

TRIM21-BINDING MOLECULES

FIELD OF THE INVENTION

The present invention relates to binding molecules comprising a TRIM21 binding moiety and a protein binding moiety for selective degradation of target proteins. The present invention also relates to compositions comprising these molecules, and their uses in therapy. The present invention also relates to TRIM21 binding molecules, and their uses.

BACKGROUND

Small molecules known as “proteolysis-targeting chimeras” (PROTACs) can be used to deplete a target protein. PROTAC molecules typically comprise a ligand, such as a small-molecule inhibitor of a protein of interest, covalently linked to a ligand of an E3 ubiquitin ligase. Upon the binding of the small-molecule inhibitor to the protein of interest, the PROTAC molecule recruits an E3 ubiquitin ligase, which can ubiquitinate the protein of interest, which targets it for subsequent degradation. PROTAC molecules have been postulated to be a potential therapy in the treatment of a number of diseases, allowing clinicians to specifically target and degrade specific proteins.

However, although PROTAC molecules are selective for the target protein, PROTAC molecules may not discriminate between the different forms of the same protein, and therefore may result in degradation of non-pathogenic forms of the protein.

Therefore, there is a need for molecules that can target the pathogenic forms of proteins. Such molecules would enable more targeted therapy. The use of more targeted protein degradation as a therapeutic strategy could minimise the off-target effects of drugs and avoid or reduce systemic drug exposure.

SUMMARY OF THE INVENTION

The present invention is directed to bifunctional molecules that comprise a TRIM21 binding moiety and a protein targeting moiety. The compounds of the invention are capable of enabling selective degradation of particular forms of the target protein due to their ability to cluster TRIM21 in close proximity to the target protein.

Accordingly, a first aspect of the invention provides a compound of formula (I)

A-L-B

(I)

or a pharmaceutically acceptable salt thereof, wherein:

A is a moiety that binds TRIM21, wherein the molecular weight of A is 1000 Da or less;

L is a linker; and

B is a moiety that binds a target protein.

The target protein is a protein that can comprise a first form (e.g. a pathogenic form) that provides multiple binding sites for B. The target protein may be a protein capable of forming an oligomeric species. When in an oligomeric form, there is provided multiple binding sites for B.

- 5 A second aspect of the invention provides a pharmaceutical composition comprising a compound of the first aspect of the invention, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

- 10 A third aspect of the invention provides an *in vitro* method of selectively degrading oligomeric or pathogenic forms of a target protein, the method comprising contacting the target protein with TRIM21 and a compound according to the first aspect of the invention or a pharmaceutically acceptable salt thereof.

- 15 A fourth aspect of the invention provides a compound having the structure of formula (IV).

A fifth aspect of the invention relates to compounds having the structure of formula (V).

- Further aspects and embodiments of the invention are described below and in the accompanying Figures.

- 20 All preferred features for the second and subsequent aspects of the invention are as for the first aspect *mutatis mutandis*.

BRIEF DESCRIPTION OF THE DRAWINGS

- 25 **Figure 1** shows thermal stabilization of Trim21 PRYSPRY (10 μ M) in the presence of Compounds 36, 37 and 38 (100 μ M) compared to DMSO only control.

- 30 **Figure 2** shows quenching of native fluorescence of Trim21 PRYSPRY upon ligand binding - compounds 36 (black circles), 37 (dark grey squares) and 38 (grey triangles). Data fitted with a 4 parameter binding model.

- 35 **Figure 3** shows a fluorescence polarization displacement assay. White circles show a titration of human IgG-Fc against Alexa-488 labelled Trim21 PRYSPRY. For displacement experiments, 0.5 μ M of IgG-Fc was used with titration of competitor compounds 36 (black circles), 37 (dark grey squares) and 38 (grey triangles).

Figure 4 shows lane view (a) and quantification (b) of TRIM21 protein levels normalised to Hsp60 protein levels and DMSO condition after RPE-1 cells were treated with DMSO or 2.5 μ M Compound

37 for 18h and whole-cell extracts harvest for capillary-based immunoblotting. Significance based on Student's t-test (two-tailed).

Figure 5 shows a schematic of assay for TRIM21-mediated degradation. Expression of the NbGFP-Fc antibody construct leads to TRIM21 recruitment to H2B-mEGFP, causing H2B-mEGFP proteasomal degradation. Compound 37 competes with NbGFP-Fc for binding to TRIM21 PRYSPRY, thus inhibiting TRIM21 recruitment and H2B-mEGFP degradation.

Figure 6 shows H2B-mEGFP fluorescence values normalised to NbGFP-Fc^{H433A} (Fc mutant that cannot bind TRIM21 PRYSPRY; control for no degradation) for each condition (DMSO or Compound 37). A value of 1 equals no degradation. (A) shows time course of degradation in cells treated with DMSO or Compound 37 at 50 μ M and 100 μ M. (B) shows a dose-response curve of H2B-mEGFP degradation in cells treated with a titration of Compound 37 or matched DMSO concentrations.

Figure 7 shows a schematic for inhibition of viral neutralization assay. Cells are infected with Adenovirus 5 (Adv5) in the presence of anti-Adv5 antibody. Normally TRIM21 will neutralize the antibody-bound virus, leading to a reduction in infection as measured by a fluorescent reporter gene (EGFP). Compounds compete with anti-Adv5 antibody for binding to TRIM21 PRYSPRY, thus inhibiting TRIM21-mediated neutralisation.

Figure 8 shows the results of an experiment as depicted in Figure 7. TRIM21 compounds dose-dependently rescue infection (increase in EGFP), demonstrating that they inhibit TRIM21 in cells.

Figure 9 shows confluence of cells after compound treatment.

Figure 10 shows thermal stabilization of Trim21 PRYSPRY in the presence of Compounds 51 (compound 37 as a mixed stereoisomer) and compound 71 (Compound 37 coupled to a short PEG linker and a chloroalkane). The figure shows that binding is only marginally affected as large thermal stabilization is still observed.

Figure 11 shows an AUC velocity experiment; The c(s) distributions show that both TRIM21 PRYSPRY (solid black line) and HaloTag (solid grey line) alone are monomeric sedimenting at 2.3 S (T, Sw,20= 2.5 S) and 2.9 S (H, Sw,20= 3.2 S) respectively with calculated masses of 22.0 and 34.4 kDa with frictional ratios of 1.143 and 1.142 respectively. The mixture alone (black dotted line) results in a single broad distribution that may be just a cumulative Guassian of the two individual unbound species (T - H). Addition of ligand at 10 μ M (black dashed line) reduces the concentration of T21 PRYSPRY and a large reduction in HaloTag with the appearance of a species sedimenting at 3.7 S (T-71-H, Sw,20= 4.1 S). This species has the approximate expected mass of a ternary complex of 57.3 kDa, assuming a frictional ratio of 1.286 (characteristic of an extended non-spherical complex).

Figure 12 shows an AUC equilibration experiment. In the absence of compound, the average mass of 30,316 Da is close to the average of the two proteins at equimolar concentrations. As the concentration of compound increases the average mass reaches a maximum at 5 μ M where the average mass is $50,482 \pm 82$ Da, which is close to the expected value for a 1:1 complex. Increasing concentration beyond this point decreases the average mass due to the binding of the compound to individual components in competition with complex formation.

Figure 13 (A) shows a schematic of cellular ternary complex assay. Cells co-expressing mCherry-TRIM21 and H2B-mEGFP-Halo. Presence of the large mCherry tag on TRIM21 prevents import into the nucleus. Upon cell division transient nuclear envelope breakdown allows access of mCherry-TRIM21 to chromatin. Nuclear localisation of mCherry-TRIM21 following cell division is indicative of ternary complex formation between mCherry-TRIM21, TRIMTAC and H2B-mEGFP-Halo. (B) and (C) show representative cells undergoing cell division. RPE-1 TRIM21 KO cells co-expressing mCherry-TRIM21 and H2B-mEGFP-Halo were treated with 1 μ M Compound 37 (B) or 1 μ M Compound 71 (C) and imaged using the IncuCyte system.

Figure 14: RPE-1 TRIM21 KO cells co-expressing H2B-mEGFP-Halo and either mCherry-TRIM21 (A), mCherry-TRIM21 Δ RB ((B); RING-Box deletion) or mCherry-TRIM21 Δ PS ((C); PRYSPRY deletion) were treated with a titration of Compound 37 or Compound 71 and imaged using the IncuCyte system. At 40h post-compound addition ternary complex was quantified by normalising the red/green overlap area (mCherry-TRIM21 positive nuclei) to total green area (total nuclei). Ternary complex formation requires the PRYSPRY domain of TRIM21. Compound 71 exhibits a bell-shaped concentration-dependence (Hook effect) for ternary complex formation.

Figure 15: (A) RPE-1 TRIM21 KO cells co-expressing mCherryTRIM21 and H2B-mEGFP-Halo were treated with a titration of the indicated compounds and ternary complex formation quantified at 48h by normalising the red/green overlap area (mCherry-TRIM21 positive nuclei) to total green area (total nuclei). (B) RPE-1 cells were plated at ~15% confluence and treated with a titration of the indicated compounds or matched DMSO controls and imaged using the IncuCyte system. Cell confluence was quantified at 72h post-treatment by normalising total cell area per image for each compound concentration to matched DMSO controls. Cells treated with 40 μ M Compound 71 or Compound 109 did not reach 100% confluence indicative of toxicity. No toxicity was observed for any compound at concentrations of 20 μ M or below.

Figure 16: RPE-1 mEGFP-Halo, RPE-1 CAV1-mEGFP-Halo and RPE-1 Cavin1-mEGFP-Halo stable cell lines were treated with the VHL-recruiting PROTAC (HaloPROTAC1) or Compounds 37, 71, 109, 110 and 111 at the indicated concentrations and imaged using the IncuCyte system for 48 hours. GFP fluorescence was quantified by normalising GFP integrated density (then product of area and mean intensity) to total cell area (phase) for each image, and then normalising to matched DMSO conditions so that a value of 1 (grey dashed line) represents no degradation. HaloPROTAC1 degrades all three

proteins, whereas TRIMTACs (Compounds 71, 109, 110 and 111) degrade oligomeric CAV1- and Cavin1-mEGFP-Halo but not monomeric mEGFP-Halo.

Figure 17: RPE-1 mEGFP-Halo, RPE-1 CAV1-mEGFP-Halo and RPE-1 Cavin1-mEGFP-Halo stable cell lines were treated with a titration of the indicated compounds for 48h and GFP fluorescence quantified using the IncuCyte system as described for Figure 16. At all concentrations tested TRIMTAC compounds (71, 109, 110 and 111) selectively degrade oligomeric CAV1- and Cavin1-mEGFP-Halo over monomeric mEGFP-Halo and exhibit a characteristic "PROTAC hook effect" concentration-dependence for degradation.

Figure 18: WT or TRIM21 KO cells stably expressing Cavin1-mEGFP-Halo (A) or CAV1-mEGFP-Halo (B) were treated with a titration of compound 71 for 48h and GFP fluorescence quantified using the IncuCyte system as described for Figure 16. Degradation of oligomeric proteins CAV1- and Cavin1-mEGFP-Halo by Compound 71 is dependent on TRIM21.

Figure 19: (A) – RPE-1 Cavin1-mEGFP-Halo cells were treated with DMSO or 2.5 μ M HaloPROTAC1 or Compound 37 for 24h and whole-cell extracts harvest for capillary-based immunoblotting. The anti-GFP immunoblot shows that in addition to full length Cavin1-mEGFP-Halo there is a smaller species likely corresponding to a cleavage product lacking the Cavin1 sequence (*-mEGFP-Halo). Treatment with HaloPROTAC1 caused a slight reduction in the Cavin1-mEGFP-Halo band and complete loss of the mEGFP-Halo band. Conversely, treatment with Compound 71 caused a substantial reduction in the Cavin1-mEGFP-Halo band, however the *-mEGFP-Halo band remained unaffected. (B) – The Cavin1-mEGFP-Halo and *-mEGFP-Halo bands were normalised to anti-actin bands and expressed as a fraction of total anti-GFP band intensity. Treatment with Compound 71 caused a significant reduction in Cavin1-mEGFP-Halo, but not *-mEGFP-Halo, protein levels. Significance based on Student's t-test (two-tailed).

Figure 20: (A) – RPE-1 Cavin1-mEGFP-Halo cells were treated with DMSO or the indicated compounds at 1.25 μ M (or 10 μ M for Compound 110) for 48hrs and imaged using the IncuCyte system. Cavin1-mEGFP-Halo exhibits bright signal at the cell membranes and some diffuse cytoplasmic fluorescence in control (DMSO) condition. (B) RPE-1 Cavin1-mEGFP-Halo cells were plated on an 8-well chambered polymer coverslip (Ibidi), images were taken before and after treatment with either DMSO or 2.5 μ M HaloPROTAC1 or Compound 71 using an Etaluma Lumascope LS720 widefield microscope equipped with a 40x apochromat 0.95NA air objective housed within a 37C 5% CO2 incubator.

Figure 21: (A) and (B) Representative examples of individual cells from Figure 20(B) pre-treatment and 24h post-treatment with 2.5 μ M HaloPROTAC1 or Compound 71. Graphs show pixel intensities along yellow line in images.

Figure 22: RPE-1 Cavin1-mEGFP-Halo cells were plated and imaged as described in Figure 20(B) 2.5h after treatment with DMSO or 2.5 μ M Compound 37 or 2.5 μ M Compound 37 + 25 μ M MG132. Graphs show pixel intensities along yellow line in images. Dashed line shows peak maximum cytosolic pixel intensity, peaks above this line represent membrane associated fluorescence.

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Figure 23: Shows the crystal structures of Trim21-PRYSPRY in complex with compounds 36 (A), 37(B) and 38 (C). Trim21 PRYSPRY is shown as a surface representation with a number of residues which form the binding site shown in stick representation (colored white) and labelled. Key residues are underlined. Ligands themselves are shown as stick representations (colored black). (B) also includes arrows (black) which indicate potential exit for developing hetero-bifunctional molecules. This is also shown in (D) using a skeletal representation of compound 37.

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Figure 24: Shows a superposition of the complex between TRIM21 and compound 37 and the complex between TRIM21 and IgG Fc (PDB: 2IWG). The antibody residues forming the natural epitope for TRIM21 are shown in white (HNHY, labelled) and compound 37 in black.

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Figure 25: RPE-1 mEGFP-FKBP(F36V) and RPE-1 Cavin-1-mEGFP-FKBP(F36V) stable cell lines were treated with Compound 414 (Cmp 414) (5 μ M) or the CRBN-recruiting (dTAG13) or VHL-recruiting (dTAGv1) PROTACs (80 nM) and GFP fluorescence quantified using the IncuCyte system as described in Figure 16. dTAG13 and dTAGv1 degrade both FKBP(F36V) fusion proteins with a preference of monomeric mEGFP-FKBP(F36V), whereas Cmp 414 degrades oligomeric Cavin1-mEGFP-FKBP(F36V) but not monomeric mEGFP-FKBP(F36V).

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Figure 26: RPE-1 mEGFP-FKBP(F36V) and RPE-1 Cavin-1-mEGFP-FKBP(F36V) stable cell lines were treated with a titration of Cmp 414, dTAG13 or dTAGv1 for 8h and GFP fluorescence quantified using the IncuCyte system as described in Figure 16. Cmp 414 degrades oligomeric Cavin-1-mEGFP-FKBP(F36V) in a concentration-dependent manner, but does not degrade monomeric mEGFP-FKBP(F36V). dTAG13 and dTAGv1 degrade both FKBP(F36V) fusion proteins with a preference of monomeric mEGFP-FKBP(F36V)

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Figure 27: WT or TRIM21 KO cells stably expressing Cavin-1-mEGFP-FKBP(F36V) were treated with a titration of Cmp 414 and images using the IncuCyte system for 8h and GFP fluorescence quantified as described in Figure 16. (A) At 5 μ M Cmp414 rapidly degrades Cavin-1-mEGFP-FKBP(F36V) in WT cells but not TRIM21 KO cells. (B) Concentration-dependent degradation of Cavin-1-mEGFP-FKBP(F36V) is dependent on TRIM21.

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Figure 28: (A) Expression construct comprises full length Myd88 c-terminally fused to the GyrB domain of *Escherichia coli* DNA gyrase. The GyrB domain dimerizes upon binding to coumermycin, a bivalent antibiotic. Coumermycin-induced GyrB dimerization induces Myd88 oligomerization and active Myddosome assembly. The mEGFP tag is included for visualization. The FKBP(F36V) domain allows

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recruitment of TRIM21 via Cmp 414. (B) U2OS cells expressing Myd88-GyrB-mEGFP-FKBP(F36V) were treated for 5h with coumermycin (100 nM) to induce Myddosome assembly, or mock treated (control). 5h post-coumermycin/mock treatment (Time 0; dotted line) cells were treated with DMSO, Cmp 414 (5 μ M) or Compound 37 (Cmp 37) (5 μ M) and GFP fluorescence imaged and quantified using the IncuCyte system as described in Figure 16. Cmp 414 does not degrade Myd88 in control-treated cells, but rapidly degrades Myd88 in coumermycin-treated cells. DMSO or Cmp 37 addition has no effect on Myd88 levels. (B) Representative images from A. In control-treated cells Myd88 is monomeric, exhibiting diffuse cytosolic distribution. Coumermycin treatment induces Myddosome assembly as observed by accumulation of cytosolic Myd88 puncta (Time -17 min). Cmp 414 degrades Myd88 puncta, but not diffuse Myd88, resulting in a partial reduction in Myd88 fluorescence in coumermycin-treated cells as quantified in A.

Figure 29: (A) Expression construct comprises full length RIPK3 N-terminally fused to mEGFP for visualisation and FKBP(F36V) for TRIM21 recruitment via Cmp 414. T/S/Z (TNF α /Smac-mimetic/ZVAD-FMK) treatment induces RIPK3 oligomerization (necrosome) to drive necroptosis. (B,C) RPE-1 cells stably expressing FKBP(F36V)-mEGFP-RIPK3 were treated with T/S/Z to induce RIPK3 oligomerization, or mock treated (control), in the presence of a titration of Cmp 414 for 8h and GFP fluorescence imaged and quantified using the IncuCyte system as described in Figure 16. Cmp 414 degrades oligomeric RIPK3 (T/S/Z) in a concentration-dependent manner, but does not degrade monomeric RIPK3 (control). TNF α was used at 20 ng/ μ l. Smac-mimetic (AZD5582) was used at 100 nM. ZVAD-FMK was used at 25 μ M.

Figure 30: RPE-1 cells expressing FKBP(F36V)-mEGFP-RIPK3 were treated with T/S/Z to induce RIPK3 oligomerization and necroptosis, or mock treated (control), in the presence of DMSO, Cmp 414 (2.5 μ M) or the RIPK3 kinase inhibitor GSK'872 (0.3 μ M) and GFP fluorescence and cell area quantified using the IncuCyte system. (A) Cmp 414 does not degrade monomeric RIPK3 in control-treated cells, but rapidly degrades oligomeric RIPK3 in T/S/Z-treated cells. (B) Representative images from A. In control-treated cells RIPK3 is monomeric, exhibiting diffuse cytosolic distribution. T/S/Z treatment induces RIPK3 oligomerization as observed by accumulation of cytosolic RIPK3 puncta. Cmp 414 degrades T/S/Z-induced RIPK3 oligomers as they assemble, resulting in a reduction in RIPK3 signal. GSK'872 inhibits RIPK3 kinase activity but does not affect RIPK3 oligomerization. (C-E) Necroptotic cell death is quantified by a reduction in total cell area. (C) Cmp 414 (2.5 μ M) partially rescues viability of T/S/Z-treated cells. (D) Representative phase images (upper panels) and segmented cell area (white, lower panels) at 48h post-treatment. (E) Quantification of cell area normalized to control/DMSO-treated cells at 48h post-treatment. Significance based on one-way Anova (*P<0.05, ****<0.0001).

Figure 31: (A) RPE-1 TRIM21 KO cells co-expressing Cavin-1-mEGFP-FKBP(F36V) and SmBiT-TRIM21 together with either LgBiT-TRIM21 or LgBiT-TUBES were plated in white 96-well plates and media changed to CO₂-independent media containing Vivazine live-cell luciferase substrate and

incubated at 37C for 30 minutes. Luminescence was measured using the Promega GloMax Discover plate reader with 2 second integration time every 2 minutes for 30 minutes prior to addition of compound 414 (5 μ M) or DMSO and for a further 90 minutes after. Luminescence values were normalized within each well to the timepoint just before compound/DMSO addition, values for compound 414-treated wells were then normalized to DMSO-treated controls. (B) RPE-1 TRIM21 KO cells co-expressing Cavin-1-mEGFP-FKBP(F36V) and SmBiT-TRIM21 together with either LgBiT-TRIM21 or LgBiT-TUBES were plated in 96-well plates and media changed to Fluorobrite media containing either compound 414 (5 μ M) or DMSO and GFP fluorescence quantified using the IncuCyte system as described in Figure 16.

DETAILED DESCRIPTION

The present disclosure describes bifunctional compounds which bind a target protein, and recruit the E3 ligase TRIM21 to promote the degradation of the target protein. The present description provides compounds which comprise a TRIM21-binding moiety that is a small molecule ligand (i.e., having a molecular weight of 1000 Da or less, such as 100 Da to 1000 Da or 100 Da to 600 Da) and a moiety that is capable of binding to the target protein (also referred to as the "protein targeting moiety"). The compounds of the invention cause molecular clustering of multiple TRIM21 molecules, triggering a process of ubiquitination that results in efficient degradation of the target protein. This enables the bifunctional compounds to selectively degrade target proteins that bind multiple copies of the compounds. This is of significant therapeutic benefit as it offers excellent potency while avoiding likely toxicity. One positive outcome of this is an improved therapeutic index.

Compounds of the invention have two criteria. First, they allow a ternary complex to form between the compounds of the invention, the target protein and TRIM21. Second, these ternary complexes form part of a higher order structure in which multiple TRIM21 molecules are brought into close proximity. The compounds of the invention are capable of activating TRIM21's enzymatic activity, leading to ubiquitination and degradation of the target protein. This makes it possible to selectively degrade proteins that are in an oligomeric form, because protein monomers allow the first but not the second criteria to be satisfied. In other words, the invention is directed to compounds, wherein the compounds are capable of forming a ternary complex with a target protein and TRIM21, wherein the ternary complex is capable of being part of a higher order structure, that leads to the activation of TRIM21.

In one embodiment the target protein can be a protein having a first form comprising two or more binding sites for the protein targeting moiety and a second form having a single binding site for the protein targeting moiety. The protein targeting moiety may bind both the first and second form of the protein. However, the compounds selectively degrade the first form of the protein, as multiple copies of TRIM21 are required to activate the protein degradation pathway. The multiple binding sites enable recruitment of multiple copies of TRIM21 to the target protein, resulting in clustering of TRIM21 that is required for TRIM21 activation.

In one embodiment the target protein can have a pathogenic form and a non-pathogenic form. The pathogenic form of the target protein may comprise a repeat domain or is an oligomeric form of the protein, e.g. is a form of the protein that provides two or more binding sites for the compound of the invention. The protein targeting moiety may bind both the pathogenic form and the non-pathogenic form of the protein. However, the compounds selectively degrade the pathogenic form of the protein, as multiple copies of TRIM21 are recruited to the target protein, enabling activation of the degradation pathway. The multiple copies of TRIM21 recruited to the target protein, result in clustering of TRIM21 that is required for TRIM21 activation.

In one embodiment the compound recruits TRIM21 to enable selective degradation by TRIM21 of the oligomeric forms of the target protein over its monomeric form.

By "selective degradation" or similar is meant that the compounds degrade one form of the protein over another. For example, "selective degradation" or similar means that the pathogenic form (e.g., the oligomeric forms) is degraded whereas the non-pathogenic form (e.g. monomeric form) of the target protein is not. Without wishing to be bound by theory, this is believed to be because TRIM21 activation is triggered by the clustering of multiple TRIM21 molecules in close proximity. Multiple compounds of the invention can bind the oligomeric form of the protein, thereby enabling multiple TRIM21s to cluster and activate the TRIM21 protein degradation pathway, leading to ubiquitination and degradation of the pathogenic form (e.g. oligomeric form) of the protein.

Therefore, the molecules of the invention have a distinct mechanism of action from known PROTACs, in that they require multiple compounds of the invention to bind the target protein and activate the protein degradation pathway. As such, they can selectively degrade the oligomeric but not monomeric forms of the same protein. This is in contrast to known PROTACs which may degrade both oligomeric and monomeric forms of the protein. In the present invention mere binding of the molecule to the target protein will not trigger efficient degradation, unless the protein is in, for example, an oligomeric form such that multiple TRIM21s can be recruited in close proximity to trigger the protein degradation pathway. The molecule of the invention may more efficiently degrade oligomers in contrast to known PROTACs.

Accordingly, the invention provides a compound of formula (I)

A-L-B

(I)

or a pharmaceutically acceptable salt thereof, wherein:

A is a moiety that binds TRIM21;

L is a linker; and

B is a moiety that binds a target protein.

The target protein is such that binding of the compounds of formula (I) to the target protein can result in clustering of TRIM21 to enable activation of TRIM21. Activation of TRIM21 can result in degradation of the target protein.

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In one embodiment the target protein is a protein that can be in a first form (e.g. a pathogenic form) that provides multiple binding sites for B. In one embodiment the target protein may be capable of forming an oligomeric species. When in an oligomeric form, there is provided multiple binding sites for B.

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The bifunctional compounds of the invention bind TRIM21 via 'A'. TRIM21 is a member of the tripartite motif-containing family of proteins, the sequence of human TRIM21 can be accessed as P19474 in the UniProt database. The sequence of human TRIM21 is set forth in SEQ ID NO: 1. Reference herein to TRIM21 is typically a reference to human TRIM21. TRIM21 has ubiquitin ligase activity and is able to initiate protein degradation via the ubiquitin-proteasome system (UPS).

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In the compounds of the invention having the structure of formula (I), 'A' is a TRIM21 binding moiety. In one embodiment 'A' binds the PRYSPRY domain of TRIM21 or variants thereof. The PRYSPRY domain is comprised of the PRY and SPRY regions at positions 286-337 and 339-465 of the human TRIM21 amino acid sequence as set forth in SEQ ID NO: 1.

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In one embodiment 'A' binds one or more of residues selected from W381, W383, D355, F369, L370, L371, F450, Y328, H368, S447, M330, D452, R364, Q395, Y393, E389 and K387 of human TRIM21 as set forth in SEQ ID NO: 1. In one embodiment 'A' binds one or more of residues selected from W381, W383, D355, F369, L370, L371, F450, Y328, H368, S447 and M330. In one embodiment 'A' binds one or more of residues selected from D452, R364, Q395, Y393, E389 and K387. In one embodiment 'A' binds at least residue W381. In one embodiment 'A' binds residues W381, W383 and D355 of TRIM 21.

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In some embodiments, 'A' binds residue D355 of TRIM21. In some embodiments, 'A' comprises a moiety capable of forming a hydrogen bond (e.g. a charged hydrogen bond) with residue D355 of TRIM21. In some embodiments, 'A' binds residue W381 of TRIM21. In some embodiments, 'A' comprises a moiety capable of hydrophobic stacking with residue W381 of TRIM21. In some embodiments, 'A' binds residue W383 of TRIM21. In some embodiments, 'A' comprises a moiety capable of hydrophobic stacking with residue W383 of TRIM21.

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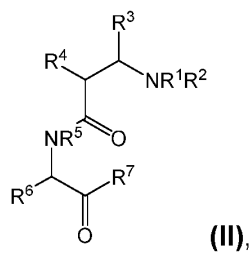
'A' can compete with IgG binding to TRIM21. In one embodiment 'A' can compete with Fc binding to TRIM21.

The binding affinity of a compound described herein to TRIM21 may be measured by the dissociation constant (K_d) value of an adduct of the compound and TRIM21 using methods known in the art (e.g., fluorescence quenching). In certain embodiments, the adduct comprises the compound and TRIM21 PRYSPRY, which are bound (e.g., non-covalently) to each other. In an embodiment, a compound of formula (I) has a K_d value for TRIM21 in the range of 0.1 nM to 1000 μ M, such as 1 nM to 1000 μ M, preferably 10 nM to 100 μ M.

The binding to specific residues can be determined by comparing the binding affinity to wild-type versus mutant forms (for example, comparing the binding affinity of wild-type TRIM21 to TRIM21 mutated at the residue of interest e.g. at D355, W381 or W383) by using a suitable biophysical technique, such as differential scanning fluorometry (DSF), isothermal titration calorimetry (ITC), or surface plasmon resonance (SPR). The contribution of the specific residue can then be directly quantified by the difference in binding energy. In addition, binding to specific residues may be demonstrated by solving a structure of the TRIM21 PRYSPRY domain in complex with the ligand to show the interactions with the residue.

As described herein, a TRIM21-binding moiety (such as 'A') targets the C-terminal PRYSPRY domain and binds within the 'SPRY' element. Specifically, it targets a concave binding surface that is surrounded by six flexible loops within the 'SPRY'. A TRIM21-binding moiety as described herein displaces the natural ligand, IgG, and is capable of competing with it for binding. The moiety functionally replaces the linear sequence of 'HNHY' found at the C-terminus of IgG. It may do so by making similar interactions as the 'HNHY' motif, such as hydrophobic stacking interactions (e.g. with W381 and W383) and charged hydrogen bonds (e.g. with D355A). A TRIM21 binder can be obtained by choosing molecules whose complexation with the PRYSPRY domain causes an increase in the PRYSPRY melting temperature (T_m), changes the intrinsic tryptophan fluorescence of residues W381 or W383 and/or which displace IgG Fc. Such methods are described in the examples.

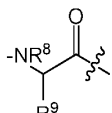
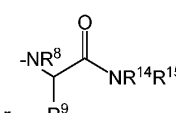
In some embodiments, A has the structure of formula (II)



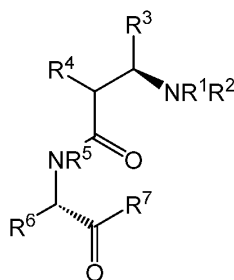
wherein:

R^1 and R^2 are each independently selected from hydrogen and C_{1-2} alkyl; or one of R^1 and R^2 is hydrogen and the other is selected from an amino acid residue, and a site of covalent attachment to L;

- R³ is selected from hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted heteroalkyl, optionally substituted heteroalkenyl, optionally substituted heteroalkynyl, optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl and optionally substituted heteroaryl, wherein R³ optionally comprises a site of covalent attachment to L;
- R⁴ is hydrogen, halo or C₁₋₂ alkyl;
- R⁵ is hydrogen or C₁₋₂ alkyl and R⁶ is optionally substituted alkyl; or R⁵ and R⁶ combine to form an optionally substituted heterocyclic ring;

- R⁷ is , a site of covalent attachment to L, or ;
- R⁸ is hydrogen or C₁₋₂ alkyl;
- R⁹ is optionally substituted alkyl;
- R¹⁴ and R¹⁵ are each independently selected from hydrogen and C₁₋₂ alkyl; and
-  in the structure of R⁷ indicates a site of covalent attachment to L;
- provided that A comprises a site of covalent attachment to L.

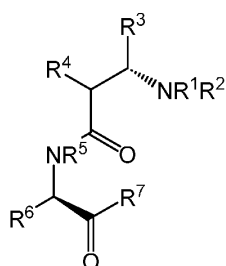
- In some embodiments, A has the following structure:



- wherein:
- R¹ and R² are each independently selected from hydrogen and C₁₋₂ alkyl; or one of R¹ and R² is hydrogen and the other is selected from an L-amino acid residue, and a site of covalent attachment to L; and



- In some embodiments, A has the following structure:



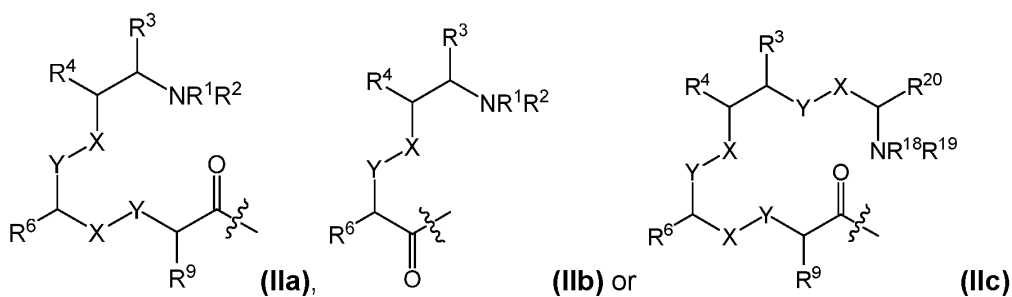
wherein:

- 5 R^1 and R^2 are each independently selected from hydrogen and C_{1-2} alkyl; or one of R^1 and R^2 is hydrogen and the other is selected from an D-amino acid residue, and a site of covalent attachment to L; and

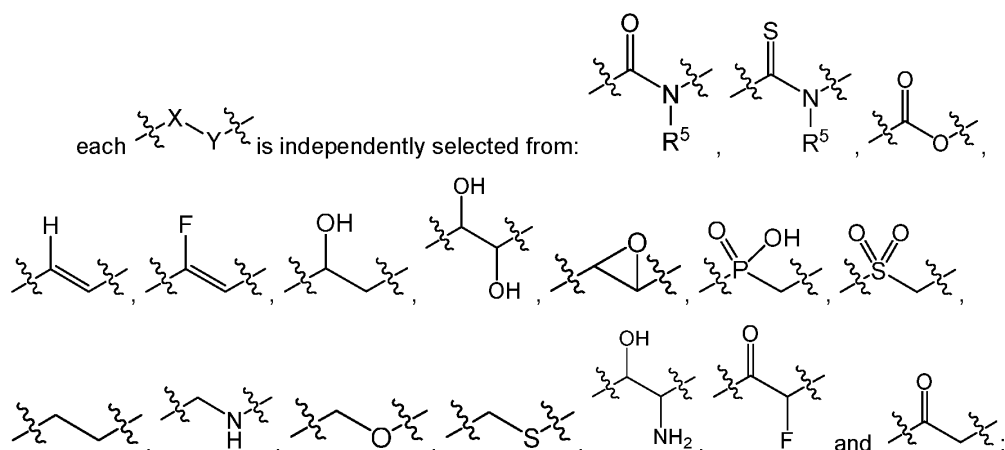


- When the structure of moiety A is based on D-amino acids, this may be beneficial to evade proteolytic enzyme cleavage.

In some embodiments, A has the structure of formula (IIa), (IIb) or (IIc):



- 15 wherein:



R^1 and R^2 are each independently selected from hydrogen and C_{1-2} alkyl; or one of R^1 and R^2 is hydrogen and the other is an amino acid residue;

R^3 is selected from hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted heteroalkyl, optionally substituted heteroalkenyl, optionally substituted heteroalkynyl, optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl and optionally substituted heteroaryl;

R^4 is hydrogen, halo or C_{1-2} alkyl;

each R^5 is independently hydrogen or C_{1-2} alkyl;

R^6 is optionally substituted alkyl;

or, when R^5 and R^6 are attached to adjacent atoms, R^5 and R^6 combine to form an optionally substituted heterocyclic ring;

R^8 is hydrogen or C_{1-2} alkyl;

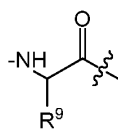
R^9 is optionally substituted alkyl;

R^{18} and R^{19} are each independently selected from hydrogen and C_{1-2} alkyl; and

R^{20} is optionally substituted alkyl.

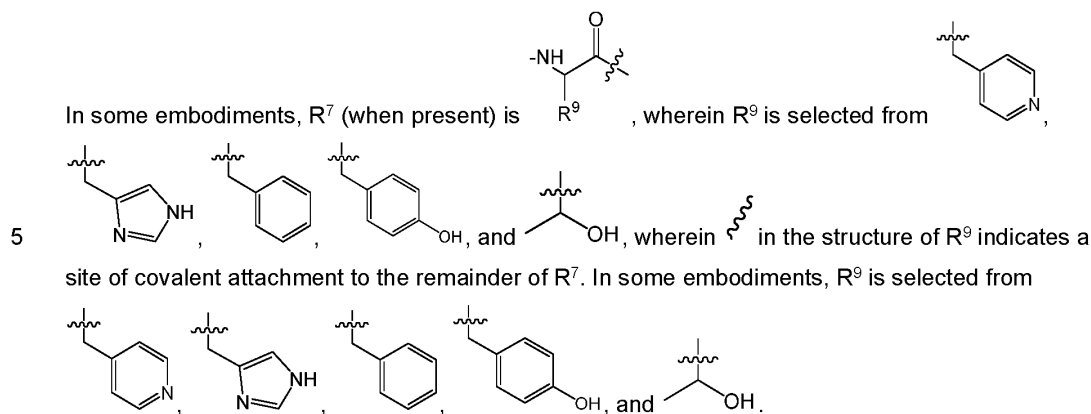
In some embodiments, R^1 , R^2 and R^4 are hydrogen.

In some embodiments, R^3 is selected from optionally substituted aryl and optionally substituted heteroaryl. For example, R^3 may be selected from furan and optionally substituted phenyl.



In some embodiments, R^7 (when present) is $\text{---NH---CH(R}^9\text{)---C(=O)---}$, wherein R^9 is selected from -alkylene- optionally substituted aryl and -alkylene- optionally substituted heteroaryl, optionally wherein R^9 is selected from $\text{---CH}_2\text{---}$ optionally substituted aryl and $\text{---CH}_2\text{---}$ optionally substituted heteroaryl. In some embodiments, R^9 is selected from -alkylene- optionally substituted aryl and -alkylene- optionally substituted heteroaryl.

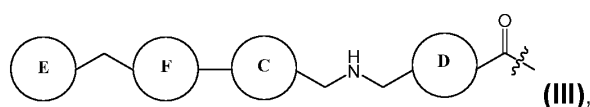
substituted heteroaryl, optionally wherein R^9 is selected from $-CH_2$ -optionally substituted aryl and $-CH_2$ -optionally substituted heteroaryl.



In some embodiments, R^9 is selected from a histidine side chain, a phenylalanine side chain, a tryptophan side chain, a tyrosine side chain, a cysteine side chain, a serine side chain, a threonine side chain, a methionine side chain, an asparagine side chain, and a glutamine side chain.

In some embodiments, R^5 and R^6 combine to form a 5-membered heterocyclic ring optionally substituted by hydroxy, nitro, cyano, halo, $-NR^{10}R^{11}$, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted $-O$ -cycloalkyl, optionally substituted $-O$ -heterocyclyl, optionally substituted $-O$ -aryl, or optionally substituted $-O$ -heteroaryl, wherein R^{10} and R^{11} are each independently H, optionally substituted alkyl, or optionally substituted cycloalkyl.

In some embodiments, A has the structure of formula (III)



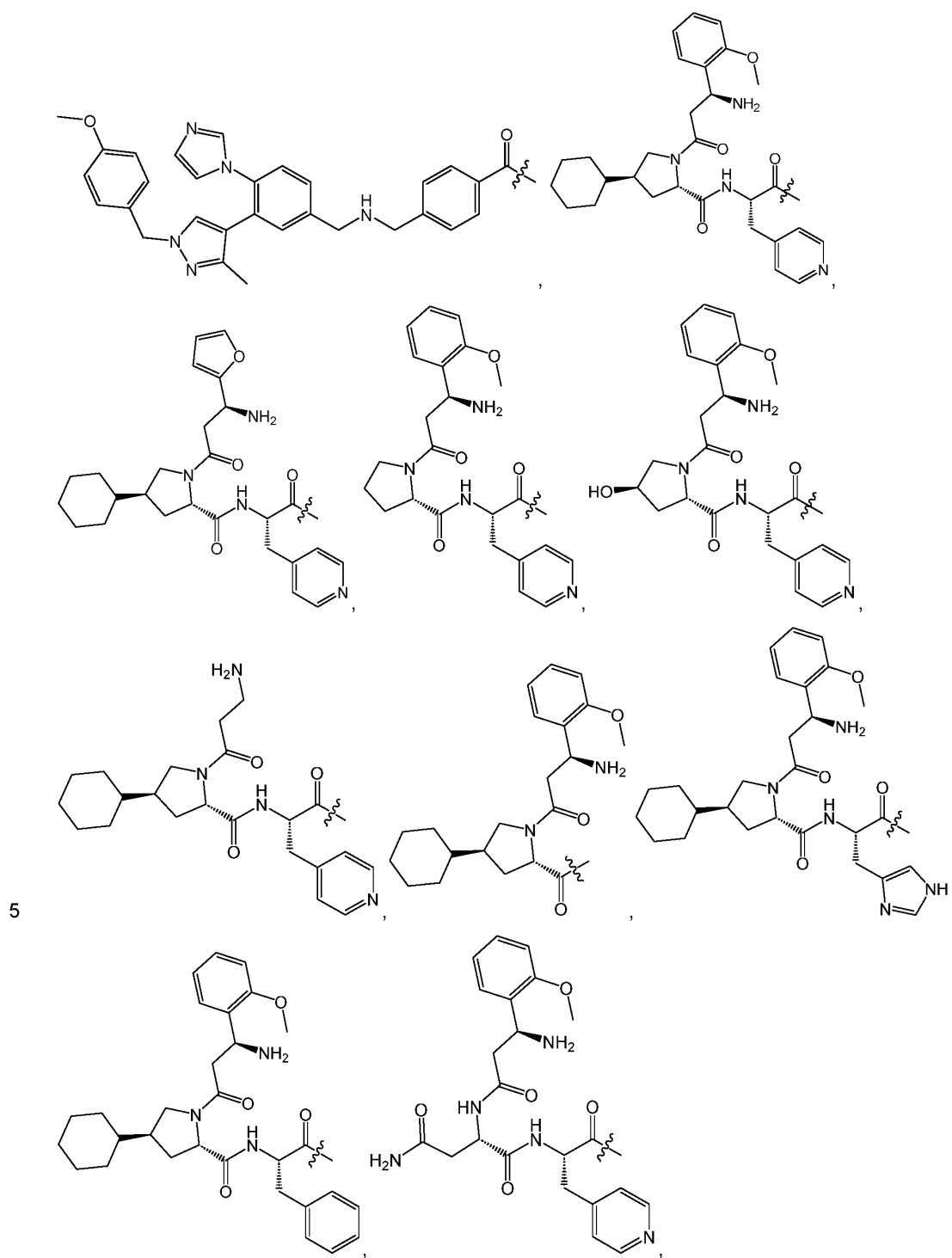
wherein:

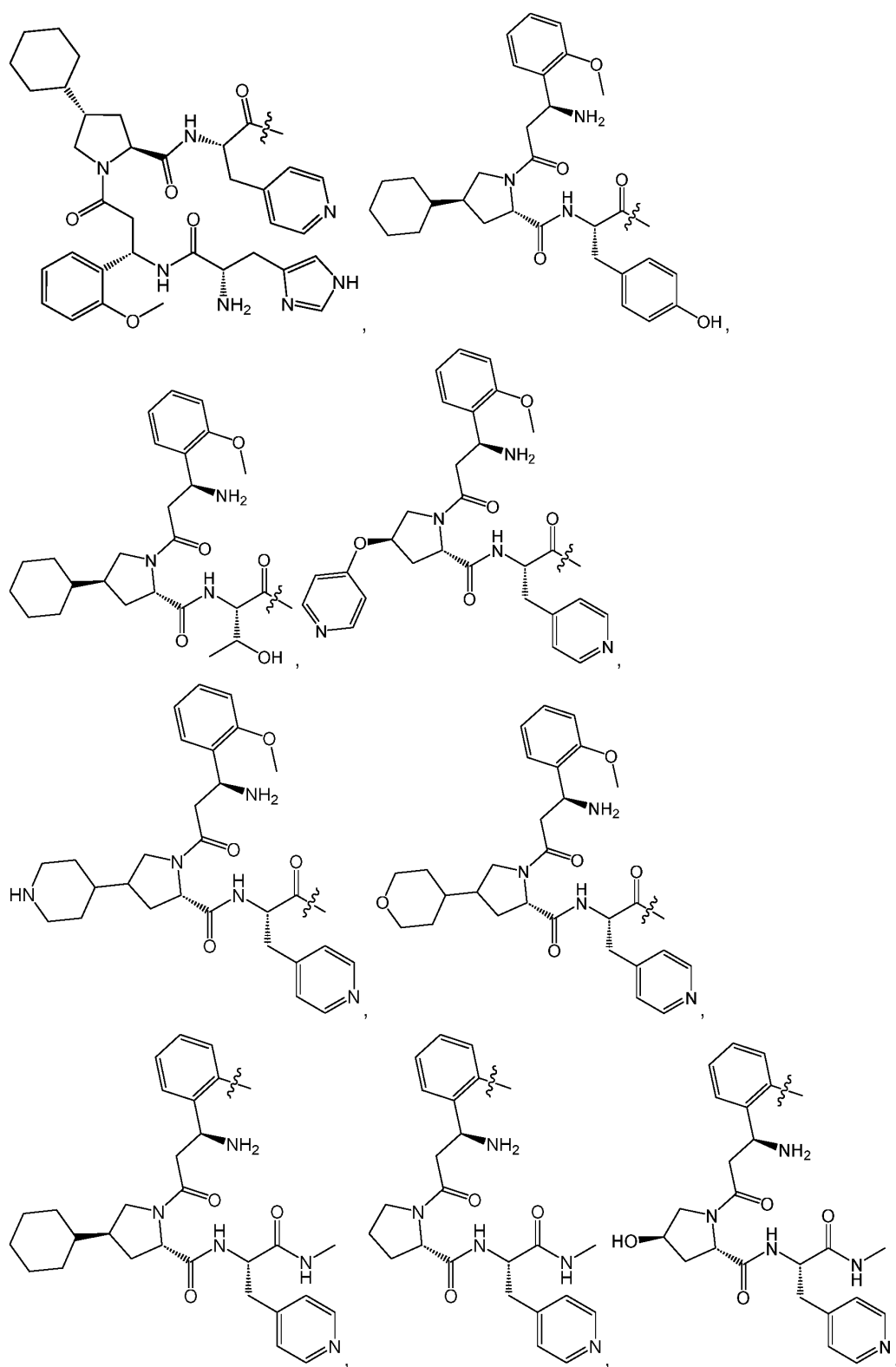
each of rings E, F, C and D is independently selected from optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl, and optionally substituted heteroaryl; and

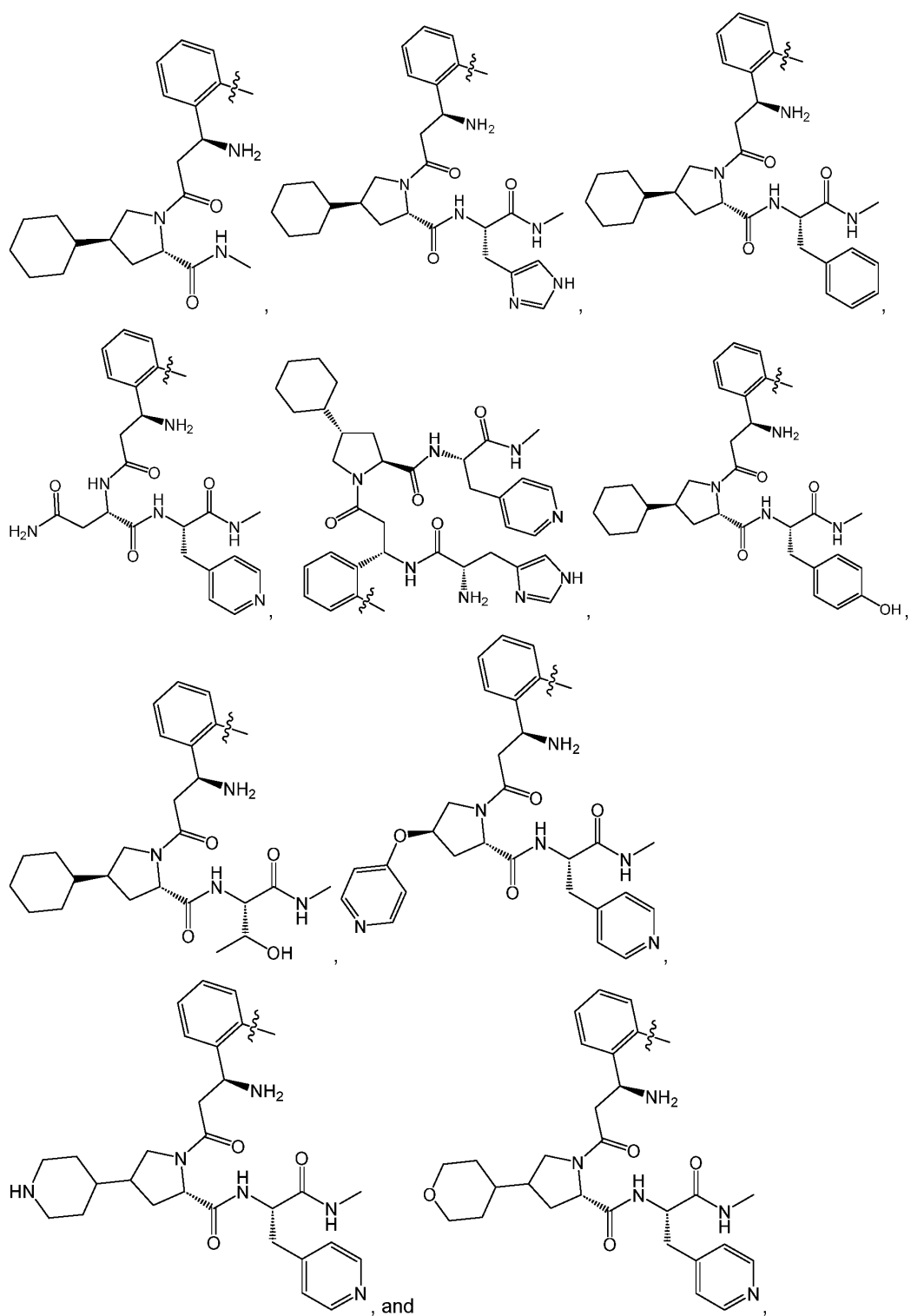
 in the structure of formula (III) indicates a site of covalent attachment to L.

In some embodiments, ring C is selected from aryl and heteroaryl, wherein the aryl or heteroaryl is substituted by aryl or heteroaryl.

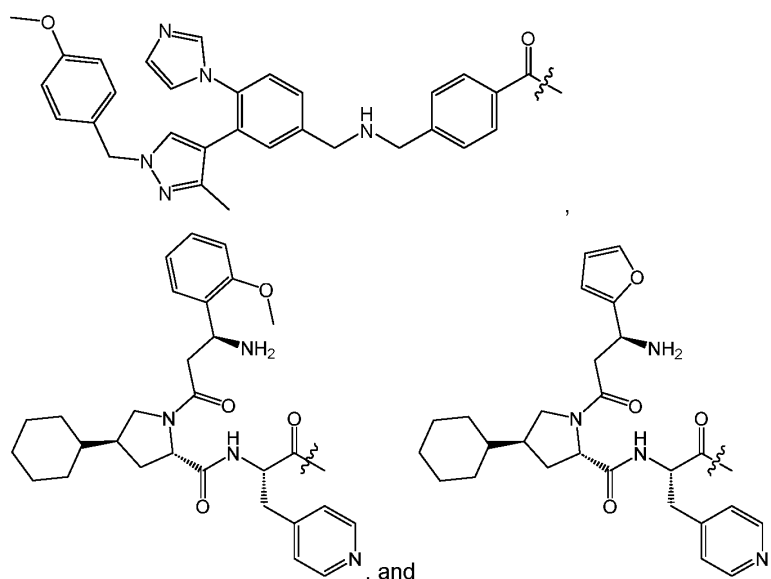
In some embodiments, A is selected from:





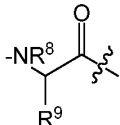


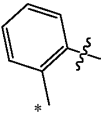
In some embodiments, A is selected from:



5 wherein  in the structure of A indicates a site of covalent attachment to L.

In the structure of formula (I), A comprises a site of covalent attachment to L. Preferably, A comprises one site of covalent attachment to L. When A has the structure of formula (II), one or more of R¹/R², R³ and R⁷ may comprise a site of covalent attachment to L. In one embodiment, one of R¹ and R² may be
 10 a site of covalent attachment to L and the other may be hydrogen. In another embodiment, R⁷ is a site

of covalent attachment to L. In another embodiment, R⁷ is  wherein  indicates a site of covalent attachment to L and R⁸ and R⁹ are as defined in formula (II). When R³ is said to be a moiety that is 'optionally substituted', this encompasses embodiments wherein the substituent is a site of covalent attachment to L. Thus, in an embodiment, R³ comprises a site of covalent attachment to L.

15 For example, R³ may be , wherein  indicates a site of covalent attachment to L and * indicates a site of covalent attachment to the remainder of A.

In an embodiment, the molecular weight of A is 1000 Da or less. In another embodiment, the molecular weight of A is in the range of 100 Da to 1000 Da. The molecular weight may be calculated
 20 based on the standard atomic weights of chemical elements as determined and published by the Commission on Isotopic Abundances and Atomic Weights (CIAAW) of the International Union of Pure and Applied Chemistry (IUPAC).

In the compounds of the invention having the structure of formula (I), B is a moiety that binds a target protein, also referred to herein as a "target protein binding moiety" or "protein targeting moiety" or similar. B is a small molecule which binds to a target protein and places/presents that protein in
5 proximity to TRIM21 such that degradation of the protein by ubiquitin ligase may occur, when there is multiple TRIM21 molecules present.

The target protein can be a protein that has a form that comprises multiple binding sites for B that can result in the clustering and activation of TRIM21, when bound by the compounds of the invention.

10

Activation of TRIM21 can be measured by methods as discussed in the Examples and known in the art. For example, using in vitro ubiquitination assays with purified TRIM21 and target protein, or using Fluorescence- (FRET) or Bioluminescence- (BRET) Resonance Energy Transfer assays or Protein
fragment Complementation Assays (PCAs) to directly measure TRIM21 clustering inside cells.

15

In one embodiment activation of TRIM21 can be determined by a NanoBiT split luciferase assay. In a NanoBiT split luciferase assay luciferase enzymatic activity is reconstituted only when the two protein fragments, LgBiT and SmBiT, are brought into close proximity. Specifically, a NanoBiT split luciferase assay may involve co-expressing in TRIM21 KO cells, two N-terminally tagged versions of TRIM21, LgBiT-TRIM21 and SmBiT-TRIM21. Compounds to be tested are added to cells. The luciferase
20 enzymatic activity is measured. The presence of luciferase activity is an indication of TRIM21 activation.

In the absence of compounds of the invention, there is no luciferase activity because TRIM21 is not clustered and thus the LgBiT and SmBiT tags do not interact. When compounds of the invention, .e.g. having the structural of formula (I) as defined herein, are present, they bind the target protein that comprises multiple binding sites for B. This results in TRIM21 clustering, which can be measured by the luciferase enzymatic activity reconstituted by the interaction of LgBiT and SmBiT tags on the N-termini of clustered TRIM21 molecules.

30

In one embodiment the target protein can having at least two distinct forms, a first form and a second form, wherein the first protein form provides multiple binding sites for 'B'. The second protein form may only provide a single binding site for 'B'. B can bind both the first and second forms of the target protein. However, the first form of the target protein enables clustering of TRIM21 about the target
35 protein when compounds having the structure of formula (I) are bound, whilst the second form does not enable clustering of TRIM21.

"Protein form" includes but is not limited to monomeric, oligomeric and multimeric forms of the protein and multicomponent complexes. The oligomer, multimer and multicomponent forms of the protein
40 comprise two or more monomeric molecules of the protein associated together.

Oligomer forms of the protein include but are not limited to multimer forms and aggregates of the protein. Oligomers include but are not limited to dimer, trimer, tetramer, pentamer, and hexamer or larger molecules, e.g. aggregates with two, three, four, five, six or more units. The oligomers may be
5 homo-oligomers or hetero-oligomers. If the oligomers are hetero-oligomers, the oligomers will comprise at least two units of the target protein, to provide multiple binding sites for 'B'.

By "multiple binding sites", it is meant that the protein form has two or more binding sites for the protein binding moiety e.g. 2, 3, 4 5, 6 or more binding sites for compounds of protein binding moiety
10 'B', i.e. multiple compounds of the invention (i.e. compounds of formula (I)) can bind the target protein form, via their respective protein binding moieties 'B'. The protein form may be a monomeric protein, or a heteromeric or homomeric oligomer of the protein.

In one embodiment the target protein can be a protein having a pathogenic and non-pathogenic form,
15 wherein the pathogenic form comprises two or more binding sites for compounds of the invention. The pathogenic form may be an oligomeric form of the protein and the non-pathogenic form may be a monomeric form of the protein. The compounds of the invention enable selective degradation of the oligomeric forms over the monomeric forms of the protein.

20 In some embodiments the target protein is a protein having a pathogenic form of the protein comprising multiple binding sites for the compound of the invention. For example, a mutated form of a protein that has multiple binding sites for the protein binding moiety, in comparison to the wild-type non-pathogenic form of the protein. The compounds of the invention enable selective degradation of the pathogenic form having multiple binding sites for the compound over a non-pathogenic form of the
25 protein that does not comprise multiple binding sites.

In some embodiments the target protein is a pathogenic form of the protein that is part of a multicomponent complex. A multicomponent complex includes, in addition to multiple subunits of the target protein, other proteins and/or compounds forming the complex.

30 As used herein the term "pathogenic form" or similar may mean that the protein is in a form or conformation that causes or is associated with a disease and therefore is present when the disease is present, including aggregation or a complex of multiple copies of the protein. The pathogenic form of the protein will have multiple binding site for the protein targeting moiety, e.g. two or more binding
35 sites. The pathogenic form will enable clustering of TRIM21 when the compounds of Formula (I) are bound. The terms "non-pathogenic form" or similar are used to refer to the normal form of the protein whose presence is not associated with disease. The non-pathogenic form may have a single binding site for the protein targeting moiety. The non-pathogenic form will not enable clustering of TRIM21 when the compounds of formula (I) are bound.

40

In some embodiments the target protein is a protein having a monomeric form and an oligomeric form, wherein the oligomeric form is associated with the presence of the disease. The compounds of the invention enable selective degradation of the oligomeric forms over the monomeric forms of the protein, due to the absence of TRIM21 clustering about the monomeric form of the target protein.

5

In some embodiments the target protein can form aggregates. For example, the target protein can include but is not limited to a protein selected from tau, synuclein (including alpha-synuclein), amyloid β , islet amyloid polypeptide (IAPP), serum amyloid A (SAA), prion proteins, misfolded transthyretin protein (TTR), TAR DNA-binding protein 43 (TDP-43), NOTCH3 receptor, mutated cystatin C, medin, dipeptide repeat protein and fused-in-sarcoma. For example, the target protein may be a protein selected from tau, synuclein (including alpha-synuclein), amyloid β , prion proteins, TAR DNA-binding protein 43 (TDP-43), dipeptide repeat protein and fused-in-sarcoma. These proteins can form aggregates or oligomers that are associated with neurodegenerative diseases or disorders. The compounds of the invention will cause selective degradation of the aggregates, as multiple compounds of the invention can bind the aggregates resulting in clustering of TRIM21.

10

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In some embodiments the target protein has expanded repeat elements when in a pathogenic form. For example, the target protein can include but is not limited to mutated huntingtin. Mutated huntingtin can comprise an expanded polyglutamine repeat and is associated with Huntington disease. The polyglutamine repeat provides multiple binding sites for the protein targeting moiety (B), which can result in clustering of TRIM21. The mutated huntingtin provides sufficient binding sites for compounds of the invention to enable cluster of TRIM21.

20

In some embodiments the target proteins are involved in signal transduction pathways in inflammation and/or cancer, preferably wherein the protein is selected from Myd88, IRAK4, IRAK2, IRAK1, TRAF6, NLRP3, RIPK3 and ASC. For example, the protein may be selected from Myd88, IRAK4, IRAK2, IRAK1, TRAF6, NLRP3, and ASC. These proteins are part of multi-component complexes that comprise multiple (e.g. two or more) units of the target protein. The multiple units of protein in the complex provide multiple binding sites for the protein targeting moiety (B), which can result in clustering of TRIM21.

25

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In some embodiments the target protein is a viral protein that forms oligomers, preferably wherein the viral protein is selected from CoV2 and influenza virus nucleoprotein. These proteins form multimers, providing multiple binding sites for the protein targeting moiety (B), which can enable clustering of TRIM21.

35

In some embodiments the target protein is a protein selected from bromodomain-containing protein 4 (BRD4), mutated gelsolin, misfolded rhodopsin and atrial natriuretic peptide. These proteins provide multiple binding sites for the protein targeting moiety (B), which can enable clustering of TRIM21.

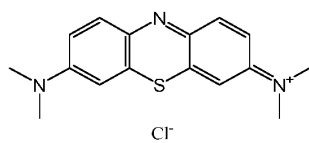
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In some embodiments the target protein is an intracellular protein.

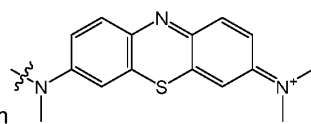
'B' may be selected from or based on known ligands of the target protein.

- 5 'B' comprises a site of covalent attachment to L. In some embodiments, B has been derivatised prior to conjugation to the remainder of the compound of formula (I) to include a site of covalent attachment to L.

10 As the skilled person will appreciate, when B is a known ligand of the target protein, it will be present in a compound of formula (I) in a modified form so as to enable covalent attachment to L. For



example, when B is methylene blue:



it can be present in a compound of formula (I) in the following form

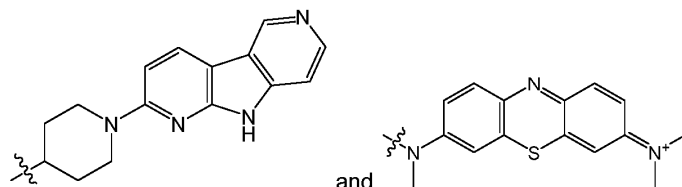
wherein indicates a site of covalent attachment to L.

- 15 In some embodiments, the target protein is tau. The Tau protein is a protein found in the central nervous system primarily in neuronal cells. In a healthy neuron, Tau binds to microtubules and regulates microtubule stability, which is critical for axonal outgrowth and neuronal plasticity. However, Tau can also form insoluble aggregates in cells. Accumulation of abnormal Tau aggregates in neurons
- 20 is an important pathological signature in multiple neurodegenerative disorders including Alzheimer's disease. As used herein, a reference to "tau" is a reference to any form of the tau protein, including normal cellular tau as well as tau assemblies, fibrils, aggregates and other abnormal conformations. It includes post-translationally modified versions of tau including phosphorylated, acetylated, glycosylated, ubiquitinated and otherwise-modified variants. It also includes mutant forms of tau,
- 25 especially mutant forms which are associated with genetically transmitted predisposition to neurodegenerative diseases. As such, in its pathogenic form Tau assembles into aggregates, also referred to as an oligomeric or multimeric form. Tau aggregates, oligomers and multimers are used to denote the same thing and are interchangeable for the purposes of the invention.
- 30 Compounds that bind to tau are known in the art. Small molecule ligands of tau are well known. For example, in Leuzy, A et al (2019) and Silva *et al* (2019). Silva *et al* (2019) proposed the use of a small molecule capable of binding tau, previously used for PET imaging of tau, to promote tau degradation. Such small molecules, such as F-AV-1451, may be useful in the context of the present invention. Various other tau PET tracers and other small molecules that bind to tau are available. Thus, when the

target protein is tau, B can, for example, be based on compounds including but not limited to PI-2014, FDDNP, AV680(T808), GTP-1, THK523, THK5105, PBB3, AV1451(T807), THK5117, THK5351, N-methyl lansoprazole, (E)-6-iodo-2-styryl-1H-benzo[d]imidazole, RO6958948, PI-2620, JNJ-067, APN-1607(PM-PBB3), MK-6240, and methylene blue.

5

In some embodiments B is selected from:



wherein  in the structure of B indicates a site of covalent attachment to L. These moieties can be used when the target protein is tau.

10

In an embodiment, the target protein is BRD4. In such embodiments, B may be selected from, for example, JQ1 and apabetalone.

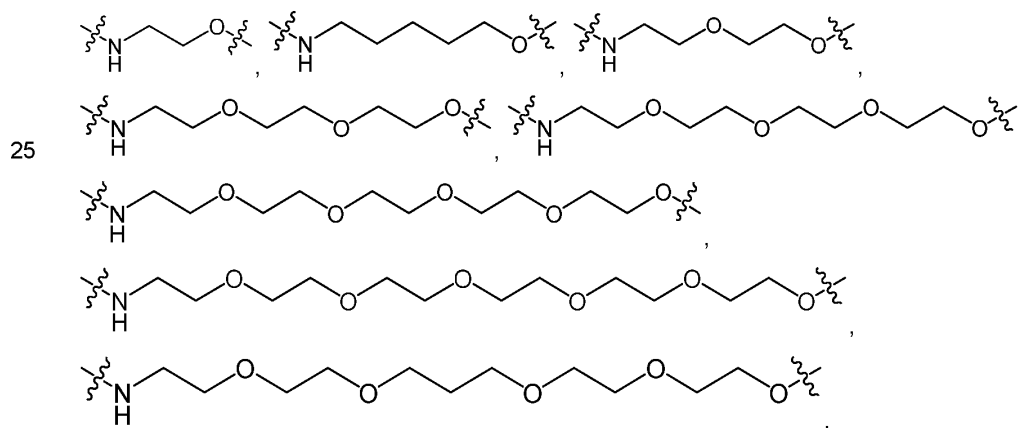
In the compounds of the invention, L is a linker covalently connecting the target protein binding moiety (B) and the TRIM21 binding moiety (A).

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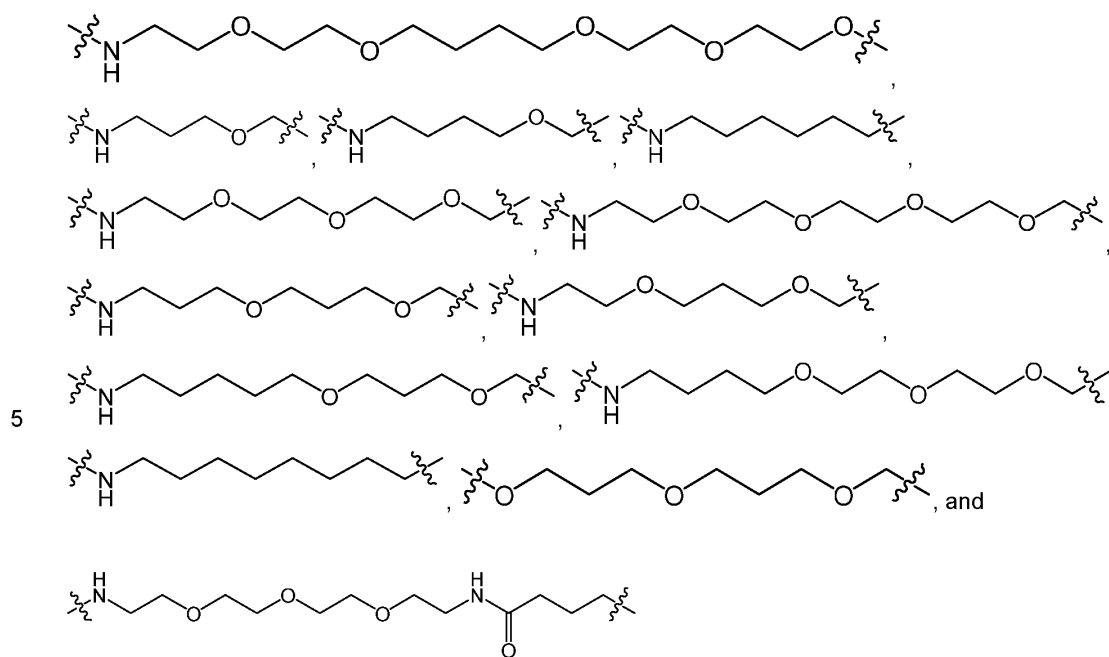
In some embodiments the linker is a bond. In some embodiments the linker is a chain of 2 to 20 carbon atoms, wherein one or more of the carbon atoms is optionally replaced by 1 to 12 ethylene glycol units and/or a heteroatom selected from O, N, S, and P. In some embodiments the linker comprises poly(ethylene glycol) (PEG), unsubstituted alkylene, substituted alkylene, or a combination thereof.

20

In some embodiments the linker is selected from:

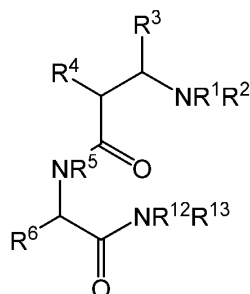


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A further aspect of the invention relates to compounds having the structure of formula (IV):



15 wherein:

R^1 and R^2 are each independently selected from hydrogen and C_{1-2} alkyl; or one of R^1 and R^2 is hydrogen and the other is an amino acid residue;

20 R^3 is selected from hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted heteroalkyl, optionally substituted heteroalkenyl, optionally substituted heteroalkynyl, optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl and optionally substituted heteroaryl;

R^4 is hydrogen, halo or C_{1-2} alkyl;

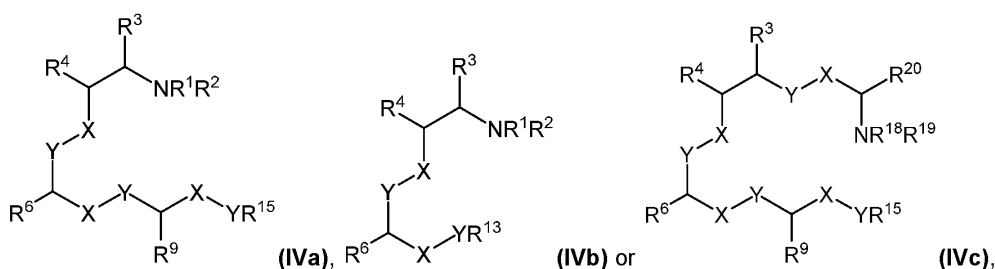
R^5 is hydrogen or C_{1-2} alkyl and R^6 is optionally substituted alkyl; or R^5 and R^6 combine to form an optionally substituted heterocyclic ring;

R^9 is optionally substituted alkyl;

- R^{12} and R^{13} are each independently selected from hydrogen and C_{1-2} alkyl; or one of R^{12} and R^{13} is hydrogen and the other is selected from optionally substituted C_{1-2} alkyl and $-CHR^9C(O)NR^{14}R^{15}$; and

R^{14} and R^{15} are each independently selected from hydrogen and C_{1-2} alkyl.

- A further aspect of the invention relates to compounds having the structure of formula (IVa), (IVb) or (IVc):



wherein:

- each $\text{---}X\text{---}Y\text{---}$ is independently selected from:
-

R^1 and R^2 are each independently selected from hydrogen and C_{1-2} alkyl; or one of R^1 and R^2 is hydrogen and the other is an amino acid residue;

- R^3 is selected from hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted heteroalkyl, optionally substituted heteroalkenyl, optionally substituted heteroalkynyl, optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl and optionally substituted heteroaryl, wherein R^3 optionally comprises a site of covalent attachment to L;

- R^4 is hydrogen, halo or C_{1-2} alkyl;
each R^5 is independently hydrogen or C_{1-2} alkyl;

R⁶ is optionally substituted alkyl;

or, when R⁵ and R⁶ are attached to adjacent atoms, R⁵ and R⁶ combine to form an optionally substituted heterocyclic ring;

R⁸ is hydrogen or C₁₋₂ alkyl;

5 R⁹ is optionally substituted alkyl;

R¹² and R¹³ are each independently selected from hydrogen and C₁₋₂ alkyl;

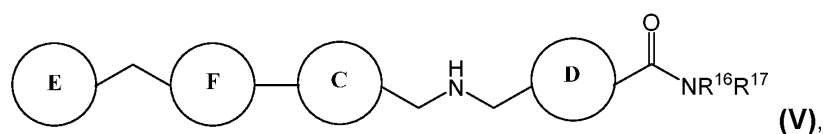
R¹⁴ and R¹⁵ are each independently selected from hydrogen and C₁₋₂ alkyl;

R¹⁸ and R¹⁹ are each independently selected from hydrogen and C₁₋₂ alkyl; and

R²⁰ is optionally substituted alkyl.

10

A further aspect of the invention relates to compounds having the structure of formula (V):



wherein:

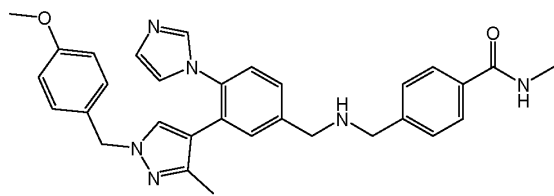
15 each of rings E, F, C and D is independently selected from optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl; and

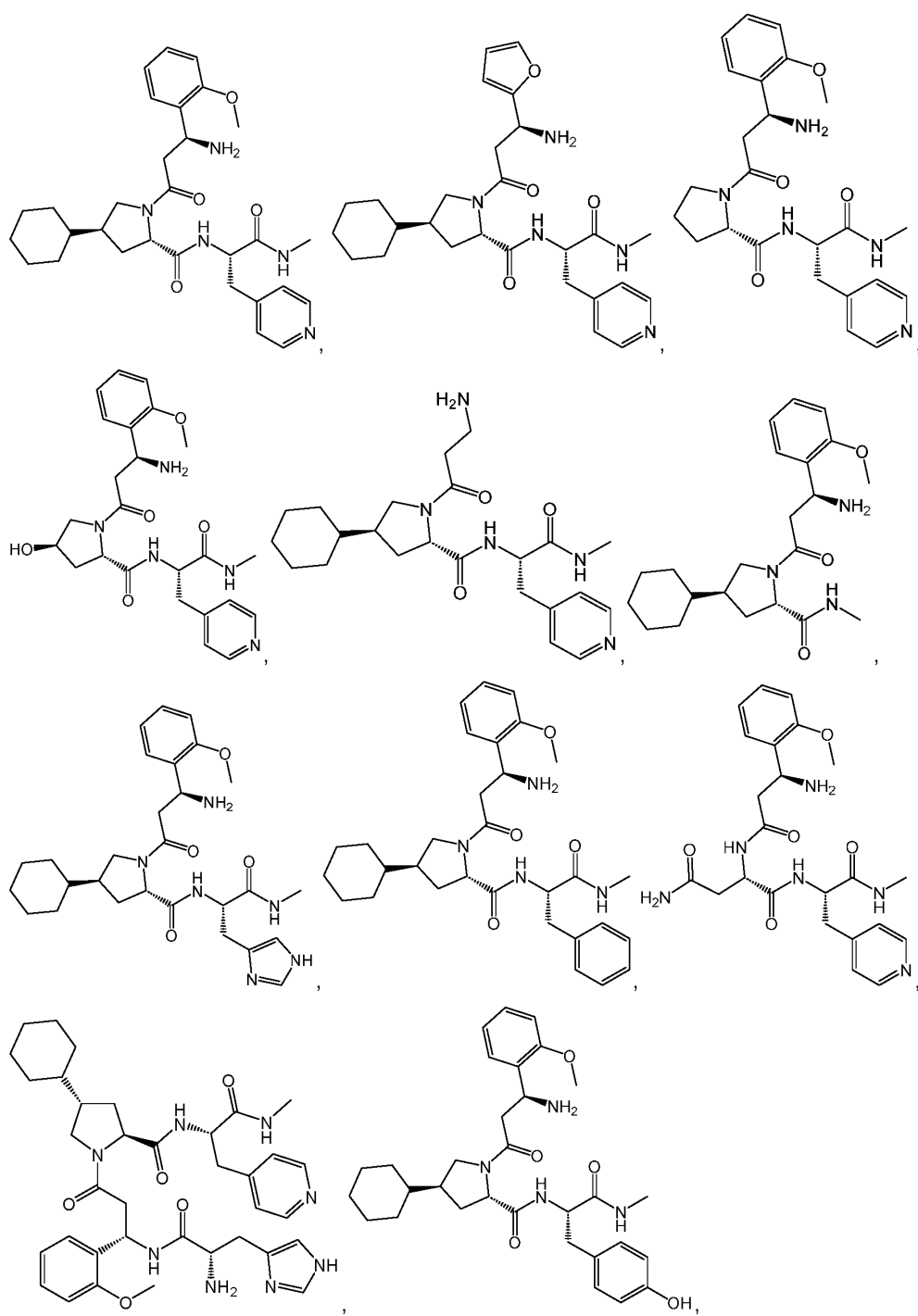
R¹⁶ and R¹⁷ are each independently selected from hydrogen and C₁₋₂ alkyl.

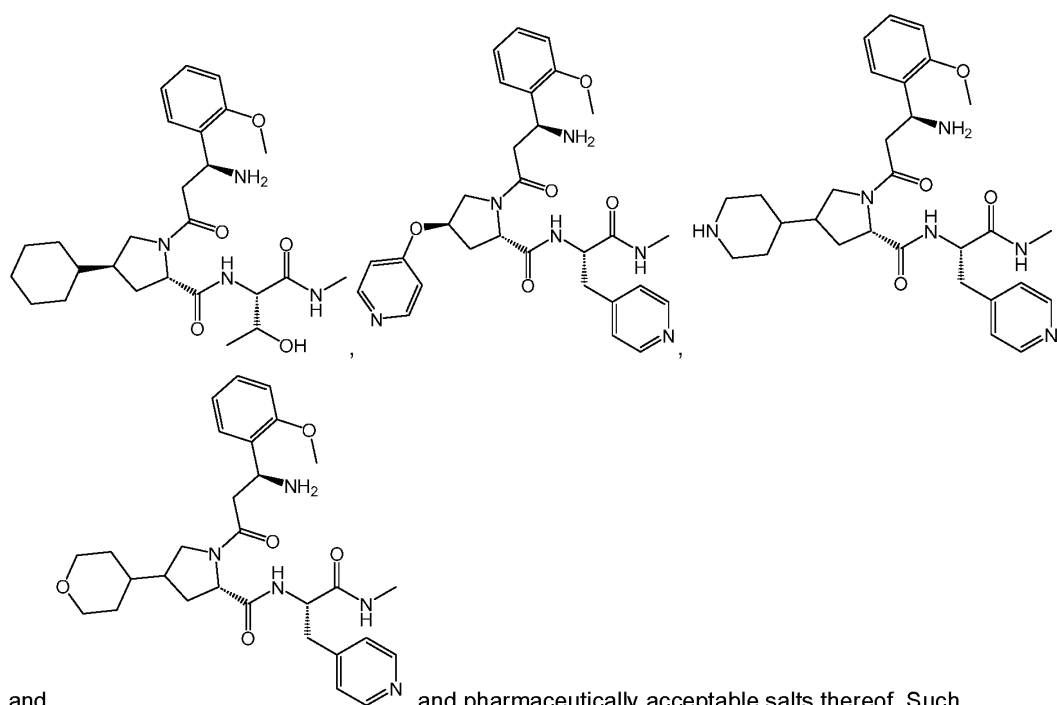
Compounds of formula (IV), (IVa), (IVb), (IVc) and (V) have the ability to bind TRIM21.

20

A compound of the invention can be selected from:







5 “Alkyl” refers to a radical of a straight-chain or branched saturated hydrocarbon group having from 1 to 20 carbon atoms (“C₁₋₂₀ alkyl”). In some embodiments, an alkyl group has 1 to 12 carbon atoms (“C₁₋₁₂ alkyl”). In some embodiments, an alkyl group has 1 to 10 carbon atoms, 1 to 6 carbon atoms, 1 to 3 carbon atoms, or 1 to 2 carbon atoms. “Alkylene” refers to an alkyl group wherein two hydrogens are removed to provide a divalent radical.

10

“Alkenyl” refers to a radical of a straight-chain or branched hydrocarbon group having from 2 to 20 carbon atoms, one or more carbon-carbon double bonds, and optionally one or more carbon-carbon triple bonds (“C₂₋₂₀ alkenyl”). In certain embodiments, alkenyl does not contain any triple bonds. In some embodiments, an alkenyl group has 2 to 10 carbon atoms, 2 to 5 carbon atoms, 2 to 3 carbon atoms, or 2 carbon atoms.

15

“Alkynyl” refers to a radical of a straight-chain or branched hydrocarbon group having from 2 to 20 carbon atoms, one or more carbon-carbon triple bonds, and optionally one or more carbon-carbon double bonds. In certain embodiments, alkynyl does not contain any double bonds. In some

20

embodiments, an alkynyl group has 2 to 10 carbon atoms, 2 to 5 carbon atoms, 2 to 3 carbon atoms, or 2 carbon atoms.

25

“Aryl” refers to a radical of a monocyclic or polycyclic 4n+2 aromatic ring system having 6–14 ring carbon atoms and zero heteroatoms provided in the aromatic ring system. In some embodiments, an aryl group has six ring carbon atoms or ten ring carbon atoms. “Aryl” also includes ring systems

wherein the aryl ring, as defined above, is fused with one or more cycloalkyl or heterocyclyl groups wherein the site of attachment is on the aryl ring. In certain embodiments, an aryl group substituted with one or more of groups selected from halo, C₁-C₈ alkyl, C₁-C₈ haloalkyl, cyano, hydroxy, C₁-C₈ alkoxy, and amino.

5

"Hetero" when used to describe a compound or a group present on a compound means that one or more carbon atoms in the compound or group have been replaced by a nitrogen, oxygen, or sulfur heteroatom. Hetero may be applied to groups such as alkyl, *e.g.*, heteroalkyl, cycloalkyl, *e.g.*, heterocyclyl, aryl, *e.g.*, heteroaryl, and the like.

10

"Heteroaryl" refers to a radical of a 5–10 membered monocyclic or bicyclic 4n+2 aromatic ring system having ring carbon atoms and 1–4 ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is independently selected from nitrogen, oxygen and sulfur ("5–10 membered heteroaryl"). Heteroaryl bicyclic ring systems can include one or more heteroatoms in one or both rings. "Heteroaryl" includes ring systems wherein the heteroaryl ring is fused with one or more cycloalkyl or heterocyclyl groups wherein the site of attachment is on the heteroaryl ring. "Heteroaryl" also includes ring systems wherein the heteroaryl ring, as defined above, is fused with one or more aryl groups wherein the point of attachment is either on the aryl or heteroaryl ring.

20

In some embodiments, a heteroaryl group is a 5–6 membered aromatic ring system having ring carbon atoms and 1–4 ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("5–6 membered heteroaryl").

25

Exemplary 5–membered heteroaryl groups containing one heteroatom include, without limitation, pyrrolyl, furanyl and thiophenyl. Exemplary 5–membered heteroaryl groups containing two heteroatoms include, without limitation, imidazolyl, pyrazolyl, oxazolyl, isoxazolyl, thiazolyl, and isothiazolyl. Exemplary 5–membered heteroaryl groups containing three heteroatoms include, without limitation, triazolyl, oxadiazolyl, and thiadiazolyl. Exemplary 5–membered heteroaryl groups containing four heteroatoms include, without limitation, tetrazolyl. Exemplary 6–membered heteroaryl groups containing one heteroatom include, without limitation, pyridinyl. Exemplary 6–membered heteroaryl groups containing two heteroatoms include, without limitation, pyridazinyl, pyrimidinyl, and pyrazinyl. Exemplary 6–membered heteroaryl groups containing three or four heteroatoms include, without limitation, triazinyl and tetrazinyl, respectively.

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In certain embodiments, a heteroaryl group substituted with one or more of groups selected from halo, C₁-C₈ alkyl, C₁-C₈ haloalkyl, cyano, hydroxy, C₁-C₈ alkoxy, and amino.

40

Non-limiting examples of heteroaryl groups include pyridinyl, pyrimidinyl, thiophenyl, thienyl, furanyl, indolyl, benzoxadiazolyl, benzodioxolyl, benzodioxanyl, thianaphthanyl, pyrrolopyridinyl, indazolyl, quinolinyl, quinoxalinyl, pyridopyrazinyl, quinazolinonyl, benzoisoxazolyl, imidazopyridinyl,

benzofuranyl, benzothienyl, benzothiophenyl, phenyl, naphthyl, biphenyl, pyrrolyl, pyrazolyl, imidazolyl, pyrazinyl, oxazolyl, isoxazolyl, thiazolyl, furylthienyl, pyridyl, pyrimidyl, benzothiazolyl, purinyl, benzimidazolyl, isoquinolyl, thiadiazolyl, oxadiazolyl, pyrrolyl, diazolyl, triazolyl, tetrazolyl, benzothiadiazolyl, isothiazolyl, pyrazolopyrimidinyl, pyrrolopyrimidinyl, benzotriazolyl, benzoxazolyl, or
 5 quinolyl. The examples above may be substituted or unsubstituted and divalent radicals of each heteroaryl example above are non-limiting examples of heteroarylene.

“Cycloalkyl” refers to a radical of a saturated cyclic hydrocarbon group having from 3 to 10 ring carbon atoms and zero heteroatoms in the non-aromatic ring system. In some embodiments, a cycloalkyl
 10 group has 3 to 8 ring carbon atoms. In some embodiments, a cycloalkyl group has 3 to 6 ring carbon atoms. In some embodiments, a cycloalkyl group has 5 to 10 ring carbon atoms. Examples of cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl.

“Heterocyclyl” or “heterocyclic” refers to a radical of a 3- to 10-membered non-aromatic ring system
 15 having ring carbon atoms and 1 to 4 ring heteroatoms, wherein each heteroatom is independently selected from nitrogen, oxygen, sulfur, boron, phosphorus, and silicon. A heterocyclyl group can either be monocyclic or a fused, bridged or spiro ring system such as a bicyclic system, and can be saturated or can be partially unsaturated. “Heterocyclyl” also includes ring systems wherein the heterocyclyl ring, as defined above, is fused with one or more cycloalkyl groups wherein the point of
 20 attachment is either on the cycloalkyl or heterocyclyl ring, or ring systems wherein the heterocyclyl ring, as defined above, is fused with one or more aryl or heteroaryl groups, wherein the point of attachment is on the heterocyclyl ring. In some embodiments, a heterocyclyl group is a 5-6 membered non-aromatic ring system having ring carbon atoms and 1-4 ring heteroatoms, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur.

25 Exemplary 3-membered heterocyclyl groups containing one heteroatom include, without limitation, azirdinyl, oxiranyl, thiorenyl. Exemplary 4-membered heterocyclyl groups containing one heteroatom include, without limitation, azetidiny, oxetanyl and thietanyl. Exemplary 5-membered heterocyclyl groups containing one heteroatom include, without limitation, tetrahydrofuranyl, dihydrofuranyl,
 30 tetrahydrothiophenyl, dihydrothiophenyl, pyrrolidinyl, dihydropyrrolyl and pyrrolyl-2,5-dione. Exemplary 5-membered heterocyclyl groups containing two heteroatoms include, without limitation, dioxolanyl, oxasulfuranyl, disulfuranyl, and oxazolidin-2-one. Exemplary 5-membered heterocyclyl groups containing three heteroatoms include, without limitation, triazoliny, oxadiazoliny, and thiadiazoliny. Exemplary 6-membered heterocyclyl groups containing one heteroatom include,
 35 without limitation, piperidinyl, tetrahydropyranyl, dihydropyridinyl, and thianyl. Exemplary 6-membered heterocyclyl groups containing two heteroatoms include, without limitation, piperazinyl, morpholinyl, dithianyl, dioxanyl. Exemplary 6-membered heterocyclyl groups containing two heteroatoms include, without limitation, triazinanyl. Exemplary 7-membered heterocyclyl groups containing one heteroatom include, without limitation, azepanyl, oxepanyl and thiepanyl. Exemplary
 40 8-membered heterocyclyl groups containing one heteroatom include, without limitation, azocanyl,

oxecanyl and thiocanyl. Exemplary 5-membered heterocyclyl groups fused to a C₆ aryl ring (also referred to herein as a 5,6-bicyclic heterocyclic ring) include, without limitation, indoliny, isoindoliny, dihydrobenzofuranyl, dihydrobenzothienyl, benzoxazolinonyl, and the like. Exemplary 6-membered heterocyclyl groups fused to an aryl ring (also referred to herein as a 6,6-bicyclic heterocyclic ring) include, without limitation, tetrahydroquinoliny, tetrahydroisoquinoliny, and the like.

Nitrogen-containing heterocyclyl groups include morpholine, piperidine (e.g. 2-piperidinyl, 3-piperidinyl and 4-piperidinyl), pyrrolidine (e.g. 2-pyrrolidinyl and 3-pyrrolidinyl), azetidine, pyrrolidone, imidazoline, imidazolidinone, 2-pyrazoline, pyrazolidine, piperazine, and N-alkyl piperazines such as N-methyl piperazine.

“Alkoxy” refers to the group –O-alkyl. In some embodiments, alkoxy is –O-C₁₋₆alkyl. In some embodiments, alkoxy is –O-C₁₋₂alkyl.

“Halo” or “halogen” refers to fluoro (F), chloro (Cl), bromo (Br), and iodo (I). In certain embodiments, the halo group is either fluoro or chloro.

“Amino acid” refers to compounds having both amino functionality and acid functionality. In some embodiments, an amino acid is a naturally occurring amino acid found in proteins. In some embodiments, an amino acid may be selected from glycine, alanine, valine, cysteine, leucine, isoleucine, serine, threonine, methionine, glutamic acid, aspartic acid, glutamine, asparagine, lysine, arginine, proline, histidine, phenylalanine, tyrosine, and tryptophan.

“Amino acid residue” refers to a portion of an amino acid after removal of a hydrogen atom from the N-terminus (–NH₂) and/or a hydroxyl group from the C-terminus (–COOH).

Exemplary carbon atom substituents include, but are not limited to, halogen, –CN, –NO₂, –N₃, –SO₂H, –SO₃H, –OH, –OR^{aa}, –N(R^{bb})₂, –SH, –SR^{aa}, –C(=O)R^{aa}, –CO₂H, –CHO, –CO₂R^{aa}, –OCO₂R^{aa}, –SO₂R^{aa}, C₁₋₁₀ alkyl, C₁₋₁₀ haloalkyl, C₂₋₁₀ alkenyl, C₂₋₁₀ alkynyl, C₃₋₁₀ cycloalkyl, 3–14 membered heterocyclyl, C₆₋₁₄ aryl, and 5–14 membered heteroaryl; each instance of R^{aa} is, independently, selected from C₁₋₁₀ alkyl, C₁₋₁₀ haloalkyl, C₂₋₁₀ alkenyl, C₂₋₁₀ alkynyl, C₃₋₁₀ cycloalkyl, 3–14 membered heterocyclyl, C₆₋₁₄ aryl, and 5–14 membered heteroaryl, or two R^{aa} groups are joined to form a 3–14 membered heterocyclyl or 5–14 membered heteroaryl ring; each instance of R^{bb} is, independently, selected from hydrogen, –OH, –OR^{aa}, –N(R^{cc})₂, –CN, –C(=O)R^{aa}, –CO₂R^{aa}, –SO₂R^{aa}, –SO₂R^{cc}, C₁₋₁₀ alkyl, C₁₋₁₀ haloalkyl, C₂₋₁₀ alkenyl, C₂₋₁₀ alkynyl, C₃₋₁₀ cycloalkyl, 3–14 membered heterocyclyl, C₆₋₁₄ aryl, and 5–14 membered heteroaryl, or two R^{bb} groups are joined to form a 3–14 membered heterocyclyl or 5–14 membered heteroaryl ring; each instance of R^{cc} is, independently, selected from hydrogen, C₁₋₁₀ alkyl, C₁₋₁₀ haloalkyl, C₂₋₁₀ alkenyl, C₂₋₁₀ alkynyl, C₃₋₁₀ cycloalkyl, 3–14 membered heterocyclyl, C₆₋₁₄ aryl, and 5–14 membered

heteroaryl, or two R^{∞} groups are joined to form a 3–14 membered heterocyclyl or 5–14 membered heteroaryl ring.

Nitrogen atoms can be substituted or unsubstituted as valency permits, and include primary, secondary, tertiary, and quaternary nitrogen atoms. Exemplary nitrogen atom substituents include, but are not limited to, hydrogen, $-OH$, $-OR^{aa}$, $-N(R^{\infty})_2$, $-CN$, $-C(=O)R^{aa}$, $-C(=O)N(R^{\infty})_2$, $-CO_2R^{aa}$, $-SO_2R^{aa}$, $-SO_2R^{\infty}$, $-SOR^{aa}$, C_{1-10} alkyl, C_{1-10} haloalkyl, C_{2-10} alkenyl, C_{2-10} alkynyl, C_{3-10} cycloalkyl, 3–14 membered heterocyclyl, C_{6-14} aryl, and 5–14 membered heteroaryl, or two R^{∞} groups attached to a nitrogen atom are joined to form a 3–14 membered heterocyclyl or 5–14 membered heteroaryl ring.

As used herein, the term “optionally substituted” is used when referring to a moiety that may be unsubstituted or substituted by one or more groups. Possible substituents include acyl, acylamino, acyloxy, alkoxy, alkoxycarbonyl, alkoxycarbonylamino, amino, substituted amino, aminocarbonyl (e.g., amido), aminocarbonylamino, aminosulfonyl, sulfonylamino, heteroaryl, aryl, aryloxy, azido, carboxyl, cyano, heterocyclyl, cycloalkyl, halogen, hydroxy, keto, nitro, thiol, $-S$ -alkyl, $-S$ -aryl, $-S(O)$ -alkyl, $-S(O)$ -aryl, $-S(O)_2$ -alkyl, and $-S(O)_2$ -aryl.

In one embodiment A is not an anti-TRIM21 antibody or antigen binding fragment thereof. In one embodiment B does not bind TRIM21, e.g. the target protein is not TRIM21. In one embodiment B is not an anti-TRIM21 antibody or antigen binding fragment thereof. In one embodiment A is not an anti-TRIM21 antibody or antigen binding fragment thereof and B is not an anti-TRIM21 antibody or antigen binding fragment thereof. In particular the compounds of the invention do not comprise an Fc or Fc region.

In embodiment B is not an anti-tau antigen or antigen binding fragment thereof. In one embodiment A is not an anti-TRIM21 antibody or antigen binding fragment thereof and B is not an anti-tau antibody or antigen binding fragment thereof.

An “antibody” as used herein includes but is not limited to, polyclonal, monoclonal, recombinant, chimeric, complementarity determining region (CDR)-grafted, single chain, bi-specific, Fab fragments and fragments produced by a Fab expression library. Such fragments include fragments of whole antibodies which retain their binding activity for the desired antigen, Fv, $F(ab')$, $F(ab')_2$ fragments, and $F(v)$ or V_H antibody fragments.

As used herein, the term “antibody fragments” refers to portions of an intact full length antibody – such as an antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab' , $F(ab')_2$, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules (e.g., scFv); multispecific antibody fragments such as bispecific, trispecific, and multispecific antibodies (e.g., diabodies, triabodies, tetrabodies); binding-domain immunoglobulin fusion proteins; camelized antibodies; minibodies; chelating recombinant antibodies; tribodies or

bibodies; intrabodies; nanobodies; small modular immunopharmaceuticals (SMIP), V_{HH} containing antibodies; and any other polypeptides formed from antibody fragments.

Antibodies and fragments thereof also encompass antibody variants and fragments thereof. Variants
5 include peptides and polypeptides comprising one or more amino acid sequence substitutions, deletions, and/or additions that have the same or substantially the same affinity and specificity of epitope binding as the antigen-specific antibody or fragments thereof

An "Fc" or "Fc region", as used herein, is the constant region of an antibody excluding the first
10 constant region immunoglobulin domain. Thus, Fc refers to the last two constant region immunoglobulin domains of IgA, IgD, and IgG, and the last three constant region immunoglobulin domains of IgE and IgM, and the flexible hinge N-terminal to these domains. For IgA and IgM, Fc may include the J chain. For IgG, Fc comprises immunoglobulin domains C_H2 and C_H3 and the hinge between C_H1 and C_H2. Although the boundaries of the Fc region may vary, the human IgG heavy
15 chain Fc region is usually defined to comprise residues C226 or P230 to its carboxyl-terminus. TRIM21 recognises the Fc domain of antibodies. However, the compounds of the invention do not comprise an Fc or Fc region as "A", i.e. the TRIM21 binding moiety of the compounds of the invention do not comprise or consist of an Fc or Fc region.

20 The term "pharmaceutically acceptable salt" refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art.

25 The inventions also provide compositions comprising the compounds of the invention. In one embodiment there is provided a pharmaceutical composition comprising a compound according to the invention, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

The compositions of the present disclosure may be formulated in a conventional manner using one or
30 more pharmaceutically acceptable carriers. Pharmaceutically acceptable carriers that may be used in these pharmaceutical compositions include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as prolamine sulfate, disodium hydrogen phosphate,
35 potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat.

In any of the aspects or embodiments described herein, the therapeutic compositions comprising the compounds described herein can be in any suitable dosage form, e.g., solid, or liquid, and configured to be delivered by any suitable route, e.g., oral, parenteral, intravenous, intraperitoneal, subcutaneous, intramuscular, etc.

5

The compounds of the invention may be used for therapy as a medicament. In some embodiments there is provided a compound having the structure of formula (I) A-L-B as described herein for use in treating a subject. In some embodiments there is provided the use of a compound having the structure of formula (I) A-L-B for use in the manufacture of medicament for the treatment of a subject. In some
10 embodiments there is provided a method for treating a subject, the method comprising administering to the subject a compound having the structure of formula (I) A-L-B. In some embodiments the invention also provides the use of the compounds having the structure of formula (I) A-L-B in the manufacture of a medicament for use in the treatment of a subject.

15 The disease to be treated will depend on the target protein. For example, in one embodiment the invention provides the compounds having the structure of formula (I) A-L-B for use in the treatment of neurodegenerative disease. The neurodegenerative disease may associated with Tau accumulation and aggregation, for example Alzheimer's Disease. In such embodiments the target protein may be tau, e.g. B may be a tau protein binding moiety.

20

The terms "treat", "treating" or "treatment" (or equivalent terms) mean that the severity of the individual's condition is reduced or at least partially improved or ameliorated and/or that some alleviation, mitigation or decrease in at least one clinical symptom is achieved and/or there is an inhibition or delay in the progression of the condition and/or prevention or delay at the onset of a
25 disease or illness.

The terms "patient", "individual" or "subject" include human and other mammalian subjects that receive either prophylactic or therapeutic treatment with the compounds described herein. Mammalian subjects include primates, e.g., non-human primates. Mammalian subjects also include laboratory
30 animals commonly used in research, such as but not limited to rabbits and rodents such as rats and mice.

In another embodiment, the present disclosure provides a method of ubiquitinating/degrading a target protein (e.g. Tau) in a cell. The method comprises administering a compound comprising the structure
35 of formula (I) A-L-B as described herein such that degradation of the target protein will occur when the target protein is placed in proximity to the ubiquitin ligase, thus resulting in degradation/inhibition of the effects of the target protein and the control of protein levels. The control of protein levels afforded by the present disclosure provides treatment of a disease state or condition, which is modulated through the target protein by lowering the level of that protein in the cells of a patient.

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The invention also relates to methods of selectively degrading oligomeric forms or pathogenic forms of a target protein, the method comprising contacting the target protein with TRIM21 and a compound having the structure of the formula (I) A-L-B, or a pharmaceutically acceptable salt thereof.

- 5 The method can be an in vivo method. Alternatively, the method can be an in vitro method, wherein the oligomeric forms of a target protein are in a sample. The method can comprise introducing the compound of the invention into the sample.

The phrase "selected from the group comprising" may be substituted with the phrase "selected from
10 the group consisting of" and vice versa, wherever they occur herein.

Similarly, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise.

- 15 The contents of all publications cited herein are incorporated herein by reference in their entirety into this application to more fully describe the state of the art to which this invention pertains.

EXAMPLES

- 20 The invention will now be described with reference to the following non-limiting examples.

Example 1: Screen to identify compounds binding to Trim21 PRYSPRY

- Construct details.** Trim21 PRYSPRY protein was expressed as an N-terminal fusion with a hexahistidine tag, which adds the residues MAHHHHHHM to the hsTrim21 PRYSPRY sequence
25 (residues 287-475). The expression and purification was performed as previously described (James et. al., 2007).

Protein name	Trim21
Protein ID	EC:2.3.2.27
Amino acid sequence	MAHHHHHHMVHITLDPDTANPWLILSEDRRQVRLGDT QQSIPGNEERFDSYPMVLGAQHFGKHYWEVDVTG KEAWDLGVCRDSVRRKGHFLSSKSGFWTIWLWNKQ KYEAGTYPQTPLHLQVPPCQVGIFLDYEAGMVSFYNIT DHGSLIYSFSECAFTGPLRPFFSPGFNDGGKNTAPLTLC PLNIGSQGSTDY (SEQ ID NO:2)
Uniprot ID	P19474
Classification	E3 Ligase
Isolation	IMAC purification and Size Exclusion Chromatography
Protein activity	Antibody binding

Structure	2IWG
Molecular weight	22.5 kDa

Capture of T21 PRYSPRY on beads. To assure the screen worked, it was necessary to capture T21 PRYSPRY protein on beads and ensure it was still active. The activity of T21 PRYSPRY can be monitored by its ability to maintain binding to IgG-Fc which is only possible when T21 PRYSPRY is in its native fold. T21 PRYSPRY was covalently coupled to magnetic carboxyl derived beads using a two step EDC-NHS protocol as detailed below.

Covalent capture of Trim21 PRYSPRY to magnetic beads

- 1) Dilute 10 μ L of MagnaBind Carboxyl beads (Catalog number: 21353) in 500 μ L MPQ in an Eppendorf and mix well.
- 2) Wash the beads in 100 μ L MPQ (by using a magnetic tube rack to collect the beads at the side of the Eppendorf and remove the current buffer with a pipette. Immediately add the listed solution and mix by brief vortexing)

3) Prepare the coupling reaction

1. Thaw an aliquot of EDC (0.4 M) and NHS (0.1 M). These are prepared in advance in MPQ and stored at -20 °C.
2. In an eppendorf, mix 20 μ L of 0.5 M MES buffer at pH 6 with 100 μ L of each EDC and NHS and mix well by pipetting.
3. Transfer 100 μ L of this reaction to the washed beads (previous solution is discarded), immediately vortex to prevent aggregation of beads and place on the shaker (20 °C, 1400 RPM)
4. Incubate for 30 minutes

4) Adding protein to activated beads

1. Prepare protein dilution: His-Trim21-PRYSPRY (15 mg/ml) is diluted to a final concentration of 10 μ g in 100 μ L (0.1 mg/ml) in HEPES buffer (20 mM HEPES (pH 7.8), 150 mM NaCl and 1 mM TCEP and stored on ice. Need 100 μ L per reaction.
2. wash the beads with HEPES buffer (100 μ L)
3. Add 100 μ L (previous solution is discarded) of protein solution to beads and immediately vortex and place on shaker.
4. Incubate 15 minutes whilst shaking (20 °C, 1400 RPM)

5) Quenching and blocking

1. Add 10 μ L of 1 M Ethanolamine-HCl pH 8.5 to the reaction, without discarding the solution!
2. Incubate for 10 minutes whilst shaking
3. Collect the flowthrough and change into 50 mM Ethanolamine-HCl pH 8.5 (100 μ L) for 15 minutes.
4. Change into any required buffer

Abbreviations. EDC: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide. NHS: N-Hydroxysuccinimide. MPQ: Milli-Q filtered water.

IgG pulldown with immobilised T21 PRYSPRY. T21 PRYSPRY, no protein or control protein were covalently coupled to magnetic carboxyl derived beads. Binding experiments were carried out in 20 mM HEPES pH 7.8, 150 mM NaCl and 1 mM TCEP. Beads were incubated with 2 μ M of IgG for 15 minutes, washed and the bound beads collected. Beads were incubated at 95 °C for 15 minute and analysed by gel electrophoresis and Coomassie staining. Additionally, for the competition experiment, ProteinAG was included at 0.1, 1 or 10 μ M during the binding incubation and otherwise processed as before.

Results. PRYSPRY-coupled beads were capable of efficiently capturing IgG. To show that capture was through specific interaction, the incubation was repeated in the presence of increasing concentrations of Protein AG, which binds the same epitope on IgG Fc as TRIM21. The ability of Protein AG to dose-dependently inhibit capture of IgG to PRYSPRY-coupled beads confirmed that interaction in this format is specific. The PRYSPRY-coupled beads were then used to capture specific ligands. Enrichment was then assessed and specific compounds chosen.

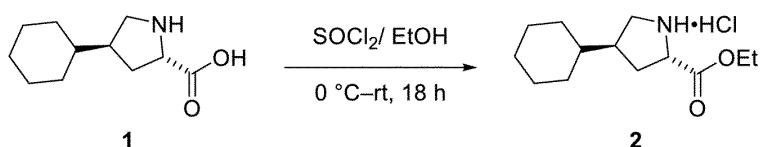
Examples of compounds recovered from the screen are detailed in Table 1 below.

Table 1. Properties of selected TRIM21 ligands

Compound	Affinity	Molecular weight / (g/mol)	Chemical name	Chemical structure
36	21 μ M	520.62	4-(((4-(1H-imidazol-1-yl)-3-(1-(4-methoxybenzyl)-3-methyl-1Hpyrazol-4-yl)benzyl)amino)methyl)-N-methylbenzamide	
37	250 nM	535.68	(2S,4S)-1-((S)-3-amino-3-(2-methoxyphenyl)propanoyl)-4-cyclohexyl-N-((S)-1-(methylamino)-1-oxo-3-(pyridin-4-yl)propan-2-yl)pyrrolidine-2-carboxamide	

38	1.8 μ M	495.61	(2S,4S)-1-((S)-3-amino-3-(furan-2-yl)propanoyl)-4-cyclohexyl-N((S)-1-(methylamino)-1-oxo-3-(pyridin-4-yl)propan-2-yl)pyrrolidine-2-carboxamide	
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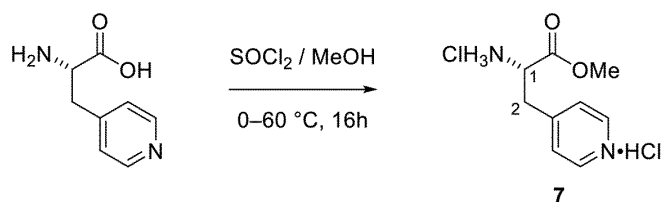
Example 2: Synthesis of N-boc protected Compound 37.



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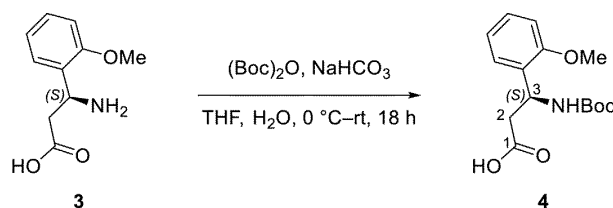
To a stirred solution of *trans*-4-cyclohexyl-L-proline (100 mg, 0.507 mmol, 1.00 equiv.) in ethanol (2.5 mL, 0.20 M) at 0 °C was added thionyl chloride (0.111 mL, 1.52 mmol, 3.00 equiv.) dropwise. The resulting reaction mixture was left to stir at room temperature for 18 h. The reaction mixture was concentrated *in vacuo* (toxic Buchi) to give desired product **2** as a white solid (130 mg, 98%). δ_{H} (400 MHz, CDCl_3) 0.84–1.38 (9H, m), 1.56–1.78 (5H, m), 1.94–2.10 (2H, m), 2.24–2.38 (1H, m), 3.06 (1H, br s), 3.75 (1H, br s), 4.28 (2H, q, J 7.1 Hz), 4.48 (1H, br s), 8.88 (1H, br s), 11.11 (1H, br s); δ_{C} (101 MHz, CDCl_3) 14.2 (CH_3), 25.9 ($2 \times \text{CH}_2$), 26.2 (CH_2), 31.6 (CH_2), 32.0 (CH_2), 33.3 (CH_2), 41.1 (CH_3), 43.1 (CH_3), 49.4 (CH_2), 59.4 (CH_3), 63.2 (CH_2), 169.1 (C); m/z (ESI) 226.1806 (MH^+ . $\text{C}_{13}\text{H}_{24}\text{NO}_2$ requires 226.1802).

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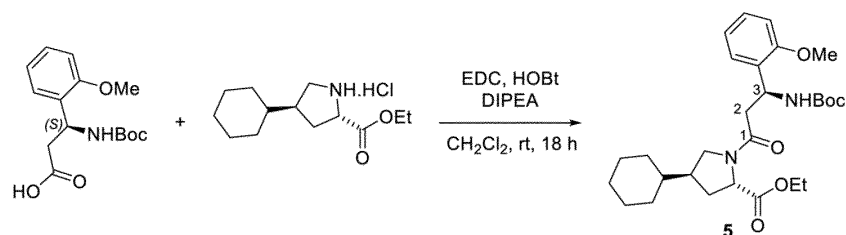


To a stirred solution of 3-(4-pyridyl)-L-alanine (100 mg, 0.602 mmol, 1.00 equiv.) in methanol (2.5 mL, 0.25 M) at 0 °C was added thionyl chloride (0.132 mL, 1.81 mmol, 3.00 equiv.) dropwise. The resulting reaction mixture was left to stir at 60 °C for 18 h. The reaction mixture was concentrated *in vacuo* (toxic Buchi) to give desired product **7** as a white solid (155 mg, 100%). δ_{H} (400 MHz, CD_3OD) 3.55 (1H, dd, J 14.5, 6.5 Hz, 2- HH), 3.63 (1H, dd, J 14.5, 7.9 Hz, 2- HH), 3.83 (3H, s, CH_3), 4.67 (1H, dd, J 7.9, 6.5 Hz, 1-H), 8.10–8.16 (2H, m, $2 \times \text{ArH}$), 8.85–8.90 (2H, m, $2 \times \text{ArH}$); δ_{C} (101 MHz, CD_3OD) 37.0 (CH_2), 53.5 (CH), 54.0 (CH_3), 129.7 ($2 \times \text{CH}$), 142.9 ($2 \times \text{CH}$), 158.3 (C), 169.4 (C); m/z (ESI) 181.0973 (MH^+ . $\text{C}_9\text{H}_{12}\text{N}_2\text{O}_2$ requires 181.0972).

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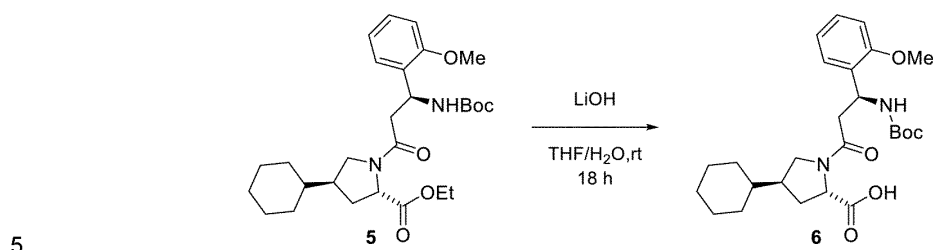


(S)-3-Amino-3-(2-methoxyphenyl)propionic acid (100 mg, 0.512 mmol, 1.00 equiv.) was dissolved in tetrahydrofuran (1.3 mL) and water (1.3 mL) and the resulting solution was cooled to 0 °C. Sodium hydrogen carbonate (129 mg, 1.54 mmol, 3.00 equiv.) was added in one portion followed by di-*tert*-butyl decarboxylate. The resulting reaction mixture was left to stir at room temperature for 18 h. The tetrahydrofuran was removed under reduced pressure and the reaction mixture was acidified to pH 1 with 1 M aqueous hydrochloric acid. The aqueous layer was extracted with dichloromethane (3 × 20 mL). The combined organic layers were dried over MgSO₄, filtered and concentrated *in vacuo* to give the desired product **4** as a white solid (131 mg, 87%); δ_{H} (400 MHz, CD₃OD) 1.42 (9H, s, 3 × CH₃), 2.63 (1H, dd, *J* 15.5, 8.8 Hz, 2-*HH*), 2.75 (1H, dd, *J* 15.5, 5.1 Hz, 2-*HH*), 3.87 (3H, s, OCH₃), 5.30 (1H, br s, 3-H), 6.90 (1H, t, *J* 7.5 Hz, ArH), 6.96 (1H, d, *J* 8.2 Hz, ArH), 7.19–7.29 (2 H, m, 2 × ArH); δ_{C} (101 MHz, CD₃OD) 28.7 (3 × CH₃), 40.7 (CH₂), 54.8 (C), 55.8 (CH), 80.2 (C), 111.8 (CH), 121.5 (CH), 128.0 (CH), 129.6 (CH), 131.2 (C), 157.4 (C), 158.0 (C), 174.8 (C); *m/z* (ESI) 318.1319 (MNa⁺. C₁₅H₂₁NNaO₅ requires 318.1312).



To a stirred solution of (S)-3-((*tert*-butoxycarbonyl)amino)-3-(2-methoxyphenyl)propanoic acid (38 mg, 0.13 mmol, 1.0 equiv.) in dichloromethane (0.4 mL) was added *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (30 mg, 0.15 mmol, 1.2 equiv.) and 1-hydroxybenzotriazole hydrate (24 mg, 0.15 mmol, 1.2 equiv.) and the resulting solution was left to stir at room temperature for 10 minutes. To the reaction mixture was added a stirred solution of (2S,4S)-ethyl 4-cyclohexylpyrrolidine-2-carboxylate hydrochloride (34 mg, 0.13 mmol, 1.0 equiv.) and *N,N*-diisopropylethylamine (0.067 mL, 0.39 mmol, 3.0 equiv.) in dichloromethane and the resulting reaction mixture was left to stir at room temperature for 18 h. The reaction mixture was concentrated *in vacuo* and purified by flash column chromatography (petroleum ether/ethyl acetate 7:3) to give desired product **5** as a white solid (55 mg, 85%). δ_{H} (400 MHz, CD₃OD) 0.78–1.46 (19H, m), 1.54–1.91 (6H, m), 1.94–2.11 (2H, m), 2.71 (1H, dd, *J* 15.0, 5.0 Hz, 2-*HH*), 2.80 (1H, dd, *J* 15.0, 7.9 Hz, 2-*HH*), 2.88–3.03 (1H, m), 3.64–3.79 (1H, m), 3.88 (3H, s, OCH₃), 4.09–4.25 (2H, m), 4.41 (1H, d, *J* 9.3, CH), 5.34 (1H, br s, 3-H), 6.92 (1H, td, *J* 7.4, 1.1 Hz, ArH), 6.95–7.00 (1H, m, ArH), 7.20–7.29 (2H, m, 2 × ArH); δ_{C} (101 MHz, CD₃OD) 14.4 (3 × CH₃), 27.1 (CH), 27.1 (CH), 27.4 (CH), 28.8 (CH₂), 32.3 (CH), 32.9 (CH), 34.2 (CH), 36.0 (C), 40.1 (CH),

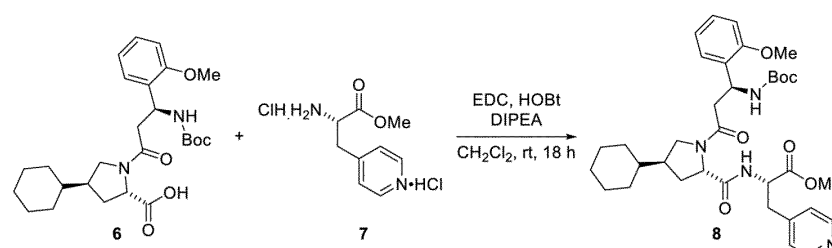
42.9 (CH), 44.9 (CH), 51.7 (CH₂), 52.5 (CH₂), 55.9 (CH₃), 60.5 (CH), 61.4 (CH), 62.3 (CH₂), 111.7 (CH), 121.6 (CH), 127.6 (C), 129.5 (CH), 129.7 (CH), 157.9 (C), 171.9 (C), 173.7 (C); *m/z* (LC-MS, ESI⁺) 503 (MH⁺, 37%); (HR-MS, ESI) 525.2943 (MNa⁺. C₂₈H₄₂N₂NaO₆ requires 525.2935).



To a stirred solution of **5** (126 mg, 0.251 mmol, 1.00 equiv.) in tetrahydrofuran (2.4 mL) and water (2.4 mL) was added lithium hydroxide hydrate (15.8 mg, 0.376 mmol, 1.50 equiv.). The resulting reaction mixture was left to stir at room temperature for 18 h. The tetrahydrofuran was removed *in vacuo* and the reaction mixture was acidified to pH 1 with 1 M aqueous hydrochloric acid. The aqueous layer was extracted with dichloromethane (3 × 30 mL). The combined organic layers were dried over MgSO₄, filtered and concentrated *in vacuo* to give the desired product **6** as a white solid (115 mg, 97%). δ_{H} (400 MHz, CD₃OD) 0.81–1.33 (7H, m), 1.41 (9H, s, 3 × CH₃), 1.53–1.93 (7H, m), 1.95–2.52 (2H, m), 2.63–3.02 (3H, m), 3.57–3.74 (1H, m), 3.87 (3H, s, OCH₃), 4.42 (1H, d, *J* 9.2 Hz, CH), 5.17–5.39 (1H, m, 3-H), 6.91 (1H, br t, *J* 7.4 Hz, ArH), 6.96 (1H, br d, *J* 8.5 Hz, ArH), 7.19–7.28 (2H, m, 2 × ArH); δ_{C} (101 MHz, MeOD) 27.1, 27.1, 27.4, 28.8, 30.7, 32.3, 32.4, 32.9, 34.3, 36.0, 40.0, 40.9, 42.9, 43.0, 43.1, 44.8, 48.3, 51.7, 52.5, 54.8, 55.8, 55.9, 60.3, 61.3, 80.3, 111.6, 111.7, 121.6, 121.7, 127.6, 128.0, 129.5, 129.7, 130.9, 131.3, 157.1, 157.3, 157.8, 171.9, 171.9, 175.4; *m/z* (LC-MS, ESI⁻) 473 (MH⁻, 100%); (HR-MS, ESI⁺) 475.2807 (MH⁺. C₂₆H₃₉N₂O₆ requires 475.2803).

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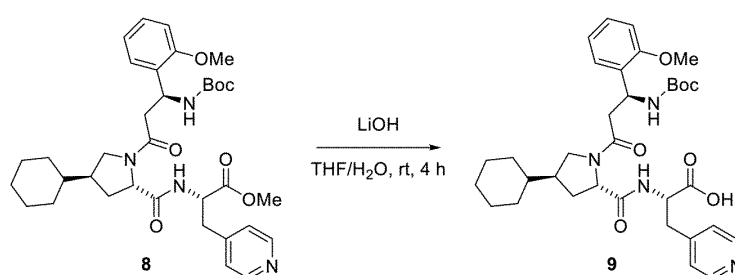
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To a stirred solution of **6** (35 mg, 0.074 mmol, 1.0 equiv.) in dichloromethane (1.0 mL) was added *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (17 mg, 0.089 mmol, 1.2 equiv.) and 1-hydroxybenzotriazole hydrate (14 mg, 0.089 mmol, 1.2 equiv.) and the resulting solution was left to stir at room temperature for 10 minutes. To the reaction mixture was added a stirred solution of **7** (16 mg, 0.074 mmol, 1.0 equiv.) and *N,N*-diisopropylethylamine (0.039 mL, 0.22 mmol, 3.0 equiv.) in dichloromethane and the resulting reaction mixture was left to stir at room temperature for 18 h. The reaction mixture was concentrated *in vacuo* and purified by flash column chromatography (dichloromethane/methanol 49:1) to give desired product **8** as a white solid (32 mg, 79%). δ_{H} (400

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MHz, CDCl₃) 0.66–1.22 (7H, m), 1.41 (9H, s, 3 × CH₃), 1.54–1.81 (5H, m), 2.18–2.36 (2H, m, 2-H₂), 2.59 (1H, dd, *J* 14.5, 5.2 Hz), 2.78–2.96 (2H, m), 3.08–3.23 (2H, m), 3.74 (3H, s, OCH₃), 3.87 (3H, s, OCH₃), 4.42 (1H, d, *J* 7.9 Hz, CH), 4.82 (1H, td, *J* 8.3, 5.4 Hz), 5.36 (1H, br s), 6.33–6.45 (1H, m, NH), 6.80–6.94 (2H, m, ArH), 6.98–7.08 (2H, m, 2 × ArH), 7.17–7.29 (2H, m, 2 × ArH), 7.61 (1H, br d, *J* 8.2 Hz, NH), 8.43–8.59 (2H, m, 2 × ArH); δ_C (101 MHz, CDCl₃) 26.0 (CH₂), 26.1 (CH₂), 26.3 (CH₂), 28.5 (3 × CH₃), 30.9 (CH₂), 31.3 (CH₂), 31.9 (CH₂), 37.4 (CH₂), 38.3 (CH₂), 41.9 (CH), 43.8 (CH), 47.3 (CH), 51.7 (CH₂), 52.3 (CH), 52.6 (CH₃), 55.5 (CH₃), 59.8 (CH), 77.4 (C), 79.5 (C), 110.4 (CH), 110.6 (C), 120.8 (CH), 124.7 (2 × CH), 127.3 (CH), 128.6 (CH), 145.7 (C), 149.7 (2 × CH), 155.1 (C), 156.4 (C), 170.9 (C), 171.4 (C); *m/z* (LC-MS, ESI⁺) 637 (MH⁺, 100%); (ESI) 659.3419 (MNa⁺. C₃₅H₄₈N₄NaO₇ requires 659.3415).



To a stirred solution of **8** (30 mg, 0.047 mmol, 1.0 equiv.) in tetrahydrofuran (0.5 mL) and water (0.5 mL) was added lithium hydroxide hydrate (3.0 mg, 0.071 mmol, 1.5 equiv.). The resulting reaction mixture was left to stir at room temperature for 4 h. The tetrahydrofuran was removed *in vacuo* and the reaction mixture was acidified to pH 1 with 1 M aqueous hydrochloric acid. The aqueous layer was extracted with dichloromethane (3 × 20 mL). The combined organic layers were dried over MgSO₄, filtered and concentrated *in vacuo* to give the desired product **9** as a white solid (20 mg, 69%). δ_H (400 MHz, CD₃OD) 0.71–2.10 (25H, m), 2.29–2.71 (2H, m), 2.74–3.15 (3H, m), 3.36–3.77 (2H, m), 3.85 (3H, s, OCH₃), 4.37 (1H, d, *J* 8.8 Hz), 4.69 (1H, dd, *J* 8.1, 5.0 Hz), 5.25–5.42 (1H, m), 6.84–7.01 (2H, m), 7.17–7.33 (3H, m), 7.39 (1H, d, *J* 5.1 Hz), 8.26–8.52 (2H, m); δ_C (101 MHz, CD₃OD) 25.7, 25.7, 26.0, 27.4, 31.0, 31.6, 33.0, 35.4, 36.4, 38.5, 41.6, 43.3, 50.7, 51.4, 54.6, 60.0, 61.3, 110.3, 110.4, 120.2, 124.8, 125.5, 128.2, 147.8, 148.5, 149.0, 156.4, 156.5, 170.7, 172.7; *m/z* (LC-MS, ESI⁺) 637 (MH⁺, 100%); (APCI) 623.3439 (MH⁺. C₃₄H₄₇N₄O₇ requires 623.3439).

To obtain Compound 37, the *N*-boc group of **9** may be removed under acidic conditions. Alternatively, **9** may be used in further synthesis to obtain bifunctional molecules.

Example 3: Structural and biophysical characterisation of TRIM21 ligands

Binding of compounds 36–38 was tested to TRIM21 PRYSPRY protein using a combination of nanoDSF (Figure 1), intrinsic tryptophan fluorescence (Figure 2) and competition fluorescence polarisation (Figure 3). The methods are described below.

NanoDSF (thermal shift assay). Thermal shift assay was performed using the nanoDSF Prometheus instrument. Assay was performed in 150 mM NaCl, 50 mM Tris pH 8, 1 mM DTT and 1% DMSO with T21 PRYSPRY at 10 μ M. Compounds were included at 100 μ M. Denaturation was performed over a range of 80 °C from 15-95 °C with a ramp of 2°C/s. Data was analysed the NanoTemper software.

Intrinsic Tryptophan fluorescence quenching. Measured using nanoDSF Prometheus instrument at 15 °C (Compound 36) or a PHERAstar plate reader at 25 °C for the other two compounds (37 and 38). Assay was performed in 150 mM NaCl, 50 mM Tris pH 8, 1 mM DTT and 1% DMSO. Compound dilutions were prepared as required (usually 2 fold dilutions) starting from 100 μ M. Protein was added from a 10X stock solution prepared in the same buffer for a final protein concentration of 8 μ M for Prometheus experiments and 0.15 μ M for PHERAstar experiments. Samples were prepared in a 384 well format (50 μ L final volume in the well) or microtubes (40 μ L reactions prepared) and left to equilibrate for 15 minutes and then the fluorescence was measured. Data were normalised and analysed in PRISM.

Fluorescence polarisation displacement assay. Experiments were performed on a PHERAstar plate reader at 25 °C in 150 mM NaCl, 50 mM Tris pH 8, 1 mM DTT, 0.01% v/v tween20 and 1% DMSO in 384 well low binding plates. Trim21-PRYSPRY-Alexa488 (50 nM) was pre mixed with human IgG Fc (5 μ M) and left to equilibrate. 5uL of this master mix was aliquoted into the 384 well plates. Compound dilutions were prepared in low binding 96 well plates, usually as 2fold-dilutions starting from 10-100 μ M. Next, 45 uL of the compound solutions was transferred to each well in the 384 well plate. The plate was centrifuged at 700g for 1 minute and left to equilibrate for 30 minutes in the dark. Finally, polarisation was read on the PHERAstar plate reader. Data were analysed in PRISM.

Crystallisation and data collection. To generate diffraction quality crystals, a solution of Trim21-PRYSPRY (15mg/ml) was mixed 50:1 with a stock solution of ligands (100 mM in DMSO), left to equilibrate for 15-30 minutes before crystallisation plates were set up. Mosquito (TTP Labtech) instrument was used to set up sparse matrix screening, using a drop size of 100 nL protein with 200 nL of precipitant. Crystals were obtained by sitting vapour diffusion at 17 °C. Diffraction quality crystals for Compound 37 and Compound 38 were obtained in 30% PEG 10000, 0.1 M TRIS HCl pH 8.5 and snap frozen in liquid nitrogen without cryoprotection. In the case of Compound 36, crystals were obtained in 20% PEG 4000, 5% iso-Propanol, 0.1 M Na Citrate, cryoprotected by supplementing the reservoir with 20% glycerol and frozen in liquid nitrogen. Data were collected at the Diamond Light Source (Didcot, UK) on beamlines I-24 (for Compound 36 and Compound 37) and I-04 for Compound 38.

Structure solution and model building. Data were processed with the CCP4i package as follows: Data were indexed, scaled and integrated using the FASTDP (Winter et al., 2011) and Xia2dials (Winter et al., 2022) pipeline. Molecular replacement was performed with PHASER using the TRIM21

PRYSPRY model 2IWG. The model was iteratively refined using REFMAC5 and COOT for model building. AceDRG was used to generate restraints for ligands.

Results. In nanoDSF experiments, the ability of compounds to bind TRIM21 was confirmed by an increase in PRYSPRY thermostability (T_m) ranging from 2-7 °C (Figure 1). In intrinsic tryptophan fluorescence experiments, it was possible to take advantage of the presence of two tryptophan residues (W381 and W383) that are present in the TRIM21 PRYSPRY binding site. Upon binding of antibody, the intrinsic fluorescence at ~ 340 nm of these tryptophan residues is quenched. Similar behaviour was observed for all three compounds (Figure 2) suggesting both that the compounds all hit the same binding site as the natural TRIM21 ligand (IgG Fc) and allowing to determine affinities. To test whether the compounds are therefore competitive with IgG a series of competition anisotropy experiments were performed with Alexa488-labelled PRYSPRY. Incubation of increasing concentrations of IgG Fc with labelled-PRYSPRY results in the formation of a complex whose larger mass induces a change in polarisation (Figure 3). To test for compound inhibition increasing concentrations of ligands were incubated with preformed IgG:PRYSPRY complex. A decrease in polarised light was observed upon titration of each ligand, indicating that they compete with IgG for binding to labelled-PRYSPRY. To provide a detailed assessment of which residues drive binding of compounds to TRIM21 three different mutants were tested in fluorescence quench experiments. For compound 36, mutation of either D355, W381 or W383 was sufficient to abolish measurable binding. For compounds 37 and 38, mutant W381A showed the weakest affinity, suggesting that this residue is the primary driver of binding (Figure 23A). To obtain atomic resolution information about TRIM21:compound binding and assess ligand complementarity x-ray structures of TRIM21 in complex with ligands 36, 37 and 38 were solved (Figures 23A-C). These structures confirmed that all three ligands access the same pocket in the PRYSPRY domain and explain why mutation of W381 in particular is crucial for interaction with ligands 37 and 38. The selected small molecule ligands also recapitulate features of the natural IgG Fc epitope in an unexpected manner, such as forming a bidentate motif that penetrates deep into the PRYSPRY pocket (Figure 24). The structures also allowed to assess possible exit vectors for building additional groups onto the TRIM21 ligands. Three different vectors were observed that could be used to extend the TRIM21 ligands, such as attaching other moieties capable of binding other proteins (Figure 23B and D).

Table 2 below shows K_D values derived from fluorescence quenching experiments with Trim21 PRYSPRY and its relevant mutants.

Table 2. Binding of TRIM21 ligands to different PRYSPRY mutants

Compound	WT K_D [μ M]	WT IC_{50} [μ M]	D355A K_D [μ M]	W381 A K_D [μ M]	W383 A K_D [μ M]
36	21	58	>100	>100	>100
37	0.25	0.38	0.25	40	0.5
38	1.8	5.5	1.5	>100	5

Example 4: TRIM21 compounds inhibit TRIM21 cellular activity

To assess the cell penetrance of TRIM21 compounds and their ability to engage the endogenous protein in situ, RPE-1 cells were treated with 2.5 μ M of Compound 37 for 18 hours and then blotted for TRIM21 protein levels. Treatment resulted in increased TRIM21 levels (Figure 4), consistent with compound stabilisation of the protein as shown by the previous biophysical data (Figures 1 and 2). To further confirm that TRIM21 compounds are cell permeable, non-toxic and capable of engaging functionally with TRIM21 two types of assay were carried out. The first assay monitors targeted protein degradation by TRIM21 while the second measures the ability of TRIM21 to neutralize viral infection in the presence of antibody.

In the first assay, mRNA encoding a protein consisting of a nanobody against EGFP (NbGFP) fused to an IgG Fc is electroporated into cells that stably express H2B-mEGFP. When the nanobody-Fc is made it binds to H2B-mEGFP and recruits endogenous TRIM21. The resulting degradation of H2B-mEGFP protein by TRIM21 is then monitored by recording cell fluorescence (Figure 5).

RPE-1 cells stably expressing H2B-mEGFP were electroporated with mRNA encoding NbGFP-Fc or NbGFPFc^{H433A} and immediately plated into media containing DMSO or Compound 37 at the concentrations indicated in Figure 6. Cells were imaged using the IncuCyte system and H2B-mEGFP fluorescence quantified by normalising GFP integrated density (then product of area and mean intensity) to total cell area (phase) for each image. H2B-mEGFP fluorescence values were then normalised to NbGFPFcH433A (Fc mutant that cannot bind TRIM21 PRYSPRY; control for no degradation) for each condition, meaning a value of 1 equals no degradation. Treatment of cells with compound 37 was sufficient to dose-dependently inhibit degradation of EGFP-H2B by TRIM21, with almost complete inhibition even after 15 hours (Figure 6).

In the second assay, cells are infected with adenovirus 5 (Adv5) in the presence of anti-adenovirus antibody. Endogenous TRIM21 can detect the incoming antibody-coated virus and degrade it, resulting in a potent block to infection as measured by expression of a virally-encoded GFP gene (Figure 7). Treatment with compounds 37 and 38 dose-dependently rescued infection, consistent with inhibition of TRIM21 activity by competing with IgG for binding (Figure 8).

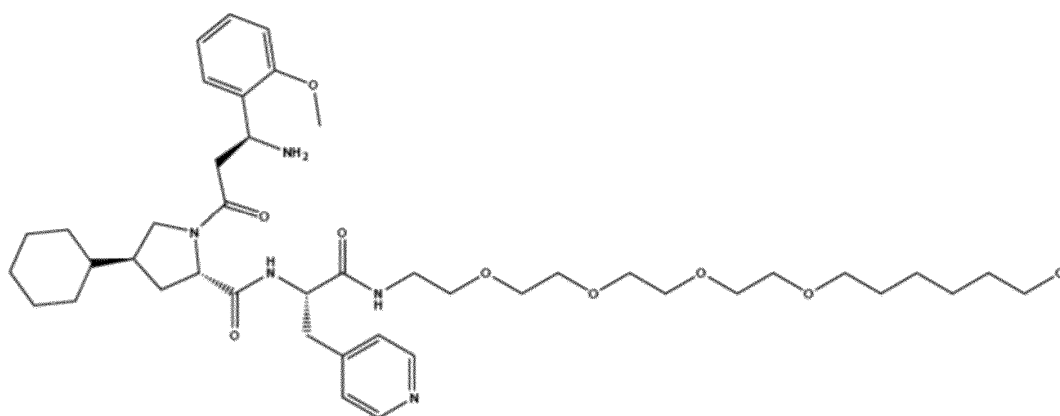
The confluence of cells treated for 48 hours was assessed to determine the toxicity of TRIM21 compounds. Confluence was largely unaffected by compound treatment (Figure 9).

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Example 5: Biophysical characterization of TrimTac and in vitro evidence of ternary complex formation

To investigate the potential for TRIM21 compounds to interact simultaneously with a second protein in addition to the PRYSPRY, a short linker and chloroalkane were appended onto compound 37 to form compound 71:

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Expression and purification. HaloTag protein was expressed as an N-terminal fusion with a hexahistidine tag, which adds the residues MAHHHHHM to the N terminus of the protein. The protein was expressed in *E. coli* (strain C41) using autoinduction media (ZYP-5052, Studier et al). Cells were lysed in 1 M NaCl, 50 mM Tris pH 8, 2 mM DTT, 10 mM Imidazole, 10% v/v BugBuster and cOmplete protease inhibitors by sonication. Lysate was cleared by centrifugation using the JA25.50 fixed angle rotor at 18000 RPM for 45 minutes. Clarified lysate was subject to IMAC affinity step using gravity flow columns packed with NiNTA agarose. After washing and elution, peak fractions were further purified using Size Exclusion Chromatography (Superdex S75 16/60) equilibrated in 50 mM Tris pH 8, 150 mM NaCl and 1 mM DTT.

AUC velocity experiment. The *c(s)* distributions show that both TRIM21 PRYSPRY (solid black line) and HaloTag (dashed black line) alone are monomeric sedimenting at 2.3 S ($Sw_{20} = 2.5$ S) and 2.9 S ($Sw_{20} = 3.2$ S) respectively with calculated masses of 22.0 and 34.4 kDa with frictional ratios of 1.143 and 1.142 respectively. The mixture alone (orange dashed line) results in a single broad distribution that maybe just a cumulative Gaussian of the two individual unbound species. Addition of ligand at 10 μ M (solid orange line) reduces the concentration of T21 PRYSPRY and a large reduction in HaloTag with the appearance of a species sedimenting at 3.7 S ($Sw_{20} = 4.1$ S). If the frictional coefficient is the same as T alone (1.143) then the calculated mass would be 48.1 kDa. This is lower than expected for a 1:1 mass of 57.3 kDa. If this species represents the complex then it would be expected to have a frictional ratio of 1.286 suggesting a slightly more extended/non-spherical conformation.

AUC equilibrium experiment. 5 μ M of TRIM PRYSPRY (22,490 Da) and Halo Tag (34,765 Da) in the absence and presence compound in 50 mM Tris HCl, pH 8.0, 150 mM NaCl, 1 mM DTT and 0.08 % (v/v) DMSO were subjected to AUC sedimentation equilibrium at 20 °C using both absorbance at 280 nm and interference optics. The data were analysed in SEDPHAT using a single exponential function to obtain an average mass. In the absence of compound, the average mass of 30,316 Da is close to the average of the two proteins at equimolar concentrations. As the concentration of compound increases the average mass reaches a maximum at 5 μ M where the average mass is $50,482 \pm 82$ Da, which is close to the expected value for a 1:1 complex. Increasing concentration beyond this point

decreases the average mass due to the binding of the compound to individual components in competition with complex formation.

Crystallisation, data collection and structure solution. To generate diffraction quality crystals, solution of HaloTag protein (13mg/ml) was mixed 50:1 with a stock solution of Compound 71 (50 mM in DMSO), left to equilibrate for 30 minutes before crystallisation plates were set up. Mosquito (TTP Labtech) instrument was used to set up sparse matrix screening, using a drop size of 100 nL protein with 100 nL of precipitant. Crystals were obtained by sitting vapour diffusion at 17 °C. Diffraction quality crystals were obtained in 25% PEG 4000, 0.1 M Na MES pH 6.5, 0.2 M Mg Chloride. Crystals cryoprotected with 20-30% glycerol and frozen in liquid nitrogen. Data were collected at the Diamond Light Source (Didcot, UK) on beamline I-04. Data were processed with the CCP4i package as follows: Data were indexed, scaled and integrated using the Xia2dials (Winter et al., 2022) pipeline. Molecular replacement was performed with PHASER using the HaloTag model from 5UY1. The model was iteratively refined using REFMAC5 and COOT for model building. AceDRG was used to generate restraints for ligands.

Model building. HADDOCK software was used to generate docked models of Trim21 PRYSPRY and HaloTag. These were inspected for appropriate orientation. COOT was used to build the model with a fitted ligand. PyMOL was used to visualise the resulting structures and models.

Results. The conjugation of a short linker and chloroalkane to compound 37 did not alter the engagement efficiency with PRYSPRY, as assessed by nanoDSF (Figure 10). It was tested whether compound 71 could simultaneously bind PRYSPRY and a second HaloTag protein by velocity analytical ultracentrifugation (AUC). Binding of compound 71 did not appreciably alter the mass of either the PRYSPRY or HaloTag protein alone (Figure 11). Moreover, the two proteins were unable to interact with each other in the absence of compound as indicated by a broadened peak with mass in-between that of both proteins. Importantly however, the addition of compound 71 to a solution containing both PRYSPRY and HaloTag resulted in the formation of a complex with the expected mass of 57 kDa (Figure 11). This result indicates that a ternary complex can be formed in vitro. These experiments were extended and the mass of the ternary complex was measured at a range of compound concentrations. The mass of the ternary complex peaked at close to the expected mass (~50 kDa) before decreasing upon further increases in compound concentration (Figure 12). This behaviour is consistent with the 'Hook Effect'; a noted phenomenon of ternary complex formation that occurs when the intermediary is added at excess and forms separate 1:1 complexes with each of the other two partners. To understand how compound 71 is mediating formation of the ternary complex an x-ray structure of the complex with HaloTag was solved. Clear density could be seen for the chloroalkane component of compound 71, located as expected in the HaloTag active site. This structure was then used together with the complex of compound 37 bound to the PRYSPRY to make a ternary complex model. This model indicates that there are no steric clashes upon ternary complex

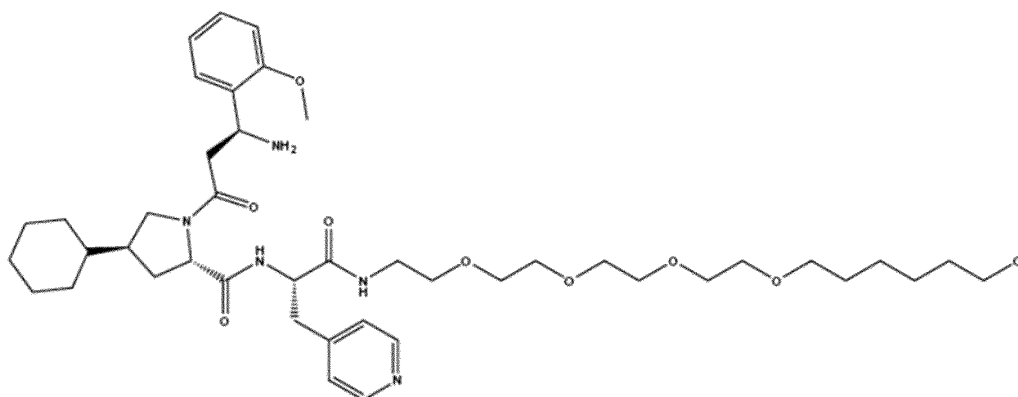
formation and that the linker between the chloroalkane and TRIM21 warheads is sufficient to allow simultaneous engagement of both protein partners.

These compounds formed comprising the TRIM21 binding moiety and the warhead directed to the target protein, are named herein as TrimTacs

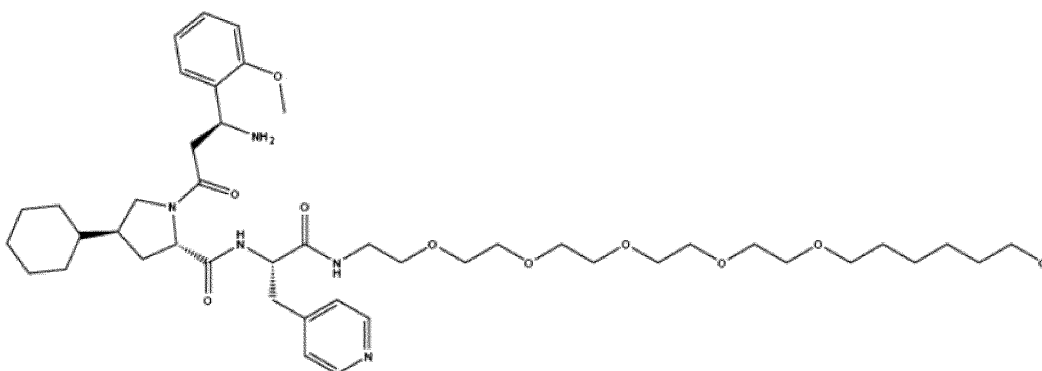
Example 6. TrimTacs form a ternary complex in cells between TRIM21 and target

To investigate whether ternary complex formation between two protein targets can be mediated by a TRIM21 ligand in living cells a fluorescent assay for colocalising ternary structures ('FACTS') was developed. In this assay, one protein (in this case TRIM21) is expressed in the cytosol labelled with a fluorescent protein tag (in this case, mCherry) and a second protein (in this case HaloTag) is expressed bound to chromatin and thus nuclear (in this case by fusing to H2B) with a fluorescent protein tag of a different colour (in this case mEGFP). Normally the two proteins do not mix and cannot form a complex. Upon cell division the nuclear envelope undergoes transient breakdown allowing mixing of the cytosol with chromatin, but unless the two proteins can form a complex they will re-segregate to the cytosol and nucleus upon nuclear membrane reformation (Figure 13A). However, in the presence of a compound capable of mediating ternary complex formation the two proteins will remain co-localised inside the nucleus. This can be readily detected by the co-localisation of mEGFP and mCherry signals. Using this assay compounds 37 and 71 were compared (with compound 37 only being capable of binding TRIM21 and compound 71 capable of binding both TRIM21 and HaloTag). No co-localisation of TRIM21 (mCherry-TRIM21) and HaloTag (H2B-mEGFP-HaloTag) was observed for either compound prior to cell division. After cell division the two proteins remained in separate compartments in the presence of compound 37 (Figure 13B). In contrast, in cells treated with compound 71, the colocalization of mEGFP and mCherry fluorescence indicated that TRIM21 remained in the nucleus with HaloTag as part of a ternary complex (Figure 13C). This experiment was repeated under a range of compound concentrations, with no evidence of a ternary complex with compound 37 at any dose. For compound 71, a dose-dependent increase in EGFP and mCherry co-localisation was observed, indicative of more ternary complex formation, until concentrations in excess of ~ 2 μ M. Ternary complex formation efficiency decreased at the highest compound 71 concentrations, consistent with a Hook Effect. To confirm these results, colocalization behaviour was compared in cells expressing either mCherry-TRIM21 lacking the RING and B Box domains (Δ RB) or lacking the PRYSPRY domain (Δ PS). Removal of the PRYSPRY domain but not the RING-B Box domains abolished ternary complex formation, consistent with the former being required for compound binding (Figure 14). Finally, a series of different TrimTac compounds were made to compare their ability to form a ternary complex and toxicity:

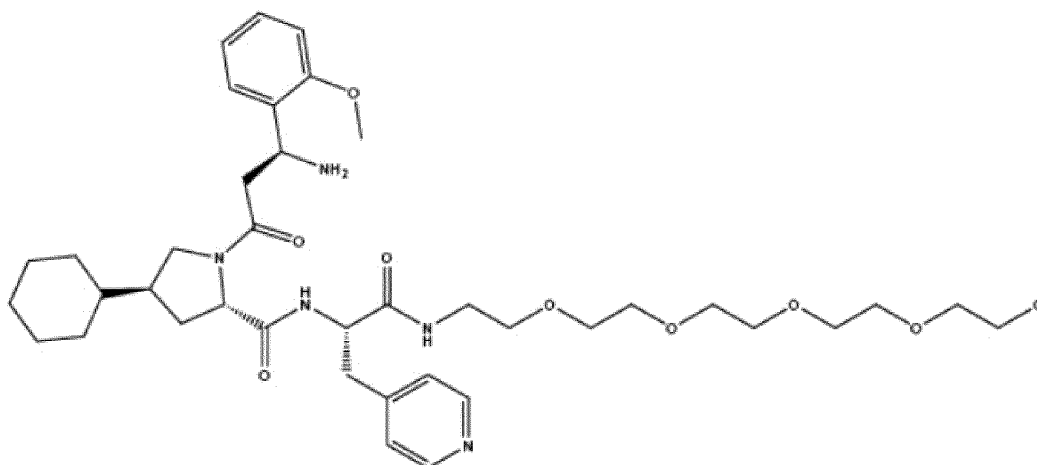
Compound 71 – 11 atom linker



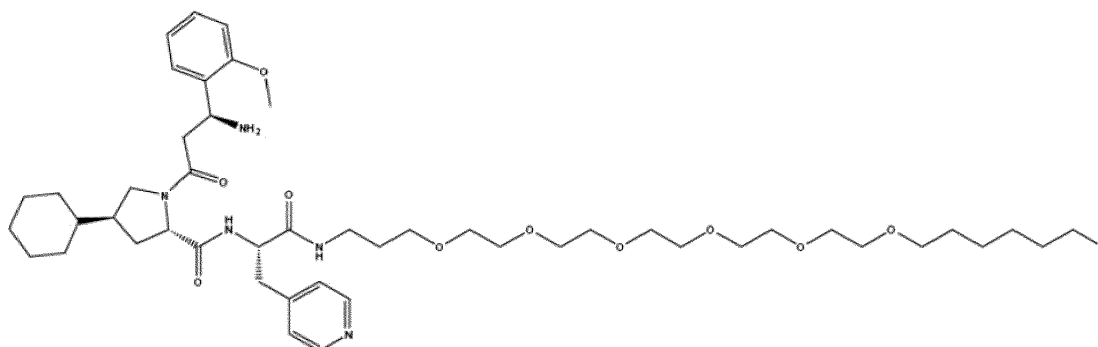
5 Compound 109 – 14 atom linker



Compound 110 – 8 atom linker



Compound 111 – 18 atom linker



- 5 RPE-1 TRIM21 KO cells co-expressing mCherryTRIM21 and H2B-mEGFP-Halo were treated with a titration of compounds 37, 71, 109, 110 and 111 and ternary complex formation quantified at 48h by normalising the red/green overlap area (mCherry-TRIM21 positive nuclei) to total green area (total nuclei). In each case the compounds were capable of mediating ternary complex formation, with a hook effect at a similar concentration (Figure 15A).

10

- RPE-1 cells were plated at ~15% confluence and treated with a titration of compounds 37, 71, 109, 110 and 111 or matched DMSO controls and imaged using the IncuCyte system. Cell confluence was quantified at 72h post-treatment by normalising total cell area per image for each compound concentration to matched DMSO controls. Cells treated with 40 μ M Compound 71 or Compound 109 did not reach 100% confluence indicative of toxicity. No toxicity was observed for any compound at concentrations of 20 μ M or below. (Figure 15B).

15

Example 7: TrimTacs selectively degrade oligomeric but not monomeric targets

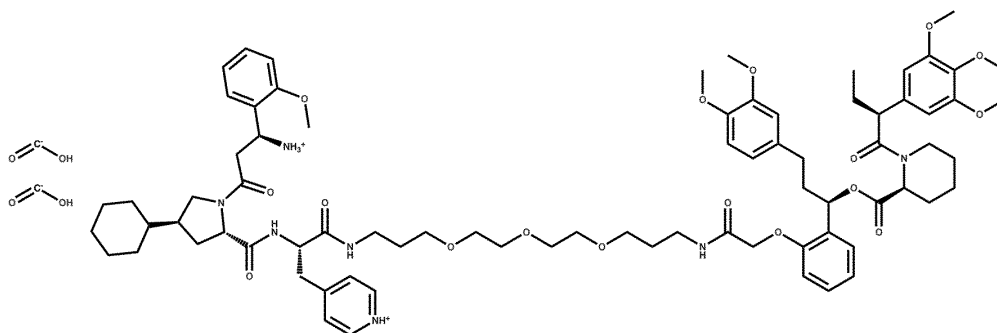
- 20 Next, the ability of TrimTacs to degrade different cellular targets was investigated: mEGFP-Halo, which is monomeric, and CAV1-mEGFP-Halo and Cavin1-mEGFP-Halo, which are oligomeric. The degradation activity of TrimTacs was compared to a canonical VHL-based PROTAC (HaloPROTAC1) and protein levels were monitored by measuring the mEGFP fluorescence. Specifically, RPE-1 mEGFP-Halo, RPE-1 CAV1-mEGFP-Halo and RPE-1 Cavin1-mEGFP-Halo stable cell lines were
- 25 treated with the VHL-recruiting PROTAC (HaloPROTAC1) or Compounds 37, 71, 109, 110 and 111 at the concentrations indicated in Figure 16 and imaged using the IncuCyte system for 48 hours. GFP fluorescence was quantified by normalising GFP integrated density (then product of area and mean intensity) to total cell area (phase) for each image, and then normalising to matched DMSO conditions so that a value of 1 (grey dashed line in Figure 16) represents no degradation. As expected,
- 30 HaloPROTAC1 efficiently degraded all three targets within 24 hours whereas compound 37, which binds TRIM21 but not a protein target, did not (Figure 16). TrimTacs 71, 109, 110 and 111 were all

capable of efficiently degrading CAV1-mEGFP-Halo and Cavin1-mEGFP-Halo. Unexpectedly however, none of the TrimTacs showed any activity against mEGFP-Halo.

To explore this further, both HaloPROTAC1 and TrimTacs were titrated over a wide-range of concentrations and substrate degradation profiles were compared. RPE-1 mEGFP-Halo, RPE-1 CAV1-mEGFP-Halo and RPE-1 Cavin1-mEGFP-Halo stable cell lines were treated with a titration of HaloPROTAC1 and compounds 37, 71, 109, 110, 111 for 48h and GFP fluorescence quantified using the IncuCyte system as described for Figure 16. HaloPROTAC1 degraded all three substrates dose-dependently, with evidence of a Hook Effect at the highest concentrations for CAV1-mEGFP-Halo and Cavin1-mEGFP-Halo (Figure 17). TrimTacs also degraded CAV1-mEGFP-Halo and Cavin1-mEGFP-Halo dose-dependently, also with a Hook Effect, but as previously showed no degradation activity against mEGFP-Halo at any concentration.

WT or TRIM21 KO cells stably expressing Cavin1-mEGFP-Halo or CAV1-mEGFP-Halo were treated
15 with a titration of compound 71 for 48h and GFP fluorescence quantified using the IncuCyte system as
described for Figure 16. The results showed that TrimTac activity was TRIM21 dependent as no
substrate degradation was observed in TRIM21 knockout cells (Figure 18).

To confirm that the selective degradation of oligomeric substrates is maintained with non-covalent
20 TRIMTACs, a short linker and AP1867 (a synthetic ligand that binds FKBP(F36V) non-covalently)
were appended to compound 37 to form compound 414:



Next, the ability of compound 414 to degrade different cellular targets was investigated: mEGFP-FKBP(F36V), which is monomeric, and Cavin1-mEGFP-FKBP(F36V), which is oligomeric. The degradation activity of compound 414 was compared to the canonical VHL-based (dTAGv1) and CRBN-based (dTAG13) PROTACs and protein levels were monitored by measuring the mEGFP fluorescence. Specifically, RPE-1 mEGFP-FKBP(F36V) and RPE-1 Cavin1-mEGFP-FKBP(F36V) stable cell lines were treated with compounds 414, dTAG13 and dTAGv1 at the concentrations indicated in Figure 25 and imaged using the IncuCyte system for 8 hours. GFP fluorescence was quantified by normalising GFP integrated density (then product of area and mean intensity) to total cell area (phase) for each image, and then normalising to matched DMSO conditions so that a value of 1 (dashed line in Figure 25) represents no degradation. As expected, dTAG13 and dTAGv1 efficiently

degraded both targets, but with a preference for monomeric mEGFP-FKBP(F36V) (Figure 25). In contrast, compound 414 efficiently degraded oligomeric Cavin-1-mEGFP-FKBP(F36V) but showed no activity against monomeric mEGFP-FKBP(F36V).

- 5 To explore this further, compounds 414, dTAG13 and dTAGv1 were titrated over a wide-range of concentrations and substrate degradation profiles were compared. Specifically, RPE-1 mEGFP-FKBP(F36V) and Cavin1-mEGFP-FKBP(F36V) stable cell lines were treated with a titration of compounds 414, dTAG13 or dTAGv1 for 8h and GFP fluorescence quantified using the IncuCyte system as described for Figure 25. dTAG13 and dTAGv1 degraded both substrates dose-
- 10 dependently, with a strong preference for monomeric substrate over oligomeric substrate (Figure 26). In contrast, compound 414 degraded oligomeric Cavin1-mEGFP-FKBP(F36V) dose-dependently, but as previously showed no degradation activity against monomeric mEGFP-FKBP(F36V) at any concentration.
- 15 Finally, WT or TRIM21 KO cells stably expressing Cavin1-mEGFP-FKBP(F36V) were treated with a titration of compound 414 for 8h and GFP fluorescence quantified using the IncuCyte system as described for Figure 25. The results showed that compound 414 activity was TRIM21-dependent as no substrate degradation was observed in TRIM21 knockout cells (Figure 27).
- 20 Taken together, the data show that TrimTacs are capable of mediating highly efficient, TRIM21-dependent substrate degradation. However, unlike VHL- and CRBN-recruiting PROTACs, TrimTacs display target selectivity wherein they can degrade oligomeric but not monomeric substrates.

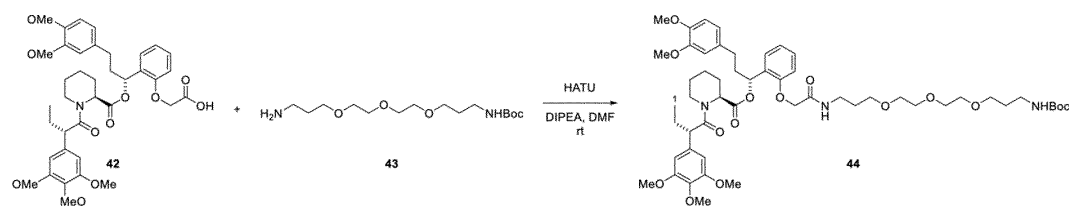
Example 8: TRIMTACs induce TRIM21 clustering and activation of ubiquitination upon binding to an oligomeric target

- 25
- To investigate the mechanism by which TRIMTACs selectively degrade oligomeric proteins, an assay was developed to detect TRIM21 clustering and activation in live cells. This assay uses the NanoBiT split luciferase system whereby luciferase activity is reconstituted only when the two protein
- 30 fragments, LgBiT and SmBiT, are brought into close proximity. Specifically, two N-terminally tagged versions of TRIM21, SmBiT-TRIM21 and LgBiT-TRIM21, were co-expressed in TRIM21 KO Cavin-1-mEGFP-FKBP(F36V) cells. Addition of compound 414 induced an immediate sharp increase in luciferase activity, suggesting that the two versions of TRIM21 were brought into close proximity by
- 35 compound 414, indicative of TRIM21 clustering upon TRIMTAC binding to the oligomeric target (Figure 31A,B). This luciferase activity peaked at 8 minutes post-compound 414 addition and then declined over next 90 minutes coinciding with Cavin-1-mEGFP-FKBP(F36V) degradation, likely representing co-degradation of TRIM21 together with the target (Figure 31A,B).

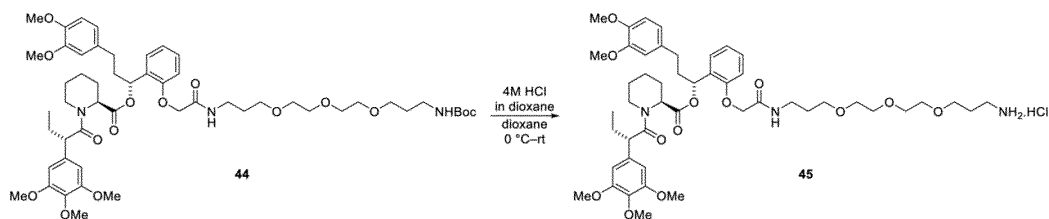
- We next adapted this assay to detect TRIMTAC-induced ubiquitination by co-expressing SmBiT-
- 40 TRIM21 together with LgBiT-TUBEs (Tandem Ubiquitin-Binding Elements) which bind to polyubiquitin

chains. Addition of compound **414** induced an immediate sharp increase in luciferase activity, suggesting that TRIMTAC induces the rapid assembly of ubiquitin chains in proximity to TRIM21 upon binding to oligomeric target (Figure 31A,B). The luciferase activity for SmBiT-TRIM21 + LgBiT-TUBEs initiated slightly later than for SmBiT-TRIM21 + LgBiT-TRIM21 and peaked at 12-16 minutes post-compound **414** addition, consistent with ubiquitin chain formation occurring after TRIM21 activation. This luciferase activity declined over the next 90 minutes coinciding with Cavin-1-mEGFP-FKBP(F36V) degradation, likely representing degradation of the polyubiquitinated species (Figure 31A,B).

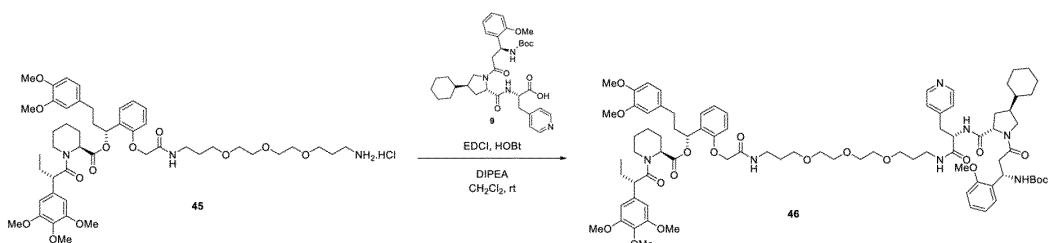
Example 9: Synthesis of a TrimTac based on Compound 37 and AP1867



- To a stirred solution of *ortho*-AP1867 (**42**) (15 mg, 0.022 mmol, 1.0 equiv.) and **43** (6.9 mg, 0.022 mmol, 1.0 equiv.) in *N,N*-dimethylformamide (0.10 mL) was added a stirred solution of 1-*[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate* (8.2 mg, 0.022 mmol, 1.0 equiv.) and *N,N*-diisopropylethylamine (0.011 mL, 0.065 mmol, 3.0 equiv.) in *N,N*-dimethylformamide (0.1 mL). The resulting reaction mixture was left to stir at room temperature in the dark for 18 h. Due to the remaining presence of **1** by TLC and NMR, a further portion of **43** (2.0 mg) and 1-*[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate* (2.0 mg) were added to the reaction mixture and this was left to stir for a further 24 h. The reaction mixture was washed with 5% aqueous lithium chloride and extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with 5% aqueous lithium chloride (2 × 20 mL), dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (0–5% methanol in dichloromethane) to give desired product **44** as a yellow oil (16 mg, 74%). δ_{H} (400 MHz, CD₃OD) 0.88 (3H, t, *J* 7.3 Hz, 1-H₃), 1.16–1.48 (11H, m), 1.55–1.83 (8H, m), 1.87–2.15 (3H, m), 2.27 (1H, d, *J* 13.2 Hz), 2.40–2.68 (3H, m), 3.06–3.29 (4H, m), 3.35–3.63 (13H, m), 3.64–3.84 (15H, m), 3.87 (1H, t, *J* 7.3 Hz), 4.13 (1H, br d, *J* 13.7 Hz), 4.38–4.68 (2H, m), 5.38–5.48 (1H, m), 6.13 (1H, dd, *J* 8.2, 5.8 Hz), 6.32–7.50 (10 H, m); δ_{C} (101 MHz, CD₃OD) 12.6, 21.9, 26.4, 27.6, 28.8, 29.3, 30.3, 30.9, 32.2, 37.6, 38.9, 45.0, 51.0, 53.4, 56.5, 56.5, 56.6, 56.7, 61.1, 61.1, 68.3, 69.8, 69.9, 70.8, 71.2, 71.5, 79.8, 106.6, 113.0, 113.1, 113.5, 113.6, 121.7, 121.8, 123.2, 128.3, 130.0, 130.5, 135.0, 136.9, 138.0, 148.8, 150.4, 154.6, 155.5, 170.6, 172.5, 174.8; *m/z* (ESI) 1018.5265 (MNa⁺. C₅₃H₇₇N₃NaO₁₅ requires 1018.5247).



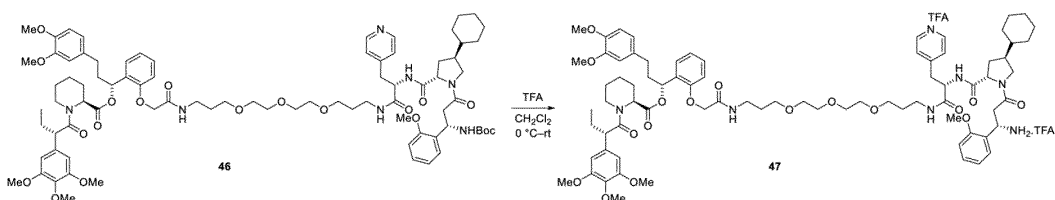
To a stirred solution of **44** (16 mg, 0.016 mmol, 1.00 equiv.) in 1,4-dioxane (0.20 mL) at 0 °C was added 4 M hydrochloric acid in 1,4-dioxane (0.20 mL). The resulting reaction mixture was left to stir at room temperature for 4 h. The reaction mixture was concentrated *in vacuo* to give desired product **45** as a colourless oil (14 mg, 93%). The product was carried forward to the next step without further purification or characterisation.



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To a stirred solution of **9** (4.0 mg, 0.0064 mmol, 1.0 equiv.) in dichloromethane (0.50 mL) was added *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (1.5 mg, 0.0077 mmol, 1.2 equiv.) and 1-hydroxybenzotriazole hydrate (1.2 mg, 0.0077 mmol, 1.2 equiv.) and the resulting solution was left to stir at room temperature for 10 minutes. To the reaction mixture was added a stirred solution of **45** (6.0 mg, 0.0064 mmol, 1.0 equiv.) and *N,N*-diisopropylethylamine (0.0025 mL, 0.019 mmol, 3.0 equiv.) in dichloromethane (0.50 mL) and the resulting reaction mixture was left to stir at room temperature for 20 h. The reaction mixture was concentrated *in vacuo* and purified by flash column chromatography (0–5% methanol in dichloromethane) to give the desired product **46** as a colourless oil (4 mg, 41%). δ_{H} (400 MHz, CD_3OD) 0.68–3.08 (51H, m), 3.10–3.26 (3H, m), 3.35–3.93 (31H, m), 3.95–4.74 (6H, m), 5.27–5.47 (2H, m), 6.13 (1H, t, J 7.0 Hz), 6.32–7.13 (11H, m), 7.14–7.87 (6H, m), 8.42 (2H, d, J 4.6 Hz); m/z (LC-MS, ESI^+) 701.1 ($[\text{M-Boc}+2\text{H}]^{2+}$, 38%).

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To a stirred solution of **46** (4.0 mg, 0.0027 mmol, 1.0 equiv.) in dichloromethane (0.10 mL) at 0 °C was added trifluoroacetic acid (0.0020 mL, 0.027 mmol, 10 equiv.) as a stock solution in dichloromethane. The resulting reaction mixture was left to stir at room temperature for 2 h. The reaction mixture was concentrated *in vacuo* to give desired product **47** as a colourless oil (4.0 mg, quant.). To prepare the compound for biological evaluation, the product was further purified by preparative HPLC (30–95% acetonitrile (+ 0.1% formic acid) in water (+ 0.1% formic acid) – desired product eluted as the di formic acid salt at tR = 13 minutes (48% acetonitrile)) and lyophilised to afford the product as a white solid (2.0 mg). δ_{H} (400 MHz, CD₃OD) 0.69–0.97 (6H, m), 0.99–1.31 (7H, m), 1.42–1.83 (16H, m), 1.86–2.12 (5H, m), 2.26 (1H, d, *J* 12.5 Hz), 2.39–2.70 (4H, m), 2.78–3.15 (6H, m), 3.13 (1H, p, *J* 1.6 Hz), 3.21–3.28 (4H, m), 3.36–3.42 (2H, m), 3.43–3.61 (11H, m), 3.64–3.71 (7H, m), 3.75 (1H, d, *J* 7.0), 3.77–3.82 (7H, m), 3.82–3.91 (4H, m), 4.14 (1H, br d, *J* 13.5 Hz), 4.37–4.50 (2H, m), 4.52–4.77 (3H, m), 5.42 (1H, s), 6.13 (1H, dd, *J* 8.2, 5.7 Hz), 6.54–6.65 (2H, m), 6.67 (1H, dd, *J* 8.2, 2.0 Hz), 6.73–6.80 (1H, m), 6.81–6.93 (2H, m), 6.94–7.11 (2H, m), 7.20–7.50 (4H, m), 8.37–8.49 (2H, m), 8.54 (1H, br s); *m/z* (LC-MS, ESI⁺) 701.1 ([M+2H]²⁺, 100%).

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Example 10: TrimTacs selectively degrade oligomeric but not monomeric proteins in the same cell

To obtain more evidence for TrimTac selectively, microscopy was used to determine where in the cell substrates are being degraded. RPE-1 Cavin1-mEGFP-Halo cells were treated with DMSO, HaloPROTAC1 at 1.25 μM , compounds 37, 71, 109, 111 at 1.25 μM , or compound 110 at 10 μM , for 48hrs and imaged using the IncuCyte system. Cavin1-mEGFP-Halo exhibited bright fluorescence at the cell membranes and some diffuse cytoplasmic fluorescence in control (DMSO) condition. Treatment with HaloPROTAC1 reduced overall fluorescence, but residual fluorescence remained concentrated at the cell membranes. Incubation with HaloPROTAC1 resulted in the degradation of protein from both membrane and cytosolic pools, although significant levels of membrane-associated substrate remained even after 48 hours. Treatment with TRIMTACs (Compounds 71, 109, 110 and 111) caused complete loss of bright fluorescence from cell membranes, but diffuse cytosolic fluorescence remained. Thus, membrane-associated form of Cavin1-mEGFP-Halo was degraded by TRIMTACs but cytosolic form was untouched.

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This was investigated further. RPE-1 Cavin1-mEGFP-Halo cells were plated on an 8-well chambered polymer coverslip (Ibidi), images were taken before and after treatment with either DMSO or 2.5 μM HaloPROTAC1 or Compound 71 using an Etaluma Lumascope LS720 widefield microscope equipped with a 40x apochromat 0.95NA air objective housed within a 37C 5% CO₂ incubator. Treatment with HaloPROTAC1 caused gradual loss of both cytosolic and membrane fluorescence over 24h. Treatment with Compound 71 caused rapid loss of membrane-associated fluorescence within 1h. It was found that after 1hr, almost all of the membrane-associated Cavin1-mEGFP-Halo had been degraded in TrimTac-treated cells, whereas even after 24hrs the cytosolic pool remained untouched (Figure 20A). In contrast, cytosolic Cavin1-mEGFP-Halo was degraded rapidly in HaloPROTAC1 treated cells but significant membrane-associated protein remained after 24hrs (Figure 20B).

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This was further corroborated by quantifying fluorescent intensities across the cell. In HaloPROTAC1-treated cells there was a decrease in protein levels across the cell (Figure 21 A), whilst in TrimTac-treated cells only the oligomeric membrane-associated protein had been degraded (Figure 21B).

5

Finally, it was tested whether TrimTacs are capable of selectively degrading oligomeric protein species when both oligomeric and monomeric versions are expressed in the same cell. RPE-1 Cavin1-mEGFP-Halo cells were treated with DMSO or 2.5 μ M HaloPROTAC1 or Compound 37 for 24h and whole-cell extracts harvest for capillary-based immunoblotting. The anti-GFP immunoblot shows that in addition to full length Cavin1-mEGFP-Halo there is a smaller species likely corresponding to a cleavage product lacking the Cavin1 sequence (*-mEGFP-Halo). Treatment with HaloPROTAC1 caused a slight reduction in the Cavin1-mEGFP-Halo band and complete loss of the mEGFP-Halo band. Conversely, treatment with Compound 71 caused a substantial reduction in the Cavin1-mEGFP-Halo band, however the *-mEGFP-Halo band remained unaffected (Figure 19 (A)). The Cavin1-mEGFP-Halo and *-mEGFP-Halo bands were normalised to anti-actin bands and expressed as a fraction of total anti-GFP band intensity. Treatment with Compound 71 caused a significant reduction in Cavin1-mEGFP-Halo, but not *-mEGFP-Halo, protein levels (Figure 19 (B)). Thus, in cells expressing both mEGFP-Halo and Cavin1-mEGFP-Halo and treated with HaloPROTAC1 there was efficient degradation of monomeric mEGFP-Halo and inefficient degradation of oligomeric Cavin1-mEGFP-Halo. In contrast, TrimTac treatment left monomeric mEGFP-Halo untouched and only degraded oligomeric Cavin1-mEGFP-Halo. As discussed herein, TrimTacs are dependent upon TRIM21 for their activity.

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RPE-1 Cavin1-mEGFP-Halo cells were plated on an 8-well chambered polymer coverslip (Ibidi), images were taken 2.5h after treatment with DMSO or 2.5 μ M Compound 37 or 2.5 μ M Compound 37 + 25 μ M MG132. Treatment with MG132 was sufficient to rescue TrimTac-mediated substrate degradation, showing that the proteasome is also required (Figure 22).

Example 11: TRIMTACs selectively degrade Myddosome-assembled Myd88

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To obtain further evidence for TRIMTAC selectivity, the degradation activity of compound 414 was tested against Myd88 either in its resting monomeric form or when Myd88 oligomerizes to assemble the Myddosome, a pro-inflammatory signalling complex. In short, an assay was established wherein Myd88 fused c-terminally to GyrB-mEGFP-FKBP(F36V) is induced to oligomerize and assemble Myddosomes by addition of the antibiotic coumermycin (Figure 28A). U2OS cells stably expressing Myd88-GyrB-mEGFP-FKBP(F36V) were treated with coumermycin (100 nM) to induce Myddosome assembly, or mock treated (control) then treated with DMSO, Cmp 414 (5 μ M) or Cmp 37 (5 μ M) and Myd88 protein levels quantified by GFP fluorescence. Cmp 414 does not degrade Myd88 in control-treated cells, but rapidly degrades Myd88 in coumermycin-treated cells. DMSO or Cmp 37 addition has no effect on Myd88 levels (Figure 28B). Microscopy images show that compound 414 rapidly degrades the oligomeric Myd88 puncta (Myddosomes) but spares the remaining diffuse Myd88 signal.

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Example 12: TRIMTACs selectively degrade Necrosome-assembled RIPK3

To obtain further evidence for TRIMTAC selectivity, the degradation activity of compound 414 was tested against RIPK3 in either its inactive resting monomeric state or when RIPK3 oligomerizes to assemble active Necrosomes, which triggers cell necroptosis. An assay was established wherein treatment of cells expressing FKBP(F36V)-mEGFP-RIPK3 with a combination of (T) TNF α , (S) Smac-mimetic and (Z) caspase inhibitor ZVAD-FMK (T/S/Z) triggers RIPK3 oligomerization and necroptosis (Figure 29A). RPE-1 cells stably expressing FKBP(F36V)-mEGFP-RIPK3 were treated with T/S/Z or mock treated (control) in the presence of DMSO or a titration of compound 414 for 8 hours. Microscopy images show that in control-treated cells RIPK3 is monomeric, exhibiting diffuse cytosolic distribution, whereas T/S/Z treatment induces RIPK3 oligomerization as observed by accumulation of cytosolic RIPK3 puncta (Figure 29B; DMSO). Compound 414 degraded oligomeric RIPK3 (T/S/Z) fluorescent puncta dose-dependently, but showed no degradation activity against monomeric diffuse RIPK3 (control) at any concentration.

To test if TRIMTAC selective degradation of oligomeric RIPK3 prevents downstream necroptosis, the activity of compound 414 was compared to the RIPK3 kinase inhibitor GSK'872. RPE-1 cells expressing FKBP(F36V)-mEGFP-RIPK3 were treated with T/S/Z to induce RIPK3 oligomerization and necroptosis, or mock treated (control), in the presence of DMSO, Cmp 414 (2.5 μ M) or GSK'872 (0.3 μ M) and GFP fluorescence (RIPK3 protein levels) and cell area (necroptotic cell death) was quantified using the IncuCyte system. Compound 414 rapidly degrades T/S/Z-induced RIPK3 oligomers as they assemble, but has no effect on monomeric diffuse RIPK3 protein levels. The RIPK3 kinase inhibitor GSK'872 does not affect RIPK3 oligomerization, similar to DMSO (Figure 30A,B). T/S/Z treatment triggers necroptosis, which can be quantified by measuring cell area from phase contrast microscopy images. As expected, the RIPK3 kinase inhibitor GSK'872 prevented necroptosis as cell area was similar to control cells at 48h. Compound 414 also partially rescued cell area in T/S/Z-treated cells, suggesting that TRIMTAC-mediated selective degradation of oligomeric RIPK3 can inhibit necroptosis.

Taken together, the data show that TrimTacs have unexpected target specificity and degrade oligomeric but not monomeric substrates. This is a property of recruiting TRIM21, as activity is lost in TRIM21 knockout cells. Thus compounds that recruit TRIM21 are not PROTACs and access a unique degradation mechanism.

This data demonstrates that the compounds of the invention (also referred to herein as TRIMTACs) are able to selectively degrade oligomeric proteins over monomeric forms of the protein.

Further embodiments of the invention are provided below:

1. A compound of formula (I)

A-L-B**(I)**

or a pharmaceutically acceptable salt thereof, wherein:

- 5 A is a moiety that binds TRIM21;
 L is a linker; and
 B is a moiety that binds a target protein.

2. The compound of clause 1 wherein the target protein can comprise a first form that provides
10 multiple binding sites for B; and/or wherein the target protein is capable of forming an oligomeric species.

3. The compound of clause 1 or 2, wherein the compound is configured to recruit TRIM21 to enable selective degradation by TRIM21 of one or more oligomeric forms of the target protein.

- 15 4. The compound of any of the preceding clauses, wherein A binds the PRYSPRY domain of TRIM21 (SEQ ID NO: 1).

5. The compound of any of the preceding clauses wherein A binds one or more of residues selected
20 from W381, W383, D355, F369, L370, L371, F450, Y328, H368, S447, M330, D452, R364, Q395, Y393, E389 and K387 of TRIM21 (SEQ ID NO: 1), preferably wherein the one or more of residues is selected from W381, W383, D355.

6. The compound of any of the preceding clauses wherein A binds residue D355 of TRIM21 (SEQ ID
25 NO: 1).

7. The compound of any of the preceding clauses wherein A comprises a moiety capable of forming a hydrogen bond with residue D355 of TRIM21 (SEQ ID NO: 1).

- 30 8. The compound of clause 7 wherein the hydrogen bond is a charged hydrogen bond.

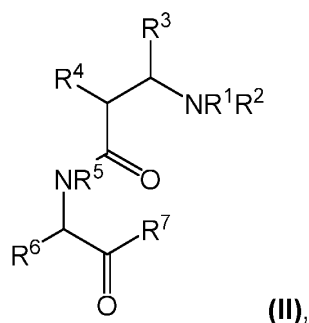
9. The compound of any of the preceding clauses wherein A binds residue W381 of TRIM21 (SEQ ID NO: 1).

- 35 10. The compound of any of the preceding clauses wherein A comprises a moiety capable of hydrophobic stacking with residue W381 of TRIM21 (SEQ ID NO: 1).

11. The compound of any of the preceding clauses wherein A binds residue W383 of TRIM21 (SEQ ID NO: 1).

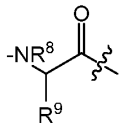
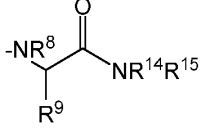
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12. The compound of any of the preceding clauses wherein A comprises a moiety capable of hydrophobic stacking with residue W383 of TRIM21 (SEQ ID NO: 1).
13. The compound of any of the preceding clauses wherein A binds residues W381, W383 and D355 of TRIM21 (SEQ ID NO: 1).
14. The compound of any of the preceding clauses, wherein A competes with IgG for binding to TRIM21.
15. The compound of any of the preceding clauses, wherein A is not an Fc region or fragment thereof.
16. The compound of any of the preceding clauses, wherein A is not an anti-TRIM21 antibody or antigen binding fragment thereof.
17. The compound of any of the preceding clauses, wherein the target protein has a pathogenic form and a non-pathogenic form, wherein the pathogenic form of the protein comprises two or more binding sites for B.
18. The compound of any of the preceding clauses, wherein B is not an anti-tau antibody or antibody binding fragment thereof.
19. The compound of any of the preceding clauses, wherein A is not an anti-TRIM21 antibody or antigen binding fragment thereof and B is not an anti-tau antibody or antigen binding fragment thereof.
20. The compound of any of the preceding clauses, wherein the molecular weight of A is 1000 Da or less.
21. The compound of any of the preceding clauses, wherein the molecular weight of A is in the range of 100 Da to 1000 Da.
22. The compound of any of the preceding clauses, wherein the compound has a K_d value for TRIM21 in the range of 0.1 nM to 1000 μ M.
23. The compound of any of the preceding clauses, wherein the compound has a K_d value for TRIM21 in the range of 1 nM to 1000 μ M.
24. The compound of any of the preceding clauses, wherein the compound has a K_d value for TRIM21 in the range of 10 nM to 100 μ M.
25. The compound of any of the preceding clauses, wherein A has the structure of formula (II)

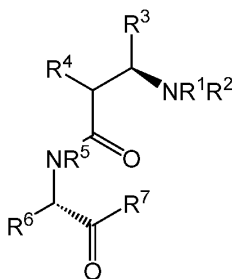


wherein:

- 5 R^1 and R^2 are each independently selected from hydrogen and C_{1-2} alkyl; or one of R^1 and R^2 is hydrogen and the other is selected from an amino acid residue, and a site of covalent attachment to L;
- R^3 is selected from hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted heteroalkyl, optionally substituted heteroalkenyl, optionally substituted heteroalkynyl, optionally substituted cycloalkyl, optionally substituted
- 10 heterocyclyl, optionally substituted aryl and optionally substituted heteroaryl, wherein R^3 optionally comprises a site of covalent attachment to L;
- R^4 is hydrogen, halo or C_{1-2} alkyl;
- R^5 is hydrogen or C_{1-2} alkyl and R^6 is optionally substituted alkyl; or R^5 and R^6 combine to form an optionally substituted heterocyclic ring;

- 15 R^7 is , a site of covalent attachment to L, or  ;
- R^8 is hydrogen or C_{1-2} alkyl;
- R^9 is optionally substituted alkyl;
- R^{14} and R^{15} are each independently selected from hydrogen and C_{1-2} alkyl; and
- 20  in the structure of R^7 indicates a site of covalent attachment to L;
- provided that A comprises a site of covalent attachment to L.

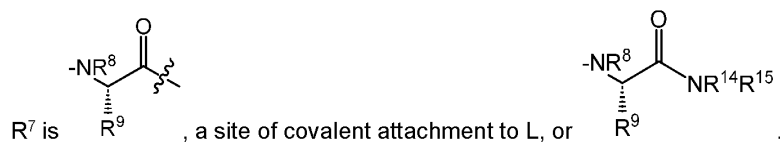
26. The compound of clause 25, wherein A has the following structure:



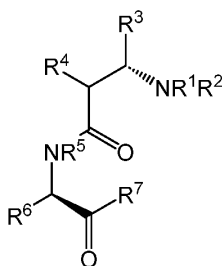
wherein:

R¹ and R² are each independently selected from hydrogen and C₁₋₂ alkyl; or one of R¹ and R² is hydrogen and the other is selected from an L-amino acid residue, and a site of covalent attachment to

5 L; and

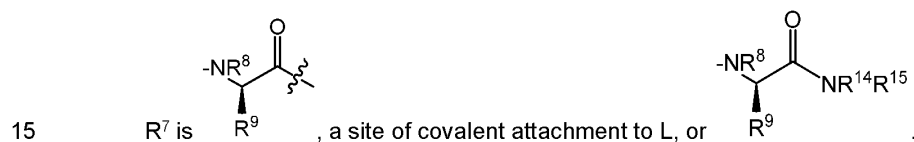


27. The compound of clause 25, wherein A has the following structure:

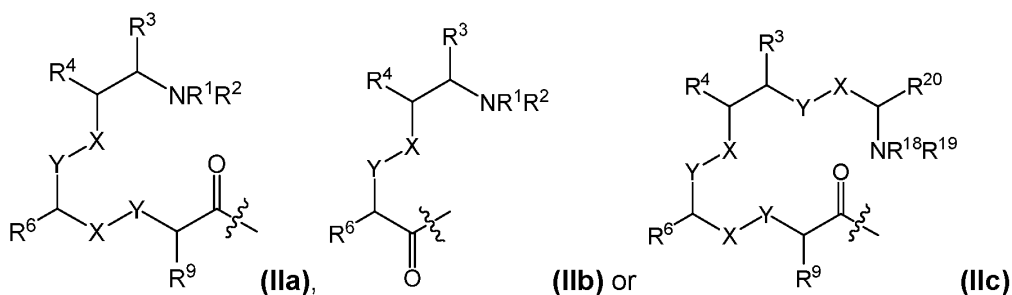


10 wherein:

R¹ and R² are each independently selected from hydrogen and C₁₋₂ alkyl; or one of R¹ and R² is hydrogen and the other is selected from an D-amino acid residue, and a site of covalent attachment to L; and

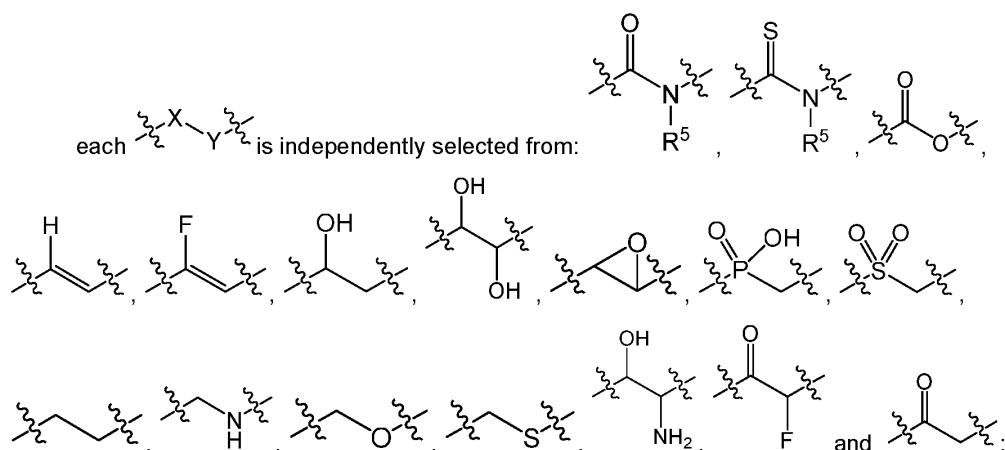


28. The compound of any of the preceding clauses, wherein A has the structure of formula (IIa), (IIb) or (IIc):



20

wherein:



R^1 and R^2 are each independently selected from hydrogen and C_{1-2} alkyl; or one of R^1 and R^2 is hydrogen and the other is an amino acid residue;

R^3 is selected from hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted heteroalkyl, optionally substituted heteroalkenyl, optionally substituted heteroalkynyl, optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl and optionally substituted heteroaryl;

R^4 is hydrogen, halo or C_{1-2} alkyl;

each R^5 is independently hydrogen or C_{1-2} alkyl;

R^6 is optionally substituted alkyl;

or, when R^5 and R^6 are attached to adjacent atoms, R^5 and R^6 combine to form an optionally substituted heterocyclic ring;

R^8 is hydrogen or C_{1-2} alkyl;

R^9 is optionally substituted alkyl;

R^{18} and R^{19} are each independently selected from hydrogen and C_{1-2} alkyl; and

R^{20} is optionally substituted alkyl.

29. The compound of clause 25-28, wherein one of R^1 and R^2 is hydrogen and the other is selected from an amino acid residue, optionally wherein the amino acid residue is selected from a histidine residue, a phenylalanine residue, a tryptophan residue, and a tyrosine residue.

30. The compound of clause 29, wherein the amino acid residue is a histidine residue.

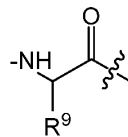
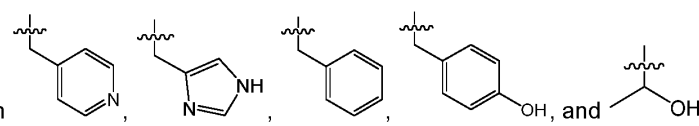
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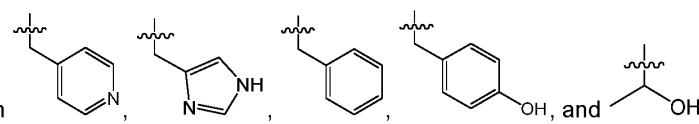
31. The compound of clause 25-28, wherein R^1 , R^2 and R^4 are hydrogen.

32. The compound of any of clauses 25-31, wherein R^3 is selected from optionally substituted aryl and optionally substituted heteroaryl.

30

33. The compound of any of clauses 25-32, wherein R^3 is selected from aryl and heteroaryl, wherein the aryl or heteroaryl is optionally substituted by one or more substituents selected from halo, C_{1-C_8} alkyl, C_{1-C_8} haloalkyl, cyano, hydroxy, C_{1-C_8} alkoxy, and amino.
- 5 34. The compound of any of clauses 25-33, wherein R^3 is selected from furan and optionally substituted phenyl.
35. The compound of any of clauses 25-34, wherein R^3 is selected from unsubstituted phenyl, phenyl substituted by C_{1-2} alkyl, phenyl substituted by C_{1-2} alkoxy, and unsubstituted 5-6 membered
10 heteroaryl.

36. The compound of any of clauses 25-27 or 29-35, wherein R^7 is , wherein:
- (a) R^9 is selected from -alkylene-optionally substituted aryl and -alkylene-optionally substituted heteroaryl, optionally wherein R^9 is selected from -CH₂-optionally substituted aryl and -CH₂-optionally substituted heteroaryl; or
- 15 (b) R^9 is selected from ,
wherein  in the structure of R^9 indicates a site of covalent attachment to the remainder of R^7 ; or
- 20 (c) R^9 is selected from a histidine side chain, a phenylalanine side chain, a tryptophan side chain, a tyrosine side chain, a cysteine side chain, a serine side chain, a threonine side chain, a methionine side chain, an asparagine side chain, and a glutamine side chain.

- 25 37. The compound of any of clauses 25-36, wherein:
- (a) R^9 is selected from -alkylene-optionally substituted aryl and -alkylene-optionally substituted heteroaryl, optionally wherein R^9 is selected from -CH₂-optionally substituted aryl and -CH₂-optionally substituted heteroaryl; or
- 30 (b) R^9 is selected from ,
or

(c) R⁹ is selected from a histidine side chain, a phenylalanine side chain, a tryptophan side chain, a tyrosine side chain, a cysteine side chain, a serine side chain, a threonine side chain, a methionine side chain, an asparagine side chain, and a glutamine side chain.

38. The compound of any of clauses 25-37, wherein R⁵ and R⁶ combine to form a 5-membered heterocyclic ring optionally substituted by hydroxy, nitro, cyano, halo, -NR¹⁰R¹¹, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted -O-cycloalkyl, optionally substituted -O-heterocyclyl, optionally substituted -O-aryl, or optionally substituted -O-heteroaryl,

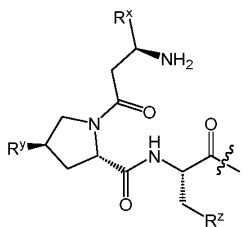
wherein R¹⁰ and R¹¹ are each independently H, optionally substituted alkyl, or optionally substituted cycloalkyl.

39. The compound of any of clauses 25-27 or 29-38, wherein R^7 is  and R^3 is , wherein  indicates a site of covalent attachment to L and * indicates a site of covalent attachment to the remainder of A.

40. The compound of any of clauses 25-27 or 29-38, wherein R⁷ is  and R³ is selected from 5-6 membered heteroaryl and phenyl, wherein the phenyl is optionally substituted by hydroxy, C₁₋₂ alkoxy or C₁₋₂ alkyl.

41. The compound of any of clauses 25-40, wherein R³ is selected from 5-6 membered heteroaryl and phenyl, wherein the phenyl is optionally substituted by hydroxy, C₁₋₂ alkoxy or C₁₋₂ alkyl.

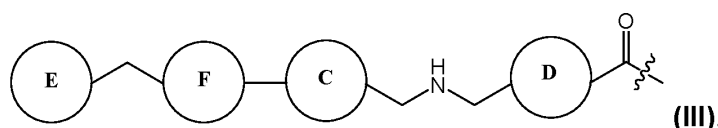
25 42. The compound of any of the preceding clauses, wherein A has the structure:



wherein each of R^x , R^y and R^z is independently selected from optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl, and optionally substituted heteroaryl.

43. The compound of clause 42, wherein R^x is selected from optionally substituted phenyl and optionally substituted 5-6 membered heteroaryl, R^y is selected from optionally substituted 5-6 membered cycloalkyl and optionally substituted 5-6 membered heterocyclyl, and R^z is selected from optionally substituted phenyl and optionally substituted 5-6 membered heteroaryl.

44. The compound of any of clauses 1-24, wherein A has the structure of formula (III)



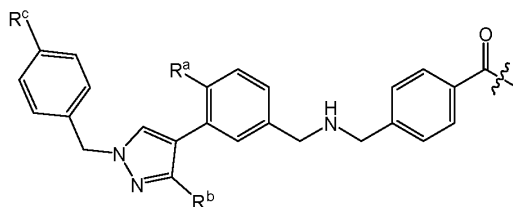
wherein:

each of rings E, F, C and D is independently selected from optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl, and optionally substituted heteroaryl; and

in the structure of formula (III) indicates a site of covalent attachment to L.

45. The compound of clause 44, wherein ring C is selected from aryl and heteroaryl, wherein the aryl or heteroaryl is substituted by aryl or heteroaryl.

46. The compound of clause any of clauses 1-34, 44 or 45, wherein A has the structure:

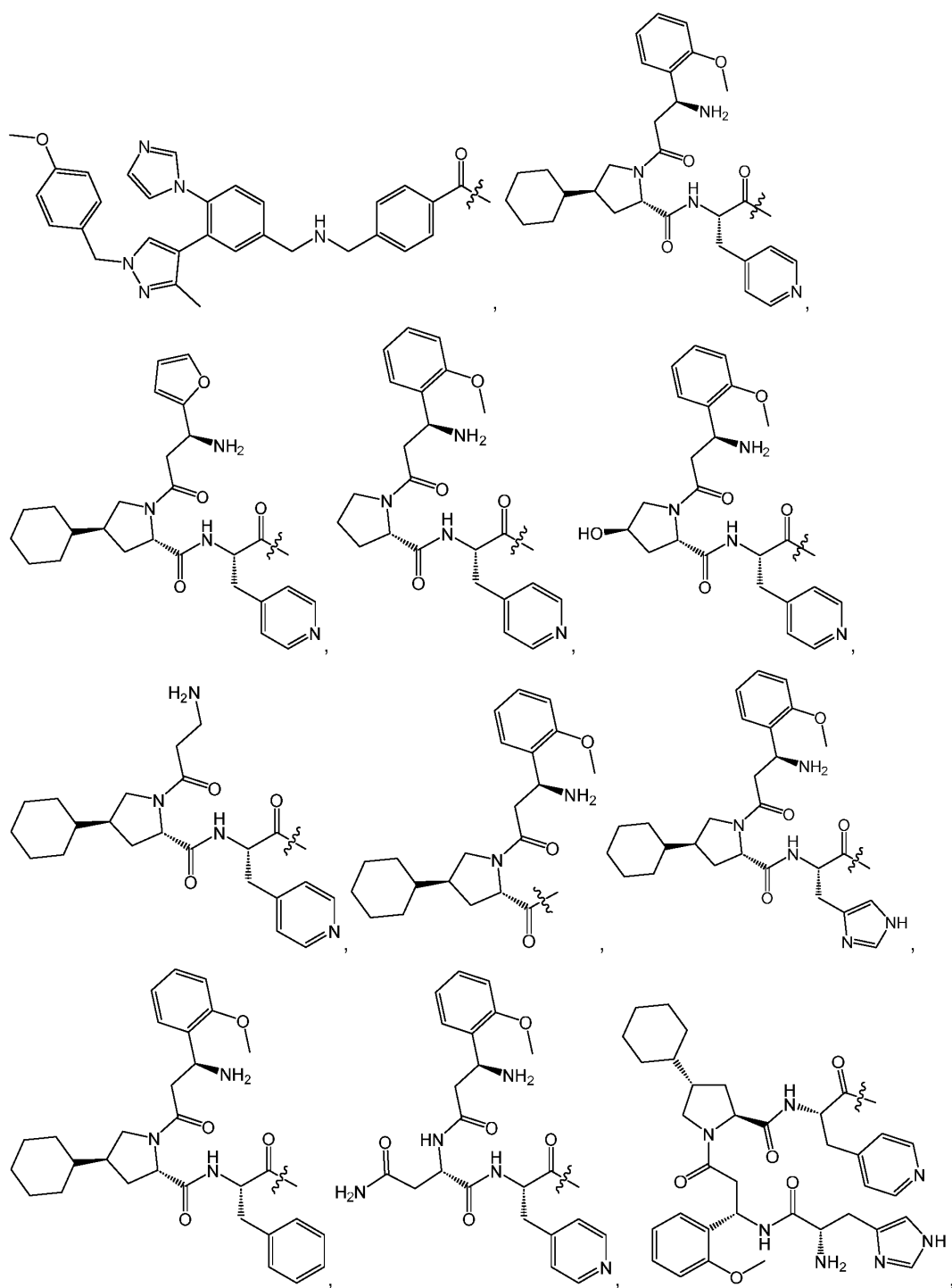


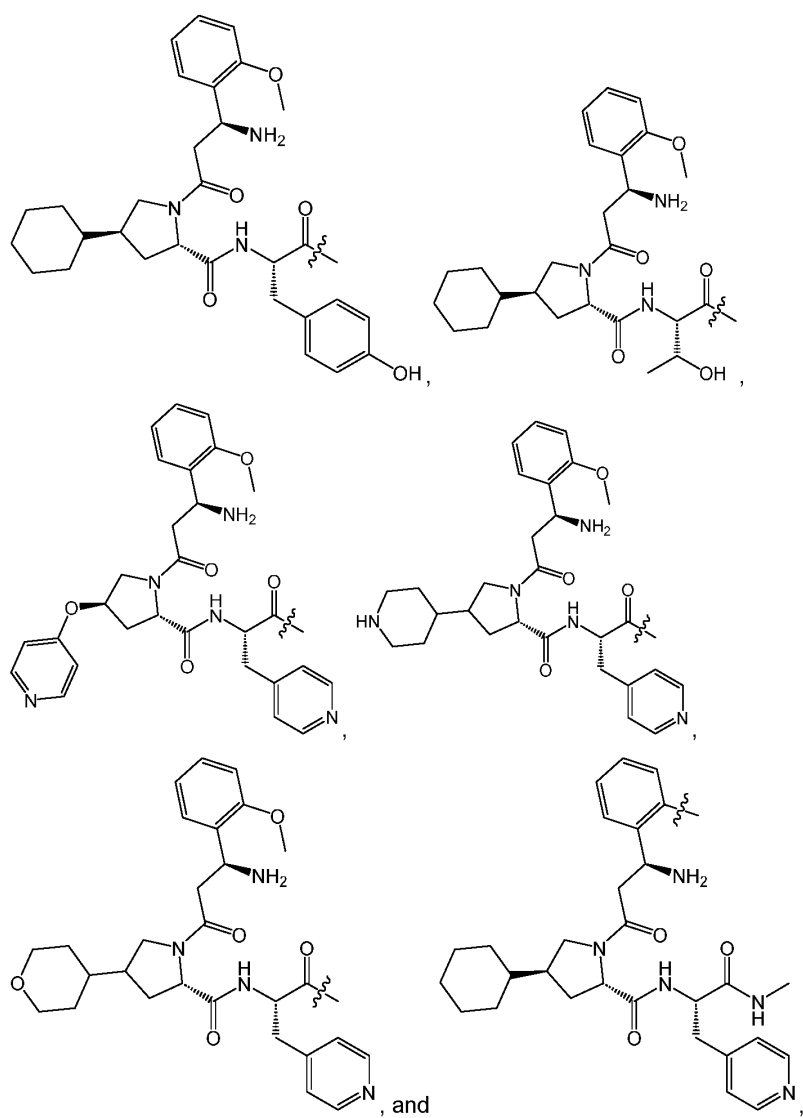
wherein R^a is selected from 5-membered heteroaryl, 6-membered heteroaryl and phenyl; R^b is selected from hydrogen, C_{1-2} alkyl, halo, and C_{1-2} alkoxy; and R^c is selected from hydrogen, C_{1-2} alkyl, halo, and C_{1-2} alkoxy.

47. The compound of clause 46, wherein R^a is 5-membered heteroaryl.

48. The compound of clause 46 or 47, wherein R^a is nitrogen-containing heteroaryl.

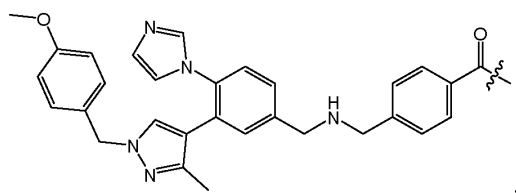
49. The compound of any of the preceding clauses, wherein A is selected from:

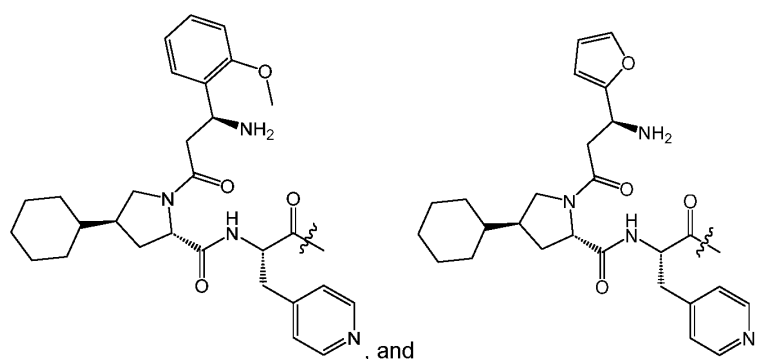




5 wherein  in the structure of A indicates a site of covalent attachment to L.

50. The compound of clause 49, wherein A is selected from:





wherein  in the structure of A indicates a site of covalent attachment to L.

51. The compound of any of the preceding clauses, wherein the target protein is selected from proteins that form aggregates.
52. The compound of any of the preceding clauses, wherein the target protein is selected from tau, synuclein, amyloid β , islet amyloid polypeptide (IAPP), serum amyloid A (SAA), prion proteins, misfolded transthyretin protein (TTR), TAR DNA-binding protein 43 (TDP-43), NOTCH3 receptor, mutated cystatin C, medin, dipeptide repeat protein and fused-in-sarcoma.
53. The compound of any of the preceding clauses, wherein the target protein is selected from tau, synuclein, amyloid β , prion proteins, TAR DNA-binding protein 43 (TDP-43), dipeptide repeat protein and fused-in-sarcoma.
54. The compound of any of the preceding clauses, wherein the target protein is tau.
55. The compound of any of the preceding clauses, wherein the target protein is selected from proteins that have expanded repeat elements.
56. The compound of any of the preceding clauses, wherein the target protein is mutated huntingtin.
57. The compound of any of the preceding clauses, wherein the target protein is selected from proteins involved in signal transduction pathways in inflammation and/or cancer.
58. The compound of any of the preceding clauses, wherein the target protein is selected from Myd88, IRAK4, IRAK2, IRAK1, TRAF6, NLRP3, RIPK3 and ASC.
59. The compound of any of the preceding clauses, wherein the target protein is selected from Myd88, IRAK4, IRAK2, IRAK1, TRAF6, NLRP3 and ASC.

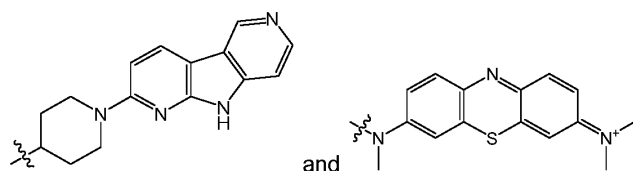
60. The compound of any of the preceding clauses, wherein the target protein is selected from viral proteins that form oligomers.

61. The compound of any of the preceding clauses, wherein the target protein is selected from CoV2 and influenza virus nucleoprotein.

62. The compound any of the preceding clauses, wherein the target protein is selected from bromodomain-containing protein 4 (BRD4), mutated gelsolin, misfolded rhodopsin and atrial natriuretic peptide.

63. The compound of any of the preceding clauses, wherein B is selected from PI-2014, FDDNP, AV680(T808), GTP-1, THK523, THK5105, PBB3, AV1451(T807), THK5117, THK5351, N-methyl lansoprazole, (E)-6-iodo-2-styryl-1H-benzo[d]imidazole, RO6958948, PI-2620, JNJ-067, APN-1607(PM-PBB3), MK-6240, and methylene blue.

64. The compound of any of the preceding clauses, wherein B is selected from:



wherein  in the structure of B indicates a site of covalent attachment to L.

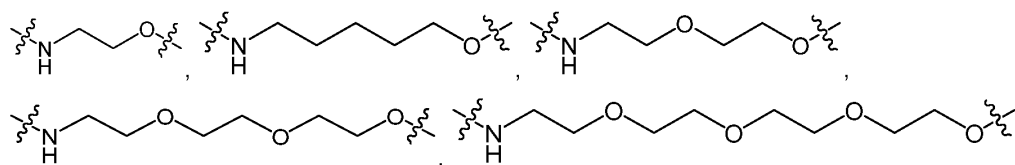
65. The compound of any of the preceding clauses, wherein the linker (L):

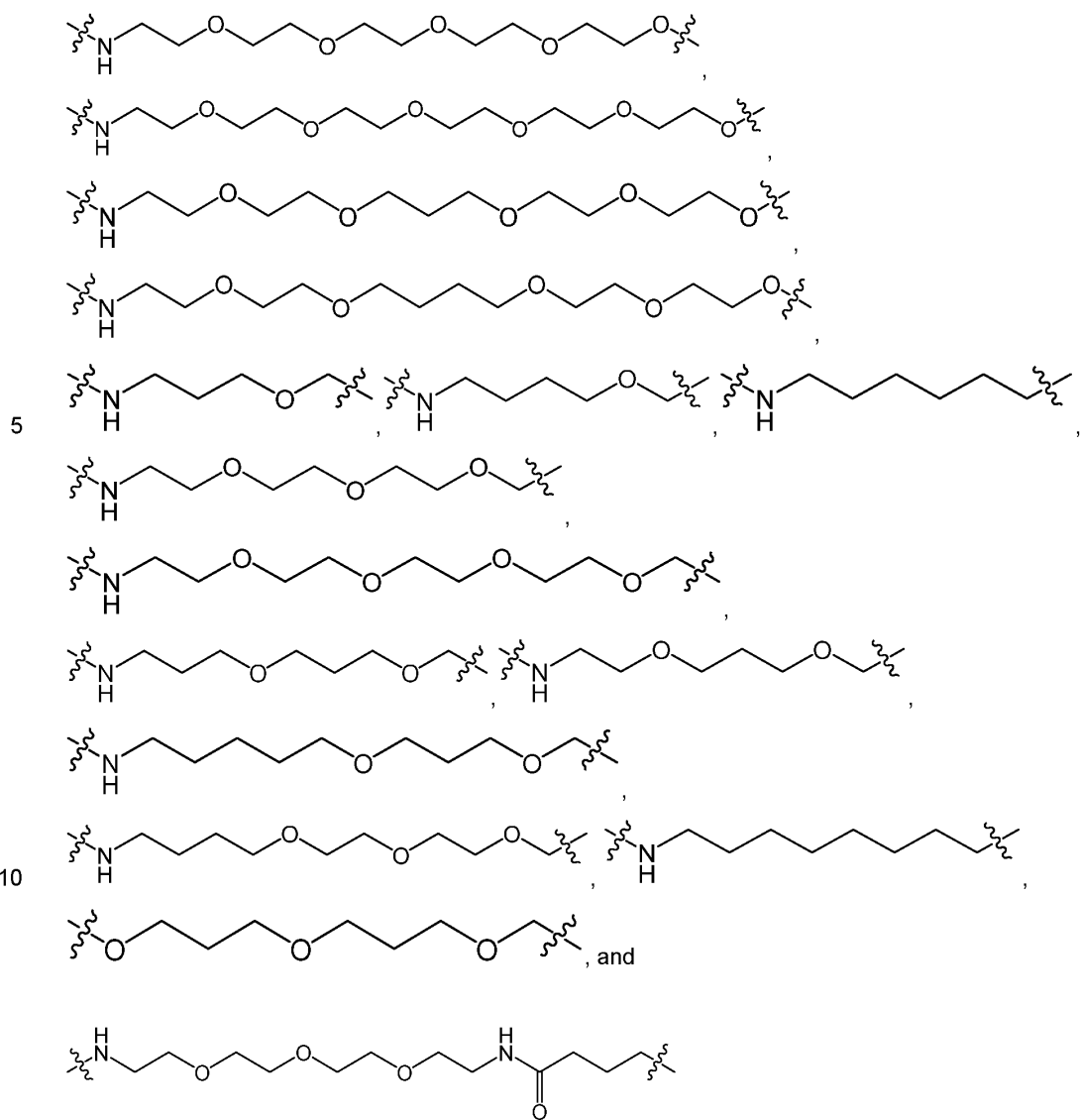
(a) is a bond; or

(b) is a chain of 2 to 20 carbon atoms, wherein one or more of the carbon atoms is optionally replaced by 1 to 12 ethylene glycol units and/or a heteroatom selected from O, N, S, and P; and/or

(c) comprises poly(ethylene glycol) (PEG), unsubstituted alkylene, substituted alkylene, or a combination thereof; and/or

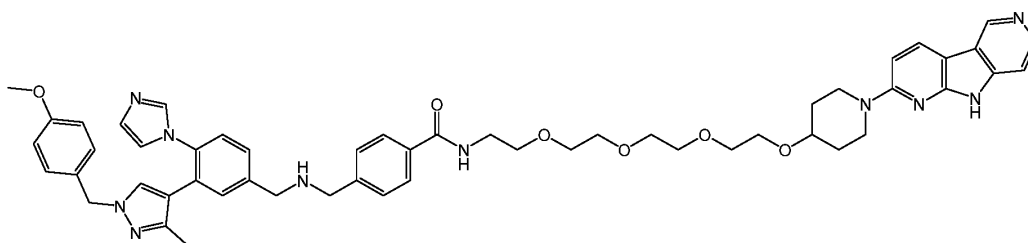
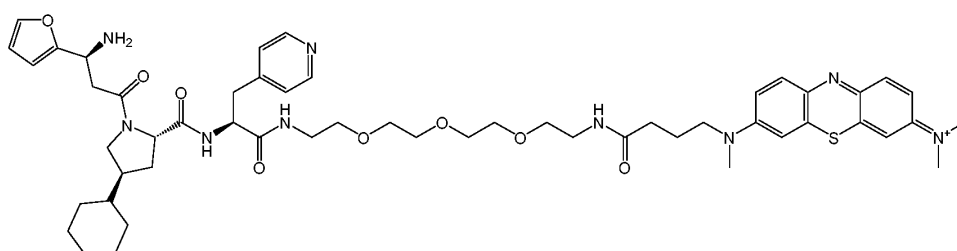
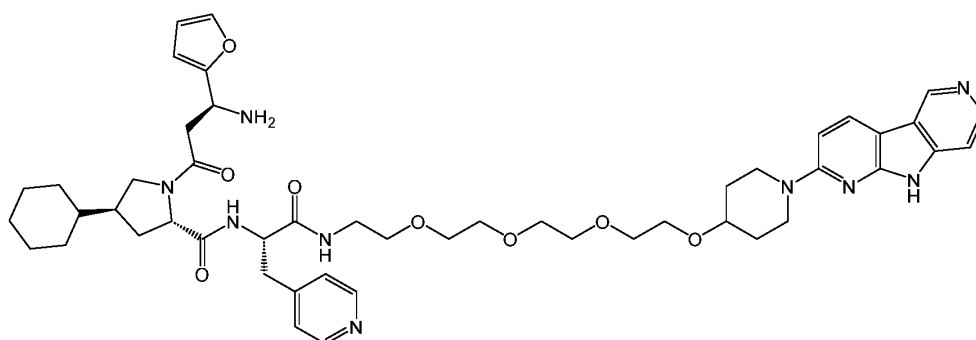
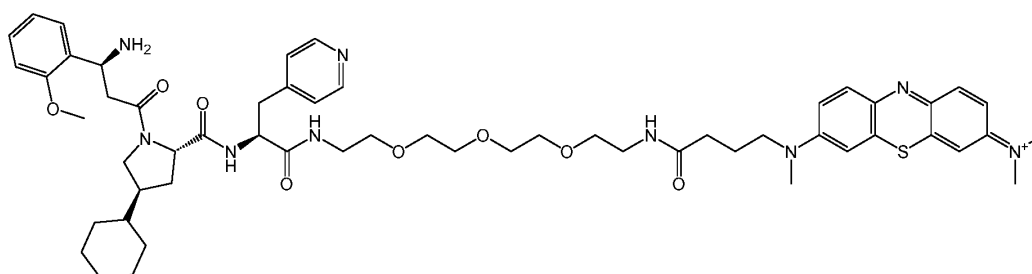
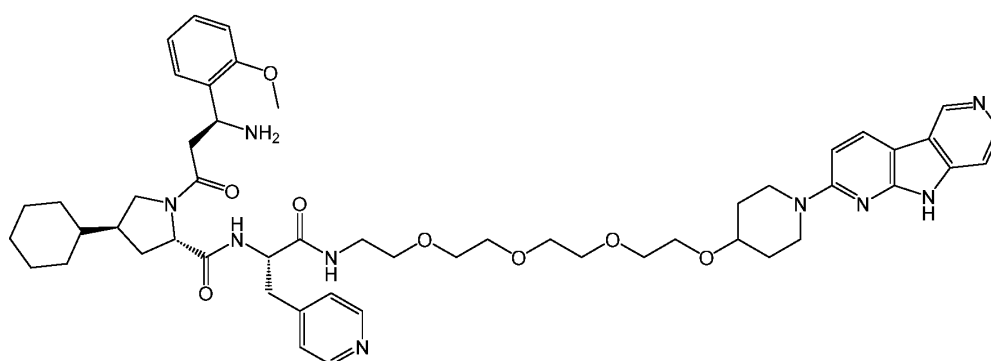
(d) is selected from:





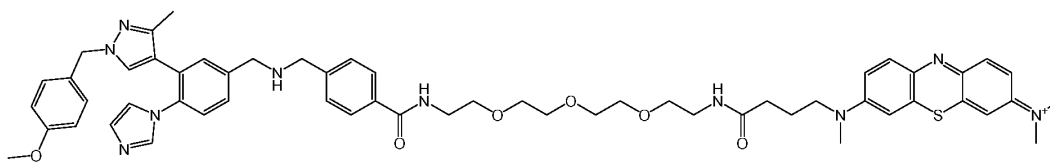
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66. The compound of any of the preceding clauses, wherein the compound is selected from:

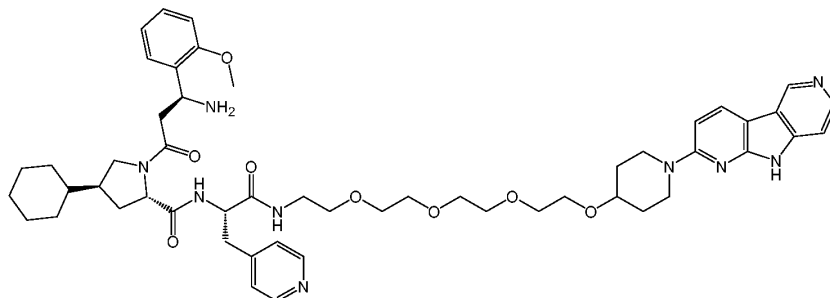


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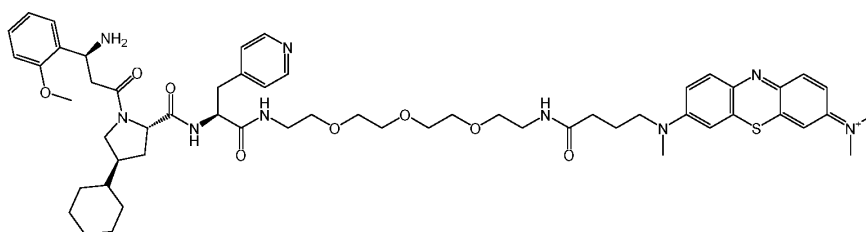
and



67. The compound of any of the preceding clauses, wherein the compound is selected from:



and



5

68. A compound of formula (I)

A-L-B

(I)

10

or a pharmaceutically acceptable salt thereof, wherein:

A is a moiety that binds TRIM21;

L is a linker; and

15

B is a moiety that binds a target protein, wherein the target protein has a first form comprising multiple binding sites for B and a second form comprising a single binding site for B.

69. The compound of clause 68, wherein A, L, B and the target protein are as defined in any of clauses 1-67.

20

70. A compound capable of simultaneously binding TRIM21 and a target protein.

71. The compound of clause 70, wherein the target protein has a first form that provides multiple binding sites for the compound.

25

72. The compound of clause 70 or 71, wherein the target protein has a first form comprising multiple binding sites for the compound and a second form comprising a single binding site for the compound.
- 5 73. The compound of any one of clauses 70-72, wherein the target protein is capable of forming an oligomeric species.
74. The compound of any one of clauses 70-73, wherein the binding enables clustering of multiple TRIM21 molecules.
- 10 75. The compound of any one of clauses 70-74, wherein the binding enables ubiquitination and degradation of the target protein.
- 15 76. The compound of any one of clauses 70-75, wherein the target protein has multiple binding sites for the compound, wherein the binding of the compound enables clustering of multiple TRIM21 molecules.
77. The compound of clause 74 or 76, wherein the clustering enables the ubiquitination and degradation of the target protein.
- 20 78. The compound of any one of clauses 70-77, wherein the target protein is as defined in any one of clauses 2, 3, 17 or 51-62.
- 25 79. The compound of any one of clauses 70-78, wherein the compound binds the PRYSPRY domain of TRIM21 (SEQ ID NO: 1).
80. The compound of any one of clauses 70-79, wherein the compound binds one or more of residues selected from W381, W383, D355, F369, L370, L371, F450, Y328, H368, S447, M330, D452, R364, Q395, Y393, E389 and K387 of TRIM21 (SEQ ID NO: 1).
- 30 81. The compound of clause 80, wherein the one or more of residues is selected from W381, W383, D355.
82. The compound of any one of clauses 70-81, wherein the compound binds residue D355 of TRIM21 (SEQ ID NO: 1).
- 35 83. The compound of any one of clauses 70-82, wherein the compound comprises a moiety capable of forming a hydrogen bond with residue D355 of TRIM21 (SEQ ID NO: 1).
84. The compound of clause 83, wherein the hydrogen bond is a charged hydrogen bond.
- 40

85. The compound of any one of clauses 70-84, wherein the compound binds residue W381 of TRIM21 (SEQ ID NO: 1).
- 5 86. The compound of any one of clauses 70-85, wherein the compound comprises a moiety capable of hydrophobic stacking with residue W381 of TRIM21 (SEQ ID NO: 1).
87. The compound of any one of clauses 70-86, wherein the compound binds residue W383 of TRIM21 (SEQ ID NO: 1).
- 10 88. The compound of any one of clauses 70-87, wherein the compound comprises a moiety capable of hydrophobic stacking with residue W383 of TRIM21 (SEQ ID NO: 1).
89. The compound of any one of clauses 70-88, wherein the compound binds residues W381, W383 and D355 of TRIM21 (SEQ ID NO: 1).
- 15 90. The compound of any one of clauses 70-89, wherein the compound competes with IgG for binding to TRIM21.
91. The compound of any one of clauses 70-90, wherein the compound is not an Fc region or
20 fragment thereof.
92. The compound of any one of clauses 70-91, wherein the compound is not an anti-TRIM21 antibody or antigen binding fragment thereof.
- 25 93. The compound of any one of clauses 70-92, wherein the compound is as defined in any one of clauses 1-69.
94. A pharmaceutical composition comprising a compound according to any of clauses 1-93, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.
- 30 95. A compound according to any of clauses 1-93, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition according to clause 94, for use in therapy.
96. A compound according to any of clauses 1-93, or a pharmaceutically acceptable salt thereof, or a
35 pharmaceutical composition according to clause 94, for use in treating a neurodegenerative disease.
97. The compound or composition for use of clause 96, wherein the neurodegenerative disease is selected from Alzheimer's disease, Parkinson's disease, and dementia.

98. A method of treating a subject having a disease or disorder, the method comprising administering a compound according to any of clauses 1-93, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition according to clause 94.

5 99. The method according to clause 98, wherein the disease or disorder is a neurodegenerative disease.

100. The use of a compound according to any of clauses 1-93, or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament for the treatment of a disease or disorder.

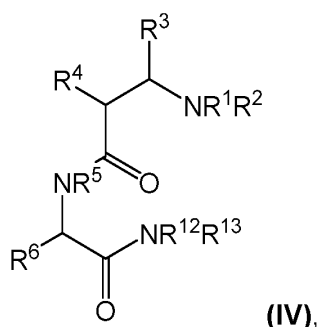
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101. The use according to clause 100, wherein the disease or disorder is a neurodegenerative disease.

102. An *in vitro* method of selectively degrading oligomeric or pathogenic forms of a target protein, the method comprising contacting the target protein with TRIM21 and a compound according to any of clauses 1-93, or a pharmaceutically acceptable salt thereof.

15

103. A compound having the structure of formula (IV)



20

wherein:

R¹ and R² are each independently selected from hydrogen and C₁₋₂ alkyl; or one of R¹ and R² is hydrogen and the other is an amino acid residue;

25

R³ is selected from hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted heteroalkyl, optionally substituted heteroalkenyl, optionally substituted heteroalkynyl, optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl and optionally substituted heteroaryl;

R⁴ is hydrogen, halo or C₁₋₂ alkyl;

30

R⁵ is hydrogen or C₁₋₂ alkyl and R⁶ is optionally substituted alkyl; or R⁵ and R⁶ combine to form an optionally substituted heterocyclic ring;

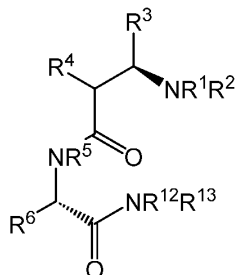
R⁹ is optionally substituted alkyl;

R^{12} and R^{13} are each independently selected from hydrogen and C_{1-2} alkyl; or one of R^{12} and R^{13} is hydrogen and the other is selected from optionally substituted C_{1-2} alkyl and $-CHR^9C(O)NR^{14}R^{15}$; and

R^{14} and R^{15} are each independently selected from hydrogen and C_{1-2} alkyl.

5

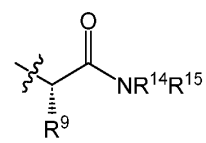
103a. The compound of clause 103, wherein the compound has the following structure:



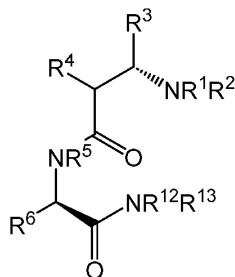
wherein:

10 R^1 and R^2 are each independently selected from hydrogen and C_{1-2} alkyl; or one of R^1 and R^2 is hydrogen and the other is an L-amino acid residue; and

R^{12} and R^{13} are each independently selected from hydrogen and C_{1-2} alkyl; or one of R^{12} and

R^{13} is hydrogen and the other is selected from optionally substituted C_{1-2} alkyl and .

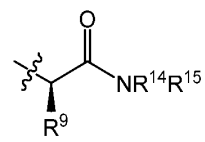
15 103b. The compound of clause 103, wherein the compound has the following structure:



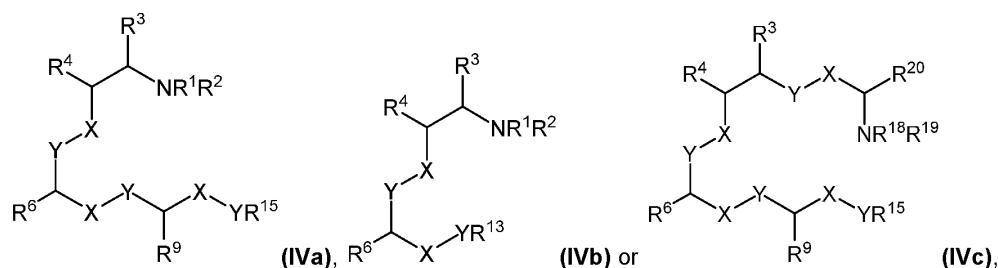
wherein:

20 R^1 and R^2 are each independently selected from hydrogen and C_{1-2} alkyl; or one of R^1 and R^2 is hydrogen and the other is a D-amino acid residue; and

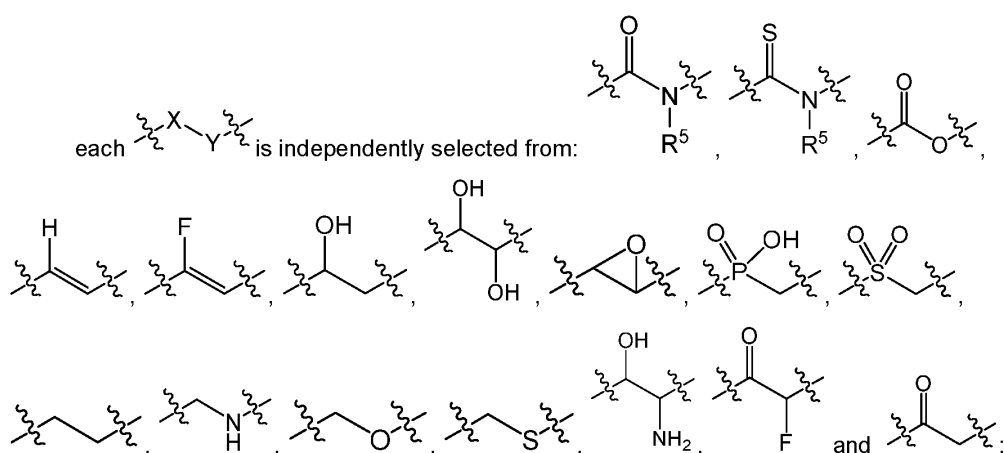
R^{12} and R^{13} are each independently selected from hydrogen and C_{1-2} alkyl; or one of R^{12} and R^{13} is

hydrogen and the other is selected from optionally substituted C_{1-2} alkyl and .

104. A compound having the structure of formula (IVa), (IVb) or (IVc):



5 wherein:



10 R^1 and R^2 are each independently selected from hydrogen and C_{1-2} alkyl; or one of R^1 and R^2 is hydrogen and the other is an amino acid residue;

R^3 is selected from hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted heteroalkyl, optionally substituted heteroalkenyl, optionally substituted heteroalkynyl, optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl and optionally substituted heteroaryl, wherein R^3 optionally comprises a site of covalent attachment to L;

15

R^4 is hydrogen, halo or C_{1-2} alkyl;

each R^5 is independently hydrogen or C_{1-2} alkyl;

R^6 is optionally substituted alkyl;

20 or, when R^5 and R^6 are attached to adjacent atoms, R^5 and R^6 combine to form an optionally substituted heterocyclic ring;

R^8 is hydrogen or C_{1-2} alkyl;

R^9 is optionally substituted alkyl;

R^{12} and R^{13} are each independently selected from hydrogen and C_{1-2} alkyl;

25 R^{14} and R^{15} are each independently selected from hydrogen and C_{1-2} alkyl;

R^{18} and R^{19} are each independently selected from hydrogen and C_{1-2} alkyl; and

R²⁰ is optionally substituted alkyl.

105. The compound of clause 103, 103a, 103b or 104, wherein one of R¹ and R² is hydrogen and the other is selected from an amino acid residue, optionally wherein the amino acid residue is selected from a histidine residue, a phenylalanine residue, a tryptophan residue, and a tyrosine residue.

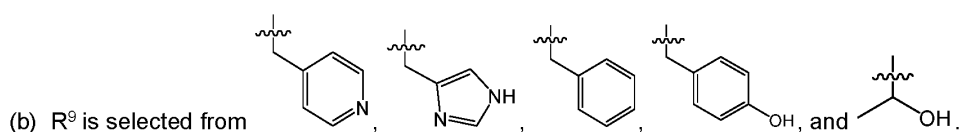
106. The compound of clause 105, wherein the amino acid residue is a histidine residue.

107. The compound of clause 103, 103a, 103b or 104, wherein R¹, R² and R⁴ are hydrogen.

108. The compound of any of clauses 103-107, wherein R³ is selected from optionally substituted aryl and optionally substituted heteroaryl, optionally wherein R³ is selected from furan and optionally substituted phenyl.

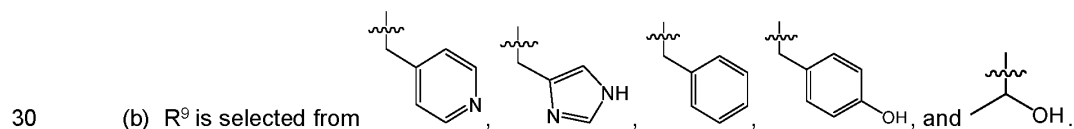
109. The compound of any of clauses 103, 103a, 103b or 105-108, wherein one of R¹² and R¹³ is hydrogen and the other is -CHR⁹C(O)NR¹⁴R¹⁵, wherein:

(a) R⁹ is selected from -alkylene-optionally substituted aryl and -alkylene-optionally substituted heteroaryl, optionally wherein R⁹ is selected from -CH₂-optionally substituted aryl and -CH₂-optionally substituted heteroaryl; or



110. The compound of any one of clauses 103-109, wherein:

(a) R⁹ is selected from -alkylene-optionally substituted aryl and -alkylene-optionally substituted heteroaryl, optionally wherein R⁹ is selected from -CH₂-optionally substituted aryl and -CH₂-optionally substituted heteroaryl; or



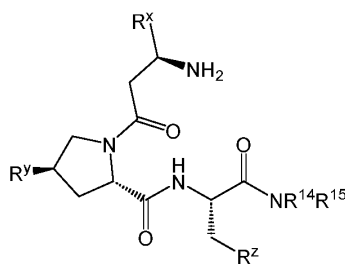
111. The compound of any of clauses 103-110, wherein R⁵ and R⁶ combine to form a 5-membered heterocyclic ring optionally substituted by hydroxy, nitro, cyano, halo, -NR¹⁰R¹¹, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted -O-cycloalkyl,

optionally substituted -O-heterocyclyl, optionally substituted -O-aryl, or optionally substituted -O-heteroaryl,

wherein R^{10} and R^{11} are each independently H, optionally substituted alkyl, or optionally substituted cycloalkyl.

5

112. The compound of any of clauses 103-111, wherein the compound has the structure:



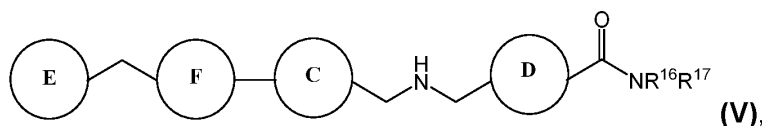
wherein each of R^x , R^y and R^z is independently selected from optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl, and optionally substituted heteroaryl.

10

113. The compound of clause 112, wherein R^x is selected from optionally substituted phenyl and optionally substituted 5-6 membered heteroaryl, R^y is selected from optionally substituted 5-6 membered cycloalkyl and optionally substituted 5-6 membered heterocyclyl, and R^z is selected from optionally substituted phenyl and optionally substituted 5-6 membered heteroaryl.

15

114. A compound having the structure of formula (V)



wherein:

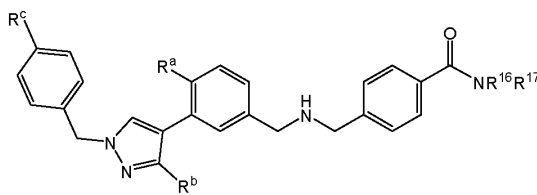
each of rings E, F, C and D is independently selected from optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl; and R^{16} and R^{17} are each independently selected from hydrogen and C_{1-2} alkyl.

20

115. The compound of clause 114, wherein ring C is selected from aryl and heteroaryl, wherein the aryl or heteroaryl is substituted by aryl or heteroaryl.

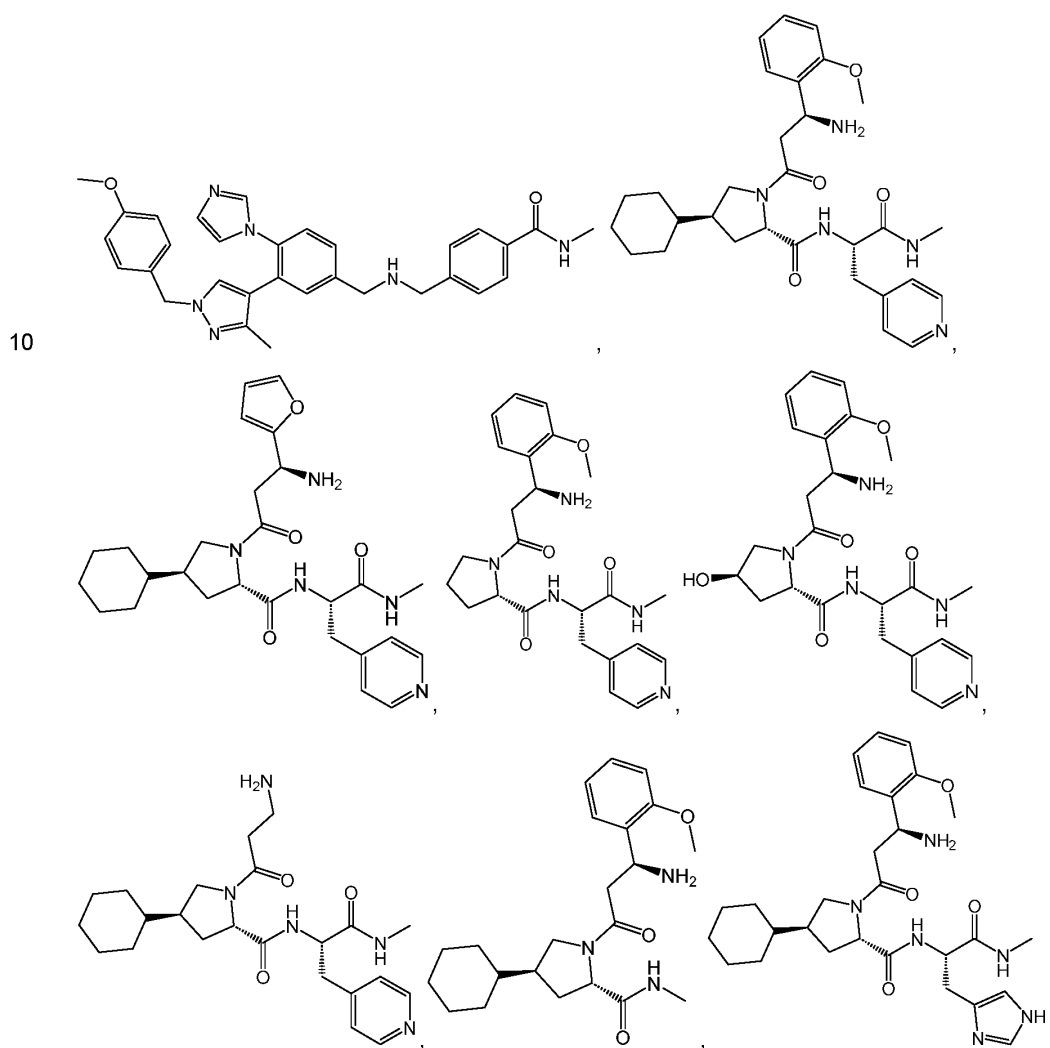
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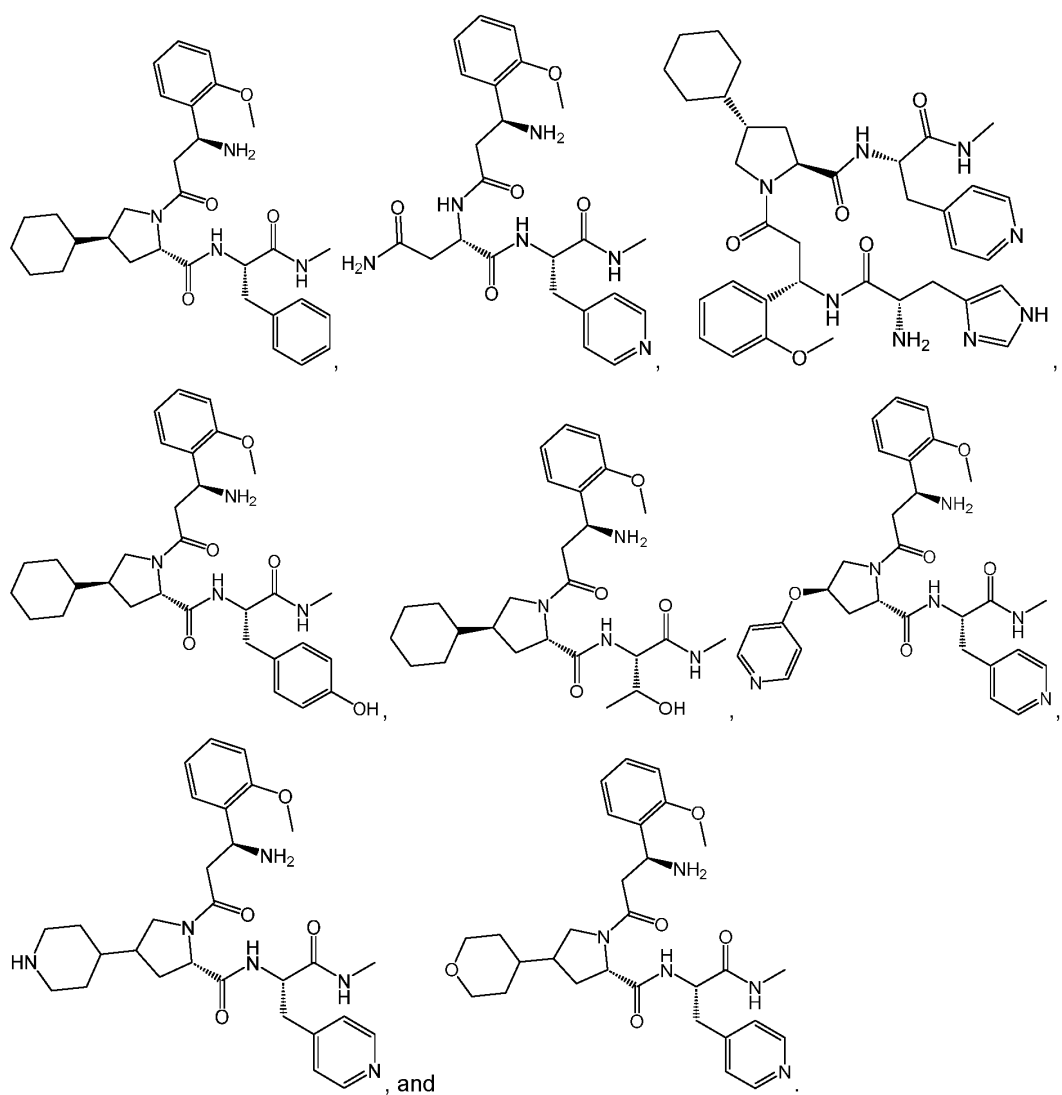
116. The compound of clause 114 or 115, wherein the compound has the structure:



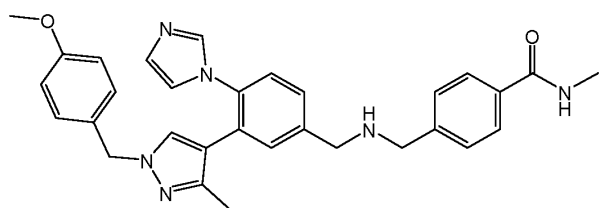
wherein R^a is selected from 5-membered heteroaryl, 6-membered heteroaryl and phenyl; R^b is selected from hydrogen, C₁₋₂ alkyl, halo, and C₁₋₂ alkoxy; and R^c is selected from hydrogen, C₁₋₂ alkyl, halo, and C₁₋₂ alkoxy.

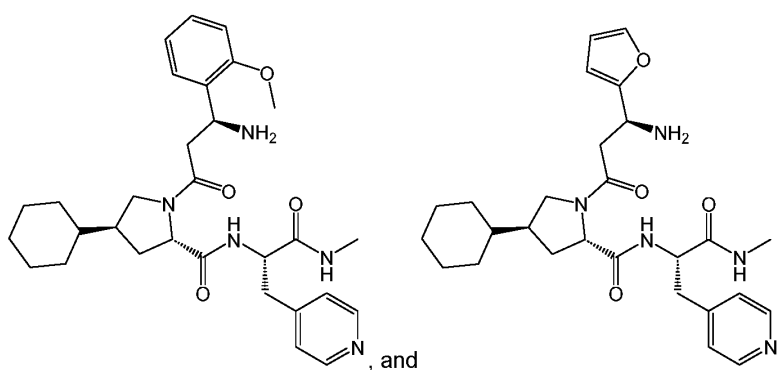
- 5 117. The compound of clause 116, wherein R^a is 5-membered heteroaryl.
118. The compound of clause 116 or 117, wherein R^a is nitrogen-containing heteroaryl.
119. The compound of any of clauses 103-118, wherein the compound is selected from:





- 5 120. The compound of clause 119, wherein the compound is selected from:



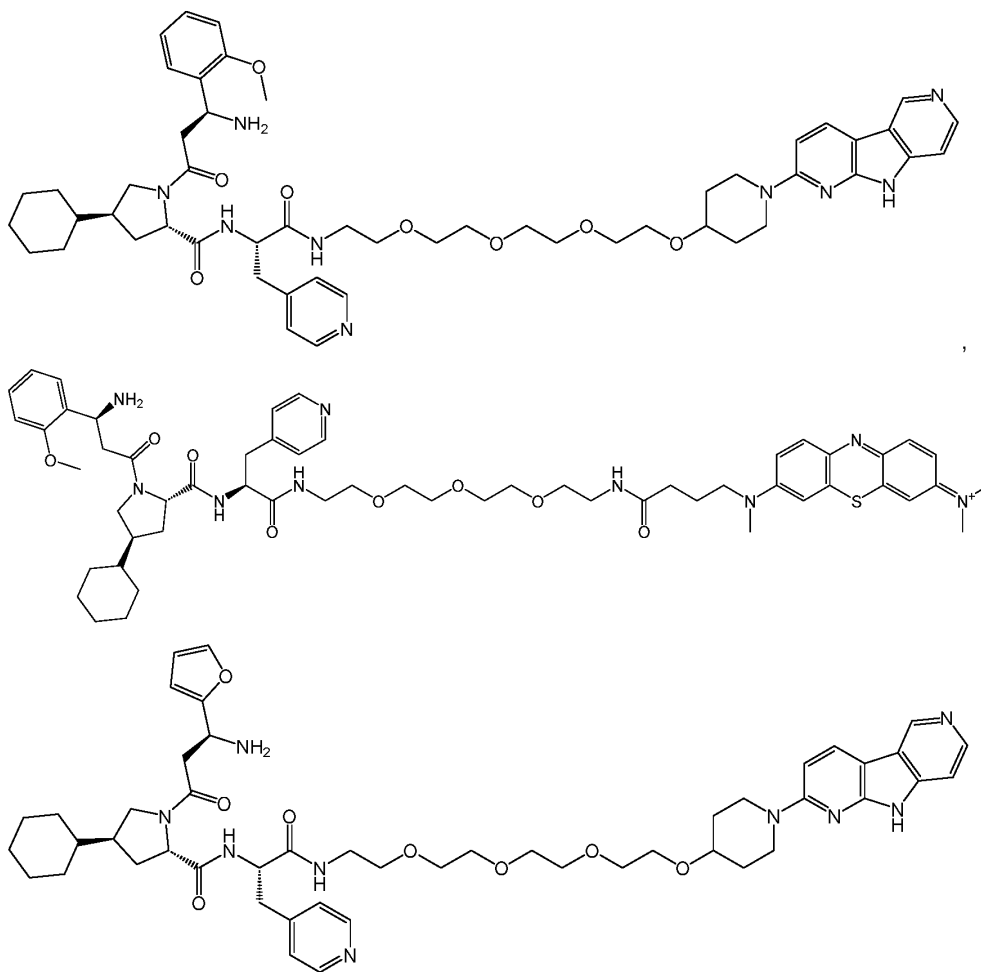


121. A method for inhibiting interaction of TRIM21 with antibodies, the method comprising contacting TRIM21 with a compound according to any of clauses 103-120.

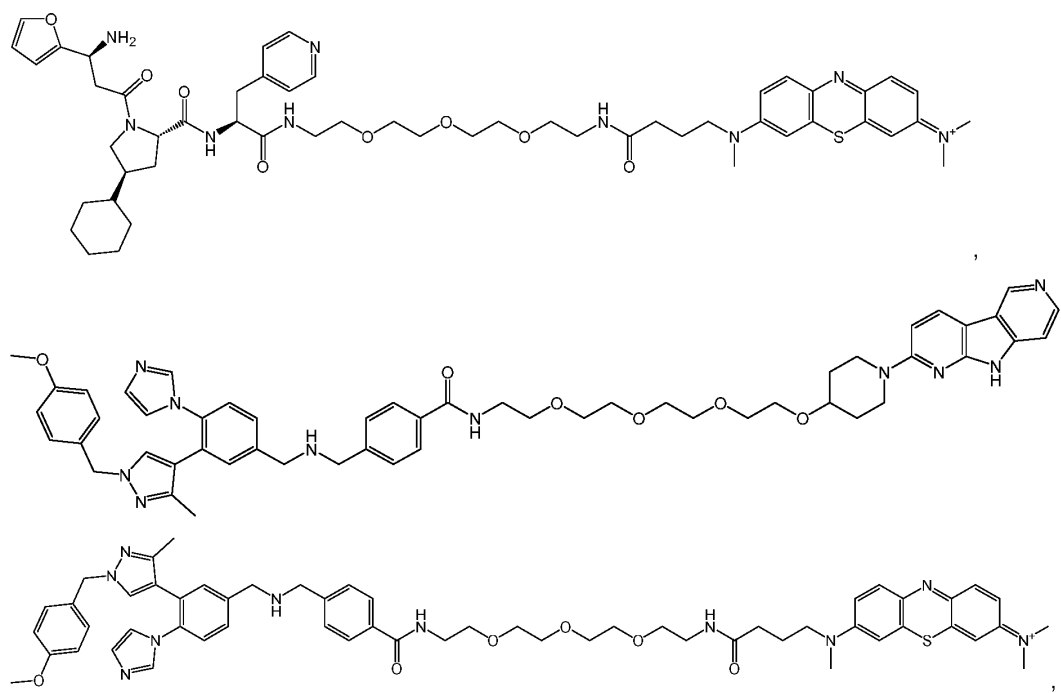
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122. The method of clause 121, wherein the method is an *in vitro* method.

123. A compound selected from:



10



and pharmaceutically acceptable salts thereof.

5

124. A pharmaceutical composition comprising a compound according to clause 123, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

125. A compound according to clause 123, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition according to clause 124, for use in therapy.

126. A compound according to clause 123, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition according to clause 124, for use in treating a neurodegenerative disease.

127. The compound or composition for use of clause 126, wherein the neurodegenerative disease is selected from Alzheimer's disease, Parkinson's disease, and dementia.

Sequence referred to herein:

20

SEQ ID NO:	Sequence	
1	MASAA RL TMMWEEV T C P I C L D P F V E P V S I E C G H S F C Q E C I S Q V G K G G S V C P V C R Q R F L L K N L R P N R Q L A N M V N N L K E I S Q E A R E G T Q G E R C A V H G E R L H L F C E K D G K A L C W V C A Q S R K H R D H A M V P L E E A Q E Y Q E K L Q V A L G E L R R K Q E L A E K L E V E I A I K R A	Human TRIM21

	DWKKTVETQKSRIHAEFVQQKNFLVEEEQRQLQELEKDEREQLRILGEKEAKLAQ QSQALQELISELDRRCHSSALELLQEVIIVLSESWNLKDLDTSPELRSVCHV PGLKKMLRTCAVHITLDPDTANPWLILSEDRRQVRLGDTQQSIPGNEERFDSYPM VLGAQHFHSGKHYWEVDVTGKEAWDLGVCRDSVRRKGHFLSSKSGFWTIWLWNK QKYEAGTYPQTPLHLQVPPCQVGIFLDYEAGMVSFYNITDHGSLIYSFSECAFTG PLRPFSPGFNDGGKNTAPLTLCPNLIGSQGSTDY	
2	MAHHHHHMHVHITLDPDTANPWLILSEDRRQVRLGDTQQSIPGNEERFDSYPM VLGAQHFHSGKHYWEVDVTGKEAWDLGVCRDSVRRKGHFLSSKSGFWTIWLW NKQKYEAGTYPQTPLHLQVPPCQVGIFLDYEAGMVSFYNITDHGSLIYSFSEC AFTGPLRPFSPGFNDGGKNTAPLTLCPNLIGSQGSTDY	Trim21 PRYSPRY with hexahistidine tag

References:

James LC *et al*, (2007) Proc Natl Acad Sci U S A. 2007104(15):6200-6205

Leuzy A *et al*, (2019) Mol Psychiatry 24, 1112–1113

5 Silva MC *et al* eLife v. 8 (2019) e45457

Studier FW *et al*, (2005). Protein Expr Purif, 41, 207-234

Winter G *et al*, (2011) Methods. 2011; 55:81–93

Winter G *et al*, (2022) Protein Sci. 2022 Jan;31(1):232-250

CLAIMS

1. A compound of formula (I)

A-L-B**(I)**

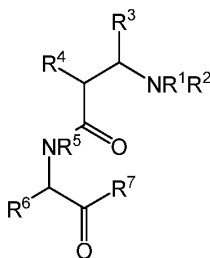
or a pharmaceutically acceptable salt thereof, wherein:

A is a moiety that binds TRIM21, wherein the molecular weight of A is 1000 Da or less;

L is a linker; and

B is a moiety that binds a target protein, wherein the target protein can comprise a first form that provides multiple binding sites for B.

2. The compound of claim 1, wherein the target protein is capable of forming an oligomeric species.
3. The compound of claim 1 or claim 2, wherein the compound is configured to recruit TRIM21 to enable selective degradation by TRIM21 of one or more oligomeric forms of the target protein.
4. The compound of any of claims 1-3, wherein A binds:
- (a) the PRYSPRY domain of TRIM21 (SEQ ID NO: 1); and/or
 - (b) one or more of residues selected from W381, W383, D355 and F369, L370, L371, F450, Y328, H368, S447, M330, D452, R364, Q395, Y393, E389 and K387 of TRIM21, preferably one or more residues selected from W381, W383 and D355.
5. The compound of any of the preceding claims, wherein A competes with IgG for binding to TRIM21.
6. The compound of any of the preceding claims, wherein A is not an antibody.
7. The compound of any of the preceding claims, wherein the compound has a K_d value for TRIM21 in the range of 0.1 nM to 1000 μ M, preferably 10 nM to 100 μ M.
8. The compound of any of the preceding claims, wherein A has the structure of formula (II)



(II),

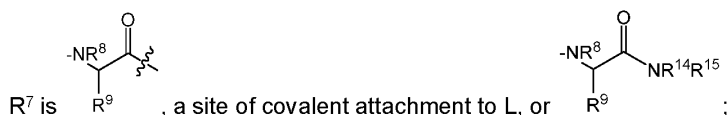
wherein:

R^1 and R^2 are each independently selected from hydrogen and C_{1-2} alkyl; or one of R^1 and R^2 is hydrogen and the other is selected from an amino acid residue, and a site of covalent attachment to L;

R^3 is selected from hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted heteroalkyl, optionally substituted heteroalkenyl, optionally substituted heteroalkynyl, optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl and optionally substituted heteroaryl, wherein R^3 optionally comprises a site of covalent attachment to L;

R^4 is hydrogen, halo or C_{1-2} alkyl;

R^5 is hydrogen or C_{1-2} alkyl and R^6 is optionally substituted alkyl; or R^5 and R^6 combine to form an optionally substituted heterocyclic ring;



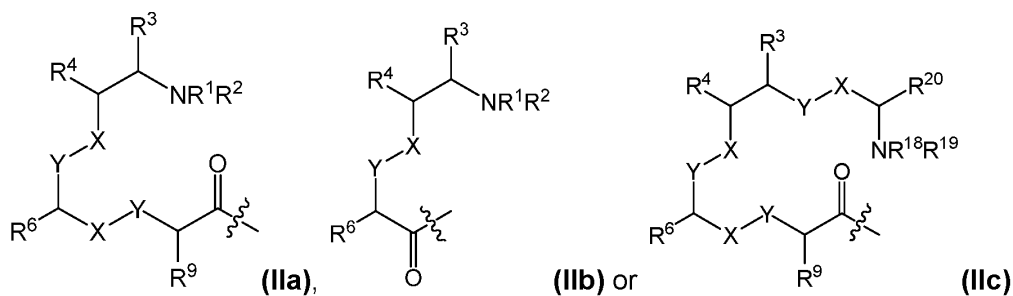
R^8 is hydrogen or C_{1-2} alkyl;

R^9 is optionally substituted alkyl;

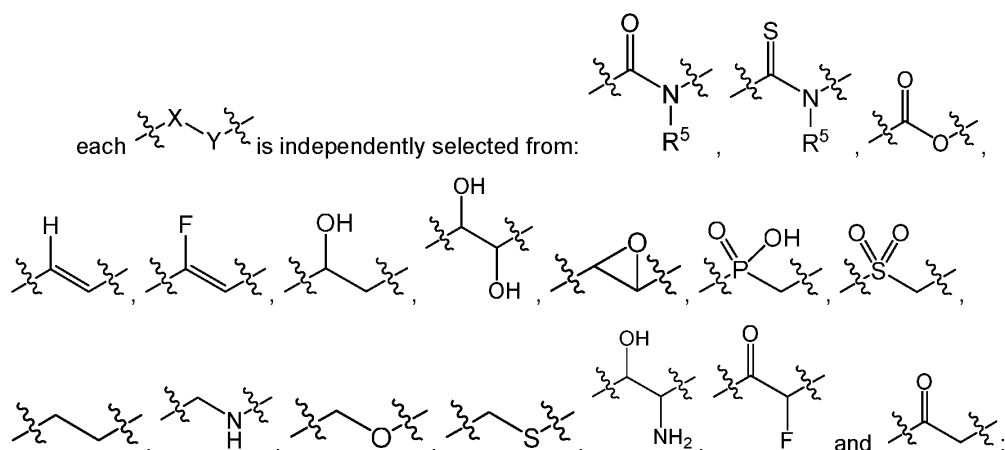
R^{14} and R^{15} are each independently selected from hydrogen and C_{1-2} alkyl; and

 in the structure of R^7 indicates a site of covalent attachment to L; provided that A comprises a site of covalent attachment to L.

9. The compound of any of the preceding claims, wherein A has the structure of formula (IIa), (IIb) or (IIc):



wherein:



R^1 and R^2 are each independently selected from hydrogen and C_{1-2} alkyl; or one of R^1 and R^2 is hydrogen and the other is an amino acid residue;

R^3 is selected from hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted heteroalkyl, optionally substituted heteroalkenyl, optionally substituted heteroalkynyl, optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl and optionally substituted heteroaryl;

R^4 is hydrogen, halo or C_{1-2} alkyl;

each R^5 is independently hydrogen or C_{1-2} alkyl;

R^6 is optionally substituted alkyl;

or, when R^5 and R^6 are attached to adjacent atoms, R^5 and R^6 combine to form an optionally substituted heterocyclic ring;

R^8 is hydrogen or C_{1-2} alkyl;

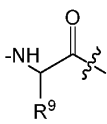
R^9 is optionally substituted alkyl;

R^{18} and R^{19} are each independently selected from hydrogen and C_{1-2} alkyl; and

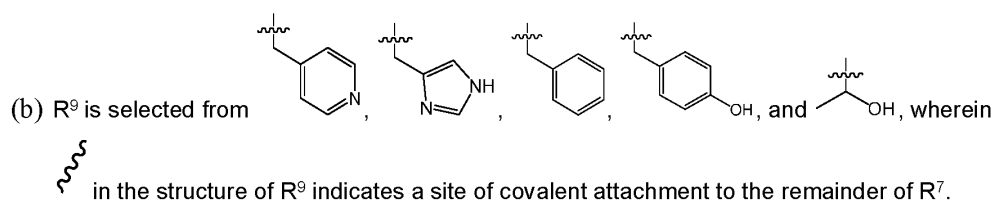
R^{20} is optionally substituted alkyl.

10. The compound of claim 8 or claim 9, wherein R^1 , R^2 and R^4 are hydrogen.

11. The compound of any of claims 8-10, wherein R^3 is selected from optionally substituted aryl and optionally substituted heteroaryl, optionally wherein R^3 is selected from furan and optionally substituted phenyl.

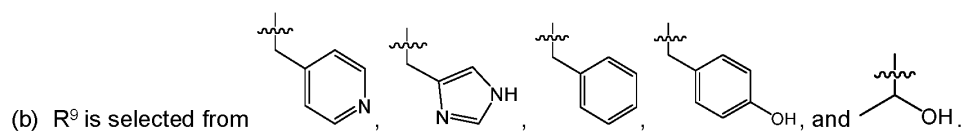
12. The compound of any of claims 8, 10 and 11, wherein R^7 is , wherein:

(a) R^9 is selected from -alkylene-optionally substituted aryl and -alkylene-optionally substituted heteroaryl, optionally wherein R^9 is selected from $-\text{CH}_2$ -optionally substituted aryl and $-\text{CH}_2$ -optionally substituted heteroaryl; or



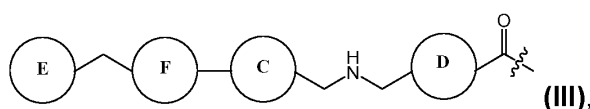
13. The compound of any of claims 9-11, wherein:

- (a) R^9 is selected from -alkylene-optionally substituted aryl and -alkylene-optionally substituted heteroaryl, optionally wherein R^9 is selected from -CH₂-optionally substituted aryl and -CH₂-optionally substituted heteroaryl; or



14. The compound of any of claims 8-13, wherein R^5 and R^6 combine to form a 5-membered heterocyclic ring optionally substituted by hydroxy, nitro, cyano, halo, -NR¹⁰R¹¹, optionally substituted alkyl, optionally substituted alkoxy, optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted -O-cycloalkyl, optionally substituted -O-heterocyclyl, optionally substituted -O-aryl, or optionally substituted -O-heteroaryl, wherein R^{10} and R^{11} are each independently H, optionally substituted alkyl, or optionally substituted cycloalkyl.

15. The compound of any of the preceding claims, wherein A has the structure of formula (III)



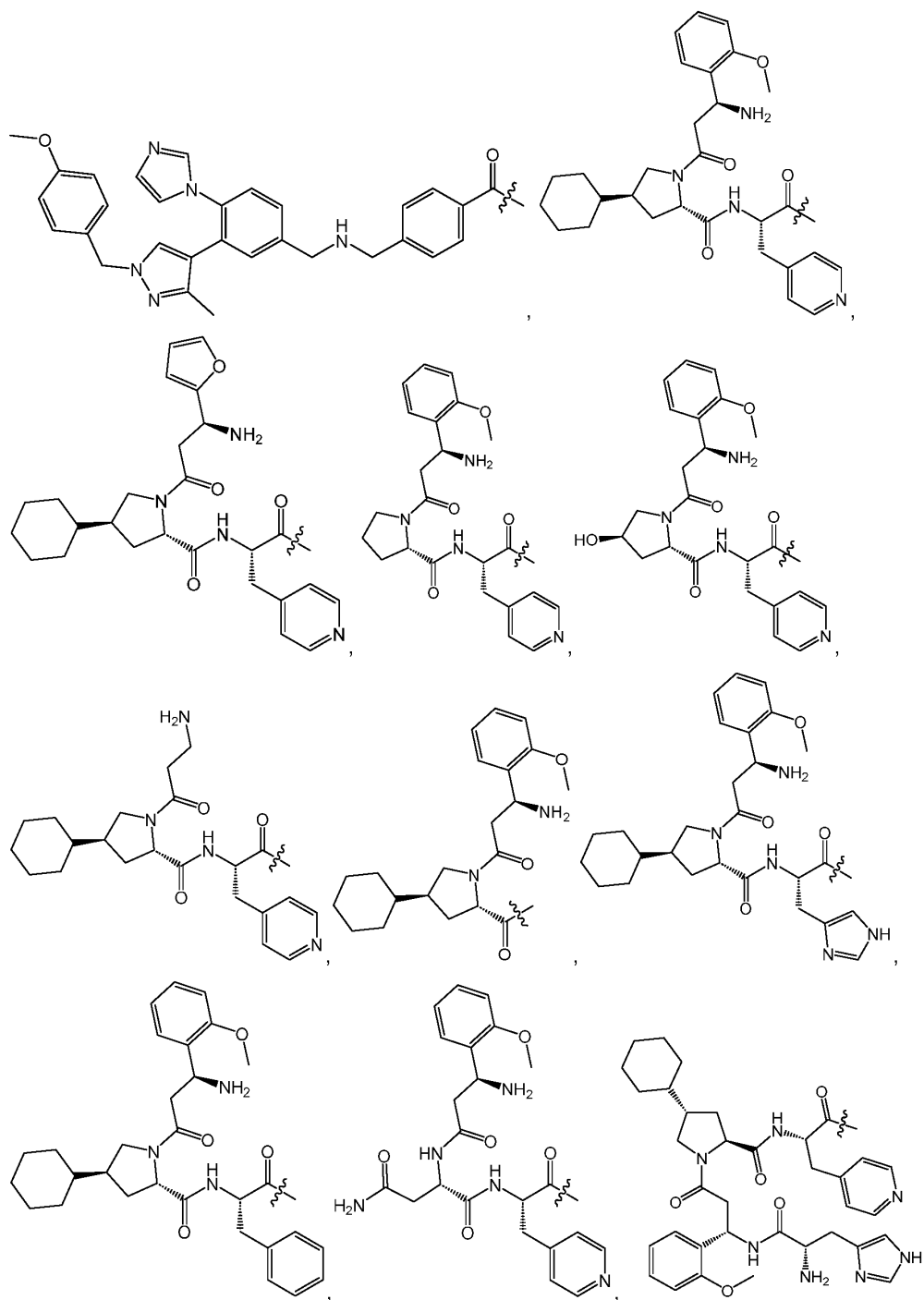
wherein:

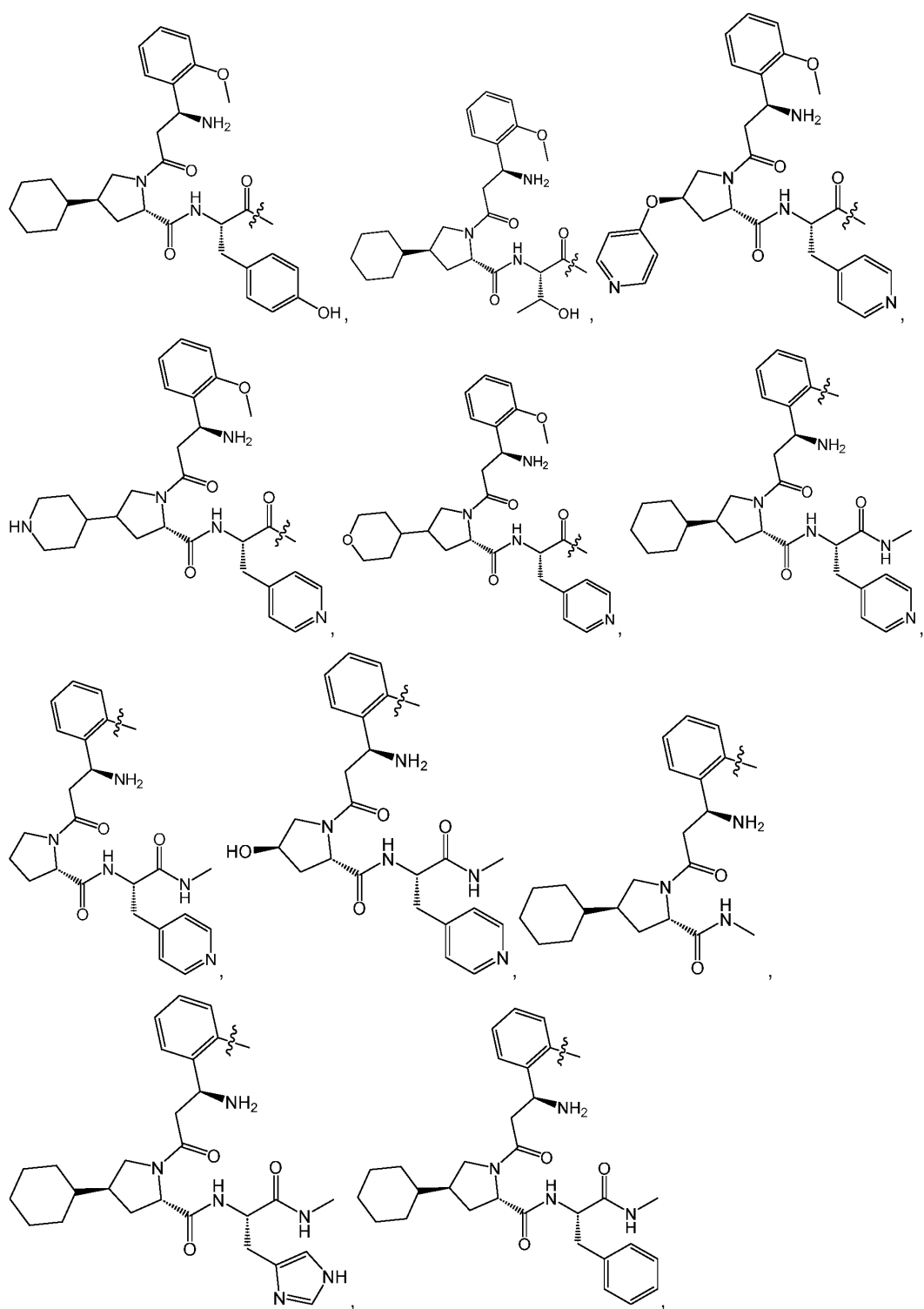
each of rings E, F, C and D is independently selected from optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl, and optionally substituted heteroaryl; and

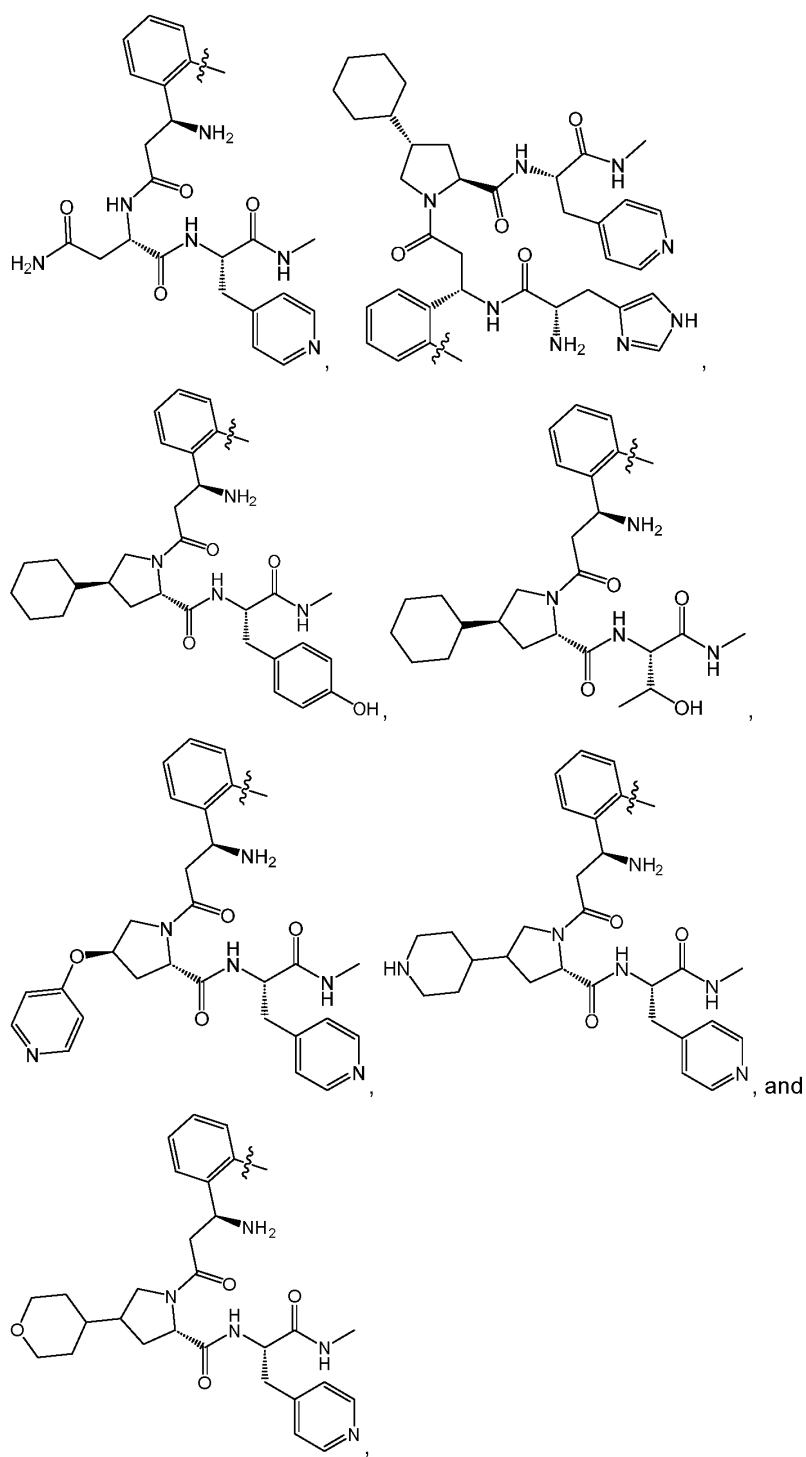
 in the structure of formula (III) indicates a site of covalent attachment to L.

16. The compound of claim 15, wherein ring C is selected from aryl and heteroaryl, wherein the aryl or heteroaryl is substituted by aryl or heteroaryl.

17. The compound of any of the preceding claims, wherein A is selected from:

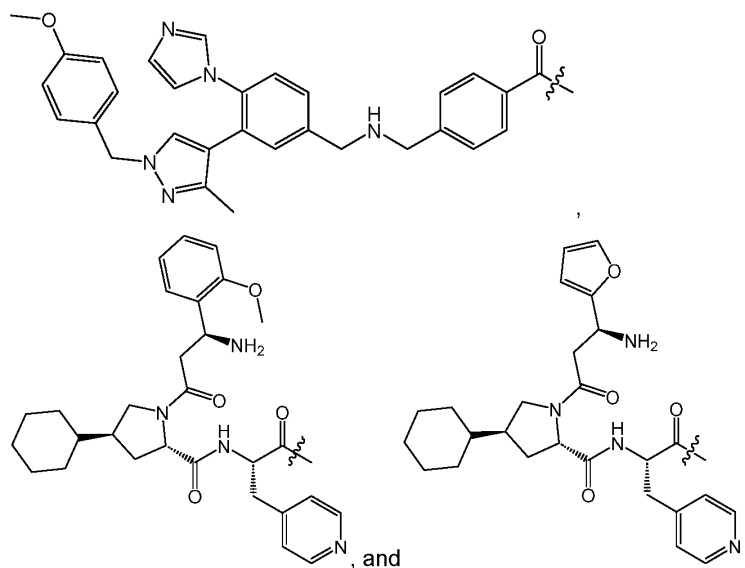






wherein  in the structure of A indicates a site of covalent attachment to L.

18. The compound of claim 17, wherein A is selected from:



wherein  in the structure of A indicates a site of covalent attachment to L.

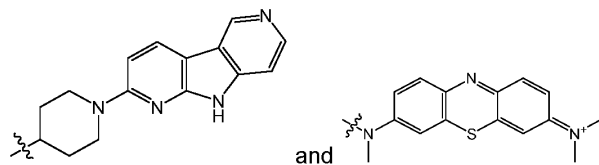
19. The compound of any of the preceding claims, wherein the target protein is selected from:

- (a) proteins that form aggregates involved in neurodegeneration, preferably wherein the protein is selected from tau, synuclein, amyloid β , islet amyloid polypeptide (IAPP), serum amyloid A (SAA), prion proteins, misfolded transthyretin protein (TTR), TAR DNA-binding protein 43 (TDP-43), NOTCH3 receptor, mutated cystatin C, medin, dipeptide repeat protein and fused-in-sarcoma, such as a protein selected from tau, synuclein, amyloid β , prion proteins, TAR DNA-binding protein 43 (TDP-43), dipeptide repeat protein and fused-in-sarcoma, preferably wherein the target protein is tau;
- (b) proteins that have expanded repeat elements, preferably wherein the protein is mutated huntingtin;
- (c) proteins involved in signal transduction pathways in inflammation and/or cancer, preferably wherein the protein is selected from Myd88, IRAK4, IRAK1, IRAK2, TRAF6, NLRP3, RIPK3 and ASC, preferably wherein the protein is selected from Myd88, IRAK4, IRAK1, IRAK2, TRAF6, NLRP3 and ASC;
- (d) viral proteins that form oligomers, preferably wherein the viral protein is selected from CoV2 and influenza virus nucleoprotein; or
- (e) a protein selected from bromodomain-containing protein 4 (BRD4), mutated gelsolin, misfolded rhodopsin and atrial natriuretic peptide.

20. The compound of any of the preceding claims, wherein B is selected from PI-2014, FDDNP, AV680(T808), GTP-1, THK523, THK5105, PBB3, AV1451(T807), THK5117, THK5351, N-methyl

lansoprazole, (E)-6-iodo-2-styryl-1H-benzo[d]imidazole, RO6958948, PI-2620, JNJ-067, APN-1607(PM-PBB3), MK-6240, and methylene blue.

21. The compound of any of the preceding claims, wherein B is selected from:



wherein  in the structure of B indicates a site of covalent attachment to L.

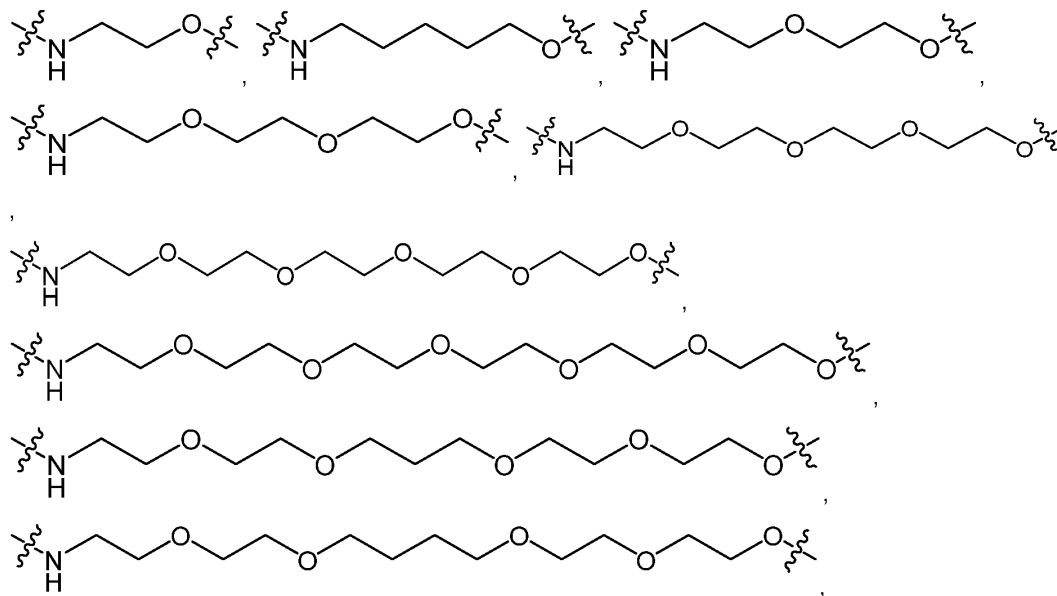
22. The compound of any of the preceding claims, wherein the linker (L):

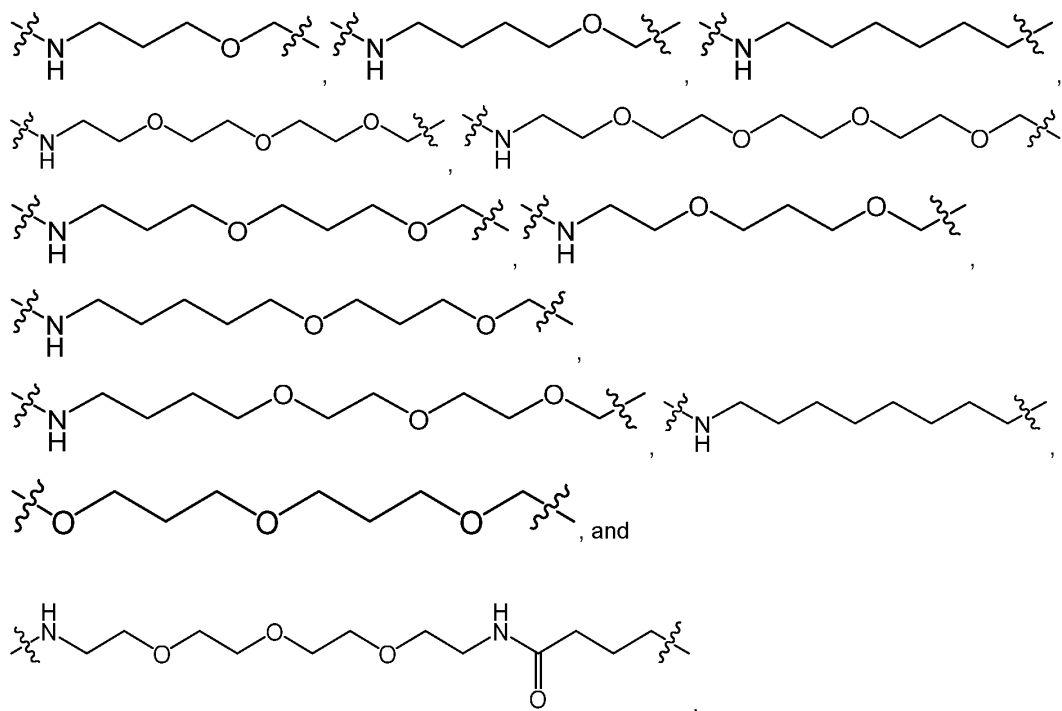
(a) is a bond; or

(b) is a chain of 2 to 20 carbon atoms, wherein one or more of the carbon atoms is optionally replaced by 1 to 12 ethylene glycol units and/or a heteroatom selected from O, N, S, and P; and/or

(c) comprises poly(ethylene glycol) (PEG), unsubstituted alkylene, substituted alkylene, or a combination thereof; and/or

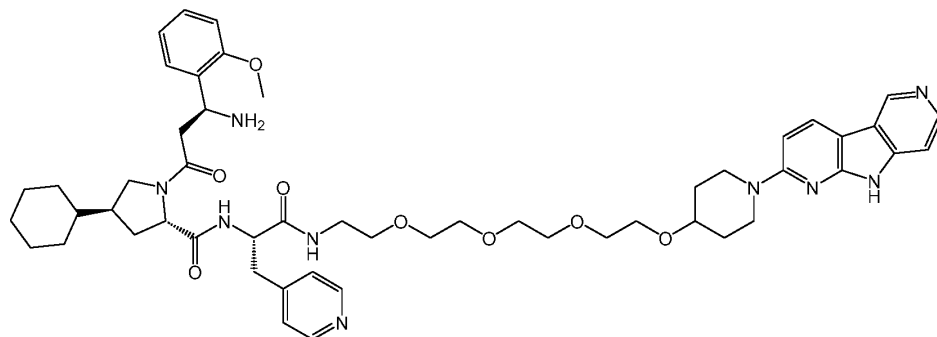
(d) is selected from:



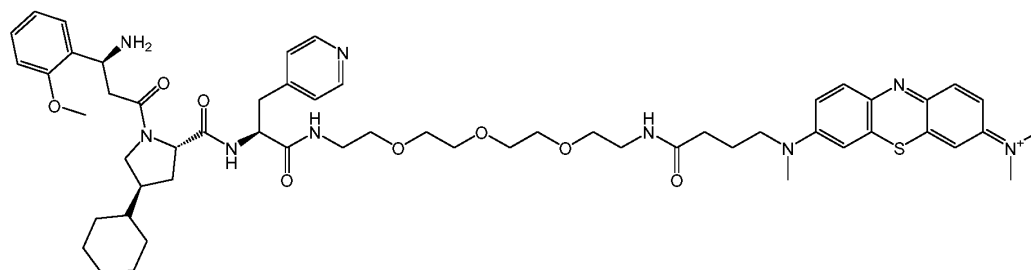


wherein each instance of  in the structure of the linker indicates covalent attachment to A or B.

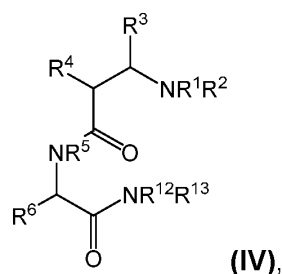
23. The compound of any of the preceding claims, wherein the compound is selected from:



and



24. A pharmaceutical composition comprising a compound according to any of the preceding claims, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.
25. A compound according to any of claims 1-23, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition according to claim 24, for use in therapy.
26. A compound according to any of claims 1-23, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition according to claim 24, for use in treating a neurodegenerative disease.
27. An *in vitro* method of selectively degrading oligomeric forms of a target protein, the method comprising contacting the target protein with TRIM21 and a compound according to any of claims 1-23, or a pharmaceutically acceptable salt thereof.
28. A compound having the structure of formula (IV)



wherein:

R^1 and R^2 are each independently selected from hydrogen and C_{1-2} alkyl; or one of R^1 and R^2 is hydrogen and the other is an amino acid residue;

R^3 is selected from hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted heteroalkyl, optionally substituted heteroalkenyl, optionally substituted heteroalkynyl, optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl and optionally substituted heteroaryl;

R^4 is hydrogen, halo or C_{1-2} alkyl;

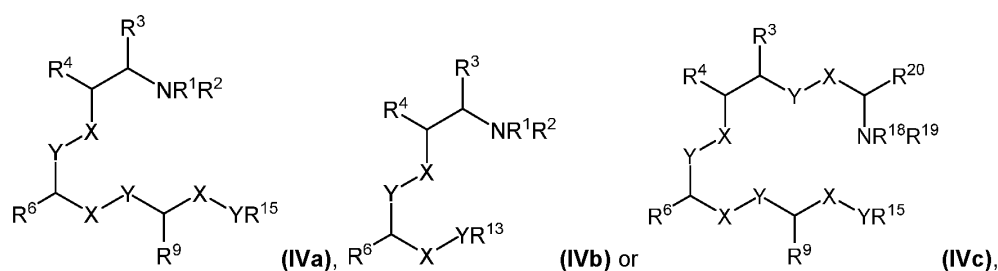
R^5 is hydrogen or C_{1-2} alkyl and R^6 is optionally substituted alkyl; or R^5 and R^6 combine to form an optionally substituted heterocyclic ring;

R^9 is optionally substituted alkyl;

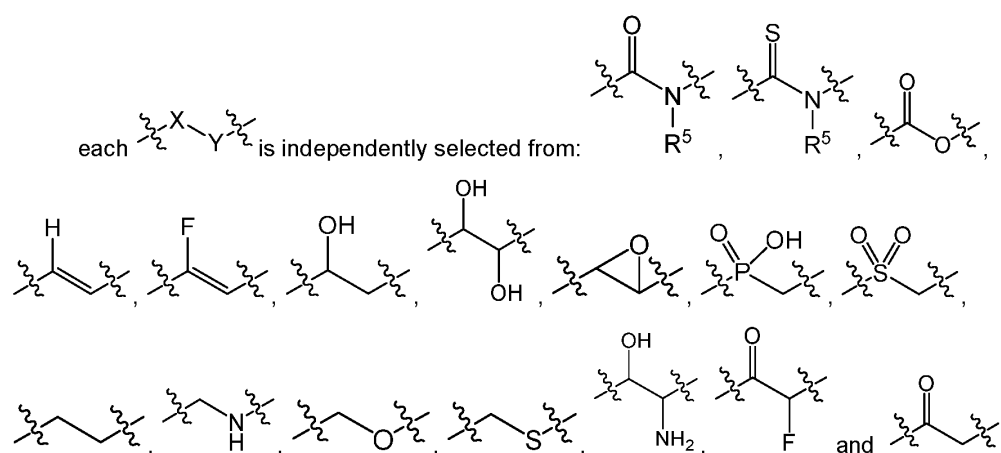
R^{12} and R^{13} are each independently selected from hydrogen and C_{1-2} alkyl; or one of R^{12} and R^{13} is hydrogen and the other is selected from optionally substituted C_{1-2} alkyl and $-CHR^9C(O)NR^{14}R^{15}$; and

R^{14} and R^{15} are each independently selected from hydrogen and C_{1-2} alkyl.

29. A compound having the structure of formula (IVa), (IVb) or (IVc):



wherein:



R^1 and R^2 are each independently selected from hydrogen and C_{1-2} alkyl; or one of R^1 and R^2 is hydrogen and the other is an amino acid residue;

R^3 is selected from hydrogen, optionally substituted alkyl, optionally substituted alkenyl, optionally substituted alkynyl, optionally substituted heteroalkyl, optionally substituted heteroalkenyl, optionally substituted heteroalkynyl, optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl and optionally substituted heteroaryl, wherein R^3 optionally comprises a site of covalent attachment to L;

R^4 is hydrogen, halo or C_{1-2} alkyl;

each R^5 is independently hydrogen or C_{1-2} alkyl;

R^6 is optionally substituted alkyl;

or, when R^5 and R^6 are attached to adjacent atoms, R^5 and R^6 combine to form an optionally substituted heterocyclic ring;

R^8 is hydrogen or C_{1-2} alkyl;

R^9 is optionally substituted alkyl;

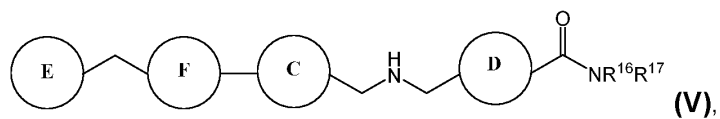
R^{12} and R^{13} are each independently selected from hydrogen and C_{1-2} alkyl;

R^{14} and R^{15} are each independently selected from hydrogen and C_{1-2} alkyl;

R^{18} and R^{19} are each independently selected from hydrogen and C_{1-2} alkyl; and

R^{20} is optionally substituted alkyl.

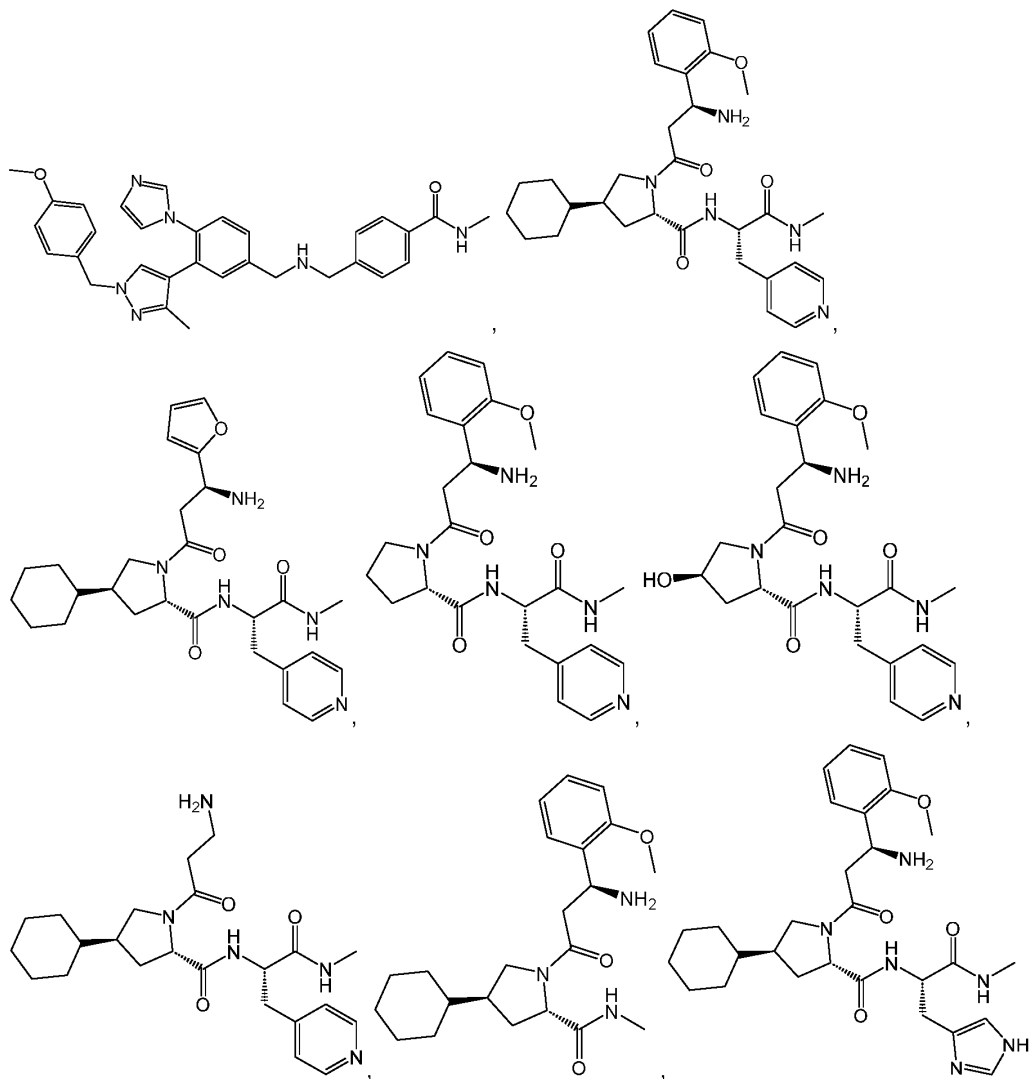
30. A compound having the structure of formula (V)

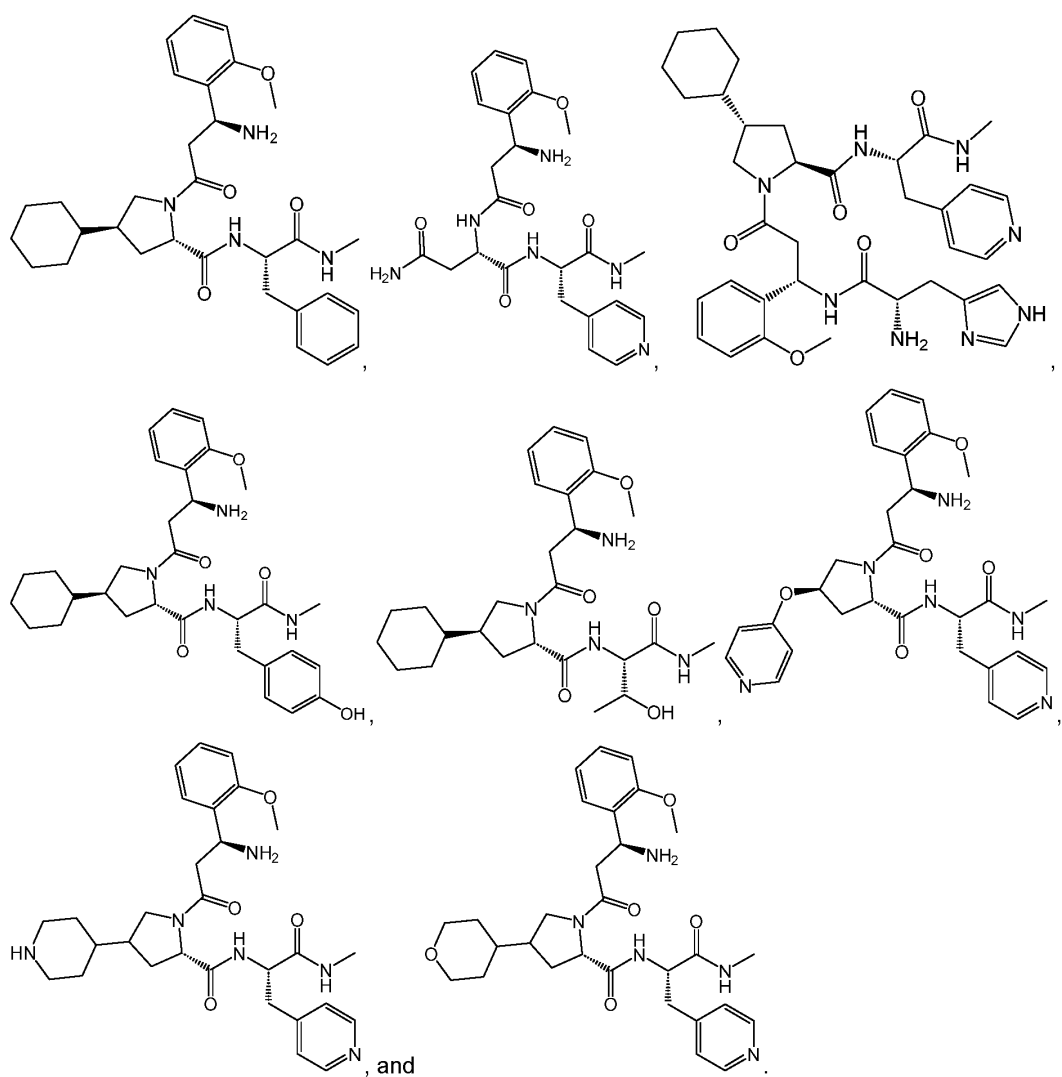


wherein:

each of rings E, F, C and D is independently selected from optionally substituted cycloalkyl, optionally substituted heterocyclyl, optionally substituted aryl, or optionally substituted heteroaryl; and R^{16} and R^{17} are each independently selected from hydrogen and C_{1-2} alkyl.

31. The compound of any of claims 28-30, wherein the compound is selected from:





32. The compound of claim 31, wherein the compound is selected from:

