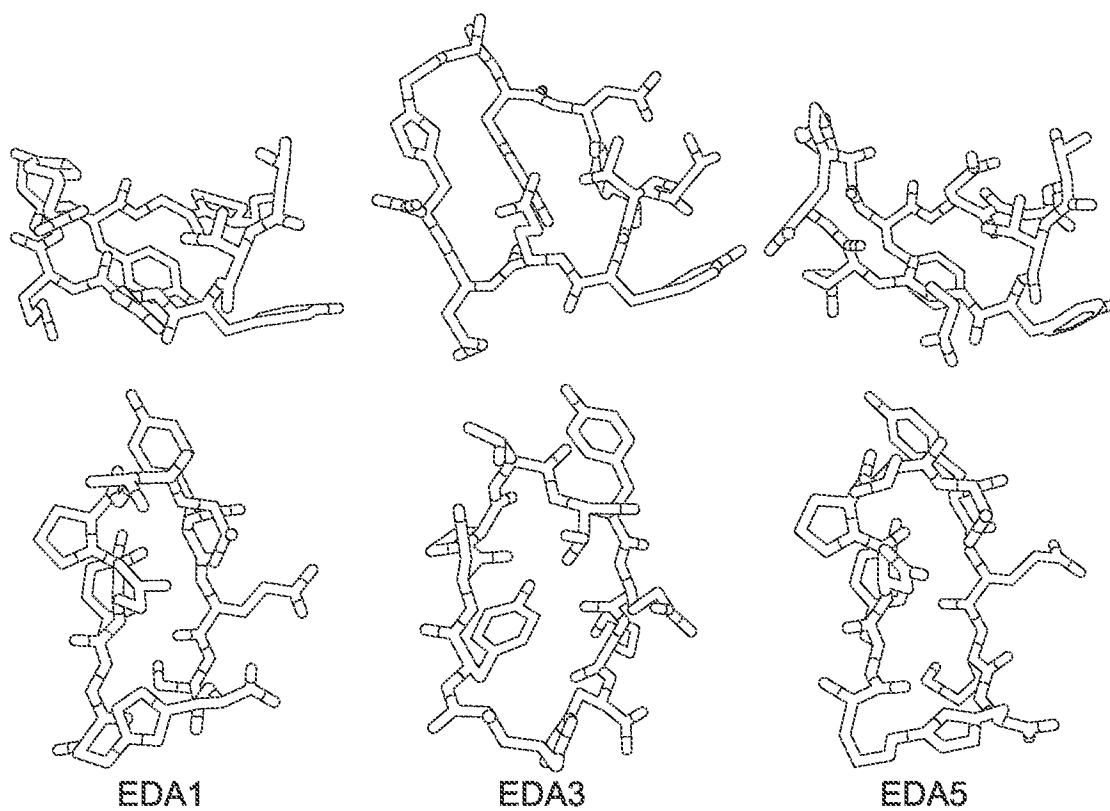


FIG. 3

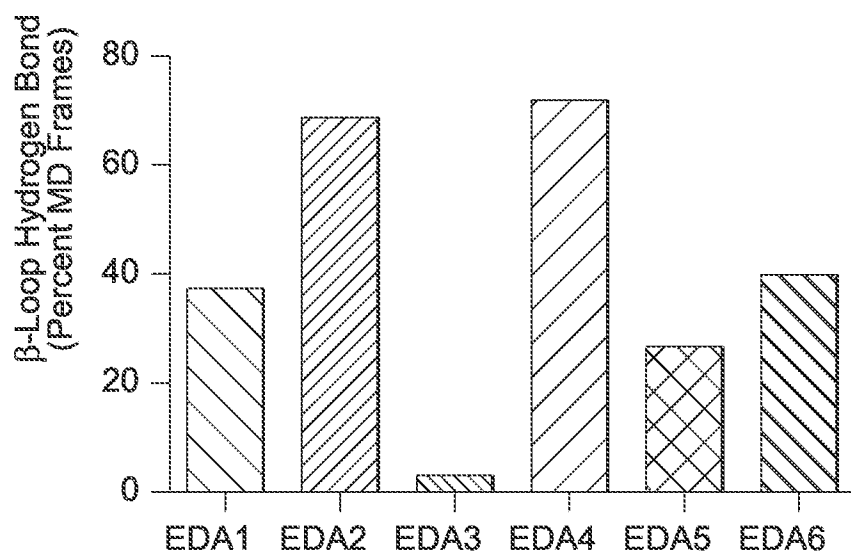


FIG. 4

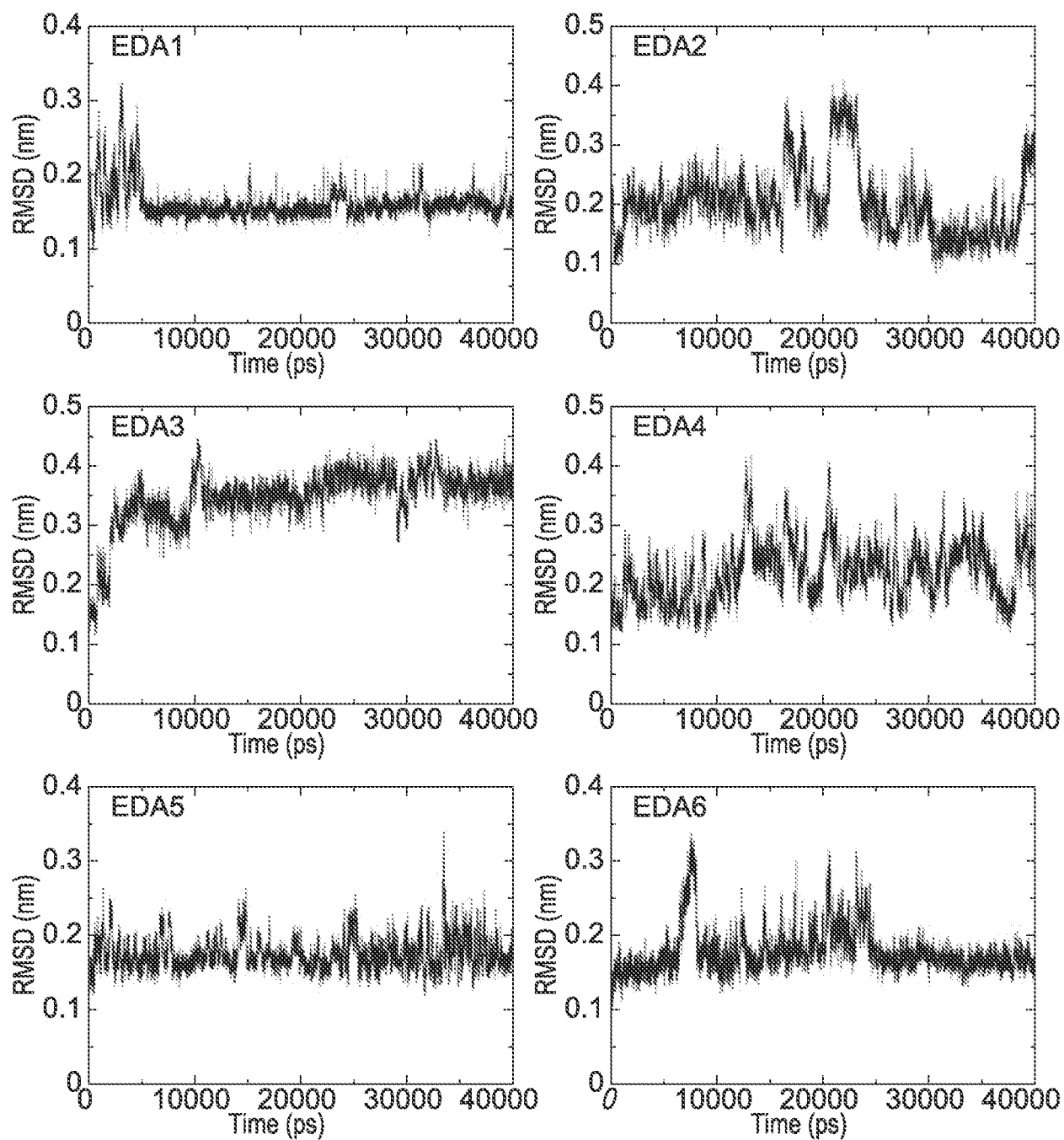


FIG. 5

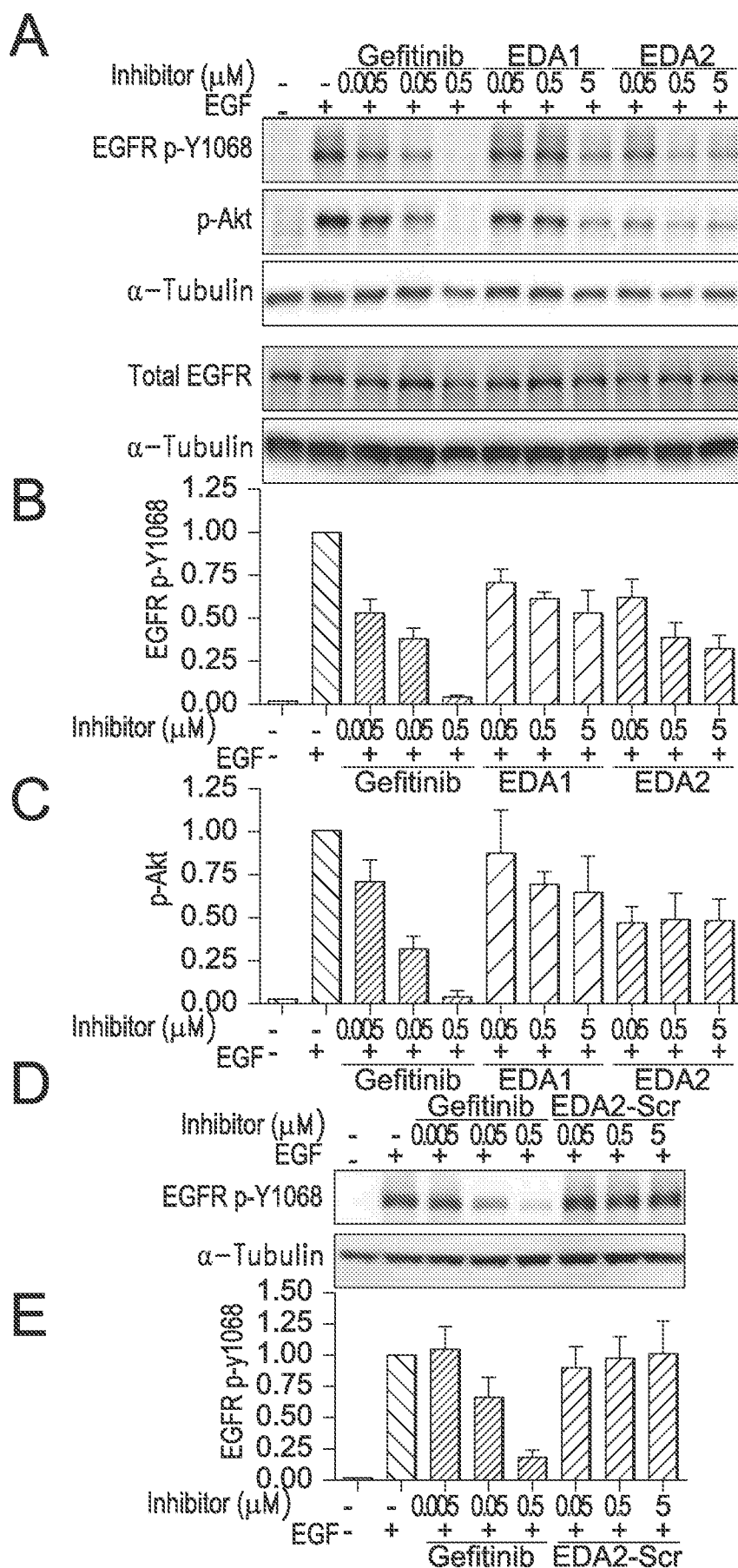


FIG. 6

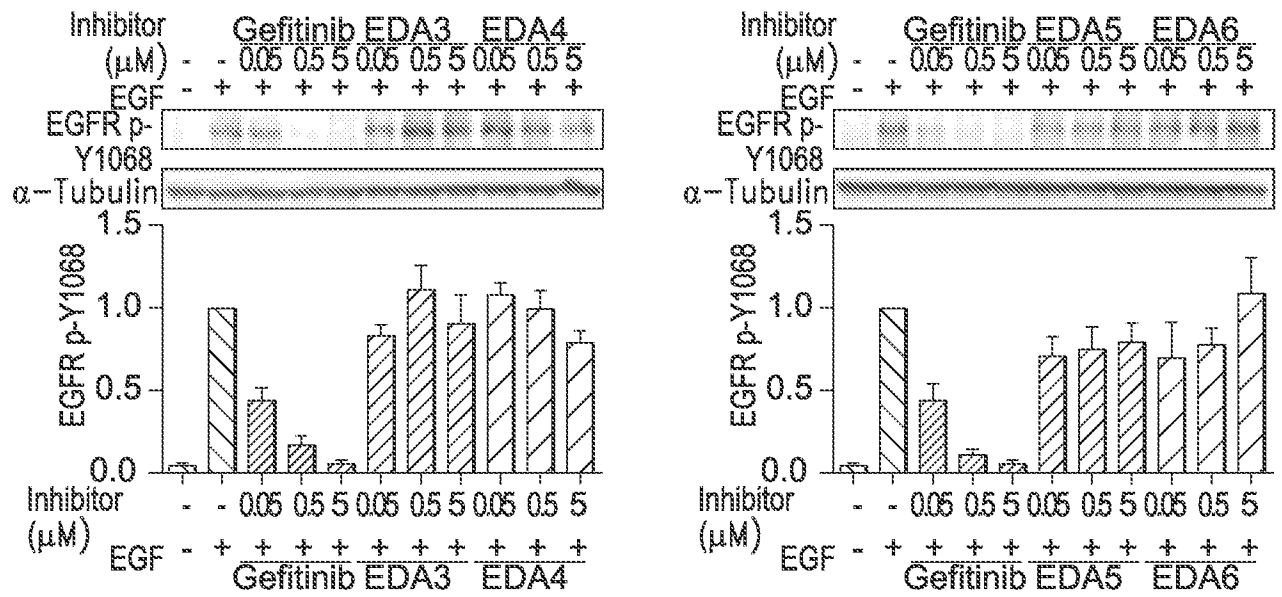


FIG. 7

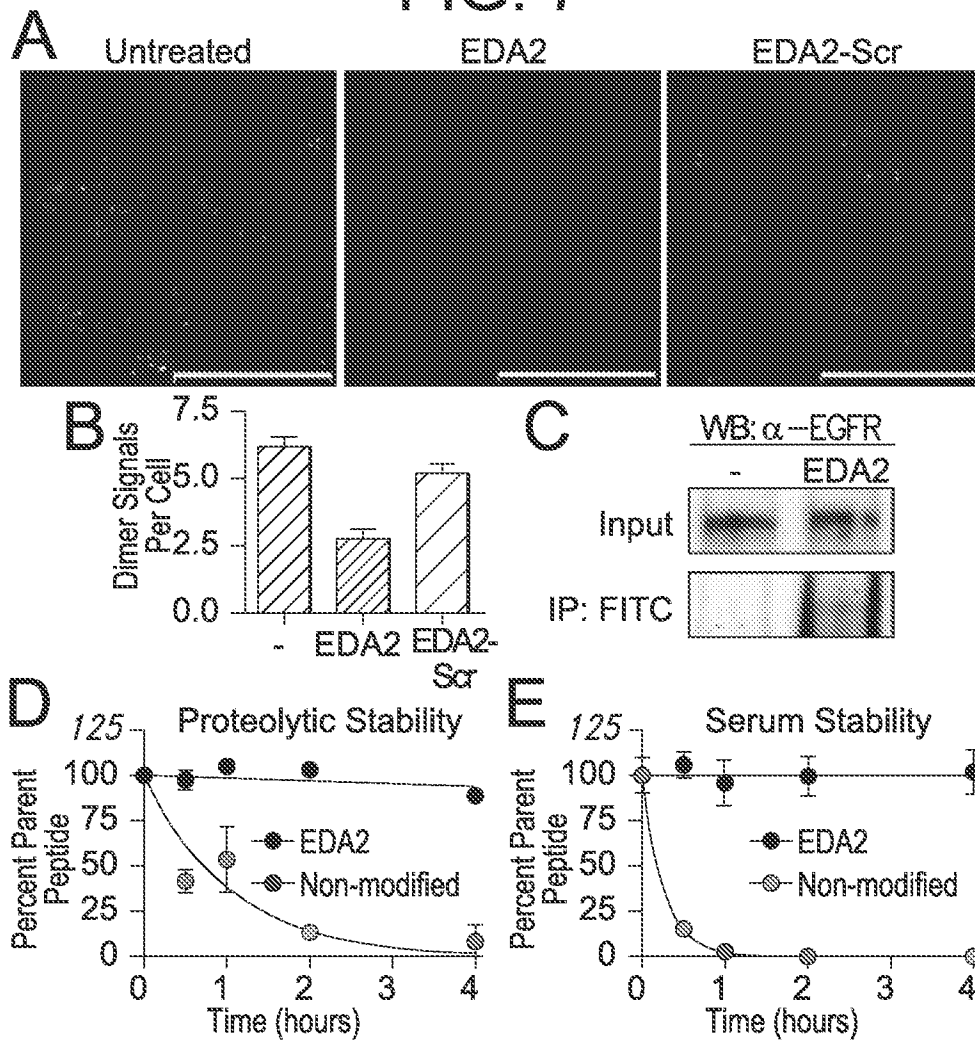


FIG. 8

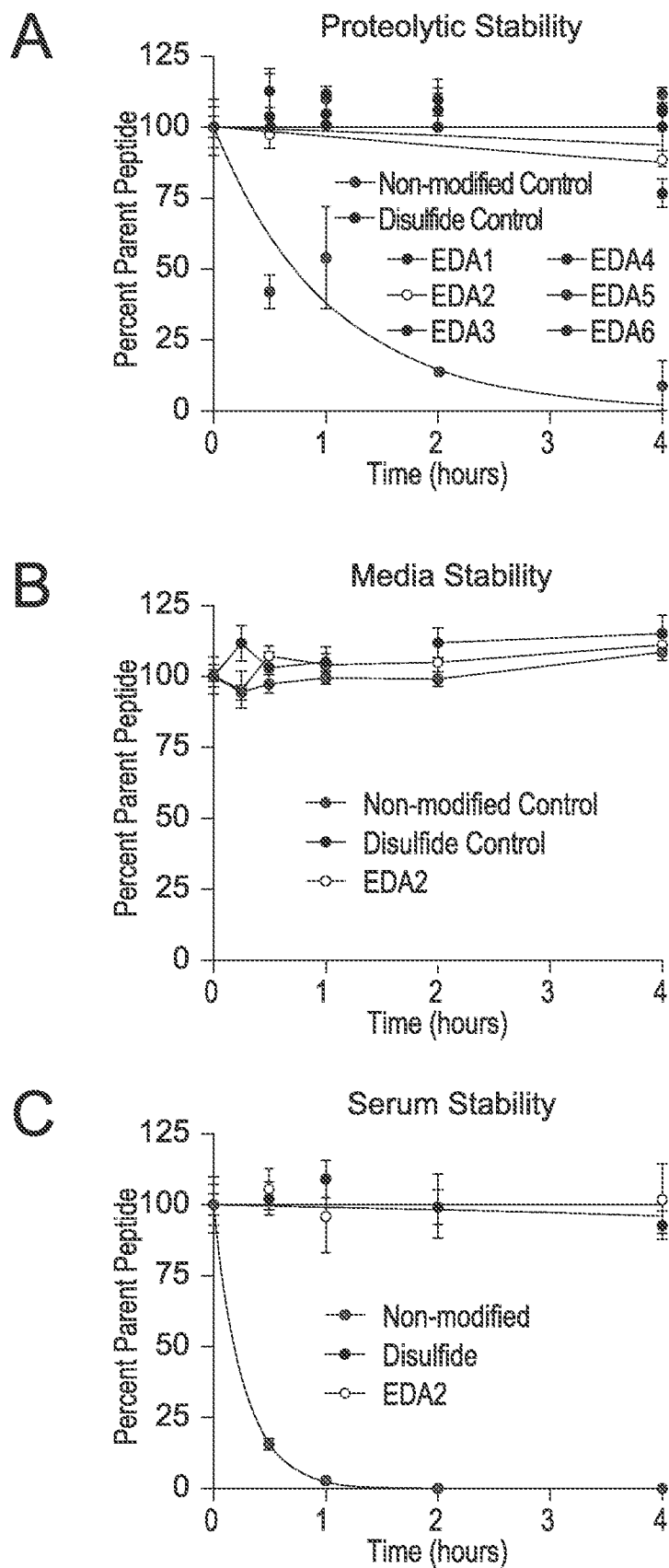


FIG. 9

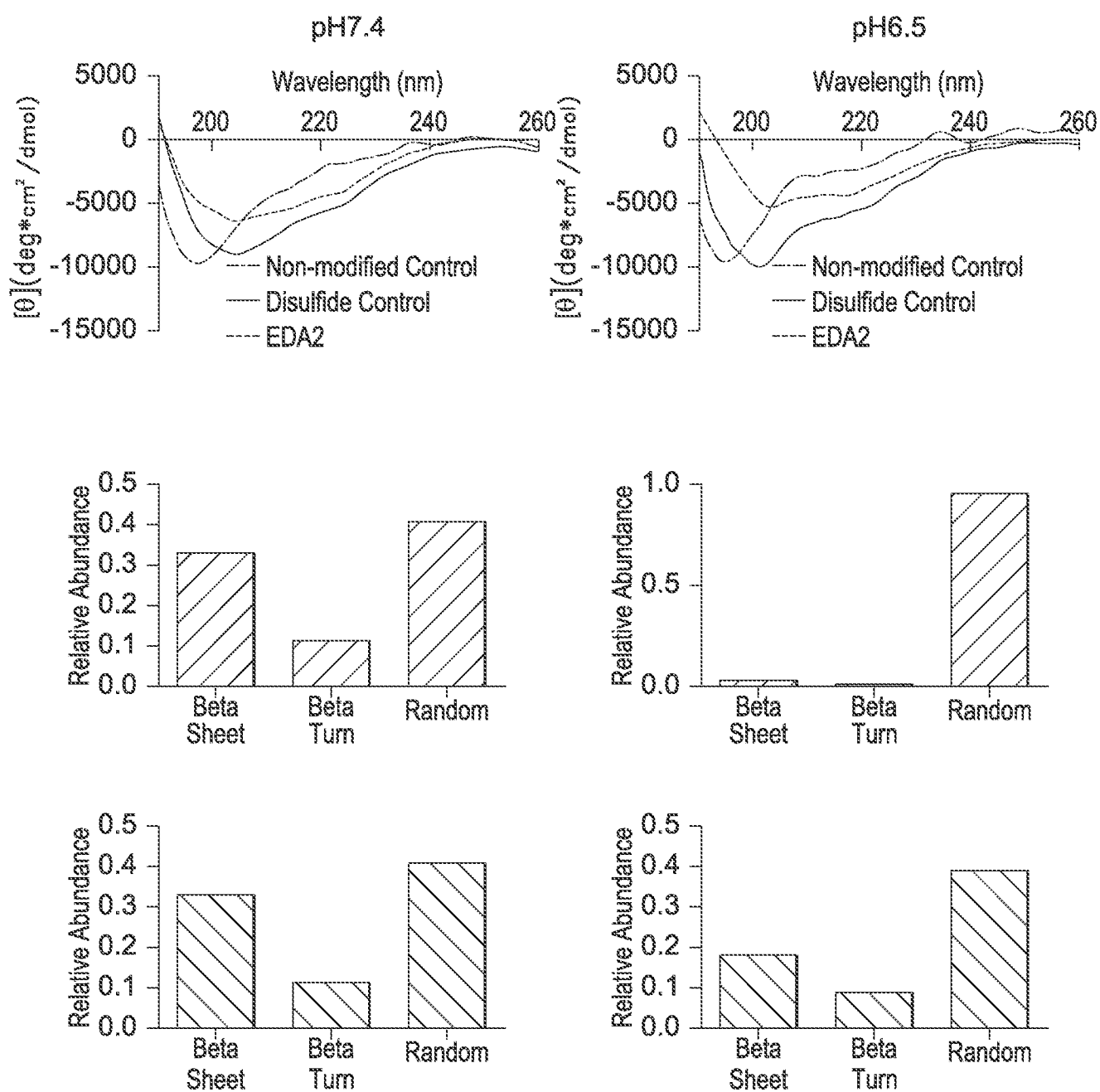


FIG. 10

Peptide	Sequence
2a	FAM-PEG- A Y N P T T Y Q Nle A
2b	FAM-PEG- C Y N P T T Y Q Nle C
2c	FAM-PEG- U Y N P T T Y Q Nle C
3a	A Y N P T T Y Q Nle A -PEG-K (FAM)
3b	C Y N P T T Y Q Nle C -PEG-K (FAM)
3c	U Y N P T T Y Q Nle C -PEG-K (FAM)
4a	A Y N P T T Y Q Nle A -PEG
4b	C Y N P T T Y Q Nle C -PEG
4c	U Y N P T T Y Q Nle C -PEG

FIG. 11

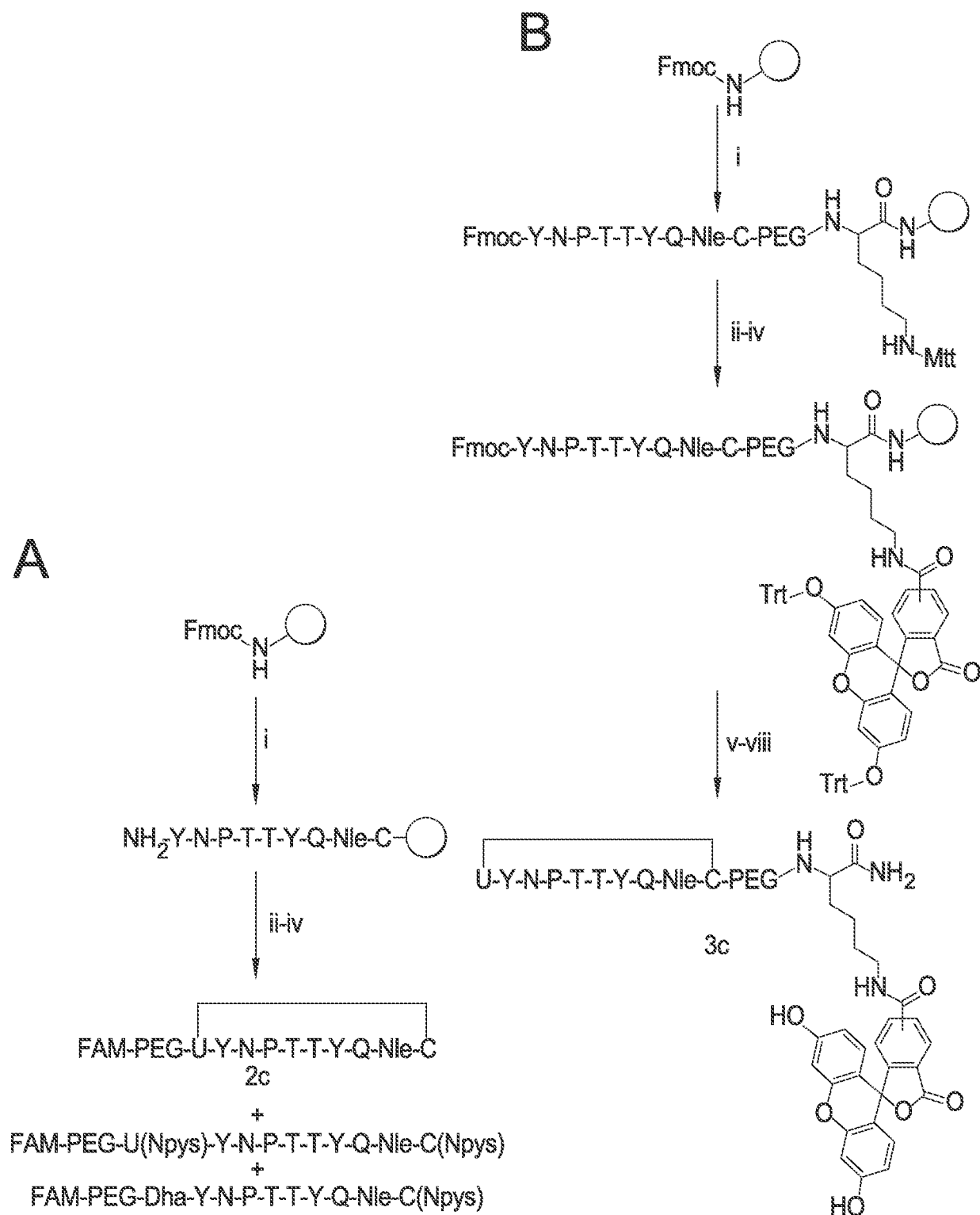


FIG. 12

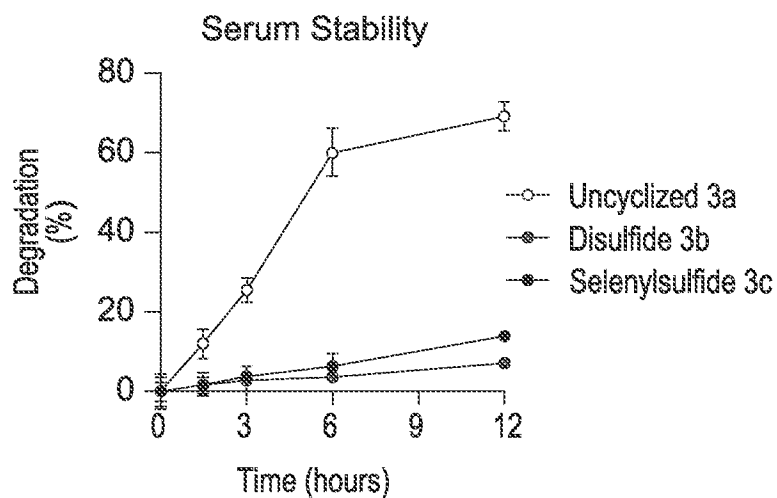


FIG. 13

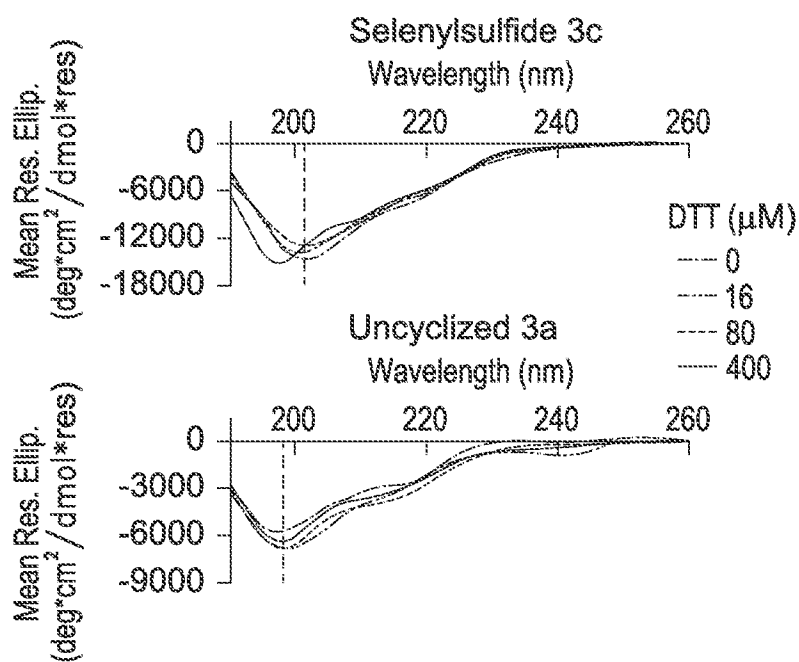
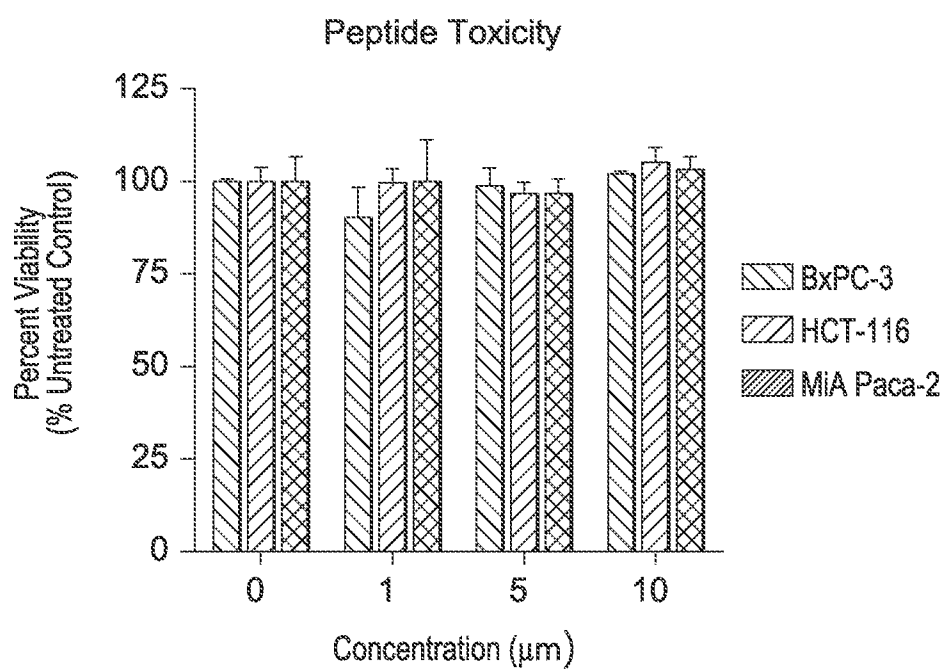


FIG. 14

**FIG. 15**

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 15/35621

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 38/04, A61K 38/00, C07K 5/00 (2015.01)

CPC - A61K 38/00

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 38/00, C07K 5/00 (2015.01)

CPC- A61K 38/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC- 530/328, 530/332, 530/323 (keyword search, terms below)

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

PubWEST (USPT, PGPB, EPAB, JPAB), Google Patents/Scholar

Search Terms Used: EGFR inhibitory peptide, cyclic peptide, Erb, peptidomimetic, mimic, norleucine, methionine, YNPPTYQM

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Mizuguchi et al. 'Inhibitory effect of a dimerization-arm-mimetic peptide on EGF receptor activation' Bioorganic and Medicinal Chemistry Letters 19 (2009) 3279-3282; pg 3279, col 2, para 3	1-3
Y	Roller et al. 'NORLEUCINE AS A REPLACEMENT FOR METHIONINE IN PHOSPHATASE-RESISTANT LINEAR AND CYCLIC PEPTIDES WHICH BIND TO P85 SH2 DOMAINS' Bioorganic and Medicinal Chemistry Letters, Vol. 4, No. 15, PP. 1879-1882, 1994; abstract	1-3
Y	US 2009/0137784 A1 (Chao et al.) 28 May 2009 (28.05.2009) para [0031], [0035], [0112], Table 1	1-3

☐ Further documents are listed in the continuation of Box C.

* 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

07 September 2015 (07.09.2015)

Date of mailing of the international search report

30 SEP 2015

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 15/35621

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.: 4-58
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:

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.:

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.