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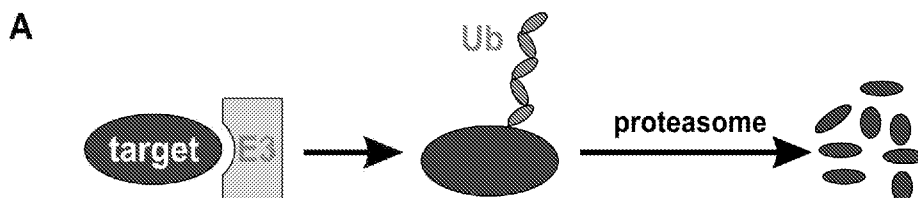
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(54) Title: COMPOSITIONS AND METHODS FOR CANCER THERAPY



FIGS. 1A

(57) Abstract: Disclosed are compounds that target receptor tyrosine kinases for degradation by ubiquitinases, and the use thereof for treating cancers.



COMPOSITIONS AND METHODS FOR CANCER THERAPY

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to U.S. Provisional Patent Application No. 62/871,516, filed July 8, 2019, the disclosure of which is expressly incorporated herein by reference.

STATEMENT REGARDING GOVERNMENT SUPPORT

[0002] This invention was made with government support under grant no. UL1TR001120 awarded by the National Center for Advancing Translational Science. The government has certain rights in the invention.

FIELD

[0003] The present disclosure relates to compositions and methods for treating cancers.

BACKGROUND

[0004] Ligand-induced selective proteolysis has emerged as a promising therapeutic means for cancer. For example, As₂O₃ triggers proteasome-mediated degradation of PML and retinoic acid receptor, which is the basis of its clinical efficacy in acute promyelocytic leukemia. Harnessing the power of the proteolytic system to degrade proteins for therapeutic benefit has been under exploration. Zhou and Howley were the first to rewire the ubiquitin-proteasome pathway, a major degradation system for selective proteolysis, to degrade normally stable proteins *in vivo* (e.g., yeast). The principle of protein knockdown strategy is based on ubiquitin-mediated proteolysis (Fig. 1A); a ubiquitin ligase (E3) recognizes its target and covalently attaches ubiquitin, a conserved 76-residue protein, onto the substrate, leading to subsequent degradation by the proteasome, a multi-subunit protease. In the last about 18 years, a number of groups manipulated different components involved in protein knockdown and demonstrated their utility mainly in tissue culture-based experiments, but largely failed to adapt the strategy in therapeutic relevant physiological settings (e.g., mouse models). The challenges encountered include poor target specificity, low cellular potency and alteration of critical cellular systems with undesirable side effects.

[0005] Recently, a group of small molecules were developed to efficiently knock down estrogen-related receptor α (ERR α), a key player in breast cancer, and led to tumor regression in the mouse model. In this PROTAC (proteolysis targeting chimera) approach, the small molecule is composed of a ligand for the target protein and a moiety that binds a ubiquitin ligase, which can trigger target

destruction. To eliminate $ERR\alpha$, an adapter molecule was designed to bring $ERR\alpha$ to a ubiquitin ligase E3 (i.e., VHL) for destruction in mammalian cells and mouse xenograft models (Figs. 1B-1D). The study overcame a major roadblock in the therapeutic potential of protein knockdown approach.

[0006] The PROTAC approach tackles two major issues in drug therapy: i) drug resistance.

Resistance often arises when tumors adapt to the drug and manage to find alternate routes to resume cell growth. A major reason behind the acquired resistance, a problem plaguing many inhibitor-based therapies, is that drug inhibition requires persistent binding of the drug and target, which provides time for cells to develop alternative routes (e.g., secondary mutations) to resume cell growth. The PROTAC adds a degradation signal that rapidly destroys the target, thereby limiting the time of drug engagement and reducing the risk that acquired defects accumulate and phenotypes are reversed. ii) difficulty in monitoring and/or blocking target activity. Many cancer targets don't have obvious enzymatic activity (e.g., kinase) that could be easily assayed *in vivo* and *in vitro*, making it hard to monitor the effects of inhibitors. In addition, whereas the molecules binding to the targets are not difficult to obtain, many of these molecules do not block the target activity. The PROTAC requires neither the direct inhibition of target activity nor the stable drug-target interaction since the target would be destroyed on contact upon drug exposure. Therefore, the PROTAC can be used on any molecule that can recognize relevant disease targets via the appendage of additional degradation moiety.

[0007] Of the two main components of the PROTAC, while the ligands for various targets have been developed and refined over the years, the degradation motifs used currently remain underdeveloped. Thus far, degradation moieties specific for a few ubiquitin ligases (e.g., VHL, cereblon, MDM2, XIAP) have been validated for triggering destruction of a variety of key cancer proteins and successfully demonstrated in mouse models for cancer (e.g., breast cancer and leukemia). However, these ubiquitin ligases (e.g., Cereblon and VHL) may not be easily adopted for other cancers, such as, brain cancer, lung cancer and kidney cancer, due to their low expression/activity in these cells. Another caveat with the degradation motifs employed is lack of degradation speed control, which can be a problem as too much or fast substrate turnover may be harmful in some cases. Moreover, it is important to reduce the sizes of PROTACs that currently are much larger than typical drugs (often less than 500 daltons), which could improve cellular permeability and potency. Therefore, what is needed is novel PROTAC molecules with reduced sizes and therapeutic methods that are more generally applicable for various cancers. The compositions and methods disclosed herein address these and other needs.

SUMMARY

[0008] In accordance with the purposes of the disclosed materials and methods, as embodied and broadly described herein, the disclosed subject matter, in one aspect, relates to compounds, compositions and methods of making and using compounds and compositions.

[0009] The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

[0010] In some aspects, disclosed herein is a compound of Formula I:

X-Y-Z

Formula I

wherein:

X is a destabilization amino acid residue;

Y is a linker; and

Z is a ligand that binds a tyrosine kinase;

or a pharmaceutically acceptable salt thereof.

[0011] In some embodiment, the destabilization amino acid residue is selected from the group consisting of Arg, His, Lys, Leu, Ile, Phe, Trp, Tyr, Cys, Asn, Asp, Glu, and Gln.

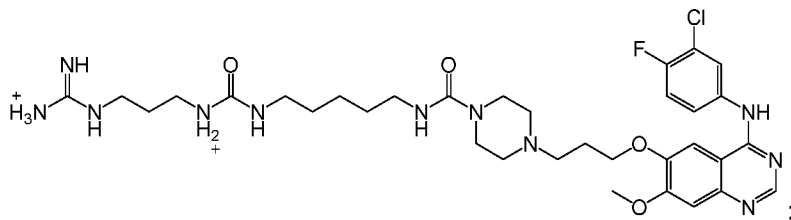
[0012] In some embodiments, the destabilization amino acid residue is Arg or His.

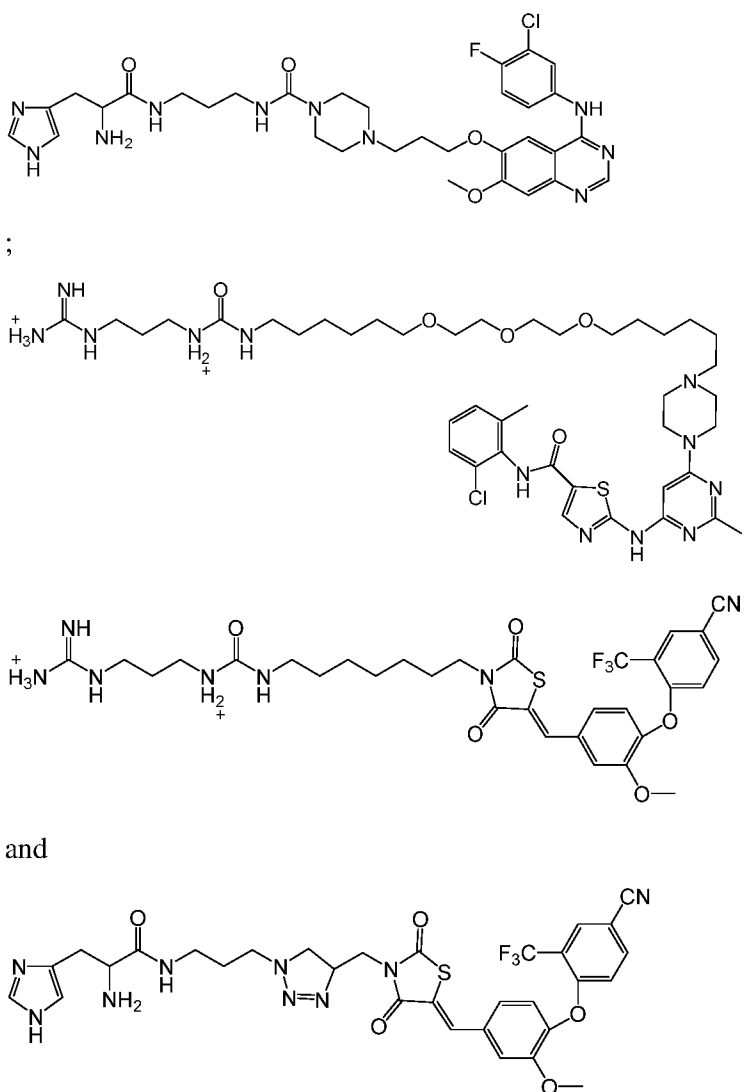
[0013] In some embodiments, the tyrosine kinase is a receptor tyrosine kinase or a non-receptor tyrosine kinase. In some embodiments, the receptor tyrosine kinase comprises estrogen-related receptor α or epidermal growth factor receptor.

[0014] In some embodiments, the ligand comprises an anti-cancer drug. In some embodiments, the anti-cancer drug comprises gefitinib, dasatinib, imatinib, crizotinib, lapatinib, or a thiazolidinedione derivative.

[0015] In some embodiments, the compound has a molecular weight no more than 1000 daltons.

[0016] In some embodiments, the compound is selected from the group consisting of





[0017] In some aspects, disclosed herein is a pharmaceutical composition, comprising: the compound of any preceding aspect; and a pharmaceutically acceptable carrier.

[0018] In some aspects, disclosed herein is a method for treating a cancer in a subject in need thereof, comprising: administering a therapeutically effective amount of the pharmaceutical composition of any preceding aspect. In some embodiments, the cancer comprises chronic myeloid leukemia, acute lymphocytic leukemia, breast cancer, or lung cancer. In some embodiments, the subject is a human.

[0019] In some aspects, disclosed herein is a method of treating a cancer in a subject in need thereof, comprising: administering a therapeutically effective amount of a compound of Formula I:

X-Y-Z

Formula I

wherein:

X is a destabilization amino acid residue;

Y is a linker; and

Z is a ligand that binds a tyrosine kinase;

or a pharmaceutically acceptable salt thereof.

[0020] Additional advantages will be set forth in part in the description that follows, and in part will be obvious from the description, or may be learned by practice of the aspects described below. The advantages described below will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive.

BRIEF DESCRIPTION OF DRAWINGS

[0021] The accompanying figures, which are incorporated in and constitute a part of this specification, illustrate several aspects described below.

[0022] **Figs. 1A-1D** show targeting estrogen-related receptor α (ERR α) for destruction. Fig. 1A shows that in ubiquitin (Ub)-mediated degradation, a Ub ligase E3 binds the substrate and put on a chain of Ub molecules (red dots), a molecular marker for the proteasome, with the help of E1 and E2 enzymes. Ub-decorated substrate is then recognized and degraded by the proteasome, a multi-subunit protease. Ub ligase E3 is the substrate recognition component of the ubiquitin system and carries out the rate-limiting step. Fig. 1B shows that VHL E3 normally does not recognize ERR α . Fig. 1C describes a group of small molecules for VHL and ERR α exist. Fig. 1D shows a chimeric PROTAC adaptor can select endogenous ERR α for destruction by the VHL pathway. The compound binds to both VHL and ERR α , bringing ERR α for VHL-mediated proteolysis. Disclosed herein are much smaller moiety (i.e., Arg) for binding to a broadly expressed potent Ubr1 E3, which leads to significant improvement.

[0023] **Figs. 2A-2D** show a number of small molecules synthesized. Fig. 2A shows Arg linked to gefitinib, a drug for EGFR-dependent cancer. Fig. 2B shows His linked to gefitinib. Fig. 2C shows Arg linked to dasatinib, a drug for BCR-ABL dependent cancer. Fig. 2D shows Arg linked to thiazolidinedione-based ligand for ERR α in breast cancer.

[0024] **Figs. 3A-3C** show that single amino acid based PROTACs induce ERR α turnover. Fig. 3A shows that structure of PROTACs Arg-T^{ERR α} and His-T^{ERR α} . Arg and His were linked to the ligand previously demonstrated specifically for ERR α . Fig. 3B shows that Arg-T^{ERR α} and His-T^{ERR α} trigger ERR α reduction in a dose-dependent manner. Varying amounts of Arg-T^{ERR α} or His-T^{ERR α}

were added to MCF-7 cells. ERR α levels were determined by western blot analysis. Equal amounts of protein extracts were used and ascertained by blotting with GAPDH antibody in the experiments. All experiments were done at least 3 times. Fig. 3C shows that ERR α stability was examined in the absence or presence of PROTACs by a protein expression shut-off assay. MCF-7 cells with or without PROTACs were treated with 200 μ g/ml cycloheximide to shut off protein synthesis. Samples were then collected at the indicated time points. Extracts were processed for immunoblotting with ERR α antibody. GAPDH serves as a loading control (lower panels).

[0025] Figs. 4A-4D show that ERR α turnover is blocked by the inhibitors of the proteasome and the N-end rule pathway. Fig. 4A and Fig. 4B show that ERR α degradation involves the proteasome. MCF-7 cells were incubated with 10 μ M Arg-T^{ERR α} or 5 μ M His-T^{ERR α} for 32 h and then mixed with DMSO control or the proteasome inhibitor MG132 (25 μ M). ERR α stability was determined after the addition of cycloheximide as described in Fig. 3C. Fig. 4C and Fig. 4D show that Arg-T^{ERR α} or His-T^{ERR α} induced ERR α reduction was reversed by dipeptides Arg-Ala and His-Ala, respectively. MCF-7 cells incubated with 10 μ M Arg-T^{ERR α} (Fig. 4C) or 5 μ M His-T^{ERR α} (Fig. 4D) were treated with dipeptides Arg-Ala (RA), His-Ala (HA) or control Ala-Ala (AA). Cell extracts were subjected to western blot analysis to determine the levels of ERR α and control GAPDH.

[0026] Figs. 5A-5D show biological effects of Arg-T^{ERR α} and His-T^{ERR α} in MCF-7 cells. Fig. 5A shows that PROTACs Arg-T^{ERR α} and His-T^{ERR α} inhibit breast cancer cell proliferation. MCF-7 cells were treated with DMSO, 10 μ M Arg-T^{ERR α} or 5 μ M His-T^{ERR α} . Cell proliferation was determined by the MTT assay after drug exposure on indicated days. Fig. 5B shows that Arg-T^{ERR α} and His-T^{ERR α} impede wound healing. Confluent monolayers of MCF-7 cells were scraped by a pipette tip to generate wounds and then treated with DMSO, 10 μ M Arg-T^{ERR α} or 5 μ M His-T^{ERR α} . Wound repair was photographed at indicated time points. Fig. 5C shows that Arg-T^{ERR α} and His-T^{ERR α} reduce breast cancer cell migration. MCF-7 cells were allowed to invade transwell chambers for 48 h in the presence or absence of PROTACs. Invaded cells were then fixed, stained and photographed. Fig. 5D shows effects of Arg-T^{ERR α} and His-T^{ERR α} on EMT markers. After the exposure to 10 μ M Arg-T^{ERR α} or 5 μ M His-T^{ERR α} , cell extracts were analyzed by western blot to examine the expression of various EMT markers including fibronectin, N-cadherin, SNAIL and E-cadherin.

[0027] Figs. 6A-6B show single amino acid based PROTACs induce BCR-Abl turnover in K562 leukemia cells. Fig. 6A shows the structure of PROTAC Arg-dasatinib. Arg was linked to the ligand dasatinib previously demonstrated specifically for Bcr-Abl, which triggers leukemia due to

Philadelphia chromosome translocation. Fig. 6B shows that Arg-dasatinib induces BCR-ABL reduction in a dose-dependent manner. Varying amounts of Arg-dasatinib or control dasatinib were added to K562 cells for 24 hours. BCR-ABL levels were determined by western blot analysis. Equal amounts of protein extracts were used and ascertained by blotting with tubulin antibody in the experiments. All experiments were done at least 3 times.

[0028] Fig. 7 shows synthesis of compound 1.3TFA (Arg-T^{ERRa} in Fig. 3A). To a solution of S1 (1.0 equiv, 0.211 mmol, 100 mg) in THF (1 mL) was added HATU (1.5 equiv, 0.316 mmol, 120 mg), DIPEA (1.5 equiv, 0.316 mmol, 52 μ L) and S2 (1.1 equiv, 0.232 mmol, 23 mg). The resulting solution was stirred at r.t. overnight. After the starting material S1 was completely consumed (monitored by TLC), THF was removed and the residue was purified by flash column chromatography to give the desired product S3 (106 mg, 90% yield) as a colorless oil. To a solution of S3 (1.24 equiv, 0.144 mmol, 80 mg) and S4 (1.0 equiv, 0.116 mmol, 53 mg) in THF (3 mL) was added CuTC (0.14 equiv, 0.016 mmol, 3 mg). The resulting mixture was stirred at r.t. overnight, then THF was removed and the residue was purified by flash column chromatography to give product S5 (49 mg, 42% yield) as a white solid. To a solution of S5 (0.018 mmol, 18.5 mg) in DCM (0.5 mL) was added TFA (0.5 mL). The resulting solution was stirred at r.t. for 1 h. Then the solution was concentrated in vacuo to afford 1 as a white solid (17.3 mg, 82% yield), which was used directly in the biological tests without further purification.

[0029] Fig. 8 shows the chemical structure of S3 in Fig. 7. $R_f = 0.5$ (EtOAc : PE = 1 : 1); HRMS-ESI (m/z) calc. for C₂₄H₄₅N₈O₇ [M+H⁺] 557.3406, found 557.3404, found 557.3404; ¹H NMR (400 MHz, CDCl₃): δ 9.40-9.32 (m, 2H), 7.14 (brs, 1H), 5.98 (d, $J = 8.8$ Hz, 1H), 4.32-4.27 (m, 1H), 4.00-3.94 (m, 1H), 3.71-3.63 (m, 1H), 3.43-3.37 (m, 1H), 3.31 (t, $J = 6.7$ Hz, 2H), 3.27-3.21 (m, 1H), 1.87-1.66 (m, 6H), 1.51 (s, 18H), 1.44 (s, 9H); ¹³C NMR (125 MHz, CDCl₃) δ 172.56, 163.17, 160.97, 155.76, 154.93, 84.43, 79.86, 79.63, 53.95, 49.32, 44.12, 37.11, 29.06, 28.66, 28.58, 28.39, 28.16, 24.70.

[0030] Fig. 9 shows the chemical structure of S5 in Fig. 7. $R_f = 0.4$ (EtOAc : PE = 3:1); HRMS-ESI (m/z): calc'd for C₄₆H₅₈F₃N₁₀O₁₁S [M+H⁺] 1015.3954, found 1015.3924; ¹H NMR (400 MHz, CDCl₃): δ 9.36-9.31 (m, 2H), 7.96 (s, 1H), 7.88 (s, 1H), 7.74 (s, 1H), 7.67 (d, $J = 8.7$ Hz, 1H), 7.22-7.15 (m, 3H), 7.12 (s, 1H), 6.76 (d, $J = 8.7$ Hz, 1H), 5.98 (d, $J = 8.4$ Hz, 1H), 5.04 (s, 2H), 4.34 (t, $J = 6.8$ Hz, 2H), 4.31-4.27 (m, 1H), 3.98-3.92 (m, 1H), 3.80 (s, 3H), 3.72-3.62 (m, 1H), 3.39-3.31 (m, 1H), 3.23-3.18 (m, 1H), 2.13-2.06 (m, 2H), 1.89-1.73 (m, 4H), 1.51 (s, 9H), 1.46 (s, 9H), 1.44 (s, 9H); ¹³C NMR (150 MHz, CDCl₃) δ 172.92, 166.95, 165.53, 163.17, 160.96, 159.01, 155.71, 154.85, 151.83, 143.57, 141.32, 137.16, 132.93, 132.51, 131.69 (q, $J = 4.9$ Hz),

123.80, 123.30, 122.29 (q, $J = 273.3$ Hz), 122.22, 120.56 (q, $J = 32.9$ Hz), 117.57, 116.61, 114.51, 105.95, 84.32, 79.89, 79.42, 56.20, 53.96, 47.89, 43.94, 36.78, 36.51, 30.51, 28.49, 28.33, 28.08, 24.64; ^{19}F NMR (565 MHz, CDCl_3) δ -62.74.

[0031] Fig. 10 shows the chemical structure of 1.3TFA in Fig. 7 (Arg- T^{ERRa} in Fig. 3A). HRMS-ESI (m/z): calc'd for $\text{C}_{31}\text{H}_{34}\text{F}_3\text{N}_{10}\text{O}_5\text{S}$ [$\text{M}+\text{H}^+$] 715.2381, found 715.2399. ^1H NMR (400 MHz, DMSO): δ 8.61 (t, $J = 5.6$ Hz, 1H), 8.34 (d, $J = 2.0$ Hz, 1H), 8.19-8.17 (d, $J = 5.0$ Hz, 3H), 8.15 (s, 1H), 8.04-8.01 (m, 2H), 7.75 (t, $J = 5.5$ Hz, 1H), 7.55 (d, $J = 2.0$ Hz, 1H), 7.40 (d, $J = 8.3$ Hz, 1H), 7.31 (dd, $J = 8.4, 2.0$ Hz, 1H), 6.93 (d, $J = 8.8$ Hz, 1H), 4.92 (s, 2H), 4.38 (t, $J = 7.0$ Hz, 2H), 3.80 (s, 3H), 3.74-3.70 (m, 1H), 3.16-3.09 (m, 4H), 2.02-1.94 (m, 2H), 1.72-1.66 (m, 2H), 1.51-1.43 (m, 2H). ^{13}C NMR (150 MHz, DMSO) δ 168.57, 166.89, 165.17, 158.79 (q, $J = 36.0$ Hz), 158.43, 156.97, 151.34, 142.92, 141.05, 138.79, 132.65, 132.30, 132.03, 123.81, 123.12, 122.98, 122.55 (q, $J = 273.6$ Hz), 121.76, 118.70 (q, $J = 31.9$ Hz), 117.59, 117.07, 116.06, 115.91 (q, $J = 290.5$ Hz), 105.44, 56.23, 52.13, 47.20, 40.17, 36.80, 36.07, 29.69, 28.33, 24.33. ^{19}F NMR (565 MHz, DMSO) δ -61.22, -74.80.

[0032] Fig. 11 shows synthesis of compound 2.2 HCL (His- T^{ERRa} in Fig. 3A). To a solution of S2 (1.1 equiv, 0.250 mmol, 25.0 mg) in DCM (1.5 mL) was added S6 (1.0 equiv, 0.227 mmol, 80.7 mg), HOBt (1.5 equiv, 0.340 mmol, 46.0 mg), DIPEA (3.0 equiv, 0.681 mmol, 112 μL) and EDCI (1.5 equiv, 0.340 mmol, 65.2 mg). The resulting solution was stirred at r.t. overnight. After the starting material S6 was completely consumed (monitored by TLC), DCM was removed. The residue was diluted with H_2O , and extracted with ethyl acetate. The combined organic layers were washed with citric acid (aq., 2 M), brine, dried over Na_2SO_4 , filtered and concentrated *in vacuo*. The crude product S7 (white solid, 57.8 mg) was used immediately in the next step without purification. To a solution of S7 (1.0 equiv, 0.132 mmol, 57.8 mg) and S4 (1.0 equiv, 0.132 mmol, 60.6 mg) in THF (3.5 mL) was added CuTC (0.1 equiv, 0.013 mmol, 2.5 mg). The resulting mixture was stirred at r.t. overnight, then THF was removed and the residue was purified by flash column chromatography to give product S8 (84.1 mg, 41% yield for 2 steps) as a white solid. Compound S8 (0.036 mmol, 32.4 mg) was dissolved in a solution of HCl in MeOH (0.5 M, 4 mL). The resulting solution was stirred at r.t. for 2 h. Then the solution was concentrated *in vacuo* to afford 2 as a yellow solid (30.5 mg, quant.), which was used directly in the biological tests without further purification.

[0033] Fig. 12 shows the chemical structure of S8 in Fig. 11. $R_f = 0.1$ (EtOAc: PE = 3:1) HRMS-ESI (m/z): calc'd for $\text{C}_{41}\text{H}_{45}\text{F}_3\text{N}_9\text{O}_9\text{S}$ [$\text{M}+\text{H}^+$] 896.3008, found 896.2998. ^1H NMR (400 MHz, CDCl_3): δ 8.01 (brs, 1H), 7.94 (s, 1H), 7.86 (s, 1H), 7.74 (s, 1H), 7.66 (dd, $J = 8.6, 1.4$ Hz, 1H),

7.20-7.09 (m, 5H), 6.76 (d, $J = 8.7$ Hz, 1H), 6.10 (brs, 1H), 5.02 (s, 2H), 4.39 (brs, 1H), 4.31-4.27 (m, 2H), 3.79 (s, 3H), 3.28-3.18 (m, 2H), 3.06-2.93 (m, 2H), 2.08-1.99 (m, 2H), 1.56 (s, 9H), 1.40 (s, 9H). ^{13}C NMR (125 MHz, CDCl_3) δ 172.07, 166.94, 165.54, 159.03, 151.87, 143.67, 141.34, 137.15, 132.92, 132.54, 131.70 (q, $J = 5.1$ Hz), 123.87, 123.83, 123.28, 122.32 (q, $J = 273.3$ Hz), 122.26, 120.64 (q, $J = 32.9$ Hz), 117.54, 116.67, 114.62, 106.02, 85.98, 80.17, 56.25, 47.50, 36.81, 36.17, 30.29, 28.38, 27.92. ^{19}F NMR (471 MHz, CDCl_3) δ -62.72.

[0034] Fig. 13 shows the chemical structure of 2.2HCL (His-T^{ERRa} in Fig. 3A) in Fig. 11. HRMS-ESI (m/z): calc'd for $\text{C}_{31}\text{H}_{29}\text{F}_3\text{N}_9\text{O}_5\text{S}$ $[\text{M}+\text{H}^+]$ 696.1959, found 696.1971. ^1H NMR (400 MHz, CD_3OD): δ 8.94 (s, 1H), 8.23 (brs, 1H), 8.11 (s, 1H), 7.98 (s, 1H), 7.85 (d, $J = 8.1$ Hz, 1H), 7.55 (s, 1H), 7.37 (s, 1H), 7.31 (s, 2H), 6.87 (d, $J = 8.7$ Hz, 1H), 5.05 (s, 2H), 4.49-4.46 (m, 2H), 4.29-4.26 (m, 1H), 3.82 (s, 3H), 3.44-3.20 (m, 4H), 2.13 (brs, 2H). ^{13}C NMR (125 MHz, CD_3OD) δ 168.66, 168.39, 166.75, 160.32, 153.13, 144.77, 139.04, 135.98, 134.18, 133.94, 132.67 (q, $J = 5.1$ Hz), 128.21, 124.64, 124.25, 123.85 (q, $J = 272.9$ Hz), 123.22, 121.06 (q, $J = 32.4$ Hz), 119.91, 118.43, 118.02, 116.45, 106.97, 56.88, 53.54, 49.91, 37.70, 36.90, 30.59, 27.74. ^{19}F NMR (471 MHz, CD_3OD) δ -63.91.

DETAILED DESCRIPTION

[0035] The materials, compounds, compositions, and methods described herein may be understood more readily by reference to the following detailed description of specific aspects of the disclosed subject matter and the Examples and Figures included therein.

[0036] Before the present materials, compounds, compositions, and methods are disclosed and described, it is to be understood that the aspects described below are not limited to specific synthetic methods or specific reagents, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only and is not intended to be limiting.

[0037] Also, throughout this specification, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which the disclosed matter pertains. The references disclosed are also individually and specifically incorporated by reference herein for the material contained in them that is discussed in the sentence in which the reference is relied upon.

[0038] Disclosed herein are compounds and methods for treating cancers. The compound is represented by X-Y-Z (Formula I) or a pharmaceutically acceptable salt thereof; wherein X is a destabilization amino acid residue Y is a linker, and Z is a ligand that binds a receptor tyrosine

kinase. This compound and/or a pharmaceutical composition comprising this compound is useful for treating various cancers by degrading a receptor tyrosine kinase in the cancer cell.

[0039] Terms used throughout this application are to be construed with ordinary and typical meaning to those of ordinary skill in the art. However, Applicant desires that the following terms be given the particular definition as defined below.

Terminology

[0040] As used in the specification and claims, the singular form "a," "an," and "the" include plural references unless the context clearly dictates otherwise. For example, the term "an agonist" includes a plurality of agonist, including mixtures thereof.

[0041] Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the disclosure are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contain certain errors necessarily resulting from the standard deviation found in their respective testing measurements. Furthermore, when numerical ranges of varying scope are set forth herein, it is contemplated that any combination of these values inclusive of the recited values may be used. Further, ranges can be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, another aspect includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another aspect. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint. Unless stated otherwise, the term "about" means within 5% (*e.g.*, within 2% or 1%) of the particular value modified by the term "about."

[0042] "Administration" to a subject includes any route of introducing or delivering to a subject an agent. Administration can be carried out by any suitable route, including oral, topical, intravenous, subcutaneous, transcutaneous, transdermal, intramuscular, intra-joint, parenteral, intra-arteriole, intradermal, intraventricular, intracranial, intraperitoneal, intralesional, intranasal, rectal, vaginal, by inhalation or via an implanted reservoir, and the like. Administration includes self-administration and the administration by another.

[0043] As used herein, the term "comprising" is intended to mean that the compositions and methods include the recited elements, but not excluding others. "Consisting essentially of" when used to define compositions and methods, shall mean excluding other elements of any essential significance to the combination. Thus, a composition consisting essentially of the elements as defined herein would not exclude trace contaminants from the isolation and purification method

and pharmaceutically acceptable carriers, such as phosphate buffered saline, preservatives, and the like. "Consisting of" shall mean excluding more than trace elements of other ingredients and substantial method steps for administering the compositions of this invention. Embodiments defined by each of these transition terms are within the scope of this invention.

[0044] "Dysregulation" refers herein refers to an abnormal level of expression or activity of a protein or gene. This can include, for example, less levels or activities than in normal healthy humans. This can also include, for example, increased levels or activities than in normal healthy humans. In some embodiments, disclosed herein comprises a cancer results from a dysregulation of a receptor tyrosine kinase.

[0045] "Inhibit," "inhibiting," and "inhibition" mean to decrease an activity, response, condition, disease, or other biological parameter. This can include but is not limited to the complete ablation of the activity, response, condition, or disease. This may also include, for example, a 10% reduction in the activity, response, condition, or disease as compared to the native or control level. Thus, the reduction can be a 10, 20, 30, 40, 50, 60, 70, 80, 90, 100%, or any amount of reduction in between as compared to native or control levels.

[0046] "Ligand" refers herein to a molecule that acts on a target in a desirable manner. Examples of actions on a target in a desirable manner include, but are not limited to binding of the target, catalytically changing the target, reacting with the target in a way which modifies/alters the target or the functional activity of the target, covalently attaching to the target as in a suicide inhibitor, facilitating the reaction between the target and another molecule. In most, but not all, instances this desirable manner is binding to the target. The ligand can be an organic compound or other chemical. The ligand can be a compound, which is obtainable from or produced by any suitable source, whether natural or artificial. The ligand can be an amino acid molecule, a polypeptide, a peptide or a chemical derivative thereof, or a combination thereof. The ligand can also be a polynucleotide molecule - which can be a sense or an anti-sense molecule. The ligand can even be an antibody. In some embodiments, the "ligand" refers to any ligand that binds to or be bound by a receptor tyrosine kinase. In some embodiments, the "ligand" refers to any ligand that binds to or be bound by a non-receptor tyrosine kinase

[0047] "Tyrosine kinases" herein refer to a family of enzymes that catalyze the phosphorylation of select tyrosine residues in the target proteins by using ATP.

[0048] "Receptor tyrosine kinases" or "RTKs" are used interchangeably herein to refer to a family of membrane receptors that phosphorylate tyrosine residues. Receptor tyrosine kinases possess an extracellular ligand binding domain, a transmembrane domain and an intracellular catalytic domain.

The extracellular domains bind cytokines, growth factors or other ligands and are generally comprised of one or more identifiable structural motifs, including cysteine-rich regions, fibronectin III-like domains, immunoglobulin-like domains, EGF-like domains, cadherin-like domains, kringle-like domains, Factor VIII-like domains, glycine-rich regions, leucine-rich regions, acidic regions and discoidin-like domains. Activation of the intracellular kinase domain is achieved by ligand binding to the extracellular domain, which induces dimerization of the receptors. A receptor activated in this way is able to autophosphorylate tyrosine residues outside the catalytic domain, facilitating stabilization of the active receptor conformation. The phosphorylated residues also serve as binding sites for proteins which will then transduce signals within the cell. Some RTKs are the high-affinity cell surface receptors for many polypeptide growth factors, cytokines, and hormones. Receptor tyrosine kinases have been shown not only to be key regulators of normal cellular processes but also to have a critical role in the development and progression of many types of cancer. Many play significant roles in development or cell division. In some embodiments, the RTK is estrogen-related receptor α . In some embodiments, the RTK is epidermal growth factor receptor. "Non-receptor tyrosine kinases" refer to a second subgroup of tyrosine kinases that are cytosolic enzymes. Non-receptor tyrosine kinases relay intracellular signals originating from extracellular receptor.

[0049] The terms "prevent," "preventing," "prevention," and grammatical variations thereof as used herein, refer to a method of partially or completely delaying or precluding the onset or recurrence of a disease and/or one or more of its attendant symptoms or barring a subject from acquiring or reacquiring a disease or reducing a subject's risk of acquiring or reacquiring a disease or one or more of its attendant symptoms.

[0050] The term "subject" refers to a human in need of treatment for any purpose, and more preferably a human in need of treatment to treat a disease or disorder, such as a cancer. The term "subject" can also refer to non-human animals, such as dogs, cats, horses, cows, pigs, sheep and non-human primates, among others.

[0051] "Pharmaceutically acceptable" component can refer to a component that is not biologically or otherwise undesirable, i.e., the component may be incorporated into a pharmaceutical formulation of the invention and administered to a subject as described herein without causing significant undesirable biological effects or interacting in a deleterious manner with any of the other components of the formulation in which it is contained. When used in reference to administration to a human, the term generally implies the component has met the required standards

of toxicological and manufacturing testing or that it is included on the Inactive Ingredient Guide prepared by the U.S. Food and Drug Administration.

[0052] "Pharmaceutically acceptable carrier" (sometimes referred to as a "carrier") means a carrier or excipient that is useful in preparing a pharmaceutical or therapeutic composition that is generally safe and non-toxic, and includes a carrier that is acceptable for veterinary and/or human pharmaceutical or therapeutic use. The terms "carrier" or "pharmaceutically acceptable carrier" can include, but are not limited to, phosphate buffered saline solution, water, emulsions (such as an oil/water or water/oil emulsion) and/or various types of wetting agents.

[0053] As used herein, the term "carrier" encompasses any excipient, diluent, filler, salt, buffer, stabilizer, solubilizer, lipid, stabilizer, or other material well known in the art for use in pharmaceutical formulations. The choice of a carrier for use in a composition will depend upon the intended route of administration for the composition. The preparation of pharmaceutically acceptable carriers and formulations containing these materials is described in, *e.g.*, *Remington's Pharmaceutical Sciences*, 21st Edition, ed. University of the Sciences in Philadelphia, Lippincott, Williams & Wilkins, Philadelphia, PA, 2005. Examples of physiologically acceptable carriers include saline, glycerol, DMSO, buffers such as phosphate buffers, citrate buffer, and buffers with other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™ (ICI, Inc.; Bridgewater, New Jersey), polyethylene glycol (PEG), and PLURONICS™ (BASF; Florham Park, NJ). To provide for the administration of such dosages for the desired therapeutic treatment, compositions disclosed herein can advantageously comprise between about 0.1% and 99% by weight of the total of one or more of the subject compounds based on the weight of the total composition including carrier or diluent.

[0054] "Pharmaceutically acceptable salt" as used herein refers to any salt that is pharmaceutically acceptable and has the desired pharmacological properties. Such salts are, for example, inorganic or organic base addition salts, or inorganic or organic acid addition salts. Each possibility represents a separate embodiment.

[0055] As used herein, the terms "treating" or "treatment" of a subject includes the administration of a drug to a subject with the purpose of curing, healing, alleviating, relieving, altering, remedying,

ameliorating, improving, stabilizing or affecting a disease or disorder, or a symptom of a disease or disorder. The terms “treating” and “treatment” can also refer to reduction in severity and/or frequency of symptoms, elimination of symptoms and/or underlying cause, and improvement or remediation of damage.

[0056] “Therapeutic agent” refers to any composition that has a beneficial biological effect.

Beneficial biological effects include both therapeutic effects, e.g., treatment of a disorder or other undesirable physiological condition, and prophylactic effects, e.g., prevention of a disorder or other undesirable physiological condition (e.g., a cancer). The terms also encompass pharmaceutically acceptable, pharmacologically active derivatives of beneficial agents specifically mentioned herein, including, but not limited to, salts, esters, amides, proagents, active metabolites, isomers, fragments, analogs, and the like. When the terms “therapeutic agent” is used, then, or when a particular agent is specifically identified, it is to be understood that the term includes the agent per se as well as pharmaceutically acceptable, pharmacologically active salts, esters, amides, proagents, conjugates, active metabolites, isomers, fragments, analogs, etc.

[0057] “Therapeutically effective amount” or “therapeutically effective dose” of a composition (e.g. a composition comprising a compound of Formula I) refers to an amount that is effective to achieve a desired therapeutic result. In some embodiment, a desired therapeutic result is the treatment of a cancer. In some embodiment, a desired therapeutic result is the treatment of chronic myeloid leukemia, acute lymphocytic leukemia, breast cancer, or lung cancer. Therapeutically effective amounts of a given therapeutic agent will typically vary with respect to factors such as the type and severity of the disorder or disease being treated and the age, gender, and weight of the subject. The term can also refer to an amount of a therapeutic agent, or a rate of delivery of a therapeutic agent (e.g., amount over time), effective to facilitate a desired therapeutic effect, such as coughing relief. The precise desired therapeutic effect will vary according to the condition to be treated, the tolerance of the subject, the agent and/or agent formulation to be administered (e.g., the potency of the therapeutic agent, the concentration of agent in the formulation, and the like), and a variety of other factors that are appreciated by those of ordinary skill in the art. In some instances, a desired biological or medical response is achieved following administration of multiple dosages of the composition to the subject over a period of days, weeks, or years.

[0058] Reference will now be made in detail to specific aspects of the disclosed materials, compounds, compositions, articles, and methods, examples of which are illustrated in the accompanying Examples and Figures.

Compounds

[0059] In some aspects, disclosed herein is a compound of Formula I:

X-Y-Z

Formula I

wherein:

X is a destabilization amino acid residue;

Y is a linker; and

Z is a ligand that binds a tyrosine kinase;

or a pharmaceutically acceptable salt thereof.

[0060] “Destabilization amino acid residue” is used herein to refer to an amino acid residue that can destabilize a protein if the amino acid residue is at the N-terminus, following the “N-end rule”. The “N-end rule” states that the stability of a protein or a peptide is largely determined by the presence of “destabilizing” or “stabilizing” N-terminal amino acids. In some embodiments, the destabilization amino acid residue can be, for example, Arg, His, Lys, Leu, Ile, Phe, Trp, Tyr, Cys, Asn, Asp, Glu, or Gln. In some embodiments, the destabilization amino acid residue is Arg. In some embodiments, the destabilization amino acid residue is His. This single, destabilizing residue (e.g. Arg or His) at the N-terminus of a protein targets the substrate to a ubiquitin ligase termed Ubr1 for rapid degradation via the N-end rule pathway.

[0061] In some embodiments, Z is a ligand binds a receptor tyrosine kinase. In some embodiments, Z is a ligand binds a non-receptor tyrosine kinase. The receptor tyrosine kinase described herein can be of any receptor tyrosine kinase. In some embodiments, the receptor tyrosine kinase comprises estrogen-related receptor α or epidermal growth factor receptor. In some embodiments, the receptor tyrosine kinase is estrogen-related receptor α . In some embodiments, the receptor tyrosine kinase is epidermal growth factor receptor. Thus, in some embodiments, the compound disclosed herein comprises a ligand binds estrogen-related receptor α . In some embodiments, the disclosed herein comprises a ligand binds epidermal growth factor receptor. In some embodiments, the non-receptor tyrosine kinase is Abelson tyrosine-protein kinase (ABL). Accordingly, in some embodiments, the compound disclosed herein comprises a ligand that binds ABL. In some embodiments, the compound disclosed herein comprises a ligand that binds BCR-ABL fusion protein.

[0062] “Estrogen-related receptor α ” or “ERR α ” refers to estrogen-related receptor α polypeptide and, in humans, is encoded by the *ESRRA* gene. In some embodiments, the estrogen-related

receptor α polypeptide or polynucleotide is that identified in one or more publicly available databases as follows: HGNC: 3471, Entrez Gene: 2101, Ensembl: ENSG00000173153, OMIM: 601998, and UniProtKB: P11474. In some embodiments, the estrogen-related receptor α is a polypeptide comprising an amino acid sequence which is at least 80% identical to SEQ ID NO:1. In some embodiments, the estrogen-related receptor α is a polypeptide comprising an amino acid sequence which is at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% identical to SEQ ID NO:1. In some embodiments, the estrogen-related receptor α is a polypeptide comprising SEQ ID NO:1.

[0063] “Epidermal growth factor receptor” or “EGFR” refers to epidermal growth factor receptor polypeptide and, in humans, is encoded by the *EGFR* gene. In some embodiments, the epidermal growth factor receptor polypeptide or polynucleotide is that identified in one or more publicly available databases as follows: HGNC: 3236, Entrez Gene: 1956, Ensembl: ENSG00000146648, OMIM: 131550, or UniProtKB: P00533. In some embodiments, the epidermal growth factor is a polypeptide comprising an amino acid sequence which is at least 80% identical to SEQ ID NO:2. In some embodiments, the epidermal growth factor is a polypeptide comprising an amino acid sequence which is at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% identical to SEQ ID NO:2. In some embodiments, the epidermal growth factor is a polypeptide comprising SEQ ID NO:2.

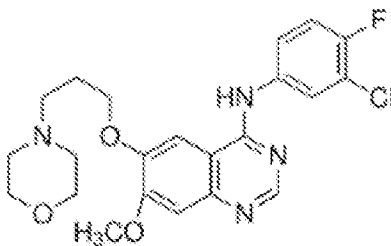
[0064] “Abelson tyrosine-protein kinase” or “ABL” refers to Abelson tyrosine-protein kinase polypeptide and, in humans, is encoded by the *ABL1* gene. In some embodiments, the Abelson tyrosine-protein kinase polypeptide or polynucleotide is that identified in one or more publicly available databases as follows: HGNC: 76, Entrez Gene: 25, Ensembl: ENSG00000097007, OMIM: 189980, UniProtKB: P00519. In some embodiments, the Abelson tyrosine-protein kinase is a polypeptide comprising an amino acid sequence which is at least 80% identical to SEQ ID NO:3. In some embodiments, the Abelson tyrosine-protein kinase is a polypeptide comprising an amino acid sequence which is at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% identical to SEQ ID NO:3. In some embodiments, the Abelson tyrosine-protein kinase is a polypeptide comprising SEQ ID NO:3.

[0065] “Breakpoint cluster region protein” or “BCR” refers to breakpoint cluster region protein and, in humans, is encoded by the *BCR* gene. In some embodiments, the breakpoint cluster region polypeptide or polynucleotide is that identified in one or more publicly available databases as follows: HGNC: 1014, Entrez Gene: 613, Ensembl: ENSG00000186716, OMIM: 151410, UniProtKB: P11274. In some embodiments, the breakpoint cluster region protein is a polypeptide

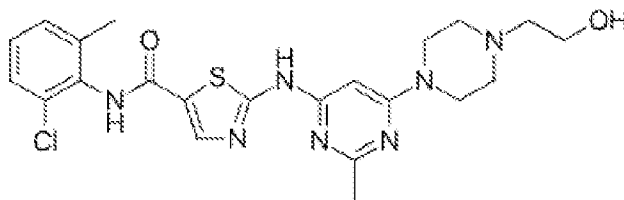
comprising an amino acid sequence which is at least 80% identical to SEQ ID NO:4. In some embodiments, the breakpoint cluster region is a polypeptide comprising an amino acid sequence which is at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% identical to SEQ ID NO:4. In some embodiments, the breakpoint cluster region is a polypeptide comprising SEQ ID NO:4.

[0066] In some embodiments, the ligand comprises an anti-cancer drug. The anti-cancer drug can be any anti-cancer drug targeting a receptor tyrosine kinase (e.g., estrogen-related receptor α or epidermal growth factor receptor). The anti-cancer drug includes, but not limited to, gefitinib, imatinib, erlotinib, sunitinib, lapatinib, trastuzumab, cetuximab, cevacizumab, nilotinib, sorafenib, crizotinib, panitumumab, or a thiazolidinedione derivative. In some embodiments, the ligand is gefitinib. In some embodiments, the ligand is imatinib. In some embodiments, the anti-cancer drug can be any anti-cancer drug targeting a non-receptor tyrosine kinase (e.g., ABL). In some embodiments, the anti-cancer drug targets BCR-ABL fusion protein. In some embodiments, the ligand is dasatinib.

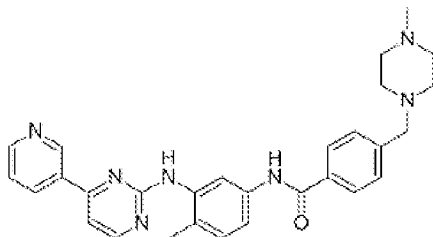
[0067] The structure of gefitinib is shown below:



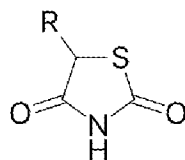
[0068] The structure of dasatinib is shown below:



[0069] The structure of imatinib is shown below:



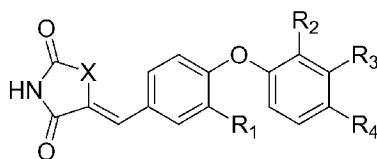
[0070] In some embodiments, the ligand is a thiazolidinedione derivative. The functional group of a thiazolidinedione is shown below:



[0071] In some embodiments, the ligand is selected from the group consisting of pioglitazone, rosiglitazone, lobeglitazone, ciglitazone, garglitazone, englitazone, netoglitazone, rivoglitazone, troglitazone, and balaglitazone.

[0072] It should be understood that the thiazolidinedione derivative can be any thiazolidinedione derivative binding a receptor tyrosine kinase, including, for example, estrogen related receptor- α .

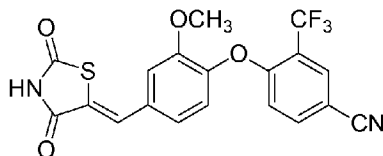
[0073] In some embodiments, the thiazolidinedione derivative can be any one of the those disclosed in Application WO2008109727A1, which is incorporated by reference herein in its entirety for its teaching of thiazolidinedione derivatives. In some embodiments, the thiazolidinedione derivative has a formula (I)



(I), wherein

R1 is halo, optionally substituted Ci-4alkyl, optionally substituted Ci-4alkoxy, or hydroxyl; R2 is selected from halo substituted Ci-3alkyl, cyano, halo, -C(O)NH₂, and -C(O)O-Ci-4alkyl, or alternatively R2 is linked together to R3 to form an aryl fused to the phenyl ring to which R2 and R3 are shown attached; R3 is H, or alternatively R3 is linked together to R2 to form an aryl fused to the phenyl ring to which R3 and R2 are shown attached; R4 is halo, cyano, -C $\equiv$ CH, halo substituted Ci-3alkyl, -C(O)O-Ci-4alkyl, -C(O)NH₂, or -S(O₂)-Ci-4alkyl; and X is S or O; or an optical isomer, enantiomer, diastereomer, cis-trans isomer, racemate, prodrug or pharmaceutically acceptable salt thereof.

[0074] In some embodiments, the structure of the thiazolidine derivative is shown below:



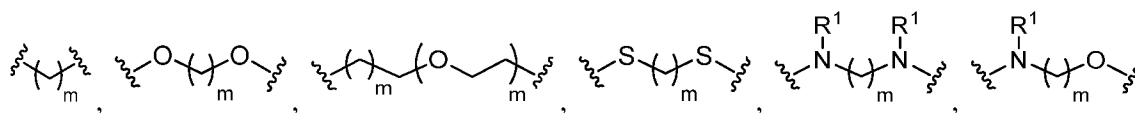
[0075] In some embodiments, the linker (Y) comprises one or more covalently connected structural unit of A (e.g., -A₁...A_n-), wherein A₁ is covalently linked to an X moiety and A_n is covalently

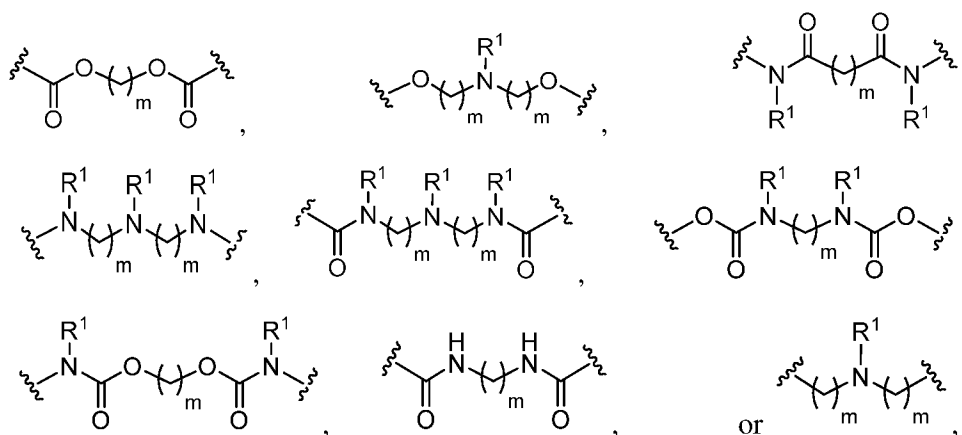
linked to a Z moiety, wherein the n is an integer greater than or equal to 0. In some embodiments, the n is greater than or equal to 1.

[0076] In some embodiments, e. g., wherein n is greater than 2, A_n is a group which is connected to a Z moiety, and A₁ and A_n are connected via structural units of A (number of such structural units of A: n-2). In some embodiments, the q is 1, the structure of the linker group X is -A₁-, and A₁ is a group which is connected to an X moiety and a Z moiety. In some embodiments, n is an integer from 1 to 100, 1 to 90, 1 to 80, 1 to 70, 1 to 60, 1 to 50, 1 to 40, 1 to 30, 1 to 20, or 1 to 10.

[0077] In some embodiments, Y can be absent (i.e., X moiety can be bound directly Z moiety). In other embodiments, Y is present. When present, the linking group can be any suitable group or moiety which is at minimum bivalent, and connects the heterocyclic rings in the nucleophile. The linking group can be composed of any assembly of atoms, including oligomeric and polymeric chains. In some cases, the total number of atoms in the linking group can be from 3 to 200 atoms (e.g., from 3 to 150 atoms, from 3 to 100 atoms, from 3 and 50 atoms, from 3 to 25 atoms, from 3 to 15 atoms, or from 3 to 10 atoms). In some embodiments, the linking group can be, for example, an alkyl, alkoxy, alkylaryl, alkylheteroaryl, alkylcycloalkyl, alkylheterocycloalkyl, alkylthio, alkylsulfinyl, alkylsulfonyl, alkylamino, dialkylamino, alkylcarbonyl, alkoxycarbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, or polyamino group.

[0078] In some embodiments, the linking group can comprise one of the groups above joined to each of the heterocyclic rings by a functional group. Examples of suitable functional groups include, for example, secondary amides (-CONH-), tertiary amides (-CONR-), secondary carbamates (-OCONH-; -NHCOO-), tertiary carbamates (-OCONR-; -NRCOO-), ureas (-NHCONH-; -NRCONH-; -NHCONR-, or -NRCONR-), carbinols (-CHOH-, -CROH-), ethers (-O-), and esters (-COO-, -CH₂O₂C-, CHRO₂C-), wherein R is an alkyl group, an aryl group, or a heterocyclic group. For example, in some embodiments, the linking group can comprise an alkyl group (e.g., a C₁-C₁₂ alkyl group, a C₁-C₈ alkyl group, or a C₁-C₆ alkyl group) bound to each heterocyclic ring via an ester (-COO-, -CH₂O₂C-, CHRO₂C-), a secondary amide (-CONH-), or a tertiary amide (-CONR-), wherein R is an alkyl group, an aryl group, or a heterocyclic group. The linking group can also comprise a polyalkoxyl group (e.g., -polyethyloxy, polypropyloxy). In certain embodiments, Y can be chosen from one of the following:





where each m is, independent of any other, an integer from 1 to 12.

[0079] In some embodiments, R^1 can be absent. In other embodiments, R^1 can be present. When present, R^1 can comprise a halogen, hydroxy, amino, cyano, azido, hydrazone, alkyl, alkenyl, alkynyl, alkoxy, aryl, heteroaryl, alkylaryl, alkylheteroaryl, cycloalkyl alkylcycloalkyl, heterocycloalkyl, alkylheterocycloalkyl, alkylthio, alkylsulfinyl, alkylsulfonyl, alkylamino, dialkylamino, alkylcarbonyl, alkoxy carbonyl, alkylaminocarbonyl, dialkylaminocarbonyl, peptidyl, polyamino, or polyalkyleneoxy group.

[0080] In some embodiments, R^1 can comprise a cationic group. In certain embodiments, the cationic group can comprise a cationic polyamino group (e.g., a polyethyleneimine segment). In other embodiments, the cationic group can comprise a cationic peptidyl group. In other embodiments, the cationic group can comprise a moiety (e.g., an alkyl group or an alkylaryl group) substituted with a cationic substituent, such as an ammonium group (e.g., a tetraalkylammonium group, an aryltrialkylammonium group, a diaryldialkylammonium group, or a triarylalkylammonium group) or a pyridinium group.

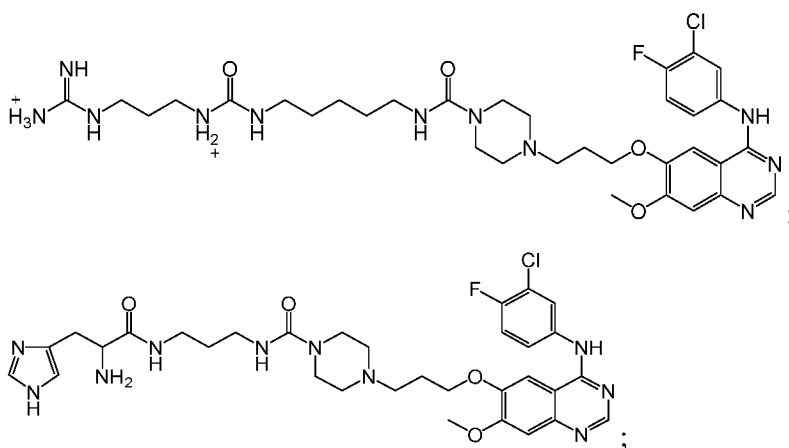
[0081] In some embodiments, R^1 can comprise a hydrophobic group. For example, in certain embodiments, R^1 can comprise an aryl group or an alkylaryl group. In some embodiments, R^1 can comprise a hydrophilic group. For example, in certain embodiments, R^1 can comprise a hydrophilic polyalkyleneoxy group (e.g., a polyethylene oxide segment).

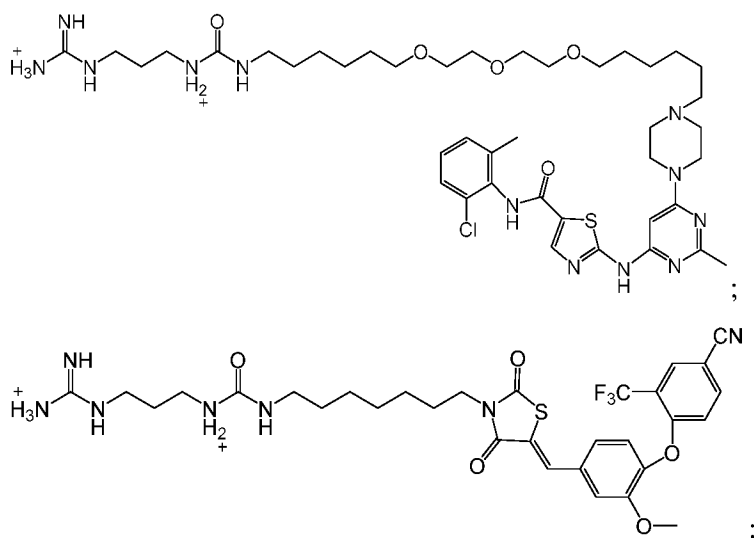
[0082] In some embodiments, R^1 can comprise a reactive functional group, such as an olefin, acetylene, alcohol, phenol, ether, oxide, halide, aldehyde, ketone, carboxylic acid, ester, amide, cyanate, isocyanate, thiocyanate, isothiocyanate, amine, hydrazine, hydrazone, hydrazide, diazo, diazonium, nitro, nitrile, mercaptan, sulfide, disulfide, sulfoxide, sulfone, sulfonic acid, sulfinic acid, acetal, ketal, anhydride, sulfate, sulfenic acid isonitrile, amidine, imide, imidate, nitron, hydroxylamine, oxime, hydroxamic acid, thiohydroxamic acid, allene, ortho ester, sulfite, enamine, ynamine, urea, pseudourea, semicarbazide, carbodiimide, carbamate, imine, azide, azo group,

azoxy group, nitroso group, N-hydroxysuccinimide ester, or maleimides. For example, in certain embodiments, R^1 can comprise an alkynyl group or an azido group. In other embodiments, R^1 can comprise a hydrazone group.

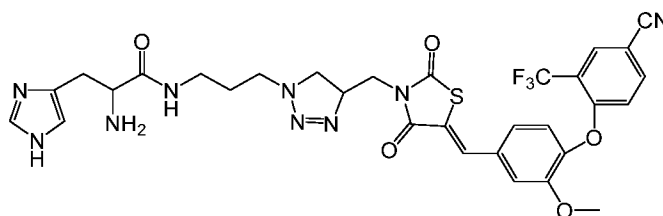
[0083] In some embodiments, the compound has a molecular weight no more than 2000 daltons, 1900 daltons, 1800 daltons, 1700 daltons, 1600 daltons, 1500 daltons, 1400 daltons, 1300 daltons, 1200 daltons, 1100 daltons, 1000 daltons, 990 daltons, 980 daltons, 970 daltons, 960 daltons, 950 daltons, 940 daltons, 930 daltons, 920 daltons, 910 daltons, 900 daltons, 890 daltons, 880 daltons, 870 daltons, 860 daltons, 850 daltons, 840 daltons, 830 daltons, 820 daltons, 810 daltons, 800 daltons, 790 daltons, 780 daltons, 770 daltons, 760 daltons, 750 daltons, 740 daltons, 730 daltons, 720 daltons, 710 daltons, 700 daltons, 690 daltons, 680 daltons, 670 daltons, 660 daltons, 650 daltons, 640 daltons, 630 daltons, 620 daltons, 610 daltons, 600 daltons, 590 daltons, 580 daltons, 570 daltons, 560 daltons, 550 daltons, 540 daltons, 530 daltons, 520 daltons, 510 daltons, 500 daltons, 490 daltons, 480 daltons, 470 daltons, 460 daltons, 450 daltons, 440 daltons, 430 daltons, 420 daltons, 410 daltons, 400 daltons, 390 dalton, 380 daltons, 370 daltons, 360 daltons, 350 daltons, 340 daltons, 330 daltons, 320 daltons, 310 daltons, 300 daltons, 290 daltons, 280 daltons, 270 daltons, 260 daltons, 250 daltons, 240 daltons, 230 daltons, 220 daltons, 210 daltons, or 200 daltons. In some embodiments, the compound has a molecular weight no more than 1000 daltons. In some embodiments, the compound has a molecular weight no more than 900 daltons.

[0084] In some embodiments, the compound is selected from the group consisting of:





and



[0085] In some aspects, disclosed herein is a pharmaceutical composition comprising the compound any preceding aspect, and a pharmaceutically acceptable carrier.

Methods of cancer therapy

[0086] Disclosed here in is a method for treating a cancer in a subject in need thereof, comprising: administering a therapeutically effective amount of a compound of Formula I:

X-Y-Z

Formula I

wherein:

X is a destabilization amino acid residue;

Y is a linker; and

Z is a ligand that binds a tyrosine kinase;

or a pharmaceutically acceptable salt thereof.

[0087] In some embodiments, the cancer results from a dysregulation of the tyrosine kinase.

“Dysregulation” refers herein refers to an abnormal level of expression or activity of a protein or gene. This can include, for example, less levels or activities than in normal healthy humans. This can also include, for example, increased levels or activities than in normal healthy humans. In some

embodiments, disclosed herein comprises a cancer results from a dysregulation of a receptor tyrosine kinase (e.g., estrogen-related receptor α or epidermal growth factor receptor) and/or a non-receptor tyrosine kinase (e.g., ABL). Thus, the present invention encompasses a method for treating any cancer having a dysregulation of the receptor tyrosine kinase and/or a non-receptor tyrosine kinase. These cancer can be, for example, lymphoma, B cell lymphoma, T cell lymphoma, mycosis fungoides, Hodgkin's Disease, myeloid leukemia, bladder cancer, brain cancer, nervous system cancer, head and neck cancer, squamous cell carcinoma of head and neck, lung cancers such as small cell lung cancer and non-small cell lung cancer, neuroblastoma/glioblastoma, ovarian cancer, skin cancer, liver cancer, melanoma, squamous cell carcinomas of the mouth, throat, larynx, and lung, cervical cancer, cervical carcinoma, breast cancer, and epithelial cancer, renal cancer, genitourinary cancer, pulmonary cancer, esophageal carcinoma, head and neck carcinoma, large bowel cancer, hematopoietic cancers; testicular cancer; colon cancer, rectal cancer, prostatic cancer, or pancreatic cancer. In some embodiments, the cancer is chronic myeloid leukemia. In some embodiments, the cancer is acute lymphocytic leukemia. In some embodiments, the cancer is breast cancer. In some embodiments, the cancer is lung cancer. In some embodiments, the cancer is kidney cancer.

[0088] In some embodiments, the subject is a human. In some embodiments, the human has a cancer. In some embodiments, the human is suspected to have a cancer.

[0089] Dosing frequency for the compound, includes, but is not limited to, at least about once every 12 months, once every 11 months, one every 10 months, once every 9 months, once every 8 months, once every 7 months, once every 6 months, once every 5 months once every 4 months, once every once every 3 months, once every 12 weeks, once every 10 weeks, once every 8 weeks, once every 7 weeks, once every 6 weeks, once every 5 weeks, once every 4 weeks, once every 3 weeks, once every 2 weeks, or weekly. In some embodiments, the dosing frequency for the compound can be, for example, about once every 10 days, once every 9 days, once every 8 days, once every 7 days, once every 6 days, once every 5 days, once every 4 days, once every 3 days, once every 2 days, once every day, two times a day, three times a day, four times a day, five times a day, or six times a day. In some embodiments, the interval between each administration is less than about a week, such as less than about any of 6, 5, 4, 3, 2, or 1 day. In some embodiments, the interval between each administration can be more than a week, such as, more than two weeks, three weeks, four weeks, five weeks, six weeks, seven weeks, eight weeks, nine weeks, ten weeks, eleven weeks, twelve weeks, three months, four months, five months, or six months. In some embodiments, the interval between each administration is constant. Administration can also be

continuous and adjusted to maintaining a level of the compound within any desired and specified range.

EXAMPLES

[0090] Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. While the invention has been described with reference to particular embodiments and implementations, it will be understood that various changes and additional variations may be made and equivalents may be substituted for elements thereof without departing from the scope of the invention or the inventive concept thereof. In addition, many modifications may be made to adapt a particular situation or device to the teachings of the invention without departing from the essential scope thereof. Such equivalents are intended to be encompassed by the following claims. It is intended that the invention not be limited to the particular implementations disclosed herein, but that the invention will include all implementations falling within the scope of the appended claims.

[0091] Cell cultures. The MCF-7 cell line (ATCC, Manassas, VA) was maintained at 37°C with 5% CO₂ in DMEM with 2 mM L-glutamine that was modified to contain 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, and 1.5 g/l sodium bicarbonate. It was also supplemented with 10% fetal bovine serum, 10 µg/ml bovine insulin, 100 units/µl penicillin/streptomycin. All drug treatment study was supplemented with 2% FBS. Functional assays including MTT, migration assays were done in 10% FBS in DMEM media

[0092] Cell proliferation assay. The (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) (MTT) method was used to measure living cells through mitochondrial dehydrogenase activity (Sigma Inc., Saint Louis, MO). Cells were plated in a 96-well, 5000 cells per 100 µl/ well. After 24 h, the cells were treated with PROTACs in fresh media. At the indicated time point, the media was removed and dimethyl sulfoxide was added as MTT solubilization solution. Absorbance was measured at 550 nm.

[0093] Western blot analysis. Cells were lysed in RIPA buffer (Sigma Inc.) with the addition of protease inhibitors tablet and phosphatase inhibitors cocktail (Sigma Inc.). Lysates were resolved by SDS– polyacrylamide gels, transferred onto nitrocellulose membranes, and then probed with antibodies as indicated. Antibodies against ERRα, E-Cadherin, N-Cadherin, Snail, and GAPDH were obtained from Cell Signaling (Danvers, MA). Fibronectin antibody was obtained from Sigma Aldrich.

[0094] Wound healing assay. Cells were allowed to grow to near confluence in six well dishes. A uniform scratch was then made down the center of the plate using a 200 microliter micropipette tip,

followed by washing twice with PBS. The same marked field of the scratch wound was photographed using an Olympus light microscope (4× objective) at the indicated time points. The width of the scratch wound was measured at three different areas with Image J software.

[0095] Migration assay. MCF-7 cells (1×10^4) were seeded on an 8 μ M pore size Thin-cert for 24 well plates (Greiner Bio-One) in serum free media. PROTAC compounds were added to the bottom chamber as chemoattractant. After 48 h, cells on the top of the membrane were removed with a cotton swab. The migrated cells at the bottom side were washed with PBS, fixed with 70% ethanol and stained using 0.1% Crystal violet to visualize the migrated cells. Migrated cells attached to the lower side of the membrane were enumerated using a light microscope at 10× magnification.

Example 1. PROTAC Molecules and the Biological Effects Thereof

[0096] A short binding motif to a potent ubiquitin ligase can greatly improve the utility of the PROTAC approach. The shortest degradation motif is single amino acid, a destabilizing residue (e.g., Arg, His, Lys, Leu, Ile) at the N-terminus of a protein that targets the substrate to a ubiquitin ligase termed Ubr1 for rapid degradation via N-end rule pathway, the first ubiquitin dependent degradation pathway identified.

[0097] Ubr1 is constitutive active and broadly expressed, making it ideally suited for this strategy. Adaptor molecules were synthesized starting with Arg and fused to a variety of cancer drugs. For example, Arg was appended to imatinib, a moiety specific for BCR-ABL protein. By linking BCR-ABL to Ubr1, the synthetic molecule can markedly reduce BCR-ABL level, which is key to chronic myeloid leukemia (CML) and acute lymphocytic leukemia (ALL). Improvements over existing strategies were achieved including: i) a small ubiquitin ligase-binding motif (i.e. Arg), markedly reducing the size of an adaptor, which increases cell permeability and efficacy; ii) the employment of Ubr1, a broadly expressed, potent ubiquitin ligase; iii) the Arg residue can be easily replaced with others (e.g. Lys, His) that changes the degradation speed due to different affinity for Ubr1, allowing optimal modulation of protein stability.

[0098] Adaptor molecules were synthesized starting with Arg or His and fused to a ligand for estrogen-related receptor α (ERR α) (Fig. 1A), a nuclear receptor that is a major regulator of several critical metabolic pathways and also is a prognostic marker of breast cancer. ERR α inhibition has been shown to reduce the proliferation of breast tumor cells *in vitro* and *in vivo*. It was found that both compounds reduced endogenous ERR α level in the MCF-7 metastatic breast cancer cell line (Fig. 1B and Fig. 1C). To assess whether ERR α decrease was due to protein turnover, the cells were treated with cycloheximide to block protein synthesis and collected the samples at various

time points for western blotting analysis. Both of these compounds triggered $ERR\alpha$ degradation (Fig. 1D).

[0099] In addition, more fusion molecules were synthesized (Figs. 2A-2D) derived from gefitinib to target EGFR in lung cancer cells (H1299, H1975, HCC827, H3255), imatinib and dasatinib to target BCR-ABL protein in leukemia cells (e.g., K562, EM3, NAML1, KCL22, LAMA84, SUP-B15), and thiazolidinedione-based ligand for $ERR\alpha$ in breast cancer cells (e.g., MCF7, MDA-MB-231). The drugs (e.g., gefitinib, imatinib, dasatinib) have been previously analyzed and are effective in target inhibition albeit resistance often occurs later developed. In addition to these novel Arg-based molecules, more molecules using chemical mimics of Arg are generated. The capacity of these fusion molecules (e.g., Arg-gefitinib chimera) in triggering rapid degradation of their targets (e.g., EGFR, BCR-ABL, $ERR\alpha$) are tested in mammalian cells as well as their physiological effects (e.g., cell proliferation, receptor signaling, apoptosis) in relevant cancer cells. In addition, their efficacy in mouse xenograft models, and ultimately in affected leukemia patients are evaluated.

[00100] The chemical structure of an example of adaptor molecules synthesized starting with Arg or His and fused to a ligand for estrogen-related receptor α ($ERR\alpha$) was shown (Fig. 3A), as introduced above (Figs. 1A-1D). It was shown herein that both compounds Arg- $T^{ERR\alpha}$ and His- $T^{ERR\alpha}$ reduced endogenous $ERR\alpha$ level in the MCF-7 metastatic breast cancer cell line (Fig. 3B). To assess whether $ERR\alpha$ decrease was due to protein turnover, the cells were treated with cycloheximide to block protein synthesis and collected the samples at various time points for western blotting analysis. Both Arg- $T^{ERR\alpha}$ and His- $T^{ERR\alpha}$ triggered $ERR\alpha$ degradation (Fig. 3C).

[00101] Whether PROTAC-induced $ERR\alpha$ degradation is mediated by the proteasome was then evaluated. Arg- $T^{ERR\alpha}$ or His- $T^{ERR\alpha}$ -triggered $ERR\alpha$ turnover was compromised upon the treatment of the proteasome inhibitor MG132 (Fig. 4A and Fig. 4B), indicating the involvement of the proteasome in $ERR\alpha$ turnover. To ascertain that $ERR\alpha$ is degraded by the N-end rule pathway, dipeptides starting with a destabilizing residue (e.g., Arg, His) were utilized to block the degradation of N-end rule substrates through their competition for the ubiquitin ligase Ubr1. It was found that the PROTAC-exposed $ERR\alpha$ level was increased upon the addition of the dipeptide Arg-Ala or His-Ala, but not the control peptide Ala-Ala bearing a stabilizing residue (Fig. 4C and Fig. 4D).

[00102] The biological effects of the PROTAC molecules were examined in MCF-7 cells. Both compounds led to decreased proliferation of MCF-7 cells (Fig. 5A). As cell migration is key to tumorigenesis, evaluation was performed regarding whether the compounds affect cell motility by the scratch wound healing method and the ThinCert cell migration assay. Both Arg- $T^{ERR\alpha}$ and

His- $T^{ERR\alpha}$ reduced MCF-7 cancer cell migration and wound repair (Fig. 5B and Fig. 5C).

Furthermore, assessment was performed to monitor the expression of several markers of epithelial-mesenchymal transition (EMT) that is crucial for cancer cell migration and invasion. PROTACs-induced $ERR\alpha$ destruction led to increased expression of E-cadherin and reduced expression of N-cadherin, SNAIL and Fibroectin (Fig. 5D), indicating EMT is repressed upon $ERR\alpha$ depletion.

Combined, these results show that Arg- $T^{ERR\alpha}$ and His- $T^{ERR\alpha}$ can effectively modulate $ERR\alpha$ and be explored for anticancer purpose. The present disclosure shows that the N-end rule based PROTACs efficiently trigger target destruction and inhibit the proliferation of breast cancer cells.

[00103] The advantage of this approach lies in the use of the simplest degradation signal - single amino acid, which can be any one of the thirteen destabilizing residues in the N-end rule pathway that in turn can lead to different degradation speed. The PROTAC method presented here overcomes limitations of existing approaches with significantly smaller molecular size of E3 targeting moiety, leading to higher permeability and better efficacy, and a modulatable potent degradation activity, allowing better control and broader application. Furthermore, the N-end rule degradation pathway is universally present and constitutively active, making it ideally suited for the PROTAC approach. This modular design expands the repertoire of limited ubiquitin pathways currently available for PROTACs and can be easily adapted for broad use in targeted protein degradation.

Example 2. Peptides, Compounds, Experimental Procedures for Synthesis and Spectroscopic data

[00104] Details of Arg- $T^{ERR\alpha}$ and His- $T^{ERR\alpha}$ syntheses are described in Figs. 7-10 and Figs. 11-13, respectively. Dipeptides Arg-Ala and His-Ala were purchased from Bachem Americas (Torrance, CA). MG132 is a proteasome inhibitor obtained from Calbiochem (Gibbstown, NJ). Cyclohexamide was purchased from Sigma-Aldrich (Saint Louis, MO).

[00105] Unless otherwise mentioned, all reactions were carried out under a nitrogen atmosphere with dry solvents under anhydrous conditions. All the chemicals were purchased commercially and used without further purification. Anhydrous THF was distilled from sodium-benzophenone, and dichloroethane and dichloromethane were distilled from calcium hydride. Yields refer to chromatographically. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated.

[00106] Reactions were monitored by Thin Layer Chromatography on plates (GF254) supplied by Yantai Chemicals (China) using UV light as visualizing agent and an ethanolic solution of phosphomolybdic acid and cerium sulfate, and heat as developing agents. If not specially

mentioned, flash column chromatography uses silica gel (200-300 mesh) supplied by Tsingtao Haiyang Chemicals (China).

[00107] NMR spectra were recorded on Brüker Advance 500 (1H 500 MHz, 13C 125 MHz) and Brüker Advance 400 (1H 400 MHz, 13C 100 MHz) and are calibrated using residual undeuterated solvent (CDCl₃ at 7.26 ppm 1H NMR, 77.16 ppm 13C NMR; pyridine-d₅ at 8.70 ppm 1H NMR, 149.6 ppm 13C NMR). The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad.

[00108] Mass spectrometric data were obtained using Brüker Apex IV FTMS using ESI (electrospray ionization) and Waters GCT (GC-MS) using EI (electron impact ionization).

[00109] It will be appreciated that variants of the above-disclosed and other features and functions, or alternatives thereof, may be combined into many other different systems or applications. Various presently unforeseen or unanticipated alternatives, modifications, variations, or improvements therein may be subsequently made by those skilled in the art which are also intended to be encompassed by the following claims.

SEQUENCES

SEQ ID NO:1

MSSQVVGIEP LYIKAEPASP DSPKGSSETE TEPPVALAPG PAPTRCLPGH
KEEEDGEGAG PGEQGGGKLV LSSLPKRLCL VCGDVASGYH YGVASCEACK
AFFKRTIQGS IEYSCPASNE CEITKRRRKA CQACRFTKCL RVGMLKEGVR
LDRVRGGRQK YKRRPEVDPL PFPGPFPAGP LAVAGGPRKT AAPVNALVSH
LLVVEPEKLY AMPDPAGPDG HLPVATLCD LFDREIVVTI SWAKSIPGFS
SLSLSDQMSV LQSVWMEVLV LGVAQRSLPL QDELAFEDL VLDEEGARAA
GLGELGAALL QLVRRQLQALR LEREYVLLK ALALANSDSV HIEDAEAVEQ
LREALHEALL EYEAGRAGPG GGAERRRAGR LLLTLPLLRQ TAGKVLAFY
GVKLEGKVPM HKLFLEMLEA MMD

SEQ ID NO:2

MRPSGTAGAALLALLAALCPASRALEEKKVCQGTSNKLTLQGTDFEDHFLSLQRMFNCEV
VLGNLEITYVQRNYDLSFLKTIQEVAGYVLIALNTVERIPLNLQIIRGNMYEYNSYALAVL
SNYDANKTGLKELPMRNLQEILHGAVRFSNNPALCNVESIQWRDIVSSDFLSNMSMDFQN
HLGSCQKCDPSCPNGSCWGAGEENCQKLTKIICAQQCSGRCRGKSPSDCCHNQCAAGCTG
PRESCLVCRKFRDEATCKDTCPLMLYNPTTYQMDVNPEGKYSFGATCVKKCPRNYVV
TDHGSCVRACGADSYEMEEDGVRKCKKEGPCRKVCNGIGIGEFKDSLSINATNIKHFKN
CTISGDLHILPVAFRGDSFHTPLDLPQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEI
IRGRTKQHGGQFSLAVVSLNITSLGLRSLKEISDGDVIISGNKNLCYANTINWKKLFGTSGQK
TKIISNRGENSCKATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKCNLLEGEPRE
FVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCVAGVMGENNTLVW
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RRRHIVRKRTLRLQLQERELVEPLTPSGEAPNQALLRILKETEFKKIKVLGSGAFGTVYKGL
WIPEGEKVKIPVAIKELREATSPKANKEILDEAYVMASVDNPHVCRLLGICLTSTVQLITQL
MPFGCLLDYVREHKDNIGSQYLLNWCVQIAKGMNYLEDRLVHRDLAARNVLVKTTPQH
VKITDFGLAKLLGAEEKEYHAEGGKVPIKWMALLESILHRIYTHQSDVWSYGVTVWELMTF
GSKPYDGIPASEISSILEKGERLPQPPICTIDVYIMVKCWMIDADSRPKFRELIIEFSKMARD
PQRYLVIQGDERMHLPSPTDSNFYRALMDEEDMDDVDADEYLIPQQGFFSSPSTSRTPLL
SSLSATSNNSTVACIDRNLQSCPIKEDSFLQRYSSDPTGALTEDSIDDTFLPVPEYINQSVPK

RPAGSVQNPVYHNQPLNPAPSRDPHYQDPHSTAVGNPEYLNTVQPTCVNSTFDSPAHWAAQ
KGS HQISLDNPDYQQDFFPKAEKPNGIFKGSTAENAEYLRVAPQSSEFIGA

SEQ ID NO:3

MLEICLKLVGCKSKKGLSSSSSCYLEEALQRPVASDFEPQGLSEAARWNSKENLLAGPSEN
DPNLFVALYDFVASGDNTLSITKGEKLRVLGYNHNGEWCEAQTKNGQGWWPSNYITPVN
SLEKHSWYHGPVSRNAAEYLLSSGINGSFLVRESESSPGQRSISLRYEGRVYHYRINTASDG
KLYVSSSESRFNTLAELVHHHSTVADGLITTLHYAPKRNKPTVYGVSPNYDKWEMERTDI
TMKHKLGGGQYGEVYEGVWKKYSLTVAVKTLKEDTMEVEEFLKEAAVMKEIKHPNLVQ
LLGVCTREPPFYIITEFMTYGNLLDYLRECNRQEVNAVVLVLYMATQISSAMEYLEKKNFIH
RDLAARNCLVGENHLVKVADFGLSRLMTGDTYTAHAGAKFPIKWTAPESLAYNKFSIKSD
VWAFGVLLWEIATYGMSPYPGIDLSQVYELLEKDYRMERPEGCEKVYELMRACWQWN
PSDRPSFAEIHQAFETMFQESSISDEVEKELGKQGVRGAVSTLLQAPELPTKTRTSRRAAEH
RDTTDVPEMPHSGKGQGESDPLDHEPAVSPLLPRKERGPPEGGLNEDERLLPKDKKTNLFS
LIKKKKKTAPTPPKRSSSFREMDGQPERRGAGEEEGRDISNGALAFPLDTADPAKSPKPSN
GAGVPNGALRESGGSGFRSPHLWKKSSLTSSRLATGEEEGGGSSSKRFLRSCSASCVPHG
AKDTEWRSVTLPRDLQSTGRQFDSSTFGGHKSEKPALPRKRAGENRSDQVTRGTVTTPPRL
VKKNEEAADDEVFKDIMESSPGSSPPNLT PKPLRRQVTVAPASGLPHKEEAGKGSALGTPAA
AEPVTPTSKAGSGAPGGTSKGPAEESRVR RHKHSSSESPGRDKGKLSRLKPAPPPPPAASAG
KAGGKPSQSPSQEAAGEAVLGAKTKATSLVDAVNSDAAKPSQPG EGLKKPVL PATPKPQS
AKPSGTPISPAPVPSTLPSASSALAGDQPSSTA FIPLISTRVSLRKTRQPPERIASGAITKGVVL
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RELQICPATAGSGPAATQDFSKLLSSVKEISDIVQR

SEQ ID NO:4

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SPGKARPGTARRPGAAASGERDDRGPASVAALRSNFERIRKGHGQPGADA EKPFYVNVE
FHHERGLVKVNDKEVSDRISSLGSQAMQMERKKSQHGAGSSVGDASRPPYRGRSSESSCG
VDGDYEDAELNPRFLKDNLIDANGGSRPPWPPLEYQPYQSIYVGGMMEGEGKGPLLRSQS
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RDKSRSPSQNSQQSFDSSSPPTPQCHKRHRHCPVVVSEATIVGVRKTGQIWPNDGEGAFHG
DADGSFGTPPGYGCAADRAEEQRRHQDGLPYIDDSPSSSPHLSSKGRGSRDALVSGALEST

KASELDLEKGLEMRKWVLSGILASEETYLSHLEALLPMKPLKAAATTSPVLTSQQIETIF
FKVPELYEIHKEFYDGLFPRVQQWSHQQRVGDLFQKLASQLGVYRAFDNYGVAMEMAE
KCCQANAQFAEISENLRARSNKDAKDPTTKNSLETLLYKPVDRVTRSTLVLHDLLKHTPAS
HPDHPLLQDALRISQNFLSSINEEITPRRQSMTVKKGEHRQLLKDSFMVELVEGARKLRHV
FLFTDLLLCTKLKKQSGGKTQQYDCKWYIPLTDL SFQMVDELEAVPNIPLVPDEELDALKI
KISQIKNDIQREKRANKGSKATERLKKKLSEQESLLLLMSPSMAFRVHSRNGKSYTFLISSD
YERAEWRENIREQQKKCFRSFSLTSVELQMLTNSCVKLQTVHSIPLTINKEDDESPGLYGFL
NVIVHSATGFKQSSNLYCTLEVDSFGYFVNKAKTRVYRDTAEPNWNEEFEIELEGSQTLRI
LCYEKCYNKTKIPKEDGESTDRLMGKGQVQLDPQALQDRDWQRTVIAMNGIEVKLSVKF
NSREFSLKRMP SRKQTGVFGVKIAVVTKRERSKVPYIVRQCVEEIERRGMEEVGIYRVSGV
ATDIQALKAAFDVNNKDVSVMSEMDVNIAIAGTLKLYFRELPEPLFTDEFYPNFAEGIALS
DPVAKESCMLNLLLSLPEANLLTFLFLLDHLKRVAEKEAVNKMSLHNLATVFGPTLLRPSE
KESKLPANPSQPITMTDSWSLEVMSQVQVLLYFLQLEAIPAPDSKRQSILFSTEV

CLAIMS

What is claimed is:

1. A compound of Formula I:

X-Y-Z

Formula I

wherein:

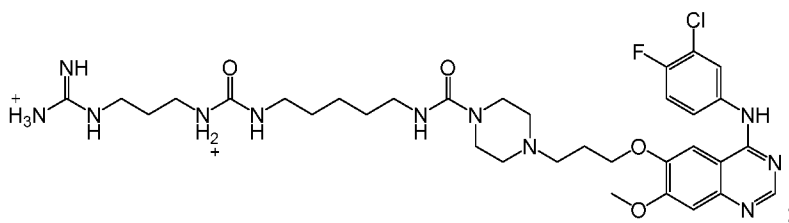
X is a destabilization amino acid residue;

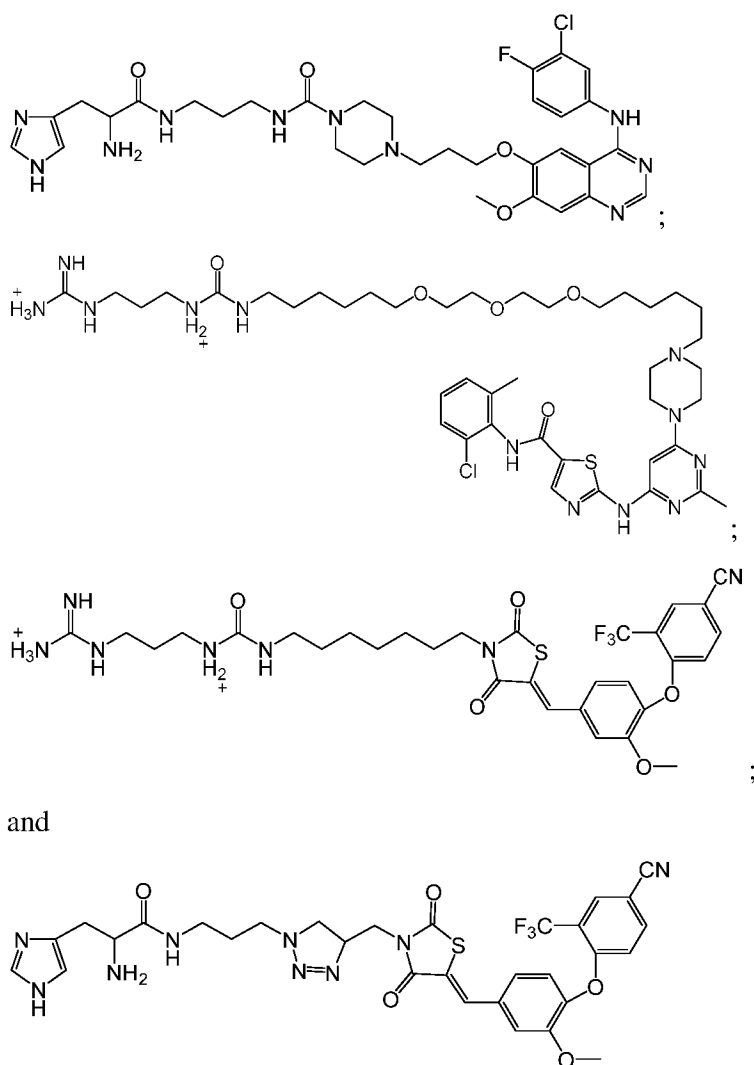
Y is a linker; and

Z is a ligand that binds a tyrosine kinase;

or a pharmaceutically acceptable salt thereof.

2. The compound of claim 1, wherein the destabilization amino acid residue is selected from the group consisting of Arg, His, Lys, Leu, Ile, Phe, Trp, Tyr, Cys, Asn, Asp, Glu, and Gln.
3. The compound of claim 2, wherein the destabilization amino acid residue is Arg or His.
4. The compound of any one of claims 1 to 3, wherein the tyrosine kinase is a receptor tyrosine kinase or a non-receptor tyrosine kinase.
5. The compound of claim 4, wherein the receptor tyrosine kinase comprises estrogen-related receptor α or epidermal growth factor receptor.
6. The compound of any one of claims 1 to 5, wherein the ligand comprises an anti-cancer drug.
7. The compound of claim 6, wherein the anti-cancer drug comprises gefitinib, dasatinib, imatinib, or a thiazolidinedione derivative.
8. The compound of any one of claims 1 to 7, wherein the compound has a molecular weight no more than 1000 daltons.
9. The compound of any one of claims 1 to 8, wherein the compound is selected from the group consisting of





10. A pharmaceutical composition comprising the compound of any one of claims 1 to 9 and a pharmaceutically acceptable carrier.
11. A method for treating a cancer in a subject in need thereof, comprising: administering a therapeutically effective amount of the pharmaceutical composition of claim 10.
12. The method of claim 11, wherein the cancer results from a dysregulation of the tyrosine kinase.
13. The method of claim 11 or 12, wherein the cancer comprises chronic myeloid leukemia, acute lymphocytic leukemia, breast cancer, or lung cancer.
14. The method of any one of claims 11 to 13, wherein the subject is a human.
15. A method for treating a cancer in a subject in need thereof, comprising: administering a therapeutically effective amount of a compound of Formula I:

X-Y-Z

Formula I

wherein:

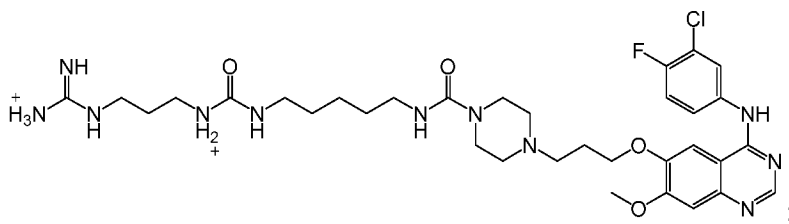
X is a destabilization amino acid residue;

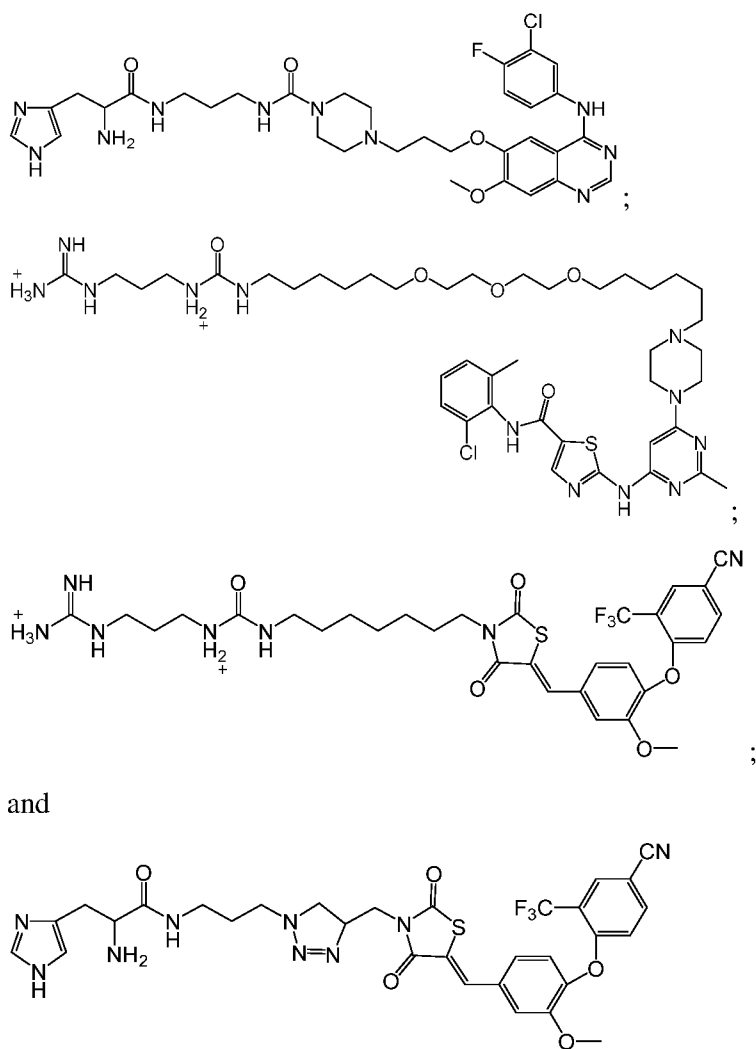
Y is a linker; and

Z is a ligand that binds a tyrosine kinase;

or a pharmaceutically acceptable salt thereof.

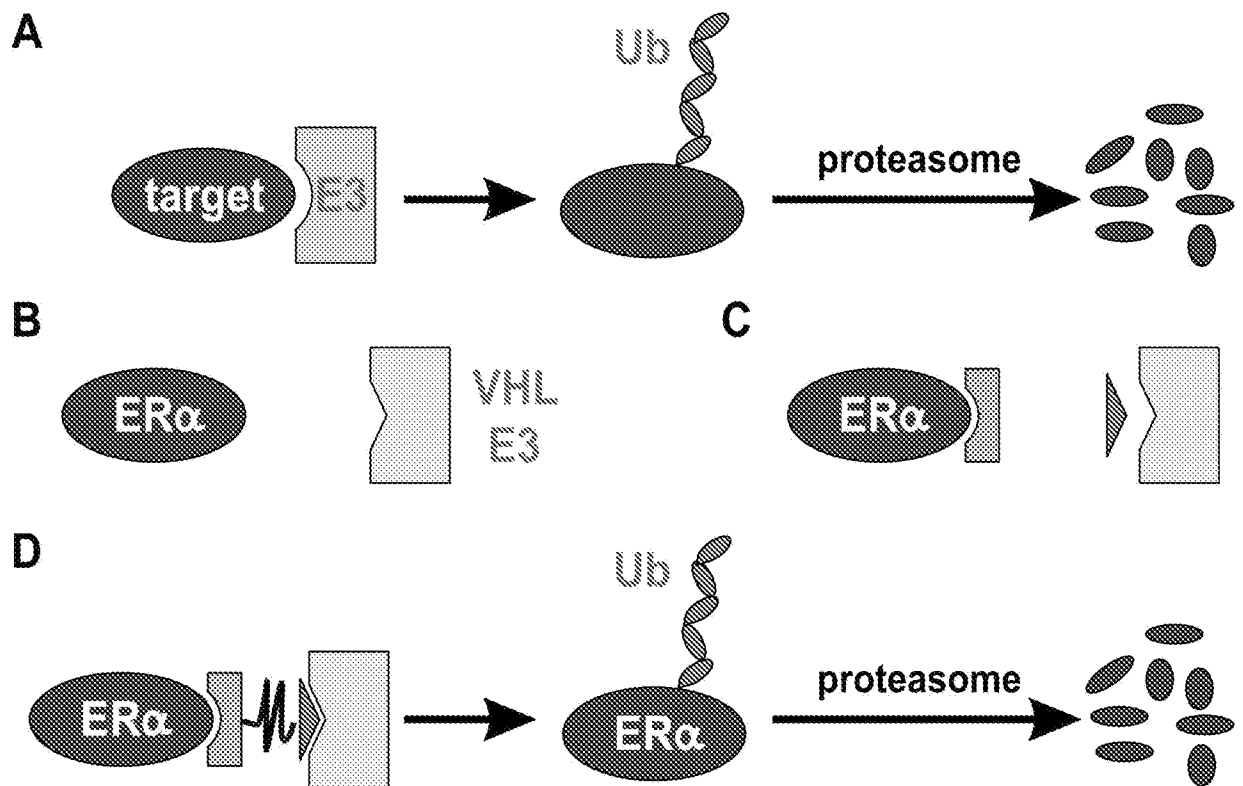
16. The method of claim 15, wherein the destabilization amino acid residue is selected from the group consisting of Arg, His, Lys, Leu, Ile, Phe, Trp, Tyr, Cys, Asn, Asp, Glu, and Gln.
17. The method of claim 16, wherein the destabilization amino acid residue is Arg or His.
18. The method of any one of claims 15 to 17, wherein the tyrosine kinase is a receptor tyrosine kinase or a non-receptor tyrosine kinase.
19. The method of claim 18, wherein the receptor tyrosine kinase comprises estrogen-related receptor α or epidermal growth factor receptor.
20. The method of any one of claims 15 to 19, wherein the ligand comprises an anti-cancer drug.
21. The method of claim 20, wherein the anti-cancer drug comprises gefitinib, dasatinib, imatinib, crizotinib, lapatinib, or a thiazolidinedione derivative.
22. The method of any one of claims 15 to 21, wherein the compound has a molecular weight no more than 1000 daltons.
23. The method of any one of claims 15 to 22, wherein the compound is selected from the group consisting of



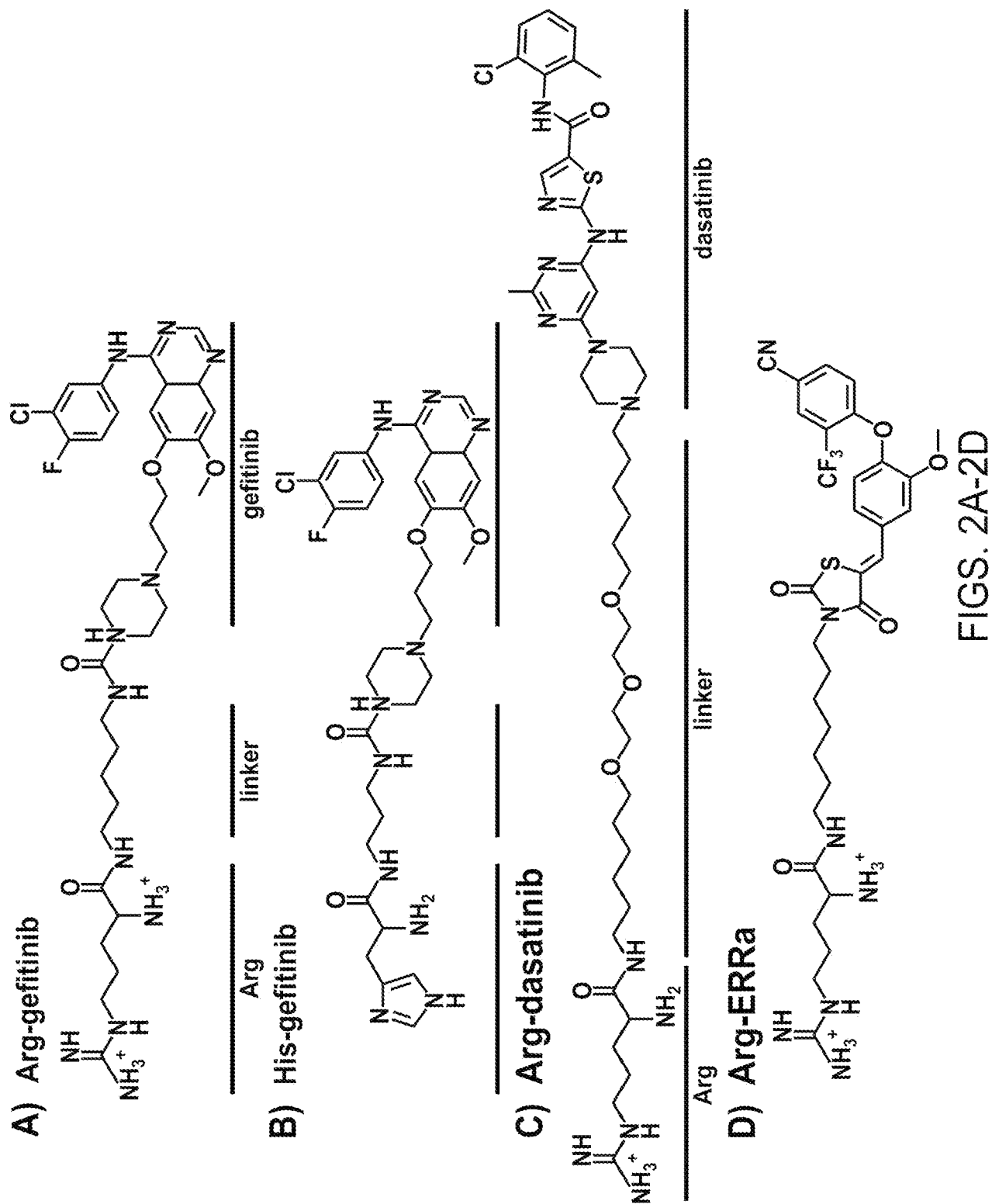


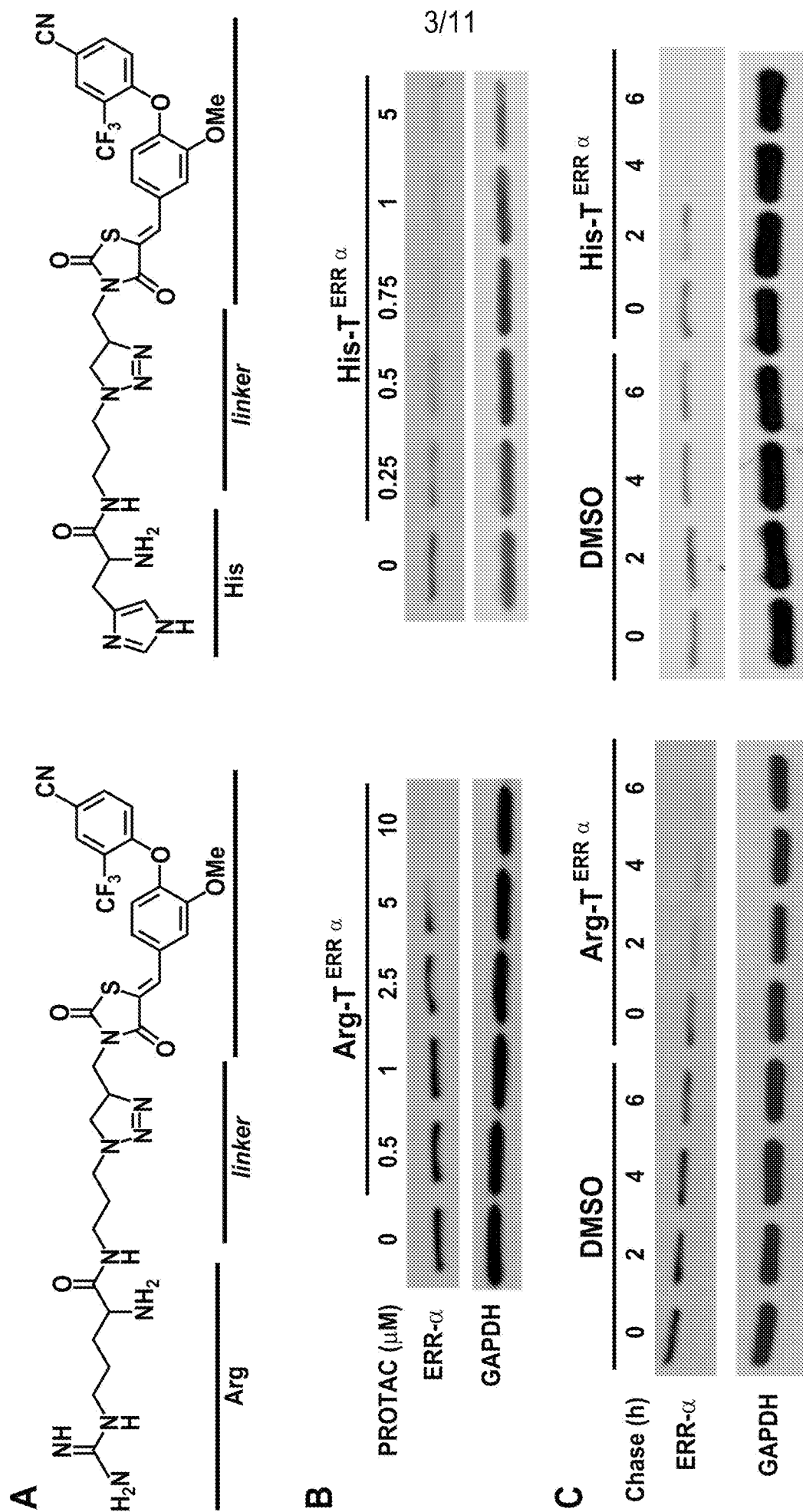
24. The method of any one of claims 15 to 23, wherein the cancer results from a dysregulation of the tyrosine kinase.
25. The method of any one of claims 15 to 24, wherein the cancer comprises chronic myeloid leukemia, acute lymphocytic leukemia, breast cancer, or lung cancer.
26. The method of any one of claims 15 to 25, wherein the subject is a human.

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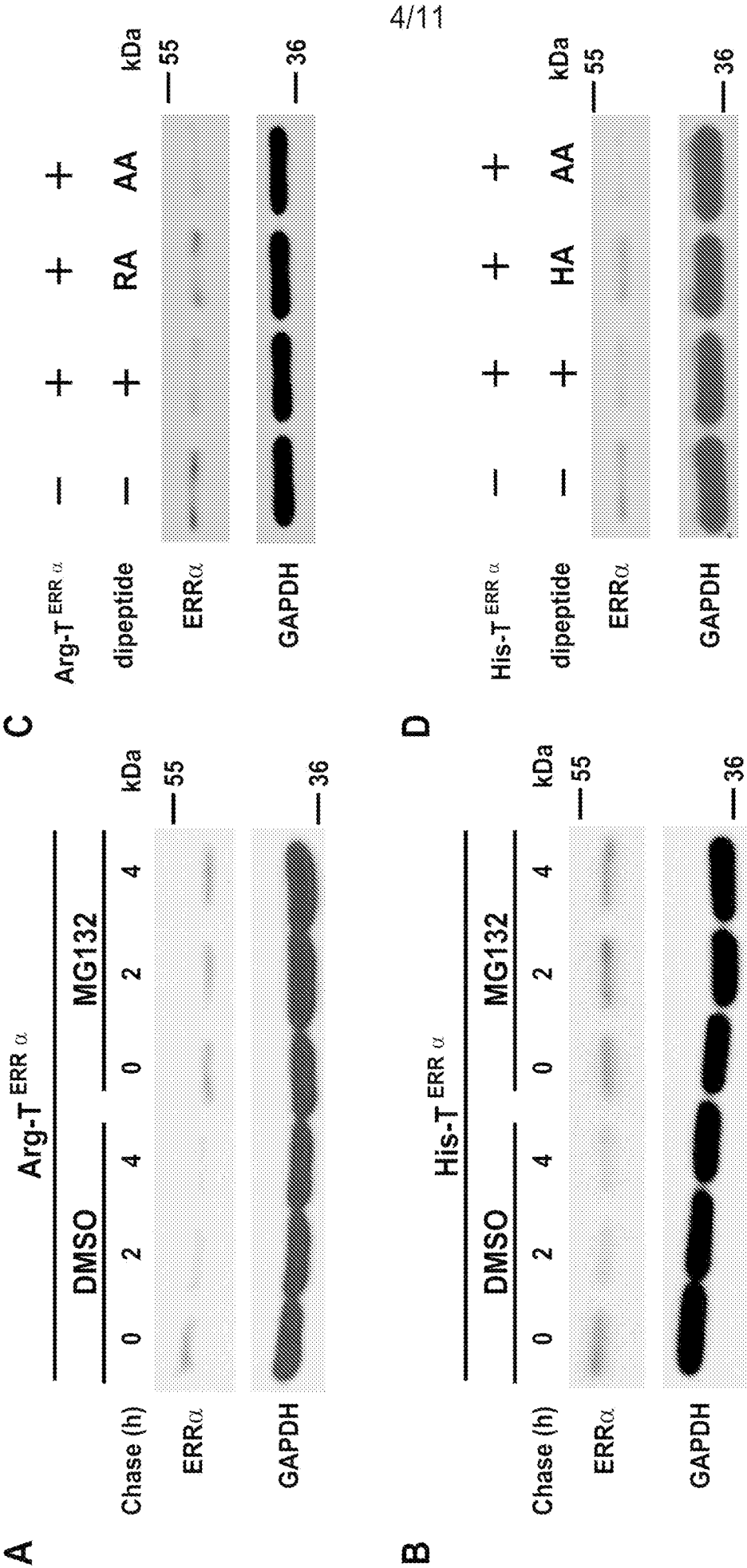
FIGS. 1A-1D





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FIGS. 3A-3C



FIGS. 4A-4D

5/11

A

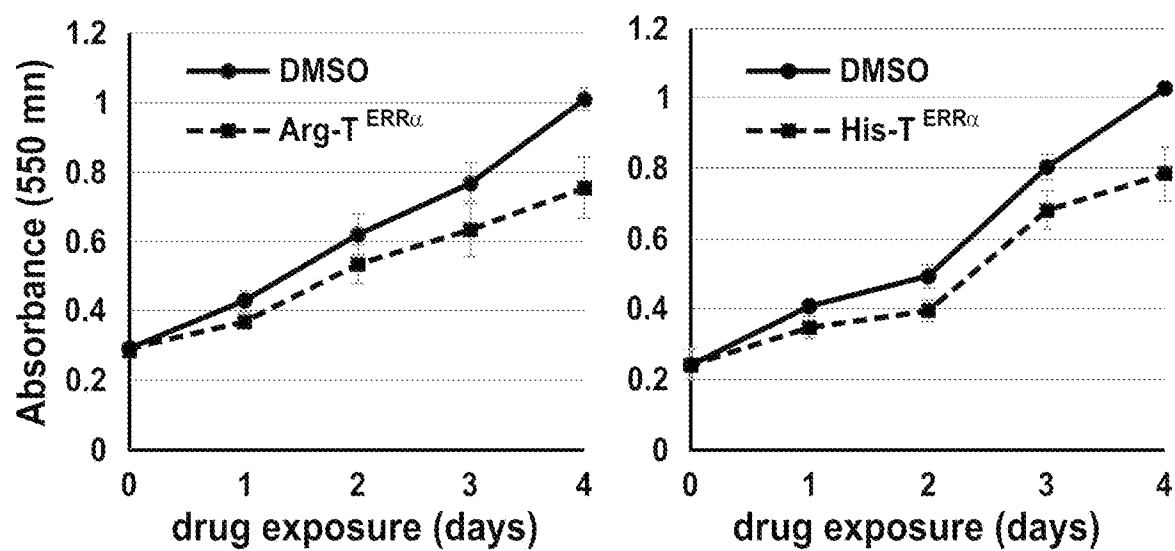
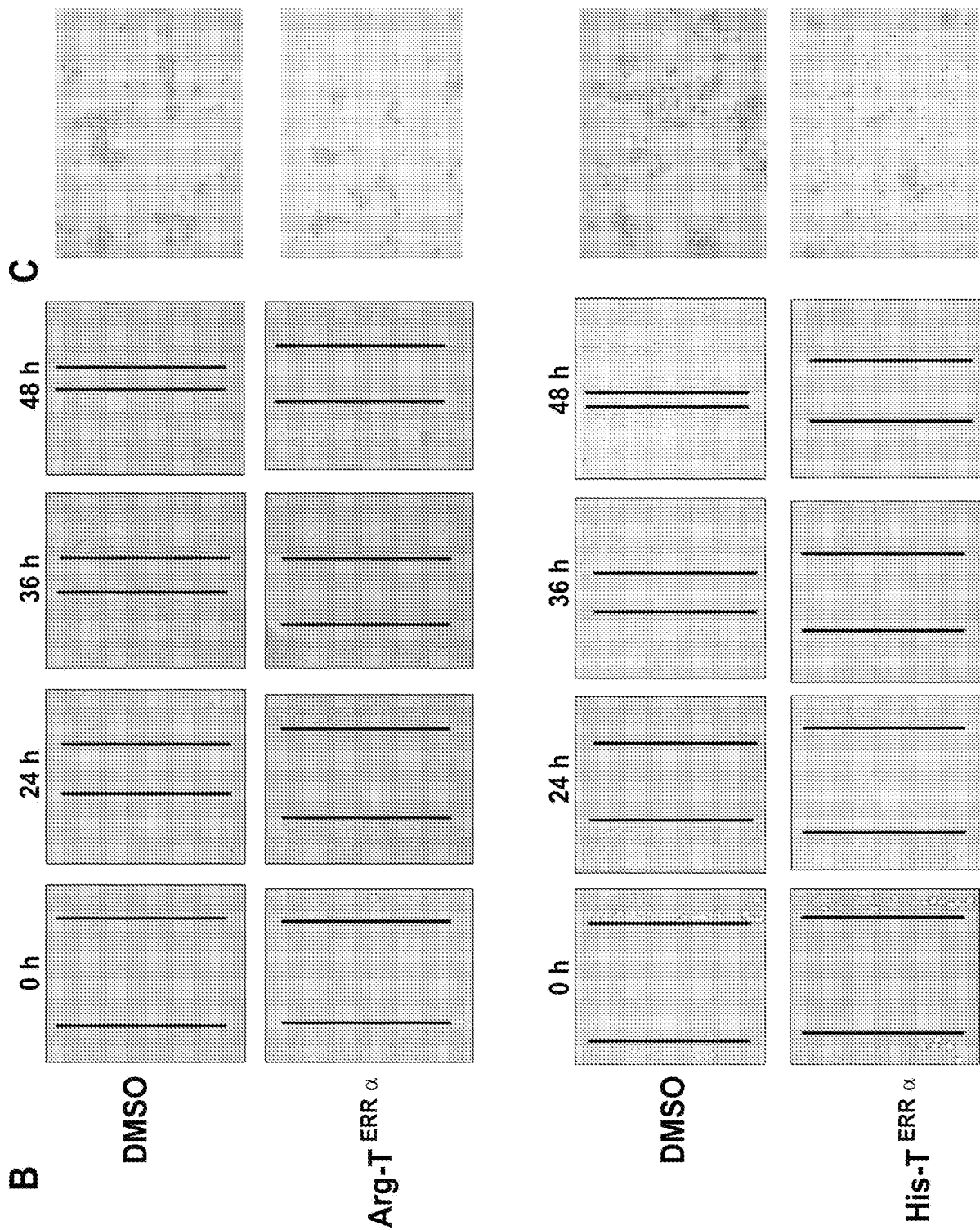


FIG. 5A



FIGS. 5B-5C

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D

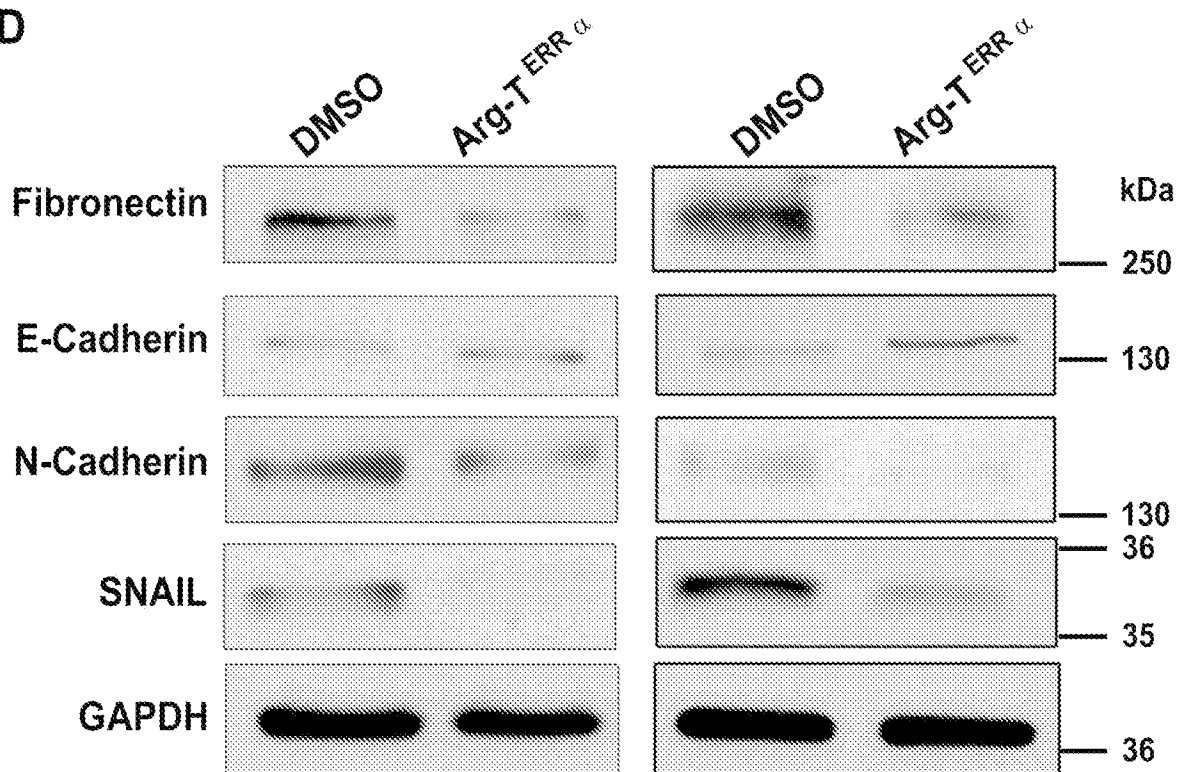
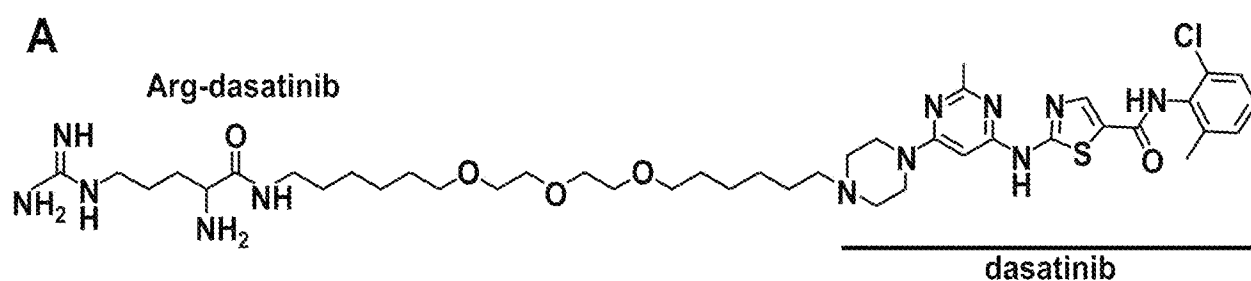
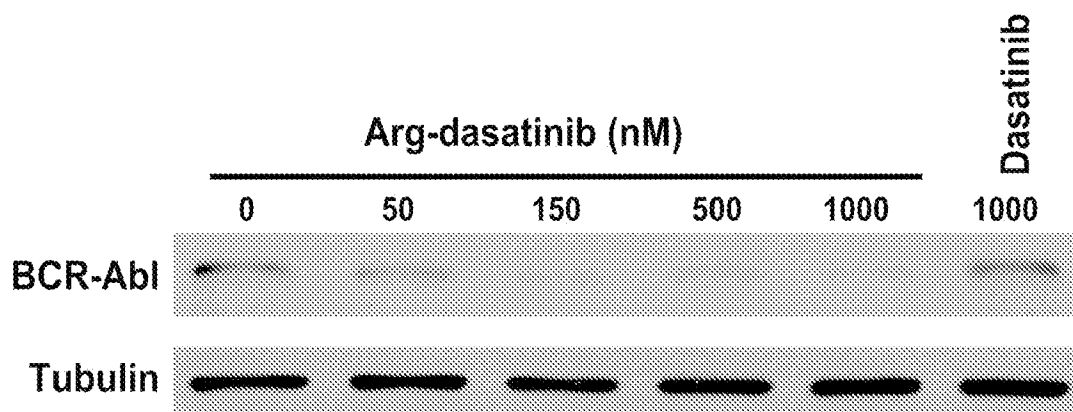


FIG. 5D



B



FIGS. 6A-6B

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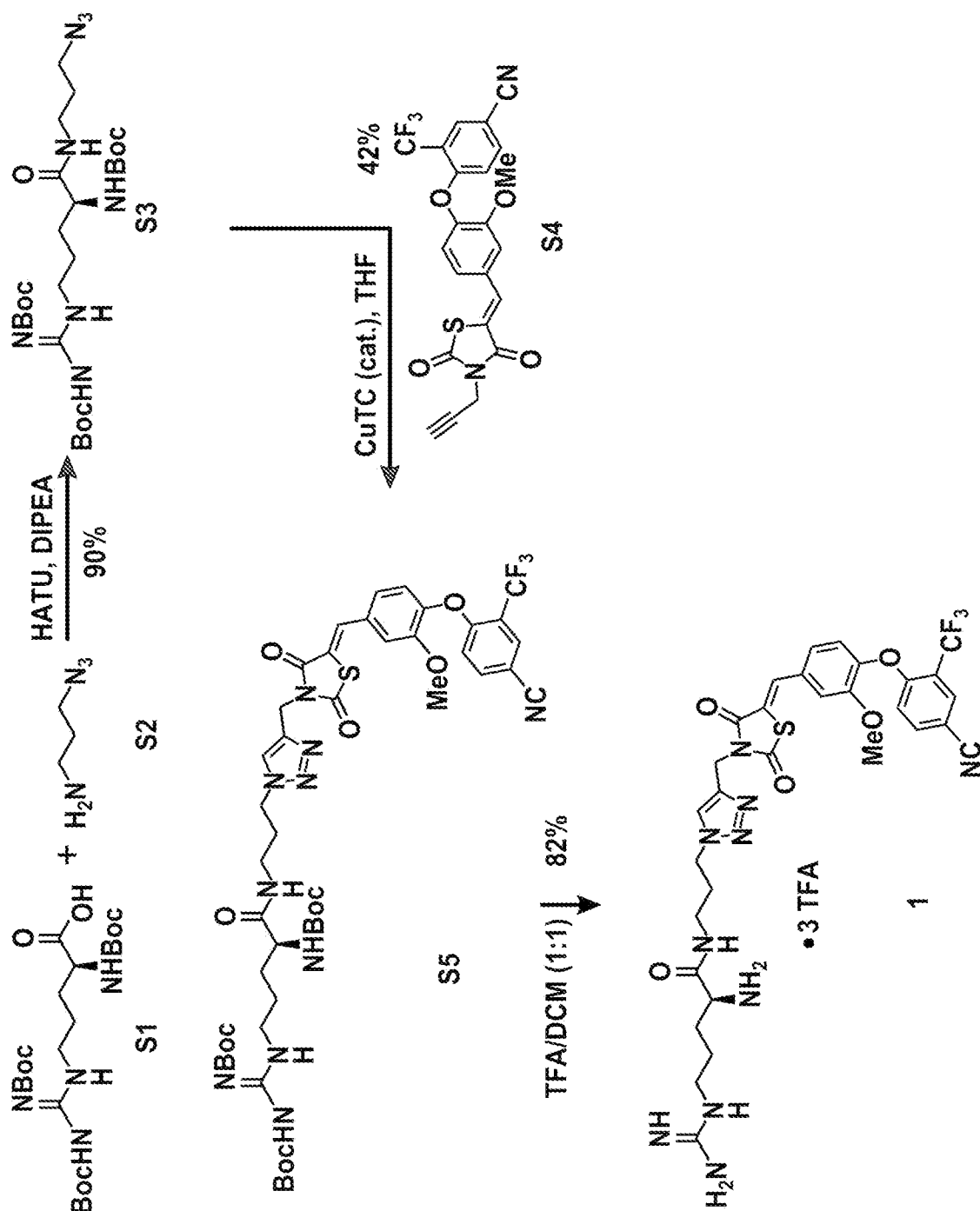


FIG. 7

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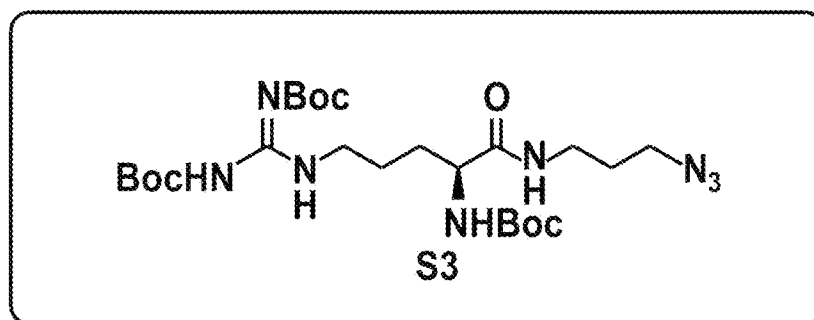


FIG. 8

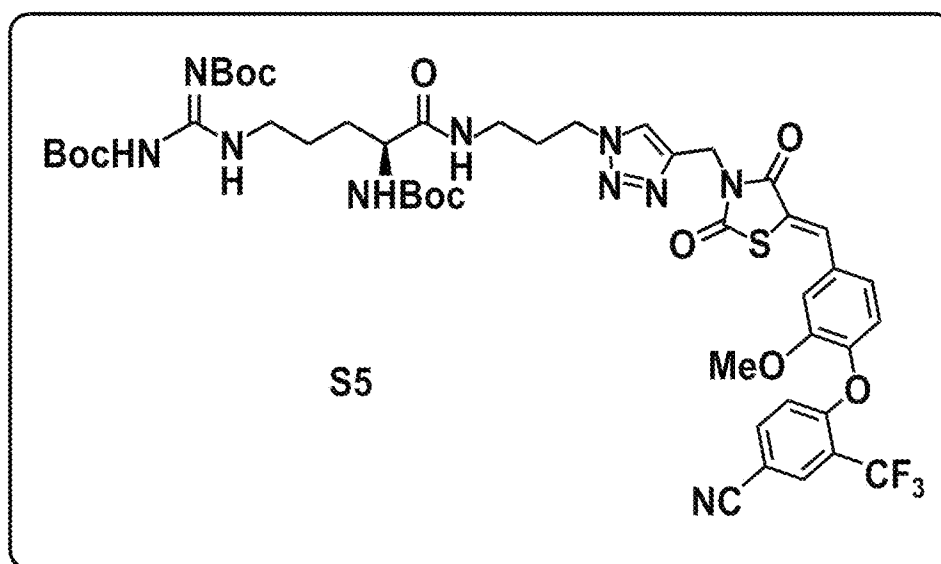


FIG. 9

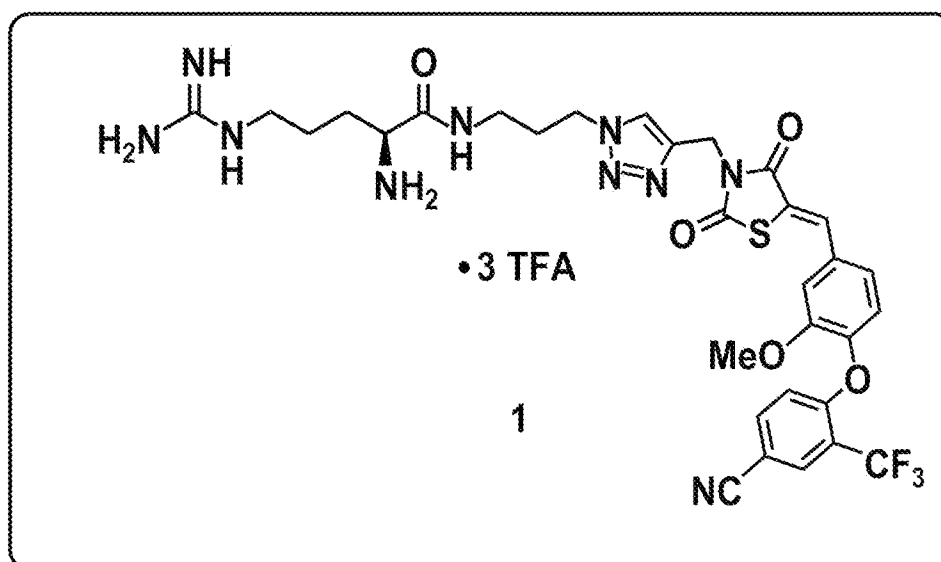


FIG. 10

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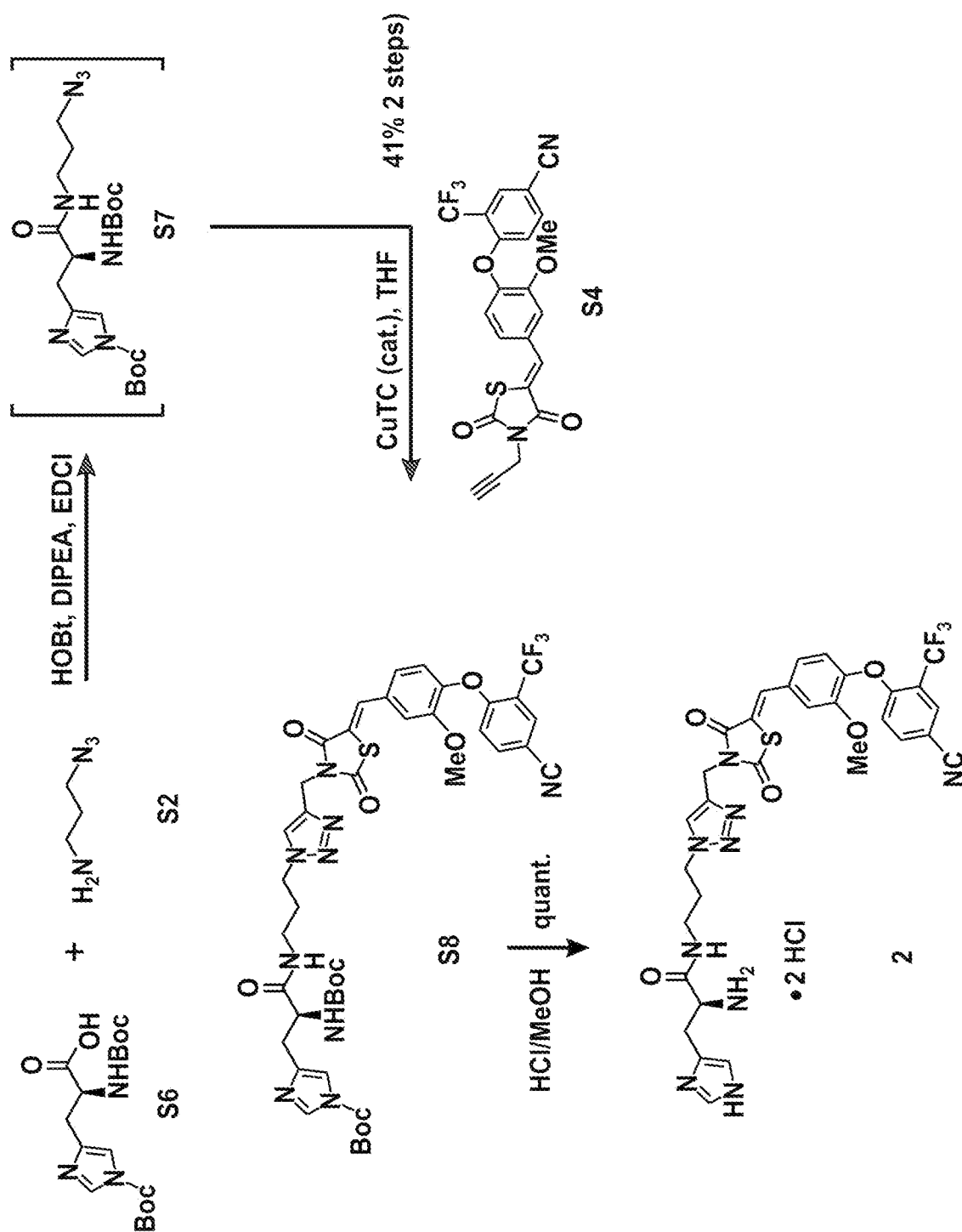


FIG. 11

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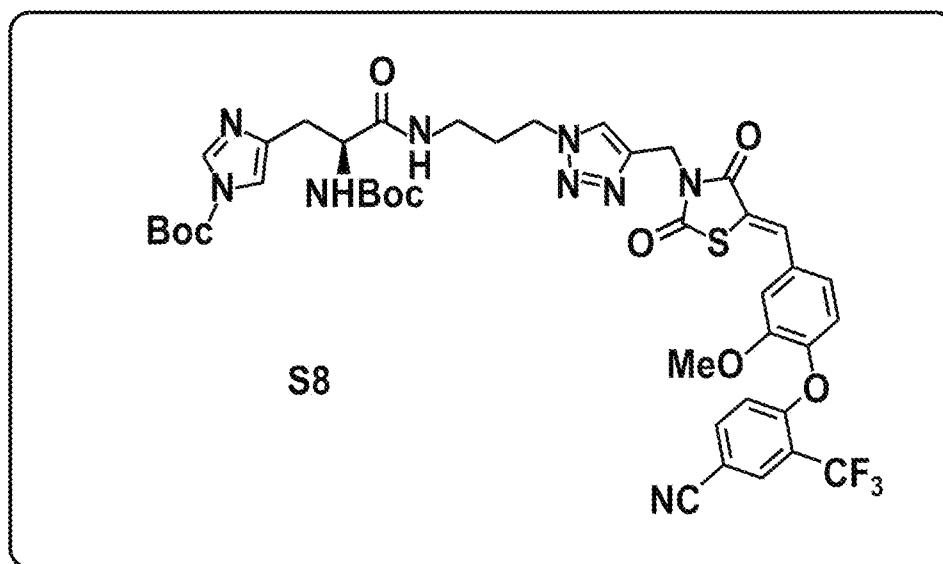


FIG. 12

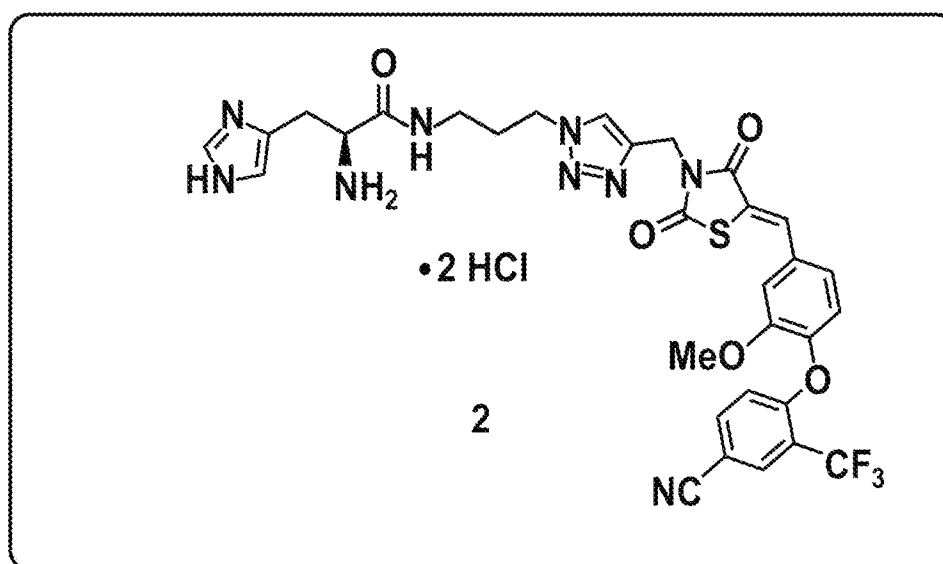


FIG. 13

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 20/41139

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 6-14 and 20-26
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:
(see supplemental sheet)

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-5

Remark on Protest

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

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 20/41139

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 31/7068; A61K 38/07 (2020.01)

CPC - A61K 31/519; A61P 35/00; C07D 471/04

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

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

See Search History document

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

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	HINEST et al. "Posttranslational protein knockdown coupled to receptor tyrosine kinase activation with phosphoPROTACs" Proceedings of the National Academy of Science, vol 110 p. 8942-8947 28 May 2013 (28.05.2013), especially p.8943, Figure 1, col 1 para 1	1-4 ----- 5
Y	BONDESON et al. "Catalytic in vivo protein knockdown by small-molecule PROTACs" Nature Chemical Biology vol 11 p. 611-617 10 June 2015 (10.06.2015), especially p. 612 col 1 Figure 1 (b), p. 612 col 1, para 1, and p. 616 col 2 para 2	5
A	US 2013/0190340 A1 (HEDSTROM et al.) 25 July 2013 (25.07.2013), entire document	1-5
A	US 2018 /0237418 A1 (ARVINAS, Inc.) 23 August 2018 (13.08.2018), entire document	1-5
A	WO 2013/106646 A2 (YALE UNIVERSITY) 18 July 2013 (18.07.2013), entire document	1-5
A	US 2002/0068063 A1 (DESHAIES et al.) 06 June 2002 (06.06.2002), entire document	1-5
A	WO 2017/197051 A1 (C4 THERAPEUTICS) 16 November 2017 (16.11.2017), entire document	1-5

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

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

"D" document cited by the applicant in the international application

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

23 September 2020 (23.09.2020)

Date of mailing of the international search report

02 DEC 2020

Name and mailing address of the ISA/US

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

Facsimile No. 571-273-8300

Authorized officer

Lee Young

Telephone No. PCT Helpdesk: 571-272-4300

Box III (Observations where Unity is Lacking)

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

Group I: Claims 1-5, drawn to a compound of Formula I.

Group II: Claims 15-19, drawn to a method of treating a cancer in a subject in need thereof, comprising administering a therapeutically effective amount of a compound of Formula I.

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

Special Technical Features

Group II requires a method of treating a cancer in a subject in need thereof, comprising administering a therapeutically effective amount of a compound of Formula I, not required by group I.

Shared Common Features

Groups I and II share the technical feature of the compound of Formula I. However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is anticipated by the article entitled "Posttranslational protein knockdown coupled to receptor tyrosine kinase activation with phosphoPROTACs" to HINES, John et al. (hereinafter HINES).

HINES discloses the compound of formula I wherein X is a destabilization amino acid residue (p.8943, Figure 1 (C), structure of compound of formula I, legend-"green polyD-arginine motif", X is the terminal D-Arg functionalized with CONH2), Y is a linker (p.8943, Figure 1 (C), Y is the portion of compound between the red decapeptide TrkA trans-autophosphorylation site and terminal D-arginine functionalized with CONH2) and Z is a ligand that binds a tyrosine kinase (p.8943, Figure 1 (C), Z is the red decapeptide sequence functionalized with NH2, Figure 1(B), and p.8943 col 1 paras 1-2- "receptor tyrosine kinase, TrkA...decapeptide sequence (Fig. 1C) whose central tyrosine is phosphorylated by TrkA following NGF binding and receptor dimerization").

As the technical feature was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

Groups I and II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.

Item 4 (contd.):

Claims 6-14 and 20-26 are determined unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).