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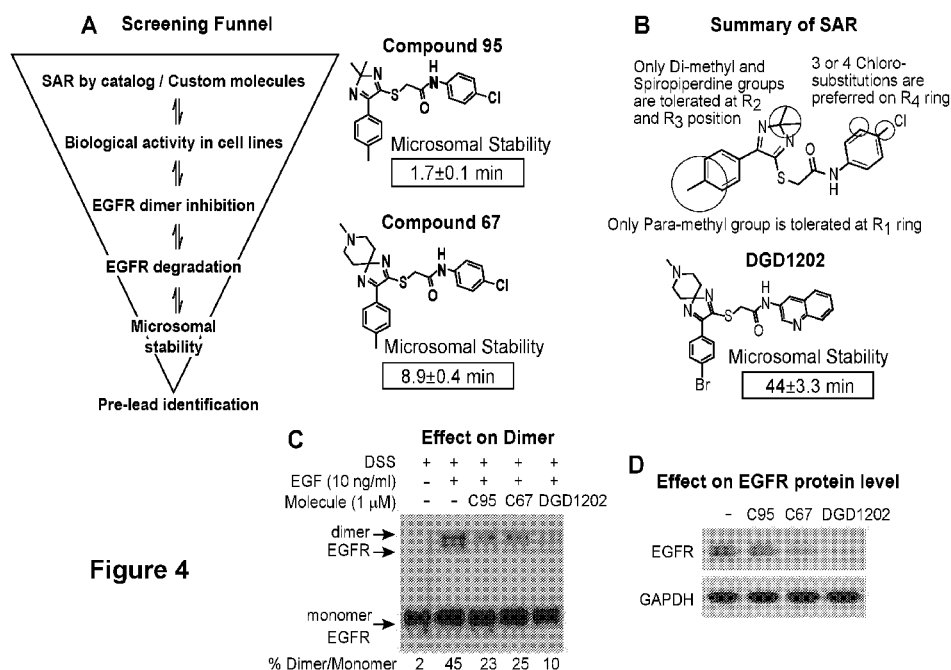


Figure 4

(57) Abstract: Provided herein are compounds that modulate EGFR and methods of using the same, for example to treat cancer.



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EGFR DIMER DISRUPTORS AND USE OF THE SAME

BACKGROUND

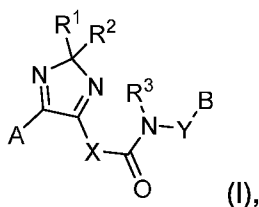
[0001] The EGFR small molecule tyrosine kinase inhibitors (TKI's) erlotinib, gefitinib, and afatinib have been most successful as single agents in the treatment of lung adenocarcinomas that have somatic mutations (such as L858R or deletion in exon 19, i.e. E746-A750) that confer sensitivity to this class of drugs, which occur in 7-20% of patients depending on ethnicity and gender(19). Unfortunately, responses rarely last more than a year because virtually all patients develop resistance to therapy (20). A third-generation irreversible inhibitor, osimertinib (AZD9291), is effective in treating naïve as well as patients who have acquired resistance to first or second generation TKIs (7). However, within a year of treatment with osimertinib, a majority of patients develop another mutation in the EGFR kinase domain (C797S), which is the drug binding site (12, 21, 22). Although several approaches to target osimertinib resistant EGFR have been reported (12, 13, 23), as of now no TKI treatment option exists for these patients with this C797S mutation. Chemotherapy is the only option.

[0002] In view of the foregoing, there exists a need for a cancer therapeutic that targets EGFR in a manner other than inhibition of EGFR tyrosine kinase activity. There also exists a need for a therapeutic that treats cancer without drug resistance developing after initial use.

SUMMARY

[0003] Provided herein are compounds and methods for modulating EGFR. More particularly, provided are modulators of EGFR and the uses of such modulators in treating or preventing diseases or disorders associated with aberrant EGFR activity, e.g., cancer.

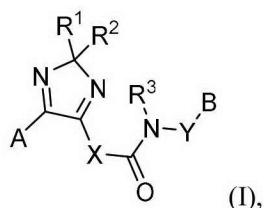
[0004] In one aspect, the disclosure provides compounds, or pharmaceutically acceptable salts thereof, of Formula I:



wherein X is O- C₀₋₆alkylene, S- C₀₋₆alkylene, or NR³- C₀₋₆alkylene, and said alkylene is X is O- C₀₋₆alkylene, S- C₀₋₆alkylene, or NR³- C₀₋₆alkylene, and said alkylene is optionally substituted with 1-3 groups independently selected from halo, N(R³)₂, and OR³; Y is C₀₋₆alkylene, and said alkylene is optionally substituted with 1-3 groups independently selected from halo, N(R³)₂, and OR³; A is C₆₋₁₀ aryl or 5-10 membered heteroaryl having 1-4 heteroatoms selected from N, O, and S, and said aryl or heteroaryl is optionally substituted with 1 to 3 R⁴; B is C₆₋₁₀ aryl, 5-10 membered heteroaryl having 1-4 heteroatoms selected

from N, O, and S, 3-8 membered cycloalkyl ring, or 3-12 membered heterocycloalkyl having 1-3 ring heteroatoms selected from O, S, and N, and said aryl, heteroaryl, cycloalkyl, or heterocycloalkyl is optionally substituted with 1 to 3 R⁵; R¹ and R² are each independently G-i-@ alkyl, or R¹ and R² together with the carbon atom to which they are attached form a 4-8 membered cycloalkyl or heterocycloalkyl ring, wherein the heterocycloalkyl ring has 1 or 2 ring heteroatoms selected from O, S, and N, and wherein said cycloalkyl ring or heterocycloalkyl ring is optionally substituted with 1-2 R⁶; each R³ is independently H or C₁₋₆ alkyl; each R⁴ and R⁵ is independently alkyl, haloalkyl, halo, or alkoxy; and R⁶ is C-i-@ alkyl, haloalkyl, (C=O)R³, (C=O)OR³, CON(R³)₂, C₀₋₃alkylene-C₃₋₈cycloalkyl, C₀₋₃alkylene-C₆₋₁₀aryl, or C₀₋₃alkylene-(5-10 membered heteroaryl having 1-4 heteroatoms selected from N, O, and S), wherein the aryl or heteroaryl is optionally substituted with 1 to 3 R⁵.

[0004a] In one aspect of the disclosure, there is provided a compound, or pharmaceutically acceptable salt thereof, having the structure of Formula I:



wherein

X is O- C₀₋₆alkylene, S- C₀₋₆alkylene, or NR³- C₀₋₆alkylene, and said alkylene is optionally substituted with 1-3 groups independently selected from halo, N(R³)₂, and OR³;

Y is C₀₋₆alkylene, and said alkylene is optionally substituted with 1-3 groups independently selected from halo, N(R³)₂, and OR³;

A is C₆₋₁₀ aryl or 5-10 membered heteroaryl having 1-4 heteroatoms selected from N, O, and S, and said aryl or heteroaryl is optionally substituted with 1 to 3 R⁴;

B is 5-10 membered heteroaryl having 1-4 heteroatoms selected from N, O, and S, and said heteroaryl optionally substituted with 1 to 3 R⁵;

R¹ and R² are each independently C₁₋₆ alkyl, or R¹ and R² together with the carbon atom to which they are attached form a 4-8 membered cycloalkyl or heterocycloalkyl ring, wherein the heterocycloalkyl ring has 1 or 2 ring heteroatoms selected from O, S, and N, and wherein said cycloalkyl ring or heterocycloalkyl ring is optionally substituted with 1-2 R⁶;

each R³ is independently H or C₁₋₆ alkyl;

each R⁴ and R⁵ is independently C₁₋₆ alkyl, C₁₋₆ haloalkyl, halo, or C₁₋₆ alkoxy; and

R⁶ is C₁₋₆ alkyl, C₁₋₆ haloalkyl, (C=O)R³, (C=O)OR³, CON(R³)₂, C₀₋₃alkylene-C₃₋₈cycloalkyl, C₀₋₃alkylene-C₆₋₁₀aryl, or C₀₋₃alkylene-(5-10 membered heteroaryl having 1-4 heteroatoms selected from N, O, and S), wherein the aryl or heteroaryl is optionally substituted with 1 to 3 R⁵

[0005] Further provided herein are methods of using the compounds disclosed to modulate EGFR. Other aspects of the disclosure include methods of using the compounds disclosed to inhibit EGFR dimerization, and methods of using the compounds disclosed to induce EGFR degradation.

[0006] Other aspects of the disclosure include a compound as disclosed herein for use in the preparation of a medicament for treating or preventing a disease or disorder associated with aberrant EGFR activity in a subject, and the use of a compound as disclosed herein in a method of treating or preventing a disease or disorder associated with aberrant EGFR activity in a subject.

BRIEF DESCRIPTION OF THE FIGURES

[0007] Figure 1 shows (left) treatment with a small peptide that inhibits EGFR dimerization affected tumor (marked with *) specific EGFR more compared to adjacent normal tissue; and (right) a schematic for degradation of EGFR. EGFR is shown by an extracellular domain connected by a transmembrane portion to a bilobular kinase domain (small blob = n-lobe, large blob = c-lobe) and a flexible C-terminal tail. EGF binding promotes active (asymmetric) dimer formation between extracellular domains and/or between n- and c-lobes of two monomers. EGFR in such a conformation remains stable and induces phosphorylation of the C-terminal tail, which promotes tumor progression. Tyrosine kinase inhibitors (e.g. osimertinib), inhibit ATP recruitment and promote inactive (symmetric) dimerization between n-lobes of two monomers. EGFR in this conformation remains in kinase-inactive conformation, but protein stability is maintained. Inactivation of EGFR activity correlates with tumor growth inhibition. The loop between the α C-helix and the b4-sheets of the c-lobe and h-helix of the n-lobe of EGFR participate in EGF-induced active dimer formation. This model assumes that Disruptin or Compound 8C binds in this pocket

[Text continued on page 3]

and interferes with EGF-induced active dimer formation. The ligand and Compound 8C bound-EGFR monomer degrades rapidly. Loss of EGFR protein correlates with cell-death. The thickness of arrow shows the effect of Compound 8C in tumor vs. normal cells.

[0008] Figure 2 shows (A) a schematic diagram of an asymmetric, active dimer of EGFR. One EGF-bound monomer of EGFR is sufficient to induce dimerization. A model of Disruptin bound to the c-lobe of EGFR (PDB Code: 2RFD) is shown in the inset and (B) hypothesized interaction between purified EGFR kinase domain and Disruptin. .

[0009] Figure 3 shows (A) the effect of Disruptin treatment on EGF induced dimerization in NCI-H1975 cells; (B) the effect of Disruptin on its target EGFR in NCI-H1975 xenografts; (C) the efficacy of Disruptin *in vivo*; and (D) the long term effect of treatment on tumor histology, EGFR expression, and mitotic index (measured by Ki-67 score).

[0010] Figure 4 shows (A) the process for pre-screening lead compounds, and the structures of two pre-lead compounds, C95 and C67; (B) the resulting SAR combined with microsomal stability (shown in the blue box), as well as the structure of the most selectively effective molecule, named Compound 8C; (C) the effect of two pre-leads and Compound 8C on EGF-stimulated EGFR dimerization in an erlotinib-resistant NCI-H1975 lung cancer cell line as described in Figure 3A (lysates were prepared and immunoblotted with anti-EGFR antibodies); and (D) the effect of selected three lead molecules (1 μ M) on steady-state EGFR protein as assessed at 24 hours post-treatment.

[0011] Figure 5 shows (A) Compound 8C competes with Disruptin for EGFR binding; (B) the effect of Compound 8C on the thermal stability of purified EGFR as confirmed by thermal stability assay; and (C) the effect of Compound 8C concentration on thermal stability of EGFR at 44° C in the presence of 0 to 10 μ M Compound 8C.

[0012] Figure 6 shows the (A) effect of Compound 8C on EGFR thermal stability in a whole cell lysate from Ba/F3-AZD cells;. (B) potency of Compound 8C as determined against osimertinib-resistant Ba/F3 cells expressing specific EGFR mutations; (C) the effect of Compound 8C on EGF induced EGFR dimerization as described in Figure 3A; (C-D). effect of Compound 8C treatment on EGFR induced dimer and EGFR protein level; (E) IC₅₀ values in response to erlotinib, osimertinib and Compound 8C as tabulated from two osimertinib resistant Ba/F3 cell lines.

[0013] Figure 7 shows (A) the specificity and potency of Compound 8C determined against a panel of osimertinib-resistant cells and compared with normal lung fibroblasts (MRC5) using the clonogenic survival assay; (B) the effect of Compound 8C concentration on EGF induced EGFR dimerization in osimertinib resistant PC9 cells as described in Figure 6; (C) the effect of Compound 8C treatment on EGFR induced dimer and EGFR protein level in PC9-AZR cells.

[0014] Figure 8 shows (A) the pharmacokinetics of a single 100 mg/kg dose of Compound 8C given intraperitoneally in nude mice bearing human NCI-H1975 tumor xenografts (>150 mm³); and (B) the pharmacokinetics of a single 100 mg/kg dose of Compound 8C given by oral gavage in nude mice bearing human NCI-H1975 tumor xenografts (>150 mm³).

[0015] Figure 9 shows (A) the basal bioluminescence and effect of pre-lead compound 95 on different time points in NCI-H1975 xenograft-bearing mice; and (B) the quantified and plotted change in bioluminescence.

[0016] Figure 10 shows the change in the average tumor volume with time of nude mice bearing UMSCC74B, a head and neck tumor model (~100 mm²) and treated with Compound 8C (30 mg/kg, daily for one week) or with vehicle (5% DMSO in PBS). Each group had at least 5 mice. Tumor volume and body weight was recorded 3-4 times a week, and is plotted. The average loss in body weight during treatment was less than 10%. Error bar represents standard error of the mean.

[0017] Figure 11 shows the effect of Compound 8C in an osimertinib resistant tumor model. Nude mice with osimertinib resistant Ba/F3 ascites tumor were treated with either vehicle, osimertinib or Compound 8C. The effect of treatment on EGFR, pEGFR and other molecules was determined by immunoblotting.

[0018] Figure 12 shows the pathological assessment of FFPE-lung PDXs in a primary tumor, 1st and 2nd xenograft of UMLCA7 and analysis of EGFR protein expression using IHC in two other PDXs. Note the abundant EGFR expression in the squamous cell carcinoma (UMLT16) but not in the large cell carcinoma (UMLT14) PDX sample (20X).

[0019] Figure 13 shows that treating a mouse pancreatic cancer model with Compound 8C showed significantly reduced propensity for developing PanIn (Pancreatic Intraepithelial Neoplasia), a type of pancreatic duct lesion. A- histological examination of pancreatic tissue shows significantly less lesion involvement in treated mice compared to a control, which is quantified in B.

[0020] Figure 14 shows that mouse xenografts bearing the UMSCC74B head and neck tumor model, treated with Compound 8C displayed significantly smaller tumors compared to control mice which received either treatment vehicle or cetuximab.

DETAILED DESCRIPTION

[0021] Although inhibition of the kinase activities of oncogenic proteins using small molecules and antibodies has been a mainstay of anticancer drug development efforts, resulting in several FDA-approved cancer therapies, the clinical effectiveness of kinase-targeted agents has been inconsistent (22, 24). EGFR has been shown to exhibit scaffold functions in addition to its tyrosine kinase activity (24-36). This is demonstrated by either

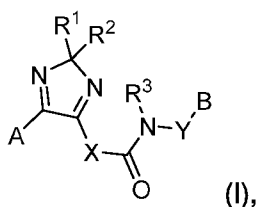
expressing a kinase-dead (KD) mutant of EGFR (e.g. K745A, V741G, and Y740F) or by expressing ErbB3 (which has no kinase activity) in Ba/F3 cells that do not express these receptors (37-39). Expression of these kinase-defective mutants promotes cell survival, indicating that these receptors can still transmit a survival signal perhaps by forming dimers, suggesting that EGFR has functions beyond kinase activity (39).

[0022] EGFR dimers are known to be relatively stable when compared to the monomers (40). Dimers are capable of generating downstream mitogenic signaling (41). Without being bound by theory, it is hypothesized that blocking EGFR dimerization would accelerate degradation of EGFR, and that this approach would be effective against tumors that are driven by TKI resistant EGFR (14, 24, 27). Briefly, it was demonstrated that EGF bound EGFR (that is phosphorylated-EGFR, prevalent in most tumors) protein stability is regulated by formation of dimers via a segment within the kinase domain of EGFR that lies between α C helix and β 4 sheets of the c-lobe and h-helix of the n-lobe of the EGFR kinase domain (15, 42). EGFR protein stability in normal cells is not primarily regulated by this dimer interface because, in the absence of EGF, EGFR does not form an asymmetric dimer (43). This difference between tumor and normal cells provides a new targetable protein-protein interaction.

[0023] To test this idea, over a dozen peptides that mimic this binding surface were generated. The most effective peptide, containing the six amino acids from the α C- β 4 loop of the EGFR, was named Disruptin(17). Disruptin is capable of inhibiting EGF-induced dimerization of EGFR. This peptide binds directly to EGFR, and this binding is not affected significantly with repeated HEPES washes compared to a control (scrambled) peptide. Although Disruptin is effective in a tyrosine kinase inhibitor (TKI) resistant lung xenograft model (14), delivery of peptides in humans remains challenging (44).

[0024] Provided herein are compounds which modulate EGFR, for example, compounds which block EGFR dimerization, induce EGFR degradation, and kill EGFR driven cells. These compounds are useful in the prevention or treatment of a variety of diseases and disorders, for example, in the treatment of cancer.

[0025] As such, provided herein are compounds, or pharmaceutically acceptable salts thereof, having the structure of Formula I:



wherein

X is O- C₀₋₆alkylene, S- C₀₋₆alkylene, or NR³- C₀₋₆alkylene, and said alkylene is

optionally substituted with 1-3 groups independently selected from halo, $N(R^3)_2$, and OR^3 ;

Y is C_{0-6} alkylene, and said alkylene is optionally substituted with 1-3 groups independently selected from halo, $N(R^3)_2$, and OR^3 ;

A is C_{6-10} aryl or 5-10 membered heteroaryl having 1-4 heteroatoms selected from N, O, and S, and said aryl or heteroaryl is optionally substituted with 1 to 3 R^4 ;

B is C_{6-10} aryl, 5-10 membered heteroaryl having 1-4 heteroatoms selected from N, O, and S, 3-8 membered cycloalkyl ring, or 3-12 membered heterocycloalkyl having 1-3 ring heteroatoms selected from O, S, and N, and said aryl, heteroaryl, cycloalkyl, or heterocycloalkyl is optionally substituted with 1 to 3 R^5 ;

R^1 and R^2 are each independently C_{1-6} alkyl, or R^1 and R^2 together with the carbon atom to which they are attached form a 4-8 membered cycloalkyl or heterocycloalkyl ring, wherein the heterocycloalkyl ring has 1 or 2 ring heteroatoms selected from O, S, and N, and wherein said cycloalkyl ring or heterocycloalkyl ring is optionally substituted with 1-2 R^6 ;

each R^3 is independently H or C_{1-6} alkyl;

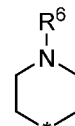
each R^4 and R^5 is independently C_{1-6} alkyl, C_{1-6} haloalkyl, halo, or C_{1-6} alkoxy; and

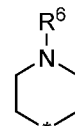
R^6 is C_{1-6} alkyl, C_{1-6} haloalkyl, $(C=O)R^3$, $(C=O)OR^3$, $CON(R^3)_2$, C_{0-3} alkylene- C_{3-8} cycloalkyl, C_{0-3} alkylene- C_{6-10} aryl, or C_{0-3} alkylene-(5-10 membered heteroaryl having 1-4 heteroatoms selected from N, O, and S), wherein the aryl or heteroaryl is optionally substituted with 1 to 3 R^5 .

[0026] In various embodiments, R^1 and R^2 are each independently C_{1-6} alkyl. In some embodiments, R^1 and R^2 are each methyl.

[0027] In various embodiments, R^1 and R^2 together with the carbon atom to which they are attached form a 4-8 membered cycloalkyl or heterocycloalkyl ring. In some embodiments, R^1 and R^2 together with the carbon atom to which they are attached form a 5 or 6 membered cycloalkyl or heterocycloalkyl ring. In some embodiments, R^1 and R^2 together with the carbon atom to which they are attached form a cyclohexyl ring.

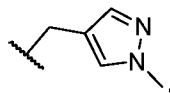
[0028] In various embodiments, R^1 and R^2 together with the carbon atom to which they



are attached form a heterocycloalkyl ring having the structure: , where * indicates the point of attachment to the rest of the compound of Formula I. In some embodiments, R^6 is C_{1-6} alkyl, C_{1-6} haloalkyl, $(C=O)R^3$, $(C=O)OR^3$, $CON(R^3)_2$, C_{0-3} alkylene- C_{3-8} cycloalkyl, C_{0-3} alkylene- C_{6-10} aryl, or C_{0-3} alkylene-(5-10 membered heteroaryl having 1-4 heteroatoms selected from N, O, and S), wherein the aryl or heteroaryl is optionally substituted with 1 to 3 R^5 . In some embodiments, R^6 is C_{1-6} alkyl, $(C=O)R^3$, $(C=O)OR^3$, or $CON(R^3)_2$. In some embodiments, R^6 is C_{1-6} alkyl. In some embodiments, R^6 is methyl, ethyl, propyl, isopropyl,

isobutyl, or isopentyl. In some embodiments, R⁶ is methyl. In some embodiments, R⁶ is deuterated. In some embodiments, R⁶ is C₁₋₆ haloalkyl. In some embodiments, R⁶ is 3,3,3-trifluoropropyl. In some embodiments, R⁶ is C₀₋₃alkylene-C₃₋₈cycloalkyl. In some embodiments, R⁶ is cyclobutyl, cyclopentyl, or cyclohexyl. In some embodiments, R⁶ is cyclobutyl or cyclopentyl. In some embodiments, R⁶ is C₀₋₃alkylene-C₆₋₁₀aryl. In some embodiments, R⁶ is benzyl. In some embodiments, R⁶ is C₀₋₃alkylene-(5-10 membered heteroaryl having 1-4 heteroatoms selected from N, O, and S), wherein the heteroaryl is optionally substituted with 1 to 3 R⁵. In some embodiments, R⁶ is C₁alkylene-(5-10 membered heteroaryl having 1-4 heteroatoms selected from N, O, and S), wherein the heteroaryl is optionally substituted with 1 to 3 R⁵. In some embodiments, R⁶ is C₀₋₃alkylene-(5-10 membered heteroaryl having 1-4 heteroatoms selected from N, O, and S), wherein the heteroaryl is substituted with 1 to 3 R⁵. In some embodiments, R⁶ is C₀₋₃alkylene-(5-10 membered heteroaryl having 1-4 heteroatoms selected from N, O, and S), wherein the

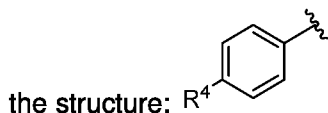
heteroaryl is unsubstituted. In some embodiments, R⁶ is

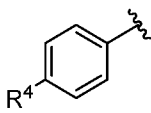


[0029] In various embodiments, A is C₆₋₁₀ aryl. In some embodiments, A is phenyl.

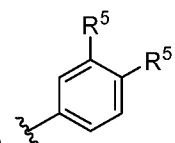
[0030] In various embodiments, B is C₆₋₁₀ aryl. In some embodiments, B is phenyl. In various embodiments, B is 5-10 membered heteroaryl having 1-4 heteroatoms selected from N, O, and S. In some embodiments, B is pyridinyl. In some embodiments, B is quinolinyl. In various embodiments, B is 3-8 membered cycloalkyl. In some embodiments, B is 5 or 6 membered cycloalkyl. In various embodiments, B is 3-12 membered heterocycloalkyl having 1-3 ring heteroatoms selected from O, S, and N.

[0031] In some embodiments, A is substituted with one R⁴. In some embodiments, A has



the structure: . In some embodiments, A is substituted with two R⁴. In some embodiments, at least one R⁴ is C₁₋₆ alkyl. In some embodiments, at least one R⁴ is methyl. In some embodiments, at least one R⁴ is halo. In some embodiments, R⁴ is bromo. In some embodiments, at least one R⁴ is C₁₋₆ alkoxy. In some embodiments, at least one R⁴ is methoxy.

[0032] In some embodiments, B is substituted with one R⁵. In some embodiments, B is

substituted with two R⁵. In some embodiments, B has the structure . In some embodiments, at least one R⁵ is halo. In some embodiments, at least one R⁵ is fluoro or chloro. In some embodiments, one R⁵ is fluoro and the other R⁵ is chloro. In some

embodiments, at least one R⁵ is C₁₋₆ alkoxy. In some embodiments, at least one R⁵ is methoxy. In some embodiments, one R⁵ is halo and the other R⁵ is C₁₋₆ alkoxy. In some embodiments, one R⁵ is chloro and the other R⁵ is methoxy.

[0033] In some embodiments, each R⁴ and R⁵ is independently C₁₋₆ alkyl, halo, or C₁₋₆ alkoxy. In some embodiments, R⁶ is C₁₋₆ alkyl, (C=O)R³, (C=O)OR³, or CON(R³)₂.

[0034] In various embodiments, X is O- C₀₋₆alkylene or S- C₀₋₆alkylene. In some embodiments, X is S- C₀₋₆alkylene. In some embodiments, X is O, S, O-CH₂-, or S-CH₂-. In various embodiments, Y is C₀₋₂alkylene. In some embodiments, Y is null or CH₂. In some embodiments, X is NR³-CH₂, O-CH₂-, or S-CH₂-, and Y is null. In some embodiments, X is NR³-CH₂, O-CH₂-, or S-CH₂-, and Y is CH₂. In some embodiments, R³ is H.

[0035] Specific compounds contemplated include those listed in Table 1, Table 2, or a pharmaceutically acceptable salt thereof:

Table 1

ID #	STRUCTURE	ID #	STRUCTURE
13		17	
14		18	
15		19	
16			

ID #	STRUCTURE
20	
21	
22	
23	
24	

ID #	STRUCTURE
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26	
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28	
29	

ID #	STRUCTURE
8A	
8B	
8C	
8D	
8E	

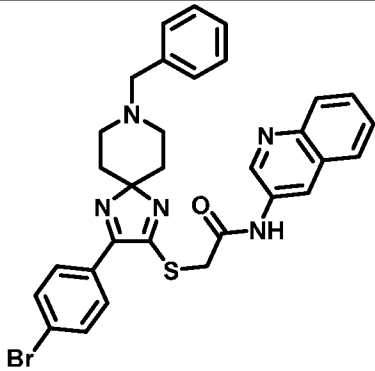
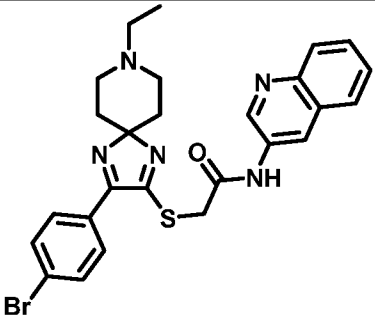
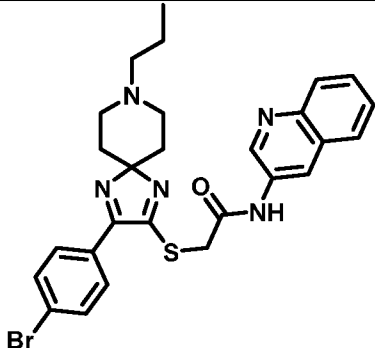
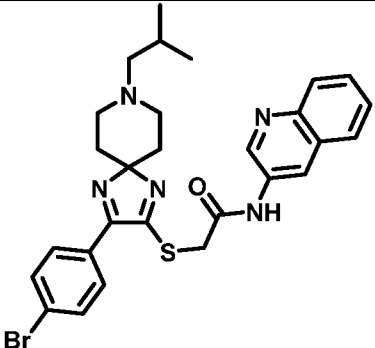
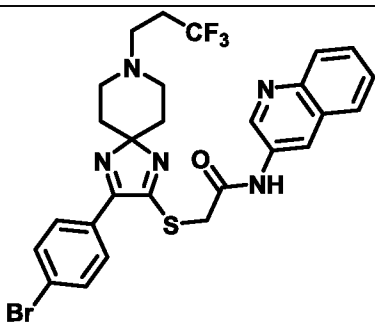
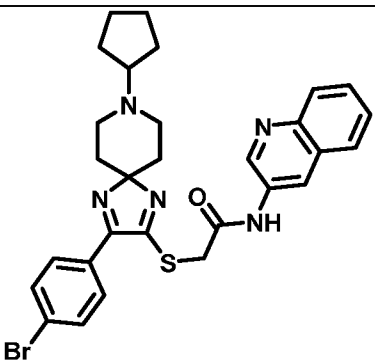
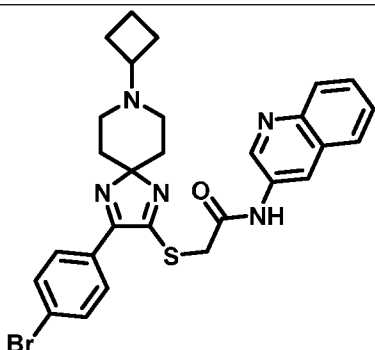
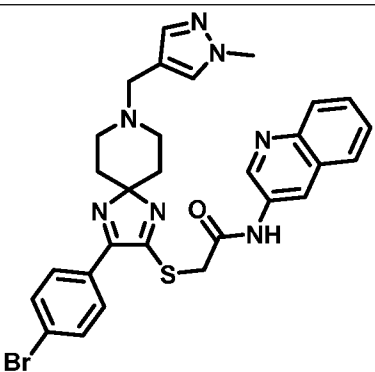
ID #	STRUCTURE
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8G	
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11	
12	

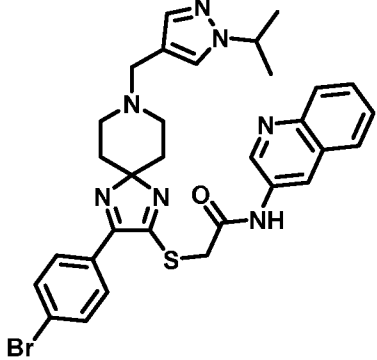
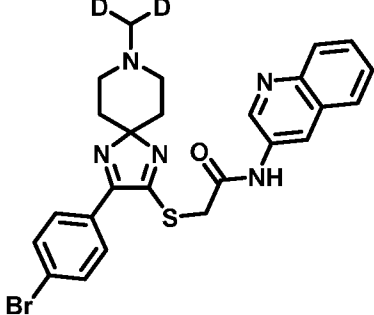
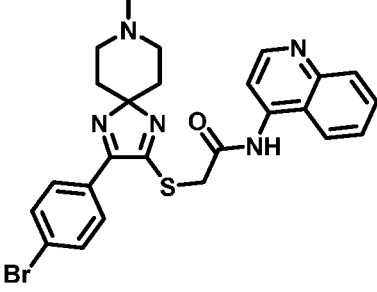
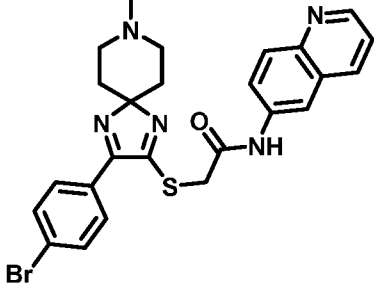
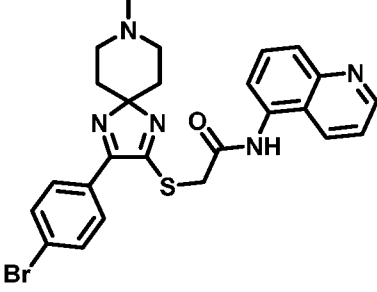
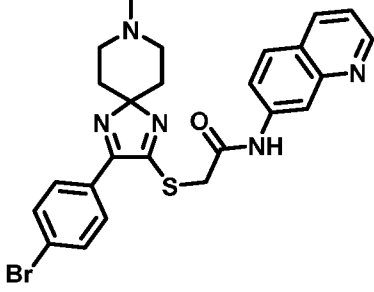
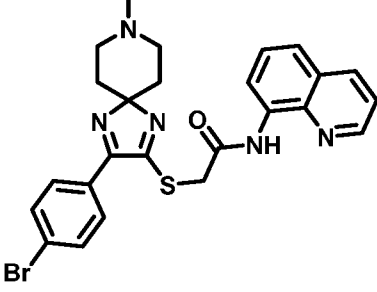
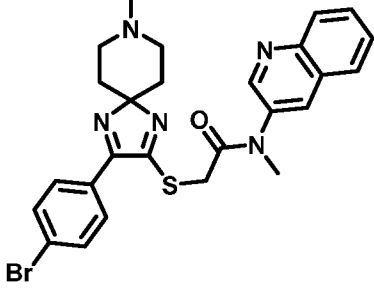
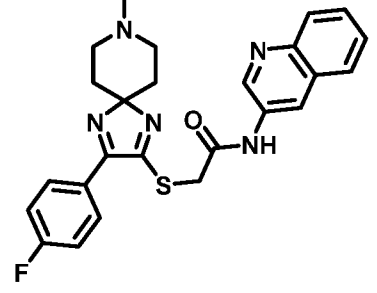
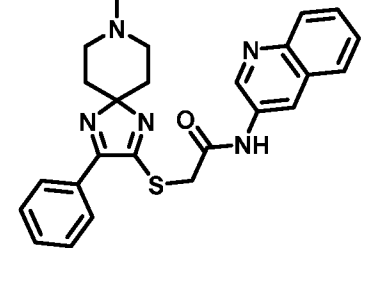
ID #	STRUCTURE
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31	
32	

ID #	STRUCTURE
3	
4	
5	

Table 2

ID#	Structure	ID#	Structure
33a		33b	

ID#	Structure	ID#	Structure
33c		33d	
33e		33f	
33g		33h	
33i		33j	

ID#	Structure	ID#	Structure
33k		33l	
34a		34b	
34c		34d	
34e		34f	
35a		35b	

ID#	Structure	ID#	Structure
35c		35d	
35e		35f	
35g		35h	
35i			

[0036] In some cases, the compound is a compound listed in Table 1, or salt thereof.

[0037] Compound 8C showed significant improvement in pharmacological properties as well as biological activity (microsomal half-life over 46 minutes, sub-micromolar IC₅₀ in the clonogenic cellular assay). Compound 8C inhibits EGF-induced EGFR dimerization, directly binds to purified EGFR, and is selectively active in EGFR-driven osimertinib resistant cell lines and in xenograft models.

Definitions

[0038] As used herein, the term “alkyl” refers to straight chained and branched saturated hydrocarbon groups containing one to thirty carbon atoms, for example, one to twenty carbon atoms, or one to ten carbon atoms. The term C_n means the alkyl group has “n” carbon atoms. For example, C_4 alkyl refers to an alkyl group that has 4 carbon atoms. C_1 - C_7 alkyl refers to an alkyl group having a number of carbon atoms encompassing the entire range (e.g., 1 to 7 carbon atoms), as well as all subgroups (e.g., 1-6, 2-7, 1-5, 3-6, 1, 2, 3, 4, 5, 6, and 7 carbon atoms). Nonlimiting examples of alkyl groups include, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl (2-methylpropyl), t-butyl (1,1-dimethylethyl), 3,3-dimethylpentyl, and 2-ethylhexyl. Unless otherwise indicated, an alkyl group can be an unsubstituted alkyl group or a substituted alkyl group.

[0039] The term “alkylene” used herein refers to an alkyl group having a substituent. For example, the term “alkylenehalo” refers to an alkyl group substituted with a halo group. For example, an alkylene group can be $-CH_2CH_2-$ or $-CH_2-$. The term C_n means the alkylene group has “n” carbon atoms. For example, C_{1-6} alkylene refers to an alkylene group having a number of carbon atoms encompassing the entire range, as well as all subgroups, as previously described for “alkyl” groups. Unless otherwise indicated, an alkylene group can be an unsubstituted alkylene group or a substituted alkylene group.

[0040] As used herein, the term “cycloalkyl” refers to an aliphatic cyclic hydrocarbon group containing three to eight carbon atoms (e.g., 3, 4, 5, 6, 7, or 8 carbon atoms). The term C_n means the cycloalkyl group has “n” carbon atoms. For example, C_5 cycloalkyl refers to a cycloalkyl group that has 5 carbon atoms in the ring. C_6 - C_8 cycloalkyl refers to cycloalkyl groups having a number of carbon atoms encompassing the entire range (e.g., 6 to 8 carbon atoms), as well as all subgroups (e.g., 6-7, 7-8, 6, 7, and 8 carbon atoms). Nonlimiting examples of cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl. Unless otherwise indicated, a cycloalkyl group can be an unsubstituted cycloalkyl group or a substituted cycloalkyl group. The cycloalkyl groups described herein can be isolated or fused to another cycloalkyl group, a heterocycloalkyl group, an aryl group and/or a heteroaryl group. When a cycloalkyl group is fused to another cycloalkyl group, then each of the cycloalkyl groups can contain three to eight carbon atoms unless specified otherwise. Unless otherwise indicated, a cycloalkyl group can be unsubstituted or substituted.

[0041] As used herein, the term “heterocycloalkyl” is defined similarly as cycloalkyl, except the ring contains one to three heteroatoms independently selected from oxygen, nitrogen, and sulfur. In particular, the term “heterocycloalkyl” refers to a ring containing a total of three to twelve atoms (e.g., 3-8, 5-8, 3-6, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12), of which 1,

2, or 3 of the ring atoms are heteroatoms independently selected from the group consisting of oxygen, nitrogen, and sulfur, and the remaining atoms in the ring are carbon atoms. Nonlimiting examples of heterocycloalkyl groups include piperidine, pyrazolidine, tetrahydrofuran, tetrahydropyran, dihydrofuran, morpholine, and the like.

[0042] Cycloalkyl and heterocycloalkyl groups can be saturated or partially unsaturated ring systems optionally substituted with, for example, one to three groups, independently selected alkyl, alkyleneOH, C(O)NH₂, NH₂, oxo (=O), aryl, alkylenehalo, halo, and OH. Heterocycloalkyl groups optionally can be further N-substituted with alkyl (e.g., methyl or ethyl), alkylene-OH, alkylenearyl, and alkyleneheteroaryl. The heterocycloalkyl groups described herein can be isolated or fused to another heterocycloalkyl group, a cycloalkyl group, an aryl group, and/or a heteroaryl group. When a heterocycloalkyl group is fused to another heterocycloalkyl group, then each of the heterocycloalkyl groups can contain three to twelve total ring atoms, and one to three heteroatoms. Unless otherwise indicated, a heterocycloalkyl group can be unsubstituted or substituted.

[0043] As used herein, the term “aryl” refers to a monocyclic or bicyclic aromatic group, having 6 to 10 ring atoms. Unless otherwise indicated, an aryl group can be unsubstituted or substituted with one or more, and in particular one to four groups independently selected from, for example, halo, alkyl, alkenyl, OCF₃, NO₂, CN, NC, OH, alkoxy, amino, CO₂H, CO₂alkyl, aryl, and heteroaryl. Aryl groups can be isolated (e.g., phenyl) or fused to another aryl group (e.g., naphthyl, anthracenyl), a cycloalkyl group (e.g. tetrahydronaphthyl), a heterocycloalkyl group, and/or a heteroaryl group

[0044] As used herein, the term “heteroaryl” refers to a monocyclic or bicyclic aromatic ring having 5 to 10 total ring atoms, and containing one to four heteroatoms selected from nitrogen, oxygen, and sulfur atom in the aromatic ring. Unless otherwise indicated, a heteroaryl group can be unsubstituted or substituted with one or more, and in particular one to four, substituents selected from, for example, halo, alkyl, alkenyl, OCF₃, NO₂, CN, NC, OH, alkoxy, amino, CO₂H, CO₂alkyl, aryl, and heteroaryl. In some cases, the heteroaryl group is substituted with one or more of alkyl and alkoxy groups. Examples of heteroaryl groups include, but are not limited to, thienyl, furyl, pyridyl, pyrrolyl, oxazolyl, triazinyl, triazolyl, isothiazolyl, isoxazolyl, imidazolyl, pyrazinyl, pyrimidinyl, thiazolyl, and thiadiazolyl.

[0045] As used herein, the term “alkoxy” or “alkoxyl” as used herein refers to a “—O-alkyl” group. The alkoxy or alkoxyl group can be unsubstituted or substituted.

[0046] As used herein, the term “therapeutically effective amount” means an amount of a compound or combination of therapeutically active compounds that ameliorates, attenuates or eliminates one or more symptoms of a particular disease or condition (e.g., cancer), or prevents or delays the onset of one of more symptoms of a particular disease or condition.

[0047] As used herein, the terms “patient” and “subject” may be used interchangeably and mean animals, such as dogs, cats, cows, horses, and sheep (e.g., non-human animals) and humans. Particular patients or subjects are mammals (e.g., humans).

[0048] As used herein, the term “pharmaceutically acceptable” means that the referenced substance, such as a compound of the present disclosure, or a formulation containing the compound, or a particular excipient, are safe and suitable for administration to a patient or subject. The term “pharmaceutically acceptable excipient” refers to a medium that does not interfere with the effectiveness of the biological activity of the active ingredient(s) and is not toxic to the host to which it is administered.

[0049] The compounds disclosed herein can be as a pharmaceutically acceptable salt. As used herein, 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. For example, S. M. Berge et al. describe pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences*, 1977, 66, 1-19, which is incorporated herein by reference. Pharmaceutically acceptable salts of the compounds of this invention include those derived from suitable inorganic and organic acids and bases. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, trifluoroacetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, glutamate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. Salts of compounds containing a carboxylic acid or other acidic functional group can be prepared by reacting with a suitable base. Such salts include, but are not limited to, alkali metal, alkaline earth metal, aluminum salts, ammonium, $N^+(C_{1-4}alkyl)_4$ salts, and salts of organic bases such as trimethylamine, triethylamine, morpholine, pyridine, piperidine, picoline, dicyclohexylamine, N,N'-dibenzylethylenediamine, 2-hydroxyethylamine, bis-(2-hydroxyethyl)amine, tri-(2-

hydroxyethyl)amine, procaine, dibenzylpiperidine, dehydroabietylamine, N,N'-bisdehydroabietylamine, glucamine, N-methylglucamine, collidine, quinine, quinoline, and basic amino acids such as lysine and arginine. This invention also envisions the quaternization of any basic nitrogen-containing groups of the compounds disclosed herein. Water or oil-soluble or dispersible products may be obtained by such quaternization. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, lower alkyl sulfonate and aryl sulfonate.

[0050] As used herein the terms "treating", "treat" or "treatment" and the like include preventative (e.g., prophylactic) and palliative treatment.

[0051] As used herein, the term "excipient" means any pharmaceutically acceptable additive, carrier, diluent, adjuvant, or other ingredient, other than the active pharmaceutical ingredient (API).

Synthesis of Compounds of the Disclosure

[0052] The compounds disclosed herein can be prepared in a variety of ways using commercially available starting materials, compounds known in the literature, or from readily prepared intermediates, by employing standard synthetic methods and procedures either known to those skilled in the art, or in light of the teachings herein. The synthesis of the compounds disclosed herein can be achieved by generally following the synthetic schemes as described in the Examples section, with modification for specific desired substituents.

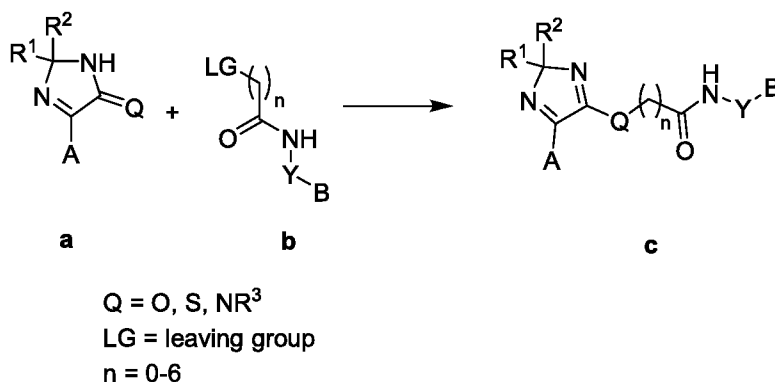
[0053] Standard synthetic methods and procedures for the preparation of organic molecules and functional group transformations and manipulations can be obtained from the relevant scientific literature or from standard textbooks in the field. Although not limited to any one or several sources, classic texts such as Smith, M. B., March, J., March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure, 5th edition, John Wiley & Sons: New York, 2001 ; and Greene, T.W., Wuts, P.G. M., Protective Groups in Organic Synthesis, 3rd edition, John Wiley & Sons: New York, 1999, are useful and recognized reference textbooks of organic synthesis known to those in the art. The following descriptions of synthetic methods are designed to illustrate, but not to limit, general procedures for the preparation of compounds of the present disclosure.

[0054] The synthetic processes disclosed herein can tolerate a wide variety of functional groups; therefore, various substituted starting materials can be used. The processes generally provide the desired final compound at or near the end of the overall process,

although it may be desirable in certain instances to further convert the compound to a pharmaceutically acceptable salt thereof.

[0055] In general, compounds of Formula (I) can be synthesized according to Scheme 1.

Scheme 1



[0056] Compounds having structure **c** can be synthesized using the procedure shown in Scheme 1. Reaction of a substituted 2,5-dihydroimidazole derivative **a** with an amide compound **b** in an appropriate solvent *e.g.*, acetonitrile, produces compounds as described herein, *i.e.*, compounds of Formula (I) having structure **c**. The amide compound **b** comprises an appropriate leaving group LG chosen based on the nature of group Q, *e.g.*, a halogen or tosylate. Appropriate further derivatization reactions of compounds having structure **c** can be selected based on the nature of substituents R¹, R², A, Y, and B.

[0057] The coupling of compounds **a** and **b** can be catalyzed by appropriate reagents selected based on the precise nature of compounds **a** and **b**. For example, when the LG of compound **b** is a halogen (*e.g.*, when LG is chloro), the coupling of compounds **a** and **b** can be catalyzed by a base *e.g.*, sodium carbonate or potassium carbonate. Occasionally, the coupling reaction may not require a catalyst.

[0058] Occasionally, before coupling with a compound having structure **b**, a compound **a** having Q selected from O, S, and NR³ can be transformed into a compound having Q selected from a different member of the group consisting of O, S, and NR³ by treatment with an appropriate reagent. For example, a compound having a structure **a** with Q = O can be transformed into a compound having a structure **a** with Q = S by treatment with a thiation reagent, *e.g.*, Lawesson's reagent or phosphorus pentasulfide. Such a compound can then be coupled with a compound having structure **b** to produce a compound described herein, *i.e.*, a compound of Formula (I) having structure **c**.

[0059] Compounds **a** and **b** can be purchased commercially or prepared by a variety of methods from commercially-available starting materials. For example, amide compounds having structure **b** can be prepared by the reaction of *e.g.* an acyl chloride with an amine.

[0060] Further derivatization reactions to transform compounds having structure **c** into other compounds disclosed herein can be selected based on the nature of the substituents R¹, R², A, Y, and B in compound **c** and the functionality desired in the derivative compound. For example, R¹ and R² together with the carbon atom to which they are attached can form a heterocycling ring, e.g. a piperidine ring, which can be further derivatized by methods known in the art (e.g., methylation, addition of protecting groups, etc.) to form a variety of other compounds of Formula (I) described herein.

Pharmaceutical formulations, dosing, and routes of administration

[0061] Further provided are pharmaceutical formulations comprising a compound as described herein (e.g., compounds of Formula I, or pharmaceutically acceptable saltsthereof) and a pharmaceutically acceptable excipient.

[0062] The compounds described herein can be administered to a subject in a therapeutically effective amount (e.g., in an amount sufficient to prevent or relieve the symptoms of a disease or disorder associated with aberrant EGFR). The compounds can be administered alone or as part of a pharmaceutically acceptable composition or formulation. In addition, the compounds can be administered all at once, multiple times, or delivered substantially uniformly over a period of time. It is also noted that the dose of the compound can be varied over time.

[0063] A particular administration regimen for a particular subject will depend, in part, upon the compound, the amount of compound administered, the route of administration, and the cause and extent of any side effects. The amount of compound administered to a subject (e.g., a mammal, such as a human) in accordance with the disclosure should be sufficient to effect the desired response over a reasonable time frame. Dosage typically depends upon the route, timing, and frequency of administration. Accordingly, the clinician titers the dosage and modifies the route of administration to obtain the optimal therapeutic effect, and conventional range-finding techniques are known to those of ordinary skill in the art.

[0064] Purely by way of illustration, the method comprises administering, e.g., from about 0.1 mg/kg up to about 100 mg/kg of a compound as disclosed herein, depending on the factors mentioned above. In other embodiments, the dosage ranges from 1 mg/kg up to about 100 mg/kg; or 5 mg/kg up to about 100 mg/kg; or 10 mg/kg up to about 100 mg/kg. Some conditions require prolonged treatment, which may or may not entail administering lower doses of compound over multiple administrations. If desired, a dose of the compound is administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms. The treatment period will depend on the particular condition, and may last one day to several months.

[0065] Suitable methods of administering a physiologically-acceptable composition, such as a pharmaceutical composition comprising the compounds disclosed herein (e.g., compounds of Formula (I)), are well known in the art. Although more than one route can be used to administer a compound, a particular route can provide a more immediate and more effective reaction than another route. Depending on the circumstances, a pharmaceutical composition comprising the compound is applied or instilled into body cavities, absorbed through the skin or mucous membranes, ingested, inhaled, and/or introduced into circulation. For example, in certain circumstances, it will be desirable to deliver a pharmaceutical composition comprising the agent orally, through injection, or by one of the following means: intravenous, intraperitoneal, intracerebral (intra-parenchymal), intracerebroventricular, intramuscular, intra-ocular, intraarterial, intraportal, intralesional, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, urethral, vaginal, or rectal. The compound can be administered by sustained release systems, or by implantation devices.

[0066] To facilitate administration, the compound is, in various aspects, formulated into a physiologically-acceptable composition comprising a carrier (e.g., vehicle, adjuvant, or diluent). The particular carrier employed is limited only by chemico-physical considerations, such as solubility and lack of reactivity with the compound, and by the route of administration. Physiologically- acceptable carriers are well known in the art. Illustrative pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions (for example, see U.S. Patent No. 5,466,468). Injectable formulations are further described in, e.g., *Pharmaceutics and Pharmacy Practice*, J. B. Lippincott Co., Philadelphia. Pa., Banker and Chalmers, eds., pages 238-250 (1982), and *ASHP Handbook on Injectable Drugs*, Toissel, 4th ed., pages 622-630 (1986)). A pharmaceutical composition comprising the compound is, in one aspect, placed within containers, along with packaging material that provides instructions regarding the use of such pharmaceutical compositions. Generally, such instructions include a tangible expression describing the reagent concentration, as well as, in certain embodiments, relative amounts of excipient ingredients or diluents (e.g., water, saline or PBS) that may be necessary to reconstitute the pharmaceutical composition.

[0067] Compositions suitable for parenteral injection may comprise physiologically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions, or emulsions, and sterile powders for reconstitution into sterile injectable solutions or dispersions. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents, or vehicles include water, ethanol, polyols (propylene glycol, polyethylene glycol, glycerol, and the like), suitable mixtures thereof, vegetable oils (such as olive oil) and injectable organic

esters such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

[0068] These compositions may also contain adjuvants such as preserving, wetting, emulsifying, and dispersing agents. Microorganism contamination can be prevented by adding various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, and the like. It may also be desirable to include isotonic agents, for example, sugars, sodium chloride, and the like. Prolonged absorption of injectable pharmaceutical compositions can be brought about by the use of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0069] Solid dosage forms for oral administration include capsules, tablets, powders, and granules. In such solid dosage forms, the active compound is admixed with at least one inert customary excipient (or carrier) such as sodium citrate or dicalcium phosphate or (a) fillers or extenders, as for example, starches, lactose, sucrose, mannitol, and silicic acid; (b) binders, as for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose, and acacia; (c) humectants, as for example, glycerol; (d) disintegrating agents, as for example, agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain complex silicates, and sodium carbonate; (e) solution retarders, as for example, paraffin; (f) absorption accelerators, as for example, quaternary ammonium compounds; (g) wetting agents, as for example, cetyl alcohol and glycerol monostearate; (h) adsorbents, as for example, kaolin and bentonite; and (i) lubricants, as for example, talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, or mixtures thereof. In the case of capsules, and tablets, the dosage forms may also comprise buffering agents. Solid compositions of a similar type may also be used as fillers in soft and hard filled gelatin capsules using such excipients as lactose or milk sugar, as well as high molecular weight polyethylene glycols, and the like.

[0070] Solid dosage forms such as tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells, such as enteric coatings and others well known in the art. The solid dosage forms may also contain opacifying agents. Further, the solid dosage forms may be embedding compositions, such that they release the active compound or compounds in a certain part of the intestinal tract in a delayed manner. Examples of embedding compositions that can be used are polymeric substances and waxes. The active compound can also be in micro-encapsulated form, optionally with one or more excipients.

[0071] Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs. In addition to the active compounds, the liquid dosage form may contain inert diluents commonly used in the art, such as water or

other solvents, solubilizing agents and emulsifiers, as for example, ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils, in particular, cottonseed oil, groundnut oil, corn germ oil, olive oil, castor oil, and sesame seed oil, glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, or mixtures of these substances, and the like.

[0072] Besides such inert diluents, the composition can also include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents. Suspensions, in addition to the active compound, may contain suspending agents, as for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar, and tragacanth, or mixtures of these substances, and the like.

[0073] Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms such as injectable solutions, drug release capsules and the like. For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration.

[0074] In jurisdictions that forbid the patenting of methods that are practiced on the human body, the meaning of "administering" of a composition to a human subject shall be restricted to prescribing a controlled substance that a human subject will self-administer by any technique (*e.g.*, orally, inhalation, topical application, injection, insertion, etc.). The broadest reasonable interpretation that is consistent with laws or regulations defining patentable subject matter is intended. In jurisdictions that do not forbid the patenting of methods that are practiced on the human body, the "administering" of compositions includes both methods practiced on the human body and also the foregoing activities.

Methods of use

[0075] The compounds described herein can modulate EGFR. In some embodiments, the compounds inhibit EGFR dimerization. In various embodiments, the compounds induce EGFR degradation.

[0076] Although EGFR has clearly been identified as an oncogene and an important molecular target in cancer, there is still a great need and opportunity for an improved approach to modulate the activity of this oncogene. Using a cell penetrating peptide that blocks dimerization (Disruptin) or siRNA, it has been shown that EGFR degradation has a profound effect on cell survival, even in TKI resistant cells (14, 23, 25, 26). Without being

bound by theory, it is hypothesized that the degradation of active-EGFR (abundant in tumors) by a small molecule that inhibits dimerization, is selective, since most normal cells do not express high levels of EGF/EGFR and therefore form symmetric dimers which are not expected to be affected by Compound 8C. This approach is effective even in TKI-resistant tumor cells because the dimer interface in the TKI resistant tumors remains intact (see Figures 1A and 1B). The approach described here is unique compared to currently approved therapies. By degrading the EGFR protein rather than simply inhibiting its kinase activity, a broad spectrum of activities has been demonstrated in preclinical models while improving the ability to target tumor tissue due to the fact that this agent affects only EGF-bound EGFR, which is abundant in tumor cells compared to normal tissue, thereby, improving the safety profile and the therapeutic window.

[0077] The approach of degrading EGFR rather than simply inhibiting its kinase activity overcomes the resistance to osimertinib that invariably develops in patients with non-small cell lung cancer. While the focus of this application is on lung cancers, additional and important clinical opportunities also exist in other cancers that are driven by EGFR, such as head & neck, colorectal, and glioblastoma. Targeted selective degradation of an oncoprotein in tumors therefore represents a novel mechanism beyond inhibition of the kinase activity, and this approach might be applicable to other oncogenic proteins (22, 25, 26, 46).

[0078] The compounds disclosed herein are particularly advantageous for the treatment or prevention of diseases or disorders caused by aberrant EGFR activity.

[0079] As used herein, "aberrant EGFR activity" refers to activity associated with mutation and overexpression of the epidermal growth factor receptor (EGFR). Such mutation and overexpression is associated with the development of a variety of cancers (Shan et al., Cell 2012, 149(4) 860-870).

[0080] Given the importance of the biological roles of EGFR, the compounds of the present disclosures are useful for a number of applications in a variety of settings. For example and most simplistically, the active agents of the present disclosures are useful for inhibiting the dimerization of EGFR in a cell. In this regard, the present disclosures provide a method of inhibiting the dimerization of EGFR in a cell. The method comprises contacting the cell with a compound of the present disclosures, or a pharmaceutically acceptable salt thereof, in an amount effective to inhibit the dimerization. In some aspects, the cell is part of an *in vitro* or *ex vivo* cell culture or *in vitro* or *ex vivo* tissue sample. In some aspects, the cell is an *in vivo* cell. In certain embodiments, the method is intended for research purposes, and, in other embodiments, the method is intended for therapeutic purposes.

[0081] Inhibition of EGFR dimerization leads to an increase in EGFR degradation. Accordingly, the present disclosures further provides a method of increasing EGFR

degradation in a cell. The method comprises contacting the cell with a compound of the present disclosures, or a pharmaceutically acceptable salt thereof, in an amount effective to increase the degradation. In some aspects, the cell is part of an *in vitro* or *ex vivo* cell culture or *in vitro* or *ex vivo* tissue sample. In some aspects, the cell is an *in vivo* cell. In certain embodiments, the method is intended for research purposes, and, in other embodiments, the method is intended for therapeutic purposes.

[0082] As shown herein, a compound that inhibits dimerization of EGFR increases tumor cell death. Thus, the present disclosures provides a method of increasing tumor cell death in a subject. The method comprises administering to the subject a compound of the present disclosure, or a pharmaceutically acceptable salt thereof, in an amount effective to increase tumor cell death.

[0083] In accordance with the foregoing, the present disclosures further provides a method of treating a cancer in a subject. The method comprises administering to the subject a compound of the present disclosures, or a pharmaceutically acceptable salt thereof, in an amount effective to treat the cancer in the subject.

[0084] As used herein, the term "treat," as well as words related thereto, do not necessarily imply 100% or complete treatment. Rather, there are varying degrees of treatment of which one of ordinary skill in the art recognizes as having a potential benefit or therapeutic effect. In this respect, the methods of treating cancer of the present disclosures can provide any amount or any level of treatment of cancer. Furthermore, the treatment provided by the method of the present disclosures may include treatment of one or more conditions or symptoms of the cancer, being treated. Also, the treatment provided by the methods of the present disclosures may encompass slowing the progression of the cancer. For example, the methods can treat cancer by virtue of reducing tumor or cancer growth, reducing metastasis of tumor cells, increasing cell death of tumor or cancer cells, and the like.

[0085] The cancer treatable by the methods disclosed herein may be any cancer, e.g., any malignant growth or tumor caused by abnormal and uncontrolled cell division that may spread to other parts of the body through the lymphatic system or the blood stream.. In some embodiments, the cancer is a cancer in which an EGFR is expressed by the cells of the cancer. In some aspects, the cancer is a cancer in which an EGFR protein is over-expressed, the gene encoding EGFR is amplified, and/or an EGFR mutant protein (e.g., truncated EGFR, point-mutated EGFR) is expressed.

[0086] The cancer in some aspects is one selected from the group consisting of acute lymphocytic cancer, acute myeloid leukemia, alveolar rhabdomyosarcoma, bone cancer, brain cancer, breast cancer, cancer of the anus, anal canal, or anorectum, cancer of the eye,

cancer of the intrahepatic bile duct, cancer of the joints, cancer of the neck, gallbladder, or pleura, cancer of the nose, nasal cavity, or middle ear, cancer of the oral cavity, cancer of the vulva, leukemia (e.g., chronic lymphocytic leukemia), chronic myeloid cancer, colon cancer, esophageal cancer, cervical cancer, gastrointestinal carcinoid tumor, Hodgkin lymphoma, hypopharynx cancer, kidney cancer, larynx cancer, liver cancer, lung cancer, malignant mesothelioma, melanoma, multiple myeloma, nasopharynx cancer, non-Hodgkin lymphoma, ovarian cancer, pancreatic cancer, peritoneum, omentum, and mesentery cancer, pharynx cancer, prostate cancer, rectal cancer, renal cancer (e.g., renal cell carcinoma (RCC)), small intestine cancer, soft tissue cancer, stomach cancer, testicular cancer, thyroid cancer, ureter cancer, and urinary bladder cancer. In particular aspects, the cancer is selected from the group consisting of: head and neck, ovarian, cervical, bladder and oesophageal cancers, pancreatic, gastrointestinal cancer, gastric, breast, endometrial and colorectal cancers, hepatocellular carcinoma, glioblastoma, bladder, lung cancer, e.g., non-small cell lung cancer (NSCLC), bronchioloalveolar carcinoma. In particular aspects, the cancer is an osimertinib-resistant cancer. In some cases, the cancer is pancreatic cancer, head and neck cancer, melanoma, colon cancer, renal cancer, leukemia, or breast cancer. In some cases, the cancer is melanoma, colon cancer, renal cancer, leukemia, or breast cancer.

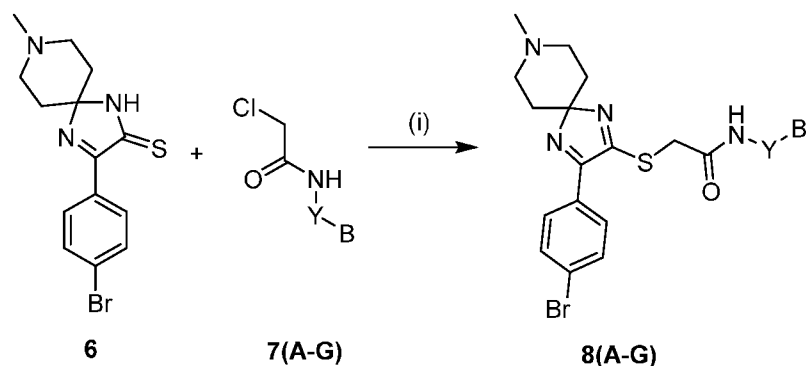
[0087] Uses of the compounds disclosed herein in the preparation of a medicament for modulating EGFR, or for treating or preventing a disease or disorder associated with aberrant EGFR activity also are provided herein.

[0088] The disclosure herein will be understood more readily by reference to the following examples, below.

[0089] In view of the many possible embodiments to which the principles of the disclosure may be applied, it should be recognized that the illustrated embodiments are only examples and should not be taken as limiting the scope of the invention.

EXAMPLES

Example 1: General Procedure A:



Reaction conditions: (i) 2M aq. K_2CO_3 , Acetonitrile, 40°C

7A 2-chloro-N-(6-methoxypyridin-3-yl)acetamide hydrochloride

7B 2-chloro-N-(6-chloropyridin-3-yl)acetamide

7C 2-chloro-N-quinolin-3-yl-acetamide

7D 2-chloro-N-(pyridin-4-yl)acetamide hydrochloride

7E 2-chloro-N-cyclohexyl-acetamide

7F 2-chloro-N-cyclopentylacetamide

7G N-benzyl-2-chloroacetamide

[0090] To a solution of 3-(4-bromophenyl)-8-methyl-1,4,8-triazaspiro[4.5]dec-3-ene-2-thione **6** in anhydrous acetonitrile was added acetamide **7A-G** (1 equiv.). The reaction mixture was warmed to 40 °C. Next, 2M aqueous potassium carbonate solution (1 equiv.) was added to the reaction mixture. The reaction was maintained at 40 °C until TLC showed loss of starting materials and new R_f spot (typically 2-6 hours). Once the reaction was complete by TLC, it was worked up. The crude reaction mixture was poured into a separatory funnel, and ethyl acetate and water were added. The organic layer was separated and then washed with brine (1 x). The organic layer was then dried over anhydrous $MgSO_4$, filtered, and the filtrate was concentrated under reduced pressure afford the crude product. The crude product was purified by flash chromatography. The crude product was loaded onto a silica column using minimal amount of ethyl acetate. The column was placed on top of another, pre-equilibrated silica column. A gradient of ethyl acetate in heptane (1-100%) or methanol in dichloromethane (0-10%) was typically used to elute the product. Product fractions were concentrated under reduced pressure to afford the desired product.

Example 2 - Synthesis of Compound 8A (2-((3-(4-bromophenyl)-8-methyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(6-methoxypyridin-3-yl)acetamide)

[0091] General procedure A was followed, using acetamide **7A**. The crude product was purified by flash chromatography with elution occurring in 10% methanol/dichloromethane solution to afford the title compound. 1H NMR (400MHz, $DMSO-d_6$) δ 10.30 (s, 1H), 8.31 (d, $J=2.29$ Hz, 1H), 7.74-7.86 (m, 5H), 6.78 (d, $J=8.87$ Hz, 1H), 4.14 (s, 2H), 3.79 (s, 3H), 2.50-2.67 (m, 4H), 2.24 (s, 3H), 1.55-1.75 (m, 4H); MS (ESI + m/z 503.10, ESI- m/z 501.10); TLC: (90:10:0.5, DCM:MeOH: NH_4OH) R_f =0.56.

Example 3 - Synthesis of Compound 8B (2-((3-(4-bromophenyl)-8-methyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(6-chloropyridin-3-yl)acetamide)

[0092] General procedure A was followed using acetamide 7B. The crude product was purified by flash chromatography with elution occurring in 10% methanol/dichloromethane solution to afford the title compound. ¹H NMR (400MHz, DMSO-d₆) δ 10.72 (s, 1H), 8.63 (s, 1H), 8.10 (br d, J=9.15 Hz, 1H), 7.85 (q, J=8.23 Hz, 4H), 7.53 (d, J=8.60 Hz, 1H), 4.24 (s, 2H), 2.56-2.81 (m, 4H), 2.27 (br s, 3H), 1.55-1.85 (m, 4H); MS (ESI + m/z 507.95, ESI- m/z 505.95); TLC: (95:5:0.5, DCM:MeOH:NH₄OH) R_f =0.17.

Example 4 - Synthesis of Compound 8C (2-((3-(4-bromophenyl)-8-methyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-3-yl)acetamide)

[0093] General procedure A was followed using acetamide 7C. The crude product was purified by flash chromatography with elution occurring in 10% methanol/dichloromethane solution to afford the title compound. ¹H NMR (400MHz, DMSO-d₆) δ 10.89 (br s, 1H), 8.95 (s, 1H), 8.67 (s, 1H), 7.96 (br d, J=8.33 Hz, 1H), 7.92 (br d, J=8.33 Hz, 1H), 7.78-7.87 (m, 4H), 7.52-7.72 (m, 2H), 4.29 (s, 2H), 2.43-2.52 (m, 4H), 2.18 (br s, 3H), 1.55-1.85 (m, 4H); MS (ESI + m/z 523.05, ESI- m/z 521.00); TLC: (90:10:0.5, DCM:MeOH:NH₄OH) R_f =0.47.

Example 5 - Synthesis of Compound 8D (2-((3-(4-bromophenyl)-8-methyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(pyridin-4-yl)acetamide)

[0094] General procedure A was followed using acetamide 7D. The crude product was purified by flash chromatography with elution occurring in 13% methanol/dichloromethane solution to afford the title compound. ¹H NMR (400MHz, DMSO-d₆) δ 10.66 (s, 1H), 8.41 (br d, J=6.13 Hz, 2H), 7.78 (q, J=8.51 Hz, 4H), 7.52 (d, J=6.04 Hz, 2H), 4.18 (s, 2H), 2.50-2.73 (m, 4H), 2.19 (br s, 3H), 1.28-1.91 (m, 4H); MS (ESI + m/z 473.05, ESI- m/z 471.05); TLC: (90:10:0.5, DCM:MeOH:NH₄OH) R_f =0.24.

Example 6 - Synthesis of Compound 8E (2-((3-(4-bromophenyl)-8-methyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-cyclohexylacetamide)

[0095] General procedure A was followed, using acetamide 7E. Following the workup procedure in general procedure A afforded the title compound without column purification. ¹H NMR (400MHz, DMSO-d₆) δ 8.04 (br d, J=7.78 Hz, 1H), 7.78 (q, J=8.63 Hz, 4H), 3.89 (s, 2H), 3.45-3.55 (m, 1H), 2.50-2.71 (m, 4H), 2.29 (s, 3H), 1.58-1.80 (m, 7H), 1.51 (br d, J=12.44 Hz, 1H), 1.05-1.28 (m, 6H); MS (ESI + m/z 478.90, ESI- m/z 476.90); TLC: (90:10:0.5, DCM:MeOH:NH₄OH) R_f =0.60.

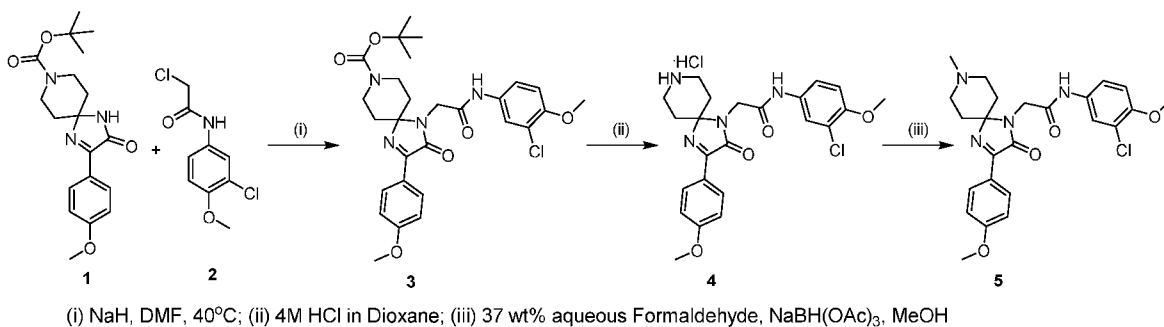
Example 7 - Synthesis of Compound 8F (2-((3-(4-bromophenyl)-8-methyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-cyclopentylacetamide)

[0096] General procedure A was followed using acetamide 7F. The crude product was purified by flash chromatography with elution occurring in 10% methanol/dichloromethane solution to afford the title compound. ¹H NMR (400MHz, DMSO-d₆) δ 8.15 (br d, J=6.77 Hz, 1H), 7.79 (q, J=8.42 Hz, 4H), 3.92-4.01 (m, 1H), 3.91 (s, 2H), 2.50-2.71 (m, 4 H), 2.32 (s, 3H), 1.58-1.82 (m, 8H), 1.45-1.54 (m, 2H), 1.38 (td, J=6.27, 12.35 Hz, 2H); MS (ESI + m/z 464.10, ESI- m/z 462.15); TLC: (90:10:0.5, DCM:MeOH:NH₄OH) R_f =0.62.

Example 8 - Synthesis of Compound 8G (N-benzyl-2-((3-(4-bromophenyl)-8-methyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)acetamide)

[0097] General procedure A was followed using acetamide 7G. The crude product was purified by flash chromatography with elution occurring in 10% methanol/dichloromethane solution to afford the title compound. ¹H NMR (400MHz, DMSO-d₆) δ 8.65 (br t, J=5.81 Hz, 1H), 7.73-7.82 (m, 4H), 7.17-7.30 (m, 5H), 4.27 (d, J=5.95 Hz, 2H), 3.99 (s, 2H), 2.50-2.73 (m, 5 H), 2.28 (s, 3H), 1.66 (br s, 3H); MS (ESI + m/z 486.00, ESI- m/z 484.10); TLC: (90:10:0.5, DCM:MeOH:NH₄OH) R_f =0.59.

Examples 9-11 - Synthesis of Compounds 3-5



Example 9 - Synthesis of Compound 3 (*tert*-Butyl 4-(2-((3-chloro-4-methoxyphenyl)amino)-2-oxoethyl)-2-(4-methoxyphenyl)-3-oxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate)

[0098] To a solution of *tert*-butyl 2-(4-methoxyphenyl)-3-oxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate, **1** in anhydrous DMF was added sodium hydride (2 equiv.). The reaction mixture was warmed to 40 °C for thirty minutes. Next, 2-chloro-N-(3-chloro-4-methoxyphenyl)acetamide hydrochloride, **2** (1.76 equiv.) was added to the reaction mixture. The reaction mixture was kept at 40 °C and stirred under a flow of N₂. After five hours, the reaction was purified although TLC indicated starting material remained. The crude reaction mixture was cooled to room temperature and then loaded onto silica. The dry loaded material was placed into a dry-load column and packed. The dry-load column was placed on top of a pre-equilibrated (1% ethyl acetate in heptane) silica column. The crude product was purified using flash chromatography eluting with a gradient of 1-100% ethyl acetate in heptane. Desired fractions (elution at 50% ethyl acetate) were collected and concentrated

under reduced pressure to afford the title compound. $^1\text{H NMR}$ (400MHz, CDCl_3) δ 8.67 (s, 1H), 8.46 (d, $J=8.97$ Hz, 2H), 7.55 (d, $J=2.56$ Hz, 1H), 7.27-7.39 (m, 1H), 6.99 (d, $J=8.97$ Hz, 2H), 6.84 (d, $J=8.87$ Hz, 1H), 4.01-4.22 (m, 2H), 3.88 (s, 3H), 3.86 (s, 3H), 3.39 (m, 2H), 2.21 (dt, $J=4.80, 12.69$ Hz, 2H), 1.50 (s, 9H), 1.19-1.40 (m, 4H); MS (ESI + m/z 501, ESI - m/z 556.15); TLC: (50:50 EA:HEP) $R_f=0.21$.

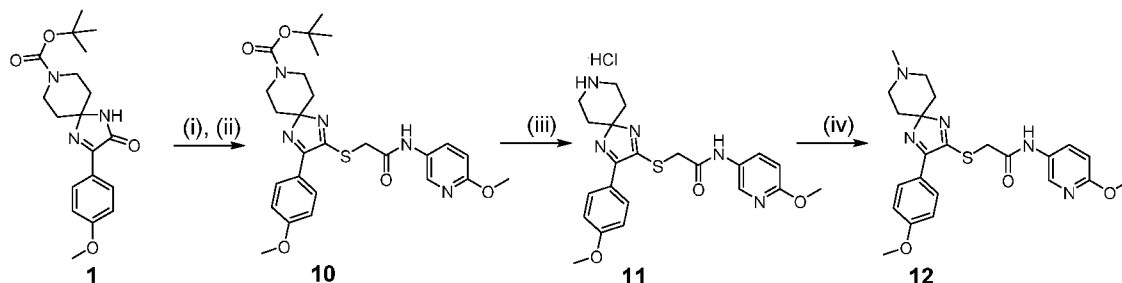
Example 10 - Synthesis of Compound 4 (N-(3-chloro-4-methoxyphenyl)-2-(3-(4-methoxyphenyl)-2-oxo-1,4,8-triazaspiro[4.5]dec-3-en-1-yl)acetamide hydrochloride)

[0099] To a solution of **3**, *tert*-butyl 4-(2-((3-chloro-4-methoxyphenyl)amino)-2-oxoethyl)-2-(4-methoxyphenyl)-3-oxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate in anhydrous dioxane was added 4M HCl in dioxane (3.3 equiv.). After one hour, no reaction was observed by TLC, so an additional portion of 4M HCl in dioxane (3.3 equiv.) was added. After one hour a new spot (baseline) was observed but starting material remained. An additional portion of 4M HCl in dioxane (3.3 equiv.) was added and the reaction mixture was stirred at 40 °C overnight. Overnight a yellow precipitate formed in the reaction mixture. The solid was filtered over a fritted funnel and rinsed with excess dioxane. The solid was transferred to a vial and dried under reduced pressure to afford the title compound. $^1\text{H NMR}$ (400MHz, DMSO-d_6) δ 10.48 (s, 1H), 9.22 (br d, $J=9.70$ Hz, 1H), 8.93 (br d, $J=10.70$ Hz, 1H), 8.39 (d, $J=8.97$ Hz, 2H), 7.78 (d, $J=2.47$ Hz, 1H), 7.45 (dd, $J=2.52, 9.01$ Hz, 1H), 7.07-7.14 (m, 3H), 4.98 (br s, 2H), 4.25 (s, 2H), 3.84 (s, 3H), 3.81 (s, 3H), 3.39-3.48 (m, 2H), 3.25-3.39 (m, 2H), 1.55 (br d, $J=13.45$ Hz, 2H); MS (ESI + m/z 457.10, ESI - m/z 455.10); TLC: (95:5:0.5, DCM:MeOH:NH₄OH) $R_f=0.03$.

Example 11 - Synthesis of Compound 5 (N-(3-chloro-4-methoxyphenyl)-2-(3-(4-methoxyphenyl)-8-methyl-2-oxo-1,4,8-triazaspiro[4.5]dec-3-en-1-yl)acetamide)

[0100] To a solution of **4**, N-(3-chloro-4-methoxyphenyl)-2-(3-(4-methoxyphenyl)-2-oxo-1,4,8-triazaspiro[4.5]dec-3-en-1-yl)acetamide hydrochloride in methanol was added aqueous formaldehyde (37 wt% solution, 7 equiv.). The reaction mixture was stirred for 5 minutes then sodium triacetoxyborohydride (3 equiv.) was added. The reaction mixture was stirred overnight at room temperature. The next morning, TLC (95:5:0.5, DCM:MeOH:NH₄OH) showed the reaction was complete with new higher R_f spot. The crude reaction was poured into a separatory funnel. To the separatory funnel was added dichloromethane. The organic layer was washed with saturated aqueous NaHCO₃ (1 x), followed by brine (1 x). The organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to afford the title compound. $^1\text{H NMR}$ (400MHz, DMSO-d_6) δ 10.15 (s, 1H), 8.34 (d, $J=8.97$ Hz, 2H), 7.74 (d, $J=2.47$ Hz, 1H), 7.39 (dd, $J=2.56, 8.97$ Hz, 1H), 7.03-7.12 (m, 3H), 4.24 (s, 2H), 3.81 (s, 3H), 3.80 (s, 3H), 2.78 (br s, 2H), 2.29 (br s, 3H), 2.15-2.26 (m, 2H) 1.13-1.33(m, 3H); MS (ESI + m/z 471.10); TLC: (95:5:0.5, DCM:MeOH:NH₄OH) $R_f=0.23$

Examples 12-14 - Synthesis of Compounds 10, 11, and 12



(i) Lawesson's Reagent, THF; (ii) **2**, **9**, 2M aq. K_2CO_3 , Acetonitrile;
 (iii) 4M HCl in Dioxane; (iv) 37 wt% aq. Formaldehyde, $NaBH(OAc)_3$, MeOH

Example 12 - Synthesis of Compound 10 (*tert*-Butyl 2-(4-methoxyphenyl)-3-((2-((6-methoxypyridin-3-yl)amino)-2-oxoethyl)thio)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate)

[0101] To a solution of *tert*-butyl 2-(4-methoxyphenyl)-3-oxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate, **1** in anhydrous THF was added Lawesson's reagent (1 equiv.). The reaction mixture was allowed to stir for 48 hours at room temperature before the crude product was purified by flash chromatography. The crude reaction mixture was dry loaded onto silica. The dry loaded material was placed into a dry-load column and packed. The dry-load column was placed on top of a pre-equilibrated (2% ethyl acetate in heptane) silica column. The crude product was purified using flash chromatography eluting with a gradient of 2-100% ethyl acetate in heptane. Desired fractions (elution at 50% ethyl acetate) were collected and concentrated under reduced pressure to afford a crude solid. The solid was triturated with a mixture of heptane, dichloromethane, and ethyl acetate (3:2:1 v/v) and filtered over a fritted funnel to afford **9** *tert*-butyl 2-(4-methoxyphenyl)-3-thio-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate. MS (ESI+ m/z 376.9, ESI- m/z 374.9); TLC: (95:0.5:0.5, DCM:MeOH:NH₄OH) R_f = 0.68.

[0102] Next, to a solution of *tert*-butyl 2-(4-methoxyphenyl)-3-thio-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate, **9** in anhydrous acetonitrile was added 2-chloro-N-(3-chloro-4-methoxyphenyl)acetamide hydrochloride, **2** (1 equiv.). The reaction mixture was warmed to 40 °C. Next, 2M aqueous potassium carbonate solution (2 equiv.) was added to the reaction mixture. The reaction continued at 40 °C overnight until TLC showed loss of starting materials and two new lower R_f spots were observed. The crude reaction mixture was poured into a separatory funnel. To the funnel was added ethyl acetate and water. Separate organic layer and then washed with brine (1 x). The organic layer was then dried over anhydrous $MgSO_4$, filtered, and the filtrate was concentrated under reduced pressure to afford the crude product. The crude product was loaded onto a silica column using a minimal amount of dichloromethane. The column was placed on top of another pre-

equilibrated silica column. A gradient of ethyl acetate in heptane (1-35%) was used to elute the product by flash chromatography. Product fractions were concentrated under reduced pressure to afford the title compound. ¹H NMR (400MHz, DMSO-d₆) δ 10.30 (s, 1H), 8.32 (d, *J*=2.38 Hz, 1H), 7.84-7.91 (m, 3H), 7.12 (d, *J*=8.97 Hz, 2H), 6.80 (d, *J*=8.97 Hz, 2H), 4.15 (s, 2H), 3.84 (s, 3H), 3.82 (s, 3H), 3.63-3.72 (m, 2H), 3.47-3.56 (m, 2H), 1.72 (br t, *J*=8.78 Hz, 2H), 1.42 (br s, 11H); MS (ESI + *m/z* 540.20, ESI- *m/z* 538.15); TLC: (50:50, EA:Hep) R_f =0.29.

Example 13 - Synthesis of Compound 11 (2-((3-(4-methoxyphenyl)-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(6-methoxypyridin-3-yl)acetamide hydrochloride)

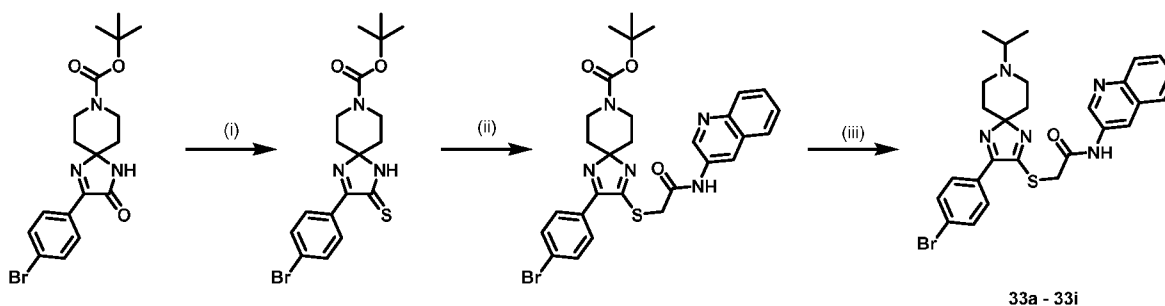
[0103] To a solution of **10**, *tert*-butyl 2-(4-methoxyphenyl)-3-((2-((6-methoxypyridin-3-yl)amino)-2-oxoethyl)thio)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate in anhydrous dioxane was added 4M HCl in dioxane (3 equiv.). After two hours, no starting materials were observed by TLC, and a new spot (baseline) was formed. A yellow precipitate formed in the reaction mixture. The solid was filtered over a fritted funnel and rinsed with excess dioxane. The solid was impure so it was loaded onto a silica column using a minimal amount of dichloromethane. A gradient of methanol in dichloromethane (0-15%) was used to elute the product via flash chromatography. Product fractions were concentrated under reduced pressure to afford the title compound. ¹H NMR (400MHz, DMSO-d₆) δ 10.40 (s, 1H), 8.45-8.60 (br s, 1H), 8.25-8.43 (m, 1H), 7.85-7.93 (m, 3H), 7.12 (d, *J*=8.87 Hz, 2H), 6.80 (d, *J*=8.78 Hz, 2H), 4.18 (s, 2H), 3.84 (s, 3H), 3.81 (s, 3H), 3.22-3.56 (m, 4H), 1.84-2.01 (m, 2H), 1.60-1.75 (m, 2H); MS (ESI + *m/z* 440.10, ESI- *m/z* 438.10); TLC: (50:50, EA:Hep) R_f =0.25

Example 14 - Synthesis of Compound 12 (2-((3-(4-methoxyphenyl)-8-methyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(6-methoxypyridin-3-yl)acetamide)

[0104] To a solution of **11**, 2-((3-(4-methoxyphenyl)-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(6-methoxypyridin-3-yl)acetamide hydrochloride in 95:5 dichloromethane:methanol was added aqueous formaldehyde (37 wt% solution, 7 equiv.). The reaction mixture was stirred for one hour then sodium triacetoxyborohydride (3 equiv.) was added. The reaction mixture was stirred overnight at room temperature. The next morning, TLC (95:5:0.5, DCM:MeOH:NH₄OH) showed the reaction was complete with new higher R_f spot. The crude reaction was poured into a separatory funnel. To the separatory funnel was added dichloromethane and water. The organic layer was separated and washed with brine (1 x). The organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure to afford the crude product. The crude product was impure so it was loaded onto a silica column using a minimal amount of dichloromethane. A gradient of methanol in dichloromethane (0-10%) was used to elute the product via flash

chromatography. Product fractions were concentrated under reduced pressure to afford the title compound. $^1\text{H NMR}$ (400MHz, DMSO-d_6) δ 10.33 (s, 1H), 8.35 (d, $J=2.38$ Hz, 1H), 7.85-7.91 (m, 3H), 7.12 (d, $J=8.02$ Hz, 2H), 6.81 (d, $J=9.06$ Hz, 1H), 4.16 (s, 2H), 3.85 (s, 3H), 3.82 (s, 3H), 2.53-2.81 (m, 4H), 2.15-2.40 (m, 2H), 1.92 (s, 3H), 1.50-1.80 (m, 2H); MS (ESI + m/z 454.10, ESI- m/z 452.10); TLC: (95:5:0.5, $\text{DCM}:\text{MeOH}:\text{NH}_4\text{OH}$) $R_f=0.22$.

Examples 15-28: Synthesis of Compounds 33a – 33l



Reaction conditions: (i) Lawesson's Reagent, THF; (ii) 2-bromo-1-(quinolin-3-yl)ethan-1-one, NEt_3 , DCM; (iii) HCl, DCM then electrophile, $\text{NaBH}(\text{OAc})_3$, DCM

[0105] General Reaction Conditions for (i): To a solution of tert-butyl 2-(4-bromophenyl)-3-oxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate (1 equiv.) was added Lawesson's reagent (0.6 equiv) in THF (0.1 M) and the reaction mixture was heated to 60 °C until the reaction was complete by TLC. The reaction mixture was concentrated onto silica gel and purified by flash column chromatography (0-100% EtOAc in hexanes) to provide tert-butyl 2-(4-bromophenyl)-3-thio-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate.

[0106] General Reaction Conditions for (ii): To a solution of tert-butyl 2-(4-bromophenyl)-3-thio-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate (1 equiv.) in DCM (0.1 M) was 2-bromo-N-(quinolin-3-yl)acetamide (1 equiv.) and triethylamine (3 equivalents). The reaction mixture was stirred for 12 hours at room temperature. The reaction mixture was diluted with DCM and washed with saturated aqueous sodium bicarbonate. The organic layer was dried, concentrated and purified by flash column chromatography (0-100% EtOAc in hexanes) to provide tert-butyl 2-(4-bromophenyl)-3-((2-oxo-2-(quinolin-3-ylamino)ethyl)thio)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate.

[0107] General Reaction Conditions for (iii): To a solution of tert-butyl 2-(4-bromophenyl)-3-((2-oxo-2-(quinolin-3-ylamino)ethyl)thio)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate (1 equiv) in DCM (0.1 M) was added 4 M HCl in dioxane (2 equiv.) and the reaction mixture was stirred at room temperature for 5 hours. The reaction mixture was concentrated. The resulting residue was taken up in THF (0.1 M) and triethylamine (5 equiv) and the appropriate electrophile (3 equiv) were added followed by $\text{NaBH}(\text{OAc})_3$ (3 equiv) and the reaction mixture was stirred for 12 hours at room temperature. The reaction mixture was

concentrated, taken up in EtOAc and washed with saturated aqueous sodium bicarbonate. The organics were dried, concentrated and purified by flash column chromatography (0-30% MeOH in DCM) to provide the desired product.

Examples 15-28

[0108] Compound 33a 2-((3-(4-bromophenyl)-8-isopropyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-3-yl)acetamide. ¹H NMR (400 MHz, DMSO-d₆) δ 10.81 (s, 1H), 8.97 – 8.90 (m, 1H), 8.00 – 7.95 (m, 1H), 7.95 – 7.90 (m, 1H), 7.89 – 7.83 (m, 2H), 7.83 – 7.77 (m, 2H), 7.71 – 7.63 (m, 1H), 7.62 – 7.55 (m, 1H), 4.26 (s, 2H), 2.73 – 2.60 (m, 4H), 1.88 – 1.71 (m, 2H), 1.62 – 1.40 (m, 2H), 0.90 (s, 3H), 0.88 (s, 3H).

[0109] Compound 33b 2-((3-(4-bromophenyl)-8-isopentyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-3-yl)acetamide. ¹H NMR (400 MHz, DMSO-d₆) δ 10.80 (s, 1H), 8.94 (d, J = 2.5 Hz, 1H), 8.67 (d, J = 2.5 Hz, 1H), 7.99 – 7.94 (m, 1H), 7.94 – 7.90 (m, 1H), 7.88 – 7.83 (m, 2H), 7.83 – 7.78 (m, 2H), 7.69 – 7.62 (m, 1H), 7.62 – 7.54 (m, 1H), 4.26 (s, 2H), 3.48 – 3.36 (m, 4H), 2.67 – 2.56 (m, 2H), 2.31 – 2.12 (m, 2H), 1.62 – 1.38 (m, 2H), 1.28 – 1.15 (m, 2H), 0.81 (d, J = 6.5 Hz, 6H).

[0110] Compound 33c 2-((8-benzyl-3-(4-bromophenyl)-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-3-yl)acetamide ¹H NMR (400 MHz, Methanol-d₄) δ 8.17 (d, J = 2.5 Hz, 1H), 7.95 (d, J = 2.5 Hz, 1H), 7.25 – 7.19 (m, 1H), 7.12 – 7.02 (m, 3H), 6.96 – 6.87 (m, 3H), 6.85 – 6.78 (m, 1H), 6.43 – 6.30 (m, 3H), 6.30 – 6.17 (m, 2H), 3.42 – 3.34 (m, 2H), 2.76 – 2.60 (m, 4H), 2.10 – 1.95 (m, 2H), 1.95 – 1.78 (m, 2H).

[0111] Compound 33d 2-((3-(4-bromophenyl)-8-ethyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-3-yl)acetamide ¹H NMR (400 MHz, DMSO-d₆) δ 10.82 (s, 1H), 8.94 (d, J = 2.5 Hz, 1H), 8.67 (d, J = 2.4 Hz, 1H), 7.97 (d, J = 8.4 Hz, 1H), 7.93 (d, J = 8.1 Hz, 1H), 7.85 (d, J = 8.5 Hz, 2H), 7.81 (d, J = 8.5 Hz, 2H), 7.69 – 7.63 (m, 1H), 7.62 – 7.53 (m, 1H), 4.27 (s, 2H), 2.66 (s, 4H), 2.48 – 2.33 (m, 2H), 1.90 – 1.40 (m, 2H), 1.02 – 0.91 (m, 3H).

[0112] Compound 33e 2-((3-(4-bromophenyl)-8-propyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-3-yl)acetamide ¹H NMR (400 MHz, DMSO-d₆) δ 10.80 (s, 1H), 8.94 (d, J = 2.5 Hz, 1H), 8.67 (d, J = 2.4 Hz, 1H), 7.99 – 7.95 (m, 1H), 7.93 (dd, J = 8.1, 1.5 Hz, 1H), 7.88 – 7.83 (m, 2H), 7.83 – 7.78 (m, 2H), 7.66 (ddd, J = 8.4, 6.8, 1.5 Hz, 1H), 7.58 (ddd, J = 8.2, 6.9, 1.3 Hz, 1H), 4.26 (s, 2H), 2.67 – 2.55 (m, 4H), 2.29 – 2.16 (m, 2H), 1.90 – 1.69 (m, 2H), 1.63 – 1.41 (m, 2H), 1.40 – 1.29 (m, 2H), 0.79 (t, J = 7.3 Hz, 3H).

[0113] Compound 33f 2-((3-(4-bromophenyl)-8-isobutyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-3-yl)acetamide ¹H NMR (400 MHz, DMSO-d₆) δ 10.80 (s, 1H), 8.95 (d, J = 2.6 Hz, 1H), 8.67 (d, J = 2.5 Hz, 1H), 8.00 – 7.94 (m, 1H), 7.94 – 7.90 (m, 1H), 7.87 – 7.83 (m, 2H), 7.83 – 7.78 (m, 2H), 7.69 – 7.62 (m, 1H), 7.62 – 7.54 (m, 1H), 4.26 (s,

2H), 2.65 – 2.52 (m, 4H), 2.06 – 1.93 (m, 2H), 1.92 – 1.75 (m, 2H), 1.72 – 1.37 (m, 3H), 0.80 (d, J = 6.5 Hz, 6H).

[0114] Compound 33g 2-((3-(4-bromophenyl)-8-(3,3,3-trifluoropropyl)-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-3-yl)acetamide 1H NMR (400 MHz, DMSO-d₆) δ 10.80 (s, 1H), 8.94 (d, J = 2.5 Hz, 1H), 8.67 (d, J = 2.4 Hz, 1H), 8.00 – 7.95 (m, 1H), 7.95 – 7.91 (m, 1H), 7.88 – 7.83 (m, 2H), 7.83 – 7.78 (m, 2H), 7.66 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 7.58 (ddd, J = 8.1, 6.9, 1.3 Hz, 1H), 4.28 (s, 2H), 2.68 – 2.54 (m, 6H), 1.96 – 1.40 (m, 6H).

[0115] Compound 33h 2-((3-(4-bromophenyl)-8-cyclopentyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-3-yl)acetamide 1H NMR (400 MHz, DMSO-d₆) δ 10.82 (s, 1H), 8.96 (s, 1H), 8.68 (s, 1H), 8.03 – 7.90 (m, 2H), 7.90 – 7.77 (m, 4H), 7.73 – 7.63 (m, 1H), 7.62 – 7.54 (m, 1H), 4.25 (s, 2H), 2.77 – 2.23 (m, 5H), 2.03 – 0.83 (m, 12H).

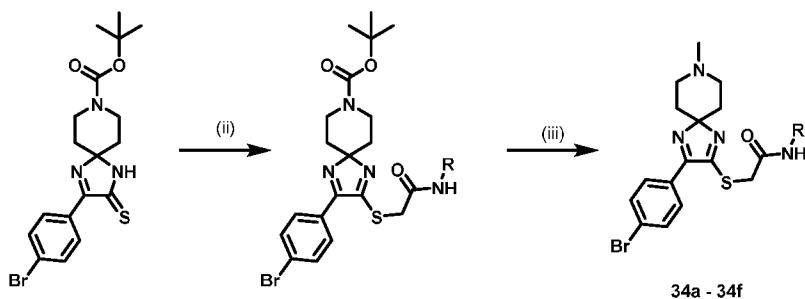
[0116] Compound 33i 2-((3-(4-bromophenyl)-8-cyclobutyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-3-yl)acetamide 1H NMR (400 MHz, DMSO-d₆) δ 10.81 (s, 1H), 8.96 (d, J = 2.5 Hz, 1H), 8.67 (d, J = 2.5 Hz, 1H), 8.00 – 7.96 (m, 1H), 7.95 – 7.90 (m, 1H), 7.88 – 7.83 (m, 2H), 7.83 – 7.77 (m, 2H), 7.66 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 7.58 (ddd, J = 8.2, 6.9, 1.4 Hz, 1H), 4.25 (s, 2H), 2.67 – 2.54 (m, 1H), 2.49 – 2.28 (m, 1H), 1.91 – 1.61 (m, 5H), 1.63 – 1.10 (m, 4H).

[0117] Compound 33j 2-((3-(4-bromophenyl)-8-((1-methyl-1H-pyrazol-4-yl)methyl)-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-3-yl)acetamide 1H NMR (400 MHz, Chloroform-d) δ 9.88 (s, 1H), 8.85 – 8.76 (m, 1H), 8.65 (d, J = 2.6 Hz, 1H), 8.11 – 8.02 (m, 1H), 7.87 – 7.77 (m, 3H), 7.70 – 7.61 (m, 3H), 7.56 (ddd, J = 8.1, 6.9, 1.2 Hz, 1H), 7.47 (d, J = 0.7 Hz, 1H), 7.37 (s, 1H), 4.00 (s, 2H), 3.91 (s, 3H), 3.62 (s, 2H), 3.04 – 2.72 (m, 4H), 2.40 – 2.07 (m, 2H), 1.78 (s, 2H).

[0118] Compound 33k 2-((3-(4-bromophenyl)-8-((1-isopropyl-1H-pyrazol-4-yl)methyl)-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-3-yl)acetamide 1H NMR (400 MHz, Chloroform-d) δ 9.89 (s, 1H), 8.80 (d, J = 2.5 Hz, 1H), 8.66 (d, J = 2.6 Hz, 1H), 8.08 – 7.98 (m, 1H), 7.87 – 7.76 (m, 3H), 7.69 – 7.63 (m, 3H), 7.56 (ddd, J = 8.2, 6.9, 1.2 Hz, 1H), 7.50 (d, J = 0.9 Hz, 1H), 7.44 (s, 1H), 4.50 (p, J = 6.7 Hz, 1H), 4.01 (s, 2H), 3.62 (s, 2H), 3.09 – 2.76 (m, 4H), 2.40 – 2.12 (m, 2H), 1.74 (d, J = 35.2 Hz, 2H), 1.53 (d, J = 6.7 Hz, 5H).

[0119] Compound 33l 2-((3-(4-bromophenyl)-8-(methyl-d₂)-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-3-yl)acetamide 1H NMR (400 MHz, DMSO-d₆) δ 10.80 (s, 1H), 8.94 (d, J = 2.5 Hz, 1H), 8.67 (d, J = 2.4 Hz, 1H), 7.97 (dd, J = 8.3, 1.1 Hz, 1H), 7.93 (dd, J = 8.1, 1.4 Hz, 1H), 7.89 – 7.83 (m, 2H), 7.83 – 7.78 (m, 2H), 7.66 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 7.58 (ddd, J = 8.1, 6.9, 1.3 Hz, 1H), 4.28 (s, 2H), 2.74 – 2.54 (m, 5H), 1.98 – 1.36 (m, 4H).

Examples 29-34: Synthesis of Compounds 34a-34f



Reaction conditions: (i) electrophile, NEt_3 , DCM; (ii) HCl, DCM then formaldehyde, $\text{NaBH}(\text{OAc})_3$, DCM

[0120] Synthesis of Compound 34a 2-((3-(4-bromophenyl)-8-methyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-4-yl)acetamide

[0121] To a solution of tert-butyl 2-(4-bromophenyl)-3-thioxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate (1 equiv) in DCM (0.1 M) was added 2-bromo-N-(quinolin-4-yl)acetamide (1 equiv) and triethylamine (3 equiv) and the reaction mixture was stirred for 10 hours. The reaction mixture was diluted with DCM and washed with saturated aqueous sodium bicarbonate. The organics were combined, dried, concentrated and purified by flash column chromatography (0-100% EtOAc in hexanes) to provide tert-butyl 2-(4-bromophenyl)-3-((2-oxo-2-(quinolin-6-ylamino)ethyl)thio)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate ^1H NMR (400 MHz, DMSO-d_6) δ 10.65 (s, 1H), 8.80 (dd, $J = 4.2, 1.7$ Hz, 1H), 8.34 (d, $J = 2.4$ Hz, 1H), 8.33 – 8.27 (m, 1H), 8.04 – 7.92 (m, 1H), 7.92 – 7.76 (m, 5H), 7.49 (dd, $J = 8.3, 4.2$ Hz, 1H), 4.27 (s, 2H), 3.70 – 3.58 (m, 2H), 3.57 – 3.46 (m, 2H), 1.74 (d, $J = 12.6, 8.4, 3.9$ Hz, 2H), 1.56 – 1.44 (m, 2H), 1.37 (s, 9H).

[0122] To a solution of tert-butyl 2-(4-bromophenyl)-3-((2-oxo-2-(quinolin-6-ylamino)ethyl)thio)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate (1 equiv) in DCM was added 4 M HCl in dioxane (2 equiv) and the reaction mixture was allowed to stir for 4 hours. The reaction mixture was concentrated. The resulting residue was taken up in THF and formaldehyde (5 equiv) and triethylamine (5 equiv) were added followed by $\text{NaBH}(\text{OAc})_3$ (3 equiv) and the reaction mixture was stirred for 12 hours. The reaction mixture was concentrated, taken up in EtOAc and washed with saturated aqueous sodium bicarbonate. The organics were dried, concentrated and purified by flash column chromatography (0-30% MeOH in DCM) to provide 2-((3-(4-bromophenyl)-8-methyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-6-yl)acetamide. ^1H NMR (400 MHz, DMSO-d_6) δ 10.67 (s, 1H), 8.80 (dd, $J = 4.2, 1.7$ Hz, 1H), 8.35 (d, $J = 2.4$ Hz, 1H), 8.29 (d, $J = 8.1$ Hz, 1H), 7.99 (d, $J = 9.1$ Hz, 1H), 7.89 – 7.77 (m, 5H), 7.49 (dd, $J = 8.3, 4.2$ Hz, 1H), 4.26 (s, 2H), 2.67 – 2.54 (m, 4H), 2.22 (s, 3H), 1.85 – 1.40 (m, 4H).

[0123] Synthesis of Compound 34b 2-((3-(4-bromophenyl)-8-methyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-6-yl)acetamide

[0124] To a solution of tert-butyl 2-(4-bromophenyl)-3-thioxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate (1 equiv) in DCM (0.1 M) was added 2-bromo-N-(quinolin-6-yl)acetamide (1 equiv) and triethylamine (3 equiv) and the reaction mixture was stirred for 10 hours. The reaction mixture was diluted with DCM and washed with saturated aqueous sodium bicarbonate. The organics were combined, dried, concentrated and purified by flash column chromatography (0-100% EtOAc in hexanes) to provide tert-butyl 2-(4-bromophenyl)-3-((2-oxo-2-(quinolin-6-ylamino)ethyl)thio)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate ¹H NMR (400 MHz, DMSO-d₆) δ 10.65 (s, 1H), 8.80 (dd, J = 4.2, 1.7 Hz, 1H), 8.34 (d, J = 2.4 Hz, 1H), 8.33 – 8.27 (m, 1H), 8.04 – 7.92 (m, 1H), 7.92 – 7.76 (m, 5H), 7.49 (dd, J = 8.3, 4.2 Hz, 1H), 4.27 (s, 2H), 3.70 – 3.58 (m, 2H), 3.57 – 3.46 (m, 2H), 1.74 (d, J = 12.6, 8.4, 3.9 Hz, 2H), 1.56 – 1.44 (m, 2H), 1.37 (s, 9H).

[0125] To a solution of tert-butyl 2-(4-bromophenyl)-3-((2-oxo-2-(quinolin-6-ylamino)ethyl)thio)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate (1 equiv) in DCM was added 4 M HCl in dioxane (2 equiv) and the reaction mixture was allowed to stir for 4 hours. The reaction mixture was concentrated. The resulting residue was taken up in THF and formaldehyde (5 equiv) and triethylamine (5 equiv) were added followed by NaBH(OAc)₃ (3 equiv) and the reaction mixture was stirred for 12 hours. The reaction mixture was concentrated, taken up in EtOAc and washed with saturated aqueous sodium bicarbonate. The organics were dried, concentrated and purified by flash column chromatography (0-30% MeOH in DCM) to provide 2-((3-(4-bromophenyl)-8-methyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-6-yl)acetamide. ¹H NMR (400 MHz, DMSO-d₆) δ 10.67 (s, 1H), 8.80 (dd, J = 4.2, 1.7 Hz, 1H), 8.35 (d, J = 2.4 Hz, 1H), 8.29 (d, J = 8.1 Hz, 1H), 7.99 (d, J = 9.1 Hz, 1H), 7.89 – 7.77 (m, 5H), 7.49 (dd, J = 8.3, 4.2 Hz, 1H), 4.26 (s, 2H), 2.67 – 2.54 (m, 4H), 2.22 (s, 3H), 1.85 – 1.40 (m, 4H).

[0126] Synthesis of Compound 34c 2-((3-(4-bromophenyl)-8-methyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-5-yl)acetamide

[0127] To a solution of tert-butyl 2-(4-bromophenyl)-3-thioxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate (1 equiv) in DCM (0.1 M) was added 2-bromo-N-(quinolin-5-yl)acetamide (1 equiv) and triethylamine (3 equiv) and the reaction mixture was stirred for 10 hours. The reaction mixture was diluted with DCM and washed with saturated aqueous sodium bicarbonate. The organics were combined, dried, concentrated and purified by flash column chromatography (0-100% EtOAc in hexanes) to provide tert-butyl 2-(4-bromophenyl)-3-((2-oxo-2-(quinolin-5-ylamino)ethyl)thio)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate.

[0128] To a solution of tert-butyl 2-(4-bromophenyl)-3-((2-oxo-2-(quinolin-5-ylamino)ethyl)thio)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate (1 equiv) in DCM was added 4 M HCl in dioxane (2 equiv) and the reaction mixture was allowed to stir for 4 hours.

The reaction mixture was concentrated. The resulting residue was taken up in THF and formaldehyde (5 equiv) and triethylamine (5 equiv) were added followed by NaBH(OAc)₃ (3 equiv) and the reaction mixture was stirred for 12 hours. The reaction mixture was concentrated, taken up in EtOAc and washed with saturated aqueous sodium bicarbonate. The organics were dried, concentrated and purified by flash column chromatography (0-30% MeOH in DCM) to provide 2-((3-(4-bromophenyl)-8-methyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-5-yl)acetamide. ¹H NMR (400 MHz, DMSO-d₆) δ 10.41 (s, 1H), 8.93 (dd, J = 4.2, 1.6 Hz, 1H), 8.52 (ddd, J = 8.6, 1.7, 0.9 Hz, 1H), 7.96 – 7.71 (m, 7H), 7.57 (dd, J = 8.6, 4.2 Hz, 1H), 4.36 (s, 2H), 2.75 – 2.56 (m, 4H), 2.25 (s, 3H), 1.89 – 1.59 (m, 4H).

[0129] Synthesis of Compound 34d 2-((3-(4-bromophenyl)-8-methyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-7-yl)acetamide

[0130] To a solution of tert-butyl 2-(4-bromophenyl)-3-thioxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate (1 equiv) in DCM (0.1 M) was added 2-bromo-N-(quinolin-7-yl)acetamide (1 equiv) and triethylamine (3 equiv) and the reaction mixture was stirred for 10 hours. The reaction mixture was diluted with DCM and washed with saturated aqueous sodium bicarbonate. The organics were combined, dried, concentrated and purified by flash column chromatography (0-100% EtOAc in hexanes) to provide tert-butyl 2-(4-bromophenyl)-3-((2-oxo-2-(quinolin-7-ylamino)ethyl)thio)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate.

[0131] To a solution of tert-butyl 2-(4-bromophenyl)-3-((2-oxo-2-(quinolin-7-ylamino)ethyl)thio)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate (1 equiv) in DCM was added 4 M HCl in dioxane (2 equiv) and the reaction mixture was allowed to stir for 4 hours. The reaction mixture was concentrated. The resulting residue was taken up in THF and formaldehyde (5 equiv) and triethylamine (5 equiv) were added followed by NaBH(OAc)₃ (3 equiv) and the reaction mixture was stirred for 12 hours. The reaction mixture was concentrated, taken up in EtOAc and washed with saturated aqueous sodium bicarbonate. The organics were dried, concentrated and purified by flash column chromatography (0-30% MeOH in DCM) to provide 2-((3-(4-bromophenyl)-8-methyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-7-yl)acetamide. ¹H NMR (400 MHz, DMSO-d₆) δ 10.69 (s, 1H), 8.85 (dd, J = 4.2, 1.8 Hz, 1H), 8.40 (d, J = 2.0 Hz, 1H), 8.28 (d, J = 8.1 Hz, 1H), 7.94 (d, J = 8.9 Hz, 1H), 7.91 – 7.84 (m, 2H), 7.83 – 7.77 (m, 2H), 7.73 (dd, J = 8.8, 2.1 Hz, 1H), 7.43 (dd, J = 8.2, 4.2 Hz, 1H), 4.28 (s, 2H), 2.67 – 2.55 (m, 4H), 2.20 (s, 3H), 1.91 – 1.38 (m, 4H).

[0132] Synthesis of Compound 34e 2-((3-(4-bromophenyl)-8-methyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-8-yl)acetamide

[0133] To a solution of tert-butyl 2-(4-bromophenyl)-3-thioxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate (1 equiv) in DCM (0.1 M) was added 2-bromo-N-(quinolin-8-yl)acetamide (1 equiv) and triethylamine (3 equiv) and the reaction mixture was stirred for 10 hours. The

reaction mixture was diluted with DCM and washed with saturated aqueous sodium bicarbonate. The organics were combined, dried, concentrated and purified by flash column chromatography (0-100% EtOAc in hexanes) to provide tert-butyl 2-(4-bromophenyl)-3-((2-oxo-2-(quinolin-8-ylamino)ethyl)thio)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate.

[0134] To a solution of tert-butyl 2-(4-bromophenyl)-3-((2-oxo-2-(quinolin-8-ylamino)ethyl)thio)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate (1 equiv) in DCM was added 4 M HCl in dioxane (2 equiv) and the reaction mixture was allowed to stir for 4 hours. The reaction mixture was concentrated. The resulting residue was taken up in THF and formaldehyde (5 equiv) and triethylamine (5 equiv) were added followed by NaBH(OAc)₃ (3 equiv) and the reaction mixture was stirred for 12 hours. The reaction mixture was concentrated, taken up in EtOAc and washed with saturated aqueous sodium bicarbonate. The organics were dried, concentrated and purified by flash column chromatography (0-30% MeOH in DCM) to provide 2-((3-(4-bromophenyl)-8-methyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-8-yl)acetamide. ¹H NMR (400 MHz, DMSO-d₆) δ 10.65 (s, 1H), 8.85 (dd, J = 4.2, 1.7 Hz, 1H), 8.60 (dd, J = 7.6, 1.3 Hz, 1H), 8.42 (dd, J = 8.3, 1.7 Hz, 1H), 7.90 – 7.79 (m, 4H), 7.69 (dd, J = 8.3, 1.4 Hz, 1H), 7.64 (dd, J = 8.3, 4.2 Hz, 1H), 7.62 – 7.56 (m, 1H), 4.34 (s, 2H), 2.50 – 2.37 (m, 4H), 2.07 (s, 3H), 1.86 – 1.17 (m, 4H).

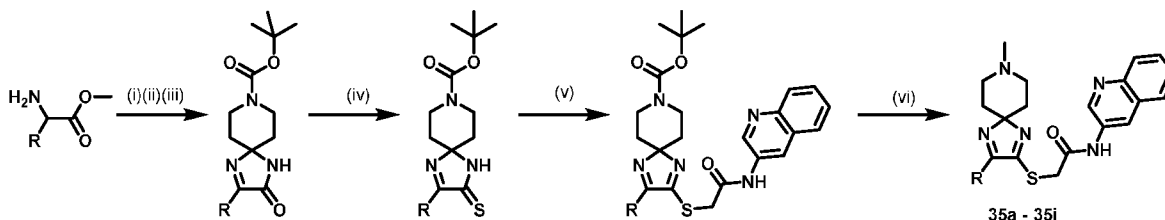
[0135] Synthesis of Compound 34f 2-((3-(4-bromophenyl)-8-methyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-methyl-N-(quinolin-3-yl)acetamide

[0136] To a solution of tert-butyl 2-(4-bromophenyl)-3-thio-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate (1 equiv) in DCM (0.1 M) was added 2-bromo-N-methyl-N-(quinolin-3-yl)acetamide (1 equiv) and triethylamine (3 equiv) and the reaction mixture was stirred for 10 hours. The reaction mixture was diluted with DCM and washed with saturated aqueous sodium bicarbonate. The organics were combined, dried, concentrated and purified by flash column chromatography (0-100% EtOAc in hexanes) to provide tert-butyl 2-(4-bromophenyl)-3-((2-(methyl(quinolin-3-yl)amino)-2-oxoethyl)thio)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate.

[0137] To a solution of tert-butyl 2-(4-bromophenyl)-3-((2-(methyl(quinolin-3-yl)amino)-2-oxoethyl)thio)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate (1 equiv) in DCM was added 4 M HCl in dioxane (2 equiv) and the reaction mixture was allowed to stir for 4 hours. The reaction mixture was concentrated. The resulting residue was taken up in THF and formaldehyde (5 equiv) and triethylamine (5 equiv) were added followed by NaBH(OAc)₃ (3 equiv) and the reaction mixture was stirred for 12 hours. The reaction mixture was concentrated, taken up in EtOAc and washed with saturated aqueous sodium bicarbonate. The organics were dried, concentrated and purified by flash column chromatography (0-30% MeOH in DCM) to provide 2-((3-(4-bromophenyl)-8-methyl-1,4,8-triazaspiro[4.5]deca-1,3-

dien-2-yl)thio)-N-methyl-N-(quinolin-3-yl)acetamide. ¹H NMR (400 MHz, DMSO-d₆) δ 9.03 (s, 1H), 8.51 (s, 1H), 8.10 (d, J = 8.5 Hz, 1H), 8.00 (dd, J = 8.4, 1.4 Hz, 1H), 7.89 – 7.63 (m, 6H), 4.01 (s, 2H), 3.37 (s, 3H), 3.30 (s, 3H), 2.65 – 2.16 (m, 7H), 1.89 – 1.18 (m, 4H).

Examples 35-43: Synthesis of Compounds 35a-35i



Reaction conditions: (i) NH₄OH, water; (ii) Boc-Piperidinone, ethanol; (iii) NBS, MeCN; (iv) Lawesson's Reagent, THF; (v) 2-bromo-1-(quinolin-3-yl)ethan-1-one, NEt₃, DCM; (vi) HCl, DCM then formaldehyde, NaBH(OAc)₃, DCM

Synthesis of Compound 35a 2-((3-(4-fluorophenyl)-8-methyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-3-yl)acetamide

[0138] Methyl amino(4-fluorophenyl)acetate HCl was stirred in 28% ammonium hydroxide (5mL/g) solution for 96 hours resulting in the formation of 2-amino-2-(4-fluorophenyl)acetamide as a white precipitate that was collected via filtration and used without further purification. To a solution of 2-amino-2-(4-fluorophenyl)acetamide (1 equiv) in ethanol (0.1 M) was added tert-Butyl 4-oxo-1-piperidinecarboxylate (1 equiv) and the reaction mixture was heated to reflux for 12 hours. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The resulting residue was dissolved in DCM (0.1 M) and N-bromosuccinimide (1 equiv) was added. The reaction mixture was stirred for 8 hours and saturated sodium bicarbonate was added. The resulting mixture was extracted with DCM. The organics were dried, concentrated and purified by FCC (0-100% EtOAc in hexanes) to provide tert-butyl 2-(4-fluorophenyl)-3-oxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate. ¹H NMR (400 MHz, DMSO-d₆) δ 10.28 (s, 1H), 8.48 – 8.37 (m, 2H), 7.41 – 7.31 (m, 2H), 3.71 – 3.50 (m, 4H), 1.79 – 1.69 (m, 2H), 1.68 – 1.56 (m, 2H), 1.44 (s, 9H).

[0139] To a solution of tert-butyl 2-(4-fluorophenyl)-3-oxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate (1 equiv.) in THF (0.1 M) was added Lawesson's reagent (0.6 equiv) and the reaction mixture was heated to 60 °C until the reaction was complete by TLC. The reaction mixture was concentrated onto silica gel and purified by flash column chromatography (0-100% EtOAc in hexanes) to provide tert-butyl 2-(4-fluorophenyl)-3-thio-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate. ¹H NMR (400 MHz, DMSO-d₆) δ 12.60 (s, 1H), 8.38 (dd, J = 8.5, 5.7 Hz, 2H), 7.34 (t, J = 8.7 Hz, 2H), 3.80 – 3.70 (m, 2H), 3.60 – 3.45 (m, 2H), 1.86 – 1.67 (m, 4H), 1.45 (s, 9H).

[0140] To a solution of tert-butyl 2-(4-fluorophenyl)-3-thioxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate (1 equiv.) in DCM (0.1 M) was 2-bromo-N-(quinolin-3-yl)acetamide (1 equiv.) and triethylamine (3 equivalents). The reaction mixture was stirred for 12 hours at room temperature. The reaction mixture was diluted with DCM and washed with saturated aqueous sodium bicarbonate. The organic layers were dried, concentrated and purified by flash column chromatography (0-100% EtOAc in hexanes) to provide tert-butyl 2-(4-fluorophenyl)-3-((2-oxo-2-(quinolin-3-ylamino)ethyl)thio)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate.

[0141] To a solution of tert-butyl 2-(4-fluorophenyl)-3-((2-oxo-2-(quinolin-3-ylamino)ethyl)thio)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate (1 equiv) in DCM (0.1 M) was added 4 M HCl in dioxane (2 equiv.) and the reaction mixture was stirred at room temperature for 5 hours. The reaction mixture was concentrated. The resulting residue was taken up in THF and triethylamine (5 equiv) and formaldehyde (3 equiv) were added followed by NaBH(OAc)₃ (3 equiv) and the reaction mixture was stirred for 12 hours at room temperature. The reaction mixture was concentrated, taken up in EtOAc and washed with saturated aqueous sodium bicarbonate. The organics were dried, concentrated and purified by flash column chromatography (0-30% MeOH in DCM) to provide 2-((3-(4-fluorophenyl)-8-methyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-3-yl)acetamide. ¹H NMR (400 MHz, DMSO-d₆) δ 10.80 (s, 1H), 8.94 (d, J = 2.5 Hz, 1H), 8.74 – 8.64 (m, 1H), 7.99 – 7.95 (m, 1H), 7.95 – 7.92 (m, 1H), 7.91 – 7.88 (m, 2H), 7.70 – 7.51 (m, 4H), 4.27 (s, 2H), 3.34 (s, 3H), 2.64 – 2.55 (m, 4H), 2.26 – 2.11 (m, 2H), 1.91 – 1.42 (m, 4H).

[0142] Synthesis of Compound 35b 2-((8-methyl-3-phenyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-3-yl)acetamide

[0143] Methyl 2-amino-2-phenylacetate hydrochloride was stirred in 28% ammonium hydroxide (5mL/g) solution for 96 hours resulting in the formation of 2-amino-2-phenylacetamide as a white precipitate that was collected via filtration and used without further purification. To a solution of 2-amino-2-phenylacetamide (1 equiv) in ethanol (0.1 M) was added tert-Butyl 4-oxo-1-piperidinecarboxylate (1 equiv) and the reaction mixture was heated to reflux for 12 hours. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The resulting residue was dissolved in DCM (0.1 M) and N-bromosuccinimide (1 equiv) was added. The reaction mixture was stirred for 8 hours and saturated sodium bicarbonate was added. The resulting mixture was extracted with DCM. The organics were dried, concentrated and purified by FCC (0-100% EtOAc in hexanes) to provide tert-butyl 3-oxo-2-phenyl-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate. ¹H NMR (400 MHz, DMSO-d₆) δ 10.26 (s, 1H), 8.47 – 8.23 (m, 2H), 7.61 – 7.48 (m, 3H), 3.70 – 3.50 (m, 4H), 1.80 – 1.68 (m, 2H), 1.68 – 1.58 (m, 2H), 1.44 (s, 9H).

[0144] To a solution of tert-butyl 3-oxo-2-phenyl-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate (1 equiv.) in THF (0.1 M) was added Lawesson's reagent (0.6 equiv) and the reaction mixture was heated to 60 °C until the reaction was complete by TLC. The reaction mixture was concentrated onto silica gel and purified by flash column chromatography (0-100% EtOAc in hexanes) to provide tert-butyl 2-phenyl-3-thioxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate. ¹H NMR (400 MHz, DMSO-d₆) δ 12.58 (s, 1H), 8.30 – 8.23 (m, 2H), 7.60 – 7.46 (m, 3H), 3.81 – 3.70 (m, 2H), 3.60 – 3.44 (m, 2H), 1.86 – 1.62 (m, 4H), 1.45 (s, 9H).

[0145] To a solution of tert-butyl 2-phenyl-3-thioxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate (1 equiv.) in DCM (0.1 M) was 2-bromo-N-(quinolin-3-yl)acetamide (1 equiv.) and triethylamine (3 equivalents). The reaction mixture was stirred for 12 hours at room temperature. The reaction mixture was diluted with DCM and washed with saturated aqueous sodium bicarbonate. The organic layers were dried, concentrated and purified by flash column chromatography (0-100% EtOAc in hexanes) to provide tert-butyl 2-((2-oxo-2-(quinolin-3-ylamino)ethyl)thio)-3-phenyl-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate.

[0146] To a solution of tert-butyl 2-((2-oxo-2-(quinolin-3-ylamino)ethyl)thio)-3-phenyl-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate (1 equiv) in DCM (0.1 M) was added 4 M HCl in dioxane (2 equiv.) and the reaction mixture was stirred at room temperature for 5 hours. The reaction mixture was concentrated. The resulting residue was taken up in THF and triethylamine (5 equiv) and formaldehyde (3 equiv) were added followed by NaBH(OAc)₃ (3 equiv) and the reaction mixture was stirred for 12 hours at room temperature. The reaction mixture was concentrated, taken up in EtOAc and washed with saturated aqueous sodium bicarbonate. The organics were dried, concentrated and purified by flash column chromatography (0-30% MeOH in DCM) to provide 2-((8-methyl-3-phenyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-3-yl)acetamide. ¹H NMR (400 MHz, DMSO-d₆) δ 10.81 (s, 1H), 8.94 (d, J = 2.5 Hz, 1H), 8.67 (d, J = 2.5 Hz, 1H), 8.02 – 7.90 (m, 4H), 7.66 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 7.58 (ddd, J = 8.1, 6.8, 1.3 Hz, 1H), 7.49 – 7.39 (m, 2H), 4.28 (s, 2H), 2.75 – 2.56 (m, 4H), 2.24 (s, 3H), 1.99 – 1.40 (m, 4H).

[0147] Synthesis of Compound 35c 2-((3-(4-chloro-3-fluorophenyl)-8-methyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-3-yl)acetamide

[0148] Methyl 2-amino-2-(4-chloro-3-fluorophenyl)acetate hydrochloride was stirred in 28% ammonium hydroxide (5mL/g) solution for 96 hours resulting in the formation of 2-amino-2-(4-chloro-3-fluorophenyl)acetamide as a white precipitate that was collected via filtration and used without further purification. To a solution of 2-amino-2-(4-chloro-3-fluorophenyl)acetamide (1 equiv) in ethanol (0.1 M) was added tert-Butyl 4-oxo-1-piperidinecarboxylate (1 equiv) and the reaction mixture was heated to reflux for 12 hours. The reaction mixture was cooled to room temperature and concentrated under reduced

pressure. The resulting residue was dissolved in DCM (0.1 M) and N-bromosuccinimide (1 equiv) was added. The reaction mixture was stirred for 8 hours and saturated sodium bicarbonate was added. The resulting mixture was extracted with DCM. The organics were dried, concentrated and purified by FCC (0-100% EtOAc in hexanes) to provide tert-butyl 2-(4-chloro-3-fluorophenyl)-3-oxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate. ¹H NMR (400 MHz, DMSO-d₆) δ 10.37 (s, 1H), 8.29 (dd, J = 10.4, 1.8 Hz, 1H), 8.20 (ddd, J = 8.4, 1.8, 0.8 Hz, 1H), 7.78 (dd, J = 8.4, 7.7 Hz, 1H), 3.69 – 3.52 (m, 4H), 1.73 (d, J = 6.8 Hz, 4H), 1.44 (s, 9H).

[0149] To a solution of tert-butyl 2-(4-chloro-3-fluorophenyl)-3-oxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate (1 equiv.) in THF (0.1 M) was added Lawesson's reagent (0.6 equiv) and the reaction mixture was heated to 60 °C until the reaction was complete by TLC. The reaction mixture was concentrated onto silica gel and purified by flash column chromatography (0-100% EtOAc in hexanes) to provide tert-butyl 2-(4-chloro-3-fluorophenyl)-3-thioxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate. ¹H NMR (400 MHz, DMSO-d₆) δ 12.58 (s, 1H), 8.30 – 8.23 (m, 2H), 7.60 – 7.46 (m, 3H), 3.81 – 3.70 (m, 2H), 3.60 – 3.44 (m, 2H), 1.86 – 1.62 (m, 4H), 1.45 (s, 9H).

[0150] To a solution of tert-butyl 2-(4-chloro-3-fluorophenyl)-3-thioxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate (1 equiv.) in DCM (0.1 M) was 2-bromo-N-(quinolin-3-yl)acetamide (1 equiv.) and triethylamine (3 equivalents). The reaction mixture was stirred for 12 hours at room temperature. The reaction mixture was diluted with DCM and washed with saturated aqueous sodium bicarbonate. The organic layers were dried, concentrated and purified by flash column chromatography (0-100% EtOAc in hexanes) to provide tert-butyl 2-(4-chloro-3-fluorophenyl)-3-((2-oxo-2-(quinolin-3-ylamino)ethyl)thio)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate. ¹H NMR (400 MHz, DMSO-d₆) δ 10.78 (s, 1H), 8.91 (d, J = 2.5 Hz, 1H), 8.71 – 8.61 (m, 1H), 8.00 – 7.95 (m, 1H), 7.95 – 7.91 (m, 1H), 7.90 – 7.83 (m, 2H), 7.81 – 7.76 (m, 1H), 7.67 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 7.58 (ddd, J = 8.2, 6.9, 1.3 Hz, 1H), 4.29 (s, 2H), 3.70 – 3.58 (m, 2H), 3.58 – 3.45 (m, 2H), 1.82 – 1.65 (m, 2H), 1.60 – 1.43 (m, 2H), 1.38 (s, 9H).

[0151] To a solution of tert-butyl 2-(4-chloro-3-fluorophenyl)-3-((2-oxo-2-(quinolin-3-ylamino)ethyl)thio)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate (1 equiv) in DCM (0.1 M) was added 4 M HCl in dioxane (2 equiv.) and the reaction mixture was stirred at room temperature for 5 hours. The reaction mixture was concentrated. The resulting residue was taken up in THF and triethylamine (5 equiv) and formaldehyde (3 equiv) were added followed by NaBH(OAc)₃ (3 equiv) and the reaction mixture was stirred for 12 hours at room temperature. The reaction mixture was concentrated, taken up in EtOAc and washed with saturated aqueous sodium bicarbonate. The organics were dried, concentrated and purified by flash column chromatography (0-30% MeOH in DCM) to provide 2-((3-(4-chloro-3-

fluorophenyl)-8-methyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-3-yl)acetamide. ¹H NMR (400 MHz, DMSO-d₆) δ 10.80 (s, 1H), 8.94 (d, J = 2.5 Hz, 1H), 8.67 (d, J = 2.5 Hz, 1H), 7.99 – 7.95 (m, 1H), 7.93 (d, J = 7.7 Hz, 1H), 7.89 – 7.82 (m, 2H), 7.80 – 7.75 (m, 1H), 7.66 (ddd, J = 8.5, 6.9, 1.5 Hz, 1H), 7.58 (ddd, J = 8.1, 6.8, 1.3 Hz, 1H), 4.29 (s, 2H), 2.63 – 2.55 (m, 4H), 2.20 (s, 3H), 1.93 – 1.38 (m, 4H).

[0152] Synthesis of Compound 35d 2-((3-(3-bromophenyl)-8-methyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-3-yl)acetamide

[0153] Methyl 2-amino-2-(3-bromophenyl)acetate was stirred in 28% ammonium hydroxide (5mL/g) solution for 96 hours resulting in the formation of 2-amino-2-(3-bromophenyl)acetamide as a white precipitate that was collected via filtration and used without further purification. To a solution of 2-amino-2-(3-bromophenyl)acetamide (1 equiv) in ethanol (0.1 M) was added tert-Butyl 4-oxo-1-piperidinecarboxylate (1 equiv) and the reaction mixture was heated to reflux for 12 hours. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The resulting residue was dissolved in DCM (0.1 M) and N-bromosuccinimide (1 equiv) was added. The reaction mixture was stirred for 8 hours and saturated sodium bicarbonate was added. The resulting mixture was extracted with DCM. The organics were dried, concentrated and purified by FCC (0-100% EtOAc in hexanes) to provide tert-butyl 2-(3-bromophenyl)-3-oxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate. ¹H NMR (400 MHz, DMSO-d₆) δ 10.33 (s, 1H), 8.54 – 8.48 (m, 1H), 8.39 – 8.25 (m, 1H), 7.80 (d, J = 8.0, 2.1, 1.1 Hz, 1H), 7.57 – 7.44 (m, 1H), 3.60 (d, J = 19.5 Hz, 4H), 1.73 (d, J = 7.4 Hz, 2H), 1.69 – 1.55 (m, 2H), 1.44 (s, 9H).

[0154] To a solution of tert-butyl 2-(3-bromophenyl)-3-oxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate (1 equiv.) in THF (0.1 M) was added Lawesson's reagent (0.6 equiv) and the reaction mixture was heated to 60 °C until the reaction was complete by TLC. The reaction mixture was concentrated onto silica gel and purified by flash column chromatography (0-100% EtOAc in hexanes) to provide tert-butyl 2-(3-bromophenyl)-3-thio-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate. ¹H NMR (400 MHz, DMSO-d₆) δ 12.63 (s, 1H), 8.51 – 8.42 (m, 1H), 8.27 (dt, J = 7.8, 1.3 Hz, 1H), 7.77 (ddd, J = 8.0, 2.1, 1.1 Hz, 1H), 7.51 – 7.44 (m, 1H), 3.82 – 3.68 (m, 2H), 3.61 – 3.43 (m, 2H), 1.86 – 1.66 (m, 4H), 1.45 (s, 9H).

[0155] To a solution of tert-butyl 2-(3-bromophenyl)-3-thio-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate (1 equiv.) in DCM (0.1 M) was 2-bromo-N-(quinolin-3-yl)acetamide (1 equiv.) and triethylamine (3 equivalents). The reaction mixture was stirred for 12 hours at room temperature. The reaction mixture was diluted with DCM and washed with saturated aqueous sodium bicarbonate. The organic layers were dried, concentrated and purified by flash column chromatography (0-100% EtOAc in hexanes) to provide tert-butyl 2-(3-bromophenyl)-3-((2-oxo-2-(quinolin-3-ylamino)ethyl)thio)-1,4,8-triazaspiro[4.5]deca-1,3-

diene-8-carboxylate. ¹H NMR (400 MHz, DMSO-d₆) δ 10.78 (s, 1H), 8.91 (d, J = 2.6 Hz, 1H), 8.70 – 8.59 (m, 1H), 8.05 (t, J = 1.8 Hz, 1H), 8.00 – 7.95 (m, 1H), 7.95 – 7.90 (m, 2H), 7.84 (ddd, J = 8.1, 2.1, 1.0 Hz, 1H), 7.67 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 7.61 – 7.54 (m, 2H), 4.28 (s, 2H), 3.71 – 3.58 (m, 2H), 3.57 – 3.46 (m, 2H), 1.80 – 1.68 (m, 2H), 1.60 – 1.42 (m, 2H), 1.38 (s, 9H).

[0156] To a solution of tert-butyl 2-(3-bromophenyl)-3-((2-oxo-2-(quinolin-3-ylamino)ethyl)thio)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate (1 equiv) in DCM (0.1 M) was added 4 M HCl in dioxane (2 equiv.) and the reaction mixture was stirred at room temperature for 5 hours. The reaction mixture was concentrated. The resulting residue was taken up in THF and triethylamine (5 equiv) and formaldehyde (3 equiv) were added followed by NaBH(OAc)₃ (3 equiv) and the reaction mixture was stirred for 12 hours at room temperature. The reaction mixture was concentrated, taken up in EtOAc and washed with saturated aqueous sodium bicarbonate. The organics were dried, concentrated and purified by flash column chromatography (0-30% MeOH in DCM) to provide 2-((3-(3-bromophenyl)-8-methyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-3-yl)acetamide. ¹H NMR (400 MHz, DMSO-d₆) δ 10.79 (s, 1H), 8.94 (d, J = 2.5 Hz, 1H), 8.67 (d, J = 2.5 Hz, 1H), 8.03 (t, J = 1.8 Hz, 1H), 7.99 – 7.93 (m, 1H), 7.93 – 7.88 (m, 2H), 7.83 (ddd, J = 8.1, 2.1, 1.0 Hz, 1H), 7.66 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 7.62 – 7.54 (m, 2H), 4.28 (s, 2H), 2.59 (s, 4H), 2.21 (s, 3H), 1.94 – 1.48 (m, 4H).

[0157] Synthesis of Compound 35e 2-((8-methyl-3-(naphthalen-2-yl)-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-3-yl)acetamide

[0158] Methyl methyl amino(2-naphthyl)acetate hydrochloride was stirred in 28% ammonium hydroxide (5mL/g) solution for 96 hours resulting in the formation of 2-amino-2-(naphthalen-2-yl)acetamide as a white precipitate that was collected via filtration and used without further purification. To a solution of 2-amino-2-(naphthalen-2-yl)acetamide (1 equiv) in ethanol (0.1 M) was added tert-Butyl 4-oxo-1-piperidinecarboxylate (1 equiv) and the reaction mixture was heated to reflux for 12 hours. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The resulting residue was dissolved in DCM (0.1 M) and N-bromosuccinimide (1 equiv) was added. The reaction mixture was stirred for 8 hours and saturated sodium bicarbonate was added. The resulting mixture was extracted with DCM. The organics were dried, concentrated and purified by FCC (0-100% EtOAc in hexanes) to provide tert-butyl 2-(naphthalen-2-yl)-3-oxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate. ¹H NMR (400 MHz, DMSO-d₆) δ 10.35 (s, 1H), 9.18 – 9.11 (m, 1H), 8.28 (dd, J = 8.6, 1.6 Hz, 1H), 8.12 – 7.96 (m, 3H), 7.68 – 7.57 (m, 2H), 3.77 – 3.64 (m, 2H), 3.64 – 3.51 (m, 1H), 1.86 – 1.73 (m, 2H), 1.73 – 1.62 (m, 2H), 1.45 (s, 9H).

[0159] To a solution of tert-butyl 2-(naphthalen-2-yl)-3-oxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate (1 equiv.) in THF (0.1 M) was added Lawesson's reagent (0.6 equiv) and the reaction mixture was heated to 60 °C until the reaction was complete by TLC. The reaction mixture was concentrated onto silica gel and purified by flash column chromatography (0-100% EtOAc in hexanes) to provide tert-butyl 2-(naphthalen-2-yl)-3-thioxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate.

[0160] To a solution of tert-butyl 2-(naphthalen-2-yl)-3-thioxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate (1 equiv.) in DCM (0.1 M) was 2-bromo-N-(quinolin-3-yl)acetamide (1 equiv.) and triethylamine (3 equivalents). The reaction mixture was stirred for 12 hours at room temperature. The reaction mixture was diluted with DCM and washed with saturated aqueous sodium bicarbonate. The organic layers were dried, concentrated and purified by flash column chromatography (0-100% EtOAc in hexanes) to provide tert-butyl 2-(naphthalen-2-yl)-3-((2-oxo-2-(quinolin-3-ylamino)ethyl)thio)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate. ¹H NMR (400 MHz, DMSO-d₆) δ 10.80 (s, 1H), 8.93 (d, J = 2.5 Hz, 1H), 8.73 – 8.64 (m, 1H), 8.59 – 8.52 (m, 1H), 8.17 – 8.09 (m, 2H), 8.09 – 7.88 (m, 4H), 7.74 – 7.63 (m, 3H), 7.62 – 7.55 (m, 1H), 4.32 (s, 2H), 3.68 (s, 2H), 3.61 – 3.46 (m, 2H), 1.88 – 1.71 (m, 2H), 1.57 – 1.43 (m, 1H), 1.39 (s, 9H).

[0161] To a solution of tert-butyl 2-(naphthalen-2-yl)-3-((2-oxo-2-(quinolin-3-ylamino)ethyl)thio)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate (1 equiv) in DCM (0.1 M) was added 4 M HCl in dioxane (2 equiv.) and the reaction mixture was stirred at room temperature for 5 hours. The reaction mixture was concentrated. The resulting residue was taken up in THF and triethylamine (5 equiv) and formaldehyde (3 equiv) were added followed by NaBH(OAc)₃ (3 equiv) and the reaction mixture was stirred for 12 hours at room temperature. The reaction mixture was concentrated, taken up in EtOAc and washed with saturated aqueous sodium bicarbonate. The organics were dried, concentrated and purified by flash column chromatography (0-30% MeOH in DCM) to provide 2-((8-methyl-3-(naphthalen-2-yl)-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-3-yl)acetamide. ¹H NMR (400 MHz, DMSO-d₆) δ 10.79 (s, 1H), 8.94 (d, J = 2.5 Hz, 1H), 8.67 (d, J = 2.5 Hz, 1H), 7.99 – 7.95 (m, 1H), 7.95 – 7.91 (m, 1H), 7.91 – 7.85 (m, 2H), 7.66 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 7.58 (ddd, J = 8.1, 6.8, 1.3 Hz, 1H), 7.16 – 7.10 (m, 2H), 4.26 (s, 2H), 3.86 (s, 3H), 3.32 (s, 3H), 2.65 – 2.53 (m, 4H), 2.24 – 2.15 (m, 2H), 1.89 – 1.29 (m, 4H).

[0162] Synthesis of Compound 35f 2-((3-(4-methoxyphenyl)-8-methyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-3-yl)acetamide

[0163] methyl amino(4-methoxyphenyl)acetate hydrochloride was stirred in 28% ammonium hydroxide (5mL/g) solution for 96 hours resulting in the formation of 2-amino-2-(4-methoxyphenyl)acetamide as a white precipitate that was collected via filtration and used

without further purification. To a solution of 2-amino-2-(4-methoxyphenyl)acetamide (1 equiv) in ethanol (0.1 M) was added tert-Butyl 4-oxo-1-piperidinecarboxylate (1 equiv) and the reaction mixture was heated to reflux for 12 hours. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The resulting residue was dissolved in DCM (0.1 M) and N-bromosuccinimide (1 equiv) was added. The reaction mixture was stirred for 8 hours and saturated sodium bicarbonate was added. The resulting mixture was extracted with DCM. The organics were dried, concentrated and purified by FCC (0-100% EtOAc in hexanes) to provide tert-butyl 2-(4-methoxyphenyl)-3-oxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate. ¹H NMR (400 MHz, DMSO-d₆) δ 10.20 (s, 1H), 8.39 – 8.29 (m, 2H), 7.11 – 7.01 (m, 2H), 3.83 (s, 3H), 3.71 – 3.48 (m, 4H), 1.78 – 1.67 (m, 2H), 1.67 – 1.54 (m, 2H), 1.44 (s, 9H).

[0164] To a solution of tert-butyl 2-(4-methoxyphenyl)-3-oxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate (1 equiv.) in THF (0.1 M) was added Lawesson's reagent (0.6 equiv) and the reaction mixture was heated to 60 °C until the reaction was complete by TLC. The reaction mixture was concentrated onto silica gel and purified by flash column chromatography (0-100% EtOAc in hexanes) to provide tert-butyl 2-(4-methoxyphenyl)-3-thioxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate.

[0165] To a solution of tert-butyl 2-(4-methoxyphenyl)-3-thioxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate (1 equiv.) in DCM (0.1 M) was 2-bromo-N-(quinolin-3-yl)acetamide (1 equiv.) and triethylamine (3 equivalents). The reaction mixture was stirred for 12 hours at room temperature. The reaction mixture was diluted with DCM and washed with saturated aqueous sodium bicarbonate. The organic layers were dried, concentrated and purified by flash column chromatography (0-100% EtOAc in hexanes) to provide tert-butyl 2-(4-methoxyphenyl)-3-((2-oxo-2-(quinolin-3-ylamino)ethyl)thio)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate. ¹H NMR (400 MHz, DMSO-d₆) δ 10.77 (s, 1H), 8.92 (d, J = 2.5 Hz, 1H), 8.70 – 8.61 (m, 1H), 7.99 – 7.93 (m, 1H), 7.93 – 7.87 (m, 2H), 7.66 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 7.58 (ddd, J = 8.1, 6.8, 1.3 Hz, 1H), 7.20 – 7.08 (m, 2H), 4.27 (s, 2H), 3.86 (s, 3H), 3.73 – 3.57 (m, 2H), 3.57 – 3.42 (m, 2H), 1.81 – 1.67 (m, 2H), 1.50 – 1.40 (m, 2H), 1.37 (s, 9H).

[0166] To a solution of tert-butyl 2-(4-methoxyphenyl)-3-((2-oxo-2-(quinolin-3-ylamino)ethyl)thio)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate (1 equiv) in DCM (0.1 M) was added 4 M HCl in dioxane (2 equiv.) and the reaction mixture was stirred at room temperature for 5 hours. The reaction mixture was concentrated. The resulting residue was taken up in THF and triethylamine (5 equiv) and formaldehyde (3 equiv) were added followed by NaBH(OAc)₃ (3 equiv) and the reaction mixture was stirred for 12 hours at room temperature. The reaction mixture was concentrated, taken up in EtOAc and washed with saturated aqueous sodium bicarbonate. The organics were dried, concentrated and purified

by flash column chromatography (0-30% MeOH in DCM) to provide 2-((3-(4-methoxyphenyl)-8-methyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-3-yl)acetamide. ¹H NMR (400 MHz, DMSO-d₆) δ 10.79 (s, 1H), 8.94 (d, J = 2.5 Hz, 1H), 8.67 (d, J = 2.5 Hz, 1H), 7.99 – 7.95 (m, 1H), 7.95 – 7.91 (m, 1H), 7.91 – 7.85 (m, 2H), 7.66 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 7.58 (ddd, J = 8.1, 6.8, 1.3 Hz, 1H), 7.16 – 7.10 (m, 2H), 4.26 (s, 2H), 3.86 (s, 3H), 2.65 – 2.53 (m, 4H), 2.20 (s, 3H), 1.89 – 1.29 (m, 4H).

[0167] Synthesis of Compound 35g 2-((3-(2-bromophenyl)-8-methyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-3-yl)acetamide

[0168] methyl 2-amino-2-(2-bromophenyl)acetate was stirred in 28% ammonium hydroxide (5mL/g) solution for 96 hours resulting in the formation of 2-amino-2-(2-bromophenyl)acetamide as a white precipitate that was collected via filtration and used without further purification. To a solution of 2-amino-2-(2-bromophenyl)acetamide (1 equiv) in ethanol (0.1 M) was added tert-Butyl 4-oxo-1-piperidinecarboxylate (1 equiv) and the reaction mixture was heated to reflux for 12 hours. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The resulting residue was dissolved in DCM (0.1 M) and N-bromosuccinimide (1 equiv) was added. The reaction mixture was stirred for 8 hours and saturated sodium bicarbonate was added. The resulting mixture was extracted with DCM. The organics were dried, concentrated and purified by FCC (0-100% EtOAc in hexanes) to provide tert-butyl 2-(2-bromophenyl)-3-oxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate. ¹H NMR (400 MHz, DMSO-d₆) δ 10.23 (s, 1H), 7.82 – 7.74 (m, 1H), 7.64 – 7.40 (m, 3H), 3.73 – 3.49 (m, 4H), 1.78 – 1.68 (m, 4H), 1.44 (s, 9H).

[0169] To a solution of tert-butyl 2-(2-bromophenyl)-3-oxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate (1 equiv.) in THF (0.1 M) was added Lawesson's reagent (0.6 equiv) and the reaction mixture was heated to 60 °C until the reaction was complete by TLC. The reaction mixture was concentrated onto silica gel and purified by flash column chromatography (0-100% EtOAc in hexanes) to provide tert-butyl 2-(2-bromophenyl)-3-thio-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate. ¹H NMR (400 MHz, DMSO-d₆) δ 12.55 (s, 1H), 7.73 (dt, J = 7.8, 0.9 Hz, 1H), 7.53 – 7.40 (m, 3H), 3.85 – 3.72 (m, 2H), 3.55 – 3.43 (m, 2H), 1.90 – 1.70 (m, 4H), 1.44 (s, 9H).

[0170] To a solution tert-butyl 2-(2-bromophenyl)-3-thio-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate (1 equiv.) in DCM (0.1 M) was 2-bromo-N-(quinolin-3-yl)acetamide (1 equiv.) and triethylamine (3 equivalents). The reaction mixture was stirred for 12 hours at room temperature. The reaction mixture was diluted with DCM and washed with saturated aqueous sodium bicarbonate. The organic layers were dried, concentrated and purified by flash column chromatography (0-100% EtOAc in hexanes) to provide tert-butyl 2-(2-bromophenyl)-3-((2-oxo-2-(quinolin-3-ylamino)ethyl)thio)-1,4,8-triazaspiro[4.5]deca-1,3-

diene-8-carboxylate. ¹H NMR (400 MHz, DMSO-d₆) δ 10.78 (s, 1H), 8.90 (d, J = 2.5 Hz, 1H), 8.65 (d, J = 2.4 Hz, 1H), 8.01 – 7.91 (m, 2H), 7.83 (dd, J = 8.2, 1.2 Hz, 1H), 7.66 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 7.63 – 7.47 (m, 4H), 4.23 (s, 2H), 3.68 – 3.53 (m, 4H), 1.83 – 1.68 (m, 2H), 1.68 – 1.54 (m, 2H), 1.40 (s, 9H).

[0171] To a solution of tert-butyl 2-(2-bromophenyl)-3-((2-oxo-2-(quinolin-3-ylamino)ethyl)thio)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate (1 equiv) in DCM (0.1 M) was added 4 M HCl in dioxane (2 equiv.) and the reaction mixture was stirred at room temperature for 5 hours. The reaction mixture was concentrated. The resulting residue was taken up in THF and triethylamine (5 equiv) and formaldehyde (3 equiv) were added followed by NaBH(OAc)₃ (3 equiv) and the reaction mixture was stirred for 12 hours at room temperature. The reaction mixture was concentrated, taken up in EtOAc and washed with saturated aqueous sodium bicarbonate. The organics were dried, concentrated and purified by flash column chromatography (0-30% MeOH in DCM) to provide 2-((3-(2-bromophenyl)-8-methyl-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-3-yl)acetamide. ¹H NMR (400 MHz, DMSO-d₆) δ 10.79 (s, 1H), 8.91 (d, J = 2.5 Hz, 1H), 8.66 (d, J = 2.5 Hz, 1H), 7.95 (ddd, J = 12.8, 8.2, 1.3 Hz, 2H), 7.88 – 7.76 (m, 1H), 7.66 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 7.62 – 7.46 (m, 4H), 4.22 (s, 2H), 2.72 – 2.53 (m, 4H), 2.24 (s, 3H), 1.94 – 1.64 (m, 4H).

[0172] Synthesis of Compound 35h 2-((8-methyl-3-(4-(trifluoromethyl)phenyl)-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-3-yl)acetamide

[0173] methyl 2-amino-2-[4-(trifluoromethyl)phenyl]acetate hydrochloride was stirred in 28% ammonium hydroxide (5mL/g) solution for 96 hours resulting in the formation of 2-amino-2-(4-(trifluoromethyl)phenyl)acetamide as a white precipitate that was collected via filtration and used without further purification. To a solution of 2-amino-2-(4-(trifluoromethyl)phenyl)acetamide (1 equiv) in ethanol (0.1 M) was added tert-Butyl 4-oxo-1-piperidinecarboxylate (1 equiv) and the reaction mixture was heated to reflux for 12 hours. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The resulting residue was dissolved in DCM (0.1 M) and N-bromosuccinimide (1 equiv) was added. The reaction mixture was stirred for 8 hours and saturated sodium bicarbonate was added. The resulting mixture was extracted with DCM. The organics were dried, concentrated and purified by FCC (0-100% EtOAc in hexanes) to provide tert-butyl 3-oxo-2-(4-(trifluoromethyl)phenyl)-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate. ¹H NMR (400 MHz, DMSO-d₆) δ 10.36 (s, 1H), 8.53 (d, J = 8.1 Hz, 2H), 7.91 (d, J = 8.1 Hz, 2H), 3.72 – 3.52 (m, 4H), 1.85 – 1.60 (m, 4H), 1.45 (s, 9H).

[0174] To a solution of tert-butyl 3-oxo-2-(4-(trifluoromethyl)phenyl)-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate (1 equiv.) in THF (0.1 M) was added Lawesson's reagent (0.6 equiv) and the reaction mixture was heated to 60 °C until the reaction was

complete by TLC. The reaction mixture was concentrated onto silica gel and purified by flash column chromatography (0-100% EtOAc in hexanes) to provide tert-butyl 2-((2-oxo-2-(quinolin-3-ylamino)ethyl)thio)-3-(4-(trifluoromethyl)phenyl)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate. ¹H NMR (400 MHz, DMSO-d₆) δ 12.66 (s, 1H), 8.58 (d, J = 2.0 Hz, 1H), 8.27 (dd, J = 8.4, 2.0 Hz, 1H), 7.80 (d, J = 8.4 Hz, 1H), 3.83 – 3.71 (m, 2H), 3.61 – 3.45 (m, 2H), 1.89 – 1.66 (m, 4H), 1.45 (s, 9H).

[0175] To a solution tert-butyl 3-thioxo-2-(4-(trifluoromethyl)phenyl)-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate (1 equiv.) in DCM (0.1 M) was 2-bromo-N-(quinolin-3-yl)acetamide (1 equiv.) and triethylamine (3 equivalents). The reaction mixture was stirred for 12 hours at room temperature. The reaction mixture was diluted with DCM and washed with saturated aqueous sodium bicarbonate. The organic layers were dried, concentrated and purified by flash column chromatography (0-100% EtOAc in hexanes) to provide tert-butyl 2-((2-oxo-2-(quinolin-3-ylamino)ethyl)thio)-3-(4-(trifluoromethyl)phenyl)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate. ¹H NMR (400 MHz, DMSO-d₆) δ 10.80 (s, 1H), 8.97 – 8.88 (m, 1H), 8.79 – 8.58 (m, 1H), 8.19 – 8.08 (m, 1H), 8.08 – 7.86 (m, 4H), 7.77 – 7.52 (m, 2H), 4.29 (s, 2H), 3.72 – 3.46 (m, 4H), 1.82 – 1.67 (m, 2H), 1.61 – 1.43 (m, 2H), 1.38 (s, 9H).

[0176] To a solution of tert-butyl 2-((2-oxo-2-(quinolin-3-ylamino)ethyl)thio)-3-(4-(trifluoromethyl)phenyl)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate (1 equiv) in DCM (0.1 M) was added 4 M HCl in dioxane (2 equiv.) and the reaction mixture was stirred at room temperature for 5 hours. The reaction mixture was concentrated. The resulting residue was taken up in THF and triethylamine (5 equiv) and formaldehyde (3 equiv) were added followed by NaBH(OAc)₃ (3 equiv) and the reaction mixture was stirred for 12 hours at room temperature. The reaction mixture was concentrated, taken up in EtOAc and washed with saturated aqueous sodium bicarbonate. The organics were dried, concentrated and purified by flash column chromatography (0-30% MeOH in DCM) to provide 2-((8-methyl-3-(4-(trifluoromethyl)phenyl)-1,4,8-triazaspiro[4.5]deca-1,3-dien-2-yl)thio)-N-(quinolin-3-yl)acetamide. ¹H NMR (400 MHz, DMSO-d₆) δ 10.80 (s, 1H), 8.94 (d, J = 2.5 Hz, 1H), 8.67 (d, J = 2.5 Hz, 1H), 8.09 (t, J = 1.2 Hz, 1H), 8.01 – 7.91 (m, 2H), 7.91 – 7.83 (m, 2H), 7.67 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 7.59 (ddd, J = 8.1, 6.8, 1.3 Hz, 1H), 4.29 (s, 2H), 2.70 – 2.57 (m, 4H), 2.23 (s, 3H), 1.98 – 1.46 (m, 4H).

[0177] Synthesis of Compound 35i tert-butyl 2-(3,4-dichlorophenyl)-3-((quinolin-3-ylcarbonyl)thio)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate

[0178] Methyl 2-amino-2-(3,4-dichlorophenyl)acetate hydrochloride was stirred in 28% ammonium hydroxide (5mL/g) solution for 96 hours resulting in the formation of 2-amino-2-(3,4-dichlorophenyl)acetamide as a white precipitate that was collected via filtration and

used without further purification. To a solution of 2-amino-2-(3,4-dichlorophenyl)acetamide (1 equiv) in ethanol (0.1 M) was added tert-Butyl 4-oxo-1-piperidinecarboxylate (1 equiv) and the reaction mixture was heated to reflux for 12 hours. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The resulting residue was dissolved in DCM (0.1 M) and N-bromosuccinimide (1 equiv) was added. The reaction mixture was stirred for 8 hours and saturated sodium bicarbonate was added. The resulting mixture was extracted with DCM. The organics were dried, concentrated and purified by FCC (0-100% EtOAc in hexanes) to provide tert-butyl 2-(3,4-dichlorophenyl)-3-oxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate. ¹H NMR (400 MHz, DMSO-d₆) δ 10.37 (s, 1H), 8.55 (d, J = 1.9 Hz, 1H), 8.29 (dd, J = 8.4, 1.9 Hz, 1H), 7.82 (d, J = 8.4 Hz, 1H), 3.70 – 3.51 (m, 4H), 1.81 – 1.58 (m, 4H), 1.44 (s, 9H).

[0179] To a solution of tert-butyl 2-(3,4-dichlorophenyl)-3-oxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate (1 equiv.) in THF (0.1 M) was added Lawesson's reagent (0.6 equiv) and the reaction mixture was heated to 60 °C until the reaction was complete by TLC. The reaction mixture was concentrated onto silica gel and purified by flash column chromatography (0-100% EtOAc in hexanes) to provide tert-butyl 2-(3,4-dichlorophenyl)-3-thioxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate. ¹H NMR (400 MHz, DMSO-d₆) δ 12.66 (s, 1H), 8.58 (d, J = 2.0 Hz, 1H), 8.27 (dd, J = 8.4, 2.0 Hz, 1H), 7.80 (d, J = 8.4 Hz, 1H), 3.83 – 3.71 (m, 2H), 3.61 – 3.45 (m, 2H), 1.89 – 1.66 (m, 4H), 1.45 (s, 9H).

[0180] To a solution tert-butyl 2-(3,4-dichlorophenyl)-3-thioxo-1,4,8-triazaspiro[4.5]dec-1-ene-8-carboxylate (1 equiv.) in DCM (0.1 M) was 2-bromo-N-(quinolin-3-yl)acetamide (1 equiv.) and triethylamine (3 equivalents). The reaction mixture was stirred for 12 hours at room temperature. The reaction mixture was diluted with DCM and washed with saturated aqueous sodium bicarbonate. The organic layers were dried, concentrated and purified by flash column chromatography (0-100% EtOAc in hexanes) to provide tert-butyl 2-(3,4-dichlorophenyl)-3-((2-oxo-2-(quinolin-3-ylamino)ethyl)thio)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate. ¹H NMR (400 MHz, DMSO-d₆) δ 10.80 (s, 1H), 8.97 – 8.88 (m, 1H), 8.79 – 8.58 (m, 1H), 8.19 – 8.08 (m, 1H), 8.08 – 7.86 (m, 4H), 7.77 – 7.52 (m, 2H), 4.29 (s, 2H), 3.72 – 3.46 (m, 4H), 1.82 – 1.67 (m, 2H), 1.61 – 1.43 (m, 2H), 1.38 (s, 9H).

[0181] To a solution of tert-butyl 2-(3,4-dichlorophenyl)-3-((2-oxo-2-(quinolin-3-ylamino)ethyl)thio)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate (1 equiv) in DCM (0.1 M) was added 4 M HCl in dioxane (2 equiv.) and the reaction mixture was stirred at room temperature for 5 hours. The reaction mixture was concentrated. The resulting residue was taken up in THF and triethylamine (5 equiv) and formaldehyde (3 equiv) were added followed by NaBH(OAc)₃ (3 equiv) and the reaction mixture was stirred for 12 hours at room temperature. The reaction mixture was concentrated, taken up in EtOAc and washed with saturated aqueous sodium bicarbonate. The organics were dried, concentrated and purified

by flash column chromatography (0-30% MeOH in DCM) to provide tert-butyl 2-(3,4-dichlorophenyl)-3-((quinolin-3-ylcarbonyl)thio)-1,4,8-triazaspiro[4.5]deca-1,3-diene-8-carboxylate. ¹H NMR (400 MHz, DMSO-d₆) δ 10.80 (s, 1H), 8.94 (d, J = 2.5 Hz, 1H), 8.67 (d, J = 2.5 Hz, 1H), 8.09 (t, J = 1.2 Hz, 1H), 8.01 – 7.91 (m, 2H), 7.91 – 7.83 (m, 2H), 7.67 (ddd, J = 8.4, 6.9, 1.5 Hz, 1H), 7.59 (ddd, J = 8.1, 6.8, 1.3 Hz, 1H), 4.29 (s, 2H), 2.70 – 2.57 (m, 4H), 2.23 (s, 3H), 1.98 – 1.46 (m, 4H).

[0182]

Pharmacokinetics Studies

Example 44 - Interaction between purified EGFR kinase domain and Disruptin

[0183] WT-EGFR kinase domain (aa696-1022, active kinase) was expressed and purified from SF9 insect cells. 100 ng pure EGFR was incubated with biotin-conjugated Disruptin, and the bound EGFR was captured using CaptAvidin beads after washing with citrate buffer. Specificity of this interaction was confirmed by co-incubating this reaction with an increasing amount of non-biotinylated Disruptin. The bound EGFR protein was released in Laemmli buffer and resolved by SDS page. Disruptin-bound EGFR was quantified using ImageJ software and shown below the blot. Competitive inhibition of Disruptin-EGFR binding by cold-Disruptin indicates that Disruptin binds directly to EGFR (see Figure 2B).

Example 45 - Effect of Disruptin treatment on EGF induced dimerization in NCI-H1975 cells

[0184] NCI-H1975 cells were treated for 1 hour with 10 μM Disruptin and an additional 30 minutes with EGF (30 ng/ml) and 30 minutes with disuccinimidyl suberate (DSS, 150 μM) to cross-link interacting proteins. Lysates were prepared and immunoblotted with anti-EGFR antibodies. (see Figure 3A).

Example 46 - Effect of Disruptin on its target EGFR in NCI-H1975 xenografts

[0185] Mice bearing NCI-H1975 xenografts were injected with Disruptin (10 mg/kg, i.p.) on days 1 and 2. On day 3 tumors were removed and prepared for immunoblotting (see Figure 3B). The effect of Disruptin on NCI-H1975 xenografts was assessed after treatment with Disruptin (10 mg/kg, i.p.) injections on two consecutive days (Monday-Tuesday) for two weeks. Tumor volume was plotted for each treatment condition. Scrambled-peptide was used as control (see Figure 3C). The long term effect of treatment on tumor histology, EGFR expression, and mitotic index (measure by Ki-67 score) was assessed by immunostaining the tumors after 2 weeks of treatment (see Figure 3D).

Example 47 - Development of Compound 8C and confirmation of biological activity

[0186] Disruptin was shown to be effective against a TKI (erlotinib) resistant NCI-H1975 xenograft model (see Figures 3A-D) (14). However, due to poor PK and other challenges in the development of peptide drugs, a drug-discovery program was undertaken to develop a

small molecule that has a similar mechanism of action but superior pharmacokinetics (see Figures 4A-D). Based on the active EGFR dimer interface, using a virtual screen, commercial library acquisition, and custom synthesis a novel series of molecules was developed that includes pre-lead compounds 95 (C95) and 67 (C67) (see Figures 4A-D). Both of these pre-lead molecules are active *in vivo* against the erlotinib-resistant NCI-H1975 xenograft model. To test the *in vivo* activity of EGFR dimer inhibition, TKI resistant NCI-H1975 EGFR reporter cells were used. These tumor xenografts show induction of bioluminescence upon loss of EGFR activity (47). A single injection of 100 mg/kg C95 led to a 2.5-fold induction of the EGFR reporter that lasted 48 hr. (see Figure 9). It was confirmed that the effect of C95 treatment on reduction in EGFR level correlates with change in bioluminescence. These data indicate that agents that can induce degradation of EGFR can be effective in TKI resistant tumors.

[0187] Compound 8C is approximately 20 times more stable compared to its pre lead (C95) in both mouse and human liver microsomes. Efficacy testing of Compound 8C in UMSCC74B, an EGFR driven, aggressive tumor model of the head and neck. Tumor bearing mice were treated with 30 mg/kg dose daily (Monday- Friday) for one week. This dose was selected based on single dose PK profile (see Figure 8A). This treatment was safe and produced significant tumor growth delay (see Figure 10).

[0188] Osimertinib resistant Ba/F3 cells mediated ascites (via i.p. injection of cells) and solid tumor models (via s.c. injection of cells) in nude mice were also used for efficacy studies. Initial studies were performed in the ascites model and the effect of Compound 8C was compared to an equal dose of osimertinib (30 mg/kg). Although treatment was initiated only after the tumor burden became high, a single 30 mg/kg dose of Compound 8C prolonged life in these mice compared to the osimertinib treated group ($p=0.072$, data not shown). The effect of treatment on EGFR was confirmed in the cells collected from the same mouse before treatment, 18 hours and 24 hours post-treatment (see Figure 11).

Example 48 - Direct interaction between EGFR kinase domain and Compound 8C

[0189] To determine if Compound 8C competes for the same binding site on EGFR, Biotin-Disruptin-Avidin coupled beads were incubated with purified EGFR in the presence or absence of Compound 8C for 15 minutes at 37° C. The agarose beads were spun down, and the non-specific protein removed by washing in citrate buffer. The bound protein was released in Laemmli buffer and resolved by SDS page. Competitive inhibition of Disruptin-EGFR binding by the Compound 8C suggests a similar mechanism of action of this molecule (see Figure 5A).

[0190] The effect of Compound 8C on thermal stability of purified EGFR was confirmed by thermal stability assay. 100 ng of purified EGFR was incubated with either DMSO or

Compound 8C (10 μ M) for 30 minutes at 4 °C. The samples were subjected to heat inactivation (20 to 44 °C) for 3 minutes. The soluble fraction was separated from aggregate by centrifugation at 13,000 RPM at 4 °C for 10 minutes. About 20 ng of soluble protein was resolved on 4-12% bis-tris gel and blotted with anti-EGFR antibodies. The EGFR band intensity as quantified by ImageJ software and plotted (see Figure 5B). The effect of Compound 8C concentration on thermal stability of EGFR at 44 °C was determined in the presence of 0 to 10 μ M Compound 8C. The soluble fraction of EGFR was quantified and plotted as described above (see Figure 5C).

[0191] The preliminary data show that Compound 8C is likely to bind to the same site on the EGFR molecule as Disruptin (see Figure 5A) and can thermally stabilize wild-type as well as osimertinib resistant (C797S) EGFR. As shown in Figures 5B and 5C, Compound 8C incubation with purified wild-type EGFR kinase domain shifts the melting curve, suggesting an interaction between EGFR and Compound 8C. To determine the effect of Compound 8C on osimertinib resistant EGFR mutant, a cell-based assay was used. Osimertinib or Compound 8C was incubated with the whole cell lysate prepared from Ba/F3 (C797S-EGFR) cells. The aliquots were heated to different temperatures in the presence of either DMSO, 10 μ M osimertinib or Compound 8C. After cooling, the samples were centrifuged, and the soluble protein fraction was collected and resolved using immunoblotting with anti-EGFR antibodies (see Figure 6A). Osimertinib was effective in shifting the melting temperature of purified WT-EGFR (see Figure 5B), but it had minimal effect on the C797S mutated EGFR protein (see Figure 6A). As expected, Compound 8C was effective in shifting the melting temperature of osimertinib resistant EGFR. These data suggest a potential interaction between Compound 8C with EGFR, even in EGFR-C797S.

[0192] Based on these data it was hypothesized that Compound 8C will remain effective in cells that have acquired resistance to osimertinib due to C797S mutation. Using these isogenic 6-Ba/F3 cell types (that includes TKI sensitive and resistant cells, Table I) it was demonstrated that Compound 8C remains active in the nanomolar range in each cell line. This is expected from this class of agents as their action is independent of EGFR kinase mutations. The effect of Compound 8C on inhibition of EGF-induced EGFR dimerization and protein expression was also confirmed in all these cells. The data shown are from the osimertinib resistant Ba/F3 cells with a C797S mutation (see Figures 6B-6E).

Example 49 - Validation of selective activity of Compound 8C in lung cancer cell lines

[0193] To test the selectivity of Compound 8C, osimertinib resistant lung cancer cell lines (PC9-AZR and HCC827-AZR), along with normal lung fibroblasts (MRC5), were treated with different concentrations, and cell survival was determined using a clonogenic survival assay (see Figure 7A and Table 3, below). These preliminary data suggest that Compound 8C

selectively kills cancer cells that are driven by EGFR. It was determined if the response to Compound 8C correlates with inhibition of EGF-induced dimerization and degradation of EGFR. For this, PC9-AZR cells were treated for 1 hour with 1 μ M Compound 8C and an additional 30 minutes with EGF (30 ng/ml) and 30 minutes with disuccinimidyl suberate (DSS, 150 μ M) to cross-link interacting proteins. Lysates were prepared and immunoblotted with anti-EGFR antibodies. Figure 7B shows that 1 μ M Compound 8C can inhibit EGFR dimerization and induce degradation of EGFR. Overall, these results confirm the findings from the Ba/F3 cells.

Example 50 - Viability assay

[0194] The viability of cells upon treatment was assessed by CellTiter-Blue® reagent following the manufacturer's protocol in RKO, UM10B, UM1, MCR5, and UMCC92 cells. Briefly, 10,000 cells were plated in 96-well plate in quadruplets. One day after seeding, cells were treated with a range of concentrations (0.1 to 30 micromolar). 3-days post-treatment, cells were incubated with the CellTiter-Blue® reagent for 4 hr. Only the viable cells convert the redox dye (reszurin) into a fluorescent product (resofurin). The emission of fluorescence (excitation 560 nm) was measured at 590 nm. The IC₅₀ value was calculated as the mean concentration of compounds required to inhibit cell proliferation as measured by the fluorescence at 590 nm by 50 percent compared to the vehicle-treated controls. Results are presented in Table 3 and Table 4, below.

Table 3

ID #	Cell Titer Blue		Clonogenic IC ₅₀ (μ M)				
	% Cell viable (24 hrs)	Conc (μ M)	RKO	UM10B	UM1	MCR5	UMCC92
13	59.4	30	25.2	6.9		335	
14	99.7	30		465		28	
15	119	30	50.7	17.2		37.0	
16	122	30	43.9	38.0		1316	
17	109	30		44.0		61.0	
18	48	30	6.7	2.5		25.9	
19	68	7.5	3.1	2.6		4.1	
20	54	15	1.8	4.0		2.4	
21	73	15	85	4.3		0.3	
22	55	7.5	0.8	0.3		2.3	0.2
23	103	30	2.2	5.3		5.4	
24	72	30	5.4	4.3		7.6	
25	78	30	3.7	2.6		69.3	2.1
26	88	30	2.6	1.1		1.3	
27	76	30	19	8.8		4.1	
28	15	15	6.5	1.0		1.3	
29	89	30	1.4	0.7		3.3	
8A	58	30	8.8		1.0		

ID #	Cell Titer Blue		Clonogenic IC ₅₀ (μM)				
	% Cell viable (24 hrs)	Conc (μM)	RKO	UM10B	UM1	MCR5	UMCC92
8B	50	15	5.3		0.8		
8C	68	3.75	1.6		0.5	18.6	0.2
8D	64	7.5	7.3		0.8		
8E	84	30	14.1		1.9		
8F	62	30	17.4		1.0		
8G	40	30	8.5		1.6		
10	81	30	21.8		1.7		
11	52	30	26		1.3		
12	78	30	34		1.2		
30	112	30		6.5		73	
31	99	30		7.7		7.8	
32	104	30	8	4		15	
3	70	30	9.8		7.8		
4	61	15	7.6		1.0		
5	60	15	11.6		0.9		34

Table 4

ID #	BAF3-EGRF LTC IC ₅₀ μM
33A	0.45
33B	0.82
33C	0.79
33D	0.71
33E	0.61
33F	0.50
33G	2.6
33H	0.85
33I	1.75
33J	1.95
33K	2.20
33L	2.85
34A	2.00
34B	2.60

ID #	BAF3-EGRF LTC IC ₅₀ μM
34C	6.50
34D	1.42
34E	4.80
34F	0.70
35A	0.56
35B	0.80
35C	0.55
35D	0.28
35E	1.13
35F	2.55
35G	5.95
35H	1.85
35I	1.57

Example 51 - *In vivo* pharmacokinetics of Compound 8C in tumor and plasma

[0195] A short-term PK study administering the agent by i.p. injection revealed that Compound 8C selectively accumulates in the tumor in spite of the fact that it is rapidly cleared from the plasma (see Figures 8A and 8B). The peak concentration of Compound 8C achieved in the tumor ($69.7 \pm 15.68 \mu\text{M}$ for ≥ 7 hours) is far greater than the concentration needed to kill 99% of TKI resistant tumor cells. The IC₅₀ and IC₉₀ for PC9-AZR, and HCC827-AZR, range from ~ 1.5 to $\sim 4 \mu\text{M}$ (see Figure 7A). It is interesting to note that the peak plasma concentration was $5.4 \pm 1.5 \mu\text{M}$ for 30 minutes. The plasma half-life of

Compound 8C is 7.96 hours, but the half-life in the tumor is much greater than 24 hours (see Figure 8A). Prolonged drug accumulation in tumors despite rapid systemic clearance is ideal for therapeutic use. The data at the 24 hour time point indicate that the agent may be suitable for daily administration. After observing selective accumulation of Compound 8C in tumors injected i.p., it was determined if Compound 8C is orally available. For oral administration, Compound 8C was formulated in 20% tween80 in PBS (v/v) after brief sonication. Mice received 100 mg/kg by oral gavage. The concentrations of Compound 8C in tumor and plasma samples at 7 hours, 15 hours and 24 hours were determined and are plotted in Figure 8B.

[0196] For intraperitoneal injection Compound 8C was formulated at 10 mg/mL in PBS with 5% DMSO by adjusting the pH to 5.5. For oral administration, a homogenous suspension was prepared in 20% tween 80 upon brief sonication. Initially, a single 100 mg/kg dose of Compound 8C was given i.p. (Figure 8A) or gavaged into nude mice bearing human NCI-H1975 tumor xenografts (>150 mm³) (Figure 8B). Mice were euthanized at time 0, 30 minutes, 1 hour, 3 hours, 7 hours, 15 hours, and at 24 hours. Tumor and plasma samples were collected. In case of mice orally gavaged, plasma and tumor samples were collected only at 7 hours, 15 hours and at 24 hours. The concentration of Compound 8C in plasma and tumor was determined and the resulting data are plotted in terms of molar concentration.

Example 52 - Validation of EGFR reporter *in vivo*

[0197] This approach was validated with compound C95. Briefly, once the tumors reached the size of about 100 mm³, mice were imaged to obtain the basal bioluminescence and effect of pre-lead compound 95 on different time points (see Figure 9A). Change in Bioluminescence was quantified and plotted (see Figure 9B). Finally, the effect of treatment on EGFR protein level was confirmed by immunoblotting after 48 hours of treatment.

Example 53 - *In vivo* activity of Compound 8C

[0198] Nude mice bearing either UMSCC74B (~100 mm²) were treated with (30 mg/kg, daily for one week) or with vehicle (5% DMSO in PBS). Each group had at least 5 mice. Tumor volume and body weight was recorded 3-4 times a week, and change in the average tumor volume with time is plotted. The average loss in body weight during treatment was less than 10%. Error bar represents standard error of the mean.

[0199] For the Compound 8C treatment group, day 0 is defined as the first day of treatment. In vehicle control mice, day 0 was defined as the day when the tumor volume was closest to the mean tumor volume in Compound 8C treatment groups on the day of treatment initiation. To assess whether tumor volume growth rates differed by treatment, mixed effect models were fit with random intercept terms at the mouse levels to account for

correlated outcomes over time within a tumor and between 2 tumors within a mouse. Monotherapy elicited a significant reduction in tumor growth rate after one week of dosing (see Figure 10). Tumor growth rates were significantly lower in the Compound 8C treatment group compared to the time-matched control group ($p=0.013$) and the overall control group ($p<0.001$).

Example 54 - Effect of Compound 8C in an osimertinib resistant tumor model.

[0200] To test the activity of Compound 8C against osimertinib resistant EGFR driven tumors, an ascites tumor model was developed using Ba/F3-AZR cells (L858R+T790M+C797S-EGFR) as reported previously (65). 5 million, BA/F3-AZR cells were injected via i.p. injection into 6-week old female nude mice. Mice developed an ascitic tumor, and a mean survival time of 20 days was observed. To test the efficacy of Compound 8C compared to osimertinib, injected 15 mice were injected with Ba/F3-AZR cells. 18 days after injection of tumor cells, mice were randomized into three groups. Mice were treated with vehicle, a single oral dose of 30 mg/kg osimertinib, or 30 mg/kg Compound 8C via i.p. injection. The health of mice was monitored and mice were euthanized according to ULAM end-stage guidelines. Although treatment was initiated after the tumor burden became high, a single 30 mg/kg dose of Compound 8C prolonged life in these mice compared to the osimertinib treated group. There was no difference noted between vehicle control and osimertinib treatment group. The difference between osimertinib and Compound 8C was calculated using log-rank test ($p=0.072$). To test the effect of treatment on target, tumor cells were collected from one mouse before treatment, 18 hours, and 24 hours post-treatment. Cells were washed in PBS and processed as described in Figure 3, and immunoblotting results showing the effect of treatment on EGFR, pEGFR and other molecules are given in Figure 11.

Example 55 - Preliminary safety test of Compound 8C in a mouse model.

[0201] A preliminary test on the safety of a daily dose of 30 mg/kg for one week was performed using C57BL6 mice. The overall health and weight of a group of 6 mice was monitored during treatment. A modest loss of 3.5 ± 2.4 % body weight after one week of treatment was observed, but mice fully recovered the weight 2-days after treatment cessation.

Example 56: NCI 60 cell line screen

[0202] The activity of Compound 8C was tested against 60 different human tumor cell lines at the National Cancer Institute, using the standard NCI 60 screening protocol. The percent growth inhibition for the top performing cell lines is given in Table 5, below.

Table 5

Panel	Cell Line	Percent Growth
Melanoma	SK-MEL-5	-96.2
Colon Cancer	HCT-116	-90.7
Melanoma	M14	-83.8
Renal Cancer	786-0	-81.7
Melanoma	UACC-62	-78.9
Melanoma	LOX IMVI	-78.5
Colon Cancer	COLO 205	-76.2
Melanoma	MALME-3M	-75.5
Melanoma	SK-MEL-28	-70.1
Colon Cancer	HT29	-67.1
Leukemia	K-562	-61.5
Melanoma	UACC-257	-61.2
Colon Cancer	HCC-2998	-51.3
Breast Cancer	MDA-MB-468	-43.8
Breast Cancer	MCF7	-42.7
Leukemia	HL-60(TB)	-40.7
Breast Cancer	MDA-MB-231/ATCC	-40.5

Example 57: Effect of Compound 8C in a pancreatic tumor model

[0203] 6-week old KC mice were treated with Compound 8C via oral gavage (30 mg/kg body weight, daily). The resulting effect on PanIn levels were observed compared to control mice which did not receive Compound 8C. Mice treated with Compound 8C showed significantly reduced propensity for developing PanIn (Pancreatic Intraepithelial Neoplasia), a type of pancreatic duct lesion, as shown in Figure 13.

Example 58: Effect of Compound 8C in a head and neck tumor model

[0204] Mouse xenographs of UMSCC74B, a head and neck tumor cell line, were treated with Compound 8C via oral gavage (30 mg/kg body weight, twice weekly). The resulting effect on tumor volume was observed compared to control mice which did not receive Compound 8C, and control mice which received cetuximab. Mice treated with Compound 8C showed significantly reduced tumor volume compared to both controls, as shown in Figure 14.

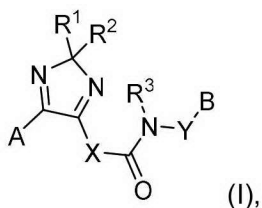
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What is Claimed:

1. A compound, or pharmaceutically acceptable salt thereof, having the structure of Formula I:



wherein

X is O- C₀₋₆alkylene, S- C₀₋₆alkylene, or NR³- C₀₋₆alkylene, and said alkylene is optionally substituted with 1-3 groups independently selected from halo, N(R³)₂, and OR³;

Y is C₀₋₆alkylene, and said alkylene is optionally substituted with 1-3 groups independently selected from halo, N(R³)₂, and OR³;

A is C₆₋₁₀ aryl or 5-10 membered heteroaryl having 1-4 heteroatoms selected from N, O, and S, and said aryl or heteroaryl is optionally substituted with 1 to 3 R⁴;

B is 5-10 membered heteroaryl having 1-4 heteroatoms selected from N, O, and S, and said heteroaryl optionally substituted with 1 to 3 R⁵;

R¹ and R² are each independently C₁₋₆ alkyl, or R¹ and R² together with the carbon atom to which they are attached form a 4-8 membered cycloalkyl or heterocycloalkyl ring, wherein the heterocycloalkyl ring has 1 or 2 ring heteroatoms selected from O, S, and N, and wherein said cycloalkyl ring or heterocycloalkyl ring is optionally substituted with 1-2 R⁶;

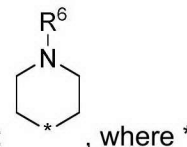
each R³ is independently H or C₁₋₆ alkyl;

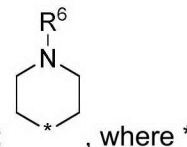
each R⁴ and R⁵ is independently C₁₋₆ alkyl, C₁₋₆ haloalkyl, halo, or C₁₋₆ alkoxy; and

R⁶ is C₁₋₆ alkyl, C₁₋₆ haloalkyl, (C=O)R³, (C=O)OR³, CON(R³)₂, C₀₋₃alkylene-C₃₋₈cycloalkyl, C₀₋₃alkylene-C₆₋₁₀aryl, or C₀₋₃alkylene-(5-10 membered heteroaryl having 1-4 heteroatoms selected from N, O, and S), wherein the aryl or heteroaryl is optionally substituted with 1 to 3 R⁵.

2. The compound or salt of claim 1, wherein R¹ and R² together with the carbon atom to which they are attached form a 4-8 membered cycloalkyl or heterocycloalkyl ring.

3. The compound or salt of claim 2, wherein R¹ and R² together with the carbon atom to



which they are attached form a heterocycloalkyl ring having the structure: , where * indicates the point of attachment to the rest of the compound of Formula I.

4. The compound or salt of claim 2 or 3, wherein R⁶ is C₁₋₆ alkyl.

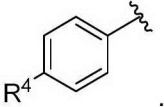
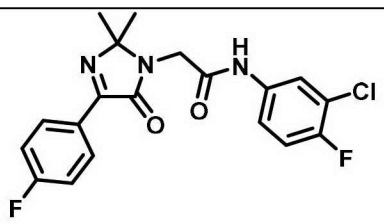
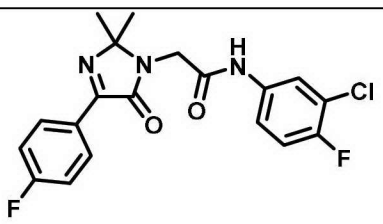
5. The compound or salt of claim 4, wherein R⁶ is methyl.
6. The compound or salt of any one of claims 1 to 5, wherein A is C₆₋₁₀ aryl.
7. The compound or salt of claim 6, wherein A is phenyl.
8. The compound or salt of any one of claims 1 to 7, wherein B is quinolinyl.
9. The compound or salt of any one of claims 1 to 8, wherein A is substituted with one R⁴.
10. The compound or salt of claim 9, wherein A has the structure: .
11. The compound or salt of any one of claims 1 to 10, wherein at least one R⁴ is halo.
12. The compound or salt of any one of claims 1 to 11, wherein X is O- C₀₋₆alkylene or S- C₀₋₆alkylene.
13. The compound or salt of claim 12, wherein X is S- C₀₋₆alkylene.
14. The compound or salt of any one of claims 1 to 13, wherein Y is C₀₋₂alkylene.
15. The compound of claim 14, wherein X is NR³-CH₂, O-CH₂-, or S-CH₂-, and Y is null.
16. The compound or salt of any one of claims 1 to 15, wherein at least one R³ is H.
17. A compound selected from those recited in Table 1 or Table 2, or a pharmaceutically acceptable salt thereof:

Table 1

ID #	Structure
13	

ID #	Structure
14	

ID #	Structure
15	
16	
17	
18	
19	
20	

ID #	Structure
21	
22	
23	
25	
26	

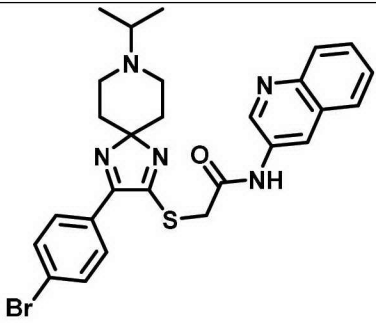
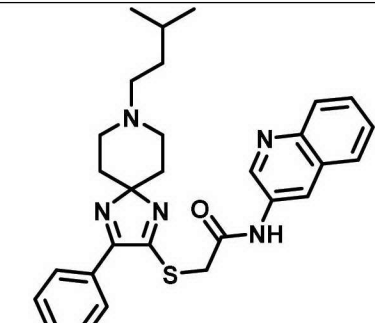
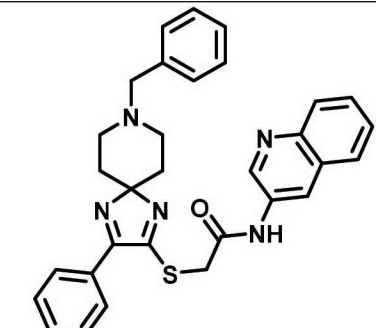
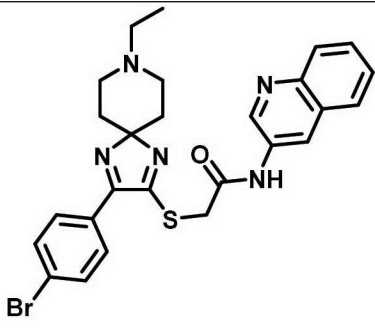
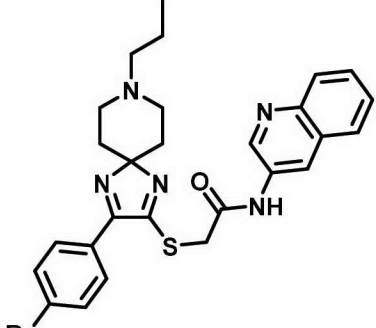
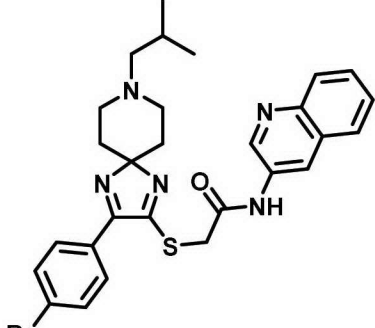
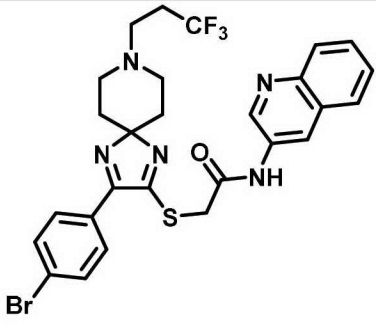
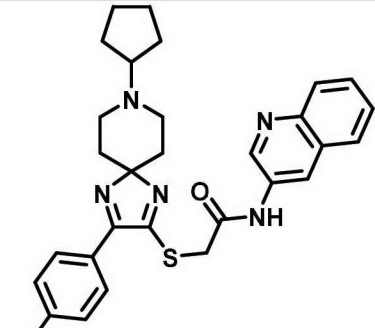
ID #	Structure
27	
28	
29	
8A	
8B	

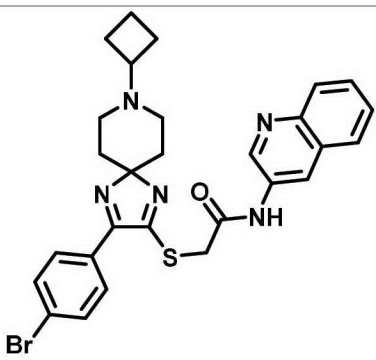
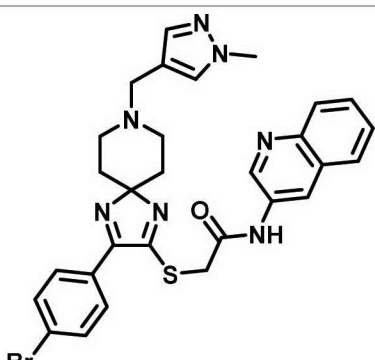
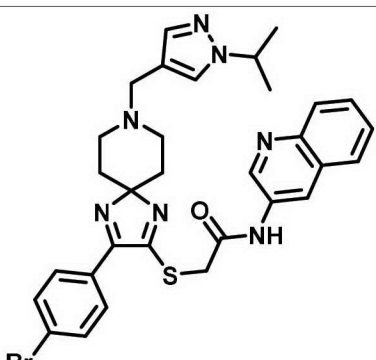
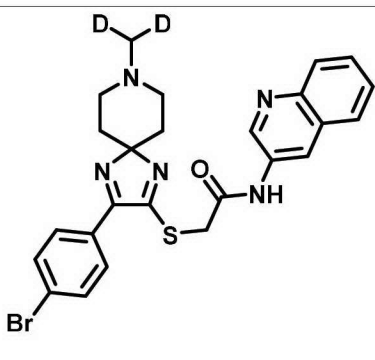
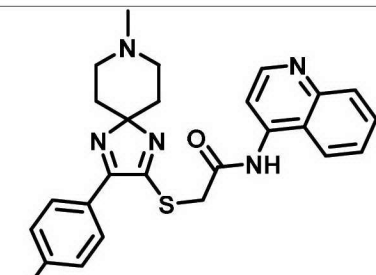
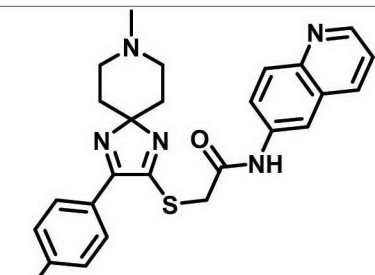
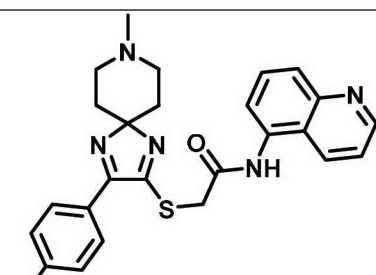
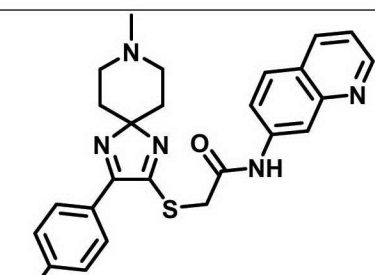
ID #	Structure
8C	
8D	
8E	
8F	
8G	

ID #	Structure
10	
11	
12	
30	
31	

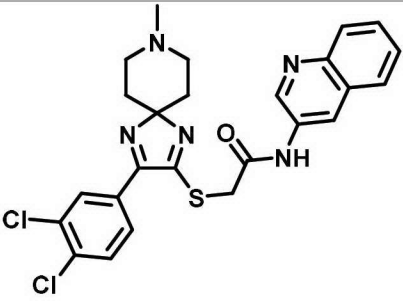
ID #	Structure
32	
3	
4	
5	

Table 2

ID#	Structure	ID#	Structure
33a		33b	
33c		33d	
33e		33f	
33g		33h	

ID#	Structure	ID#	Structure
33i		33j	
33k		33l	
34a		34b	
34c		34d	

ID#	Structure	ID#	Structure
34e		34f	
35a		35b	
35c		35d	
35e		35f	
35g		35h	

ID#	Structure	ID#	Structure
35i			

18. A pharmaceutical composition comprising the compound or salt of any one of claims 1 to 17 and a pharmaceutically acceptable carrier or excipient.
19. A method of treating or preventing a disease or disorder associated with aberrant EGFR activity, comprising administering to a subject in need thereof a therapeutically effective amount of the compound or salt of any one of claims 1 to 18.
20. Use of the compound or salt of any one of claims 1 to 18, in the manufacture of a medicament for treating or preventing a disease or disorder associated with aberrant EGFR activity.
21. The method or the use of claim 19 or 20, wherein the disease or disorder is cancer.

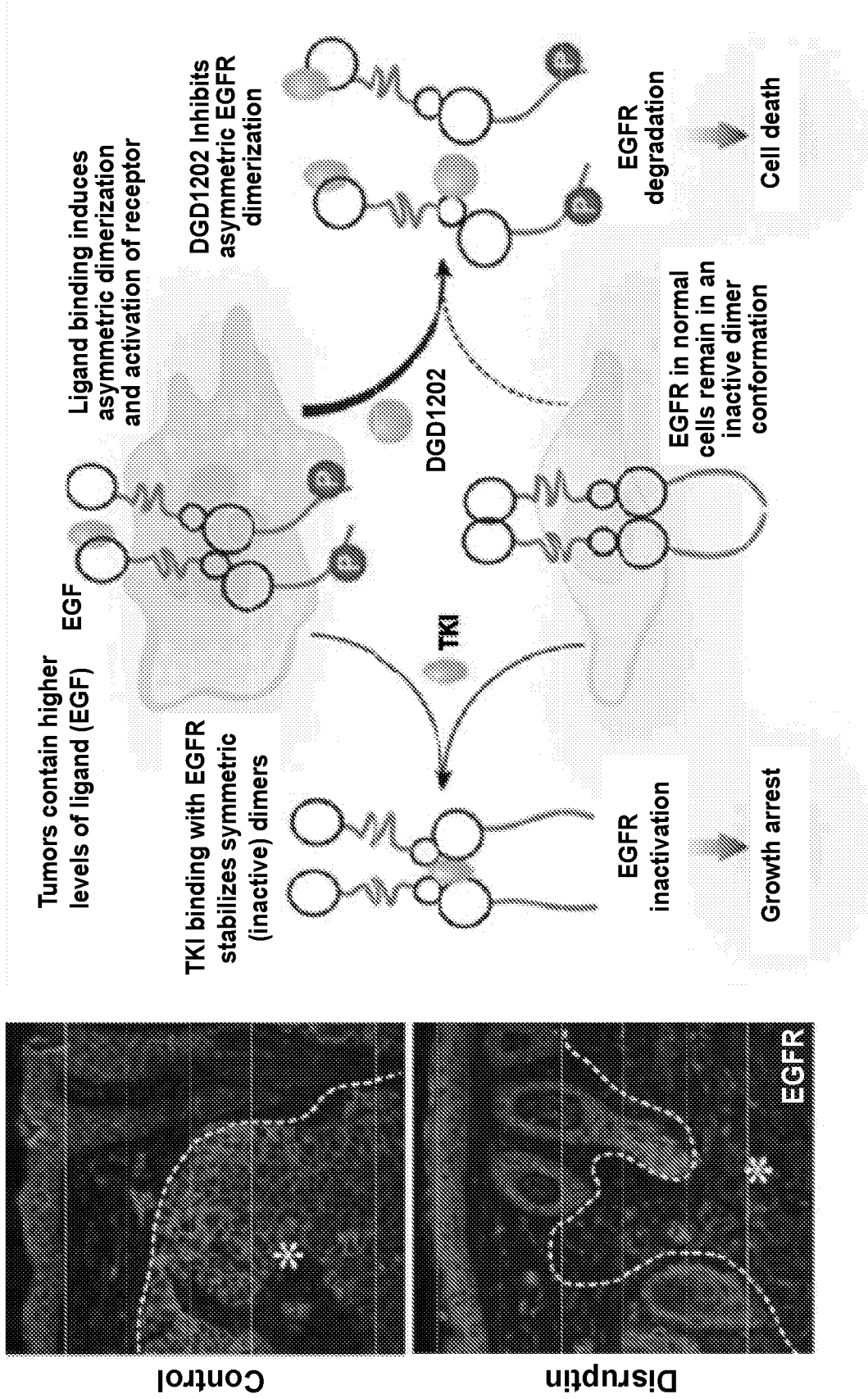


Figure 1

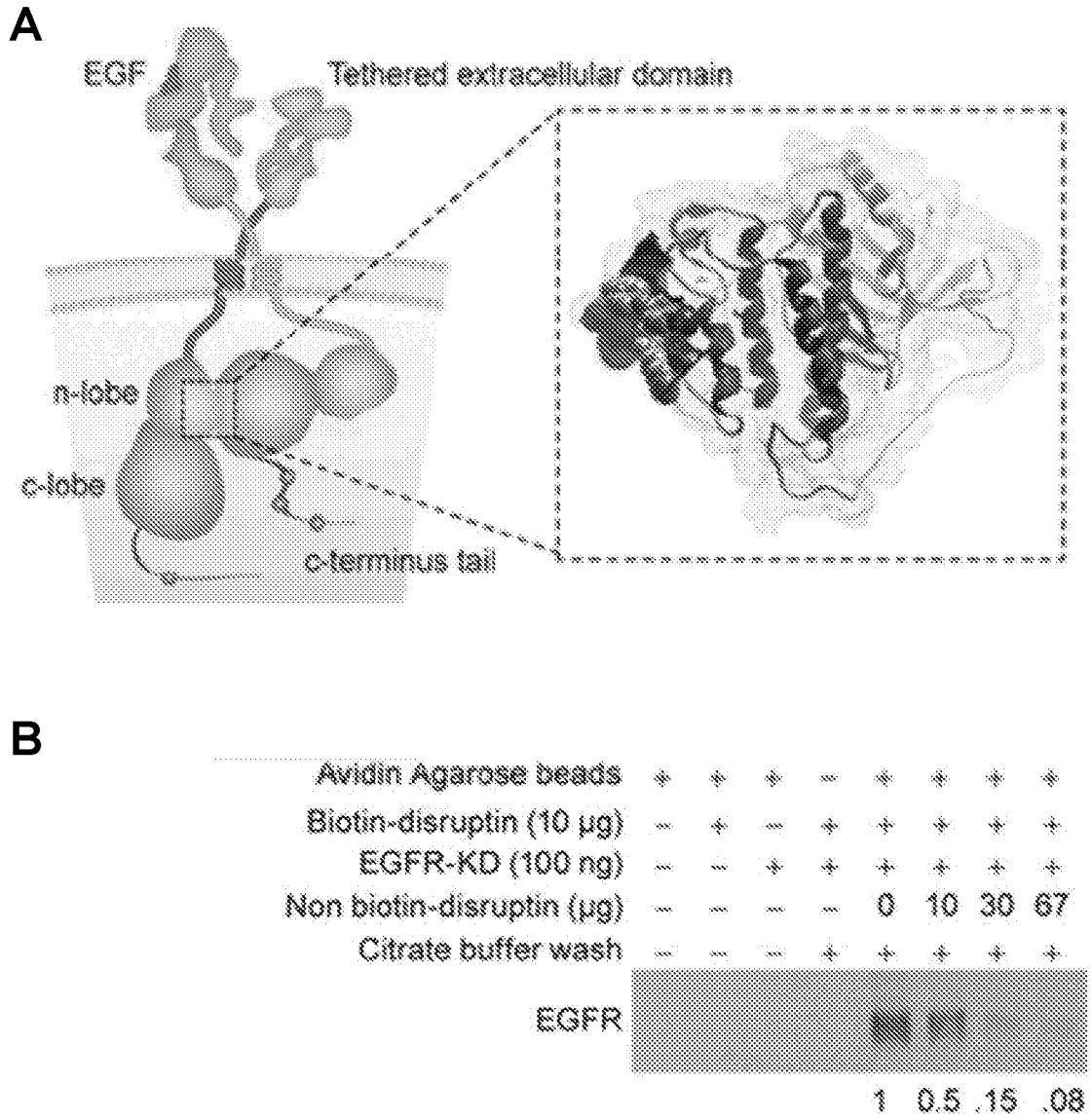


Figure 2

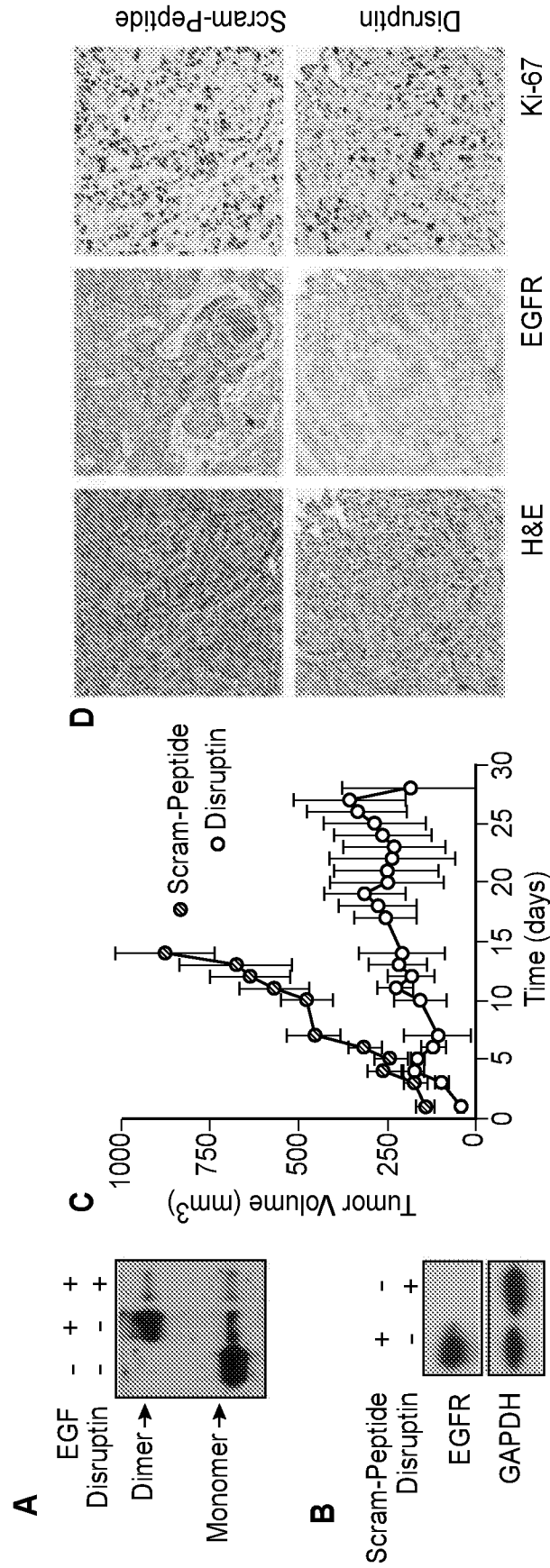


Figure 3

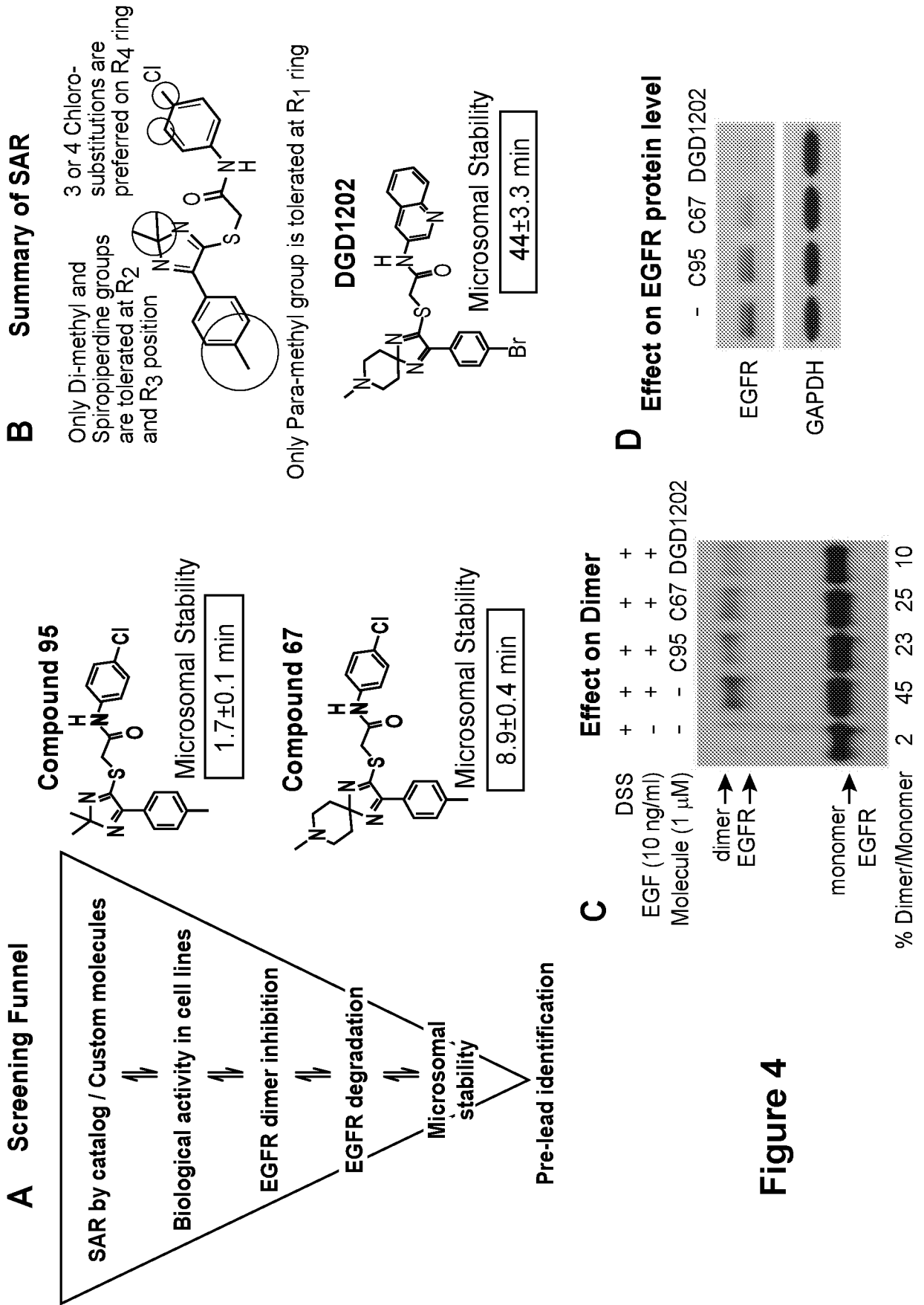
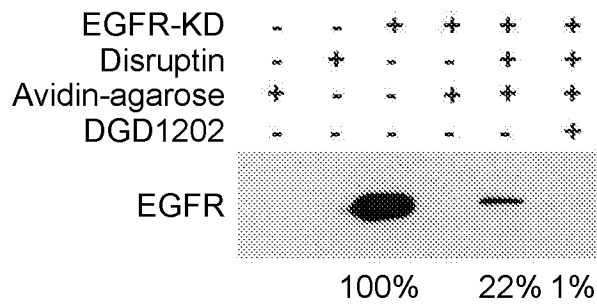


Figure 4

A DGD1202 competes with Disruptin for EGFR binding



DGD1202 enhances thermal stability of purified EGFR

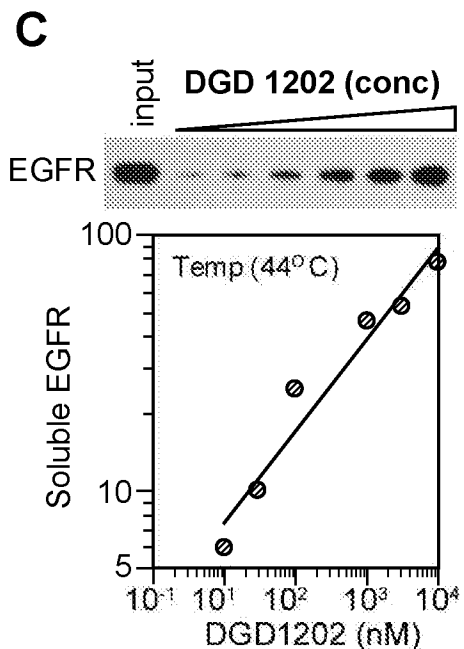
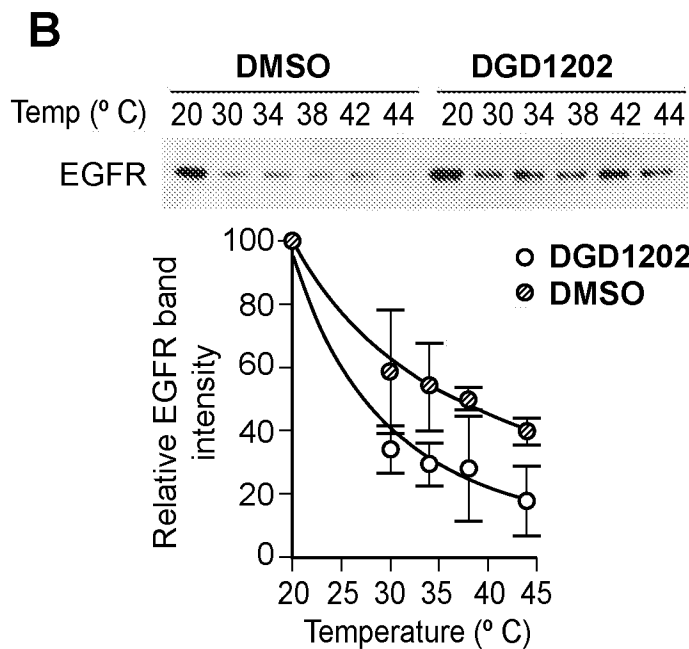


Figure 5

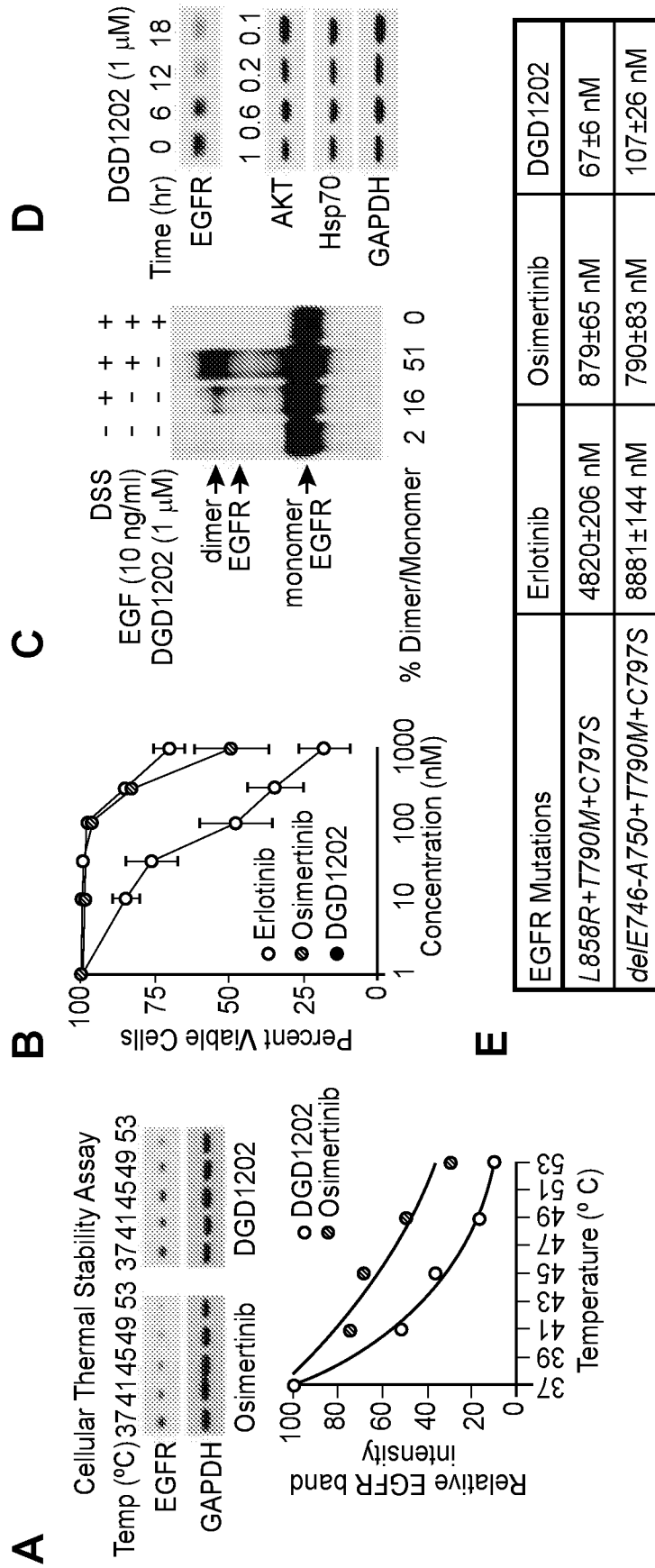


Figure 6

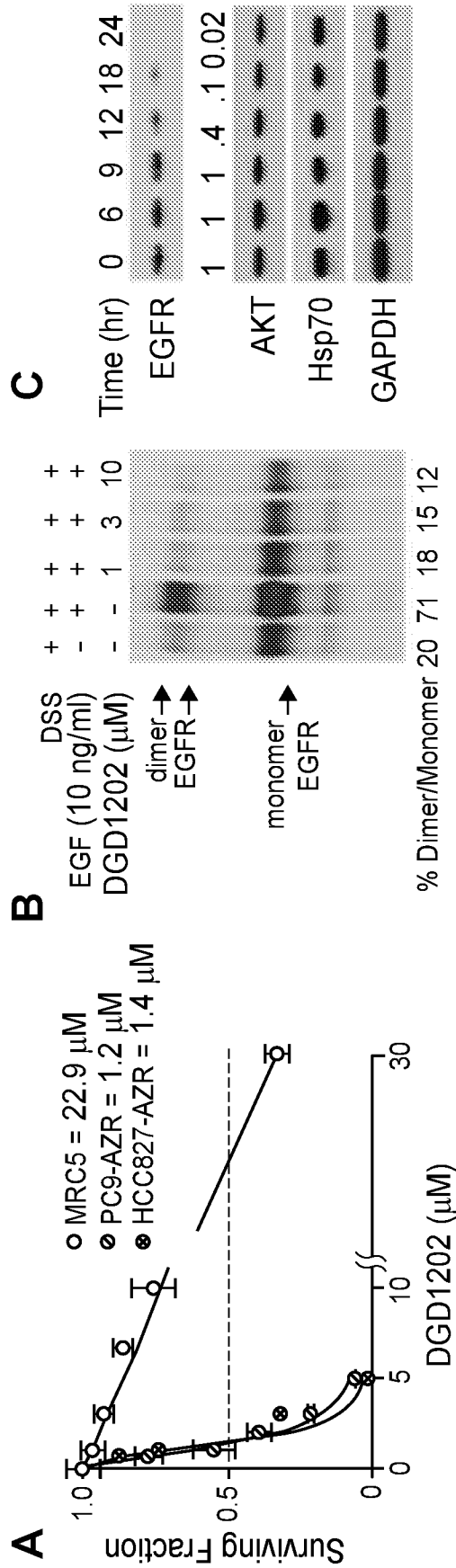


Figure 7

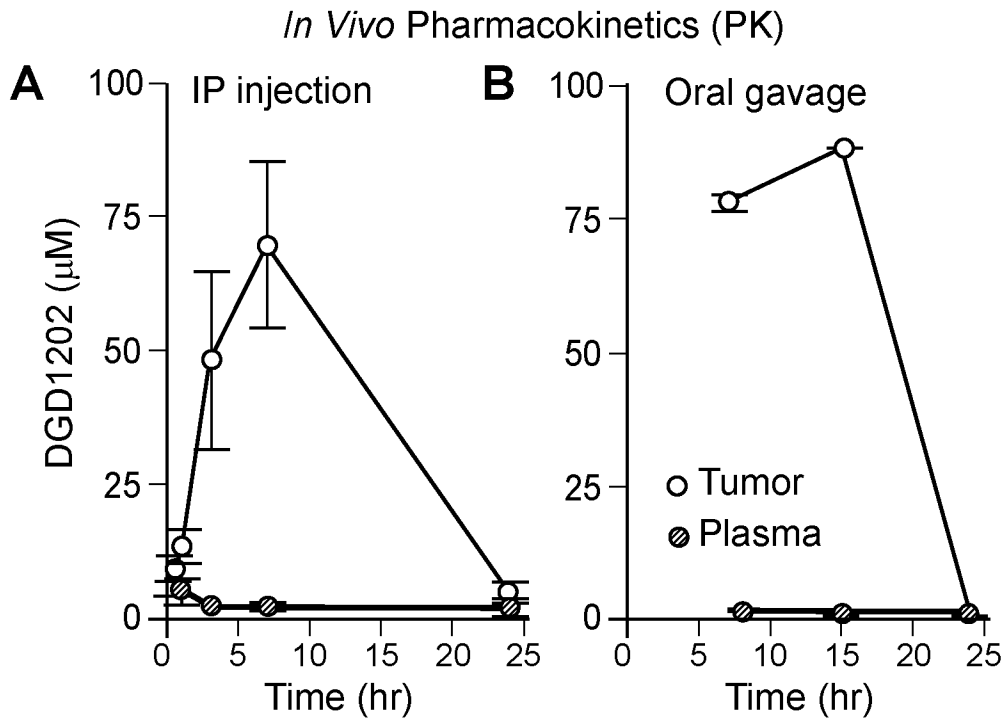


Figure 8

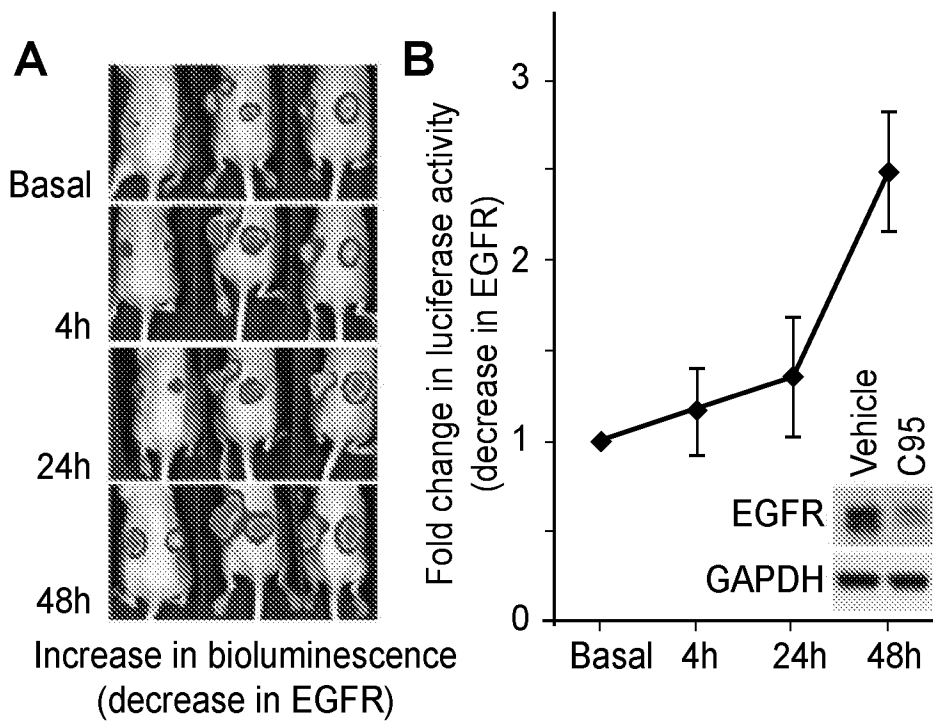


Figure 9

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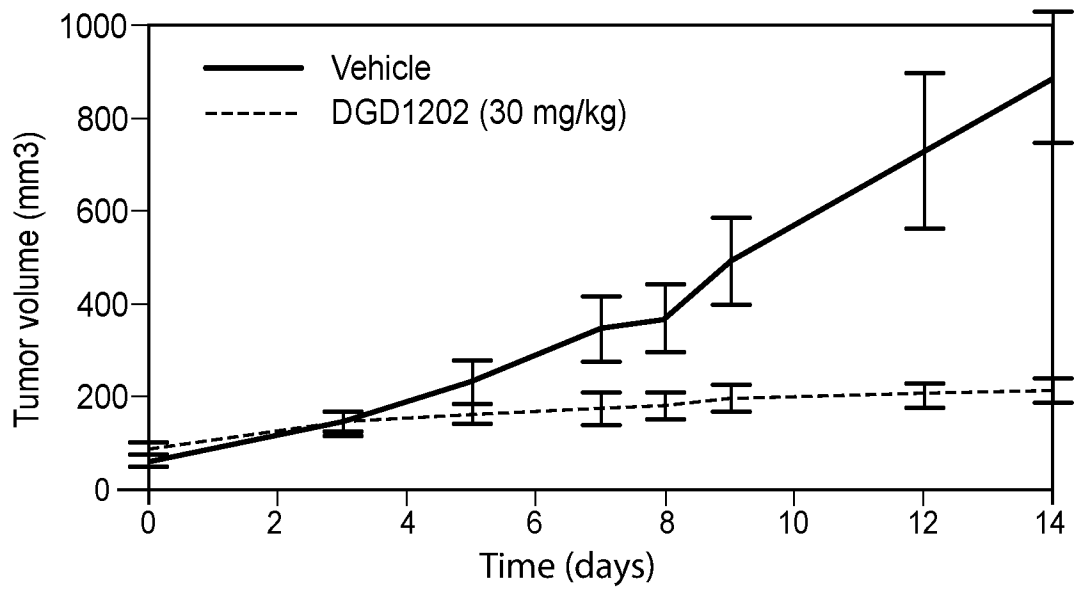


Figure 10

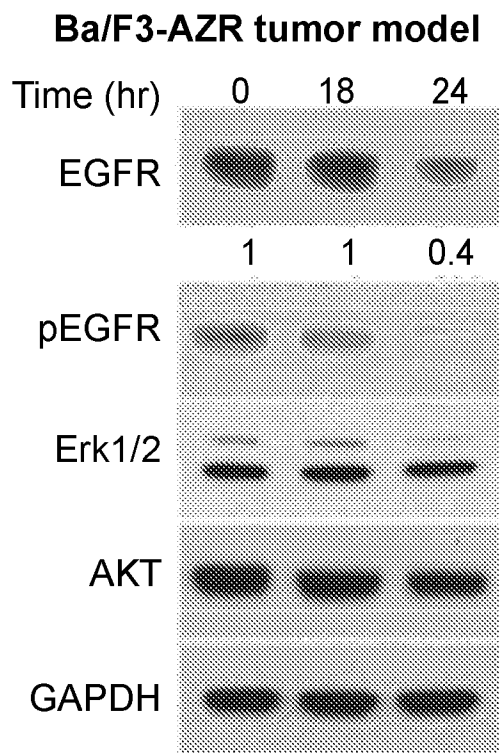


Figure 11

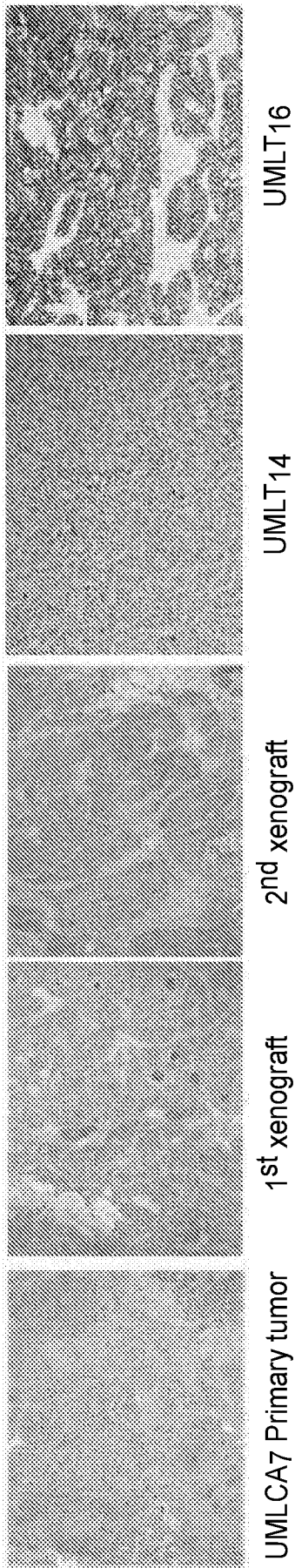


Figure 12

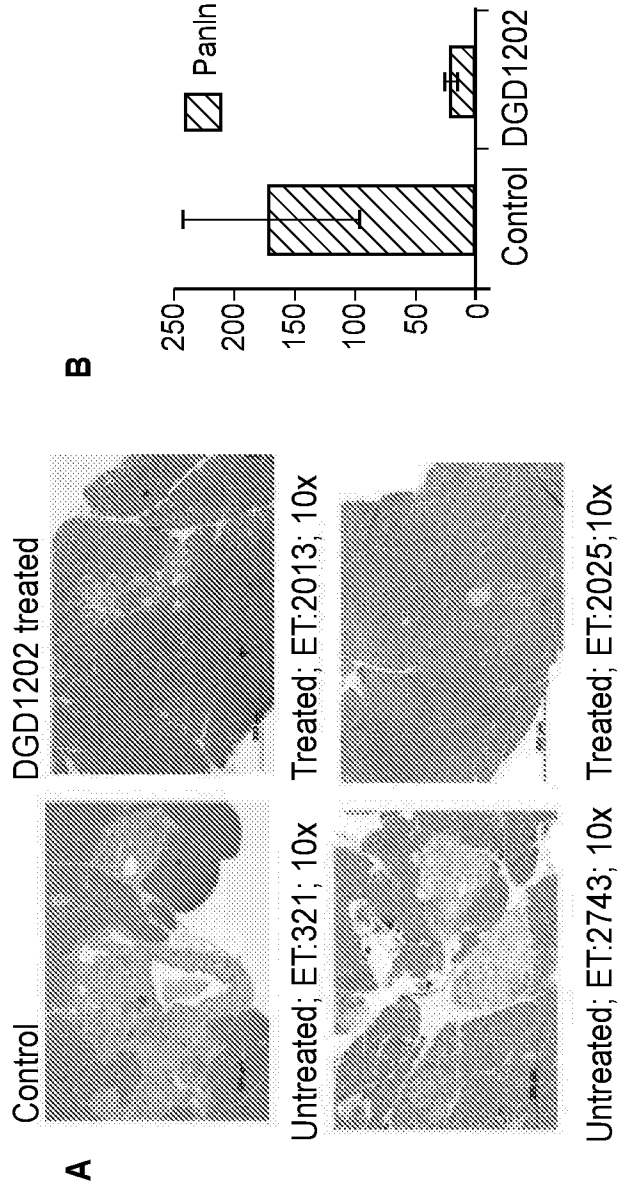


Figure 13

11/11

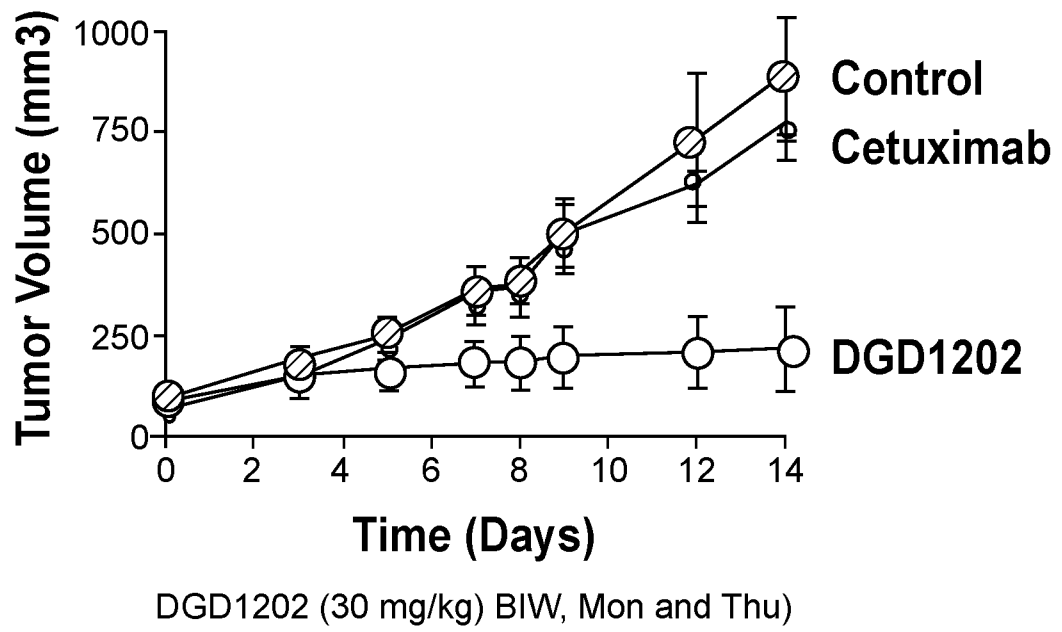


Figure 14