

## PEPTIDE NANOSPONGES FOR DRUG DELIVERY

### CROSS-REFERENCE TO RELATED APPLICATIONS

**[0001]** The present application claims the priority benefit of U.S. Provisional Patent Application Serial No. 63/542,052, filed October 2, 2023, entitled PEPTIDE NANOSPONGES FOR DRUG DELIVERY, incorporated by reference in its entirety herein.

### FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

**[0002]** This invention was made with government support under 75N91023C00008 awarded by the National Institutes of Health. The government has certain rights in the invention.

### SEQUENCE LISTING

**[0003]** The following application contains a sequence listing submitted electronically as a Standard ST.26 compliant XML file entitled "SequenceListing\_61012.xml," created on October 2, 2024, as 27,342 bytes in size, the contents of which are incorporated herein.

### TECHNICAL FIELD

**[0004]** The present disclosure describes peptide nanosponges, wherein the peptide nanosponges comprise a plurality of peptide-based building blocks that each comprise a core; a peptide block; a linking amino acid or peptide covalently linking the peptide block to the core; a capping moiety covalently attached to an N-terminal amino acid of the peptide block; a therapeutically active compound covalently attached to one or more amino acids of the peptide block; and a signaling sequence.

### BACKGROUND

**[0005]** The following description of the background of the present technology is provided simply as an aid in understanding the present technology and is not admitted to describe or constitute prior art to the present technology.

**[0006]** Many therapeutic agents for cancer treatment exhibit significant side effects including damage to healthy tissue because the therapeutic agent is administered systemically and has systemic effects. These systemic effects can cause such pain and discomfort that in some cases the

dose of the therapeutic agent administered must be reduced or the administration of the therapeutic agent stopped all together. Accordingly, a significant need exists for more targeted administration of therapeutic agents specifically to the affected tissues.

[0007] Colon cancer is the second-leading cause of cancer-related deaths in the US. Recent advances in early diagnosis and treatment are not curative in patients with advanced disease. The standard of care includes three different combination regimens of chemotherapy, each one associated with significant toxicity. At least 50% of colon cancer patients on these treatments develop metastases. Other combination therapies include immunotherapies, but these suffer from the development of resistance and increased adverse effects. Therefore, there continues to be a high unmet medical need to improve colon cancer treatment.

[0008] Idiopathic pulmonary fibrosis (IPF) is a rare disease with the adjusted incidence estimates (per 10,000 of the population) ranging from 0.35 to 1.30 in Asia-Pacific countries, 0.09 to 0.49 in Europe, and 0.75 to 0.93 in North America. IPF is a devastating disease with a 5-year survival of 20-40% (chronic and irreversible). There are no curative treatments for clinical IPF. Immunosuppression therapy for IPF was examined in a Phase III trial (PANTHER) using combinatorial therapy (prednisone, azathioprine, and N-acetylcysteine) and demonstrated no clinical survival benefit and was surprisingly associated with increased treatment-related serious adverse events. Additional therapies such as Interferon gamma-1 $\beta$  therapy and sildenafil have similarly failed to demonstrate improvement in lung function, overall survival benefit, or improvement in functional outcome studies when examined in randomized, placebo-controlled trials. While the pathobiology of IPF remains poorly understood, the current understanding is that repeated subclinical injury (i.e., smoking, trauma) directly impacts alveolar epithelium leading to cell death, persistent cell turnover, inflammation, and fibroblast recruitment in genetically susceptible individuals. The identification of a peri-vascular niche indicates that expression of CXCR7 on pulmonary capillary epithelial cells can lead to epithelial cell regeneration, reduced inflammation, and suppression of tissue fibrosis. Furthermore, this process leads to an imbalance between the epithelial-to-mesenchymal ratio of cells leading to tissue fibrosis through increased extracellular matrix deposition. This biology has led to development of new agents that target epithelial fibroblast microenvironmental interactions with a new class of drugs (pirfenidone, nintedanib). While these treatments represent a step forward, issues with patient drug tolerability,

lack of significant improvement in lung function, and no improvement in quality of life limit their utility. Therefore, the need to develop novel agents that improve the therapeutic index is necessary.

**[0009]** Cancer cells with deletions or mutations in the tumor suppressor p53 (p53 deficiency) are related to osteosarcoma (OS). OS has a US incidence around 1,000. OS is commonly found in children and adolescents with 60% of cases occurring in patients who are between 10 and 20 years of age, accounting for 2% of childhood cancers, and cases are increasing, presumably due to increases in the pediatric population. Survival of OS remains low (5-year survival of 70% for non-metastasized disease, 30% for metastasized), primarily as a result of its highly metastatic and drug-resistant nature and demonstrates the high unmet need for these patients. OS is characterized by malignant mesenchymal cells producing osteoid or immature bone which arises from the medullary cavity and grows toward the cortex and adjacent soft tissue. This bone formation results in swelling and intense pain which is severe enough to wake sleeping patients. Unfortunately, for over 30 years, there has been little progress developing treatments to address the unmet need for these patients.

#### SUMMARY

**[0010]** The current disclosure is directed to a peptide nanosponge comprising: a core; a peptide block; a linking amino acid or peptide covalently linking the peptide block to the core; a capping moiety covalently attached to a terminal amino acid of the peptide block; a therapeutically active compound covalently attached to one or more amino acids of the peptide block; and optionally, a signaling sequence.

**[0011]** In one or more embodiments, the disclosure concerns peptide nanosponges comprising a plurality of self-assembled peptide building blocks, wherein said plurality of self-assembled peptide building blocks comprises a first peptide building block comprising: a branched polymeric core comprising at least three arms; a first block co-peptide of 50 amino acids or less covalently linked via its C-terminus to one of said arms, wherein said first block co-peptide comprises a first peptide block and a second peptide block which is different from the first peptide block; a lipid-based capping moiety having a molecular weight of 400 Da or less covalently attached to an N-terminal amino acid of the first block co-peptide; and a therapeutically active compound covalently attached via a cleavable linkage to one or more amino acids of the first block co-peptide.

Advantageously, the block co-peptide is covalently attached to the core such that it is resistant to enzymatic cleavage from the core.

[0012] The disclosure is also directed to a peptide building blocks for nanosponges comprising: a core; a peptide block; a linking amino acid or peptide covalently linking the peptide block to the core; a capping moiety covalently attached to a terminal amino acid of the peptide block; a therapeutic peptide; and a “don’t eat me” sequence.

[0013] Additionally, the disclosure is directed to a peptide building blocks for nanosponges comprising: a core; a peptide block; a linking amino acid or peptide covalently linking the peptide block to the core; a capping moiety covalently attached to a terminal amino acid of the peptide block.

[0014] The disclosure also is directed to methods of treating a disease or disorder in a patient in need thereof comprising administering the peptide nanosponge described herein in a therapeutic dose to the patient, as well as pharmaceutical compositions, medicaments and uses of the peptide nanosponges to treat diseases or disorders.

[0015] Other objects and features will be in part apparent and in part pointed out hereinafter.

#### BRIEF DESCRIPTION OF THE DRAWINGS

[0016] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0017] FIG. 1A is a cartoon illustration of one exemplary embodiment of the general components of a peptide-based building block for use in forming supramolecular aggregates (nanosponges).

[0018] FIG. 1B is a cartoon illustration of a further exemplary embodiment of the general components of a peptide-based building block for use in forming supramolecular aggregates (nanosponges).

[0019] FIG. 1C is a cartoon illustration of a further exemplary embodiment of the general components of a peptide-based building block for use in forming supramolecular aggregates (nanosponges).

[0020] FIG. 2 is a schematic illustration of a peptide-based building block that could be used for delivering a DCKL1 inhibitor compound.

[0021] FIG. 3 is a schematic illustration of a peptide-based building block that could be used for delivering a MK2 inhibitor compound.

[0022] FIG. 4 is a schematic illustration of a peptide-based building block that could be used for delivering a cytotoxic agent against p53 deficient cells.

[0023] FIG. 5 shows TEM images of nanosponges assembled. A) TEM image for assembled S10K20CG-based (SEQ ID NO: 18) nanosponge labeled with cholesterol; size average around 96 nm; and B) TEM image for assembled D10K20CG-based (SEQ ID NO:20) nanosponge labeled with cholesterol; size average around 77nm.

[0024] FIG. 6 shows (Top) Representative colony formation images of HUCCT1 cells treated with Gemcitabine (Gem), IA-DC-103, DCLK3a, IA-DC-125 for 48h with IC50 and ½ IC50 as previously determined by hexosaminidase assay. (Bottom) Quantification of colony number and size. Data are represented as mean number and size from 3 independent experiments, \* p<0.05.

[0025] FIG. 7 shows (Top) Representative colony formation images of HUH28 cells treated with Gemcitabine (Gem), IA-DC-103, DCLK3a, IA-DC-125 for 48h with IC50 and ½ IC50 as previously determined by hexosaminidase assay. (Bottom) Quantification of colony number and size. Data are represented as mean number and size from 3 independent experiments, \* p<0.05.

[0026] FIG. 8 shows (Left) Representative spheroid formation images of HUCCT1 cells treated with Gemcitabine (Gem), IA-DC-103, DCLK3a, IA-DC-125 at IC50, ½ IC50 and ¼ IC50 doses, as previously determined by hexosaminidase assay. (Right) Quantification of spheroid number. Data are represented as the mean from 3 independent experiments, \* p<0.05

[0027] FIG. 9 is an immunoblot showing that TGFβ1 can stimulate collagen 1 protein expression in BJ(hTERT).

[0028] FIG. 10 is an immunoblot showing that a nanosponge carrier without any conjugated small molecule drug can induce p38 MAPK pathway activation and is cytotoxic at 50 μM and that the nanosponge backbone can induce collagen production.

[0029] FIG. 11 is an immunoblot showing the PF-3655022 (naked drug, without attachment to a nanosponge) and PF-3655022-nanosponge dose response curve.

[0030] FIG. 12 is an immunoblot showing HSP27 phosphorylation in BJ(hTERT) and CAF 067 following MK2 inhibitor treatment.

[0031] FIG. 13 is an immunoblot showing MK-Inh-1 small molecule drug conjugate or by itself does not inhibit MK2 phosphorylation in a dose response fashion, *in vitro*.

[0032] FIG. 14 is a TEM image of a D10K20 (SEQ ID NO:21) nanosponge where water-filled nanovesicles are discernible as darker spots within the nanosponge.

[0033] FIG. 15 shows a graph of percent calculated of PCZ loaded in nanosponge (marked with star).

[0034] FIG. 16 is a graph showing the concentration versus percent proliferation of PDAC cells for prochlorperazine (PCZ) and prochlorperazine-nanosponge agents.

[0035] FIG. 17 shows images that are representative of a cell uptake assay showing PCZ-nanosponge uptake in MiaPaCa-2 and S2-007 cells where the white arrows show uptake.

[0036] FIG. 18 show images of PCZ-nanosponge uptake in KPCC-orthotopic tumors in C57BL/6 mice (*Ex-Vivo* IVIS imaging).

[0037] FIG. 19 shows graphs and blot images for the activity of peptides tested in HEK293T (wildtype) and HEK293T-PKD1-KO-c4 (CRISPR-generated) cell lines.

[0038] FIG. 20 shows images of organs in mice treated after plectin-nanosponge uptake in PDAC-bearing mice after overnight incubation.

[0039] FIG. 21 shows images and heat map from *ex vivo* IVIS imaging (fluorescent) imaging confirming specific uptake of plectin-nanosponge in PDAC tumor only.

[0040] Corresponding reference characters indicate corresponding parts throughout the drawings.

#### DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

[0041] One aspect of this disclosure is focused on developing a widely applicable peptide nanosponge drug delivery system. The peptide nanosponges are characterized as water-soluble sponge-like supramolecular assemblies or aggregates comprising a plurality of individual peptide-based building blocks (e.g., 1,000-50,000 building blocks) that can dynamically self-assemble, disassemble, and reassemble into small clusters to facilitate site-selective delivery and uptake of their therapeutic payload into cells. The nanosponges can be used to deliver a variety of active agents covalently bound via cleavable linkages to the peptide-based building blocks and/or noncovalently sequestered within the interstitial spaces of the assemblies. The assemblies are rationally designed, but unlike previous peptide nanosponge versions do not contain cleavable linkages throughout the assembly network, such as consensus sequences that can be readily cleaved by various enzymes within the body. The assemblies are designed to associate with or be

preferentially taken up the targeted cell type into the early endosome, and further are designed to escape the endosome and be released into the cytoplasm where enzymes, such as esterases or proteases, cleave the linkage via which the active agent is tethered thereby quickly releasing the active agent in a targeted vicinity. The remainder of the nanosponge matrix will be more slowly broken down (biodegraded) via normal physiological processes, such as phagocytosis by macrophages or enzymatic degradation, etc. that naturally clear foreign materials and cellular debris *in vivo*.

**[0042]** The peptide nanosponge is generally nontoxic to tissues and can be developed to target particular cells or tissues that are the object of the particular treatment. In some cases, a therapeutic small molecule or peptide can be covalently bound to the peptide nanosponge via cleavable linkages, and then the bond broken once the peptide nanosponge reaches the target cells or tissue. In this way, an effective treatment for various conditions can be delivered with fewer side effects that can result from systemic administration of the same therapeutic compound.

**[0043]** Another aspect of this disclosure is focused on developing novel “nanosponge” linked small molecule DCLK1 inhibitors for treating cholangiocarcinoma (CCA), a rare, heterogenous, and aggressive malignancy of the bile ducts that presents at a late stage with poor outcomes. The incidence of CCA increases with age with 70-72 years as the median age of onset. A 5-Year survival of CCA is 13% (median survival 7 months), primarily due to non-resectable tumors and advanced disease at the time of initial diagnosis. Incidence of CCA is increasing and has been estimated at more than 10,865 US incidence in 2022. Cytotoxic chemotherapy is currently the standard of care for CCA patients treated perioperatively. First line standard of care is based on gemcitabine plus cisplatin, and recently approved targeted agents have done little to improve the high unmet need for transformative treatments for CCA patients where surgery is considered the only effective treatment but is often not possible.

**[0044]** Doublecortin Calmodulin-like Kinase 1 (DCLK1) is a serine-threonine kinase and is a novel therapeutic target in oncology. Our team has shown that both knockdown of DCLK1 expression and small molecule DCLK1 inhibitors suppress xenograft tumor growth in mice. In order to maximize the efficacy and safety of DCLK1 inhibitors, the inhibitors will be delivered to the site of the tumors in the biliary tract using a rationally designed peptide nanosponge technology. Taken together these two unique technologies offer the possibility of administering breakthrough treatment directly to the liver and changing the treatment paradigm for CCA patients.

**[0045]** In general, with reference to FIG. 1A, the peptide nanosponge comprises a plurality of peptide-based building blocks 10. The specific components used in each building block are variable, however, each building block 10 will generally comprise a multi-armed core 12; a linear peptide sequence 14 having its C-terminal end 14a covalently attached to each arm of the core 12, directly or via a cysteine residue as a spacer, and its N-terminal end 14b typically capped with a capping moiety 16 (but could be some other kind of terminal moiety, as discussed below). The peptide sequence 14 is a block co-peptide comprising two or more distinct peptide blocks covalently attached to the core 12 via a linking amino acid (C) and spacer residue (G) covalently linking the peptide block 14 at its C-terminus 14a to the core 12. The linear peptide sequence 14 is preferably free of any enzymatic consensus sequences and also is preferably not attached to the core 12 via any cleavable consensus sequences. That is, the peptide building block itself is preferably free any cleavable linkages or enzymatic consensus sequences, and is therefore resistant to enzymatic degradation.

**[0046]** In more detail, as shown in FIG. 1B, the block co-peptide sequence 14 includes a capping moiety 16 covalently attached to a terminal amino acid of the peptide block 14. For nanosponge assembly, it is preferred that 50% or more of the building block arms in the assembly composition should comprise lipid-based capping moieties 16. In other words, of the total arms of all peptide-based building blocks present in a nanosponge, at least half of the arms include lipid-based capping moieties 16 to sufficient drive building block assembly into the supramolecular aggregates the solution. As also shown in FIG. 1B, the block co-peptide sequence 14 includes a therapeutically active compound or other active agent 18 covalently attached to one or more amino acids of the peptide block 14 (and preferably the second N-terminal block of the block co-peptide sequence) via a cleavable linkage. That is, the cleavable linkages in these novel peptide-based building blocks are used only for attaching the active agent 18 to the peptide block 14, and preferably present nowhere else in the nanosponge or building block. Cleavable linkages for attaching the active agent 18 are preferably esterase cleavable bonds and/or short (8 residues or less) enzymatic consensus sequences, preferably esterase consensus sequences.

**[0047]** Each peptide block 14 can have two or more therapeutically active compounds 18 attached thereto, as described in more detail below. In some embodiments, as shown in Fig. 1C, the building block can include a signaling sequence or targeting moiety 20 covalently attached to the core 12 via a respective linkage 22 on one or more of the arms (depending on the number of arms).

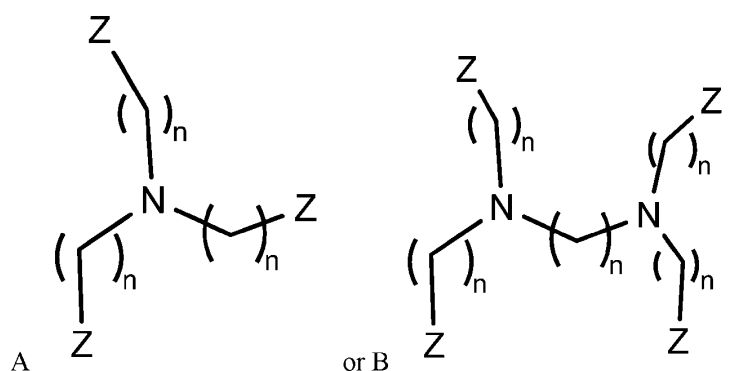
Preferably, the linkage 22 that connects the signaling sequence or targeting moiety 20 is different from the peptide sequence used for the block co-peptide 14 used to attach the lipid capping moiety 16 and active agent 18 to the core.

**[0048]** In some embodiments, the signaling sequence 20 is a “don’t eat me” sequence, an “eat me” sequence, or a targeting moiety. The signaling sequence 20 may also be itself a detectable moiety (e.g., fluorescent label) or it may further include a detectable label conjugated to a secondary signaling sequence. In some embodiments, the signaling sequence 20 may itself be a therapeutic agent. That is, the signaling sequence could be a targeting moiety that preferentially hones the nanosponge to a particular cell type or target receptor, and then also is able to block, bind, or otherwise interact with the target in a therapeutic manner. Such embodiments are exemplified in the working examples using MK2 inhibitors.

**[0049]** The individual building blocks can be designed and synthesized to have the same or different sequence characteristics and moieties on each arm of the core. For example, the exemplary building block depicted in FIG. 1B comprises (consists of) a trigonal core on which all three arms are attached the same block co-peptide 14, active agent 18, and capping moiety 16. Alternatively, as illustrated in FIG. 1C, the exemplary building block comprises (consists of) a trigonal core having two arms from which peptide blocks 14 with active agents 18 coupled thereto extend, and one arm coupled with a signaling sequence 20 via its respective linker 22. In one or more embodiments, the peptide nanosponges are a composite comprising two or more different types of peptide-based building blocks. For example, the peptide nanosponges can be assembled from a plurality of peptide-based building blocks having therapeutic moieties coupled thereto in combination with a plurality of peptide-based building blocks having signaling sequences. In this manner, the resulting nanosponge assembly matrix is characterized by a plurality of individual branched building blocks associating with one another to form a three-dimensional network that defines open spaces (i.e., cavities, voids, and pores) throughout the matrix (i.e., internal and external open spaces). The branched arms and peptide sequences of the assembled building blocks together create the interconnected network of the matrix, which defines the open spaces of the matrix, with the pores being those open spaces or holes making up the interstitial spaces between branched arms and peptide sequences.

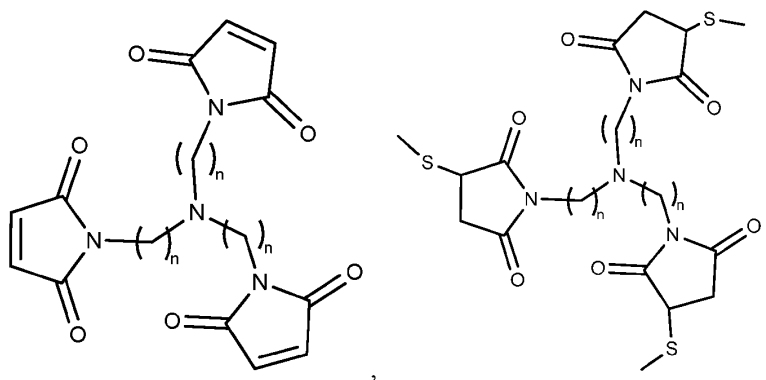
**[0050]** In one or more embodiments, the core is a branched structure that can have three or more “arms” terminating in functional groups that can covalently link the core to a respective

peptide sequence. In general, the cores are preferably dendrimer-like branched or hyperbranched polymeric structures of 800 Da or less in size. In one or more embodiments, the core can comprise three or more maleimide groups, three or more carboxylate groups, three or more amide groups, or three or more hydroxyl groups as illustrated by Formula A or B:

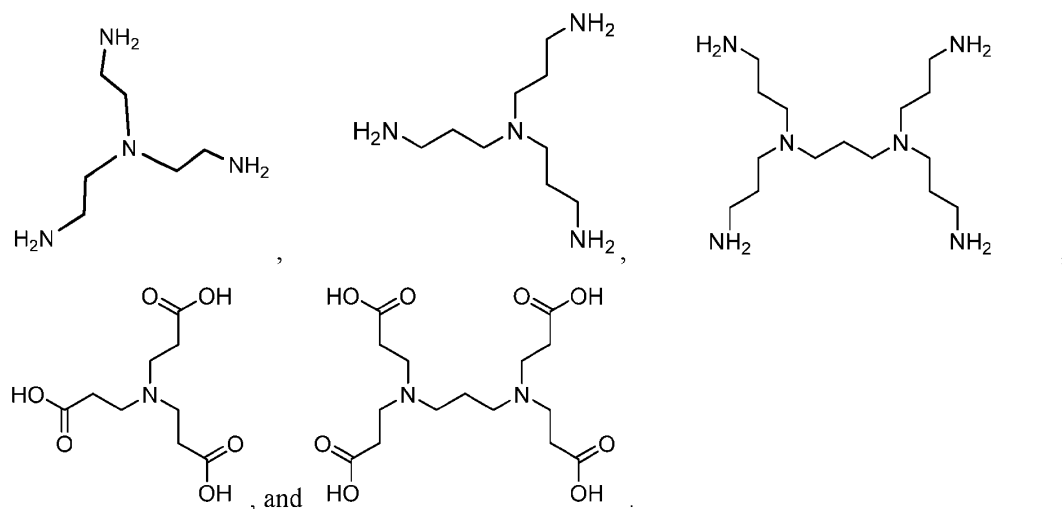


where  $n$  is 2 to 5, preferably 2 to 3, preferably 3; and  $Z$  is a maleimide,  $-NH_2$ ,  $-COOH$ , or  $-OH$ . In some cases,  $Z$  itself represents a nitrogen with further branching.

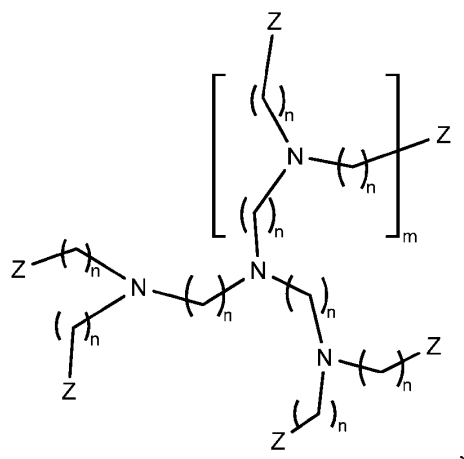
**[0051]** In one or more embodiments, the peptide nanosponge building blocks can have the core be derived from a trimaleimide compound or analogs thereof:



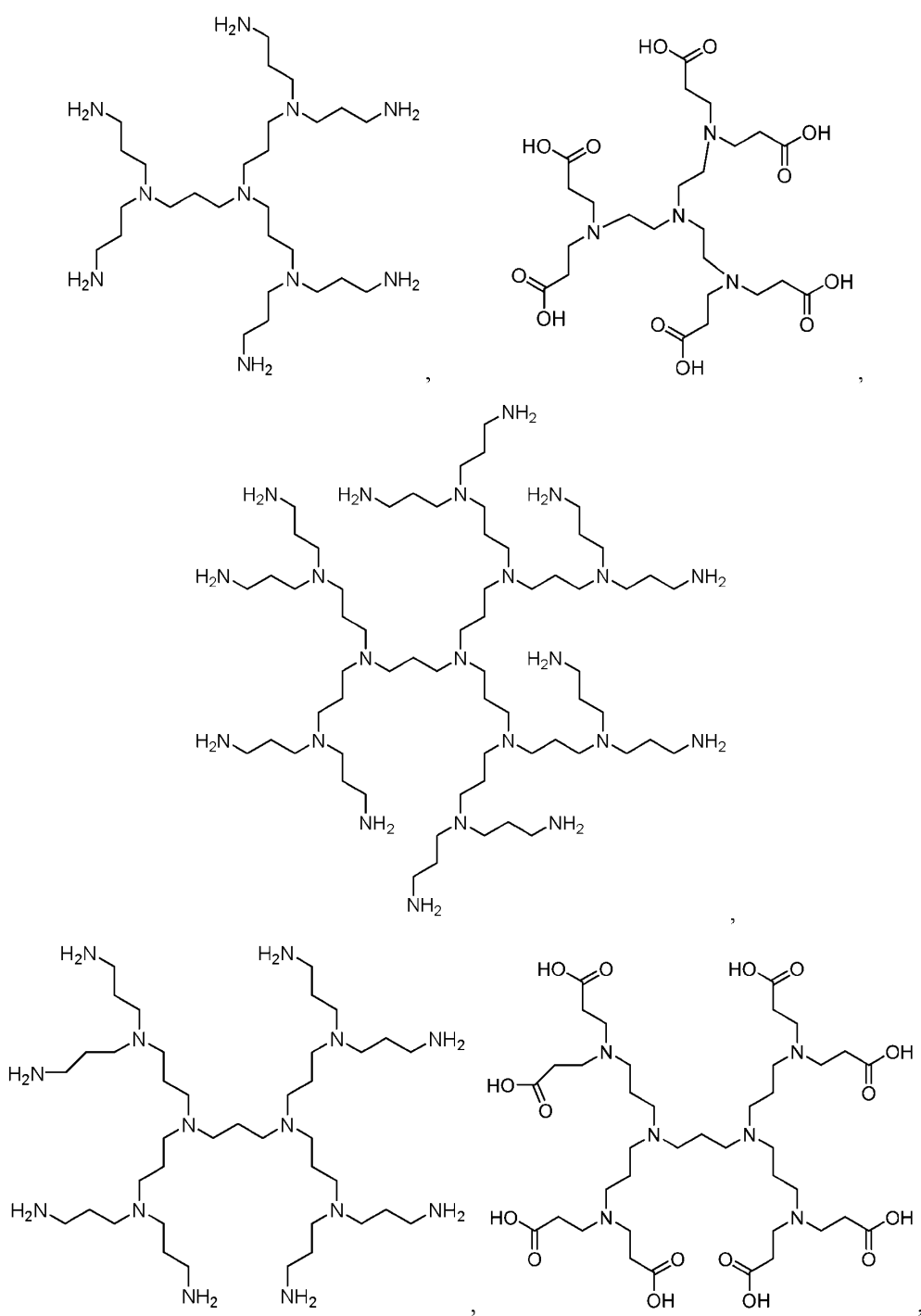
where  $n$  is as defined above. Suitable core linkers include other analogs such as:

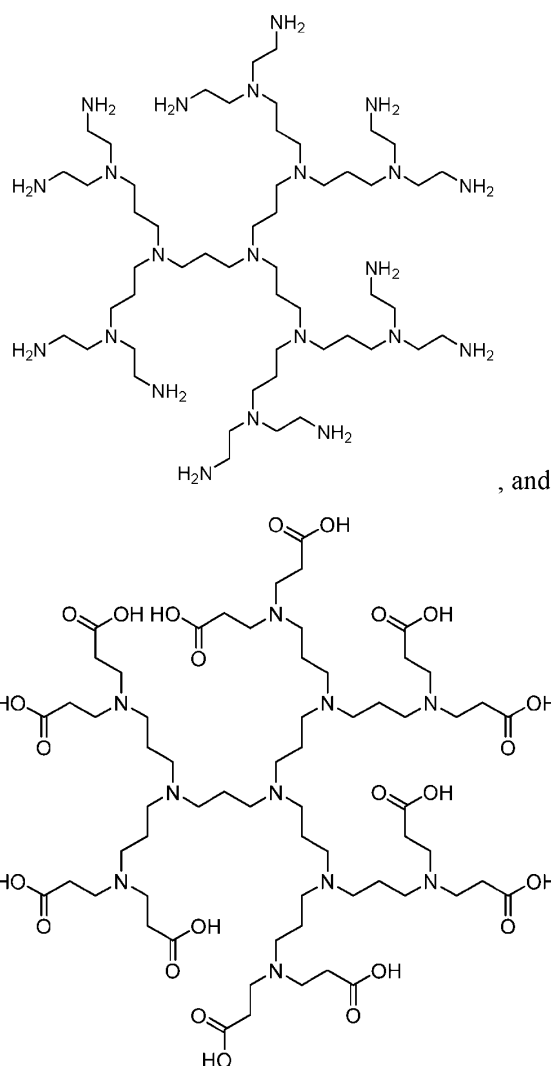


Hyperbranched dendrimers with repeating units are also envisioned including:



where  $n$  and  $Z$  are defined as above and  $m$  is 1-5. Examples of such hyperbranched polymeric cores include, without limitation:





As illustrated above, a variety of branched polymeric cores can be envisioned, having 3 or more terminal amine groups. In such embodiments, the terminal -COOH group of the peptide sequence can be linked to the terminal -NH<sub>2</sub> to form a very stable amide bond at each arm of the core. The skilled person will recognize that the -NH<sub>2</sub> groups will be converted into amides with attached peptide chains. Again, the branched polymeric cores can be synthesized with arms terminating in other suitable functional groups, including COOH as also illustrated, or maleimide groups, amide groups, or hydroxyl groups, for covalent bonding to the peptide sequences.

[0052] The peptide sequences can be directly attached to the core and/or can be attached via short spacers of one to two amino acids. For example, the peptide building blocks can have a linking amino acid or peptide comprising cysteine. Yet another aspect of the disclosure, the peptide building blocks described herein can have the linking peptide comprise cysteine with a glycine spacer residue at the C-terminus. In general, a glycine residue will be included at the C-terminus of the entire peptide sequence, including the linker cysteine, as an artifact from peptide synthesis. Other linking amino acids or peptides include glutamate, aspartate, serine, threonine, tyrosine, lysine, histidine, and the like, provided they are capable of forming a covalent bond with the C-terminal end of block co-peptide. That is, the block co-peptide is preferably directly or indirectly attached to the core in a manner that is not specifically cleavable, i.e., is not an enzyme consensus sequence, and is preferably resistant to enzymatic cleavage and not readily cleaved or broken down *in vivo* at least until after the active agent (which *is* attached via a readily cleavable sequence) is released. However, it will be appreciated, that, as with most biodegradable matrices, the nanosponge network will eventually be broken down through normal physiological processes used by the body to clear dead cells, debris, and foreign matter *in vivo*.

[0053] The peptide sequence comprises a block co-peptide comprising a first peptide block and a second peptide block which is different from the first peptide block. The first peptide block can be covalently attached to the linking amino acid or spacer peptide. The second peptide block can be covalently attached to the first peptide block. Block co-peptide sequences comprising a third peptide block can also be envisioned. In general, the peptide sequence has an overall length of 50 amino acids or less, preferably 5 to 40 amino acids, and preferably 10 to 35 amino acids.

[0054] Preferably, the first block which makes up the C-terminal end of the sequence and is attached to the core (directly or via a linker or spacer) is selected from amino acids having positively charged side chains, such as lysine, histidine, arginine, and the like. Preferably, the first block is a homopeptide of 10 to 25 amino acid residues, preferably 15 to 20 amino acid residues, and more preferably consists of lysine residues. When a peptide building block has two or more arms comprising a block co-peptide, it is preferred that the same peptide sequence be used for both block co-peptides, and more preferably that each peptide sequence is positioned the same distance (e.g., via a linker or peptide spacer) from the core to enhance stability of the structure.

[0055] Preferably the second block which makes up the N-terminal end of the sequence (and is connected to the first block) is selected from amino acids having side chains terminating in -

COOH or -OH groups, namely serine, aspartic acid (or aspartate), tyrosine, glutamic acid (or glutamate), threonine, as well as non-natural amino acids having amine, hydroxyl or carboxylic acid functions, and the like. Preferably, the second block is a homopeptide of 5 to 15 amino acid residues, more preferably 5 to 10 amino acid residues, and more preferably consists of serine or aspartic acid. In some embodiments, the second block consists of the same type of amino acid residue having side chains terminating in -COOH or -OH groups, namely serine, aspartic acid (or aspartate), tyrosine, glutamic acid (or glutamate), threonine, as well as non-natural amino acids having amine, hydroxyl or carboxylic acid functions (i.e., all serines, all tyrosines, etc.), but the selected amino acids are separated by inert spacer residues (e.g., alanines) to facilitate spacing of the placement of the active agent along the chain.

**[0056]** The peptide sequence is designed such that it does not include and is preferably free of an enzyme consensus sequence (whether in the middle of or at the N-terminal or C-terminal end of the peptide block).

**[0057]** That is, the only cleavable linkages in the building block are the linkages tethering the active agent to the block co-peptide. Suitable consensus sequences for attaching the active agent preferably consist of 2 to 8 amino acid residues. Consensus sequences are well known in the art for being selective for proteases (e.g., caspases, matrix metalloproteinases, cathepsins, calpains, and the like. Alternatively or additionally, the active agent can be attached cleavable chemical linkages, such as ester linkages which are cleaved by esterases found throughout the body. Again, combinations of consensus sequences and chemical linkages can be designed into the building block to modulate how slowly or quickly the drug is released after administration.

**[0058]** The second peptide block preferably has a covalently attached capping moiety. Preferably, the capping moiety is a lipid moiety having a molecular weight of 400 Da or less. In one or more embodiments, the capping moiety comprises cholesterol (386 Da). Other types of lipids include betulinic acid, saturated and unsaturated fatty acids, adamantane, and the like, of 400 Da or less. Alternatively, the peptide nanosponges can have the capping moiety be cholic acid when the nanosponge is designed to treat cholangiocarcinoma.

**[0059]** In one or more embodiments, the signaling sequence can be covalently attached directly or indirectly to the core via its own respective peptide linkage (that is different from the block co-peptide sequences described above).

**[0060]** Signaling sequences can be “eat me” sequences, “don’t eat me” sequences, targeting sequences, detectable labels, and/or combinations thereof. The signaling sequence can, for example, be a signaling sequence for CD44 comprising sHPWSYLWTQQAs (SEQ ID NO:1); a plectin-targeting sequence comprising KTLPTPG (SEQ ID NO:2); a signaling sequence for EGFR comprising sYHWYGYTPENVIGsG (SEQ ID NO:3); a signaling sequence for LDLR comprising sCMPRLRGCGAGsG (SEQ ID NO:4); a signaling sequence for CD46 comprising sLPGTICKRTMLDGLNDYCTGsG (SEQ ID NO:5); a signaling sequence for SIRPα “don’t eat me” comprising kGNYTCEVTELSREGKTVIELKsG (SEQ ID NO:6); a signaling sequence for SIRPα “don’t eat me” comprising kGNYTCEVTELSREGKTVIELKk (SEQ ID NO:7); a signaling sequence for SIRPα “eat me” comprising 4N1: RFYVVMWK (SEQ ID NO:8) and 7N3 FIRVVMYEGKK (SEQ ID NO:9); a signaling sequence for CD206 “shut down M2 polarization” (rp-182) comprising KFRKAFKRFFGsG (SEQ ID NO:10); a signaling sequence for Mucosal Membrane: WTAS comprising PLKWPGKKKKGKPGKRKEQEKKRRTRG (SEQ ID NO:11). As noted, certain types of sequences can be considered both signaling sequences and therapeutic sequences. The peptide nanospheres described herein can be used to deliver therapeutic peptides for polycystic kidney disease comprise TAFGASLFVPPSHVQFIG (P17) (SEQ ID NO:12), TAFGASLFVPPSHVQFIGGKKKKKG (KP17) (SEQ ID NO:13), or TAFGASLFVPPSHVQFIGDDDDDDG (DP17) (SEQ ID NO:14).

**[0061]** The peptide building blocks can be used to deliver a wide variety of therapeutically active agents, which are bound via cleavable bonds to the block co-peptide sequence, and preferably to the second (N-terminal) block. In general, depending on the length of block, multiple active agents can be attached. For example, for a second block consisting of 10 amino acid residues, up to 5 active agents can be attached, e.g., to every other serine in the block. Alternatively, the peptide block can include spacers (e.g., alanines) between each serine residue and the block could carry 10 active agents attached to each serine.

**[0062]** The peptide nanospheres can be used to deliver a wide variety of therapeutically active agents including, without limitation, a DCLK1 inhibitor, a MK2 inhibitor, a cytotoxic agent against p53 deficient cells, a MIF inhibitor, a SMAD phosphorylation inhibitor, a dopamine receptor inhibitor, or a combination thereof. The active agents can be attached to the peptide building blocks. Alternatively, active agents can also be sequestered within the interstitial spaces of the

assembled nanosponge for controlled release and delivery as the nanosponge assembly is broken down. This is particularly advantageous for lipophilic active agents.

[0063] The peptide nanosponges can have the therapeutically active agent be covalently attached to one or more amino acid residues in the second peptide block.

[0064] Rationally designed peptide nanosponges in the working examples feature trigonal building blocks (e.g., tris-maleimides) to which other peptides can be tethered via cysteine-mediated Michael addition. This strategy provides a great flexibility when targeting cells *in vitro* and especially *in vivo*.

[0065] These peptide-based building blocks then form supramolecular aggregates (nanosponges) with each other characterized by a flexible three-dimensional matrix in which the assembled building blocks and their respective branched structures cooperate to form the matrix network having interstitial open spaces. This is driven by the interaction of the hydrophobic components of the peptide building blocks (e.g., cholesterol, cholesterol-derivatives (such as cholic acid), hydrophobic peptide sequences or hydrophobic dyes) in the reaction solution which comprises an aqueous or organic solvent system. The assembled matrix can dynamically flex and bend and disassemble into smaller clusters and reassemble depending upon the environment and conditions being applied to it (e.g., *in vivo* through the cellular uptake process).

[0066] The flexible structure of peptide nanosponges permits their tailored design.

[0067] Additionally, cleavage sequences for any of the approximately 600 human proteases can be used to tether the active agents to respective peptide-based building blocks described herein. Also, cleavage sequences for groups of proteases (e.g. MMPs, cathepsins, etc.) can be incorporated into the nanosponges depending on the desired target.

[0068] The peptide nanosponges can be used to deliver a wide variety of therapeutically active agents, including small molecules, therapeutic peptides, and the like. In general, any active agent with a size of < 2nm (longest axis) or less could be delivered using the nanosponges.

#### **DCLK1 Inhibitors**

[0069] The peptide nanosponges described herein can have the therapeutically active agent comprise a DCLK1 inhibitor.

[0070] Doublecortin Like Kinase 1 (DCLK1) is considered a cancer stem cell (CSC) marker. The DCLK1-signaling pathway involves AKT phosphorylation at Thr308 which leads to Notch1

activation. Notch1 activates transcription factor Pregnane X Receptor (PXR) expression. PXR induces cancer cell proliferation and metastasis and regulates the expression of multidrug resistance protein 1 and other proteins involved in drug metabolism including cytochrome P4503A4 (one of six enzymes responsible for the metabolism of approximately 90% of all small molecule drugs). Oral administration of representative small molecule DCLK1 inhibitors demonstrated *in vivo* antitumor activity in a mouse colon cancer xenograft model. Although we were able to demonstrate that inhibition of DCLK1 was associated with antitumor response, the chemical scaffold from which lead candidates were generated is not pharmaceutically tractable. The nanosponge linked to small molecule DCLK1 inhibitors could allow for delivery directly at the tumor site in the colon to enhance antitumor activity while “protecting” small molecule DCLK1 inhibitors from potential metabolism.

**[0071]** Doublecortin-like kinase 1 (DCLK1). DCLK1 is a cancer stem cell (CSC) marker. CSCs are rare and difficult to isolate and identify cells within the tumor mass. Multiple methods used to identify CSCs include sphere-forming ability, dye exclusion due to overexpression of efflux pumps, intracellular enzyme activity and expression of cell surface markers (3, 4). Gordon and colleagues were the first to identify DCLK1 in the intestine. DCLK1 is confirmed as a CRC and pancreatic cancer stem cell marker. DCLK1 is a member of the calmodulin-like protein kinase superfamily. The full-length encoded protein contains two N-terminal doublecortin (DCX) domains, which bind microtubules and regulate microtubule polymerization, a C-terminal serine/threonine kinase domain that has homology to Ca<sup>2+</sup>/calmodulin-dependent protein kinase, and a serine/proline-rich domain in between the doublecortin and the protein kinase domains. DCLK1 is predominantly transcribed from a second promoter in colon cancer cells. AKT1 peptide surrounding Thr308 interacts with kinase domain of DCLK1 protein, a short form that lacks the N-terminal DCX domains. DCLK1 knock out mouse models suppress colon polyps in APC<sup>min</sup>/+ mice but do not affect normal intestine. These data suggest that DCLK1+ cells mark CSCs, not normal stem cells. An important consequence of this finding is that targeting DCLK1 will specifically target CSCs while sparing normal proliferating cells.

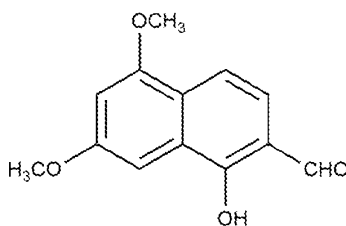
**[0072]** DCLK1 has been shown to phosphorylate the microtubule-associated protein MAP7D1. Using *in silico* modeling, AKT1 was identified surrounding threonine at position 308 (IKDGATMKTFGTP (SEQ ID NO:15)). Using AutoDock Vina, and the crystal structure of the kinase domain of DCLK1 (PDB ID 5JZN), it was observed that the AKT1 peptide surrounding

Thr308 interacts with the DCLK1 kinase domain (binding energy of -5.7 kcal/mol). DCLK1 amino acids targeted for AKT1 interaction include ASP398, ASP475, GLU515, and THR552. We confirmed the interaction using a magnetic relaxation assay and an immunoprecipitation-coupled western blot assay. An in vitro assay using recombinant DCLK1 and the AKT peptide also confirmed significant phosphorylation of the AKT substrate by DCLK. This phosphorylation was lost when DCLK1 was denatured.

**[0073]** In one or more embodiments, the DCLK1 inhibitor is gemcitabine.

**[0074]** It has been demonstrated that lead candidate MRL16 inhibits colon cancer growth by inhibiting proliferation at the G2/M phase of the cell cycle, inducing apoptosis and suppressing stemness by inhibiting spheroid growth. MRL16 suppressed DCLK1-mediated phosphorylation of AKT1 at Thr308, resulting in downstream suppression of Notch signaling and PXR. *In vivo* mouse xenograft studies with MRL16 subsequently demonstrated antitumor activity providing evidence that DCLK1 is a viable CRC therapeutic target. Combining inhibition of this novel drug target with a novel approach to selectively deliver DCLK1 inhibitors to CRC tumors, DCLK1 LLC aims to improve treatment outcomes by enhancing efficacy and reducing toxicity. Described herein is the synthesis of novel nanosponge-linked DCLK1 inhibitors and evaluation of the agents in vitro. This novel nanosponge drug delivery platform has not been previously employed for the treatment of CRC.

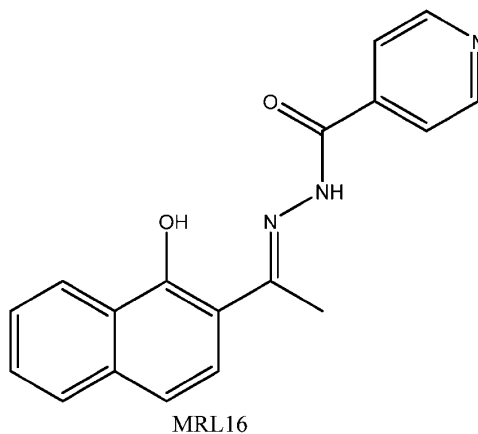
**[0075]** Researchers at the University of Kansas Medical Center isolated marmelin (1-hydroxy-5,7-dimethoxy-2-naphthalene-carboxaldehyde, MRL) from *Aegle marmelos*, an Ayurveda treatment for gastrointestinal cancers.



Marmelin (MRL), MW 232.23

**[0076]** From this scaffold, MRL16, was selected for target validation studies based on its early ADMET properties summarized in Table 1. Despite limited aqueous solubility and moderate in vitro metabolic stability, the compounds proved excellent probes for proof of principle studies to validate DCLK1 as a therapeutic target. MRL16 significantly interacts with the kinase domain,

with a binding energy of -7.9 kcal/mol, with the key amino acid being VAL468.



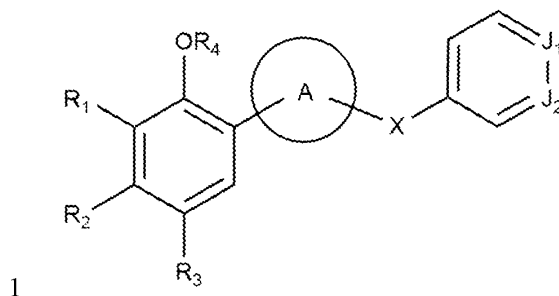
MRL16

MRL 16, MW 305.33

[0077] DCLK1 is a protein that marks quiescent stem cells in colon cancer. Stem cells support spheroid growth. We performed spheroid assays with HCT116 cells where DCLK1 is knocked down using specific shRNA. 500 cells were plated in an ultra-low-attachment 12-well dish in a medium devoid of serum but containing essential factors for spheroid growth. A significant reduction in spheroid formation compared to controls was observed. Cells were then treated with MRL16 (0.5  $\mu$ M) and showed a significant reduction in primary and secondary colonosphere growth. Secondary spheroids are developed from primary spheroids. In secondary spheroids, no additional drug is added, relying on the number of CSCs that survived when primary spheroids were treated. MRL16 did not invoke a significant reduction in cells lacking DCLK1, confirming the analogs suppress DCLK1+ CSCs to inhibit spheroid growth.

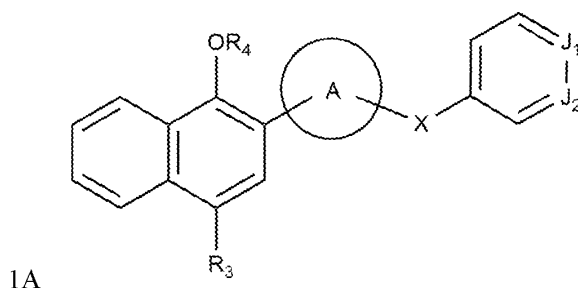
[0078] FIG. 2 shows one embodiment contemplated for the nanosponge for delivery of the DCLK1 inhibitors wherein the trimaleimide structure is the core that is attached to a first peptide block of 20 lysine residues and a second peptide block of 10 residues of serine or aspartic acid (although the second block could also be threonine, aspartate, or glutamic acid/glutamate). Additionally, two of the blocks are capped with a lipid cap (cholesterol) and the other position is attached to a signaling sequence that can be either a sequence targeting CD44 or targeting SIRP $\alpha$  ("don't eat me" sequence). Depending on the peptides used for the second 10-residue block, the DCLK1 inhibitor can be covalently attached to the hydroxyl groups of serine or threonine residues to form a cleavable carbonate bond or covalently attached to the carboxylic acid groups of aspartic acid or glutamic acid groups to form cleavable ester bonds.

[0079] The DCLK1 inhibitors can have a structure corresponding to Formula 1



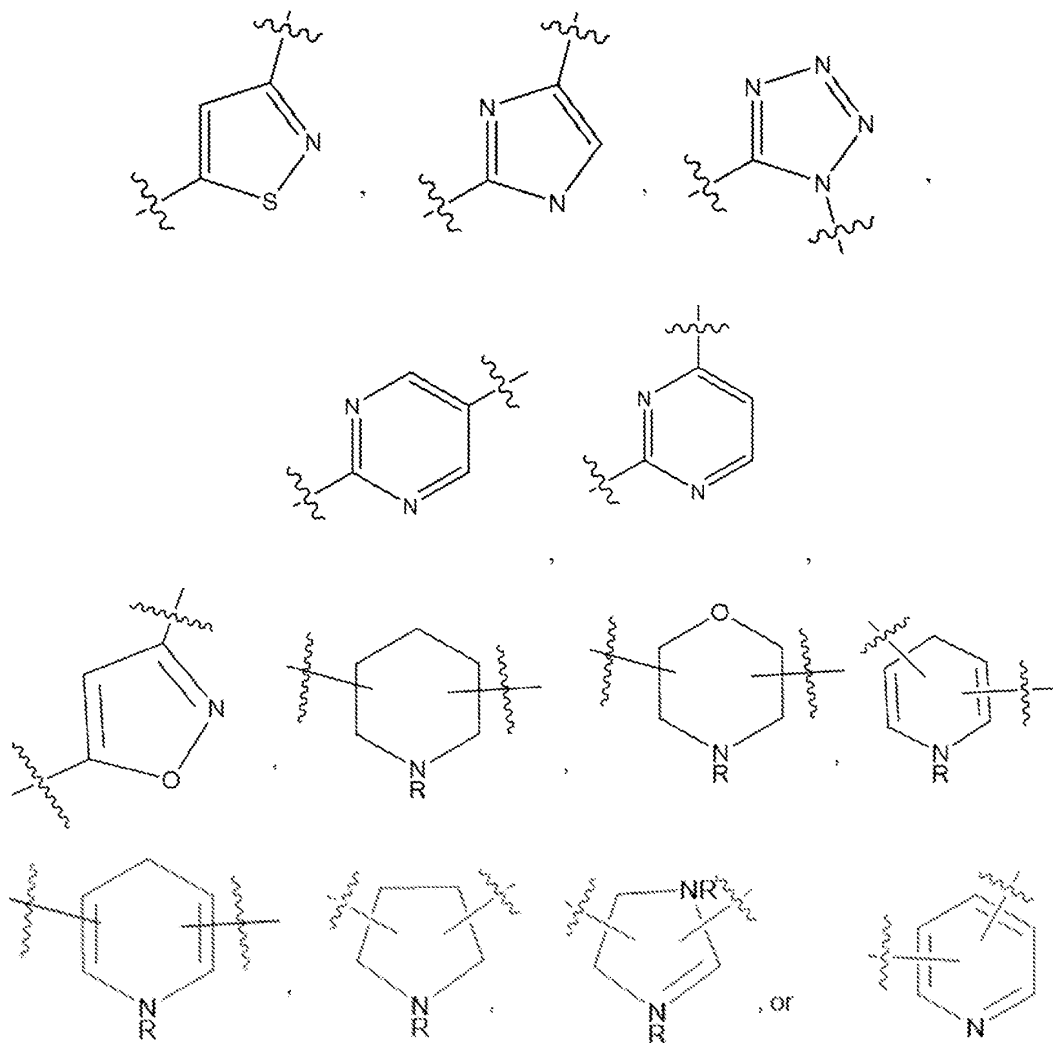
wherein  $A$  is a 5- or 6-membered heterocycle, preferably a nitrogen-containing heterocycle, preferably a nitrogen-containing heteroaryl group;  $R_1$ ,  $R_2$ , and  $R_3$  are each independently selected from -H, -O, -N, -S, -F, -Cl, -OH, -OCH<sub>3</sub>, NO<sub>2</sub>, alkyls (e.g., C<sub>1</sub>-C<sub>12</sub>, preferably C<sub>1</sub>-C<sub>6</sub> alkyls), and alkaryls (e.g., C<sub>1</sub>-C<sub>12</sub> alkyl-substituted C<sub>3</sub>-C<sub>6</sub> aryls), or  $R_1$  and  $R_2$  together with the carbons they are attached to form a fused 5- or 6-membered ring with the adjacent 6-membered ring to which  $R_1$  and  $R_2$  are bound;  $R_4$  is H or -CH<sub>3</sub>;  $X$  is -C(O)-, -NHC(O)-, -C(O)NH-, or -NHC(O)NH-; and  $J_1$  and  $J_2$  are each independently -CH- or -N-, and preferably with the proviso that at least one of  $J_1$  and  $J_2$  is -N-.

[0080] Additionally, the DCLK1 inhibitors can have the structure corresponding to Formula 1A

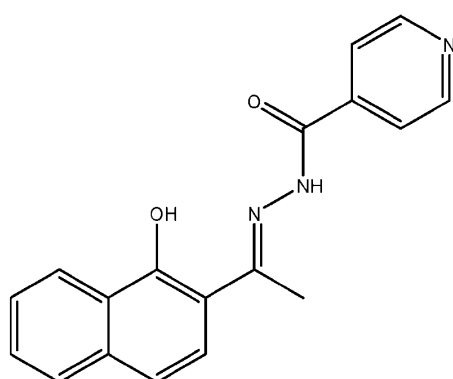


wherein  $A$  is a 5- or 6-membered heterocycle, preferably a nitrogen-containing heterocycle, preferably a nitrogen-containing heteroaryl group;  $R_3$  is independently selected from -H, -O, -N, -S, -F, -Cl, -OH, -OCH<sub>3</sub>, NO<sub>2</sub>, alkyls (e.g., C<sub>1</sub>-C<sub>12</sub>, preferably C<sub>1</sub>-C<sub>6</sub> alkyls), and alkaryls (e.g., C<sub>1</sub>-C<sub>12</sub> alkyl-substituted C<sub>3</sub>-C<sub>6</sub> aryls);  $R_4$  is H or -CH<sub>3</sub>;  $X$  is -C(O)-, -NHC(O)-, -C(O)NH-, or -NHC(O)NH-; and  $J_1$  and  $J_2$  are each independently -CH- or -N- and preferably with the proviso that at least one of  $J_1$  or  $J_2$  is -N-.

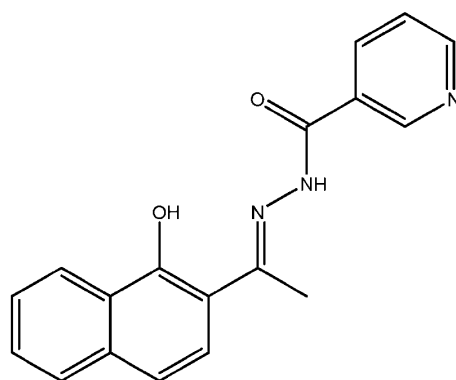
[0081] The DCLK1 inhibitors having the structure corresponding to Formula 1 or Formula 1A wherein A is a 5- or 6-membered heterocycle, preferably a nitrogen-containing heterocycle, having one of the following structures:



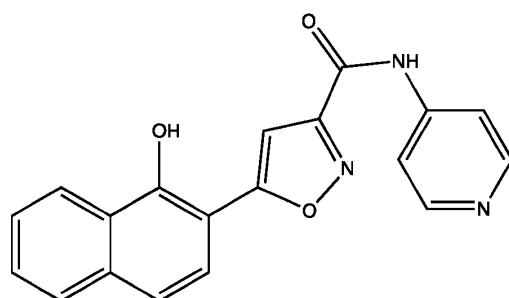
[0082] In one or more embodiments, the DCLK1 inhibitors can also have the following structures



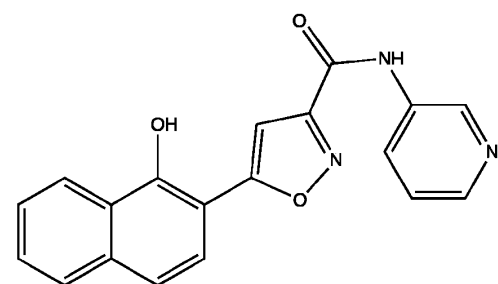
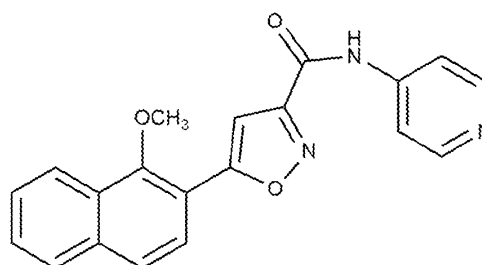
MRL16



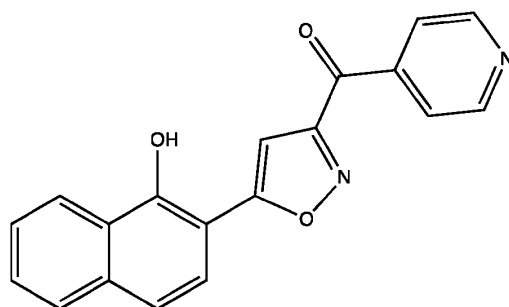
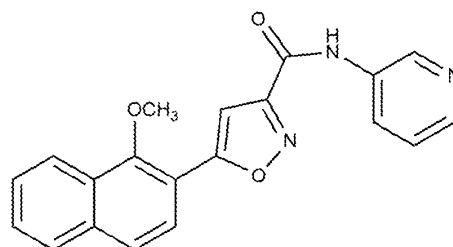
MRL17



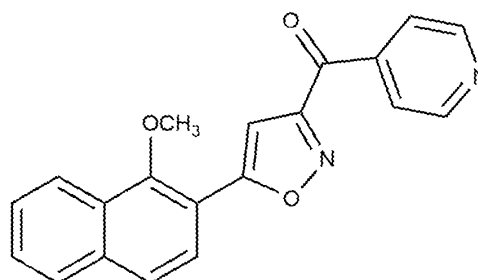
DCLK3

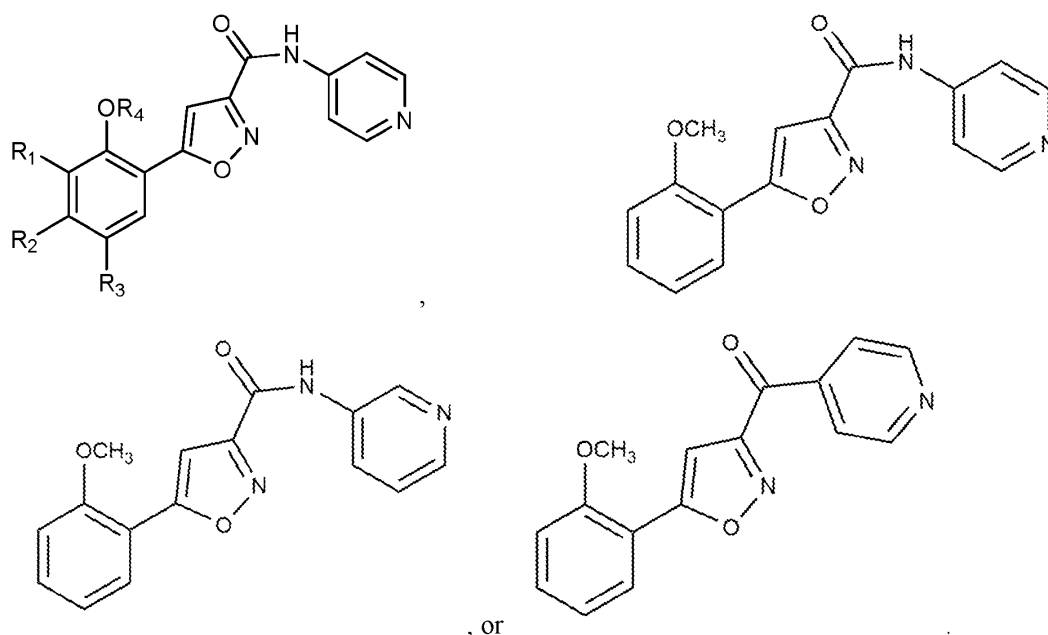


DCLK3A



DCLK4





### MK2 Inhibitors

[0083] Further, the peptide nanosponges can be used to deliver the therapeutically active agent comprising an MK2 inhibitor.

[0084] MK2 pathway is important for inflammatory cytokine production and mesenchymal gene expression. Preclinical work demonstrates higher levels of phosphorylated MK2 (p-MK2) (active MK2) in IPF patients compared to normal lung patients. Inhibition of MK2 in vitro leads to reduced TGF $\beta$ 1-mediated myofibroblast development and extracellular matrix deposition. Furthermore, inhibition of MK2 could prevent bleomycin-induced pulmonary injury and inflammation in mice. However, there are no FDA-approved MK2 inhibitors and early generation small molecule MK2 inhibitors lack “druggability” because of lack of enzymatic ATP-pocket selectivity and poor water solubility lead to unrealistically high EC50 levels needed to achieve therapeutic benefit

[0085] Suppression of the MK2 pathway using a small molecule pharmaceutical linked to a nanosponge will allow delivery of the small molecule directly to the cell which will lead to downregulated activation via disruption of the p38 $\alpha$ -MK2 interaction which will in turn

downregulate idiopathic pulmonary fibrosis inflammatory cytokine production and EMT activation thereby improving overall IPF progression.

**[0086]** Known MK2 inhibitors that have poor aqueous solubility or those that are easily degraded by endogenous plasma proteases can advantageously be conjugated to the peptide nanosponges described herein. The nanosponge platform can allow for increased drug solubility; can be modified to prevent degradation (increasing drug access); or further modified to allow delivery of the payload to the target cell-tissue with localized drug release at the site of disease.

**[0087]** We recognize that IPF and fibrotic lung disorders are already established in our clinical patients. We also acknowledge that current bleomycin lung injury models assess the ability to prevent acute pneumonitis and do not evaluate whether they can reverse established soft tissue fibrosis. Our proposal will specifically examine a cohort of mice that have sustained an initial bleomycin injury (>60 days following bleomycin treatment), have established damaged fibrotic lung injury, and whether delivery of our MK2 inhibitor drug conjugate can reverse/reduce established lung fibrotic injury. This is an innovative experimental approach which will more uniquely address the clinical paradigm we are proposing to study.

**[0088]** Using known MK2 inhibitors, we have synthesized the first three nanosponge-linked MK2 inhibitors complex (“drug conjugates”) demonstrating proof of principle that our drug development platform is feasible. Subsequently, we have demonstrated these first-generation compounds can inhibit TGFβ1-induced myofibroblast development via reduction in p-MK2, αSMA, collagen, and fibronectin protein expression levels. Building on these results, we will perform iterative improvements-modifications of the drug conjugates and retest their ability to inhibit myofibroblast development and activation. Once we have identified the best drug conjugate, this will be advanced to *in vivo* animal studies. First, we will establish a maximally tolerated dose of the drug conjugate. Next, we will determine the optimal dose delivery schedule (1 vs 2 weeks of drug delivery). Finally, we will examine the impact of the optimal drug conjugate using both an acute inflammatory pneumonitic model followed by chronic fibrotic injury model.

**[0089]** FIG. 3 illustrates design components of peptide nanosponges for the treatment of pulmonary fibrosis. FIG. 3A shows three MK2-blockers (PF-3644022, MK2-IN-1, and MK2-IN-4). FIG. 3B shows variable structure of the peptide nanosponges. The signaling and therapeutic sequence YARAAARQARAKALARQLGVAA (SEQ ID NO:16) and a CD47-targeting “don’t eat me sequence” (on/on, on/off, off/on), the three small molecule MK2-blockers and the D<sub>10</sub> and

S<sub>10</sub> blocks will be alternated, resulting in 18 novel nanosponges. FIG. 3C shows alternate chemical connections of the MK2-blockers via a tertiary amide bond to D<sub>10</sub> of the block co-peptide ((SEQ ID NO:21) or a urethane bond to S<sub>10</sub> of the block co-peptide (SEQ ID NO:19).

**[0090]** Peptide nanosponges offer the opportunity to deliver covalently attached drugs to biologic targets. According to results from coarse-grained molecular dynamics (MD) simulations, the cholesterol units of the nanosponges form hydrophilic nanodomains. Water-solvent filled nanoholes are present in their structures as well. The integration of fluorescent labels (i.e., rhodamine B) is feasible, because they can be covalently attached to the N-terminal end of the K<sub>20</sub>S<sub>10</sub> (SEQ ID NO:19) building blocks. As shown in FIG. 3, all three MK2 inhibitors (PF-3644022, MK2-IN-1, and MK2-IN-4) feature secondary amine groups that will be utilized to attach them either to S<sub>10</sub> blocks via esterase-cleavable urethane bonds, or to D<sub>10</sub> blocks by means of protease-cleavable amide bonds. We anticipate that urethane bonds will be faster cleaved upon entering of the nanosponges into the cytoplasm than peptide bonds. This permits the optimization of pharmacokinetics. Up to 10 MK2-inhibitors will be tethered to each nanosponge building block. Quantitation will be performed by means of <sup>1</sup>H-NMR. Targeting of activated myofibroblasts will be achieved by utilizing a peptide that service as both a signaling and therapeutic sequence YARAAARQARAKALARQLGVAA (SEQ ID NO:16). To enhance the circulation time of the nanosponges, the “Don’t Eat Me” peptide sequence kGNYTCEVTELSREGKTVELKk (SEQ ID NO:7) will be incorporated into the nanosponge structure. This peptide sequence mimics the binding motif of regulatory protein alpha (SIRPα) in phagocytes. This event triggers the auto-protective response of CD47. Both peptide sequences bear D-amino acids at both terminal ends to prevent rapid proteolytic degradation while in circulation. We anticipate synthesizing 12-15 iterative novel nanosponges featuring the three MK2-inhibitors tethered via urethane or amide bonds. The nanosponges will be equipped with either a signaling or a “don’t eat me” sequence, or both to optimize drug delivery. We will synthesize 500 mg of each nanosponge.

**[0091]** In certain embodiments, MK2 pathway inhibition using a small molecular pharmaceutical conjugated to the nanosponge will reduce radiation induced fibrosis by reducing inflammatory cytokine production and mesenchymal gene activation. In one or more embodiments, Apatanib or another MK2 inhibitor will be linked to the nanosponge moiety via amide and esterase-cleavable bonds. Compounds will be synthesized in an iterative process and evaluated in in vitro cell based screens. One lead compound will then be evaluated in an in vivo pulmonary lung fibrosis model

where we will establish the maximum tolerated dose, assess optimal drug delivery schedule, and its ability to reduce acute pneumonitic lung injury and chronic late fibrotic lung disorders.

[0092] In certain embodiments, MK2 pathway inhibition using a small molecular pharmaceutical conjugated to the nanosponge will reduce radiation induced fibrosis by reducing collagen-1 deposition and inflammatory cytokine production. In one or more embodiments, Apatanib or another MK2 inhibitor will be linked to different nanosponge backbones via amide and esterase cleavable bond. Conjugated compounds will be iteratively developed and tested using in vitro cell and protein-based screens. One lead compound will be advanced to in vivo biologic studies to determine a maximum tolerated dose and pharmacokinetic and pharmacodynamic distribution of drug at two doses (MTD and ½ MTD).

#### **Cytotoxic agents effective against p53 deficient cells**

[0093] Also, the peptide nanosponges can have the therapeutically active agent comprise a cytotoxic agent against p53 deficient cells. Although KU-D2 and KU-D2F comply with the Lipinski requirements for effective drug delivery, they feature three benzylic groups that are prone to effective oxidation, leading to poor pharmacokinetics. Attachment to a rationally designed peptide nanosponge will protect the drugs against oxidation by its supramolecular structure. Furthermore, targeting peptide sequence has been proven successful in facilitating targeted drug delivery.

[0094] The development of small molecule-nanosponge linked compounds that will selectively release the active moiety in the tumor cells with high potency and selectivity. One analog has already demonstrated proof of principle in animal models of osteosarcoma (OS), when dosed intraperitoneally, with undetectable adverse effects, demonstrating that proper delivery of this compound and analogs may be a viable strategy for the treatment of OS. Although exhibiting excellent activity, the systemic exposure to chemotherapy is a concern of long-term dosing, particularly in the pediatric population. Using the “nanosponge” technology to allow for local exposure will enable higher doses of small molecules to be administered to the site of the tumor with lower risk of toxicity. The synthesis of a series of 16 combinations of the lead compound KU-D2F and three analogs, which will be linked to the four different nanosponges via esterase-cleavable bonds. Compounds will be synthesized in an iterative process and evaluated in *in vitro*

screens. Two lead nanosponge/small molecule assemblies will then be evaluated in *in vivo* OS mouse models.

[0095] FIG. 4 shows the design components of nanosponges for osteosarcoma treatment. FIG. 4A shows four analogs of KU-D2F. FIG. 4B shows variable structures of the nanosponges. The signaling sequences from CD47 and CD49, and the D<sub>10</sub> and S<sub>10</sub> blocks (SEQ ID NO:19 or 21) will be alternated whereas the d8 signaling sequences will remain at the shown position. FIG. 4C shows alternate chemical connections via ester bond to D<sub>10</sub> and carbonate bond to S<sub>10</sub> (SEQ ID NO:19 or 21).

[0096] The synthesis of four p53 synthetic lethality inducers (KU-D2F\* and three analogs) that will be linked to four types of nanosponges. KU-D2F\* differs from the parent KU-D2F by the presence of a phenolic group instead of a phenyl-methyl-ether. The former will permit the attachment to either aspartic acid (D10) or serine (S10) blocks of the nanosponges. Three analogs of KU-D2F\* will be synthesized. The four analogs will be tethered to four conceptionally different nanosponges. We will combine two DEM signaling sequences derived from CD47 and CD49 with two different chemical attachments (ester bonds vs. carbonate bonds) of the four analogs. This will permit optimized drug delivery to OS as well as subsequent release of the DEM inducers utilizing esterase activity in the cytoplasm.

[0097] The synthesis of the nanosponge/small molecule assemblies will be followed by *in vitro* studies required to recognize the two best embodiments, which will then be tested *in vivo*. The nanosponge-linked analogs will release the active small molecule to the cytoplasm of the tumor cells. Execution of the aims described in this proposal will generate novel nanosponge-linked compounds that maintain the active small molecule moieties.

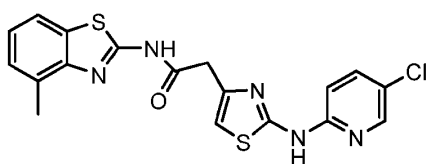
#### **MIF Inhibitors**

[0098] The peptide nanosponges can have the therapeutically active agent comprise a MIF inhibitor.

[0099] In one example, the peptide nanosponges can have a trigonal linker (e.g., tris(maleimide)) covalently attached to a signaling sequence for EGFR comprising sYHWYGYTPENVIGsG (SEQ ID NO:3); a signaling sequence SIRP $\alpha$  "don't eat me" comprising kGNYTCEVTELSREGKTVELKsG (SEQ ID NO:6), and a S<sub>10</sub>K<sub>20</sub>C (SEQ ID NO:17) covalently attached to the trigonal linker through the cysteine residue and capped with cholesterol covalently

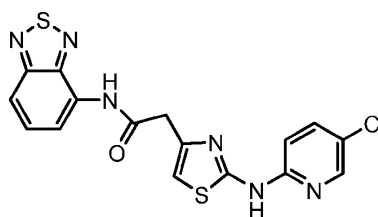
attached to the serine peptide block and having a MIF inhibitor covalently attached to the amino acid residue(s) of the S<sub>10</sub>K<sub>20</sub> (SEQ ID NO:19) peptide block. The particular nanosponges can have some combination of these elements as long as there is a peptide block that allows formation of a supramolecular structure and amino acid residues allowing the covalent attachment of the MIF inhibitor compound.

**[0100]** In some embodiments, the compounds of the present invention that can be used as MIF1/MIF2 inhibitors are shown as follows:



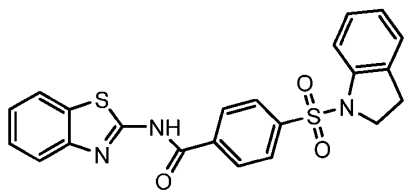
2-(2-((5-chloropyridin-2-yl)amino)thiazol-4-yl)-N-(4-methylbenzo[d]thiazol-2-yl)acetamide

KU0180931; F5950-0872; Compound 2



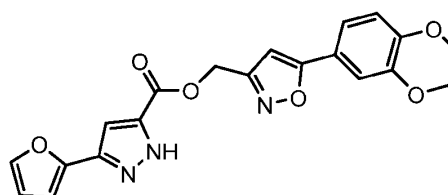
N-(benzo[c][1,2,5]thiadiazol-4-yl)-2-(2-((5-chloropyridin-2-yl)amino)thiazol-4-yl)acetamide

KU0180903; F5950-0730; Compound 6



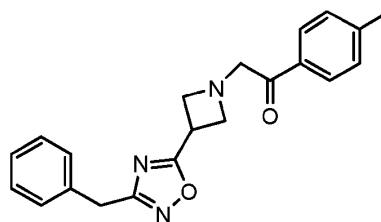
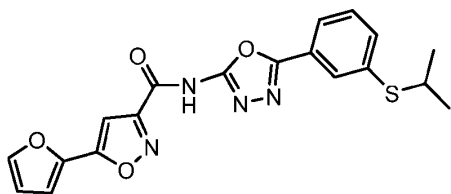
N-(benzo[d]thiazol-2-yl)-4-(indolin-1-ylsulfonyl)benzamide

KU0168339; F0725-0226; Compound 14

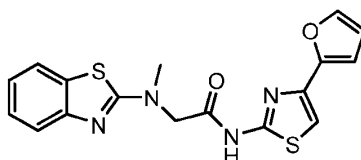


(5-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)isoxazol-3-yl)methyl 3-(furan-2-yl)-1H-pyrazole-5-carboxylate

KU0171533; F2496-3431; Compound 30



5-(furan-2-yl)-N-(5-(3-(2-(3-(3-benzyl-1,2,4-oxadiazol-5-yl)azetidin-1-yl)-1-(p-tolyl)ethan-1-one  
(isopropylthio)phenyl)-1,3,4-oxadiazol-2-yl)isoxazole-3-carboxamide  
KU0178573; F5773-3144; Compound 42



2-(benzo[d]thiazol-2-yl(methyl)amino)-N-(4-(furan-2-yl)thiazol-2-yl)acetamide  
KU0182093; F6225-0003; Compound 74

### Compositions and Methods of Treatment

**[0101]** Depending on the active agent and condition being treated, the nanosponges can be formulated for various routes of delivery and modalities of treatment. Therapeutic compositions are described herein which comprise a nanosponge (or a plurality of nanosponges) dispersed in a suitable delivery vehicle or carrier for administration to a subject. The therapeutic compositions comprise a therapeutically effective amount of peptide nanosponge with active agent dispersed or suspended in a pharmaceutically acceptable carrier. In one or more embodiments, the compositions can comprise a mixture of two or more different types of nanosponges and/or different types of active agents. For example, it is contemplated that nanosponges carrying therapeutically active agents could be co-administered with nanosponges carrying detectable labels for monitoring and visualizing delivery of the nanosponges to the targeted area.

**[0102]** A pharmaceutically acceptable carrier or vehicle is any carrier suitable for *in vivo* administration. As used herein, the term “pharmaceutically acceptable” means not biologically or otherwise undesirable, in that it can be administered to a subject without excessive toxicity, irritation, or allergic response, and does not cause unacceptable biological effects or interact in a deleterious manner with any of the other components of the composition in which it is contained. A pharmaceutically-acceptable carrier would be selected to minimize any degradation of the nanosponge (at least during storage) and to minimize any adverse side effects in the subject.

**[0103]** Pharmaceutically-acceptable ingredients include those acceptable for veterinary use as well as human pharmaceutical use. For example, pharmaceutically-acceptable carriers suitable for

mucosal administration include aqueous solutions such as, water, buffered solutions, commercially available proprietarily formulated carriers and adjuvants, naturally occurring mono, di-, and polysaccharides, carbohydrates, sugars, polymers, proteins, and the like, including any of the following including mixtures thereof: celluloses, derivatives thereof, and microcrystalline forms thereof such as methyl cellulose, ethyl cellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, sodium carboxymethyl cellulose, and the like; other naturally derived polysaccharides and polymers such as sodium alginate, gelatin, chitosan, collagen, hyaluronic acid, dextran; mono or oligosaccharides such as D-mannitol, sorbitol, glucose, lactose, fructose, inositol, sucrose, amylose, and the like; dextrans such as  $\alpha$ -,  $\beta$ - or  $\gamma$ -cyclodextrin, dimethyl- $\beta$ -cyclodextrin, dextrin; native starches and their derivatives such as hydroxyethyl or hydroxypropyl starch and carboxymethyl starch; as well as gums such as gum arabic, tragacanth gum and glucomannan. Suitable carriers can further include proteins, such as serum proteins, casein, albumin, and the like.

**[0104]** Additional components of the compositions may suitably include excipients such as stabilizers, preservatives, diluents, emulsifiers, and lubricants. For oral delivery, flavoring agents or palatability enhancers can also be included in the compositions.

**[0105]** Other ingredients may be included in the composition, such as adjuvants, other active agents, preservatives, buffering agents (e.g., histidine, phosphates), salts, and other pharmaceutically-acceptable ingredients.

**[0106]** In one or more embodiments, the pharmaceutically-acceptable carrier comprises a combination of one or more of the above-described vehicles, and preferably is configured for enhancing delivery via the desired route. For example, for mucosal delivery the formulation can include is enteric coated for protecting the nanosponge against premature gastrointestinal degradation. The nanosponges can be formulated in a variety of solid or liquid dosage forms. For example, the nanosponges can advantageously be dried, e.g., lyophilized, spray-dried, etc., to create as shelf-stable powder. Powders can be used for inhalation, packaged in gel caps, compressed into tablets, and the like. Such powders can include one or more inert carriers or vehicles to facilitate free-flowing powder and reduce aggregation or clumping. Powders can also be reconstituted into a liquid form for various routes of administration, e.g., via nebulizer, injection, IV, oral, ocular, otic, or nasal drops or sprays, oral gavage, and the like. The nanosponges can be formulated as sublingual or buccal tablets, drops, lozenges, and the like. The nanosponges

can be formulated as topical creams, gels, ointments. The nanosponges can be formulated as suppositories, bladder installations, and the like.

**[0107]** As used herein, a “therapeutically effective” amount or “therapeutic dose” refers to the amount or dosage that will elicit the biological or medical response of a tissue, system, or subject that is being sought by a researcher or clinician, such as to elicit some desired therapeutic or prophylactic effect as against the disease or condition depending upon the active agent delivered. One of skill in the art recognizes that an amount may be considered therapeutically “effective” even if the disease, condition, or symptom is not totally eradicated or prevented, but it or its symptoms and/or effects are improved or alleviated partially in the subject (e.g., reduction in tumor volume, etc.).

**[0108]** The disclosure is also directed to a method of treating a disease or disorder in a subject in need thereof comprising administering the peptide nanosponges or compositions disclosed herein in a therapeutic dose to the patient.

**[0109]** The methods of treating a disease or disorder described herein, wherein the disease or disorder is colon cancer, cholangiocarcinoma, a genitourinary cancer, a gynecologic cancer, a gastrointestinal cancer, lymphoma, melanoma or skin cancer, a head and neck cancer, lung cancer, intestinal cancer, kidney cancer, pancreatic cancer, breast cancer, stomach cancer, leukemia, bone cancer, thyroid cancer, brain cancer, a glioma, idiopathic pulmonary fibrosis (IPF), head and neck fibrosis, osteosarcoma, or bladder cancer; further, wherein the disease or disorder is colon cancer; additionally, wherein the disease or disorder is idiopathic pulmonary fibrosis (IPF); and also, wherein the disease or disorder is osteosarcoma.

**[0110]** As noted, the peptide nanosponges can be enterically coated in order to have the nanosponges pass without hydrolysis into the gastrointestinal tract.

**[0111]** The peptide nanosponges described herein can be administered via virtually any route of administration, depending on the condition to be treated, including direct application to the site to be treated or systemic administration. Non-limiting routes of administration include intraperitoneally, intravenously, intramuscularly, subcutaneously, intradermally, orally, buccally, sublingual, topically, intranasally, rectally, intraosseously, or parenterally, and the like

**[0112]** The nanosponge composition may be administered by one or more dosages, which dosages can be administered, by the same or different route, to achieve the desired prophylactic or therapeutic effect. In some embodiments, the nanosponge composition can be provided in unit

dosage form in a suitable container. The term “unit dosage form” refers to a physically discrete unit suitable as a unitary dosage for human or animal use. Each unit dosage form may contain a predetermined amount of the nanosponge (and/or other active agents) in the carrier calculated to produce the desired effect. In other embodiments, the nanosponge composition can be provided separate from the carrier (e.g., in its own vial, ampule, sachet, or other suitable container) for on-site mixing before administration to a subject. A kit comprising the nanosponge composition is also disclosed herein. The kit further comprises instructions for administering the nanosponge composition to a subject, including reconstituting it if needed. The nanosponge can be provided as part of a dosage unit, already dispersed in a pharmaceutically-acceptable carrier, or it can be provided separately from the carrier. The kit can further comprise instructions for preparing the nanosponge for administration to a subject.

**[0113]** Additional advantages of the various embodiments of the invention will be apparent to those skilled in the art upon review of the disclosure herein and the working examples below. It will be appreciated that the various embodiments described herein are not necessarily mutually exclusive unless otherwise indicated herein. For example, a feature described or depicted in one embodiment may also be included in other embodiments, but is not necessarily included. Thus, the present invention encompasses a variety of combinations and/or integrations of the specific embodiments described herein.

**[0114]** As used herein, the phrase “and/or,” when used in a list of two or more items, means that any one of the listed items can be employed by itself or any combination of two or more of the listed items can be employed. For example, if a composition is described as containing or excluding components A, B, and/or C, the composition can contain or exclude A alone; B alone; C alone; A and B in combination; A and C in combination; B and C in combination; or A, B, and C in combination.

**[0115]** The present description also uses numerical ranges to quantify certain parameters relating to various embodiments of the invention. It should be understood that when numerical ranges are provided, such ranges are to be construed as providing literal support for claim limitations that only recite the lower value of the range as well as claim limitations that only recite the upper value of the range. For example, a disclosed numerical range of about 10 to about 100 provides literal support for a claim reciting “greater than about 10” (with no upper bounds) and a claim reciting “less than about 100” (with no lower bounds).

[0116] Having described the disclosure in detail, it will be apparent that modifications and variations are possible without departing from the scope of the invention defined in the appended claims.

## EXAMPLES

[0117] The following examples set forth methods in accordance with the invention. It is to be understood, however, that following non-limiting examples are provided by way of illustration and nothing therein should be taken as a limitation upon the overall scope of the invention.

### EXAMPLE 1

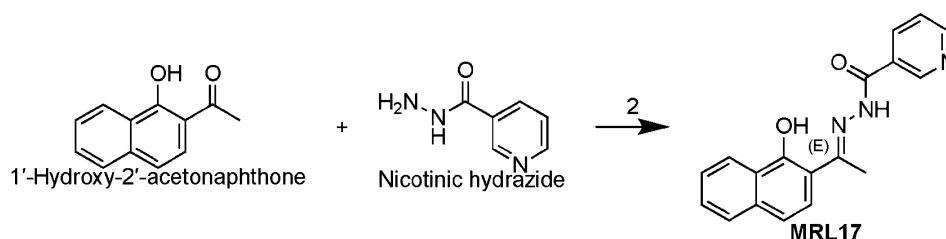
#### DCLK AND MRL COMPOUND SYNTHETIC PROCEDURES

[0118] Example 1A: Preparation of **MRL16**



[0119] To a dry round bottom flask is added 1'-hydroxy-2'-acetonaphthone (1 eq.) and isonicotinic hydrazide (1 eq.) followed by 0.5 mL of concentrated HCl and 5 mL of methanol. The reaction mixture is then heated to 60°C and stirred for 4 hours. During this time precipitate may form. If precipitate does not form after this time, the reaction flask is allowed to cool slowly to RT and then placed in the freezer to induce recrystallization of product. After either the reaction time or recrystallization, the mixture is filtered and the solid is washed with DCM. The yellow solid is isolated, and no further purification was needed.

[0120] Example 1B: Preparation of **MRL17**

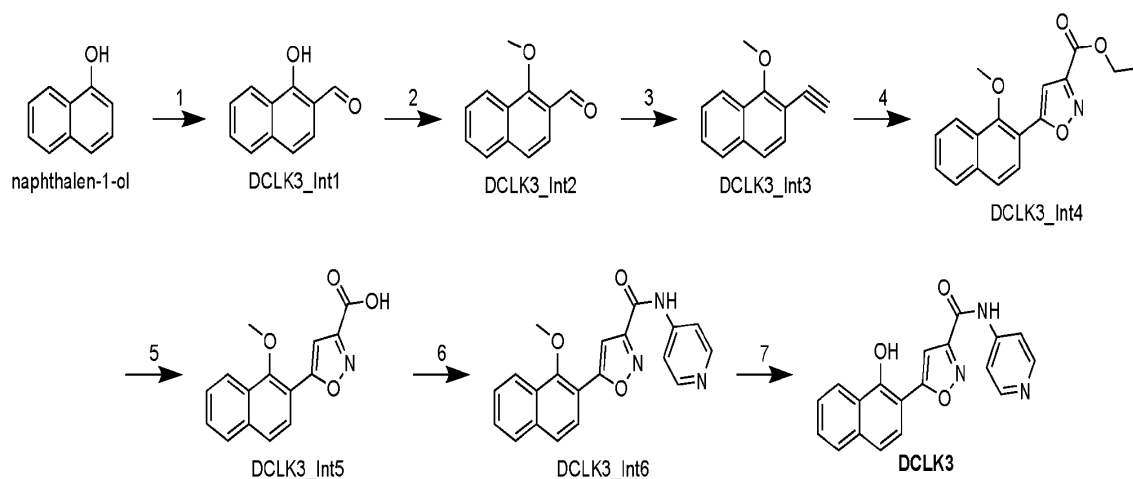


[0121] To a dry round bottom flask is added 1'-hydroxy-2'-acetonaphthone (1 eq.) and nicotinic hydrazide (1 eq.) followed by 0.5 mL of concentrated HCl and 5 mL of methanol. The

reaction mixture is then heated to 60°C and stirred for 4 hours. During this time precipitate may form. If precipitate does not form after this time, the reaction flask is allowed to cool slowly to RT and then placed in the freezer to induce recrystallization of product. After either the reaction time or recrystallization, the mixture is filtered and the solid is washed with DCM. The yellow solid is isolated, and no further purification was needed.

[0122] It is important to note for the synthesis of both MRL compounds, it is likely that the *Z* isomer also forms but in trace amounts due to steric hindrance of the *Z* isomer. As such only the *E* isomer is being shown.

[0123] Example 1C: Preparation of **DCLK3**



[0124] Step 1: Naphthalen-1-ol (7.2 g, 50 mmol) was dissolved in EtOH (28 mL), and a mixture of NaOH (14.4 g, 36.0 mmol) and H<sub>2</sub>O (30 mL) was made and added dropwise. The mixture is then heated to 75°C. Once at the desired temperature, chloroform (6.5 mL, 80 mmol) was added dropwise, and the reaction is heated for 2-3 hours. Upon the completion of the reaction (monitored by TLC), the mixture was acidified with 50mL of 1N HCl, and extracted with DCM. The organic layer is washed with brine and dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, concentrated, and purified via silica gel flash column chromatography using hexane: ethyl acetate gradient.

[0125] Step 2: To a mixture of DCLK3\_Int1 (1 eq.) and K<sub>2</sub>CO<sub>3</sub> (1 eq.) in 10 mL of THF: DMF (10:1) under argon is iodomethane (3 eq.) added dropwise at RT. the mixture is then stirred for overnight. After overnight, water (15 mL) was added to the mixture. The aqueous phase was extracted with ethyl acetate, washed with brine, dry with sodium sulfate. The organic phase was

filtered and concentrated, and the crude product was purified via silica column chromatography using hexane: DCM gradient.

**[0126]** Step 3: To a mixture of DCLK3\_Int2 (1 eq.) and potassium carbonate (1.4 eq.) in 5 mL of MeOH, under argon, is added dimethyl (1-diazo-2-oxopropyl) phosphonate (1 eq.) in one portion at RT. This mixture is stirred at RT for overnight, TLC to monitor reaction. After overnight, the reaction mixture is concentrated and purified via silica column chromatography using hexane: DCM gradient.

**[0127]** Step 4: To a mixture of DCLK3\_Int3 (1 eq.) and ethyl 2-chloro-2-(hydroxyimino) acetate (2.2 eq) dissolved in 5 mL of ethyl acetate is  $\text{KHCO}_3$  (2 eq.) added. The mixture is flushed under argon and stirred at RT for overnight. After overnight, heptane (5 mL) was added and allowed to stir for an additional 5 minutes. The mixture was filtered using heptane to aid in the filtration. The filtrate is concentrated and purified via silica column chromatography using hexane: DCM gradient.

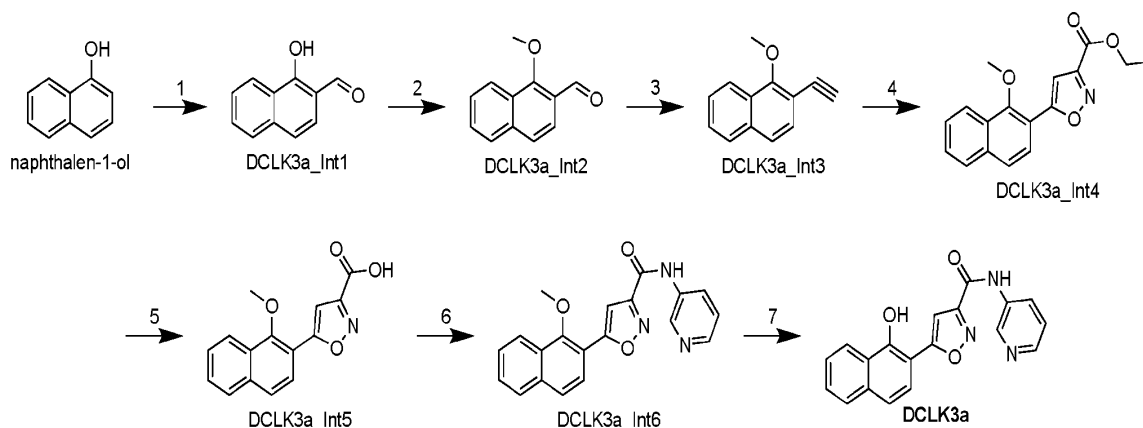
**[0128]** Step 5: To a mixture of DCLK3\_Int4 (1 eq) in MeOH (48 eq) was added 1N NaOH (79 eq). The mixture was stirred at RT for overnight. After overnight, the reaction mixture was further diluted with water (~25 mL) and adjusted the pH to 2~3 using 1M HCl and stirred for an additional hour. Within the hour, a white precipitate was generated. The mixture was then filtered and washed with water. The precipitate was collected and dried under reduced pressure at 50°C.

**[0129]** Step 6: DCLK\_Int5 (1.0 eq.), 4-aminopyridine (2.0 eq.) and DIPEA (5.0 eq.) were dissolved in 1 mL of DMF, flushed under argon and placed in a dry ice: acetonitrile bath in the dark. To this was added a solution of T<sub>3</sub>P in EA (50 wt%, 2.0 eq.) dropwise while keeping the internal temperature between -30°C and -10 °C. Following the dropwise addition, the resulting mixture was warmed to -10°C and stirred for an hour. After, the reaction mixture was warmed to 0°C, and water was added. Ethyl acetate was added to the resulting suspension. The layers were separated and the organic layer was washed with aqueous  $\text{K}_2\text{CO}_3$  solution (5 wt %), and dried over magnesium sulfate followed by filtration and concentration to yield product. This procedure was changed for DCLK3a and DCLK4.

**[0130]** Step 7: DCLK\_Int6 (1 eq.) was dissolved in 1 mL of DCM, placed in an ice bath, and flushed under argon. While at 0°C, 1M  $\text{BCl}_3$  in DCM (68 eq.) is added dropwise. Post dropwise addition, the reaction mixture is kept in the ice bath for overnight. After overnight, the reaction mixture is quenched via the addition of ice water (5 mL) and stirring for an additional hour. The

reaction mixture is then further diluted with water and washed multiple times with DCM. The organic layer is isolated, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated to yield **DCLK3**. No further purification was needed.

**[0131]** Example 1D: Preparation of **DCLK3a**

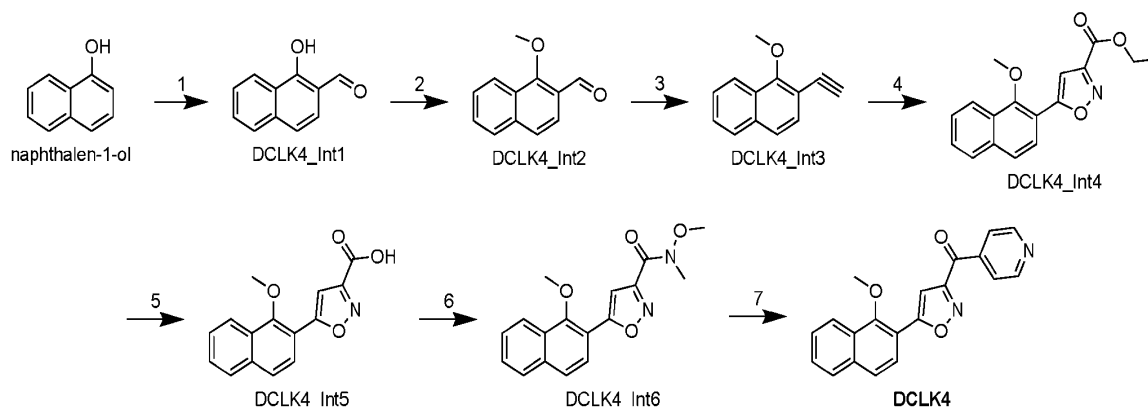


**[0132]** Steps 1-5 for **DCLK3a** are the same as the ones for **DCLK3**.

**[0133]** Step 6: A mixture of DCLK3a\_Int5 (1 eq.), 3-aminopyridine (1.5 eq.), triethylamine (3.4 eq.) in 3 mL of DCM is flushed under argon and placed in an ice bath in the dark. While at 0°C, a solution of T<sub>3</sub>P in ethyl acetate (50 wt%, 1.7 eq.) is added dropwise while stirring. Post dropwise addition the reaction mixture is allowed to stir at 0°C for an additional hour. This is then allowed to warm to RT and left to stir for 3-4 days in the dark. TLC (5: 95 Methanol: DCM) to monitor the reaction. After the reaction time, the mixture is further diluted with DCM and the organic layer is washed with water (x2), saturated sodium bicarbonate (x1) and brine (x1). The organic layer is isolated, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The crude residue was purified via silica column chromatography using DCM: Methanol gradient (99:1 → 90:10).

**[0134]** Step 7: DCLK\_Int6 (1 eq.) was dissolved in 1 mL of DCM, placed in an ice bath, and flushed under argon. While at 0°C, 1M BCl<sub>3</sub> in DCM (68 eq.) is added dropwise. Post dropwise addition, the reaction mixture is kept in the ice bath for overnight. After overnight, the reaction mixture is quenched via the addition of ice water (5 mL) and stirring for an additional hour. The reaction mixture is then further diluted with water and washed multiple times with DCM. The organic layer is isolated, dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated. The crude residue was purified via silica column chromatography using DCM: Methanol (99:1) to yield **DCLK3a**.

**[0135]** Example 1E: Preparation of **DCLK4**



[0136] Steps 1-5 are the same as for **DCLK3**.

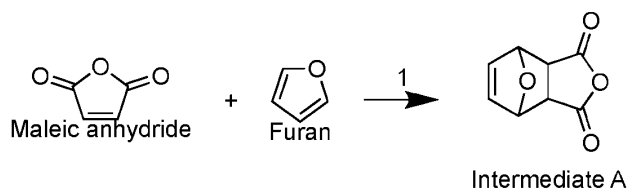
[0137] Step 6: A mixture of DCLK4\_Int5 (1 eq.), N,O-Dimethylhydroxylamine hydrochloride (1.5 eq.), triethylamine (3.4 eq.) in 3 mL of DCM is flushed under argon and placed in an ice bath in the dark. While at 0°C, a solution of T<sub>3</sub>P in ethyl acetate (50 wt%, 1.7 eq.) is added dropwise while stirring. Post dropwise addition the reaction mixture is allowed to stir at 0°C for an additional hour. This is then allowed to warm to RT and left to stir for 3-4 days in the dark. TLC (5: 95 Methanol: DCM) to monitor the reaction. After the reaction time, the mixture is further diluted with DCM and the organic layer is washed with water (x2), saturated sodium bicarbonate (x1) and brine (x1). The organic layer is isolated, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated to yield crude product. It is important to note that DCLK4\_Int6 is light sensitive and must be kept in the dark during synthesis and storage. This will quickly degrade if not handled properly. Purification efforts of this all resulted in degradation. As such, DCLK4\_Int6 is used crude.

[0138] Step 7: To a suspension of 4-pyridylmagnesium bromide in THF, which was freshly prepared by treating 4-iodopyridine (16.6 mmol, 6.5 eq.) in 55 mL of THF with 16.6 mL of 1.0M ethyl magnesium bromide at room temperature, was added DCLK4\_Int6 (1 eq.) in 3.5 mL of THF dropwise. After 2 hours, the reaction was quenched with 1.0 mL of acetic acid and sat. NH<sub>4</sub>Cl, and then extracted with 2 x ethyl acetate at 0-5 °C. The organic layer extracts were dried over MgSO<sub>4</sub>, filtered, concentrated by rotary evaporator. The residue was suspended in ethyl acetate, filtered, and washed the filter cake with ethyl acetate, to give a light-yellow solid as the first crop of product. The filtrate was concentrated with silica gel to dryness, providing a free-flowing solid, which was dry loaded onto a column of silica gel wet with heptane. The column was sequentially eluted with

ethyl acetate: heptane (1:3, 1:2 and 1:1), the resulting product fractions were combined and distilled under vacuum at 35 °C to give the second crop of product as a white solid.

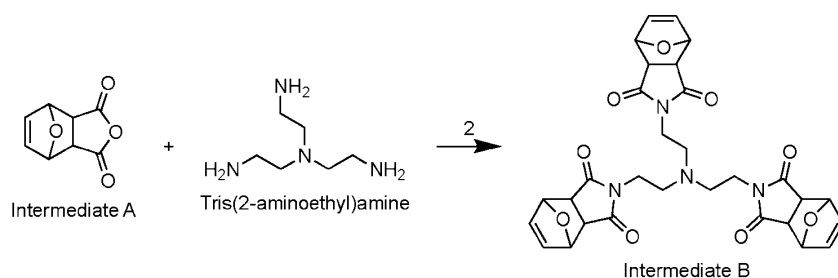
## EXAMPLE 2

### TRIMALEIMIDE LINKER (CORE) SYNTHESIS



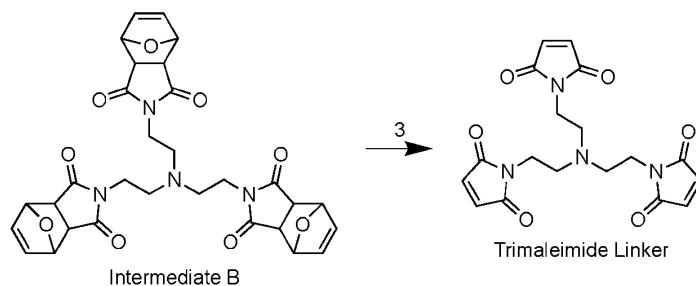
**[0139]** Step 1: To a dry round bottom flask is maleic anhydride (1 eq.) added and dissolved in 5-10 mL of tetrahydrofuran. To this mixture is furan (1 eq.) added dropwise at RT while stirring. Post addition the reaction mixture is stirred overnight at RT. During this time, a precipitate may form. After overnight, the reaction mixture is filtered and the precipitate is washed with hexane (3x5mL), collected, and dried *in vacuo*. The filtrate is heated, cooled to RT, and placed in a freezer to induce recrystallization of the product.

**[0140]** If no precipitate forms overnight, 15mL of hexane is added to the reaction mixture. The change in polarity may cause product to precipitate out. If precipitate is formed, filter, collect and dry the precipitate *in vacuo*. If no precipitate generates, subject the mixture to recrystallization as mentioned. Collect the recrystallized product as mentioned before via vacuum filtration. No further purification is necessary.



**[0141]** Step 2: To a dry round bottom flask is intermediate A (5.2 eq) added and dissolved in 10 mL of methanol. This mixture is placed in an ice bath and stirred. A solution of tris(2-aminoethyl) amine (1 eq.) in methanol (3 mL) is prepared and added to cold mixture dropwise. After dropwise addition, the resulting mixture is stirred at 0°C for 5 minutes, and then allowed to warm to RT and stirred for 30 additional minutes. After this time, the reaction is heated to reflux (70°C) for 4 hours.

This is then allowed to cool to RT slowly and placed in a freezer overnight to induce recrystallization of product. This is then filtered, the solid washed with cold methanol (3x5mL), isolated and dried *in vacuo*. No further purification is necessary.



[0142] Step 3: To a dry round bottom flask is intermediate B (1 eq.) added and suspended in 10 mL of toluene. This mixture is heated to reflux (115°C) for overnight. After, the reaction mixture is concentrated, and the product is isolated and further dried *in vacuo* overnight. After the product is collected and no further purification is necessary.

### EXAMPLE 3

#### SOLID PHASE PEPTIDE SYNTHESIS PROTOCOL

[0143] All peptides mentioned in Table 1 and 2 below were synthesized following the Fmoc (*N*-(9-fluorenyl)methoxycarbonyl) solid phase peptide synthesis procedure (Wang H., Udukala D. N., Samarakoon T. N., Basel M. T., Kalita M., Abayaweera G., Manawadu H., Malalasekera A., Robinson C., Villanueva D., Maynez P., Bossmann L., Riedy E., Barriga J., Wang N., Li P., Higgins D. A., Zhu G., Troyer D. L., and Bossmann, S. H. Nanoplatfoms for Highly Sensitive Fluorescence Detection of Cancer-Related Proteases. *Photochemistry & Photobiology Sciences*. **2014**, 13:231–240 and Duro-Castano A., Conejos-Sanchez I., and Vicent M. Peptide-Based Polymer Therapeutics. *Polymers*. **2014**, 6(2):515-551). Briefly, a 2-ClTrt (2-chlorotrityl) resin containing the first amino acid in the peptide sequence (Gly in this case), was first swelled in dichloromethane (DCM) for 20 minutes and then washed with *N,N*-dimethylformamide (DMF) for 1 minute. After washing, a solution containing Fmoc-protected amino acid (resin:amino acid, 1:3 molar ratio) and O-Benzotriazole-*N,N,N',N'*-tetramethyl-uronium-hexafluoro-phosphate (HBTU) as a coupling agent (resin:HBTU, 1:29 molar ratio) was prepared in a 1:23 diisopropylethylamine (DIEA):DMF solution and was added to the resin and allowed to swirled for 30 minutes for the coupling reaction. Each amino acid was added two times to enhance addition

of amino acid to the peptide chain. Before moving to the next amino acid, the last Fmoc-protected amino acid was deprotected using a 20% diethylamine solution in DMF, followed by 5 washes with DMF to remove any excess diethylamine present. Each deprotection and addition of an amino acid is a repeated cycle during peptide synthesis until all amino acids on the peptide sequence chain have been added. After synthesis was completed, some peptides were labeled with cholesterol, cholic acid, and cleaved unlabeled. For cholesterol and cholic acid additions, these were coupled on the N-terminal end of the peptide using 1,1'-Carbonyldiimidazole (CDI) as the coupling reagent. Cholesterol was coupled following 5 additions within 24-hours reaction, while cholic acid was coupled following a total of 7 additions within 24-hours reaction. Cleaving of completed sequences (labeled or unlabeled) is performed using trifluoroacetic acid incubated for 3 hours. After the 3-hr cleaving reaction, peptide is precipitated in cold ether, collected via centrifugation (4,000 xg for 5 min), and washed with cold ether for a total of 4-6 times. Cleaved peptides were then dried and kept under argon at -20°C. S<sub>10</sub>K<sub>20</sub> (SEQ ID NO: 19) and D<sub>10</sub>K<sub>20</sub> (SEQ ID NO:21) block co-peptides were prepared that included a C-terminal linking amino acid (cysteine) and spacer residue (glycine, from resin synthesis).

**Table 1.** Peptide sequences synthesized for DCLK1 nanosponges

| Peptide sequence/name                                                              | Calculated molecular weight (g/mol) |
|------------------------------------------------------------------------------------|-------------------------------------|
| “Don’t eat me” sequence unlabeled<br><i>k</i> *GNYTCEVTELSREGKTVIELKCK <i>k</i> *G | 2786.224                            |
| S <sub>10</sub> K <sub>20</sub> CG unlabeled (SEQ ID NO:18)                        | 3612.478                            |
| D <sub>10</sub> K <sub>20</sub> CG unlabeled (SEQ ID NO:20)                        | 3892.582                            |
| Cholesterol - S <sub>10</sub> K <sub>20</sub> CG (SEQ ID NO:18)                    | 4025.128                            |
| Cholesterol - D <sub>10</sub> K <sub>20</sub> CG (SEQ ID NO:20)                    | 4305.232                            |
| Cholic acid - S <sub>10</sub> K <sub>20</sub> CG (SEQ ID NO:18)                    | 4047.048                            |
| Cholic acid - D <sub>10</sub> K <sub>20</sub> CG (SEQ ID NO:20)                    | 4327.152                            |

*k*\*: *D*-Lys amino acid

**Table 2.** Peptide sequences synthesized for MK2 nanosponges

| Peptide sequence/name | Calculated molecular weight (g/mol) |
|-----------------------|-------------------------------------|
|-----------------------|-------------------------------------|

|                                                                 |          |
|-----------------------------------------------------------------|----------|
| Rhodamine B – EGFR<br><i>s</i> **YHWYGYTPENVIG <i>s</i> **CG    | 2394.108 |
| Cholesterol - S <sub>10</sub> K <sub>20</sub> CG (SEQ ID NO:18) | 4025.128 |
| Cholesterol - K <sub>20</sub> CG (SEQ ID NO:23)                 | 3176.266 |

*s*\*\* : D-Ser amino acid

#### EXAMPLE 4

##### NANOSPONGE ASSEMBLY AND COUPLING OF SMALL MOLECULE PROTOCOLS

[0144] The trimaleimide linker used to assemble nanosponges contains three -NH<sub>2</sub> groups, serving as the anchor to link up to 3 different peptides, which determined the molar ratio to use for assembly. Block co-peptides S/D<sub>10</sub>K<sub>20</sub>CG were used (SEQ ID NO:18 or SEQ ID NO:20). Nanosponges assembled in the presence of the “Don’t eat me peptide” sequence consisted in a 1:2:1 ratio of linker : cholesterol-block co-peptide : “Don’t eat me peptide”. On the other hand, for nanosponges assembled without the “Don’t eat me peptide”, the ration consisted in 1:3 linker : cholesterol/cholic acid- block co-peptide. And lastly, when 3 different peptides were coupled to the same central linker (MK2 nanosponges), the ratio was kept at a 1:1:1:1 for all. In detail, prior to assembly, 1X Phosphate Buffered Saline (PBS, pH 7.2) and anhydrous DMF were degassed with argon. In separate vials, tris-maleimide linker was dissolved in 10-15  $\mu$ L of degassed DMF and peptides were dissolved together in 1-2 mL of degassed PBS. Both solutions were then combined and stirred for 24 hours at room temperature under argon atmosphere (deoxygenated). After 24 hours, nanosponge was transferred to a dialysis membrane tubing (3,500 Da MWCO) to carry dialysis against water for 1-2 hours in order to remove unbound components. Nanosponge was then lyophilized overnight to dry and stored at -20°C. FIG. 5A shows TEM images of assembled S10K20CG-based (SEQ ID NO:18) nanosponge labeled with cholesterol; size average around 96 nm. FIG. 5B shows TEM image for assembled D10K20CG-based (SEQ ID NO:20) nanosponge labeled with cholesterol; size average around 77nm.

[0145] The nanosponges were compared to previous constructs made with cholesterol-(peptide)<sub>3</sub>-trimaleimide units consisting of a trigonal maleimide linker to which homopeptides (either all K or D) of variable lengths (n = 5, 10, 15, 20), SEQ ID NO:23 or 24, and a consensus sequence for executioner caspases (DEVDGC, SEQ ID NO:26) were added via Michael addition.

[0146] Small molecules were coupled to the nanosponges using CDI or HBTU (only for D<sub>10</sub>K<sub>20</sub>CG nanosponges (SEQ ID NO:20)) as the coupling reagents. Briefly, for DCLK1 small molecules, 10% (per weight) of each small molecule and CDI were dissolved in anhydrous DMF and stirred for 10 min at room temperature. In a separate vial, nanosponge was dissolved in DMF and solution was transferred to small molecule-CDI reaction solution; reaction was stirred for 24 hours at room temperature. Similarly, 10% of each MK2 inhibitors (PF3644022, MK2-IN-1, and MK2-IN-3) and CDI were dissolved in DMSO and reacted for 10 min, followed by the addition of nanosponge dissolved in DMF and reaction carried for 24 hours at room temperature. Dialysis was repeated on the nanosponge-small molecule complex for 1 hour to remove any unbound components, followed by lyophilization overnight to dry. Gemcitabine has also been linked to the nanosponge and shown *in vitro* and *in vivo* activity. Using the nanosponge technology to allow for local exposure will enable higher doses of Gemcitabine to be administered to the site of the tumor with lower risk of toxicity and limiting systemic exposure and thus slowing resistance. Scale-up synthesis of the nanosponge linked Gemcitabine and will be optimized to complete *in vivo* efficacy and release characterization of the material. Table 3 and 4 summarize all compounds assembled for each project.

**Table 3.** Nanosponge-small molecule assembled for DCLK1

| Compound  | Nanosponge | SEQ ID NO: | Cholesterol | Cholic acid | Don't eat me sequence | Small molecule coupled |
|-----------|------------|------------|-------------|-------------|-----------------------|------------------------|
| IA-DC-101 | S10K20CG   | 18         | X           |             | Yes                   | MRL16                  |
| IA-DC-102 | S10K20CG   | 18         | X           |             | Yes                   | MRL17                  |
| IA-DC-103 | S10K20CG   | 18         | X           |             | Yes                   | Gemcitabine            |
| IA-DC-104 | S10K20CG   | 18         | X           |             | No                    | MRL16                  |
| IA-DC-105 | S10K20CG   | 18         | X           |             | No                    | MRL17                  |
| IA-DC-106 | S10K20CG   | 18         | X           |             | No                    | Gemcitabine            |
| IA-DC-107 | D10K20CG   | 20         | X           |             | Yes                   | MRL16                  |
| IA-DC-108 | D10K20CG   | 20         | X           |             | Yes                   | MRL17                  |
| IA-DC-109 | D10K20CG   | 20         | X           |             | Yes                   | Gemcitabine            |
| IA-DC-110 | D10K20CG   | 20         | X           |             | No                    | MRL16                  |

|           |          |    |   |   |     |             |
|-----------|----------|----|---|---|-----|-------------|
| IA-DC-111 | D10K20CG | 20 | X |   | No  | MRL17       |
| IA-DC-112 | D10K20CG | 20 | X |   | No  | Gemcitabine |
| IA-DC-113 | S10K20CG | 18 |   | X | No  | - control - |
| IA-DC-114 | S10K20CG | 18 |   | X | No  | Gemcitabine |
| IA-DC-115 | S10K20CG | 18 |   | X | No  | MRL17       |
| IA-DC-116 | S10K20CG | 18 |   | X | No  | DCLK3       |
| IA-DC-117 | S10K20CG | 18 | X |   | No  | - control - |
| IA-DC-118 | S10K20CG | 18 | X |   | No  | DCLK3       |
| IA-DC-119 | D10K20CG | 20 |   | X | No  | - control - |
| IA-DC-120 | D10K20CG | 20 |   | X | No  | DCLK3       |
| IA-DC-121 | S10K20CG | 18 |   | X | No  | DCLK3A      |
| IA-DC-122 | S10K20CG | 18 | X |   | No  | DCLK3A      |
| IA-DC-123 | D10K20CG | 20 |   | X | No  | DCLK3A      |
| IA-DC-124 | D10K20CG | 20 | X |   | No  | - control - |
| IA-DC-125 | S10K20CG | 18 |   | X | Yes | DCLK3A      |

**Table 4.** Nanosponge-small molecule assembled for MK2 inhibitors

| Nanosponge                                                                                                      | Small molecule coupled |
|-----------------------------------------------------------------------------------------------------------------|------------------------|
| RhodamineB-EGFR : Cholesterol-S10K20CG : Cholesterol-K20CG<br>(SEQ ID NO: 22) : (SEQ ID NO:18) : (SEQ ID NO:23) | PF3644022              |
| RhodamineB-EGFR : Cholesterol-S10K20CG : Cholesterol-K20CG<br>(SEQ ID NO: 22) : (SEQ ID NO:18) : (SEQ ID NO:23) | MK2-IN-1               |
| RhodamineB-EGFR : Cholesterol-S10K20CG : Cholesterol-K20CG<br>(SEQ ID NO: 22) : (SEQ ID NO:18) : (SEQ ID NO:23) | MK2-IN-3               |

**EXAMPLE 5****NANOSPONGE IC<sub>50</sub> AND PROPERTIES**

[0147] Compounds were screened using a colorimetric hexosaminidase assay. Briefly, cells are plated in 96 well plates. Cells are then treated with increasing doses of compound for 48 hours. Media was aspirated and cells were incubated with substrate buffer containing p-nitrophenol-N-acetyl-beta-D-glucosaminidase. Plates were read at an absorbance of 405 nm.

[0148] Viability was calculated as a percentage of absorbance of treatment relative to control. The resulting IC<sub>50</sub> data is shown in Table 5.

**Table 5. Nanosponge IC<sub>50</sub>**

| Nanosponge | Small molecule coupled | HUCCT1 48hr IC <sub>50</sub> | HUH28 48hr IC <sub>50</sub> |
|------------|------------------------|------------------------------|-----------------------------|
| IA-DC-101  | MRL16                  | 7 $\mu$ M                    | 10 $\mu$ M                  |
| IA-DC-102  | MRL17                  | 12 $\mu$ M                   | 25 $\mu$ M                  |
| IA-DC-103  | Gemcitabine            | 0.5 $\mu$ M                  | 17 $\mu$ M                  |
| IA-DC-104  | MRL16                  | 3 $\mu$ M                    | 20 $\mu$ M                  |
| IA-DC-105  | MRL17                  | 13 $\mu$ M                   | 7 $\mu$ M                   |
| IA-DC-106  | Gemcitabine            | 0.7 $\mu$ M                  | 8 $\mu$ M                   |
| IA-DC-107  | MRL16                  | 6 $\mu$ M                    | 10 $\mu$ M                  |
| IA-DC-108  | MRL17                  | 7 $\mu$ M                    | 12 $\mu$ M                  |
| IA-DC-109  | Gemcitabine            | 0.5 $\mu$ M                  | 22 $\mu$ M                  |
| IA-DC-110  | MRL16                  | 12 $\mu$ M                   | 9 $\mu$ M                   |
| IA-DC-111  | MRL17                  | 15 $\mu$ M                   | 8 $\mu$ M                   |
| IA-DC-112  | Gemcitabine            | 0.625 $\mu$ M                | 40 $\mu$ M                  |
| IA-DC-113  | - control -            | 52 $\mu$ M                   | 25 $\mu$ M                  |
| IA-DC-114  | Gemcitabine            | 1 $\mu$ M                    | 30 $\mu$ M                  |
| IA-DC-115  | MRL17                  | 25 $\mu$ M                   | 35 $\mu$ M                  |
| IA-DC-116  | DCLK3                  | NA                           | 25 $\mu$ M                  |
| IA-DC-117  | - control -            | NA                           | 32 $\mu$ M                  |
| IA-DC-118  | DCLK3                  | 40 $\mu$ M                   | 80 $\mu$ M                  |
| IA-DC-119  | - control -            | 50 $\mu$ M                   | 30 $\mu$ M                  |
| IA-DC-120  | DCLK3                  | 50 $\mu$ M                   | 40 $\mu$ M                  |
| IA-DC-121  | DCLK3A                 | 17 $\mu$ M                   | 50 $\mu$ M                  |
| IA-DC-122  | DCLK3A                 | 35 $\mu$ M                   | 70 $\mu$ M                  |
| IA-DC-123  | DCLK3A                 | 20 $\mu$ M                   | NA                          |
| IA-DC-124  | - control -            | 9 $\mu$ M                    | 80 $\mu$ M                  |

[0149] For colony formation, cells were plated in 6-well plates. Cells were then treated with test articles at IC50, and ½ IC50 doses for 48h. Media is then aspirated and replaced with complete media without drug. Colonies are then allowed to grow for 2 weeks before staining, counting, and size determination using ImageJ software.

[0150] For spheroid formation, cells were plated in 24-well low attachment plates. Spheroids were allowed to form for 72 hours before the addition of test articles at IC50, ½ IC50, and ¼ IC50 doses. Spheroids were allowed to grow for an additional 96 hours before imaging and quantification.

[0151] FIG. 6 (top) shows representative colony formation images of HUCCT1 cells treated with Gemcitabine (Gem), IA-DC-103, DCLK3a, IA-DC-125 for 48h with IC50 and ½ IC50 as previously determined by hexosaminidase assay. (bottom) Quantification of colony number and size. Data are represented as mean number and size from 3 independent experiments, \* p<0.05.

[0152] FIG. 7 (top) shows representative colony formation images of HUH28 cells treated with Gemcitabine (Gem), IA-DC-103, DCLK3a, IA-DC-125 for 48h with IC50 and ½ IC50 as previously determined by hexosaminidase assay. (bottom) Quantification of colony number and size. Data are represented as mean number and size from 3 independent experiments, \* p<0.05.

[0153] FIG. 8 shows (left) representative spheroid formation images of HUCCT1 cells treated with Gemcitabine (Gem), IA-DC-103, DCLK3a, IA-DC-125 at IC50, ½ IC50 and ¼ IC50 doses, as previously determined by hexosaminidase assay. (Right) Quantification of spheroid number. Data are represented as the mean from 3 independent experiments, \* p<0.05.

[0154] The calculated molecular weights (g/mol), log P, calculated solubility (mol/L), experimental solubility (mg/mL), measured dynamic light scattering (DLS) in nm, polydispersity index (PDI), and measured zeta potential (mV) are shown in Table 6 for selected nanosponges.

**Table 6. Nanosponge Properties**

| Nanosponge                  | Calculated<br>MW<br>(g/mol) | LogP  | Calculated<br>solubility<br>(Mol/L) | Experimental<br>solubility<br>(mg/mL) | Measured<br>DLS<br>(nm) | PDI   | Measured<br>zeta<br>potential<br>(mV) |
|-----------------------------|-----------------------------|-------|-------------------------------------|---------------------------------------|-------------------------|-------|---------------------------------------|
| <b>Previous Constructs:</b> |                             |       |                                     |                                       |                         |       |                                       |
| (Cholesterol-K20DEV DGC     | 11224.714                   | -0.64 | 3.16                                | >400                                  | 218.18                  | 0.292 | 40.31                                 |

| Nanosponge                                                                                                         | Calculated<br>MW<br>(g/mol) | LogP  | Calculated<br>solubility<br>(Mol/L) | Experimental<br>solubility<br>(mg/mL) | Measured<br>DLS<br>(nm) | PDI   | Measured<br>zeta<br>potential<br>(mV) |
|--------------------------------------------------------------------------------------------------------------------|-----------------------------|-------|-------------------------------------|---------------------------------------|-------------------------|-------|---------------------------------------|
| )3-linker*,<br>SEQ ID NO:25                                                                                        |                             |       |                                     |                                       |                         |       |                                       |
| Cholesterol-<br>D20DEV DGC<br>) , SEQ ID<br>NO:24- 2-<br>(NLS<br>– CGGPKKK<br>RKVG G)1-<br>linker, SEQ ID<br>NO:27 | 8303.019                    | -3.08 | 182                                 | >100                                  | 266.40                  | 0.584 | -17.97                                |
| (Cholesterol-<br>D10K20<br>DEV DGC)3-<br>linker, SEQ ID<br>NO:28                                                   | 14677.38                    | -1.60 | 9.54                                | >780                                  | 324.07                  | 0.336 | 14.63                                 |
| <b>New Constructs:</b>                                                                                             |                             |       |                                     |                                       |                         |       |                                       |
| (Cholesterol-<br>D10K20CG)3-<br>linker, SEQ ID<br>NO:20                                                            | 13302.096                   | -1.28 | 7.41                                | >800                                  | 583.39                  | 0.314 | 32.69                                 |
| (Cholesterol-<br>S10K20CG)3-<br>linker, SEQ ID<br>NO:18                                                            | 12461.784                   | -1.30 | 8.12                                | >850                                  | 467.18                  | 0.335 | 25.55                                 |

| Nanosponge                                               | Calculated<br>MW<br>(g/mol) | LogP | Calculated<br>solubility<br>(Mol/L) | Experimental<br>solubility<br>(mg/mL) | Measured<br>DLS<br>(nm) | PDI | Measured<br>zeta<br>potential<br>(mV) |
|----------------------------------------------------------|-----------------------------|------|-------------------------------------|---------------------------------------|-------------------------|-----|---------------------------------------|
| (Cholesterol-<br>K20CG)3-<br>linker. SEQ ID<br>NO:23     |                             | 0.83 | -0.13                               |                                       |                         |     |                                       |
| (Cholic Acid-<br>D10K20CG)3-<br>linker, SEQ ID<br>NO:20  |                             | 10.7 | -1.65                               |                                       |                         |     |                                       |
| (Cholic Acid -<br>S10K20CG)3-<br>linker, SEQ ID<br>NO:18 |                             | 12.3 | -1.71                               |                                       |                         |     |                                       |

The calculated solubility and log P were performed via a segmented approach using SWISSADME [www.swissadme.ch/](http://www.swissadme.ch/).

\*A consensus sequence for executioner caspases (DEVDGC, SEQ ID NO:26).

[0155] Additional nanosponges have been synthesized or are proposed for synthesis (Table 7).

**Table 7. Additional nanosponges.**

| Nanosponge                                                                                                                                                     | Small molecule<br>coupled |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------|
| Cholesterol- D <sub>10</sub> K <sub>20</sub> CG (SEQ ID NO:20)<br>Rhodamine B-CD44 (kHPWSYLWTQQAkGCG) (SEQ ID NO:29)                                           | Prochlorperazine          |
| Cholesterol- S <sub>10</sub> D <sub>20</sub> CG: (SEQ ID NO:30)<br>Don't eat me signal peptide:<br>Therapeutic peptide P17 (TAFGASLFVPPSHVQFIG) (SEQ ID NO:12) | none                      |
| Cholesterol- S <sub>10</sub> D <sub>20</sub> CG: (SEQ ID NO:30)<br>Don't eat me signal peptide:                                                                | none                      |

|                                                                                                                                                                          |                                |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------|
| Therapeutic peptide KP17 (TAFGASLFVPPSHVQFIGGKKKKKG)<br>(SEQ ID NO:13)                                                                                                   |                                |
| Cholesterol-S <sub>10</sub> D <sub>20</sub> CG: (SEQ ID NO:30)<br>Don't eat me signal peptide:<br>Therapeutic peptide DP17 (TAFGASLFVPPSHVQFIGGDDDDDG)<br>(SEQ ID NO:14) | none                           |
| Cholesterol-S <sub>10</sub> K <sub>20</sub> CG (SEQ ID NO:18)<br>CD47 or CD49 signaling peptide                                                                          | p53 inhibitor<br>(KU-compound) |
| Cholesterol- S <sub>10</sub> K <sub>20</sub> CG (SEQ ID NO:18)<br>CD206 targeting peptide:<br>Don't eat me signal peptide                                                | Ingenol-3-<br>Angelate         |

**EXAMPLE 6****NANOSPONGE-MK2 INHIBITOR DATA**

**[0156]** Cell culture. BJ(hTERT) and patient-derived cancer associated fibroblasts (CAF) 036 and 067 were obtained from the University of Colorado Denver Anschutz Cancer Pavillion from Dr. Antonio Jimeno. BJhTERT cells were cultured and maintained in DMEM (Invitrogen) supplemented with 10% FBS and 1% penicillin/streptomycin. All cells tested negative for mycoplasma. Cell confluency was measured using Countess II FL hemacytometer. Cells were treated with a log dilution of MK2 inhibitor (PF-3644022), MK2 inhibitor 1 (MK2i1), MK2i+nanosponge, or nothing (control). Cells were stimulated with 2ng/mL TGFβ1. MK2i and MK2i+nanosponge were added 2 h prior to stimulation with TGFβ1. Cells were grown an additional 48 h from stimulation at which time they were harvested for protein and mRNA analysis. Our protocol was adapted from an established IPF study [PMID: 23470623]. CAF 036 and 067 were treated in a similar fashion to BJ(hTERT).

**[0157]** Immunoblotting. At 48 hours, cells were lysed in ice cold RIPA buffer (ThermoFisher) with 1x concentration protease and phosphatase inhibitor (Pierce). Protein concentration was determined using standard BCA protein assay (ThermoFisher). Proteins were separated by gradient SDS-PAGE and 25 µg total protein was loaded per lane. Gels were transferred to a PVDF membrane using iBlot2 (Invitrogen). The following antibodies were used: MK2 (Cell Signaling Technologies #12155; clone D1E11; 1:10,000 dilution), pMK2 (Cell Signaling Technologies

#3007; Thr334; Clone 27B7; 1:10,000 dilution), p-MK2 (ThermoFischer; PA5-12619, 1:10,000 dilution), HSP27 (Cell Signaling technologies; #2402; clone G31, 1:10,000 dilution), pHSP27 (Cell Signaling Technologies; #9707; Ser82, clone D1H2F6, 1:3,000 dilution). Standard horse radish peroxidase conjugated secondary antibodies were used and detected using Super Signal West Femto (ThermoFisher).

[0158] We observed with constant TGF $\beta$ 1 stimulation, a time dependent increase and steady state expression of collagen 1 by immunoblot (FIG. 9). We attempted to immunoblot  $\alpha$ -SMA. However, this protein cannot be detected as the immortalization of these cells through TERT prevents expression (data not shown). After treatment with the nanosponge backbone alone, it was concluded that higher concentrations (50uM) are cytotoxic and would also induce the p38-MK2 pathway. It was also observed that the nanosponge alone will induce collagen production (FIG. 10). We performed a drug dose response study and tested both the MK2 inhibitor, PF-3644022, and showed that PF-3644022 could reduce MK2 phosphorylation better than the PF-3644022-nanosponge conjugate (PF conjugate) (FIG. 11). The nanosponge conjugate drug only reduced collagen expression at 50  $\mu$ M and was also found to be cytotoxic (FIG. 11). Next, we performed another drug dose response study evaluating the impact of PF-3644022 vs PF conjugate and immunoblotted for p-HSP27, a known downstream phosphorylation target for MK2. PF-3644022 reduced BJ(hTERT) HSP27 phosphorylation 1 order of magnitude lower concentration compared to PF-conjugate. In contrast, we also examined patient CAF 067 which demonstrated a dose response effect with PF conjugate and was better with suppressing HSP27 phosphorylation over PF-3644022 drug alone (FIG. 12).

[0159] MK2i1 was not shown to decrease MK2 phosphorylation, nor impact downstream targets, either as the naked drug or conjugate (FIG. 13). In conclusion, PF-3644022 is the more effective MK2 inhibitor, but increased inhibitory efficacy of conjugation with the nanosponge is inconclusive. As PF-3644022 is extremely hydrophobic, the nanosponge carrier has improved drug solubilization compared to the naked drug alone.

## EXAMPLE 7

### NANOSPONGE-PROCHLORPERAZINE DATA

[0160] **Nanosponge-prochlorperazine (PCZ) Synthesis.** In order to increase drug accumulation and potency of PCZ to translate to clinics, nanosponges were designed and loaded with PCZ. The

peptide-based nanosponges for advanced drug delivery comprised of a trigonal tris-maleimide linker, a D<sub>10</sub>K<sub>20</sub>CG (SEQ ID NO:20) peptide building block label with cholesterol and CD44-targeting peptide sequence (sHPWSYLWTQQAAs (SEQ ID NO:1)) labeled with rhodamine B for drug delivery to PDAC. For *in vivo* experiments, the “Don’t eat me” peptide sequence kGNYTCEVTELSREGKTVIELKk (SEQ ID NO:7) (bearing D-lysines at both terminals ends to prevent rapid proteolytic degradation) was included in order to enhance the circulation time of the nanosponges in blood. For *in vivo* designed nanosponges, the molar mass used for assembly of nanosponges was as follows, 5 (cholesterol labeled D<sub>10</sub>K<sub>20</sub>CG, (SEQ ID NO:20)) : 1 (trimalimide linker): 1 (rhodamine B labeled CD44): 1 (“Don’t eat me” peptide”). Upon CD44 receptor-mediated endocytosis, the nanosponges can quickly escape into the cytoplasm. After assembling nanosponges, these were loaded with prochlorperazine dimeleate salt (PCZ), 10% per weight.

**[0161] Characterization.** PCZ nanosponges were characterized using TEM, and an exemplary TEM image is shown in FIG. 14. Percent loaded of PCZ in nanosponge was also determined. A calibration curve was developed for PCZ drug alone, and then a known concentration of PCZ-nanosponge was measured using 320nm absorbance and plotted into the calibration curve to calculate amount and percentage of PCZ loaded in nanosponge. Results demonstrated that nanosponge was loaded with 10.12% of PCZ, as shown in FIG. 15.

**[0162] Cell and animal experiment results.**

**[0163] Effect of PCZ nanosponges on the proliferation of pancreatic cancer cells.** We treated pancreatic cancer cells MiaPaCa-2 cells with prochlorperazine and its nanoparticles from 0-30  $\mu$ M concentrations. We recorded the cell viability using hexosaminidase activity at 24 h, 48 h and 72 h. We observed that both prochlorperazine and its nanosponges inhibited the growth of the pancreatic cancer cell line in a dose and time-dependent manner (FIG. 16).

**[0164] Cell uptake assay of prochlorperazine nanosponges in pancreatic cancer cells.** To confirm the uptake, we treated MiaPaCa-2 and S2-007 cells with PCZ nanosponges, fixed them, stained them with DAPI (blue nucleus) and phalloidin (green cytoplasm), and imaged them using confocal microscopy. Rhodamine B is tagged to nanoparticles that were imaged red. We found a significant uptake of nanoparticles PDAC cells (FIG. 17).

**[0165] *In vivo* uptake.** To confirm nanosponge uptake in mice by tracking accumulation of nanoparticles in PDAC tumors in mice using IVIS, we injected rhodamine B-labeled PCZ nanosponges in KPCC-orthotopic tumors carrying C57BL/6 mice at a dose of 10 mg/kg

intravenously and imaged *Ex-Vivo* after 24 h. We observed a significant uptake of PCZ nanosponges in the tumors (FIG. 18).

## EXAMPLE 8

### NANOSPONGES FOR PKD TREATMENT

**[0166] Synthesis.** Nanosponges were designed for polycystic kidney disease (PKD) treatment by using modified peptide fragments from the stalk sequence in the polycystin-1 (PC1) C-terminal fragment, which are, P17 (TAFGASLFVPPSHVQFIG (SEQ ID NO:12)), KP17 (TAFGASLFVPPSHVQFIGGKKKKKG (SEQ ID NO:13)), DP17 (TAFGASLFVPPSHVQFIGDDDDDDG (SEQ ID NO:14)). These nanosponges consisted of a trigonal tris-maleimide linker, a D10K20CG (SEQ ID NO:20) peptide building block labeled with cholesterol, the short PC1 CTF fragment (P17, KP17, and RP17), and a “don’t eat me” peptide sequence (kGNYTCEVTELSREGKTVIELKk (SEQ ID NO:7)), with a molar ratio of 1:2:1:1 respectively.

**[0167] Characterization.** Each of the PC1 CTF peptides were characterized using LC-MS, which showed characteristic peaks confirming each of the P17 (calculated MW 1875.16 g/mol, 96% purity), KP17(calculated MW 2630.12 g/mol, 98% purity), and DP17 peptides (calculated MW 2564.71 g/mol, 95% purity) (graphs not shown).

**[0168] Cell experiments.** P17, KP17, and DP17 peptides were tested at a concentration of 1mM for each in HEK293T (wildtype) and HEK293T-PKD1-KO-c4 (CRISPR-generated) cell lines and incubated overnight for ~20 hours. KP17 peptide demonstrated to have the highest activity in comparison to all other peptides and controls tested in both cell lines, followed by P17 fragment peptide. The results are shown in FIG. 19.

## EXAMPLE 9

### APATANIB AS MK2 INHIBITOR

**[0169]** Multiple models demonstrate that TGF $\beta$ 1-mediated fibrosis is caused by activated myofibroblast that signals through the TGF $\beta$ 1R-p38-MK2 signaling axis and direct inhibition of MK2 can prevent myofibroblast development and subsequent fibrotic injury in idiopathic pulmonary fibrosis and cardiac fibrosis injury models. Furthermore, activation of pro-angiogenic

signaling processes have similarly been shown to promote soft tissue fibrosis in liver, bowel and lung injury models *in vivo*.

[0170] While screening for novel MK2 inhibitors using our p38 $\alpha$ -MK2 high throughput screening assay, we identified the VEGFR2 inhibitor, Apatanib. Our preliminary findings demonstrated that Apatanib could inhibit MK2 phosphorylation (preventing MK2 activation), reduce HSP27 phosphorylation (downstream target of MK2) and subsequently reduce collagen-1 protein production. However, there are no FDA-approved MK2 inhibitors and early generation small molecule MK2 inhibitors lack “druggability” because of lack of enzymatic ATP-pocket selectivity and poor water solubility lead to unrealistically high EC50 levels needed to achieve therapeutic benefit.

EXAMPLE 10

NANOSPONGES FOR PDAC TREATMENT TARGETING PLECTIN

[0171] **Synthesis.** In order to increase nanosponge accumulation in pancreatic ductal adenocarcinoma (PDAC), a nanosponge targeting plectin was designed. Plectin nanosponge consisted of a trigonal tris-maleimide linker, a S<sub>10</sub>K<sub>20</sub>CG (SEQ ID NO:18) peptide building block label with cholesterol and plectin-targeting peptide sequence (sHPWSYLWTQQAs, (SEQ ID NO:1)) labeled with rhodamine B to track and confirm delivery into the tumor site. For *in vivo* experiments, the “*Don’t eat me*” peptide sequence kGNYTCEVTELSREGKTVIELKk (SEQ ID NO:7). For *in vivo* designed nanospoenges, the molar mass used for assembly of nanospoenges was as follows, 2 (cholesterol-labeled S<sub>10</sub>K<sub>20</sub>CG, (SEQ ID NO:18)) : 1 (trimaleimide linker): 1 (rhodamine B labeled plectin): 1 (“Don’t eat me” peptide”).

[0172] **Characterization.** Characterization of S10K20CG (SEQ ID NO:18) using DLS and zeta potential

| Nanosponge                                                          | DLS (nm) | PDI   | Zeta potential (mV) |
|---------------------------------------------------------------------|----------|-------|---------------------|
| S <sub>10</sub> K <sub>20</sub> CG-based nanosponge, (SEQ ID NO:18) | 218..18  | 0.292 | 25.55               |

[0173] **Cell and animal experiments.** PDAC bearing mice, were treated with plectin-nanosponge after tumor was developed and had grown for over 3 weeks. After IP injecting plectin-nanospoenges in mice and incubating overnight, mice were sacrificed and various organs and tumor tissues were removed and imaged *ex vivo* to determine localization of plectin-nanosponge. IVIS imaging

confirmed specific uptake of plectin-nanosponge in tumor site only, with no uptake in other healthy organs. The results are shown in FIG. 20 and FIG. 21.

**[0174]** When introducing elements of the present disclosure or the preferred embodiments(s) thereof, the articles "a", "an", "the" and "said" are intended to mean that there are one or more of the elements. The terms "comprising", "including" and "having" are intended to be inclusive and mean that there may be additional elements other than the listed elements.

**[0175]** In view of the above, it will be seen that the several objects of the disclosure are achieved and other advantageous results attained.

**[0176]** As various changes could be made in the above methods without departing from the scope of the disclosure, it is intended that all matter contained in the above description shall be interpreted as illustrative and not in a limiting sense.

## CLAIMS:

1. A peptide nanosponge comprising a plurality of self-assembled peptide building blocks, wherein said plurality of self-assembled peptide building blocks comprises a first peptide building block comprising:
  - a branched polymeric core comprising at least three arms;
  - a first block co-peptide of 50 amino acids or less covalently linked via its C-terminus to one of said arms, wherein said first block co-peptide comprises a first peptide block and a second peptide block which is different from the first peptide block;
  - a lipid-based capping moiety having a molecular weight of 400 Da or less covalently attached to an N-terminal amino acid of the first block co-peptide; and
  - a therapeutically active compound covalently attached via a cleavable linkage to one or more amino acids of the first block co-peptide.
2. The peptide nanosponge of claim 1, wherein said branched polymeric core is a dendrimer-like branched or hyperbranched polymeric structures of 800 Da or less.
3. The peptide nanosponge of claim 1 or claim 2, wherein said arms of said branched polymeric core terminate in functional groups for peptide bonding selected from the group consisting of maleimide groups, carboxylate groups, amide groups, and hydroxyl groups, wherein said first block co-peptide is resistant to cleavage from said branched polymeric core.
4. The peptide nanosponge of any one of claims 1 to 3, comprising a cysteine and/or glycine spacer residue between said block co-peptide and said arm.
5. The peptide nanosponge of any one of claims 1 to 4, wherein the core is derived from a tris(maleimide) compound or analog thereof.
6. The peptide nanosponge of any one of claims 1 to 5, wherein said first peptide block consists of amino acids having positively charged side chains.

7. The peptide nanosponge of claim 6, wherein said first peptide block consists of amino acids selected from the group consisting of lysine, histidine, and arginine.
8. The peptide nanosponge of claim 6, wherein said first peptide block is a homopeptide consisting of 10 to 25 lysine residues.
9. The peptide nanosponge of any one of claims 1 to 8, wherein said second peptide block consists of natural or non-natural amino acids having side chains terminating in amine, hydroxyl or carboxylic acid groups.
10. The peptide nanosponge of claim 9, wherein said second peptide block consists of amino acids selected from the group consisting of serine, aspartic acid (or aspartate), tyrosine, glutamic acid (or glutamate), and threonine.
11. The peptide nanosponge of claim 9, wherein said second peptide block is a homopeptide consisting of 5 to 15 amino acids.
12. The peptide nanosponge of any one of claims 1 to 11, wherein the second peptide block is covalently attached to first block peptide.
13. The peptide nanosponge of any one of claims 1 to 12, said first peptide building block further comprising a signaling sequence covalently attached to a different arm of said branched polymeric core than said block co-peptide.
14. The peptide nanosponge of claim 13, wherein said signaling sequence is attached to said different arm via a respective peptide linkage, wherein said signaling sequence is selected from the group consisting of targeting moieties, detectable labels, "don't eat me" sequences, "eat me" sequences, and combinations thereof.
15. The peptide nanosponge of claim 13 or 14, wherein the signaling sequence comprises a signaling sequence for CD44 comprising sHPWSYLWTQQA<sub>s</sub> (SEQ ID NO:1); a plectin-

targeting sequence comprising KTLLPTPG (SEQ ID NO:2); a signaling sequence for EGFR comprising sYHWYGYTPENVIGsG (SEQ ID NO:3); a signaling sequence for LDLR comprising sCMPRLRGCGAGsG (SEQ ID NO:4); a signaling sequence for CD46 comprising sLPGTICKRTMLDGLNDYCTGsG (SEQ ID NO:5); a signaling sequence for SIRPα “don’t eat me” comprising kGNYTCEVTELSREGKTVELKsG (SEQ ID NO:6); a signaling sequence for SIRPα “don’t eat me” comprising kGNYTCEVTELSREGKTVELKk (SEQ ID NO:7); a signaling sequence for SIRPα “eat me” comprising 4N1: RFYVVMWK (SEQ ID NO:8) or 7N3 FIRVVMYEGKK (SEQ ID NO:9); a signaling sequence for CD206 “shut down M2 polarization” (rp-182) comprising KFRKAfKRFFGsG (SEQ ID NO:10); a signaling sequence for Mucosal Membrane: WTAS comprising PLKWPGKKKKGKPGKRKEQEKKRRTRG (SEQ ID NO:11).

16. The peptide nanosponge of any one of claims 1 to 15, wherein the lipid-based capping moiety comprises cholesterol.

17. The peptide nanosponge of any one of claims 1 to 15, wherein the lipid-based capping moiety comprises cholic acid.

18. The peptide nanosponge of any one of claims 1 to 17, wherein the therapeutically active agent is selected from the group consisting of a DCLK1 inhibitor, an MK2 inhibitor, a cytotoxic agent against p53 deficient cells, an MIF inhibitor, or a combination thereof.

19. The peptide nanosponge of any one of claims 1 to 18, wherein said plurality of self-assembled peptide building blocks further comprises a second peptide building block different from said first peptide building block and comprising:

- a branched polymeric core comprising at least three arms;
- a second block co-peptide of 50 amino acids or less covalently linked via its C-terminus to one of said arms, wherein said second block co-peptide comprises a first peptide block and a second peptide block which is different from the first peptide block;
- a lipid-based capping moiety having a molecular weight of 400 Da or less covalently attached to an N-terminal amino acid of the first block co-peptide; and

a signaling sequence covalently attached to a different arm of said branched polymeric core than said block co-peptide.

20. The peptide nanosponge of claim 19, wherein said signaling sequence is attached to said different arm via a respective peptide linkage that is not a block co-peptide.

21. A pharmaceutical composition comprising a plurality of peptide nanosponges according to any one of claims 1 to 20 dispersed in a pharmaceutically-acceptable carrier.

22. A method of treating a disease or disorder in a subject comprising administering a therapeutically-effective amount of a peptide nanosponge according to any one of claims 1 to 20 or a pharmaceutical composition thereof to a subject in need thereof.

23. The method of claim 22, wherein the disease or disorder is colon cancer, cholangiocarcinoma, a genitourinary cancer, a gynecologic cancer, a gastrointestinal cancer, lymphoma, melanoma or skin cancer, a head and neck cancer, lung cancer, intestinal cancer, kidney cancer, pancreatic cancer, breast cancer, stomach cancer, leukemia, bone cancer, thyroid cancer, brain cancer, a glioma, idiopathic pulmonary fibrosis (IPF), head and neck fibrosis, osteosarcoma, or bladder cancer.

24. The method of claim 23, wherein the cancer is colon cancer or a cholangiocarcinoma.

25. A medicament for treating a disease or disorder in a subject in need thereof, the medicament comprising a therapeutically-effective amount of a peptide nanosponge according to any one of claims 1 to 20.

26. Use of a peptide nanosponge according to any one of claims 1 to 20 to treat a disease or disorder in a subject in need thereof.